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A morphological and molecular study in the *Deschampsia cespitosa* complex (Poaceae; Poeae; Airinae) in northern North America[1]

Jorge O. Chiapella[2], Veronica L. DeBoer[3,4], Guillermo C. Amico[5], and Joseph C. Kuhl[3,6]

[2]Laboratorio Molecular, IMBIV-Universidad de Córdoba—CONICET, Campus Ciudad Universitaria, Velez Sarsfield 1611, X5016GCA Córdoba, Argentina; [3]U. S. Department of Agriculture, Agricultural Research Service, Subarctic Agricultural Research Unit, Palmer, Alaska 99645 USA; [4]Scientific Crime Detection Laboratory, 5500 East Tudor Rd., Anchorage, Alaska 99507 USA; and [5]Laboratorio Ecotono, INIBIOMA (CONICET-Universidad Nacional del Comahue), Quintral 1250, R8402GAL, Bariloche, Argentina

- *Premise of the study:* In the North American Arctic, the existence of one or several taxa closely related to *Deschampsia cespitosa* var. *cespitosa* has remained a puzzle for many years. Extreme morphological variation, lack of clear limits between alleged forms, and an extended geographic range often render identification keys incomplete, and raise the question of how many species this taxon represents.
- *Methods:* Morphological and molecular analysis, including multivariate statistics, ITS and the cpDNA marker *trnK-rps16*, was conducted on *D. cespitosa* var. *cespitosa* and related taxa using 201 herbarium specimens from northern North America (Alaska, Canada, and Greenland). Fifty-three morphological characters were recorded from all specimens, while sequences were retrieved from 167 specimens.
- *Key results:* Results show that *Deschampsia cespitosa* (L.) P. Beauv. var. *cespitosa*, *D. cespitosa* subsp. *alpina* (L.) Tzvelev, *D. cespitosa* subsp. *beringensis* (Hultén) W. E. Lawr., *D. brevifolia* R. Br., *D. cespitosa* (L.) P. Beauv. subsp. *glauca* (Hartm.) C. Hartm., *D. mackenzieana* Raup, *D. cespitosa* subsp. *orientalis* Hultén, and *D. pumila* (Griseb.) Ostenf. differed significantly in a few morphological variables, but molecularly are a closely related group with several sequences and haplotypes that are nearly identical.
- *Conclusions:* Overall, the evidence points to the existence of a single species, *Deschampsia cespitosa*. The occurrence of slightly different morphological types related to specific geographical distributions allows recognition of three additional taxa at the infraspecific level, *D. cespitosa* subsp. *alpina*, *D. cespitosa* subsp. *beringensis*, and *D. brevifolia*. All studied taxa showed morphological variation in a gradient, suggesting the existence of phenotypic plasticity.

Key words: Arctic; *Deschampsia*; morphology; North America; Poaceae; ITS; *trnK-rps16*.

Historically, the Arctic has presented plant taxonomists a dilemma: how many closely related taxa exist for any given species, additional species, and/or subspecies? The study of Arctic grasses has repeatedly been confounded by how to clearly and consistently resolve the circumscription of species, a consequence of high morphological variation and broad geographic distribution, frequently extending over vast circumpolar ranges. Arctic grass taxonomy is further confounded by different interpretations among European, North American, and Russian botanists, resulting in conflicting taxonomies for the Arctic flora, and lack of a concise list of species (Brysting et al., 2004). The analysis of molecular data together with morphology has provided new approaches and often the recognition of single, extremely variable species [e.g., *Arctagrostis latifolia* (Aiken and Lefkovitch, 1990), *Festuca brachyphylla* (Aiken et al., 1994), and *Dupontia fisherii* (Brysting et al., 2004)].

In addition to a circum-arctic distribution (Hultén, 1962), *Deschampsia* P. Beauv. also occurs in the southern hemisphere where scattered populations thrive along the Andes in Argentina and Chile south of 30°S (Chiapella and Zuloaga, 2010) and Australia and New Zealand (Edgar, 1993). *Deschampsia cespitosa* var. *cespitosa*, the most abundant and widespread variety, is a perennial, self-incompatible, tussock plant with high morphological variation attributed to phenotypic plasticity (Seliskar, 1985a, b). The two common cytotypes in North America, consisting of 26 and 52 chromosomes (Kawano, 1963, 1966; Rothera and Davy, 1986), do not show a specific relationship to a particular morphotype.

In the past researchers used the classical "ecotype" concept (Turesson, 1922), i.e., in the context of "common garden" transplantation studies (Lawrence, 1945; Ward, 1969; Pearcy and Ward, 1972), to provide a framework to organize the

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[6]Author for correspondence (e-mail: jkuhl@uidaho.edu); present address: Department of Plant, Soil, and Entomological Sciences, University of Idaho, P.O. Box 442339, Moscow, Idaho 83844 USA

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extreme variation shown by the species. The array of different forms vary gradually and have sympatric distribution areas; a common puzzle has been to determine whether variability can be better accounted for as a single species with several infraspecific taxa, or several closely related species. This situation reflects the old (and not fully resolved) conflict of how to accommodate the plastic nature of morphological variation into the categories (or hierarchical ranks) of traditional taxonomical schemes, which are always rigid. The delimitation of taxa in a complex of species, in which variation is gradual and borders fuzzy as in the *D. cespitosa* complex in Central Europe (Chiapella, 2000), should be related to an independent factor such as distribution. By considering morphological variation together with geographic distribution (sensu DuRietz, 1930) it might be possible to construct a framework to accommodate continuous variation in defined geographic regions.

Selection of geographical constraints for this study was not readily obvious. The scope of the work focused originally in Alaska, but *D. cespitosa* var. *cespitosa* has a widespread distribution throughout the arctic, subarctic, and continental regions of North America, although with varying abundance, extending over major vegetation zones, tundra, boreal forests, and Rocky Mountains (Barbour and Billings, 2000). The Arctic is traditionally considered to extend northward of the boreal tree line (Yurtsev, 1994; Elvebakk et al., 1999), but the establishment of this line is problematic since the mean height of several tree species gradually shrinks poleward (Britton, 1967; Viereck and Little, 2007). So instead of focusing on a particular area, the study was extended to include all forms in the Arctic and subarctic or boreal zone, because there is no clear separation among those. Victorin in his *Flore Laurentienne* (1964, p. 30) used a comparable rationale, by asserting that the vegetation unit called subarctic forest changed gradually into a coniferous forest and that studying each separately would be partial. In our case, the immediate consequence was extending the range of the study to cover Greenland, most of Canada (primarily northern specimens), Alaska, and a few specimens from eastern Russia.

Early treatments on Arctic plants included forms related to *Deschampsia cespitosa* and foreshadowed problems in species delimitation (Brown, 1823; Vasey, 1892, p. 45); in particular Brown (1823, p. 3) mentioned that "I have also experienced also much greater difficulty than I had anticipated in determining many of the species; arising either from their extremely variable nature". Brown (1823, p. 33) also described *D. brevifolia*. Among the many botanists who treated *Deschampsia* in the Arctic region and neighboring areas, Eric Hultén clearly stands out as the most prolific. In 1927, he started a series of studies on the Arctic flora with an account of the plants of the Kamchatcka Peninsula, where he described two taxa, *D. beringensis* and *D. cespitosa* subsp. *orientalis*. Hultén (1927) distinguished *D. beringensis* from *D. cespitosa* var. *cespitosa* mainly by its larger size, longer and glabrous leaves, and the spikelets being often 3-4 flowered; he noted that the newly described taxon was already observed by Trinius (1820) as "similar" to *D. bottnica* (=*D. cespitosa* subsp. *Bottnica*), a European taxon found on the coasts of the Gulf of Bothnia. *Deschampsia cespitosa* subsp. *orientalis* was described as the widespread form of *D. cespitosa* in eastern Siberia, which differed in being smaller and having more contracted panicles. The geographic distribution was also noted as different, since the taxon is restricted to Kamchatcka and the Kuriles. In the following years, he produced floras for the Aleutian Islands (Hultén, 1937) and Alaska (Hultén, 1941, 1968). In his work on Alaska, Hultén (1941) accounted for five species, *D. beringensis*, *D. danthonioides*, *D. elongata*, *D. flexuosa* (*Avenella flexuosa*), and *D. cespitosa* with two varieties (*D. cespitosa* var. *cespitosa* and *D. cespitosa* var. *glauca*) and one subspecies (*D. cespitosa* subsp. *orientalis*), differentiated by plant height, the width of basal leaves, and the insertion point of the awn.

Other authors who studied *Deschampsia* in northern regions included Porsild (1955), who accepted only one species, *D. brevifolia*; Böcher et al. (1968) reported five species for Greenland (*D. cespitosa* var. *cespitosa*, *D. alpina*, *D. brevifolia*, *D. pumila*, and *D. flexuosa*); Scoggan (1978) accounted six species for Canada (*D. cespitosa* var. *cespitosa*, *D. alpina*, *D. danthonioides*, *D. elongata*, *D. atropurpurea* [*Vahlodea atropurpurea*], and *D. flexuosa*); Porsild and Cody (1980) reported four taxa for continental northwestern Canada (*D. cespitosa* var. *cespitosa*, *D. brevifolia*, *D. mackenzieana*, and *D. pumila*). The most recent treatment of *Deschampsia* (for North America north of Mexico) was done by Barkworth (2007), who accepted eight taxa: *D. cespitosa* (with three subspecies, *D. cespitosa* subsp. *cespitosa*, *D. cespitosa* subsp. *beringensis*, and *D. cespitosa* subsp. *holciformis*), *D. alpina*, *D. danthonioides*, *D. elongata*, *D. mackenzieana*, *D. sukatschewii*, and *D. flexuosa*).

