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## Recognition of *Bromus Richardsonii* and *B. Ciliatus*: Evidence from Morphology, Cytology, and DNA Fingerprinting (Poaceae: Bromeae)

Paul M. Peterson  
*Smithsonian Institution*

Jacques Cayouette  
*Agriculture and Agri-Food Canada*

Yasas S. N. Fernandez  
*Agriculture and Agri-Food Canada*

Bruce Coulman  
*Agriculture and Agri-Food Canada*

Ralph E. Chapman  
*Smithsonian Institution*

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RECOGNITION OF *BROMUS RICHARDSONII* AND *B. CILIATUS*: EVIDENCE FROM MORPHOLOGY, CYTOLOGY, AND DNA FINGERPRINTING (POACEAE: BROMEAE)

PAUL M. PETERSON

*Department of Systematic Biology—Botany, National Museum of Natural History  
Smithsonian Institution, Washington, DC 20560-0166  
e-mail: peterson.paul@nmnh.si.edu*

JACQUES CAYOUE

*Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada  
Central Experimental Farm, Wm Saunders Bldg., Ottawa, ON K1A 0C6, CANADA  
e-mail: cayouettej@EM.AGR.CA*

YASAS S. N. FERDINANDEZ AND BRUCE COULMAN

*Forage Crop Breeding and Genetics, Agriculture and Agri-Food Canada  
Saskatoon Research Centre, 107 Science Place  
Saskatoon, SK S7H 2V3, CANADA  
e-mail: ferdinandezy@EM.AGR.CA, coulmanb@EM.AGR.CA*

AND

RALPH E. CHAPMAN

*Applied Morphometrics Laboratory, ADP EG-15 NHB  
National Museum of Natural History  
Smithsonian Institution, Washington, DC 20560-0136  
e-mail: chapman.ralph@nmnh.si.edu*

ABSTRACT

Since our goal was to determine characteristic differences between *Bromus richardsonii* and *B. ciliatus*, a discriminate analysis (DA), principal components analysis (PCA), multidimensional scaling (MDS), bivariate analysis, and an amplified fragment length polymorphisms (AFLP) analysis were undertaken on 93 herbarium specimens and 31 field-collected populations. A cytological survey of *B. ciliatus*, *B. richardsonii*, and *B. mucroglumis* confirm previous reports that the first species is diploid ( $2n = 14$ ) and the latter two are tetraploid ( $2n = 28$ ). All taxa were correctly classified in the DA and important characters for each of the species were identified. *Bromus richardsonii* has lemmas with scattered hairs on the lower half between the midnerve and margins [glabrous in *B. ciliatus*], anthers (1.2) 1.6–2.7 (3.4) mm long [(0.9) 1–1.4 (1.6) mm long in *B. ciliatus*], second glumes (7.8) 8.9–11.3 (13.2) mm long [(6.2) 7.1–8.5 (9.5) in *B. ciliatus*]; and basal sheaths with dense, short to medium hairs [glabrous or with long hairs in *B. ciliatus*]. The PCA easily separated *B. ciliatus* and *B. richardsonii* into two well-defined groups and MDS mirrored the principal components analysis but displayed more overlap of individuals between the two groups. The AFLP-derived UPGMA dendrogram separated 154 individuals into two distinct clusters, one consisting entirely of *B. ciliatus* individuals and the other consisting of *B. richardsonii* individuals with six individuals of *B. mucroglumis* embedded within. Our study clearly indicates that there are distinctive morphological, cytological, and genetic differences to distinguish *B. richardsonii* and *B. ciliatus* as separate species.

RESUMEN

Puesto que nuestra meta era determinar diferencias características entre *Bromus richardsonii* y *B. ciliatus*, se emprendieron el análisis discriminado (DA), el análisis de los componentes principales (PCA), el escalamiento multidimensional (MDS), el análisis bivalente, y un análisis de los polimorfismos de la longitud de fragmentos amplificados (AFLP) en 93 especímenes de herbario y 31 poblaciones naturales. Una encuesta citológica de *B. ciliatus*, *B. richardsonii*, y *B. mucroglumis* confirman previos informes que la primera especie es diploide ( $2n = 14$ ) y las últimas dos son tetraploides ( $2n = 28$ ). Todos taxones fueron correctamente clasificados en el DA e importantes caracteres para cada una de las especies fueron identificados. *Bromus richardsonii* presenta lemas con pelos dispersos en

la parte baja entre el nervio medio y las márgenes [glabra en *B. ciliatus*], anteras (1,2) 1,6–2,7 (3,4) milímetros de largo [(0,9) 1–1,4 (1,6) milímetros de largo en *B. ciliatus*], glumas secundarias (7,8) 8,9–11,3 (13,2) milímetros de largo [(6,2) 7,1–8,5 (9,5) en *B. ciliatus*]; y vaina basal con pelo denso, corto a medio [glabra o con pelos largos en *B. ciliatus*]. El PCA separó fácilmente a *B. ciliatus* y *B. richardsonii* en dos grupos bien definidos y el MDS también reflejaba el análisis de los principales componentes pero mostraba más individuos superpuestos entre los dos grupos. El dendrograma UPGMA derivado del AFLP separó 154 individuos en dos grupos marcados, uno consistiendo enteramente de individuos de *B. ciliatus* y el otro consistiendo de individuos de *B. richardsonii* con seis individuos de *B. mucroglumis* implantados. Nuestro estudio indica claramente que hay marcadas diferencias morfológicas, citológicas y genéticas para distinguir a *B. richardsonii* y *B. ciliatus* como especies separadas.

**Key words:** Amplified fragment length polymorphisms (AFLP), Bromaceae, *Bromus*, cytology, discriminant analysis (DA), DNA fingerprinting, morphology, multidimensional scaling (MDS), phenetics, Poaceae, Pooideae, principal components analysis (PCA).

## INTRODUCTION

*Bromus* L. is a large genus of at least 150 species that occur throughout the world primarily in temperate regions (Clayton and Renvoize 1986; Pavlick 1995; Watson and Dallwitz 1992). Along with *Boissiera* Hochst. ex Steud. and *Littledalea* Hemsl., *Bromus* has been placed in its own tribe, Bromaceae, characterized by fused leaf sheath margins more than ¼ their length (Clayton and Renvoize 1986; Soreng and Davis 1998). More recent classifications of the Poaceae align the Bromaceae near the Triticeae (both tribes in subfamily Pooideae) by possessing simple endosperm starch grains and a hairy ovary with free style branches (Hsiao et al. 1999; Macfarlane and Watson 1982; Soreng and Davis 1998; Soreng et al. 2000). *Bromus* section *Bromopsis* Dumort., which includes *Bromus ciliatus* L. and *Bromus richardsonii* Link, is comprised of at least 26 species in North America (including Mexico; NA), six species in Central America (all shared with NA), and 12 species indigenous to South America (14 in total, 2 shared with NA) for a total of 38 species in the Western Hemisphere (Pavlick 1995; Planchuelo and Peterson 2000; Pohl and Davidse 1994; Soderstrom and Beaman 1968; Wagnon 1952). This section (*Bromus* sect. *Bromopsis*) contains mostly perennial (often short-lived) species that have 1(3)-nerved lower glumes, 3(5)-nerved upper glumes, and spikelets that are terete (not laterally compressed) before anthesis (Pavlick 1995; Wagnon 1952). Our purpose is to clarify the species boundary between *B. ciliatus* and *B. richardsonii*.

*Bromus ciliatus* is wide-ranging species often associated with wet meadows, streambanks, bogs, thickets, and occasionally talus slopes and roadsides. It occurs in the Aleutians Islands of Alaska and the Northwest Territories to Newfoundland, Canada south to Maryland, North Carolina, Tennessee, Illinois, Nebraska, Colorado to southern California (Pavlick 1995; Wagnon 1952). It is a diploid with a chromosome number of  $2n = 14$ , as established by Wagnon (1952) who counted 67 individuals mostly collected from the Great Lakes-New England region where it is abundant.

Later reports from Canada and the United States confirmed the diploid status of *B. ciliatus* (Armstrong 1981, 1983; Bowden 1960; Gervais 1979; Löve and Löve 1964; Mitchell and Wilton 1965; Schulz-Shaeffer 1956, 1960; Wilton 1965). Wilton (1965) also reported a diploid count from a Russian collection. Reports of tetraploid *B. ciliatus* from Wyoming (Reeder 1977) and the western United States (Barnett 1955; Elliot 1948, 1949) are probably in error and referable to *B. richardsonii*. A sterile triploid hybrid ( $2n = 21$ ), apparently between *B. ciliatus* and *B. richardsonii*, with intermediate morphological characteristics was discovered in Alaska (Mitchell and Wilton 1965). *Bromus ciliatus* differs from *B. richardsonii* by possessing lemmas that are glabrous or scabrous on the back (although the margins are hairy), lacking a tuft of hairs at the summit of the sheath, and having anthers that are less than 2 mm long (Mitchell 1967; Mitchell and Wilton 1965; Pavlick 1995; Wagnon 1952).

Much confusion surrounded the recognition of *B. richardsonii* in the last century (Mitchell and Wilton 1965), and the name has been treated as a synonym of *B. ciliatus* by Allred (1993), Arnou (1987), Cronquist et al. (1977), Gould and Moran (1981), Hitchcock (1935), Hitchcock and Chase (1951), Soderstrom and Beaman (1968), and Wilken and Painter (1993). Scoggan (1978) and Scholz (1994) treated *B. richardsonii* as a synonym of *B. canadensis* Michx., whereas Boivin (1967, 1981) and Kartesz (1999) recognized *B. ciliatus* var. *richardsonii* (Link) Boivin. Only Shear (1900), Hitchcock (1913), Wootton and Standley (1915), Wagnon (1952), Mitchell and Wilton (1965), Mitchell (1967) and more recently Pavlick (1995) have recognized *B. richardsonii* as a distinct species.

