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MOLECULAR INSIGHTS INTO THE TAXONOMY OF *GLYCERIA* (POACEAE: MELICEAE) IN NORTH AMERICA[1]

IAN G. WHIPPLE,[2] MARY E. BARKWORTH,[2] AND BRADLEY S. BUSHMAN[3,4]

[2]Utah State University Department of Biology, 5305 Old Main Hill, Logan, Utah 84322 USA; and
[3]USDA-ARS Forage and Range Research Lab, 695 N 1100 E, Logan, Utah 84322-6300 USA

Eighteen *Glyceria* species grow in the United States and Canada, with 16 being native to the region. We used data from morphology and three chloroplast DNA intergenic regions to address taxonomic questions concerning *Glyceria* in North America, particularly the status of *G. declinata*, *G. occidentalis*, *G. fluitans*, *G. striata*, and *G. elata* in western North America. The chloroplast data confirmed the presence of two European species, *G. declinata* and *G. fluitans*, in western North America. *Glyceria occidentalis* was exceptional among the taxa studied in having chloroplast genotypes that fell into two different clades, one of which contained *G. fluitans* and the other the North American species *G. leptostachya*. The morphological data showed *G. occidentalis* to be intermediate between *G. fluitans* and *G. leptostachya* with respect to their distinguishing characters. Based on these results, we hypothesize that *G. occidentalis* consists of hybrids between *G. fluitans* and *G. leptostachya*. *Glyceria elata* and *G. striata*, which have sometimes been treated as a single species, had different chloroplast genotypes, supporting their recognition as distinct taxa. DNA data from all three intergenic regions would be needed for unequivocal identification of the non-hybrid species examined.

Key words: chloroplast DNA; *Glyceria*; taxonomy.

Glyceria (Poaceae: Meliceae) includes approximately 40 species (Shu, 2006). Eighteen species grow in the United States and Canada, 16 being native to the region. Two of the native species, *G. borealis* and *G. striata* are transcontinental in their distribution; the remaining species are western, growing between the Pacific Coast and the Rocky Mountains, or eastern, growing almost entirely between 100°W and the Atlantic Coast. Church (1949) placed the species in three sections: sects. *Glyceria*, *Striatae*, and *Hydropoa*. *Glyceria* sect. *Glyceria* is the most distinct of the three. Its members have elongate, terete spikelets and winged paleas rather than the oval, dorsally flattened spikelets that characterize sects. *Hydropoa* and *Striatae*. Members of sect. *Glyceria* also differ from species of the other sections in growing in association with still or slowly moving water rather than along the banks of streams and ditches with evidently moving water. Sect. *Hydropoa* can be distinguished from sect. *Striatae* by its ovoid, rather than obovoid, caryopses and flatter lemmas (Church, 1949).

Hitchcock (1935, 1951) provided a key to all the species of *Glyceria* in the contiguous United States, which has served as the basis for the treatments in almost all subsequent regional floras. Attempts to modify his key for use in volume 24 of *Flora of North America* (Barkworth et al., 2007) proved impossible, primarily because of the morphological overlap within two groups of species: (1) *G. declinata*, *G. occidentalis*, and *G. fluitans* and (2) *G. striata* and *G. elata*. This study was designed to provide a contemporary morphological and molecular assessment of the historical morphological delineation that had been developed for these five species. Additional North American taxa were included to provide a context for the results obtained. We used cpDNA intergenic primers because of polyploidy in *Glyceria* and the poor quality of DNA obtainable from the herbarium specimens. Our hypothesis was that the cpDNA data would place specimens of the same species in a monophyletic clade. In the next paragraphs, we review in more detail three specific questions.

Question 1—Is *G. declinata* present in North America? *Glyceria declinata* is a European taxon that Hitchcock (1951) distinguished from other species, including *G. occidentalis*, on the basis of its small stature, boat-shaped leaf tips, and narrow (2–3 mm wide) leaf blades. He stated that it was present in California, Nevada, and New York. European floras (e.g., Hylander, 1953; Holub, 1980), while agreeing that *G. declinata* is a relatively short species (to 90 cm tall), generally rely on its lobed lemma tips and bifid paleas for identification. Church (1949) reported that *G. declinata* is diploid, whereas *G. occidentalis* is tetraploid.

Questions concerning the presence of *G. declinata* in western North America arose when Holmgren and Holmgren (1977) stated that the morphological variation of *G. declinata* from the Intermountain Region of western North America fell within the range of the native *G. occidentalis*. Consequently, they treated *G. occidentalis* as a species with both diploid and polyploid phases and stated that *G. declinata* was not found in the Intermountain Region. More recently, Leppig examined living populations of *G. declinata* and *G. occidentalis* in California in conjunction with herbarium specimens from many U.S. herbaria. Like Holmgren and Holmgren (1977), Leppig concluded that the two species were indistinguishable in North America (G. Leppig, unpublished data), but he did not

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[4] Author for correspondence (e-mail: sbushman@cc.usu.edu)

TABLE 1. Chloroplast DNA primers and informative characters examined to estimate relationships of *Glyceria* taxa.