The disagreements concerning North American *Deschampsia* extend well beyond North America; for example, Aiken et al. (1995) considered *D. sukatschewii* (Popl.) Roshev. to be the correct name for the species fomerly known as *D. pumila* (Griseb.) Ostenf. The Russian botanist Nina S. Probatova (Institute of Biology & Soil Science, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia, personal communication), however, considers *D. sukatschewii* presence in North America as doubtful, since it is usually found in the Transbaicalian-Amur river basin; plants corresponding to its description are rarely found in far eastern locations such as Sakhalin. In addition, Hultén (1968, p. 112) regards *D. sukatschewii* as a synonym of *D. cespitosa* subsp. *orientalis*, which has a main distribution area in northern Siberia, Kamchatka, and the Kuriles (Chiapella and Probatova, 2003) and reaches only the shores of Alaska and Arctic Canada. Another nomenclature predicament surrounds the existence of a morphologically identifiable form of *Deschampsia* corresponding to the epithet *pumila*. Nomenclatural problems in *Deschampsia* in North America are extensive, and we will deal with them in a future work, but the present contribution aims at clarifying the existence of one or more species, specifically the question: how many taxa exist in the region comprised from Greenland, through Canada to Alaska? With this aim, we conducted a morphological analysis and a phylogenetic study of nuclear ITS and plastid marker *trnK-rps16* sequences to recognize taxa differing morphologically and/or molecularly from the common, widespread *D. cespitosa* var. *cespitosa*.

MATERIALS AND METHODS

Taxon sampling—After a preliminary assessment of approximately 800 herbarium specimens of *Deschampsia* from Greenland, Canada, Alaska, and eastern Russia belonging to ALA, CAN, C, and S (Holmgren et al., 1990), 201 specimens with fully mature plants were selected, covering the region mentioned above (Appendix S1; see Supplemental Data with the online version of the article). The specimens were treated as operational taxanomic units (OTUs), representing the following taxa (including both recognized and unrecognized by Barkworth, 2007): *D. cespitosa* (L.) P. Beauv. var. *cespitosa* (67 specimens; identified using keys from Hitchcock et al., 1969); *D. cespitosa* subsp. *alpina* (L.) Tzvelev (11 specimens; keys in Tzvelev, 1976: 413); *D. cespitosa* subsp.

beringensis (Hultén) W. E. Lawr. (27 specimens; keys in Lawrence 1945; Hultén, 1968: 110, and Tzvelev, 1976: 415); *D. brevifolia* R. Br. (38 specimens; key in Hultén, 1968, p. 110 and Tzvelev, 1976, p. 414); *D. cespitosa* (L.) P. Beauv. subsp. *glauca* (Hartm.) C. Hartm. (20 specimens; keys in Wiggins and Thomas, 1962, p. 73; Hultén, 1968, p. 110, and Tzvelev, 1976, p. 414); *D. mackenzieana* Raup (3 specimens), *D. cespitosa* subsp. *orientalis* Hultén (12 specimens; keys in Hultén, 1968, p. 110; Tzvelev, 1976, p. 415, and Koyama, 1987, p. 150); and *D. pumila* (Griseb.) Ostenf. (23 specimens; key in Hultén, 1968, p. 110). A taxon probably related, *D. holciformis* J. Presl, was not included because its main distribution area on the Pacific coast of the continental United States is south of the primary area of study.

Morphological analysis—Fifty-five characters (variables) were selected after study of identification keys and published descriptions in floras, revisions, and studies on *Deschampsia* in North America (Hultén, 1927, 1937, 1941, 1962, 1968; Polunin, 1940, 1959; Lawrence, 1945; Hitchcock, 1951; Tieszen and Bonde, 1967; Hitchcock et al., 1969; Ward, 1969; Pearcey and Ward, 1972; Scoggan, 1978; Koch, 1979; Porsild and Cody, 1980; Purdy and Bayer, 1995; Barkworth, 2007), Europe (Paunero, 1955; Kawano, 1963, 1966; Chrtek and Jirasek, 1965; Clarke, 1980; Hedberg, 1986; Conert, 1987; Chiapella, 2000), and South America (Chiapella and Zuloaga, 2010) and includes most characters used in grass descriptions such as culm height and branching, size of panicles, shape, size and hairiness of glumes and lemmas, length of the awns, and point of insertion in the lemmas, occurrence of vivipary (pseudovivipary), among other characters (complete list in Table 1). The length of lemmas, paleas, and awns in spkelets of *D. alpina* were not recorded due to the extreme deformation that is common in plants with pseudovivipary and are recorded as missing data. Character traits included both quantitative and qualitative variables. Measurements of spikelets, ligules, and leaf width were measured with a digital caliper and transferred directly into a spreadsheet. All morphological data are in Appendix S2 (see Supplemental Data with the online version of the article). Analyses applied bivariate and multivariate methods. The variation within and among the putative taxa with respect to individual quantitative characters was examined by comparing box-and-whisker plots, featuring median values, first and third quartiles, and extreme values range. Dispersion diagrams were done between two pairs of variables, panicle length/plant height, and awn length/lemma length (first of each pair as *x*-coordinate, second as *y*-coordinate). The nonparametric Mann–Whitney *U* test (MW test) for pairs of samples (Sokal and Rohlf, 1995) was carried out between *D. cespitosa* var. *cespitosa* and each of the considered taxa to detect deviations. Finally, the multivariate method nonmetric multidimensional scaling (NMDS) (Kruskal, 1964; Legendre and Legendre, 1998) was used on a data matrix with quantitative variables log transformed and standardized to examine the relationships of all OTUs. Nonmetric multidimensional scaling is an ordination method that differs from principal component analysis (PCA) and principal coordinate analysis (PCOORDA) in that distances among OTUs in the scatter plot have a monotone relationship to distances among the original objects (Kruskal, 1964) and that results in dissimilar objects being farther apart and similar objects clustering together (Legendre and Legendre, 1998, p. 444). Analysis is performed in an iterative manner after an initial configuration of points, usually obtained by PCA or PCOORDA, and computes a statistic called *Stress*, which measures the goodness of fit of the distances displayed in the plot to the monotone function of the distances of the OTUs in the original data. The statistic ranges between 0 (perfect fit) and 1 (poorest fit); Kruskal (1964) suggests the following values of *Stress* measurement: 0.00, perfect; 0.05, excellent; 0.10, good; 0.20, fair; and 0.40, poor. The number of dimensions included in the analysis has a direct influence on the *Stress* statistic (i.e., the more dimensions the less *Stress* indicating better fit) (Hartman, 1988). In our case, two dimensions were included. The default settings of NTSYS (*Stress* 1 and '*Mono*') were used. Mann–Whitney test and NMDS were done with the program NTSYS-pc (Rohlf, 1986, 1998), box-and-whiskers plots, dispersion diagrams and regressions with Infostat (2008).

DNA extraction, PCR, and sequencing—Genomic DNA was extracted from leaf samples of herbarium specimens using DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). Amplification of double-stranded DNA was carried out using a standard polymerase chain reaction (PCR) in 25-μL reactions containing 2.5 μL template DNA, 0.625 units of PrimeSTAR HS DNA polymerase (Takara Bio USA, Madison, Wisconsin, USA), 5 μL of 5× buffer, 0.2 mmol/L dNTPs, and 1.2 μmol/L amplification primers. Chloroplast primers *trnK5*′r and *rps*16-4547mod were used to amplify the *trnK-rps16* intergenic region and 3′ end of *rps*16 (*trnK-rps16*; Kress et al., 2005). The PCR program included 31 cycles of 15 s at 94°C; 5 s at initial 50°C (0.3°C increase per cycle), 3 min at 72°C; and a final extension of 10 min at 72°C. PCR products were

TABLE 1. Characters used in morphological analyses: continuous variation (characters 1, 5, 6, 7, 8, 12, 13, 18, 19, 26, 27, 30, 31, 36, 37, 44, 49), two-state characters (2, 16, 20, 21, 25, 29, 35, 38, 46, 47, 48, 50, 54) and multistate discontinuous characters (3, 4, 9, 10, 11, 14, 15, 17, 22, 23, 24, 28, 32, 33, 34, 39, 40, 41, 42, 43, 45, 51, 52, 53, 55).

