Phylogenetic Relationships Among Puccinellia and Allied Genera of Poaceae as Inferred from Chloroplast DNA Restriction Site Variation

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Phylogenetic relationships among *Puccinellia* and allied genera of Poaceae as inferred from chloroplast DNA restriction site variation[1]

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A cladistic analysis of chloroplast DNA restriction site variation among accessions of *Catabrosa* P. Beauv., *Phippsia* (Trin.) R. Br., *Sclerochloa* P. Beauv., and *Puccinellia* Parl. resolved a monophyletic *Puccinellia*, with *Sclerochloa* as its sister group, *Phippsia* the sister of the *Puccinellia* + *Sclerochloa* clade, and *Catabrosa* situated more distantly. These results suggest that the taxonomic fusion of *Phippsia* and *Puccinellia*, which has been proposed in light of the existence of natural hybrids between them (currently recognized as the nothogenus ×*Pucciphippsia* Tsvelev), would yield a grouping that would not be monophyletic unless *Sclerochloa* also was included. The set of restriction site characters that resolve these relationships provides minimal support for species groupings within *Puccinellia*, and the groupings that are resolved are inconsistent in some cases with species boundaries as determined by morphology and isozymes.

The grass subfamily Pooideae, with about 150 genera, includes nearly one-fourth of all genera of grasses (MacFarlane and Watson, 1982; Watson, Clifford, and Dallwitz, 1985; Clayton and Renvoize, 1986). This subfamily, representing one of about five major radiations of grasses, is most diverse in temperate to Arctic and alpine regions, where its species far outnumber those of other subfamilies. Within Pooideae, *Puccinellia* Parl. is a moderately speciose genus, with perhaps 25 (Hitchcock, 1969) to 80 (Clayton and Renvoize, 1986) to more than 100 species (Tsvelev, 1983) recognized. *Puccinellia* usually occurs in saline soils, in both coastal and inland habitats. Its geographic distribution is principally limited to the Northern Hemisphere, where it occurs from the middle latitudes to the high Arctic, with a few species occurring in South America, Africa, and Australia, predominantly at high latitudes. *Puccinellia* is included in tribe Poeae (formerly Festuceae: Stebbins and Crampton, 1956; Decker, 1964; Clayton and Renvoize, 1986), and like most genera in this tribe, and in the more inclusive Pooideae, it bears spikelets with relatively short glumes and two to several florets.

In Arctic regions of North America and Eurasia, *Puccinellia* often co-occurs with *Phippsia* (Trin.) R. Br., a genus of perhaps one to three species distributed principally at these high latitudes (cf. Hultén, 1964) with a few disjunct localities in alpine habitats farther south (Weber, 1952). *Phippsia* differs from *Puccinellia* in bearing just one floret per spikelet. The existence of natural hybrids between these two genera (Hedberg, 1962; Tsvelev,

1983) has suggested that they are closely related; these sterile hybrids have been assigned to the nothogenus ×*Pucciphippsia* Tsvelev. Löve (1970) and Löve and Löve (1975a, b, 1981), noting the existence of the intergeneric hybrids, and suggesting that the morphological differences between *Puccinellia* and *Phippsia* are minor, proposed the fusion of these two genera into a single genus, which for reasons of nomenclatural priority would bear the name *Phippsia*.

The relationship between *Phippsia* and *Puccinellia* is not the only problematic area of generic delimitation that involves *Puccinellia*. Striking similarities in morphology between *Puccinellia* and *Glyceria* R. Br. have complicated the taxonomic histories of these two genera. Church (1949) documented several morphological and cytological differences between *Glyceria* and *Puccinellia*. His analysis also revealed that much of the difficulty in differentiating these two genera was attributable to a small group of species that resemble *Glyceria* in gross morphology, but share a set of technical attributes (e.g., lodicule structure, base chromosome number) with *Puccinellia*. Church delimited this assemblage as *Torreyochloa* Church, but Clausen (1952) suggested that the genus did not warrant recognition, and recommended that its species be included within *Puccinellia* (see review by Davis, 1991). Stebbins and Crampton (1956) assigned *Glyceria* to tribe Meliceae, and *Torreyochloa*, along with *Puccinellia*, to the Poeae (also see Stebbins, 1956; Decker, 1964).

A phylogenetic analysis of chloroplast DNA restriction site variation in Pooideae (Soreng, Davis, and Doyle, 1990) substantiated the assignment of *Glyceria* to Meliceae, and *Puccinellia* to Poeae, while placing *Torreyochloa* in yet a third tribe, Aveneae (*Phippsia* was not represented in that study). The authors noted that the morphological similarities among these genera, in contrast to the many differences among them in micromorphological, anatomical, chromosomal, and now restriction site characters, was not a case of character conflict, because the phylogenetic structure suggested by the chloroplast DNA anal-

TABLE 1. Accessions of *Puccinellia* and related genera sampled for chloroplast DNA restriction site variation. Isozyme species within *P. nuttalliana* are numbered (see text), and multiple accessions within species (within isozyme species of *P. nuttalliana*) are differentiated by letters following their name. Country of origin is listed for each accession: for those from Canada and the United States, province or state (respectively) also is listed.