Primer	Sequence	Total characters[a] (no.)	Informative SNPs[b] (no.)	Informative indels (no.)	Reference
trnHF	CGCGCATGGTGGATTCACAATCC	614	19	4	Sang et al., 1997
psbA3′f	GTTATGCATGAACGTAATGCTC				Sang et al., 1997
trnC	CCAGTTCAAATCTGGGTGTC	791	61	17	Demsure et al., 1995
petN1r	CCCAAGCAAGACTTACTATATCC				Lee and Wen, 2003
trnK5′r	TACTCTACCRTTGAGTTAGCAAC	614	59	7	Johnson and Soltis, 1995
rps16-4547mod	AAAGGKGCTCAACCTACARGAAC				Kress et al., 2005
Total		2019	139	28	

[a] Total characters includes nucleotide sequence and insertions/deletions (indels).
[b] SNPs, single nucleotide polymorphisms.

include European specimens of *G. declinata*. Thus we wished to determine if the European *G. declinata* is present in North America and whether or not it is distinct from *G. occidentalis*. The question is of particular interest because *G. declinata*, but not *G. occidentalis*, is thought to be invading vernal pools in California (Rogers, 1998).

Question 2—Are *G. occidentalis* and *G. fluitans* distinct taxa? *Glyceria fluitans* is a European species that is established in eastern North America, but its status in western North America is less clear. It differs from other European species in having relatively large florets, spikelets, and anthers and acute lemma apices. It also differs from all eastern North American species, but not the western species *G. occidentalis*, in these respects. A. S. Hitchcock (1935, 1951) included both *G. fluitans* and *G. occidentalis* in his treatment, but many specimens cannot be unequivocally identified using his descriptions and keys. C. L. Hitchcock (1969), on the other hand, considered that *G. fluitans* was not present in the Pacific Northwest, specimens so identified belonging to *G. occidentalis*. He explained how he considered the two species to differ, but many specimens could not be identified with confidence using his criteria. Thus, we wished to determine whether *G. fluitans* is molecularly distinct from *G. occidentalis* and, if so, whether it is present in western North America.

Question 3—Are *G. striata* and *G. elata* distinct taxa? According to C. L. Hitchcock (1969), *G. elata* tends to be a larger and have fleshier culms and wider leaves than *G. striata*, but they are very hard to tell apart in the herbarium. Arnow (1993, p. 830) treated them as a single species, stating that "the characters traditionally used to separate [*G. elata*] from [*G. striata*] are quantitative, overlapping, and by no means consistently correlated." On the other hand, Ruiz de Esparza and Maze (1997), after conducting phenetic analyses of several morphological characters, concluded that the two species were distinct. In addition, many botanists in Washington and Oregon favor the traditional treatment of recognizing both species as distinct (D. Giblin, University of Washington, personal communication). Thus we were interested in determining whether molecular data would support recognition of *G. striata* and *G. elata* as distinct taxa.

MATERIALS AND METHODS

Plant materials—The plant materials used for the molecular studies consisted of 87 samples representing 16 species of *Glyceria* and four samples of *Melica bulbosa*, the taxon used as an outgroup. The samples were obtained from herbarium specimens belonging to HSC, ID, JEPS, OSC, UBC, UTC, WTU, and WVA (herbarium codes from Holmgren et al., 1990). The samples originated from 14 U.S. states, three Canadian provinces, Sweden, Denmark, and Australia. An additional two fresh samples of *G. occidentalis* were collected specifically for this study. Collection and voucher information is provided in Appendix 1. The specimens were identified by Barkworth, based on morphological criteria, prior to inclusion in the current study.

Measurements of lemma and anther lengths were conducted on 186 herbarium specimens of *G. occidentalis*, *G. fluitans*, and *G. leptostachya*, which included those in the molecular studies. As lemma and anther lengths are correlated variables ($r = 0.88$, $P < 0.01$), multivariate analysis of variance of both measurements was conducted for the overall species effect with the GLM procedure and MANOVA statement of SAS (SAS Institute, 2003). For the overall species effect model, four species were defined: *G. fluitans* from North America with 38 samples, *G. fluitans* from Europe with 51 samples, *G. occidentalis* with 56 samples, and *G. leptostachya* with 41 samples. A test for homogeneity among covariances was performed with the DISCRIM procedure, resulting in significant covariances ($P < 0.001$). Therefore, multivariate contrasts to simultaneously compare mean anther and lemma lengths were computed using the MIXED procedure (Westfall et al., 1999).

DNA extraction, PCR amplification, purification, and sequencing—DNA was extracted from herbarium tissue using the Qiagen DNeasy 96 plant kit (Valencia, California, USA) according to the manufacturer's instructions. Quantity and quality of DNA was estimated from spectrophotometry and resolution on 1% agarose gels. Primers for three chloroplast intergenic regions were used in this analysis: *trnH-psbA*, *trnC-ycf6*, and *trnK-rps16* (Table 1). Amplification of DNA used a standard *Taq* polymerase (Promega, Madison, Wisconsin, USA) and Platinum High-Fidelity polymerase (Invitrogen, Carlsbad, California, USA). The PCR conditions were 94°C for 90 s, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 80 s, with a final extension at 72°C for 7 min. The PCR products were purified prior to sequencing with ExelaPure 96-well plates (Edge Biosystems, Gaithersburg, Maryland, USA). Sequencing reactions used BigDye version 3.1 (Applied Biosystems, Foster City, California, USA), and dye terminator removal was done with Performa v3 96-well plates (Edge Biosystems, Gaithersburg, Maryland, USA).