Morphological character		Character states
1	Plant height	(mm)
2	Tillering	intravaginal (0), extravaginal (1)
3	Nodes of fertile shoot	(no.)
4	Nodes of panicle	(no.)
5	Length of panicle	(mm)
6	Width of panicle	(mm)
7	Length 1st panicle internode	(mm)
8	Length 2nd panicle internode	(mm)
9	Ramifications of 1st node of panicle	(no.)
10	Ramifications of 2nd node of panicle	(no.)
11	Scabrousness of secondary ramifications	glabrous (0), few (1), abundant (2)
12	Length of blade of penultimate leaf	(mm)
13	Width of penultimate leaf	(mm)
14	Length of longest basal blade leaf	(mm)
15	Width of longest basal blade leaf	(mm)
16	Scabrousness of veins of penultimate leaf-abaxial side	glabrous (0), few (1), abundant (2)
17	Scabrousness of veins of penultimate leaf-adaxial side	glabrous (0), few (1), abundant (2)
18	Nature of margin of leaf	membranous (0), scarious (1)
19	Scabrousness of leaf sheaths	glabrous (0), few (1), numerous (2)
20	Height of leaf node	(mm)
21	Length of ligule	(mm)
22	Nature of ligule	membranous (0), scarious (1), mixed (2)
23	Shape of ligule apex	sharp (0), other (1)
24	Color of spikelets	purple-violaceous (0), greenish (1), golden- yellow (2)
25	Florets per spikelet	1 (0), 2 (1), 3 or more (2)
26	Pseudovivipary	absent (0), present (1)
27	Hairiness of rachilla	glabrous (0), few (1), abundant (2)
28	Shape of glume 1	lanceolate (0), narrowly lanceolate (1)
29	Length of glume 1	(mm)
30	Width of glume 1	(mm)
31	No. of nerves of glume 1	(no.)
32	Shape of glume 2	lanceolate (0), narrowly lanceolate (1)
33	Length of glume 2	(mm)
34	Width of glume 2	(mm)
35	No. of nerves of glume 2	(no.)
36	Scabrousness of veins of glumes	absent (0), only midvein (1), all veins (2)
37	Scabrousness between veins	absent (0), few (1), abundant (2)
38	Margin of glumes	membranous (0), scarious (1)
39	Length of lemma 1	(mm)
40	Width of lemma 1	(mm)
41	Nature of lemma	membranous (0), scarious (1)
42	Relative size of apical teeth	all similar (0), lateral larger (1), central larger (2)
43	No. of apical teeth	(no.)
44	No. of nerves of lemma 1	(no.)
45	Scabrousness of nerves of lemma 1	glabrous (0), few (1), numerous (2)
46	Scabrousness between nerves	glabrous (0), few (1), numerous (2)
47	Length of awn	(mm)
48	Insertion of awn	basal 1/3 (0), medium 1/3 (1), superior 1/3 (2)
49	Nature of awn	straight (0), bended (1)
50	Nature of awn II	not twisted (0), twisted (1)
51	Scabrousness of awn	absent (0), present (1)
52	Length of palea 1 (mm)	(mm)
53	Nature of palea 1	membranous (0), hyaline (1)
54	Shape of palea dorsum	Bi-keeled (0), other (1)
55	Scabrousness of nerves of palea 1	glabrous (0), few (1), abundant (2)

cleaned using Agencourt AMPure Kit (Beckman Coulter, Fullerton, California, USA) according to the manufacturer's protocol. The poly A tailing procedure was conducted using a 10 μL reaction containing 7 μL purified PCR product, 1× Platinum *Taq* PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dATP, and 5 units Platinum *Taq* DNA Polymerase (Invitrogen, Carlsbad, California, USA) incubated for 1 min at 94°C and 20 min at 68°C. Ligation and transformation used 2 μL of poly A tailed product with the pGem-T Easy Vector System (Promega, Madison, Wisconsin, USA) according to the manufacturer's protocols. White colonies were selected and plasmids were purified using the QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer's protocol. Presence of the correct-sized insert, ~625 bp for the *trnK-rps*16 region, was verified after digestion with *Eco*RI restriction enzyme and electrophoresis. Glycerol stocks of plasmids containing *trnK-rps16* fragments were sent to the Research Technical Support Facility at Michigan State University (East Lansing, Michigan, USA) for extraction and sequencing. Plasmids were extracted using an Agencourt CosMCPrep (Beckman Coulter) on a Beckman Coulter Biomex FX pipetting robot. The cleaned products were used in cycle-sequencing 1/8 reaction with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, California, USA) using M13 18-mer forward and reverse primers. Reactions were cleaned with Agencourt CleanSEQ (Beckman Coulter) and sequenced in an ABI 3730xl DNA Analyzer with a 50 cm array. Samples were resequenced as necessary to recover clearly defined base calls for forward and reverse reads.

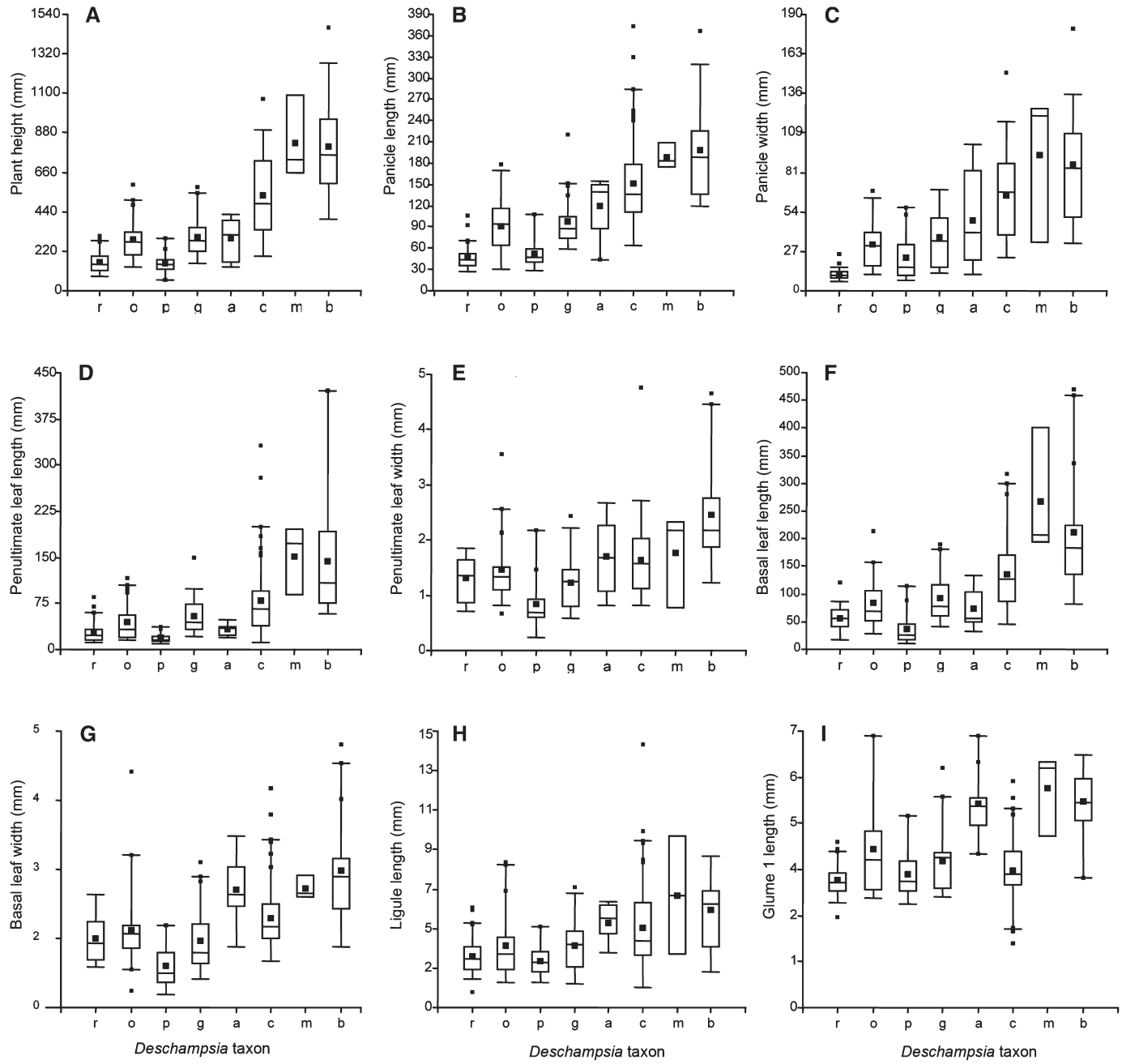

Fig. 1. Box-and-whisker comparative plots of quantitative morphological characters of (c) *Deschampsia cespitosa* var. *cespitosa*, (a) *D. cespitosa* subsp. *alpina*, (b) *D. cespitosa* subsp. *beringensis*, (r) *D. brevifolia*, (g) *D. cespitosa* subsp. *glauca*, (m) *D. mackenzieana*, (o) *D. cespitosa* subsp. *orientalis*, and (p) *D. pumila*. Box: upper (75%) and lower (25%) quartiles; horizontal line in box: median value; point in box: mean; whiskers: upper (95%) and lower (5%) quartiles; points outside box: extreme values. All measurements are in millimeters.