*Bromus richardsonii* is found in open woods, canyons, exposed slopes, along creeks, trails, and roadsides in western North America from Southern Alaska (Little Susitna Valley) and Yukon, British Columbia south to western Texas, then west to Arizona, northern Mexico, and southern California (Mitchell 1967; Mitchell and Wilton 1965; Pavlick 1995; Wagnon 1952). During field collecting in 1998 the second au-

thor also discovered it in the Cypress Hills of southern Saskatchewan and adjacent Alberta. Unlike *B. ciliatus*, *B. richardsonii* is a confirmed tetraploid ( $2n = 28$ ), as established by Armstrong (1981, 1983), Mitchell and Wilton (1965), Ward and Spellenberg (1988) and Wagnon (1952), who surveyed 28 individuals from Alaska, Arizona, British Columbia, Colorado, New Mexico, and Wyoming. *Bromus richardsonii* can be separated from *B. ciliatus* by having lemmas with short appressed hairs on the back (in addition to hairy margins), a tuft of hairs at the summit of the sheath, and anthers that are generally more than 2 (2–3.5) mm long (Mitchell 1967; Mitchell and Wilton 1965; Pavlick 1995; Wagnon 1952).

The autecology of *B. ciliatus* and *B. richardsonii* also differs. Cayouette et al. (1997) found differences in plant height, winter survival, phenology (heading and maturity), clump diameter, and seed yield. Individuals of *B. richardsonii* had fewer and more erect branches, denser foliage, glaucous-green leaves (yellow-green leaves in *B. ciliatus*), and greater rust resistance than individuals of *B. ciliatus* (Cayouette and Coulman unpubl.).

*Bromus mucroglumis* Wagnon, a species ranging from southwestern New Mexico to the San Francisco Mountains of Arizona south to Chihuahua and Sonora, Mexico, and then known from the Sierra de la Laguna (Baja California Sur, Mexico), was included in this study since it may be a possible introgressive hybrid with *B. richardsonii* (Wagnon 1950, 1952). After counting seven individuals from Arizona and Baja California Sur, Wagnon (1952) concluded that *B. mucroglumis* is also a tetraploid at  $2n = 28$ . Since its original description by Wagnon in 1950, *B. mucroglumis* has not generally been recognized as a distinct species until recently resurrected by Pavlick (1995), Scholz (1994), and Kartesz (1999). We have only a cursory sampling of *B. mucroglumis* and expect to more fully investigate its relationship with other members of *Bromus* section *Bromopsis* in the future.

The present study was undertaken to clarify the distinctness of *B. richardsonii* and *B. ciliatus* by studying cytology, ecology, morphology, and molecular markers. The investigation involved: (1) determining chromosome numbers; (2) a numerical analysis based on morphological characters from a field study and herbarium specimens, and (3) a molecular study using AFLP markers to distinguish individuals and populations.

#### MATERIALS AND METHODS

##### Plant Material

Herbarium vouchers and seeds were collected from 31 localities for *B. ciliatus*, *B. mucroglumis*, and *B. richardsonii* (Appendix 1; Fig. 1). Field collections

were made from Quebec and Ontario in the east, Saskatchewan, Alberta, and British Columbia in the west, then south to California and Arizona. Most of the collections obtained between 1993 and 1999 were gathered for germplasm screening for forage potential and ecovar development at the Agriculture and Agri-Food Saskatchewan Research Center, Plant Gene Resources of Canada and Ducks Unlimited Canada (Cayouette and Coulman unpubl. data). In addition, 62 herbarium specimens were selected from throughout the range of *B. ciliatus* and *B. richardsonii* including some type material: New York, *Heady 768* (DAO), neotype of *B. ciliatus* L.; Newfoundland, *Despréaux s.n.* (P), holotype of *B. denseciliatus* Steud. (= *B. ciliatus*—Cayouette in prep.); Kurile Islands, *Ohwi 1080* (US), isotype of *B. yezoensis* Ohwi (= *B. ciliatus*); and Arizona, *Wagnon 1520* (US), isotype of *B. mucroglumis* Wagnon (see Appendix 1 and Fig. 1 for complete list of samples and locations). Identification of all specimens used in this study was determined prior to the analyses.

##### Cytology

Cytological observations were carried out on living material from seeds collected in the field between 1993 and 1999 and grown in the Department of Botany greenhouse at the Museum Support Center (see Appendix 1). One specimen per population was used for cytological study. Young root tips were pretreated in cold water and kept at 4°C for at least 18 h to condense the chromosomes (Darbyshire et al. 1992) before being fixed in Farmer's fixative (glacial acetic acid: absolute ethanol, 1:3) for 12–24 h and then stored in 70% ethanol. Staining was in alcoholic hydrochloric acid-carmin for 6 days at room temperature (Snow 1963). Excised root tips were squashed in 45% glacial acetic acid mixed with a few drops of glycerin. Representative cells were counted and photographed with an Olympus BH-2 using Kodak TMAX film. Chromosome counts were made on 1–6 cells from each collection.

##### Morphological Data and Analyses

Data Matrix: Data were available for 20 morphological characters (Table 1, characters 2–21) and a single ecological character (elevation, character 1 in Table 1). For the multivariate analyses only characters 5–18 were used; the other characters exhibited high levels of missing data and would have affected those analyses too strongly. The other morphological characters (2–4, 19–21), as well as elevation, were investigated separately in bivariate scatter plots with other characters, where missing data have no effect other than not to be plotted. A complete set of 93 operational taxonomic units  $\times$  21 characters is available from the first author upon request.



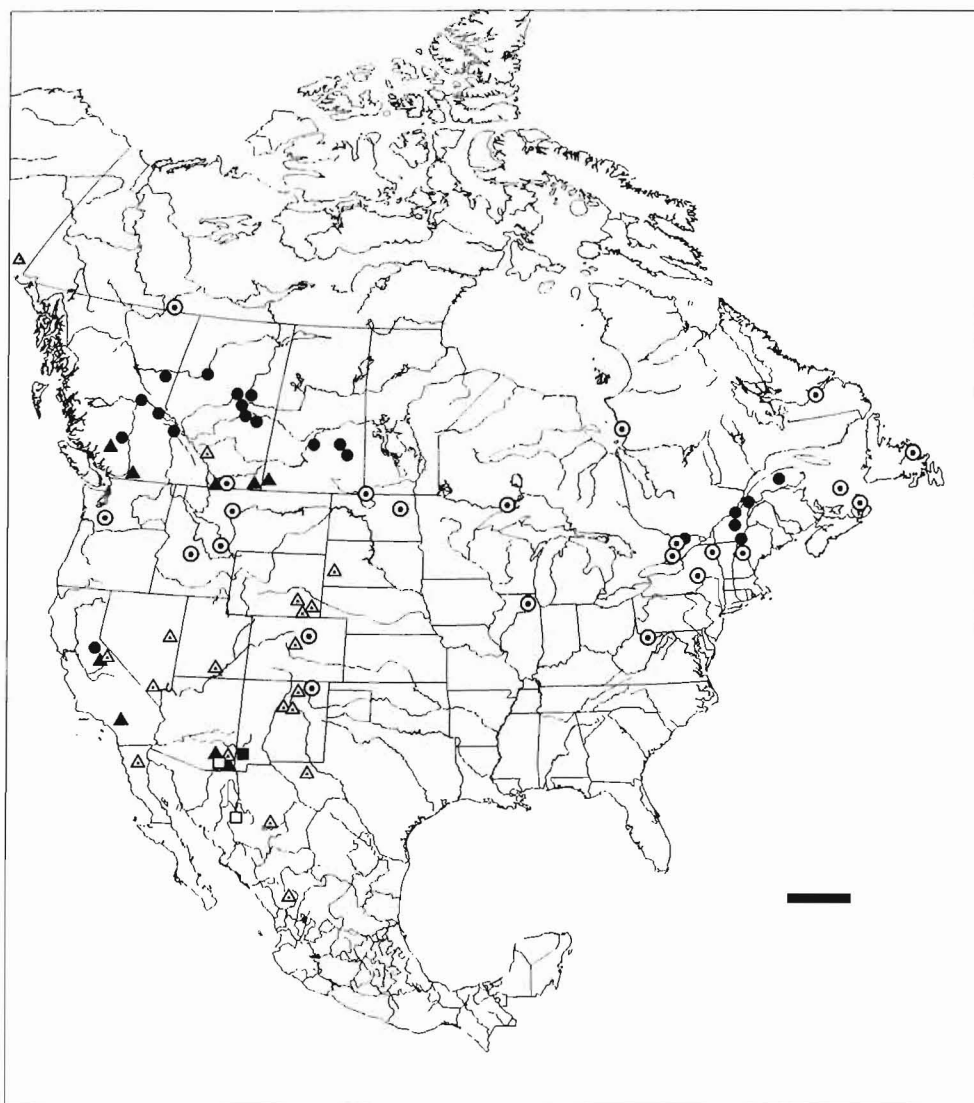


Fig. 1. Location of specimens used in this study. Circles = *Bromus ciliatus*; triangles = *Bromus richardsonii*; squares = *Bromus mucroglumis*. Solid symbols indicate field collection and chromosome determination ( $2n = 14$  for all *B. ciliatus*; and  $2n = 28$  for all *B. mucroglumis* and *B. richardsonii*). Scale bar = 500 km.

Three forms of multivariate analysis were applied to the morphological data for characters 5–18; a Discriminant Analysis (DA), a Principal Components Analysis (PCA), and a Non-Metric Multidimensional Scaling (MDS). First, a discriminant analysis was performed using SYSTAT (Version 9, 1999, SPSS, Inc.) and this included specimens from all three taxa. *Bromus mucroglumis* was not included in the subsequent analyses because it was well separated from the other taxa in the results of the DA, and because the number of specimens available for this study was small compared with the number available for the other two taxa. Instead, the general ordinations were run to identify variation patterns for these two well-represented species. The PCA was performed using the program PC-ORD (Version 4, McCune and Mefford 1999) using a correlation matrix of standardized data for the vari-

ables, and three axes were interpreted as having variation relevant to this study. The choice of using the correlation matrix based on standardized data was to give each character the same contribution to the overall variance in the analysis. The mixture of presence-absence and measurement data is undesirable for either DA or PCA (Davis 1986) and this led to the application of MDS, also using PC-ORD, based on a matrix of Euclidean distances between the specimens. For most data, MDS has been demonstrated as the optimum technique for finding the optimum ordination in two or three dimensions (Clarke and Warwick 1994; McCune and Mefford 1999).