Sequence alignment and analyses—Sequences from the 91 samples were aligned for each of the three regions separately using Sequencher software (Gene Codes, Ann Arbor, Michigan, USA). Insertions or deletions (indels) were coded using the "simple" method proposed by Simmons and Ochoterena (2001). Identical haplotypes were combined, and phylogenetic analysis was conducted on the reduced set of 41 sequences. The three primer-amplified regions were combined into one contiguous sequence for analysis, and heuristic searches were conducted using PAUP* version 4.0b10 (Swofford, 2001). A maximum parsimony search, including indels, was conducted with nearest neighbor interchange (NNI) branch swapping. Branch support for the tree was obtained with bootstrap analysis with 1000 pseudoreplicates of the data. All searches were conducted with MulTrees on and Steepest Descent off. A strict consensus tree with bootstrap values is reported. A search was also conducted with tree-bisection-reconnection (TBR) branch swapping, which resulted in the same strict consensus tree as NNI.

RESULTS

The *trnH-psbA3′f*, *trnC-petN1r*, and *trnK-rps16* cpDNA intergenic spacer primer sets were used for analysis (Table 1). For the *trnH-psbA3′f*-amplified region, 23 (3.7%) characters were variable and parsimoniously informative. Nineteen of the 23 characters were single nucleotide polymorphisms (SNPs), and four were indels. The *trnC-petN1r* primer set provided 79 (10%) informative characters, with 61 SNPs and 17 indels. Region *trnK-rps16* had 66 (10.7%) informative characters, of which 59 were SNPs and seven were indels. In total there were 2019 characters, including indels, of which 167 (8.3%) were parsimoniously informative.

The consensus tree showed three strongly supported (bootstrap values = 100) clades within *Glyceria* (Fig. 1) that corresponded completely with Church's (1949) three sections. Within the clade corresponding to sect. *Glyceria*, there were three strongly supported (bootstrap values $\geq$ 96) subclades. One of these contained the transcontinental, diploid North American *G. borealis*, three eastern North American tetraploids, *G. acutiflora*, *G. septentrionalis*, *G. arkansana*, and the Australian *G. australis*. The second contained *G. declinata*, *G. fluitans*, *G. notata*, and three of the 10 *G. occidentalis* samples. The *G. declinata*, *G. fluitans*, and *G. notata* species are all European taxa (Hitchcock, 1935, 1951; Church 1949), with the former a diploid and the latter two tetraploids. Each of *G. declinata* and *G. fluitans* were represented in the study by samples from both Europe and western North America. Despite this, all the *G. declinata* samples had exactly the same chloroplast genotype. The samples of *G. fluitans* were only slightly less uniform: nine of the 10 shared an identical chloroplast genotype, the remaining sample, which came from California, differed by only one SNP. The third subclade in sect. *Glyceria* contained the remaining six *G. occidentalis* samples and the western North American tetraploid *G. leptostachya*.

The results for *G. occidentalis* suggested two alternatives: either the samples came from morphologically extreme specimens of *G. leptostachya* and *G. fluitans*, or *G. occidentalis* consists of hybrids between these two species. The most reliable measurable morphological characters for distinguishing among *G. leptostachya*, *G. fluitans*, and *G. occidentalis* were lemma length and anther length, and both variables were significant overall among the three species (Table 2). Most measurements for lemma and anther length in *G. occidentalis* were between those for *G. leptostachya* and *G. fluitans*, although there was some overlap, chiefly with *G. fluitans* (Fig. 2). Multivariate contrasts showed no significant difference in the characters between western North American and European specimens of *G. fluitans*, but the contrasts among each of the three species were significantly different (Table 2).

The clade corresponding to sect. *Striatae* (Fig. 1) had four subclades. The samples of *G. canadensis* formed the only strongly supported (bootstrap = 96) subclade. The two other taxa represented by more than one specimen, *G. elata* and *G. striata*, formed distinct subclades with bootstrap support of 55 and 81, respectively. The only sample of *G. striata* that came from an eastern North American specimen had a chloroplast genotype identical to that of three specimens from Idaho. The single *G. melicaria* sample was sister to the other subclades within the sect. *Striatae* clade. Section *Hydropoa* was represented by five samples of two taxa: three of *G. grandis* and two of *G. maxima*. The two taxa formed separate clades with bootstrap support of 83.

TABLE 2. Multivariate analysis of variance of anther and lemma length measurements on 186 herbarium specimens of *Glyceria fluitans*, *G. occidentalis*, and *G. leptostachya*.

Statistic	Sample size	*F*	df Num, Den	*P*
Overall species effect	186	168.92	6, 362	<0.0001
Pre-defined contrasts				
European vs. American *G. fluitans*	89	0.92	2, 185	0.4002
G. fluitans vs. *G. occidentalis*	145	314.80	2, 185	<0.0001
G. fluitans vs. *G. leptostachya*	130	853.98	2, 185	<0.0001
G. leptostachya vs. *G. occidentalis*	97	178.02	2, 185	<0.0001

Notes: Num = numerator, Den = denominator.

DISCUSSION

Chloroplast DNA data supported the sectional delineations proposed by Church (1949), and, with the notable exception of *G. occidentalis*, they also supported recognition of each of the other taxa in our study. Haplotypes of *G. occidentalis* were present in two monophyletic clades, one belonging to *G. leptostachya* and the other to *G. fluitans*. This suggests two parsimonious hypotheses; one being that *G. occidentalis* is a hybrid of reticulate origin between *G. leptostachya* and *G. fluitans*, the other that it consists of extremes of variation within the two taxa.