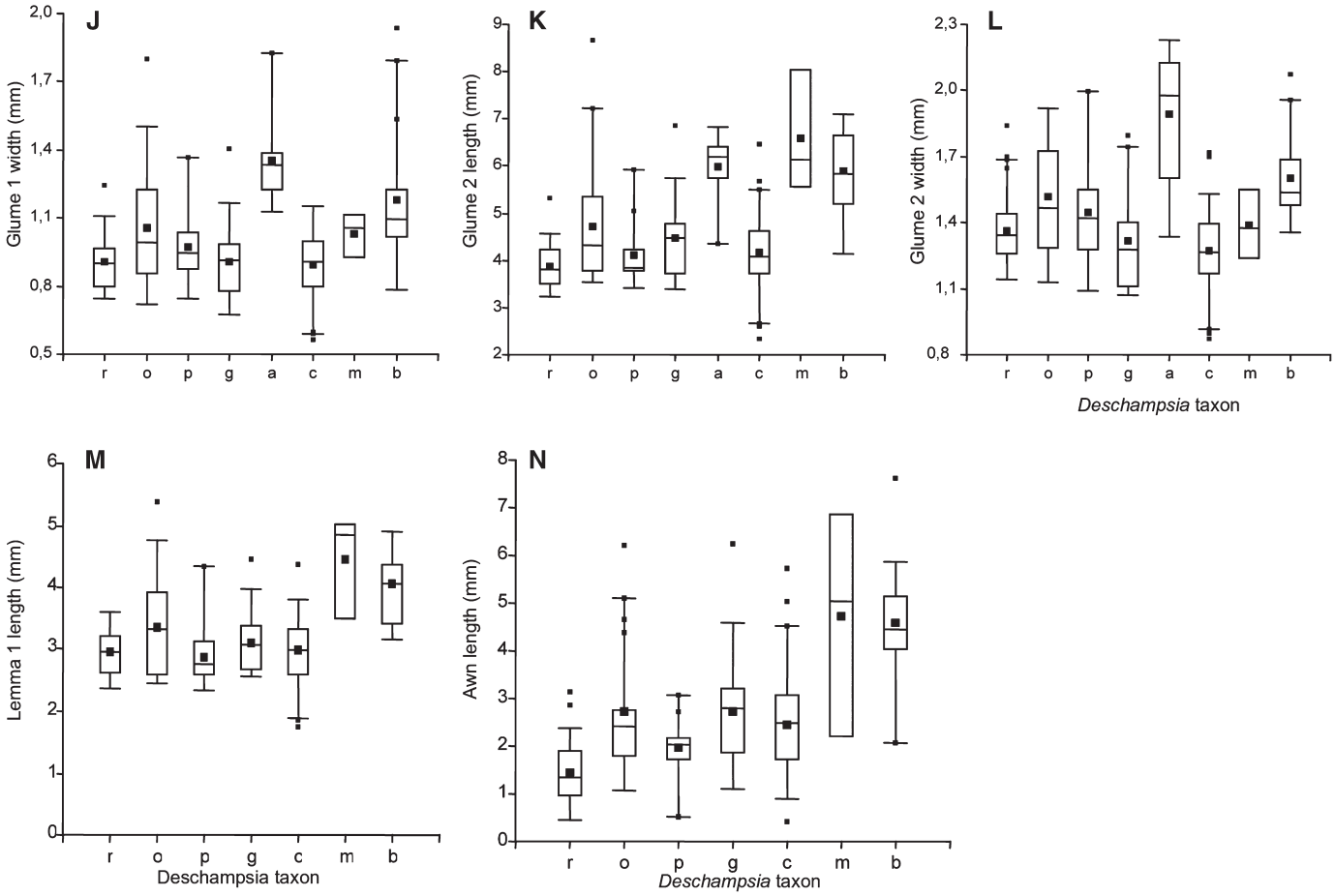

Fig. 1. Continued.

The entire ITS region was amplified with primers ITS 5 and ITS 4 (White et al., 1990) using reaction conditions and PCR settings listed above. Fragment size, ~547 bp, was verified by electrophoresis prior to sequencing. The ITS PCR products were processed through Sephadex G-50 (Sigma, Ronkonkoma, New York, USA) columns and sent for direct sequencing using primers ITS4 and ITS5 (White et al., 1990). Chromatograms for both *trnK-rps16* and ITS were edited and assembled with Sequencher version 4.8 (GeneCodes, Ann Arbor, Michigan, USA). Each sample is the consensus of the forward and reverse sequencing results, and all discrepancies were manually edited according to IUPAC ambiguity codes. ITS sequences from all samples were shortened to remove low quality base calls on the 5′ and 3′ ends by 37 and 15 bp, respectively. Sequences were submitted to GenBank (accession numbers HQ114285–HQ114562).

Alignment and phylogenetic analyses—Sequences were aligned manually using BioEdit (Hall, 1999). Alignments for *trnK-rps16* and ITS can be found in Appendices S3 and S4, respectively (see Supplemental Data with the online version of the article). Maximum parsimony (MP) and Bayesian Markov chain Monte Carlo inference (BI; Yang and Rannala, 1997) analyses were used to estimate evolutionary relationships among trnK-rps16 haplotypes and ITS genotypes. Only one individual representing each haplotype/genotype was used to conduct MP analysis with the program PAUP* version 4b10 (Swofford, 2002) and BI analysis with the program MrBayes (Ronquist and Huelsenbeck, 2003). We executed MP using the heuristic search option, with 1000 random additions keeping up to 100 trees in each replicate; the branch-swapping algorithm was tree-bisection-and-reconnection (TBR) for all separate and combined analyses. Nodal support was assessed using the nonparametric bootstrap (BS; Felsenstein, 1985) with 1000 pseudoreplicates using a heuristic search with nearest neighbor interchange option for branch swapping. For BI, the best fitting model of sequence evolution was determined using the program MrModeltest (Nylander et al., 2004). The hierarchical likelihood ratio test (Felsenstein, 1988) selected the HKY85 (Hasegawa et al., 1985) model, which was then used for BI analysis. We executed BI in two independent analyses, each with four chains, for five million generations each. Trees and parameters were saved every 100 generations, producing 50 000 trees. Starting model parameters were assigned a uniform prior probability distribution except for the base frequencies where a Dirichlet distribution was assigned. In concatenated analysis, the estimates between partitions were unlinked, thus allowing each to vary independently. The run was set to stop if topological convergence was reached between the two runs, which was determined by the presence of a standard deviation in split frequencies that was lower than 0.01 (discarding 25% as burn-in). Upon run completion, inspection of the likelihood scores vs. generation plots showed that these scores had always reached stationary before the first 25% of the samples; thus, discarding this fraction as burn-in was conservative.

Parsimony network—The haplotype network was constructed using the program TCS version 1.3 (Clement et al., 2000). The network with probabilities above the parsimony limit (0.95) was selected. We used both chloroplast and nuclear regions analyzed together to construct the haplotype network.

Analysis of molecular variance—Analysis of molecular variance (AMOVA) was performed with combined ITS and *trnK-rps16* data to examine genetic relationships between the infraspecific taxa of *Deschampsia* and among geographic regions. A matrix with haplotype data were analyzed with the program Arlequin (Excoffier et al., 2005), using the Kimura (1980) two-parameter distance. Fixation indices significance was tested with a nonparametric approach after 1000 permutations. On the basis of grouping results of morphological data, we decided to test haplotype frequency from *D. cespitosa* subsp. *alpina* against the other taxa. The data were further analyzed to assess whether the genetic variability was related to the different alleged taxa.

RESULTS

Morphological analysis—Four recorded characters were excluded prior to analysis because of the lack of variation: character 2, tillering (always intravaginal); character 18, margin of leaves (always membranous); character 37, scabrousness between veins (always glabrous); and character 51, scabrousness of awns (always scabrous). Trends in variation of quantitative characters are shown in Fig. 1. The results of the Mann–Whitney test are given in Table 2, and among the main results, it shows that (1) plant height differed significantly in most of the paired comparisons, except between *D. cespitosa* var. *cespitosa* and *D. mackenziana*, where differences were nonsignificative; (2) panicles differed significantly in length and width between *D. cespitosa* var. *cespitosa* and all other taxa, except with *D. mackenzieana* and *D. cespitosa* subsp. *alpina* (Fig. 1A, Table 2). No difference was found between *D. cespitosa var. cespitosa* and *D. mackenzieana* in panicle length or width (Fig. 1A, 1B; Table 2). Glumes differed significantly between *D. cespitosa* var. *cespitosa* and *D. cespitosa* subsp. *alpina*, *D. cespitosa* subsp. *beringensis* and *D. brevifolia*, (Fig. 1I–1L, Table 2), although the lower glume width (Fig. 1J) was not different. *Deschampsia cespitosa* subsp. *orientalis* differed only in glume width but not in length, while *D. mackenzieana* differed in length but not in width. *Deschampsia cespitosa* subsp. *glauca* presented no significant difference in glume length with *D. cespitosa* var. *cespitosa* (Fig. 1I–1K, Table 2). Lemmas had a low degree of differentiation, with significative differences only between *D. cespitosa* var. *cespitosa* and *D. cespitosa* subsp. *beringensis* and *D. mackenzieana* (Fig. 1M, Table 2) and the width between *D. cespitosa* var. *cespitosa* and *D. cespitosa* subsp. *orientalis*. No other comparisons showed significant differences (*P* values in Table 2). Lemmas and glumes of *D. cespitosa* subsp. *alpina* (Fig. 1J–1M) are larger than most of other studied taxa.

A linear relationship between panicle length and plant height and awn length and lemma 1 length was found in all taxa and is depicted in dispersion diagrams in Appendices S5A and S5B (see Supplemental Data with the online version of the article).

Analysis using NMDS was performed on a similarity matrix of 201 OTUs × 51 characters using an initial configuration created with PCOORDA (graph not shown). The initial input matrix went through 40 iterations before reaching the minimum *Stress* value. A two-dimensional scatter plot with *Stress* coefficient S= 0.19 indicated a fit between fair and good (Fig. 2). It shows specimens belonging to *D. cespitosa* var. *cespitosa*, *D. cespitosa* subsp. *alpina*, *D. cespitosa* subp. *beringensis*, and *D. brevifolia* grouping more or less together; *D. cespitosa* subsp. *alpina* departs from *D. cespitosa* var. *cespitosa* while *D. cespitosa* subsp. *beringensis* and *D. brevifolia* showed overlap with *D. cespitosa* var. *cespitosa* and a small overlap between them. All other taxa (*D. cespitosa* subsp. *glauca*, *D. cespitosa* subsp. *orientalis*, *D. mackenziana*, *D. pumila*) showed complete overlap with *D. cespitosa* var. *cespitosa*.