Final evaluation of the two general ordinations was done by calculating a cophenetic correlation coefficient (Sneath and Sokal 1973) and plotting the final, inferred distances between the specimens against the

Table 1. Characters and their states scored for the analysis of *Bromus ciliatus*, *B. mucroglumis*, and *B. richardsonii*.

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1. Elevation (m)
2. Chromosome number: 0—diploid; 1—tetraploid
3. Basal sheath vestiture: 0—glabrous or with long hairs; 1—dense, short or medium hairs
4. Basal sheath crown vestiture: 0—same as sheath; 1—hairs longer and crowded
5. Top culm node: 0—glabrous; 1—hairy
6. Top culm sheath: 0—glabrous; 1—hairy
7. Top blade collar: 0—glabrous; 1—hairy
8. Top sheath margin: 0—glabrous; 1—hairy, same as sheath; 2—long hairs different from sheath
9. Top culm blade abaxial vestiture: 0—glabrous; 1—hairy
10. Top culm blade adaxial vestiture: 0—glabrous; 1—hairy
11. Glume 1 length (mm)
12. Glume 1 vestiture: 0—smooth or scabrous; 1—hairy
13. Glume 2 length (mm)
14. Glume 2 apex: 0—unawned; 1—mucronate; 2—short awned
15. Glume 2 vestiture: 0—glabrous; 1—hairy
16. Lemma 2 vestiture pattern: 0—margin area only; 1—not restricted to margin area
17. Lemma 2 central nerve basal half: 0—smooth or scabrous; 1—hairy
18. Palea 2 vestiture pattern between keels: 0—smooth or scabrous; 1—hairy
19. Anther length (mm)
20. Caryopsis length (mm)
21. Caryopsis color: 0—light to amber brown; 1—dark amber to purple

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starting Euclidean distance matrix, although this method was also used in an exploratory manner to compare the results of the MDS and PCA.

### DNA Fingerprinting

For the AFLP analysis seeds from the 31 localities were grown in the Department of Botany greenhouse at the Museum Support Center and harvested when young (at two months of age). Four to six leaf blades from four to six individuals of each accession were harvested and lyophilized prior to DNA extraction. From each sample two leaves were placed in a 2 ml microcentrifuge tube with two 3 mm glass beads. The tubes were placed on a horizontal shaker until the leaf tissue was ground to a fine powder and the DNA was extracted using DNeasy<sup>TM</sup> Plant Mini Kit (QIAGEN Inc.) according to the manufacturer's directions. Total genomic DNA was quantified by fluorimetry using Hoechst 33258 stain (Sigma Chemical Co, St. Louis, USA).

Four *EcoRI*:*MseI* primer combinations were used in this study. The AFLP method was performed as described by Vos et al. (1995) using the AFLP<sup>TM</sup> Analysis System I provided by Life Technologies. This general protocol included four main steps: (1) restriction digestion of 250 ng of genomic DNA with *EcoRI* and *MseI* restriction enzymes and ligation of adapters

to the restriction fragments in order to create primary templates for pre-amplification; (2) pre-amplification of the primary templates with AFLP primers with an additional single nucleotide at the 3' end; (3) selective amplification was performed with  $\gamma^{33}\text{P}$ -labeled *EcoRI* primers having three selective nucleotides at the 3' end (AGG, ACG, AAG) in combination with *MseI* primers having three selective nucleotides at the 3' end (CTC, CTG, CGC, CAC); and (4) the amplification products were separated on 5% polyacrylamide gels for 2.30 h at 80 W. The gels were transferred to Whatman paper and dried on a gel dryer for 2 h at 80°C. Gels were exposed to Kodak BIOMAX film at -80°C for 1–7 days depending on the signal intensity.

AFLP products were scored as present (1) or absent (0) on autographs to create a binary matrix. Euclidean distance matrices between individuals and locations were computed by using NTSYS-PC 2.01 (Rohlf 1997). Dendrograms were constructed using unweighted pair-group method of arithmetic averages (UPGMA) cluster analysis using the Euclidean distance matrices. The goodness of fit of each of the dendrograms to the relevant Euclidean distance matrices were assessed using cophenetic correlation coefficients (Sneath and Sokal 1973) using the NTSYS MxComp function. A complete set of data is available from Y. S. N. Fernandez upon request.

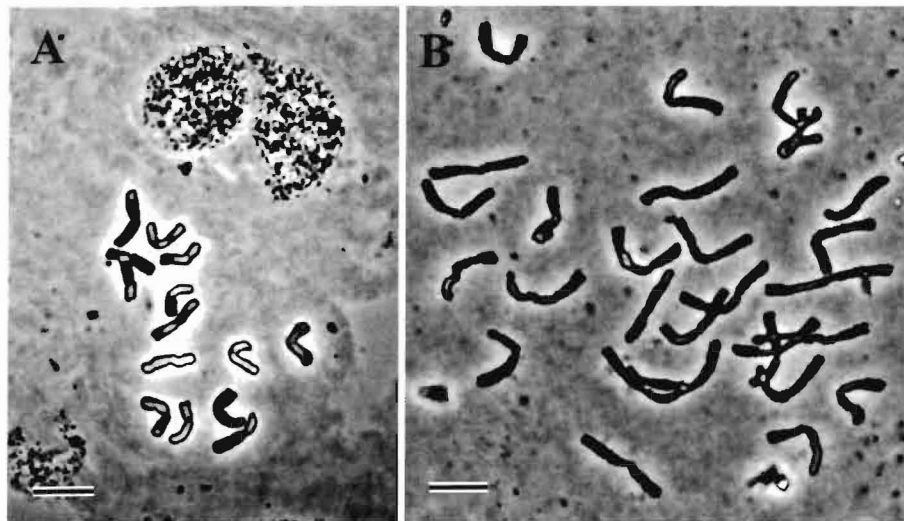
## RESULTS

### Cytology

Chromosome counts are given for 36 specimens (Appendix 1). Thirty-one came from our vouchers and the rest from voucher collections of previous studies (Cayouette, Wojtas, and Fillion unpubl. data; Wagnon 1952). All twenty-two counts for *B. ciliatus* were diploid  $2n = 14$ , whereas all 12 counts of *B. richardsonii* and both counts of *B. mucroglumis* were tetraploid  $2n = 28$  (Fig. 2). These mitotic counts were made at late prophase, metaphase, and/or anaphase. Satellites were observed for both diploid and tetraploid cytotypes.

### Discriminant Analysis

Despite some characters being binary and therefore not optimal for DA (Davis 1986), the results obtained were excellent in that all specimens (93 specimens, see Appendix 1 for locations) from all three taxa were correctly classified using characters 5 through 18. DA axis I easily discriminated between the two main taxa, *B. ciliatus* (47 specimens) and *B. richardsonii* (42 specimens), and DA axis II separated *B. mucroglumis* (4 specimens) from the other two species (Fig. 3; Table 2). The three most important characters and their states (presence for binary characters, magnitude for lengths) that served to separate each taxon from the others are



### *B. ciliatus* $2n = 14$    *B. richardsonii* $2n = 28$

Fig. 2. Photomicrograph of mitotic chromosomes. A. Metaphase of *Bromus ciliatus*,  $2n = 14$  (Cayouette 8439-4). B. Late prophase of *Bromus richardsonii*,  $2n = 28$  (Cayouette 8446). Line scale = 6.5  $\mu\text{m}$ .

as follows: for *B. ciliatus*, abaxially hairy top culm blade (9), hairy top culm node (5), and hairy top culm sheath (6); *B. mucroglumis*, glume 1 length with a mean of 6.60 mm (12), lemma 2 with hairs not restricted to the margin area (16), and adaxially hairy top culm blade (10); *B. richardsonii*, lemma 2 with hairs not restricted to margin area (16), hairy top sheath margin (8), and glume 2 length with a mean of 10.10 mm (13).

The mean and standard deviation for all quantitative characters are given in Table 3. *Bromus richardsonii* generally occurs in montane habitats at 2213 m (mean), whereas *B. ciliatus* is found on the lower slopes and plains at 650 m (mean). Glume 2 length

(13), anther length (19), and caryopsis length (20) also appear to be useful characters to discriminate between *B. ciliatus*/*B. richardsonii* since their standard deviations when added or subtracted from the means do not overlap.

#### Principal Components Analysis

Using characters 5 through 18, the first three PC's accounted for 60.4% of the total variation in the data. The eigenvector information and general PC data are given in Table 4. The first axis is by far the largest, accounting for just under 40% of the total variance. It has positive loadings for all lengths and negative loadings for most binary characters denoting hairiness (characters 5, 6, and 9). The position of each specimen

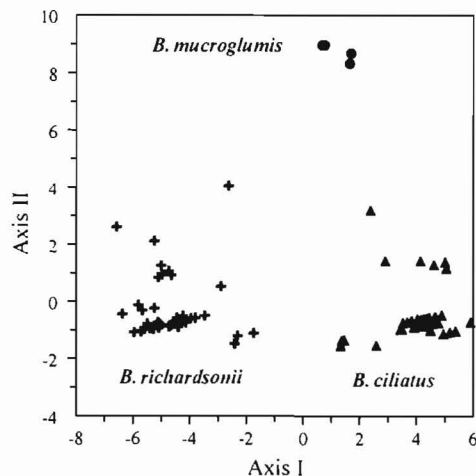


Fig. 3. Discriminant analysis of morphological data (characters 5–18) for *Bromus ciliatus* ( $\blacktriangle$ ), *B. mucroglumis* ( $\bullet$ ), and *B. richardsonii* ( $+$ ). The scores of all individuals are projected onto the two-dimensional space defined by axis I and axis II.

Table 2. Summary of the canonical discriminant functions (DF) used to generate Figure 3.

	DF 1	DF 2
5. Top culm node	1.7861	1.3403
6. Top culm sheath	1.2547	-0.2048
7. Top blade collar	0.6239	0.0088
8. Top sheath margin	-0.8149	0.2548
9. Top culm blade abaxial vestiture	2.8925	0.7336
10. Top culm blade adaxial vestiture	-0.1714	2.2392
11. Glume 1 length (mm)	0.0935	-0.0017
12. Glume 1 vestiture	0.4525	3.1472
13. Glume 2 length (mm)	-0.4947	-0.0844
14. Glume 2 apex	-0.3281	-0.0766
15. Glume 2 vestiture	-0.1823	0.0315
16. Lemma 2 vestiture pattern	-3.0406	2.2910
17. Lemma 2 central nerve basal half	0.1560	-0.1823
18. Palea 2 vestiture pattern between keels	0.9632	1.6594

Table 3. Mean, standard deviation (SD), and number of individuals (n) measured in parentheses for all quantitative characters (characters 1, 11, 13, 19, 20).