Species	Accession	Collection location[a]
1. *Sesleria insularis* Sommier ssp. *sillingeri* (Deyl) Deyl	PI 253719	Yugoslavia
2. *Catabrosa aquatica* (L.) Beauv. A	*Soreng 3407*	Canada (Quebec)
3. *Catabrosa aquatica* (L.) Beauv. B	*Soreng 3861*	Turkey
4. *Phippsia algida* (Solander) R. Br.	*Soreng 3520*	USA (Montana)
5. *Sclerochloa dura* (L.) Beauv. A	*Martin s.n.*	USA (Virginia)
6. *Sclerochloa dura* (L.) Beauv. B	*Soreng 3862*	Turkey
7. *Puccinellia distans* (L.) Parl.	*Davis 558-1*	Canada (Alberta)
8. *Puccinellia fasciculata* (Torrey) E. P. Bicknell	*Davis 395-3*	USA (Utah)
9. *Puccinellia festuciformis* (Host.) Parl.	*Soreng 3763*	Greece
10. *Puccinellia howellii* J. Davis	*Davis 526-11*	USA (California)
11. *Puccinellia lemmonii* (Vasey) Scribn.	*Davis 109-88-34*	USA (Oregon)
12. *Puccinellia limosa* (Schur) Holmberg	PI 251164	Yugoslavia
13. *Puccinellia lucida* Fern. & Weath. A	*Davis 610-A-24*	Canada (Nova Scotia)
14. *Puccinellia lucida* Fern. & Weath. B	*Soreng 3412*	Canada (New Brunswick)
15. *Puccinellia nuttalliana* (J. A. Schultes) A. S. Hitchc., isoz. sp. 1 A	*Davis 542-3*	USA (Alaska)
16. *Puccinellia nuttalliana* (J. A. Schultes) A. S. Hitchc., isoz. sp. 1 B	*Davis 563-1*	USA (North Dakota)
17. *Puccinellia nuttalliana* (J. A. Schultes) A. S. Hitchc., isoz. sp. 2	*Davis 271-5*	USA (California)
18. *Puccinellia nuttalliana* (J. A. Schultes) A. S. Hitchc., isoz. sp. 3	*Davis 525-19*	USA (Oregon)
19. *Puccinellia nuttalliana* (J. A. Schultes) A. S. Hitchc., isoz. sp. 5	*Davis 516-3*	USA (Montana)
20. *Puccinellia nuttalliana* (J. A. Schultes) A. S. Hitchc., isoz. sp. 6	*Davis 394-23*	USA (Utah)
21. *Puccinellia parishii* A. S. Hitchc.	*Davis & Manos 568-15*	USA (New Mexico)
22. *Puccinellia phryganodes* (Trin.) Scribner & Merr.	*Belsky s.n.*	USA (Alaska)
23. *Puccinellia poecilantha* (C. Koch) Grossh. A	PI 311722	Turkey
24. *Puccinellia poecilantha* (C. Koch) Grossh. B	PI 384942	Iran
25. *Puccinellia poecilantha* (C. Koch) Grossh. C	PI 384944	Iran
26. *Puccinellia pumila* (Vasey) A. S. Hitchc. A	*Davis 325-3*	USA (Alaska)
27. *Puccinellia pumila* (Vasey) A. S. Hitchc. B	*Davis 333-16*	USA (Alaska)

[a] PI = United States Department of Agriculture Plant Introduction Station.

ysis is consistent with the retention by these three genera of a basic spikelet morphology that may be plesiomorphic for the Pooideae. The analysis resolved three major clades within Poeae, one of them comprising *Puccinellia, Catabrosa* P. Beauv., *Sclerochloa* P. Beauv., and *Sesleria* Scop. (each represented by a single accession). Within this group, *Sesleria* was resolved as the sister group of a clade that included the other three, relationships among which were not resolved. *Catabrosa*, comprising one or perhaps two species, is widely distributed in freshwater habitats. *Sclerochloa*, another small genus, includes perhaps three species, distributed in southwestern Eurasia, with *S. dura* (L.) P. Beauv. now widely naturalized in North America.