Sequence variation in Deschampsia—Amplification and sequencing yielded different numbers of readable sequences for the two regions; the *trnK-rps16* yielded 167 sequences, while 98 were recovered from the ITS region. The *trnK-rps16* spacer, including outgroups, had 636 aligned positions, while the ITS region including the outgroups had 548 aligned positions. The alignment contained few gaps that were unambiguously aligned and were treated as missing data in the phylogenetic analyses. Information on variable, parsimony informative and uninformative sites are given in Table 3.

A total of 36 haplotypes were recognized for *Deschampsia* in the *trnK-rps16* spacer. The ITS region was less variable than the chloroplast, with only nine haplotypes and no obvious relationships between them. The nuclear region added only three haplotypes, totaling 39, when both regions were combined (Appendix S6; see online Supplemental Data). A combined parsimony analysis of the haplotypes was constrained to 100 000 trees. Using both MP and BI analyses, the *D. cespitosa* group obtained strong support, but without internal resolution (Fig. 3).

The combined haplotype parsimony network shows two haplotypes (H01 and H04) as being common (Fig. 4) and widely distributed (Fig. 5A). Haplotypes H01 and H04 differ by 3 bp

TABLE 2. Mann–Whitney test results for paired comparisons among *Deschampsia cespitosa* var. *cespitosa* and each studied taxa of the complex.

Character	Comparison of *Deschampsia* taxa						
	C-A	C-B	C-G	C-M	C-O	C-P	C-R
Plant height	*** (0.0009)	*** (0.0003)	*** (0.0001)	ns (0.0595)	*** (0.0001)	*** (0.0001)	*** (0.0001)
Panicle length	ns (0.1778)	** (0.0061)	*** (0.0001)	ns (0.1035)	*** (0.0001)	*** (0.0001)	*** (0.0001)
Panicle width	ns (0.0876)	* (0.0228)	*** (0.0001)	ns (0.2050)	*** (0.0001)	*** (0.0001)	*** (0.0001)
Penultimate leaf length	*** (0.001)	** (0.0013)	ns (0.0993)	* (0.0384)	* (0.0044)	*** (0.0001)	*** (0.0001)
Penultimate leaf width	ns (0.7088)	*** (0.0001)	ns (0.0057)	ns (0.6051)	ns (0.1704)	*** (0.0001)	* (0.0140)
Basal longest leaf length	** (0.0014)	*** (0.0008)	** (0.0038)	* (0.0252)	*** (0.0007)	*** (0.0001)	*** (0.0001)
Basal longest leaf width	** (0.0044)	*** (0.0002)	** (0.0036)	* (0.0369)	ns (0.0686)	*** (0.0001)	* (0.0038)
Ligule length	ns (0.1871)	* (0.0416)	ns (0.1626)	ns (0.2430)	ns (0.0630)	* (0.0023)	*** (0.0004)
Lower glume length	*** (0.0001)	*** (0.0001)	ns (0.4062)	(0.0080)	ns (0.0917)	ns (0.4707)	** (0.0400)
Lower glume width	*** (0.0001)	*** (0.0001)	ns (0.9309)	ns (0.0952)	* (0.0095)	ns (0.1336)	ns (0.9757)
Upper glume length	*** (0.0001)	*** (0.0001)	ns (0.1903)	** (0.0051)	ns (0.2009)	ns (0.5393)	* (0.0177)
Upper glume width	*** (0.0001)	*** (0.0001)	ns (0.7442)	ns (0.2637)	*** (0.0005)	* (0.0202)	* (0.0163)
Lower lemma length	na	*** (0.0001)	ns (0.4599)	* (0.0107)	ns (0.1464)	ns (0.2292)	ns (0.7662)
Lower lemma width	na	*** (0.0001)	ns (0.3303)	ns (0.8024)	** (0.0025)	ns (0.0753)	ns (0.0567)
Lower palea length	na	*** (0.0001)	ns (0.3616)	* (0.0301)	ns (0.1785)	ns (0.4476)	ns (0.3424)
Awn length	na	*** (0.0001)	ns (0.2710)	ns (0.0954)	ns (0.7137)	* (0.0429)	*** (0.0001)

Notes: D. cespitosa in North America. C-A: *Deschampsia cespitosa* var. *cespitosa–D. cespitosa* subsp. *alpina*; C-B: *D. cespitosa* var. *cespitosa–D. cespitosa* subsp. *beringensis*; C-G: *D. cespitosa* var. *cespitosa–D. cespitosa* subsp. *glauca*; C-M: *D. cespitosa* var. *cespitosa–D. mackenzieana*; C-O: *D. cespitosa* var. *cespitosa–D. cespitosa* subsp. *orientalis*; C-P: *D. cespitosa* var. *cespitosa–D. pumila*; C-R: *D. cespitosa* var. *cespitosa–D. brevifolia*. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns, not significant; na, not applicable.

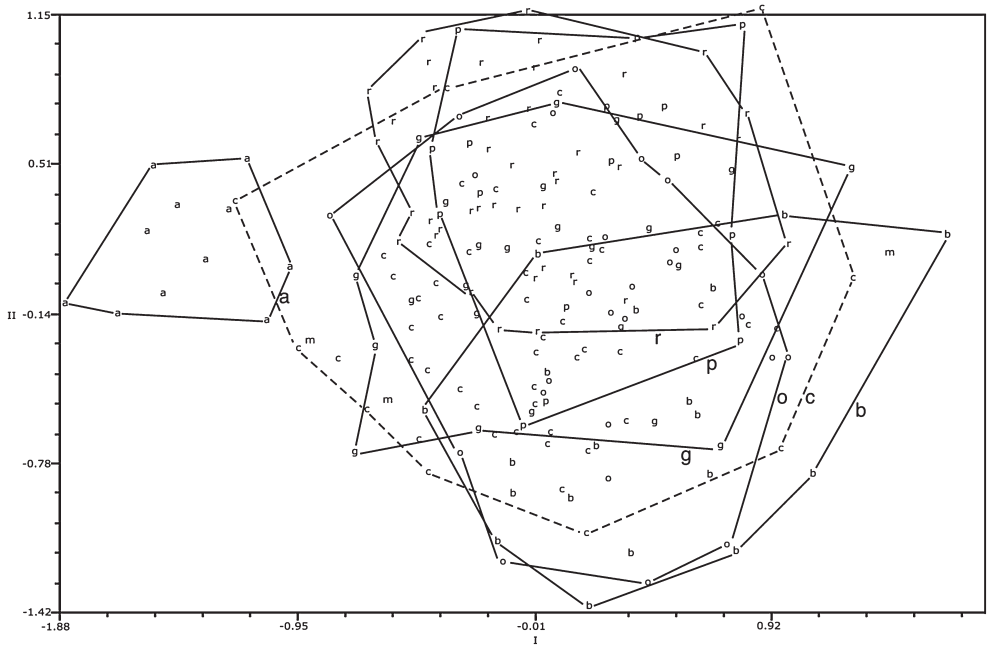

Fig. 2. Nonmetric multidimensional scaling two-dimensional scatterplot of the OTUs. The values on the axes represent standard deviates of the normalized scores. Dashed line: (c) *Deschampsia cespitosa* var. *cespitosa*; solid lines: (a) *D. cespitosa* subsp. *alpina*, (b) *D. cespitosa* subsp. *beringensis*, (m) *D. mackenzieana*, (g) *D. cespitosa* subsp. *glauca*, (o) *D. cespitosa* subsp. *orientalis*, (p) *D. pumila*, and (r) *D. brevifolia*.

and are comprised of 61 and 50 specimens per haplotype, respectively. Most of the other haplotypes were obtained from only one specimen, the exceptions being H12 and H34 (three specimens), H02 and H03 (four specimens,) and H06 and H10 (six specimens). These less-common haplotypes were mapped in Appendix S7 (see online Supplemental Data). None of the 39 haplotypes found for the combined data set characterized any of the seven infraspecific taxa of *Deschampsia cespitosa* previously defined. Different infraspecific taxa shared haplotypes, and no haplotype is unique to an infraspecific taxon. These data suggest that the considered taxa form a homogenous group.

The AMOVA showed no statistical differences between the alleged taxa (Table 4). In this analysis, the greatest percentage of the variation (96%) was attributed to within populations (i.e., within each infraspecific taxon). These data suggest that, for the sequences evaluated here, the infraspecific taxa within *D. cespitosa* do not represent genetically defined lineages.

DISCUSSION

Dividing a plasticity gradient across northern North America—To answer the question of how many closely related taxa exist for *Deschampsia cespitosa* in northern North America, we collected morphological and molecular data. Molecular results using two widely used sequences suggest the tentative recognition of a single species, *D. cespitosa*, with four infraspecific taxa to label forms attached to specific geographic regions. The other studied taxa showed significant morphological overlap with *D. cespitosa* var. *cespitosa* (Table 2) and nearly identical DNA sequences.

Our results support the perception of *Deschampsia cespitosa* as a grass with a plastic reaction norm to environmentally induced variation as suggested by Seliskar (1985a, b; Fusco and Minelli, 2010). In particular Seliskar (1985b) reported significant differences in exomorphological features in a 40 m long transect, while anatomical features remained unchanged (Seliskar, 1985b, p. 1351). These results agree with those presented here in that both data sets showed a response to environmental factors according to the concept of modular plasticity by DeKroon et al. (2005), who described phenotypic plasticity in plants not as whole-plant response, but as the summation of individual plant parts, in which single parts of individual plants showed different environmental induced plasticity. In the present case, characters like plant height, panicle and leaf length, showed more noticeable variation (Fig. 1A–1H) compared to the size of floral parts (1I–1M). In the absence of significant genetic differences as inferred from sequence data, the observed morphological variation could be explained by phenotypic plasticity. *Deschampsia cespitosa* var. *cespitosa* and allies in Arctic North America have been extensively studied (see introduction) by authors that used the morphological species concept (or morphospecies, or classical phenetic species concept) to delimit taxa (Sokal, 1973), which in an accepted form states "species are the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means" (Cronquist, 1978, p. 15). Under this framework, entities differing in the key

TABLE 3. Summary information for the molecular data set and descriptive statistics from the parsimony analyses.