	<i>B. ciliatus</i> Mean $\pm$ SD (n)	<i>B. richardsonii</i> Mean $\pm$ SD (n)	<i>B. mucroglumis</i> Mean $\pm$ SD (n)
1. Elevation (m)	640 $\pm$ 650 (45)	2213 $\pm$ 644 (41)	1944 $\pm$ 157 (4)
11. Glume 1 length (mm)	6.24 $\pm$ 0.67 (47)	7.98 $\pm$ 1.09 (42)	6.60 $\pm$ 0.65 (4)
13. Glume 2 length (mm)	7.79 $\pm$ 0.74 (47)	10.10 $\pm$ 1.16 (42)	8.15 $\pm$ 0.99 (4)
19. Anther length (mm)	1.21 $\pm$ 0.18 (43)	2.05 $\pm$ 0.53 (40)	1.70 $\pm$ 0.32 (4)
20. Caryopsis length (mm)	6.68 $\pm$ 0.50 (34)	8.72 $\pm$ 1.01 (19)	7.35 $\pm$ 1.34 (2)

along PCI, therefore, is a general indication of its hairiness (more hairy to the negative side, less hairy to the positive side) and size (larger specimens to the positive side, smaller ones to the negative). The plot of PCI vs. PCII (Fig. 4) shows that specimens belonging to *B. richardsonii* have larger glumes (both 1 & 2) than those belonging to *B. ciliatus*, but the latter tend to be much more hairy, providing a definite separation between the taxa. The characters that have negative high loadings, and thus are characteristic of the hairy and shorter-glumed taxon *B. ciliatus* (characters 5, 6, and 9) show hairs that occur on the top culm node, top culm sheath, and the top culm blade abaxial surface; seen also in the DA. The other two PCs reflect variation with *B. richardsonii*, with PCII (Fig. 4, 5) loading highly for top sheath margin (8), glume 1 vestiture (12), glume 2 vestiture (15) and palea 2 vestiture pattern (18) and PC III (Fig. 6) loading negatively for top blade collar (7), top sheath margin (8) and glume 2 apex (14) and most highly positive for lemma 2 central nerve basal half (17). The general geometry of the variance of the three dimensions, as shown (Fig. 4–6), is for a small sphere of specimens in negative space for PCI with near-zero values for the other two axes for *B. ciliatus*, and a terete-ring for *B. richardsonii* in the positive space of PCI and showing large variation

in PCs II and III, with relatively few near-zero values for those axes; a small sphere of *B. ciliatus* in front of a terete-ring of *B. richardsonii*. These data argue for a detailed investigation of variation within the species *B. richardsonii*.

#### Multidimensional Scaling Analysis

The most robust ordination method available for these data is MDS (Fig. 7). All the *B. ciliatus* individuals form a tight cluster in the upper left of the plot and these are somewhat surrounded by four individuals of *B. richardsonii*. One individual outlier of *B. ciliatus* near the upper center of the plot is depicted within the more open cluster of *B. richardsonii* individuals. The overlap between the two species is greater than in the PCA plots, which might suggest the method was less successful, but the cophenetic correlation for the MDS ( $r = 0.98$ ) is very much better than that for the PCA ( $r = 0.76$ ), even allowing one more dimension to the PCA. Bivariate plots of the inferred versus starting distances show the MDS to be much more representative of the true relationships inferred by the Euclidean distance matrix. Overall, the differences between the taxa are still quite distinct, although a few specimens of *B. richardsonii* do overlap within the

Table 4. Eigenvector loadings for characters 5–18 on the first three principal components (PC). Relative eigenvalues, percent of variance, and cumulative percent of variance are listed below.

	PC 1	PC 2	PC 3
5. Top culm node	–0.3531	0.1868	–0.0845
6. Top culm sheath	–0.3236	0.1708	–0.0115
7. Top blade collar	0.2591	0.2157	–0.3854
8. Top sheath margin	0.0017	0.3941	–0.5725
9. Top culm blade abaxial vestiture	–0.3680	0.1355	0.1226
10. Top culm blade adaxial vestiture	–0.1063	0.0146	0.1402
11. Glume 1 length	0.3409	–0.1477	0.0904
12. Glume 1 vestiture	0.1596	0.5264	0.2613
13. Glume 2 length	0.3513	–0.1367	–0.0287
14. Glume 2 apex	0.2412	0.1447	–0.3641
15. Glume 2 vestiture	0.2261	0.3426	0.2980
16. Lemma 2 vestiture pattern	0.3867	–0.1069	0.0207
17. Lemma 2 central nerve basal half	0.1646	0.1426	0.3968
18. Palea 2 vestiture pattern	0.0754	0.4774	0.1589
Eigenvalue	5.563	1.503	1.394
% of variance	39.733	10.734	9.968
Cumulative % of variance	39.733	50.467	60.425

smaller dispersion area of *B. ciliatus* and one of the latter individuals is within the more-dispersed region of *B. richardsonii*.

### Bivariate Comparisons

Further investigation is possible for those variables represented by continuous data (the lengths and elevation) through bivariate comparisons. This especially helped in examining trends in those variables not used in the multivariate analyses; specimens with missing data were simply excluded from the graphics and statistics. These bivariate graphs each contain four comparisons (Fig. 8–15). The data are shown for each taxon using different symbols and correlation coefficients were calculated for each pair of variables for *B. ciliatus* and *B. richardsonii*. *Bromus mucroglumis* was represented by too few specimens to be analyzed.

The effect of elevation on glume length (Fig. 8, 9) clearly shows that *B. richardsonii* has larger glumes (higher values for the y-axes) and is typically found at higher elevation (to the right in the graphs), when compared with *B. ciliatus*, reinforcing the results of the summary statistics and multivariate analyses. Neither taxon shows a significant correlation of glume length with elevation. However, if all *Bromus* data are combined, there is an apparent, highly significant correlation ( $P \ll 0.001$ ) in both cases, a theme that will be seen in most of the bivariate graphs. This suggests potential utility in looking at data in a more combined form for an analysis of allometry in the genus by including additional species. However, with only two species with any significant sample sizes, it also suggests extreme caution in that the apparent, highly significant correlations may simply be an artifact of the positions of two uncorrelated distributions of individual taxa rather than a trend for the genus.

The most straight-forward morphometric comparison is that of the two glume lengths (Fig. 10) which show highly correlated ( $P \ll 0.001$ ) trends in both individual taxa, and as combined data. The slopes of the reduced major axes are sub-parallel (approximately 1.1 for *B. ciliatus* and 1.06 for *B. richardsonii*) showing that glume 2 increases in length slightly quicker than glume 1 length in both species, also reflected in the higher values for glume 2 length. The combined analyses show a slightly higher slope of 1.135.

The next step was to analyze variation in two continuous measurements (anther length and caryopsis length) which could not be used in the multivariate analyses as a result of missing data. First comparison of these two variables (Fig. 11) with each other shows the expected smaller sample sizes for all taxa, the expected larger size of *B. richardsonii*, and non-significant correlations for both species. Their combined data again show the highly significant correlation ( $P \ll$

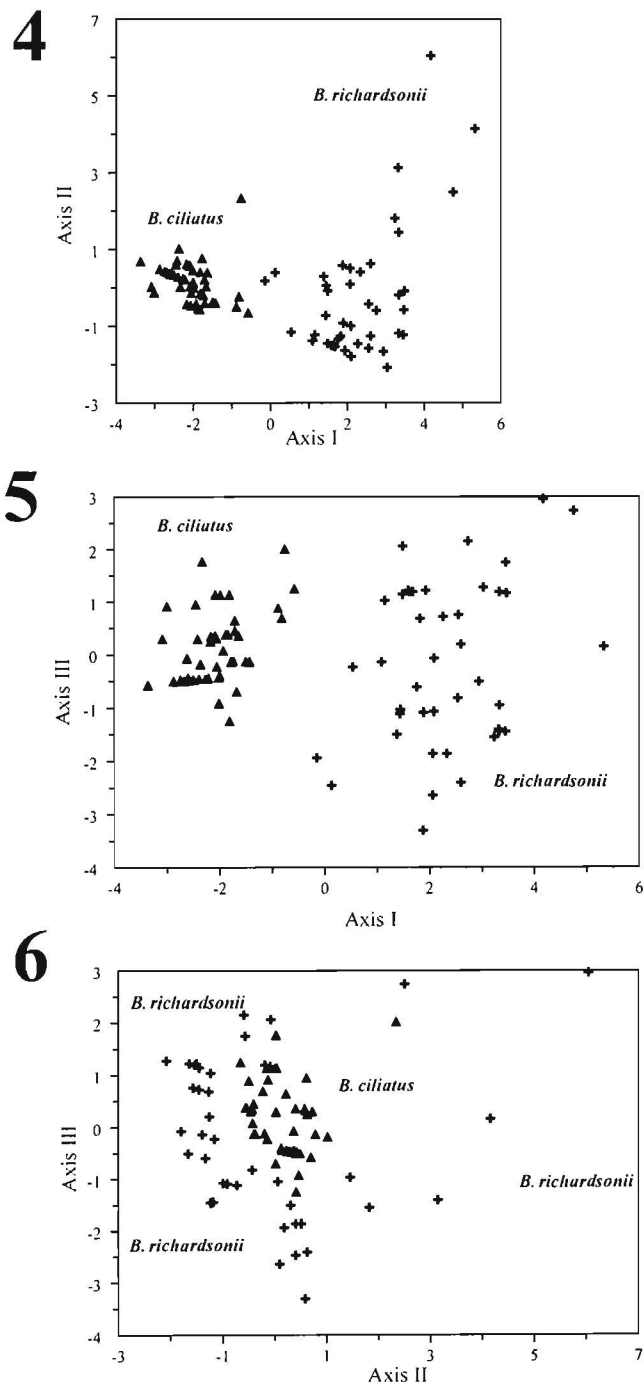


Fig. 4–6. Principal components analysis of morphological data (characters 5–18) for *Bromus ciliatus* ( $\blacktriangle$ ) and *B. richardsonii* (+). The scores of all individuals are projected onto the two-dimensional space ( $r = 0.76$ ).—4. Axis I (accounts for 39.7% of the variation) and axis II (accounts for 10.7% of the variation).—5. Axis II and axis III (accounts for 10.0% of the variation).—6. Axis I and axis III.