Glyceria leptostachya, *G. occidentalis*, and *G. fluitans* are tetraploids (Church, 1949), with the former two native to western North America (for their distributions, see http://herbarium.usu.edu/webmanual/) and the latter native to Europe. It is unknown when *G. fluitans* was first introduced to western North America; the oldest specimens in our sample were 70 yr old. Morphologically, *G. leptostachya* and *G. fluitans* differ most in their anther and lemma lengths (cf. e.g., Hylander, 1953; Hitchcock, 1969; Holub, 1980) and in the shape of their lemma apices. *Glyceria occidentalis* has lemma and anther lengths that are intermediate between them, but there is some overlap (Fig. 2). In general, *G. occidentalis* is also intermediate in the shape of its lemma apex, but considerable variation may exist within the panicles of each taxon. No other characters were found to be effective in distinguishing the taxa in this study. Interpreting *G. occidentalis* as representing extremes of variation within *G. fluitans* and *G. leptostachya* would require expanding the range of morphological variation in both species. For *G. fluitans*, it would require expanding the range beyond that reported for Europe and China (Holub, 1980; Shu, 2006). We consider that the chloroplast and morphological data favor interpretation of *G. occidentalis* as a series of natural hybrids between *G. leptostachya* and *G. fluitans*.

Sympatric hybridization is well documented in plants (Rieseberg and Wendel, 2004), and intermediate morphological characteristics in hybrids are not uncommon (Grant, 1981). Interspecific hybrids are presumed to backcross with one and/or the other parental species, such that new combinations of genes will result from introgression and further reticulation (Barton, 2001). Nascent progeny from hybridization may also

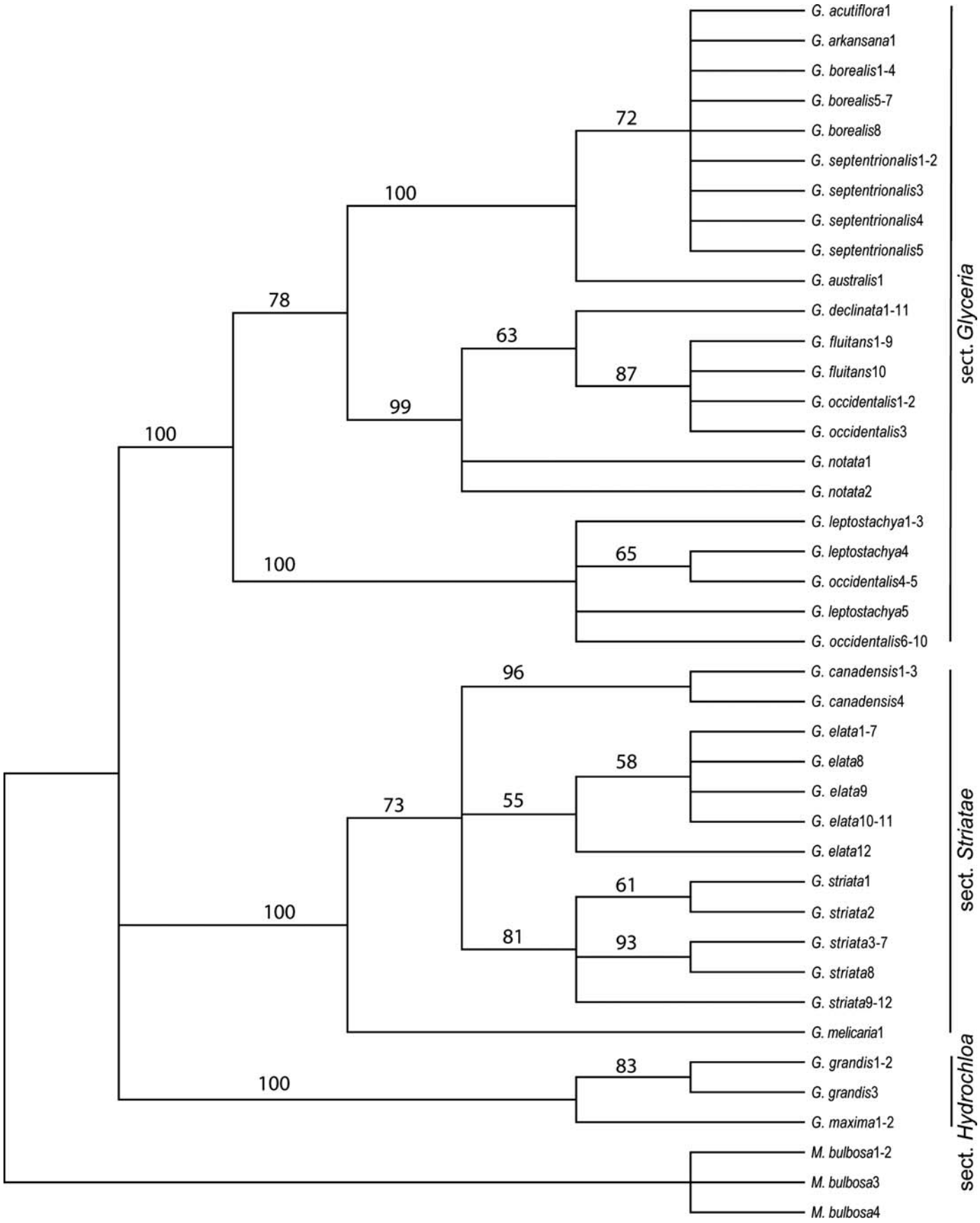

Fig. 1. Strict consensus tree, with bootstrap values for nodal support, of 16 *Glyceria* taxa with *Melica bulbosa* as an outgroup.