Partition	Aligned length	PIC	PUC	Trees	Length	CI	RI
trnK-rps16	636	3	20	6	80	0.97	0.99
ITS	548	6	2	180	43	1.00	1.00
All	1184	9	22	100 000 [a]	156	0.75	0.80

Notes: PIC = parsimony informative characters; PUC = parsimony uninformative characters; CI = consistency index; RI = retention index.

[a] Maximum trees were set to 100 000.

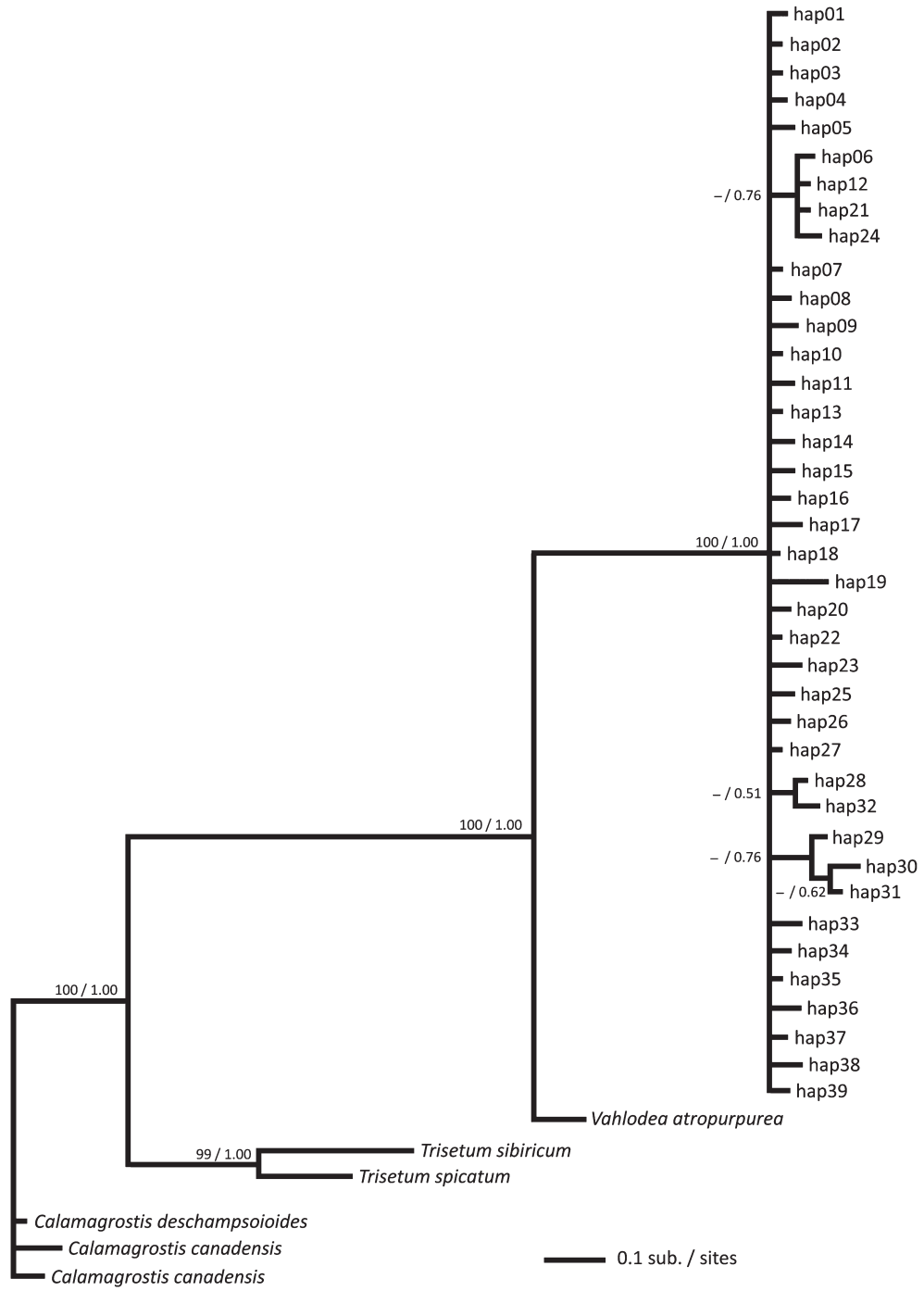

Fig. 3. Phylogram obtained from Bayesian inference (BI) of the combined *trnK-rps16* intergenic spacer and ITS nuclear region of the 39 haplotypes obtained from *Deschampsia cespitosa* and related taxa. Numbers above branches represent MP bootstrap values (100 pseudoreplicates) followed by BI posterior probabilities. Specimen haplotypes are listed in Appendix S1.

characters of traditional floras (e.g., plant height, length of panicles, leaf length) (Fig. 1, Table 2) have often attained species status. In our study, three taxa (*D. cespitosa* subsp. *alpina*, *D. cespitosa* subsp. *beringensis*, and *D. brevifolia*) differed statistically in some morphological characters (Table 2), and additionally have rather different geographic distributions (Fig. 5B). The other entities (*D. cespitosa* subsp. *glauca*, *D. cespitosa* subsp. *orientalis*, *D. pumila*) had minimal or no significant differences, and a single case (*D. mackenziana*) had a limited geographical distribution, but most morphological characters did not differ significantly from those *D. cespitosa* var. *cespitosa*, while sequence data did not yield differences from that taxon. Recognizing taxonomical entities should be based upon easily observable characters and constitutes the basis for floras (Stuessy, 1990, p. 172). A survey of 104 monographs on 126 genera encompassing 1790 species revealed that the majority

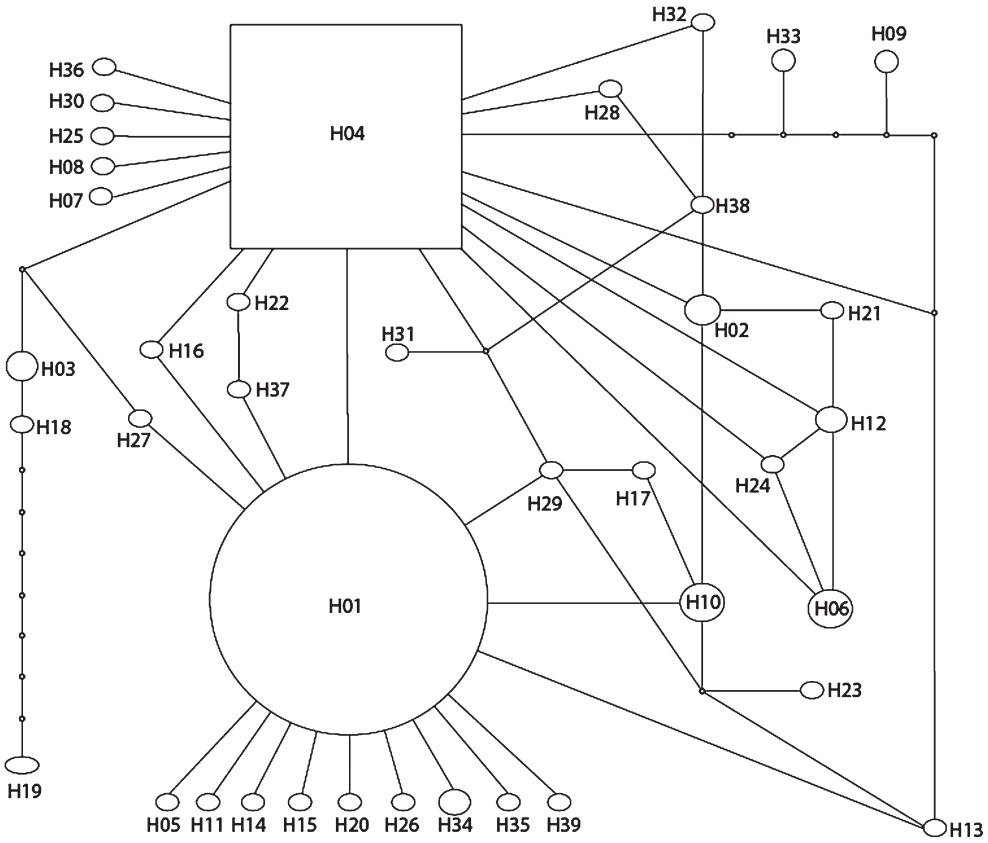

Fig. 4. Parsimony haplotype network of cpDNA *trnK-rps16* intergenic spacer and ITS nuclear region for *Deschampsia cespitosa* and related taxa. The size of the haplotype is proportional to the number of taxa included, such that haplotypes H01 and H04 are the two most common haplotypes, solid-circle and crossed-circle symbols in Fig. 5A, respectively. Specimen haplotypes are listed in Appendix S1.

of monographers indicated using the morphological species concept (McDade, 1995, p. 612). In this context, the characters used to define taxa are usually measurements, all of which are easily assessed and have been widely employed, might have overemphasized the recognition of entities that are essentially part of a continuous gradient of variation in *D. cespitosa* (see Fig. 1).