0.001) seen for the other graphs. Second, anther length and caryopsis length were then compared with the two glume lengths in all four combinations (Fig. 12–15), with similar results for each. Each shows the same size relationships between the two taxa, the significant cor-

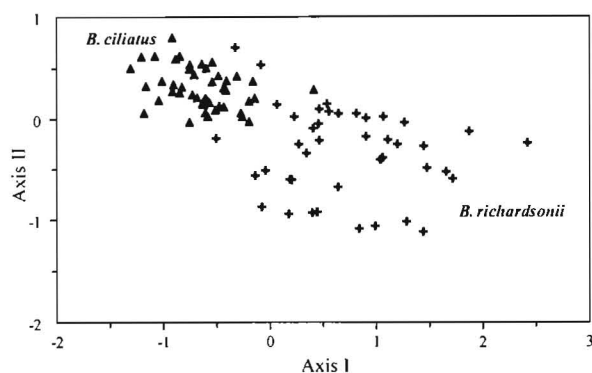


Fig. 7. Multidimensional scaling of morphological data (characters 5–18) for *Bromus ciliatus* (▲) and *B. richardsonii* (+). The scores of all individuals are projected onto the two-dimensional space defined by axis I and axis II ( $r = 0.98$ ).

relation in combined form ( $P \ll 0.001$ ), and non-significant correlations within individual species. The two exceptions are significant correlations between caryopsis length and both glume lengths for *B. richardsonii* (glume 1— $P < 0.02$ ; glume 2— $P < 0.05$ ). In both cases the slopes of the regression lines show glume lengths increasing more slowly than caryopsis lengths (slopes of reduced major axes approximately 0.949 and 0.948).

### Other Characters

Basal sheath vestiture (3) appears to be a very good character since all 44 specimens of *B. ciliatus* were glabrous or had long hairs whereas 35 out of 38 specimens (92%) of *B. richardsonii* had dense, short or medium hairs. All 40 specimens of *B. ciliatus* had glabrous or long basal hairy sheath crowns (4) whereas 8/34 (24%) specimens of *B. richardsonii* had hairs that were longer and crowded. Finally, caryopsis color (21) is somewhat subjective since color can be very hard to quantify, particularly when seen by more than one person. However, 31/34 (91%) specimens of *B. ciliatus* had light to amber brown fruits and 14/19 (74%) of specimens of *B. richardsonii* had dark amber to purple fruits.

### AFLP Analysis

In total four primer combinations produced 413 bands (Table 5). In each primer combination the number of bands ranged from 77 to 136. Of all the bands from each primer combination only 15 were monomorphic. The percentage of polymorphic bands for each primer combination ranged from 92% to 98%. From each of the four primer combinations used, an

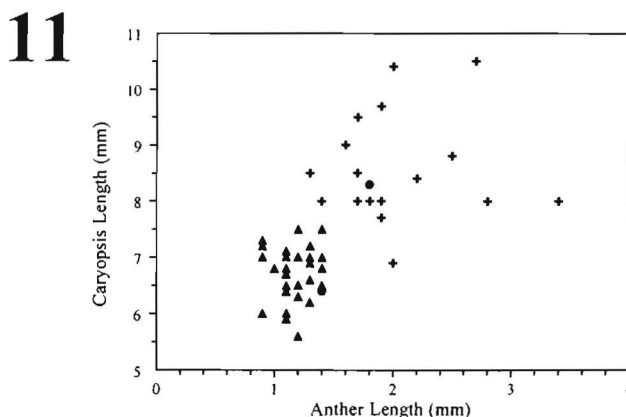
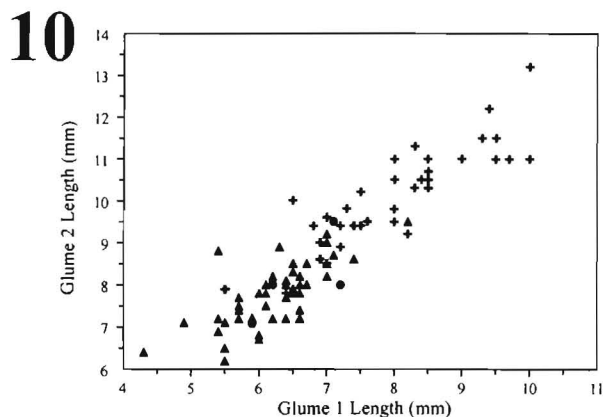
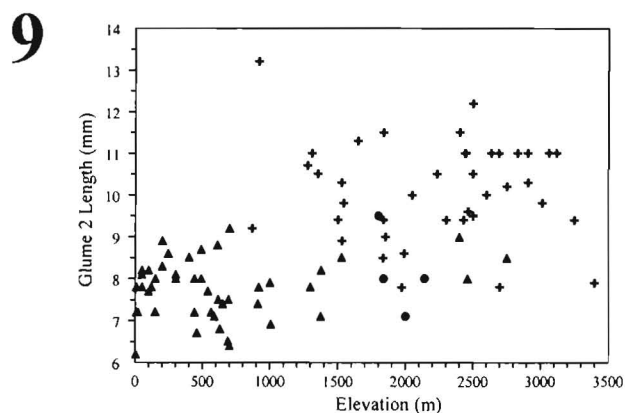
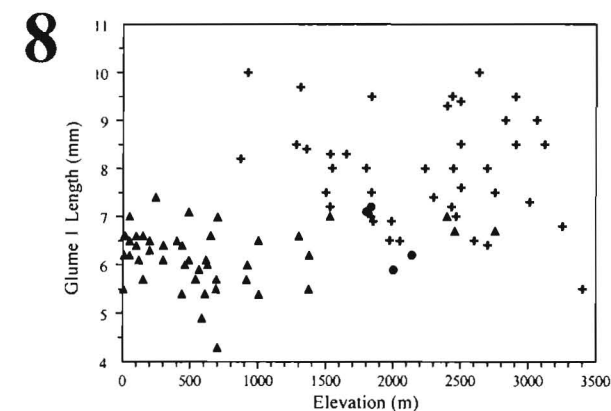


Fig. 8–11. Bivariate comparisons of morphological and ecological data for *Bromus ciliatus* (▲), *B. mucroglumis* (●), and *B. richardsonii* (+).—8. Elevation (character 1) versus glume 1 length (character 11).—9. Elevation versus glume 2 length (character 13).—10. Glume 1 length versus glume 2 length.—11. Anther length (character 19) versus caryopsis length (character 20).



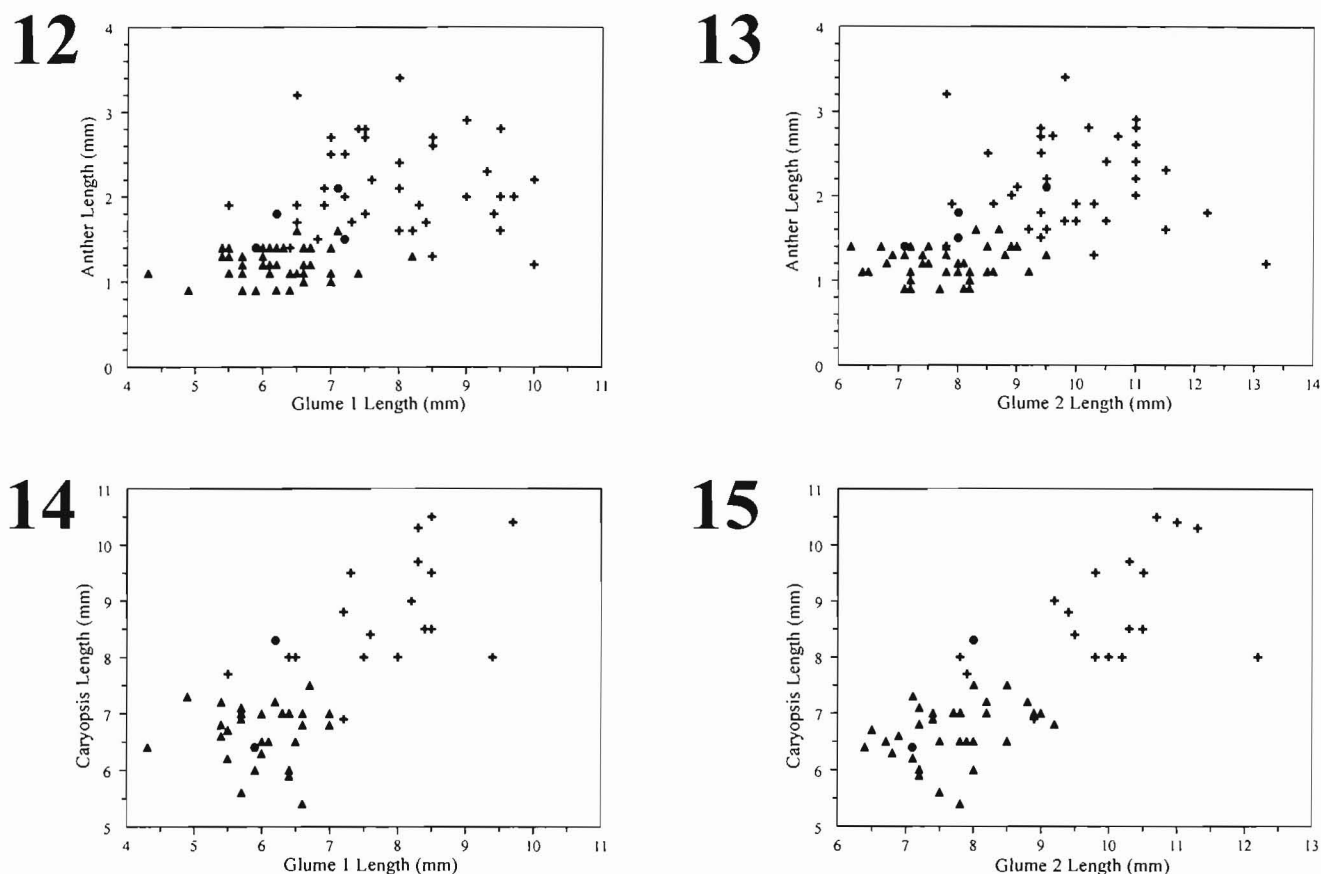


Fig. 12–15. Bivariate comparisons of morphological data for *Bromus ciliatus* (▲), *B. mucroglumis* (●), and *B. richardsonii* (+).—12. Glume 1 length (character 11) versus anther length (character 19).—13. Glume 2 length (character 13) versus anther length.—14. Glume 1 length versus caryopsis length (character 20).—15. Glume 2 length versus caryopsis length (character 20).

average of 20 polymorphic bands were used in the analysis. These bands were selected on the basis of robustness of the band, and ease of scoring without any ambiguity. There were many other polymorphic bands that were not used in this analysis because of difficulty in scoring.