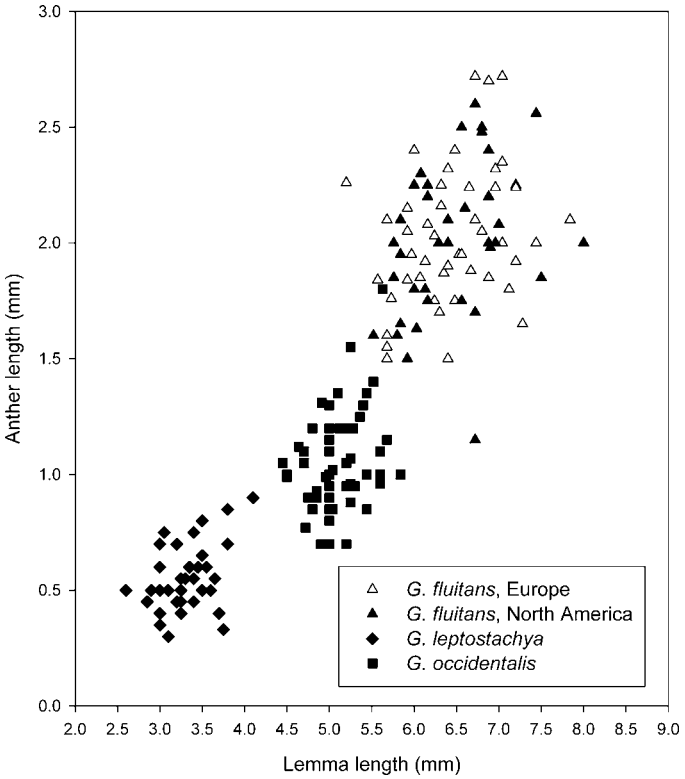

Fig. 2. Bivariate plot of lemma and anther length measurements for *Glyceria occidentalis* and the two species with which it shares cpDNA haplotypes.

increase in fitness and expand their distribution (Grant, 1981). The two chloroplast haplotypes and the extended range of *G. occidentalis* are not inconsistent with a reticulate origin, but further studies, including hybridization and analysis of nuclear molecular characters, are needed to confirm or reject the hybrid hypothesis.

Glyceria declinata, which Holmgren and Holmgren (1977) suggested was confused with *G. occidentalis*, formed a separate branch from the two *G. occidentalis*-containing clades. Although the collections of the *G. declinata* were sampled on two continents, no polymorphisms were found among the specimens for any of the chloroplast regions in our study. *Glyceria declinata* is usually shorter and more decumbent than *G. occidentalis*, its panicle branches tend to be shorter, straighter, and have fewer spikelets than those of *G. occidentalis*, and its lemmas have two more or less equal lobes on either side of the tip rather than inconspicuous, unequal lobes (see images at http://herbarium.usu.edu/webmanual/). The morphological and cpDNA data support both recognition of *G. declinata* as a distinct species and its presence in western North America, and the cpDNA primer sets used in this study can be useful for *G. declinata* identification in areas where it is thought to be invasive. In particular, the trnC-petN1r primer set easily distinguishes *G. declinata* from *G. leptostachya*, and the trnK-rps16 primer set distinguishes *G. declinata* from *G. occidentalis*.

The eastern North American species of sect. *Glyceria* (*G. acutiflora*, *G. arkansana*, and *G. septentrionalis*) formed a clade with the transcontinental, diploid *G. borealis*. *Glyceria arkansana* and *G. septentrionalis* are sometimes treated as conspecific varieties (Yatskievych, 1999). Our results are consistent with their recognition as distinct taxa but our sampling of these two taxa was not adequate to make a strong statement as to their taxonomic treatment. Similarly, no conclusions can be drawn concerning the relationship of the Australian species, *G. australis*, to other members of the genus in the absence of a global sampling of the genus.

Our data show *G. striata* and *G. elata* species as separate subclades (Fig. 1). Several SNPs were detected between the two species with the *trnK-rps16* primer set. The *trnC-petN1r*- and *trnH-psbA*-amplified regions showed no polymorphisms between the two. Nevertheless, the combination of cpDNA polymorphisms from *trnK-rps16*, the observations of field botanists, and the phenetic study of Ruiz de Esparza and Maze (1997) concur in supporting recognition of *G. striata* as distinct from the western North American taxon, *G. elata*.

Primer sets used in this study were a subset of those examined by Kress et al. (2005) in plant "barcoding" efforts. The primer sets amplified cpDNA regions short enough to be tractable with poor-quality DNA obtainable from herbarium specimens. Kress et al. (2005) proposed that the *trnH-psbA* primer set was most suitable for plant barcoding, but they broadly surveyed the plant kingdom and included only two grasses. For the *Glyceria* species, *trnH-psbA* was the least variable of the three primer sets and was unable to provide high bootstrap support for some subclades. Our results support the use of the chloroplast intergenic primer sets proposed by "barcoding" efforts, but suggest that a combination of primer sets will be necessary for species distinction.