Lack of sequence resolution causes reexamination of morphological and geographical data—The combination of congruent morphological and molecular data sets is a common practice to boost confidence in resulting phylogenies (Weller and Sakai, 1999). However, in the case of incongruent data sets, molecular data showing nearly no variation in DNA sequences and morphological data with statistically significant differences in a number of characters leads to the evaluation of which data set is more informative. Computer simulations of character-state evolution have shown that the maximum probability of retrieving an accurate phylogeny for a given number of taxa is higher with molecular than with morphological data (fig. 8 in Givinish and Systma, 1997, p. 327); in our case, the low amount of informative variation in the molecular set might be indicative of a young, rapidly evolving group (Weller and Sakai, 1999). Although morphological data seem to be more prone to be affected by homoplasy, excluding them from phylogenetic analysis would not result in better-resolved phylogenies (Donoghue and Sanderson, 1992). Our results provide the first widespread view of the *Deschampsia cespitosa* species complex in North America, and while ITS may show problems of concerted evolution (Small et al., 2004), it proved to be useful in several levels of taxonomic utility (Baldwin et al., 1995) and in a previous study of *Deschampsia* (Chiapella, 2007). The intergenic spacer, *trnK-rps16*, was noted as highly variable by Daniell et al. (2006) and reported to have higher levels of variation than more traditional chloroplast regions (Kress et al., 2005; Shaw et al., 2007). The similarity of sequences and the result of AMOVA in the present case rules out the existence of more than one species, but the existence of groups bounded by some morphological distinctiveness attached to particular geographic areas indicates variation that could be worth recognizing. Morphological data suggest that *D. cespitosa* subsp. *alpina*, *D. cespitosa* subsp. *beringensis*, and *D. brevifolia* differ from the common *D. cespitosa var. cespitosa* (Fig. 2). The existence of intermediate morphs linking these forms with *D. cespitosa var. cespitosa* may of course hamper this differentiation, but the alleged taxa have rather defined geographic distributions with *D. brevifolia* normally found in the Canadian Arctic Islands (Aiken et al., 1995) and *D. cespitosa* subsp. *beringensis* along the Pacific coast of Alaska and Canada (Hultén, 1968) (Fig. 5B). The distribution of *D. cespitosa* subsp. *alpina* is more difficult to assess; for the area considered in this study, it is found in Greenland and northeastern Canada, but this taxon is also found in northwestern Europe (Hedberg, 1986; Chiapella, 2000). The ability to produce different morphotypes in response to different environmental conditions has been described for populations of *D. cespitosa* at high elevations, which showed limited growth and

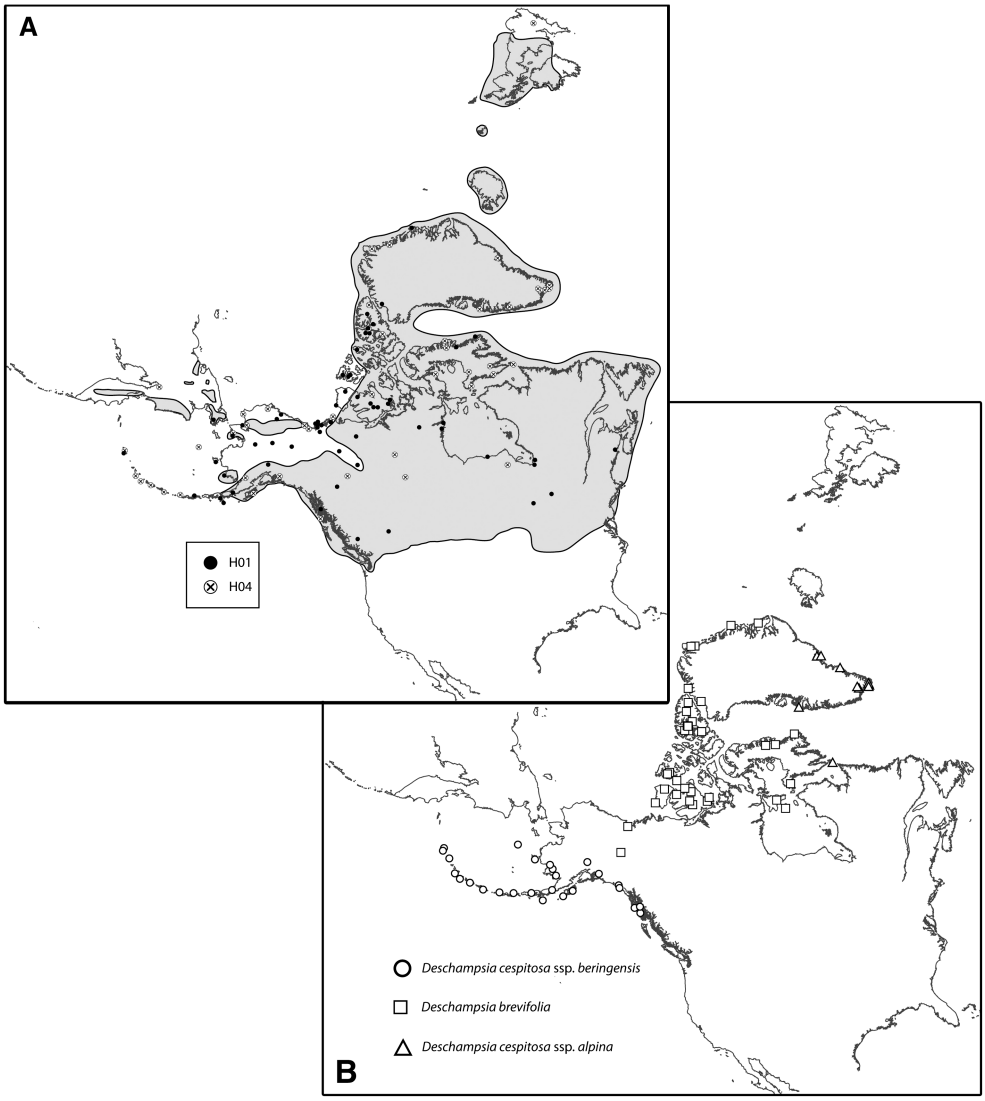

Fig. 5. Distribution maps of North American *Deschampsia*. (A) Common haplotypes H01 and H04, shading indicates last glacial maximum; (B) accepted taxa: *D. cespitosa* subsp. *beringensis*, *D. brevifolia*, and *D. cespitosa* subsp. *alpina*.

earlier anthesis in relation to low-elevation populations when grown in similar environments (Pearcey and Ward, 1972). Similarly, *D. cespitosa* shows modular morphological plasticity in plant height and panicle size induced by contrasting environments, the harsh conditions of the Arctic and the milder conditions of the Pacific coast. The decision on whether to keep a single variable species or to split it into several more coherent taxa can be done based upon results of numerical taxonomic analysis (Gilmartin et al., 1986; Gornall, 1997). Results of the MW test show that *D. cespitosa* var. *cespitosa* differs significantly from *D. cespitosa* subsp. *beringensis* in 16 characters, and in 12 from *D. brevifolia* (Table 2). Multivariate analysis shows (Fig. 3) that although the overlap with *D. cespitosa* var. *cespitosa* is extensive, both *D. cespitosa* subsp. *beringensis* and *D. brevifolia* tend to break away. Because the variation found throughout the studied taxa is mostly gradual, the species concept to be applied should accommodate a situation where DNA sequence data supports a single clade, but morphology points to groups differing slightly in one or more variables. Helbig et al. (2002) have provided a guide to follow the diagnosability of taxa, suggesting taxon identification relies upon recognition of particular states in diagnostic variables, or sets of variables. Gornall (1997) suggested that at least two independent variables should have discontinuous variation to recognize specific status. The putative taxa analyzed seems to fit in the framework provided by the phylogenetic species concept (Nixon and Wheeler, 1990, p. 220), since all taxa showed continuous variation in most of the variables analyzed (i.e., plant height, glumes length and width, and lemma length, Fig. 1) and were not resolved by the sequence data. The subtle discontinuities showed by *D. cespitosa* subsp. *alpina*, *D. cespitosa* subsp. *beringensis*, and *D. brevifolia* (Fig. 1), plus the geographical discontinuity in distribution (Fig. 5B), would suffice to recognize infraspecific taxa in spite of the trend of continuous variation in a single, molecularly defined clade.

Patchy genetic structure in patchy environments—Environmental heterogeneity in continental-scale regions could produce a

TABLE 4. Results of AMOVA among all considered taxa.