The UPGMA dendrogram divides all 154 individuals into two distinct clusters, one consisting entirely of *B. ciliatus* individuals and a second consisting of *B. richardsonii* and *B. mucroglumis* individuals (Fig. 16). The cophenetic correlation coefficient for this plot is very robust at  $r = 0.98$ . Within this second cluster, all six individuals of *B. mucroglumis* form a cluster embedded in the *B. richardsonii* group. There is no overlap of individuals between species. Within each species, individuals from one location usually clustered with each other.

A second UPGMA dendrogram using a matrix of 31 locations also shows two distinct clusters corresponding to *B. ciliatus* and *B. mucroglumis/B. richardsonii* groups (Fig. 17). Because all individuals of a population are combined one would expect a lower cophenetic correlation coefficient for this plot ( $r = 0.89$ ). The single population of *B. mucroglumis* from the Chiricahua Mountains again fell within the *B. ri-*

*chardsonii* group. Within species, different collections from similar geographic areas (province or area of country) tend to cluster together. However, there were some exceptions, for example an Alberta collection of *B. ciliatus* (LAM-c-AB) clustered with a Québec (GRA1-c-QC) collection.

#### DISCUSSION

Our chromosome data confirmed that *B. ciliatus* is diploid and *B. richardsonii* and *B. mucroglumis* are tetraploid (Armstrong 1981, 1983; Mitchell and Wilton 1965; Wagnon 1952; Cayouette et al. 1997; Cayouette, Wojtas, and Fillion unpubl. data; Ward and Spellenberg 1988). Triploid cytotypes ( $2n = 21$ ) were not encountered. The presence of small pin-head satellites on some chromosomes of *B. ciliatus* and *B. richardsonii* has been previously documented (Armstrong 1981, 1983; Gervais 1979; Schulz-Schaeffer 1956, 1960; Wilton 1965), but their numbers vary according to the cytotypes and to the interpretations of the authors. Satellite observation is sometimes obscured by various techniques used to shorten chromosomes (Armstrong 1983; Gervais 1979). Armstrong (1981) considered their presence and quantity important when assessing

Table 5. AFLP markers generated among *Bromus ciliatus*, *B. richardsonii*, and *B. mucroglumis*.

+3 primer pair <i>EcoRI</i> : <i>MseI</i>	Total bands	Total monomorphic bands	Total polymorphic bands	Polymorphism rate (%)
AGG:CTC	108	2	106	98
ACG:CTG	92	7	85	92
AGG:CGC	77	1	76	98
AAG:CAC	136	5	131	96

genomic relationships within section *Bromopsis*. Our observations confirmed the presence and often the quantity of satellited chromosomes, however, even if they appeared to be more numerous in the tetraploid cytotypes, the satellites could not be properly studied because of our chromosome shortening technique.

The morphological traits identified as distinctive for *B. richardsonii* and *B. ciliatus* clearly distinguish these as two separate species. However, since there were only four individuals of *B. mucroglumis* included in this study, and these appear intermediate between clusters of *B. ciliatus* and *B. richardsonii* when comparing characters 1, 11, 13, 19, and 20 amongst each other (Fig. 8–15, elevation/glume 1 or 2 length; glume 1/glume 2 length; anther length/caryopsis length; glume 1 or 2 length/anther or caryopsis length), it would be premature to conclude that *B. mucroglumis* is a distinct species. The DA was 100% successful at classifying all three species, the PCA easily separated *B. ciliatus* and *B. richardsonii* into two well-defined groups, and the MDS also mirrored the PCA but displayed more overlap of individuals between the two groups. The latter technique (MDS) appears to be much better at representing the Euclidean distance matrix since the cophenetic correlation was high ( $r = 0.98$ ) as compared to the PCA ( $r = 0.76$ ). In general, the bivariate analyses show the new characters acting much like the continuous data used in the multivariate analyses; i.e., the two glume lengths. Each shows a strong size-based discrimination between *B. ciliatus* and *B. richardsonii*, although comparisons amongst these variables shows weaker or no correlations between the variables compared with the two glume lengths.

Clearly more intense sampling of *B. mucroglumis* is needed to ascertain whether or not this entity is indeed a distinct species. As mentioned in the introduction, Wagnon (1950, 1952) indicated that “most of the variation [of *B. mucroglumis*] is apparently in the direction of *B. richardsonii*, thus suggesting introgression with this species.” During our most recent collecting trip (September/October of 2000) the first two authors observed *B. mucroglumis* from the Chiricahua Mountains (Arizona) as having fairly consistent morphological features in which to separate it from *B. richardsonii*, also from the same locality. These were hairy, first (character 12) and second (15) glumes, hairy, top culm blades both upper (10) and lower surfaces (9),

hairy top culm nodes (5), and long hairy top sheath margins (8). Hairy first glumes and upper top culm blades were identified earlier in our DA as being important for *B. mucroglumis*. However, when we collected and observed other specimens of *B. mucroglumis* (apparently) and *B. richardsonii* from Sonora, Chihuahua, and Durango, Mexico, hairiness of the top culm blade, nodes, and top sheath margins became less reliable features to differentiate between these taxa. We hope to clarify the relationship of these two species in future studies.

The high percentage of polymorphic AFLP bands (92%–98%) and low number of monomorphic bands observed in this study are indicative of a highly outcrossing species that are widely distributed geographically. Both of these latter characteristics are found in *B. ciliatus* and *B. richardsonii*. In a diversity study of wheat (self-pollinating) cultivars, the AFLP marker polymorphism rate varied from 1.2% to 26.1% (Barrett and Kidwell 1998). Whereas, in maize (cross-pollinating but intensely selected) the range was 43% to 63% (Marsan et al. 1998). There were two types of polymorphic markers observed in this study: (1) AFLP markers that were common to all three species but present in different frequencies and (2) AFLP markers that were specific to a species. This combination of common and species-specific markers was previously observed in a study conducted on cultivated brome-grass species, *B. inermis* Leyss. and *B. riparius* Rehm (Ferdinandez 1999).

The ability to distinguish closely related groups by AFLP markers, are well documented in other genera (Fuentes et al. 1999; Powell et al. 1996) and proved decisive in our study. The large numbers of polymorphic bands yields large amounts of information to assess genetic diversity between individuals and populations of *B. ciliatus* and *B. richardsonii*. The dendrogram was able to cluster the 154 individuals into two distinct groups. The genetic relationships determined by the AFLP markers are consistent with the chromosomal differences and morphological conclusions. The clustering of *B. mucroglumis* within the *B. richardsonii* group indicates a close genetic relationship between the two species. Similar results were observed in a phylogenetic study conducted on tall fescue using RFLPs (Xu and Sleper 1994). In this study genotypes from the same species with similar chromosome num-