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APPENDIX 1. The scientific names, voucher information, and GenBank accession numbers for taxa used in this study. The names and their authors follow the International Plant Names Index (http://www.ipni.org/) and are listed in descending rank order. Information on voucher specimens is presented in the following order: **Number** in fig. 1, herbarium code, and specimen accession number; collection locality; *collector's name and collection number*—GenBank accession numbers for *trnC-ycf6*, *trnH-psbA*, and *trnK-rps16*. Herbarium abbreviations: UBC = University of British Columbia, HSC = Humboldt State University, ID = University of Idaho, JEPS = University of California-Berkeley, OSC = Oregon State University, UTC = Utah State University, WVA = West Virginia University.

Poaceae Barnhart (Grass family)
Meliceae Endl.
Glyceria R. Br.
Glyceria R. Br. sect. ***Glyceria***

Glyceria acutiflora Torr. (**1**) WVA 106032; USA, West Virginia, Monroe Co.; *T.F. Wieboldt*, *C.E. Stevens 9996*—DQ665369, DQ665460, DQ665551.

Glyceria arkansana Fernald (**1**) ID 63496; USA, Louisiana, Grant Parish; *R.D. Thomas, J.W. Parker 28980*—DQ665370, DQ665461, DQ665552.

Glyceria australis C.E. Hubb (**1**) UTC 209438; Australia, New South Wales; *R.G. Coveny, P. Hind 16041*—DQ665371, DQ665462, DQ665553.

Glyceria borealis (Nash) Batch. (**1**) ID 113741; USA, Idaho, Idaho Co.; *A.C. Sondenaa 98*—DQ665372, DQ665463, DQ665554. (**2**) ID 111555; USA, Idaho, Fremont Co.; *S. Markow 10774*—DQ665373, DQ665464, DQ665555. (**3**) ID 97792; USA, Idaho, Teton Co.; *R. Bursik 1512*—DQ665374, DQ665465, DQ665556. (**4**) ID 117398; USA, Wisconsin, Oconto Co.; *R.W. Freckmann 28155*—DQ665375, DQ665466, DQ665557. (**5**) ID 130656; USA, Washington, Pend Oreille Co.; *M. Mancuso 2546*—DQ665376, DQ665467, DQ665558. (**6**) ID106397; USA, Idaho, Bonner Co.; *R. Bursik 1772*—DQ665377, DQ665468, DQ665559. (**7**) ID 96515; USA, Idaho, Idaho Co. *R. Bursik 319*—DQ665378, DQ665469, DQ665560. (**8**) ID 126295; USA, Nevada, Washoe Co.; *A. Tiehm 14047*—DQ665379, DQ665470, DQ665561.

Glyceria declinata Bréb. (**1**) UTC 239413; Denmark, Zealand, Ermelunden; *M.E. Barkworth, N. Jacobsen, S. Frederiksen s.n.*—DQ665384, DQ665475, DQ665566. (**2**) UTC 239439; Denmark, Zealand, Ermelunden; *M.E. Barkworth, N. Jacobsen, S. Frederiksen s.n.*—DQ665386, DQ665477, DQ665568. (**3**) OSC 167872; USA, Oregon, Benton Co.; *R. R. Halse 3404*—DQ665387, DQ665478, DQ665569. (**4**) HSC 10053; USA, California, Humbolt Co.; *L. Ahart 7987*—DQ665388, DQ665479, DQ665570. (**5**) HSC 94245; USA, California, Sacramento Co.; *G.F. Hrusa 10935*—DQ665389, DQ665480, DQ665571. (**6**) JEPS 94779; USA, California, Butte Co.; *L. Ahart 7987*—DQ665390, DQ665481, DQ665572. (**7**) JEPS 96625; USA, California, Mariposa Co.; *D. W. Taylor 15919*—DQ665391, DQ665482, DQ665573. (**8**) JEPS 103521; USA, California, Nevada Co.; *L. Ahart 9673*—DQ665392, DQ665483, DQ665574. (**9**) JEPS 98452; USA, California, Butte Co.; *L. Ahart 8377*—DQ665393, DQ665484, DQ665575. (**10**) JEPS 95269; USA, California, Stansilaus Co.; *M.W. Flesher*—DQ665385, DQ665476, DQ665567.