Source	df	Sum of squares	Variance components	Percentage of variation
Group A: cespitosa-alpina, Group B: brevifolia-pumila-mackenzieana-beringensis-glauca-orientalis				
Among groups	1	0.332	–0.0170	–4.40
Among populations within groups	6	5.589	0.03406	8.81
Within populations	160	59.120	0.36950	95.59
Total	167	65.042	0.38656	
Group A: cespitosa-beringensis, Group B: alpina-brevifolia-glauca-mackenzieana-orientalis-pumila				
Among groups	1	0.484	–0.01120	–2.87
Among populations within groups	6	5.437	0.03128	8.03
Within populations	160	59.120	0.36950	94.84
Total	167	65.042	0.38958	
Group A: cespitosa-brevifolia, Group B: alpina-beringensis-glauca-mackenzieana-orientalis-pumila				
Among groups	1	2.221	0.01696	4.23
Among populations within groups	6	3.701	0.01402	3.50
Within populations	160	59.120	0.36950	92.26
Total	167		65.042	0.40048
Group A: cespitosa-glauca, Group B: alpina-beringensis-brevifolia-mackenzieana-orientalis-pumila				
Among groups	1	0.884	–0.00571	–1.46
Among populations within groups	6	5.038	0.02784	7.11
Within populations	160	59.120	0.36950	94.35
Total	167	65.042	0.39163	
Group A: cespitosa-mackenzieana, Group B: alpina-beringensis-brevifolia-glauca-orientalis-pumila				
Among groups	1	0.797	0.01086	–2.79
Among populations within groups	6	5.125	0.03028	7.79
Within populations	160	59.120	0.36950	95.01
Total	167	65.042	0.38892	
Group A: cespitosa-orientalis, Group B: alpina-beringensis-brevifolia-glauca-mackenzieana-pumila				
Among groups	1	1.288	0.00007	0.02
Among populations within groups	6	4.634	0.02441	6.20
Within populations	160	59.120	0.36950	93.79
Total	167	65.042	0.39398	
Group A: cespitosa-pumila, Group B: alpina-beringensis-brevifolia-glauca-mackenzieana-orientalis				
Among groups	1	0.744	–0.00789	–2.02
Among populations within groups	6	5.178	0.02915	7.46
Within populations	160	59.120	0.36950	94.56
Total	167	65.042	0.39076	

relatively large mosaic of habitats in many different ecosystems, with biotic or abiotic conditions varying sharply over short distances (Forman, 1995). The Arctic and sub-Arctic zone encompasses vast extensions of plains, hills, and high mountainous regions. The vision of an environment that is "uniformly and predictable harsh" has been questioned by Murray (1987, 1997), who instead portrays these environments as landscapes with a complex topography, irregular terrains, and varying moisture gradients. For instance, elevation ranges from sea level at the Arctic coastal plains of northern Alaska, to nearly 2700 m a.s.l. in the Brooks Range or 2000 m a.s.l. in eastern Ellesmere Island's mountain system and adjacent Axel Heiberg Islands (Edlund and Alt, 1989). The plant communities and terrains are a "kaleidoscopic patchwork of disturbance intensities, ranging from essential stability to activity in the soil so intense that no plants can survive in it. Further, this patchwork … changes with time, at rates that differ with slope, exposure, length of season, water supply and soil textures..." (Raup, 1969, cited in Murray 1987, p. 247). The plateaus are covered with Arctic tundra vegetation, where grasses and sedge meadows are found in soils with poor drainage, while well-drained soils bear shrub-dominated communities (Edlund and Alt, 1989). The wind-swept snow interaction with vegetation, mainly shrubs (Essery and Pomeroy, 2004), further contributes to enhance the environmental heterogeneity by generating different spatial pattern variations in snow depth, that lead to strong variations in water balance on small scales (Marsh et al., 1995; Essery and Pomeroy, 2004).

Environmental heterogeneity is one of the main causes for genetic heterogeneity (Linhart and Grant, 1996) and, in the present case, renders almost impossible the generation of a pattern to relate haplotypes to a given geographic area. The uniform regional profile of the two most common haplotypes, H01 and H04, account together for 111 of 168 specimens with haplotypes. The two haplotypes extend over heavily glaciated, glacial margins and nonglaciated regions (Fig. 5A). Avise et al. (1987, p. 509) suggest that populations showing phylogenetic continuity and lack of effective spatial separation have benefited from recent historical interconnections through gene flow.

A patchy environment would offer many different opportunities for colonizing species since environmental gradients are associated with genetic variation in plant populations (Zangerl and Bazzaz, 1984). The genetic structure of some North American *Deschampsia* populations was assessed by isozymes and

RAPDs (Gehring and Linhart, 1992; Bush and Barret, 1993; Purdy and Bayer, 1995; Nkongolo et al., 2001). In particular, Gehring and Linhart (1992) studied genetic variation using isozymes along a moisture gradient; their results show most of the variation, however, was found among subpopulations, resulting in a patchy distribution pattern. In a similar way, the genetic structure revealed in the current study is patchy as most of it was found within infraspecific taxa. Haplotypes are not related to any particular geographic distribution. Patterns of haplotype distribution may be interpreted as clues for past events of migration into glaciated areas; widely distributed haplotypes might be the result of expansion from more than one source or spread from refugial areas after a glaciation event (Prentice et al., 2008). Haplotypes H01 and H04 were present in 111 individuals, most of them *D. cespitosa* var. *cespitosa* but also in *D. cespitosa* subsp. *alpina* (8), *D. cespitosa* subsp. *beringensis* (17), D. *brevifolia* (27), *D. cespitosa* subsp. *glauca* (13), *D. mackenzieana* (2), *D. cespitosa* subsp. *orientalis* (4), and *D. pumila* (9). Of the two most numerous haplotypes in North America, H04 is shared with accesions of eastern Russia (Kamchatka) and Great Britain. The other widespread haplotype, H01, was found in the Arctic, southern Canada, Greenland, and Alaska, showing a high number of haplotypes in and around Alaska. Haplotype H04 is uniformly distributed from Russia through North America to England, while H01 is largely confined to North America, suggesting that it is a continental-derived type (a single specimen was found in eastern Russia). A set of low-frequency haplotypes (differing by a single nucleotide polymorphism) are found mainly in Alaska and in continental Canada, with a few in the Arctic Islands (Appendix S7).

Although patchy genetic structure has been associated with limited seed and/or pollen dispersal (Linhart et al., 1981), this seems not to be the case for *Deschampsia*, whose disseminules have been found to be wind-dispersed and to be able to survive and germinate after landing on the ice surface of St. Mary's Glacier (Bonde, 1969). In wind-pollinated grasses, gene flow due to airborne pollen in the direction of prevailing winds can reach 21 km (Watrud et al., 2004); since *Deschampsia* is found in open environments such as the alpine tundra, the combination of strong winds and low vegetation structure might result in relatively frequent long-distance pollen dispersal events, which could result in low genetic differentiation.

Among the alleged taxa considered, the $2n = 52$ *D. mackenzieana* has the smallest geographic distribution, being restricted to sand dunes at Lake Athabasca in northwestern Saskatchewan (Raup and Argus, 1982), and has been treated as different from the common $2n = 26$ *D. cespitosa* var. *cespitosa* by Barkworth (2007). Purdy and Bayer (1995) reported it to be genetically distinct from *D. cespitosa* var. *cespitosa* on the basis of 13 isoenzymes. The sequences included in this study (one from the type specimen) showed no difference with *D. cespitosa* var. *cespitosa*, while differences in morphology were moderate (Table 2). Genetically different populations of *D. cespitosa* var. *cespitosa* exist also in northern Ontario (Nkongolo et al., 2001), while all populations were found to be $2n = 26$. Rothera and Davy (1986) studied the cytology of *D. cespitosa* var. *cespitosa* in Britain, finding two cytotypes ($2n = 26$ and $2n = 52$) that were morphologically indistinguishable, although a tendency was detected for the $2n = 26$ plants to be in ancient woodlands, while $2n = 52$ plants were found where invasions have occurred. In agreement, we consider the morphological differences very subtle and the genetical differences reported not conclusive, since this level of genetic differentiation in populations of *D. cespitosa* var. *cespitosa* is not unusual (Nkongolo et al., 2001). The treatment of *D. mackenzieana* as a separate taxon is at the present not supported.

***Conclusion and perspectives in* Deschampsia**—The simplest approach based on our data is to recognize a single taxon, *D. cespitosa* var. *cespitosa* with three additional infraspecific taxa corresponding to *D. cespitosa* subsp. *alpina*, *D. cespitosa* subsp. *beringensis*, and *D. brevifolia* to label the distinguishable forms attached to defined geographic distributions. Two of these have been already treated as infraspecific taxa, namely, *D. cespitosa* subsp. *alpina* and *D. cespitosa* subsp. *beringensis*, while the third has been treated either as a subspecies of *D. cespitosa* or as a separate species, *D. brevifolia*; lowering the taxonomic rank from species to subspecies has the undesired byproduct of having to coin a new name, since the available combination at the subspecies level, *D. cespitosa* subsp. *brevifolia* (R.Br.) Tzvel., is illegitimate (nomenclatural changes arising from this work will be addressed in a future contribution). All other studied taxa showed significant morphological overlap with *D. cespitosa* var. *cespitosa* in the multivariate analysis and nearly identical DNA sequences and are therefore reduced to synonyms of that taxon. However, we do not exclude the possibility that additional studies with more markers, particularly low-copy, highly variable nuclear markers (Mason-Gamer et al., 1998), in combination with cytogenetic analysis, might provide some basis for their recognition. At present, based upon the available morphological and sequence data, it is impossible to grant them recognition. Quantitative characters (Fig. 1) show gradient-like variation and suggest how different parts of this gradient have received different names in the past. The low variability revealed by sequence data and the morphological overlap of the three taxa suffices to deny them recognition at the species level. The lack of genetic structuring exposed in this work leaves unanswered questions related to *Deschampsia* and major glaciation events in North American, which could be addressed in the future with methods such as amplified fragment length polymorphisms (AFLPs) or intersimple sequence repeats (ISSRs). The present case offers an opportunity to reassess the conventional species concepts, since DNA sequence homogeneity and morphological gradient-like variation of species complexes across extensive geographic regions have not been analyzed in reviews (Mayden, 1997; Sites and Marshall, 2004). Finally, this case might provide insight into the interface between population genetics, systematics, and evolution. A thorough understanding of the genetic structure of North American *Deschampsia* could provide a sound taxonomical arrangement of the genus and its relationships to historical events such as glaciation.

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