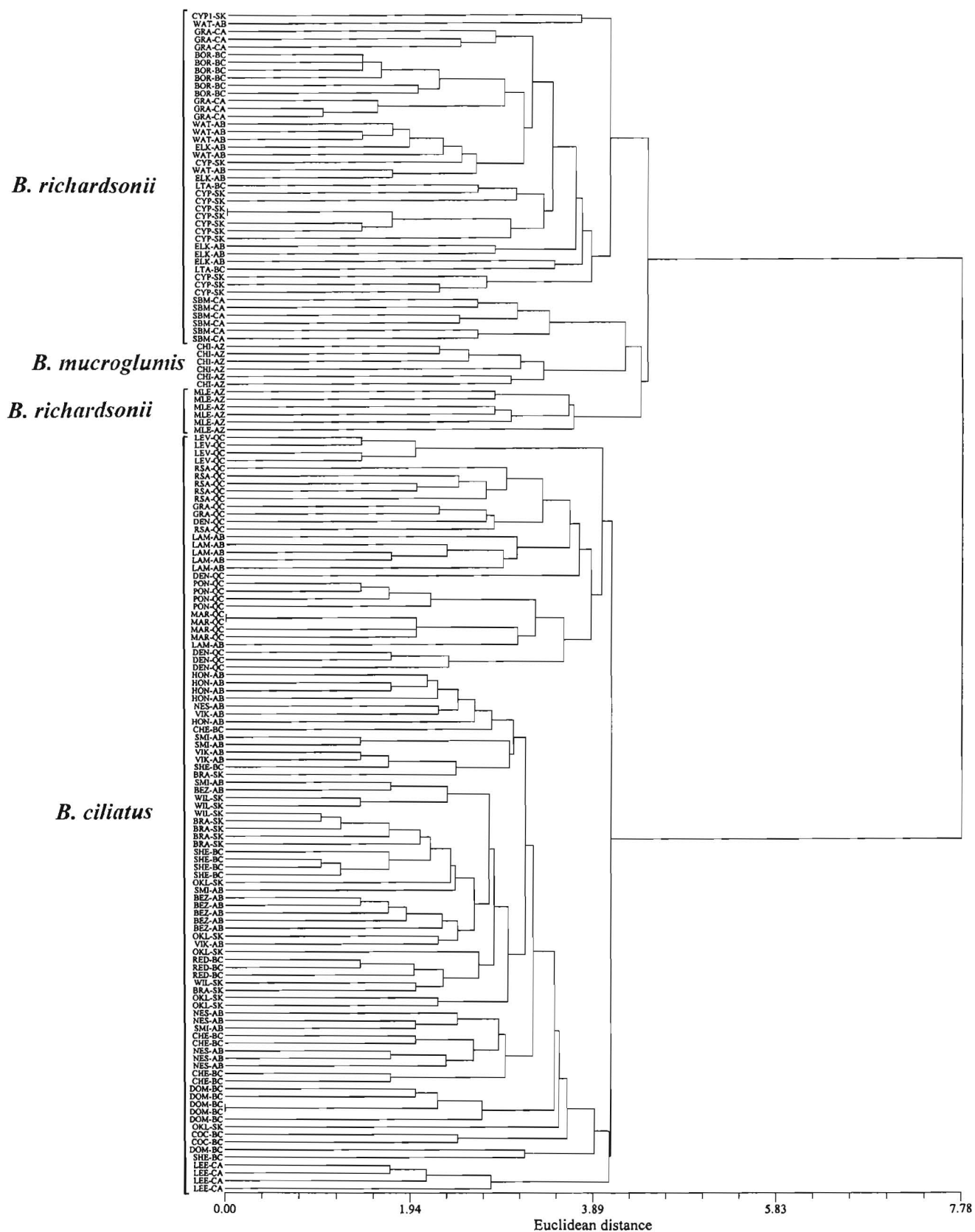


Fig. 16. Genetic relationships of 154 individuals of *Bromus ciliatus* (100 total), *B. mucroglumis* (6 total), and *B. richardsonii* (48 total) as depicted by a UPGMA dendrogram ( $r = 0.98$ ). Geographical identifiers (see Appendix 1) followed by state abbreviations are given for each individual.

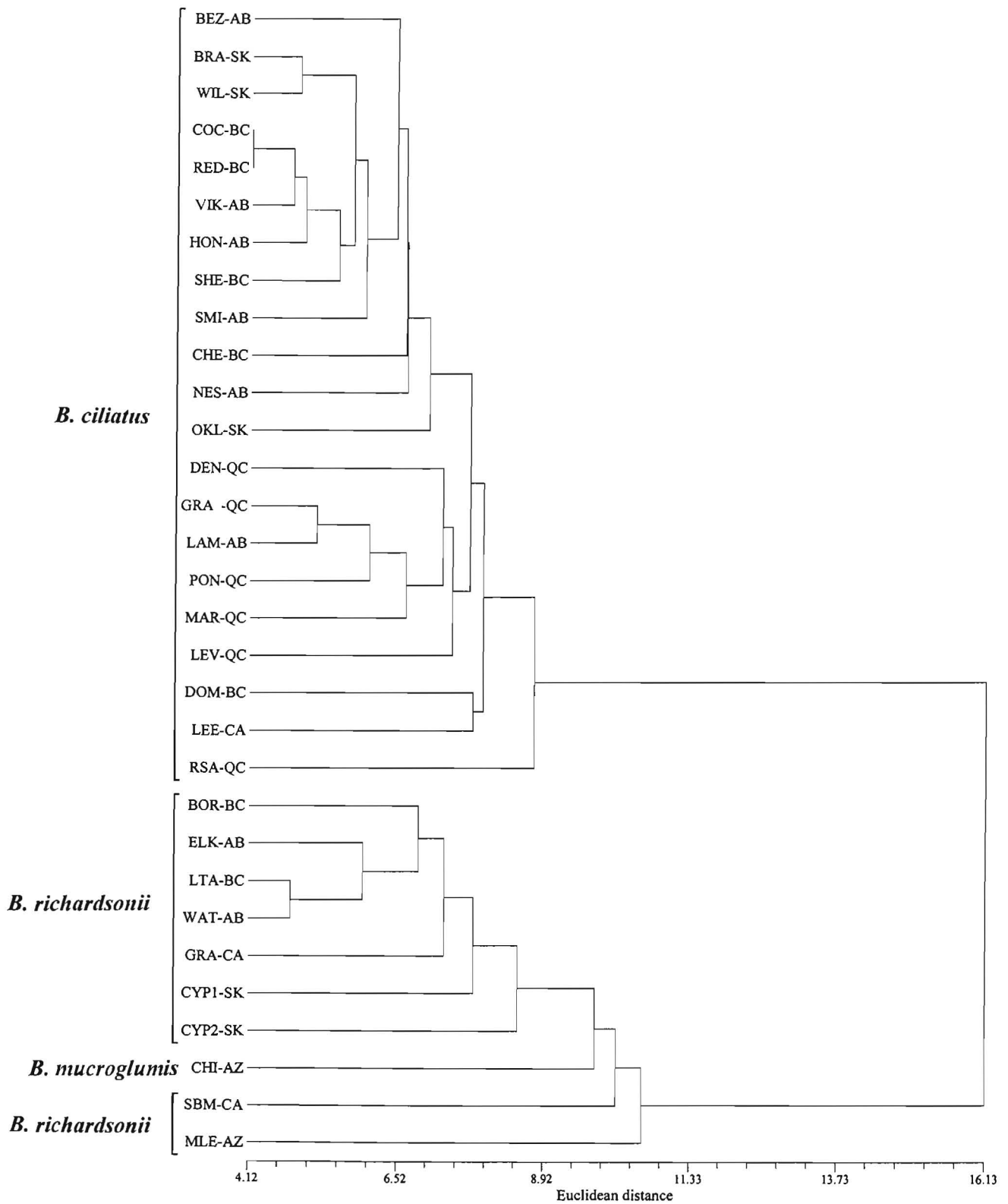


Fig. 17. Genetic relationships of 31 populations of *Bromus ciliatus* (21 total), *B. mucroglumis* (1 total), and *B. richardsonii* (9 total) as depicted by a UPGMA dendrogram ( $r = 0.89$ ). Geographical identifiers (see Appendix 1) followed by state abbreviations are given for each population.

bers grouped in the same cluster. However, because there is only one population of *B. mucroglumis* included in this study we are unable to adequately resolve its relationship with *B. richardsonii* at this time.

Despite overlap of some individuals between locations the majority of the individuals from a particular location clustered together. It may be possible to differentiate closely related individuals with greater resolution by increasing the number of polymorphic markers. However, it is also possible that clustering of individuals from different locations is a result of genomic similarity rather than the inability of the AFLP markers to determine the differences. If the former is true, increasing the number of markers will only heighten the detectable differences and will not alter the fact that certain individuals from one location are genetically more similar to individuals from another location. In the present study, it appears that it is possible to differentiate closely related individuals collected from the same location and of the same species.

### Taxonomic Treatment

Since it appears that *B. ciliatus* and *B. richardsonii* are distinct species, the following key using morphological features is given to separate these taxa. The characters used in this key are listed in descending order for ease of use.

1. Lemmas usually only ciliate along margins; anthers (0.9) 1–1.4 (1.6) mm long; second glumes (6.2) 7.1–8.5 (9.5) mm long; basal sheaths glabrous or with long hairs; top culm blades with hairs on the upper surface; top culm nodes usually hairy; caryopses (5.4) 6.2–7.2 (7.5) mm long; top culm sheath usually hairy . . . . . *B. ciliatus*
2. Lemmas ciliate along margin and with scattered hairs on the lower half between the midnerve and margins; anthers (1.2) 1.6–2.7 (3.4) mm long; second glumes (7.8) 8.9–11.3 (13.2) mm long; basal sheaths with dense, short or medium hairs; top culm blades glabrous on the upper surface; top culm nodes usually glabrous; caryopses (6.9) 7.7–9.7 (10.5) mm long; top culm sheaths glabrous . . . . . *B. richardsonii*

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## APPENDIX 1

List of the *Bromus* vouchers used for morphological, cytological and molecular studies. ##) = sample number; C = chromosome number voucher, CM = chromosome and molecular voucher, and geographical identifier (preceded by a dash) used in Figure 16 and 17; *Bromus ciliatus*:  $2n = 14$ ; *B. mucroglumis* and *B. richardsonii*:  $2n = 28$ . \* not used for morphological data.

*Bromus ciliatus*: CANADA. Alberta: 86) Athabasca River, 8 Sep 1998, *J. Cayouette* 8482 (DAO) CM-SMI; 87) Bezanson, 9 Sep 1998, *J. Cayouette* 8485 (DAO) CM-BEZ; 85) Hondo, 8 Sep 1998, *J. Cayouette* 8479 (DAO) CM-HON; 83) Lamont, 7 Sep 1998, *J. Cayouette* 8475 (DAO) CM-LAM; 84) Nestow, 7 Sep 1998, *J. Cayouette* 8478 (DAO) CM-NES; 82) Viking, 6 Sep 1998, *J. Cayouette* 8474 (DAO) CM-VIK; 40) Waterton Lake Park, Crooked Creek, 16 Aug 1953, A. J. Breitung 17619 (US); British Columbia: 88) Chetwind, 10 Sep 1998, *J. Cayouette* 8494 (DAO) CM-CHE; 64) Cooper Creek, 14 Sep 1993, *J. Cayouette* 7615 (DAO) CM-COC; 90) Dome Creek, 11 Sep 1998, *J. Cayouette* 8497 (DAO) CM-DOM; 91) Red Pass, 11 Sep 1998, *J. Cayouette* 8499 (DAO) CM-RED; 89) Shelley, 11 Sep 1998, *J. Cayouette* 8495 (DAO) CM-SHE; Labrador