Glyceria fluitans (L.) R. Br. (**1**) UTC 239457; Sweden, Skåne; *M.E. Barkworth, B. Salomon s.n.*—DQ665406, DQ665497, DQ665588. (**2**) UTC 239445; Denmark, Zealand, Rye Gaard Dyrehave; *M.E. Barkworth, N. Jacobsen, S. Frederiksen s.n.*—DQ665409, DQ665500, DQ665591. (**3**) UTC 239450; Denmark, Zealand, Lystrop Skov; *M.E. Barkworth, N. Jacobsen, S. Frederiksen s.n.*—DQ665410, DQ665501, DQ665592. (**4**) UTC 239425; Denmark, Zealand, Ermelunden; *M.E. Barkworth, N. Jacobsen, S. Frederiksen s.n.*—DQ665411, DQ665502, DQ665593. (**5**) UBC 202291; Canada, British Columbia; *F. Lomer s.n.*—DQ665412, DQ665503, DQ665594. (**6**) HSC 93934; USA, California, Humboldt Co.; *G. Leppig, S. Leppig 1125*—DQ665413, DQ665504, DQ665595. (**7**) HSC 93744; USA, California, Humboldt Co.; *G. Leppig, S. Leppig, K. Neander 791*—DQ665414, DQ665505, DQ665596. (**8**) WTU 325658; USA, Washington, King Co.; *R. del Moral s.n.*—DQ665415, DQ665506, DQ665597. (**9**) UTC 219196; USA, California; Humboldt Co., *G. Leppig 243*—DQ665407, DQ665498, DQ665589. (**10**) HSC 92863; USA, California; *G. Leppig 243*—DQ665408, DQ665499, DQ665590.

Glyceria leptostachya Buckley (**1**) OSC 166730; USA, Oregon, Polk Co.; *R.R. Halse 2612*—DQ665419, DQ665510, DQ665601. (**2**) HSC 10073; USA, Washington, Grays Harbor Co.; *R. Spellenberg*—DQ665420, DQ665511, DQ665602. (**3**) OSC 166732; USA, Oregon, Polk Co.; *R.R. Halse 2612*—DQ665421, DQ665512, DQ665603. (**4**) OSC 170143; USA, Oregon, Benton Co.; *E.R. Alverson 1150*—DQ665422, DQ665513, DQ665604. (**5**) OSC 126756; USA, Washington, Grays Harbor Co.; *R. Spellenberg*—DQ665423, DQ665514, DQ665605. (**6**) UTC 243596; USA, Oregon, Jackson County; *B. Wilson 11236*.—DQ665433, DQ665524, DQ665615;

Glyceria notata Chevall (1) UTC 239422; Denmark, Zealand; *M. E. Barkworth, N. Jacobsen, S. Frederiksen s.n.*—DQ665427, DQ665518, DQ665609. (**2**) UTC 239427; Denmark, Zealand; *M.E. Barkworth, N. Jacobsen, S. Frederiksen s.n.*—DQ665428, DQ665519, DQ665610.

Glyceria* ×*occidentalis (Piper) J.C. Nelson (**1**) UBC 219226; Canada, British Columbia; *F. Lomer 96–117*—DQ665429, DQ665520, DQ665611. (**2**) UTC 243683; USA, Washington, Pacific Co.;

P. Zika 22273—DQ665431, DQ665522, DQ665613. (**3**) UBC 195463; Canada, British Columbia; *F. Lomer s.n.*—DQ665432, DQ665523, DQ665614. (**4**) ID 44951; USA, Idaho, Benewah Co.; *W.H. Baker 15729*—DQ665434, DQ665525, DQ665616. (**5**) ID 97666; USA, Idaho, Coeur d'Alene Co.; *R. Bursik 1283*—DQ665435, DQ665526, DQ665617. (**6**) ID 97788; USA, Idaho, Coeur d'Alene Co.; *R. Bursik 401*—DQ665436, DQ665527, DQ665618. (**7**) OSC 83783; USA, Oregon, Coos Co.; *J.R. Thienes*—DQ665437, DQ665528, DQ665619. (**8**) UBC 106285; USA, Oregon, Benton Co.; *L.R.J. Dennis, F.H. Smith 2528*—DQ665438, DQ665529, DQ665620. (**9**) UTC 243685; USA, Washington, Pacific Co., *P. Zika 22275*—DQ665430, DQ665521, DQ665612.

Glyceria septentrionalis Hitchc. (**1**) WVA 84540; USA, West Virginia, Tucker Co.; *W.N. Grafton 6-16-93*—DQ665439, DQ665530, DQ665621. (**2**) WVA 84913; USA, West Virginia, Greenbriar Co.; *W.N. Grafton 9-23-95*—DQ665440, DQ665531, DQ665622. (**3**) WVA 13175; USA, West Virginia, Genesee Co.; *M.A. Leupold Jun 26 1980*—DQ665441, DQ665532, DQ665623. (**4**) WVA 84541; USA, West Virginia, Randolph Co.; *W.N. Grafton 9-26-95*—DQ665442, DQ665533, DQ665624. (**5**) WVA 84096; USA, West Virginia, Greenbriar Co.; *W.N. Grafton 9-23-95*—DQ665443, DQ665534, DQ665625.

Glyceria sect. ***Hydropoa***. (Dumort.) Dumort.

Glyceria grandis S. Watson (**1**) UTC 172685; USA, Idaho, Ada Co.; *B. Ertter, J. Strachan 3956*—DQ665416, DQ665507, DQ665598. (**2**) UTC 212256; USA, Colorado, Moffet Co.; *W.A. Weber 14328*—DQ665417, DQ665508, DQ665599. (**3**) UTC 120348; USA, Colorado, Gunnison Co.; *H.H. Hall 421*—DQ665418, DQ665509, DQ665600.

Glyceria maxima (Hartm.) Holmb. (**1**) UTC 239456; Sweden, Skåne; *M. E. Barkworth, B. Salomon s.n.*—DQ665424, DQ665515, DQ665606; (**2**) UTC 239454; Denmark, Zealand; *M. E. Barkworth, N. Jacobsen, S. Frederiksen s.n.*—DQ665425, DQ665516, DQ665607.