(Nfld.): 57) Goose Bay, 19 Aug 1950, *J. M. Gillett & W. I. Findlay* 5802 (US); **Manitoba**: 41) Brandon, 18 Jul 1896, *J. Macoun* 13042 (US); **Newfoundland**: 59) Terra Nova, [ca. 1828, prob. *J. M. Despréaux s. n.*] comm. [unicavit] Lenormand (P) (Type of *Bromus denseciliatus* Steud.); 1) Gander District, Great Rattling Brook, 17 Aug 1960, *E. Rouleau* 6313 (US); **Northwest Territories**: 38) Liard River, 15 mi SW of Fort Liard, 21 Jul 1961, *W. J. Cody & K. M. Spicer* 11508 (US); **Nova Scotia**: 58) Indian Cove, 19 Aug 1941, *C. A. & U. F. Weatherby* 7088 (US); **Ontario**: 47) Kaladar, 4 Aug 1947, *W. G. Dore & H. A. Senn* 47674 (US); 49) Packenham Twp., Cedar Hill Road, 18 Sep 1991, *J. Cayouette & S. J. Darbyshire* 7107-2 (DAO) C; 48) Thunder Bay District, 1 mi E of Port Arthur, 2 Aug 1950, *C. E. Garton* 1233 (US); **Québec**: 72) Coleraine, Mont du Granite, 20 Aug 1997, *J. Cayouette & G. Hall* 8222 (DAO) CM-GRA1; 74) Lévis, 8 Oct 1997, *J. Cayouette & N. Lavoie* 8272 (DAO) CM-LEV; 51) Magdalen Islands, Coffin Island, 17 Aug 1912, *M. L. Fernald, B. Long & H. St. John* 6932 (US); 73) Notre-Dame-des-Bois, Mont du Marbre, 21 Aug 1997, *J. Cayouette & A. Thériault* 8237 (DAO) CM-MAR; \*Pontiac-Station, 15 Oct 1998, *J. Cayouette, J. Labrecque, A. Sabourin & D. Paquette* 8582 (DAO) CM-PON; 92) St-Denis-sur-Mer, 25 Oct 1998, *J. Cayouette* 8589 (DAO) CM-DEN; 71) St-Joseph-des-Monts, 18 Aug 1996, *J. Cayouette* 8045 (DAO) CM-RSA; 50) Vieux Comptoir, James Bay, 26–28 Aug 1944, *A. Dutilly & E. Lepage* 13405 (US); **Saskatchewan**: 77) Brancepeth, 28 Aug 1998, *J. Cayouette* 8440 (DAO) CM-BRA; 76) Okla, 27 Aug 1998, *J. Cayouette* 8439 (DAO) CM-OKL; 75) Willowbrook, 27 Aug 1998, *J. Cayouette* 8429 (DAO) CM-WIL; **JAPAN**. **Hokkaido**, 63) Nemuro, 10 Sep 1931, *J. Ohwi s. n.* (US) (sub *Bromus yesoensis*); 62) Hokkaido, Nakkenshi, 13 Aug 1937, *K. Uno* 21462 (US) (sub *B. yesoensis*); **RUSSIA**, formerly **JAPAN**. **Kuriles**: 61) Umanose, Shillotan Island, 30 Jul 1931, *J. Ohwi* 1080 (US) (isotype of *Bromus yesoensis*); **U.S.A.** **California**: 68) Lee Vining River, 9 Sep 1994, *J. Cayouette & S. J. Darbyshire* 7966 (DAO) CM-LEE; **Colorado**: 31) Sheephorn Divide, 1 Sep 1898, *Shear & Bessey* 1548 (US); **Idaho**: 24) Lake, 26 Aug 1916, *H. J. Rust* 900 (US); **Illinois**: 42) Elgin, 9 Aug 1919, *H. C. Benke* 3664 (US); **Montana**: 25) Shields River, above Wilsall, 23 Aug 1916, *W. N. Suksdorf* 88 (US); 26) Wetzell, Aug 1900, *Griffiths & Lang* 271 (US); **New Hampshire**: 55) Mount Washington, Oakes Gulf, 26 Aug 1917, *A. S. Hitchcock* 16041 (US); **New Mexico**: 18) vicinity of Ute Park, 6 Sep 1916, *P. C. Standley* 14421 (US); **New York**: 60) Newcomb, Huntington Wildlife Forest Station, 21 Jul 1939, *H. F. Heady* 768 (DAO) (neotype of *Bromus ciliatus*); 52) Oriskany, 1841, *G. Vasey s. n.* (US); **North Dakota**: 35) Wood Lake, Tokio, 1 Aug 1940, *O. A. Stevens* 478 (US); **Washington**: 21) Big Klickitat River, 1 Sep 1903, *J. S. Cotton* 1485 (US); **West Virginia**: 56) Canaan Valley, Blackwater River, 22 Jul 1943, *H. A. Allard* 10874 (US).

**Bromus mucroglumis**: **MEXICO**. **Chihuahua**: 5) 9 mi E of Yecora on HWY 16, 13 Oct 1992, *P. M. Peterson & C. R. Annable* 12479 (US); **U.S.A.** **Arizona**: 70) Chiricahua Mountains, 18 Sept. 1994, *J. Cayouette & S. J. Darbyshire* 7985 (DAO) CM-CHI; 13) South Cave Creek, 28 Jul 1948, *Goodding, Locke & Johnson s. n.* (Wagnon 1520) (US) C (isotype of *Bromus mucroglumis*); 3) Huachuca Mountains, 9 Sep 1893, *F. X. Holgnir* 2164 (US).

**Bromus richardsonii**: **CANADA**. **Alberta**: 80) Elkwater, Cypress Hills Provincial Park, 30 Aug 1998, *J. Cayouette* 8451 (DAO) CM-ELK; 39) Vermilion Mt., vicinity of Banff, 25 Jul 1899, *W. C. McCalla* 2303 (US); 81) Waterton Lakes National Park, Cameron Creek, 4 Sep 1998, *J. Cayouette* 8460 (DAO) CM-WAT; **British Columbia**: 66) Lake Borgeson, 16 Sep 1993, *J. Cayouette* 7622 (DAO) CM-BOR; 65) Lake Taseko, 16 Sep 1993, *J. Cayouette* 7621 (DAO) CM-LTA; **Saskatchewan**: 78) Cypress Hills Provincial Park, 30 Aug 1998, *J. Cayouette* 8446 (DAO) CM-CYP1; 79) Idem, *J. Cayouette* 8449 (DAO) CM-CYP2; **MEXICO**. **Baja California**: 43) Sierra San Pedro Martir, 27 Aug 1988, *P. M. Peterson, C. R. Annable, R. F. Thorne & R. D. Noyes* 5147 (US); 44) Cerro Observatorio, 28 Aug 1988, *P. M. Peterson, C. R. Annable, R. F. Thorne & R. D. Noyes* 5244 (US); 54) La Encantada, 20 Sep 1930, *I. L. Wiggins* 4960 (US); 53) 5 mi NW of La Grulla, 17 Sep 1930, *I. L. Wiggins & D. Demaree* 4873 (US); **Chihuahua**: 46) 27.6 mi NW of Rocheachi, 19 Sep 1991, *P. M. Peterson, C. R. Annable & J. Valdes-Reyna* 10798 (US); **Durango**: 45) 16.5 m. W of El Salto on HWY 40, 29 Sep 1988, *P. M. Peterson & C. R. Annable* 6041 (US); **U.S.A.** **Alaska**: 20) Little Susitna Valley, 14 mi N of Palmer, 13 Sep 1963, *W. W. Mitchell* 1105 (US); **Arizona**: 7) Chiricahua Mountains, Bear Creek Trail, 7 Sep 1988, *P. M. Peterson & C. R. Annable* 5502 (US); 9) Huachuca Mountains, 17 Oct 1893, *E. A. Mearns* 2579 (US); 12) Idem, Carr Canyon, 29 Sep 1945, *F. W. Gould & H. S. Haskell* 3323a (US); 10) Idem, Garden Canyon, 24 Aug 1948, *E. Kurtz & H. Haskell* 268 (Wagnon 1542) (US) C; 8) Mount Graham, 12 Aug 1934, *T. H. Kearny & R. H. Peebles* 9915 (US); 14) San Francisco Mts., near Flagstaff, 27 Jul 1901, *J. B. Leiberger* 5749 (US); 4) Santa Catalina Mts., Bear Canyon, 15 Sep 1994, *S. J. Darbyshire & J. Cayouette* 4598 (DAO); 69) Idem, Mt. Lemmon, 15 Sep 1994, *J. Cayouette & S. J. Darbyshire* 7974 (DAO) CM-MLE; 11) Idem, Soldier's Camp, 25 Jul 1948, *M. C. Young* 519 (Wagnon 1515) (DAO) C; **California**: 6) Inyo Co., Big Pine Creek, 31 Aug 1931, *R. Hoffmann* 583 (US); 93) John Muir Wilderness, Lamark Trail below Grass Lake, 28 Sep 1999, *P. M. Peterson, R. Blackburn & M. R. Peterson* 14842 (US) CM-GRA; 67) San Geronio Mts., S of Barton Flats, 2 Sep 1994, *J. Cayouette & S. J. Darbyshire* 7951 (DAO) CM-SBM; **Colorado**: 2) Capital City, 12 Jul 1966, *Sponberg* 66-123 (US); 29) Moraine Park, 25 Jul 1948, *H. K. Wagnon* 1519 (DAO) C; 30) Eagle Co., Redcliff, 22 Jul 1898, *Shear & Bessey* 1284 (1433) (US); **Nevada**: 22) Ely, Duck Creek valley, 13 Aug 1913, *A. S. Hitchcock* 872 (DAO); 23) Kyle Canyon, Charleston Mts., 27 Aug 1957, *P. H. Raven* 11282 (US); **New Mexico**: 16) Cimarron Canyon, 29 Aug 1964, *Weatherwax* 2990 (DAO); 19) Sancha Mts., 4 Aug 1910, *E. O. Wootton s. n.* (US); 15) Santa Fe Canyon, 7 Sep 1928, *M. A. Talbot* 721 (US); 17) vicinity of Ute Park, 30 Aug 1916, *P. C. Standley* 14035 (US); **South Dakota**: 36) Black Hills, Harney Peak trail, 11 Aug 1924, *H. E. Hayward* 369 (US); 37) 5 mi E of Pringle Ranger Station, 20 Jul 1948, *A. A. Beetle* 9040 (US); **Texas**: 28) Davis Mts., Limpia Canyon near Mt. Livermore, 27 Aug 1939, *L. C. Hinckley* 1218 (US); **Utah**: 27) Dixie Ntn. Forest, Posy Lake, 13 Aug 1965, *N. H. Holmgren, J. L. Reveal & C. LaFrance* 2459 (DAO); **Wyoming**: 32) 2 mi SW of Arlington, 11 Sep 1992, *P. M. Peterson & C. R. Annable* 11949 (US); 33) NW of Cheyenne, Pole Mts., 28 Jul 1940, *J. R. Swallen* 6575 (US); 34) Cooper Hill, 22 Aug 1897, *A. Nelson* 4407 (US).