Glyceria sect. ***Striatae*** **G.L. Church**

Glyceria canadensis (Michx.) Trin. (**1**) UTC 138271; USA, Wisconsin, Douglas Co.; *R.G. Koch 6091*—DQ665380, DQ665471, DQ665562. (**2**) UTC 161595; Canada, Quebec; *W.G. Dore 24313*—DQ665381, DQ665472, DQ665563. (**3**) UTC 136175; USA, New Hampshire, Cheshire Co.; *D.E. Boufford*—DQ665382, DQ665473, DQ665564. (**4**) UTC 114052; USA, Wisconsin, Bayfield Co.; *L.E. Hibbert 69*—DQ665383, DQ665474, DQ665565.

Glyceria elata (Nash) Hitchc. (**1**) ID 119040; USA, Idaho, Clearwater Co.; *C.A. Richardson 483*—DQ665394, DQ665485, DQ665576. (**2**) ID 97425; USA, Idaho, Bonner Co.; *D. Henderson 5707*—DQ665398, DQ665489, DQ665580. (**3**) ID 97938; USA, Idaho, Latah Co.; *M. Curto, L. Allen 551*—DQ665399, DQ665490, DQ665581. (**4**) UTC 191790; USA, Utah, Beaver Co.; *A. Taye 2929*—DQ665400, DQ665491, DQ665582. (**5**) ID 102852; USA, Idaho, Idaho Co.; *S. Riley 60*—DQ665401, DQ665492, DQ665583. (**6**) ID 97764; USA, Montana, Missoula Co.; *R. Bursik 493*—DQ665402, DQ665493, DQ665584. (**7**) ID 97304; USA, Idaho, Lemhi Co.; *D. Henderson 7545*—DQ665403, DQ665494, DQ665585. (**8**) ID 118908; USA, Idaho, Clearwater Co.; *C.A. Richardson 476*—DQ665404, DQ665495, DQ665586. (**9**) ID 59949; USA, Idaho, Clearwater Co.; *R.W. Steele 1 July 1970*—DQ665405, DQ665496, DQ665587. (**10**) ID 39226; USA, Idaho, Shoshone Co.; *W.H. Baker 15394*—DQ665395, DQ665486, DQ665577. (**11**) ID 97556; USA, Idaho, Lemhi Co.; *D. Henderson 5823*—DQ665396, DQ665487, DQ665578. (**12**) ID 96374; USA, Idaho; *D. Henderson, A. Cholewa 7396*—DQ665397, DQ665488, DQ665579.

Glyceria melicaria (Michx.) F.T. Hubb. (**1**) UTC 232391; Canada, New Brunswick; *W.G. Dore 15222*—DQ665426, DQ665517, DQ665608.

Glyceria striata (Lam.) Hitchc. (**1**) ID 107501; USA, Idaho, Idaho Co.; *R. Bursik, S. Spence 1682*—DQ665444, DQ665535, DQ665626. (**2**) ID 119171; USA, Idaho, Lemhi Co.; *S. Crockett 306*—DQ665448, DQ665539, DQ665630. (**3**) UTC 217497; USA, Utah, Cache Co., *M.E. Barkworth, C. Hsiao 94–037*—DQ665449, DQ665540, DQ665631. (**4**) ID 16820; USA, Idaho, Nez Perce Co.; *W.H. Baker 7411*—DQ665450, DQ665541, DQ665632. (**5**) ID 81031; USA, Idaho, Butte Co.; *D. Henderson, A. Cholewa 6375*—DQ665451, DQ665542, DQ665633. (**6**) UTC 210741; USA, Utah, Rich Co.; *M.E. Barkworth, J. Hughes, D. Welker 124*—DQ665452, DQ665543, DQ665634 (**7**) ID 92830; USA, Idaho, Idaho Co.; *B.H.M. Mooers 808*—DQ665453, DQ665544, DQ665635. (**8**) ID 97957, USA, Idaho, Idaho Co.; *M. Curto, L. Allen 571*—DQ665454, DQ665545, DQ665636. (**9**) ID 115056; USA, Idaho, Owyhee Co.; *M. Mancuso 1467*—DQ665455, DQ665546, DQ665637. (**10**) ID 119418; USA, Idaho, Custer Co.; *S. Crockett, D. Henderson 186*.—DQ665445, DQ665536, DQ665627 (**11**) UTC 228794; USA, New York, Onondaga Co.; *F.B. Gaffney*—DQ665446, DQ665537, DQ665628. (**12**) ID 113714; USA, Idaho, Idaho Co.; *A.C. Sondenaa 28*—DQ665447, DQ665538, DQ665629.

Melica L.

Melica bulbosa Geyer ex. Thurb (**1**) UTC 151446; USA, Idaho, Lincoln Co.; *A.B. Nicholson 75-7*—DQ665456, DQ665547, DQ665638. (**2**) UTC 151448; USA, Idaho, Custer Co.; *C.J. Brown 73-289*—DQ665457, DQ665548, DQ665639. (**3**) UTC 151450; USA, Idaho, Twin Falls Co.; *W.J. Sanders 53*—DQ665458, DQ665549, DQ665640. (**4**) UTC 151449; USA, Idaho, Cassia Co.; *W.J. Sanders 57*—DQ665459, DQ665550, DQ665641.