With *Glyceria* and *Torreyochloa* placed more distantly, relationships among *Puccinellia, Catabrosa, Sclerochloa,* and *Phippsia* have remained unresolved. The present analysis was initiated to determine phylogenetic relationships among these four genera, and specifically to test the monophyly of *Puccinellia*, that is, to determine if the origins of any of the other three might lie among species of *Puccinellia*. No monographic treatment that delineates natural groupings within *Puccinellia* is available. To determine whether any of the other genera is nested within *Puccinellia*, several species considered representative of diversity within the genus were included in the analysis, some as more than one accession. This sampling was extended to include multiple isozyme species within the North American *P. nuttalliana* complex (Davis and Manos, 1991). *Sesleria*, having been resolved as a close relative of *Puccinellia, Catabrosa*, and *Sclerochloa*, was used as the outgroup for the analysis.

MATERIALS AND METHODS

Twenty-seven individuals were established from seed or transplanted from collection sites, and grow in the Cornell University greenhouses (Table 1); voucher specimens are deposited in the Bailey Hortorium herbarium. Plants were placed in a shaded chamber for the final 24 hr prior to harvesting to reduce starch content. Total DNA was extracted from 0.5–2 g of fresh or previously frozen (−70 C) leaf and stem material using Doyle and Doyle's (1990) modification of the hexadecyltrimethylammonium bromide (CTAB) isolation method of Saghai-Maroof et al. (1984).

Digestions were conducted with five restriction endonucleases (*Bam*H I, *Bgl* II, *Dra* I, *Hae* III, and *Hind* III) according to instructions provided by the supplier, Gibco/Bethesda Research Lab (G/BRL; Gaithersburg, MD). DNA fragments obtained from the digests were size-fractionated electrophoretically in agarose gels stained with ethidium bromide, with combined *Hind* III and *Pst* I digests of lambda DNA as standards. To maximize the separation and resolution of restriction fragments, gels of different concentrations were used, depending on expected site-frequencies for each enzyme; concentrations used were 0.8% (*Hind* III), 0.9% (*Bam*H I, *Bgl* II), and 1.3% (*Dra* I, *Hae* III). Once separated, restriction fragments were transferred to a Zetaprobe-GT nylon membrane (G/BRL) using the Southern (1975) blotting procedure. Selected cloned fragments from *Pennisetum americanum* (pMC; Thomas et al., 1984), *Triticum aestivum* (Tr; Bowman et al., 1981), *Nicotiana tabacum* (N; Sugiura et al., 1986),

and *Vigna radiata* (MB; Palmer and Thompson, 1981) cpDNA recombinant libraries were used as heterologous probes for restriction fragment detection (Fig. 1). Permission to use these clones was kindly provided by J. Rawson (British Petroleum America, Cleveland, OH: *Pennisetum*), C. Bowman (Institute of Plant Science Research, Cambridge, England: *Triticum*), and J. Palmer (Indiana University, Bloomington, IN: *Nicotiana* and *Vigna*). Probing was targeted toward the detection of variation in the Large Single Copy (LSC) and Small Single Copy (SSC) regions of the chloroplast genome, where most variation previously detected within tribe Poeae occurs (Soreng, Davis, and Doyle, 1990; Davis and Soreng, in press; Soreng and Davis, unpublished data). Three clones that hybridize partially or wholly with DNA in the Inverted Repeat regions (IR) also were used. Probing in the area within the LSC region in which grass chloroplast genomes exhibit three inversions relative to the orientation in most other plants (Palmer and Thompson, 1982; Howe, 1985; Quigley and Weil, 1985; Howe et al., 1988; Hiratsuka et al., 1989; Shimada and Sugiura, 1989, 1991; Downie and Palmer, 1992; Doyle et al., 1992) was conducted exclusively with *Triticum* and *Pennisetum* clones (Fig. 1). Hybridization was conducted overnight at 68 C in the solution described by Bernatzky and Tanksley (1986). The membranes then were exposed to X-ray film (Kodak XAR-5) and stripped for rehybridization with successive probes (Palmer, 1986).

Observed restriction fragment length polymorphisms interpretable as site polymorphisms were coded as binary data representing site presence/absence (Tables 2, 3). Sizes of all fragments are reported as estimated from autoradiographs (e.g., Table 2, character 7), not as calculated by adding sizes of smaller constituent fragments. For three digests (*Bam*H I, *Bgl* II, and *Hin*d III), interpretation of fragment patterns was facilitated by reference to existing restriction-site maps (Soreng and Davis, unpublished data).

Cladistic relationships among chloroplast genomes were analyzed with *Hennig86* (Farris, 1988). Three separate analyses were conducted, using successively more inclusive subsets of the terminals, to determine the effects of including multiple terminals with identical scores, except for the occurrence of missing values, for informative characters (i.e., those with scores of 0 for at least two terminals and 1 for at least two terminals). The first analysis included only one representative of each of four groups of taxa with identical scores for all nonmissing informative characters (see Results). This analysis included 11 terminals, few enough to use command *ie** (implicit enumeration, guaranteed to find all most-parsimonious trees). The second analysis included all terminals in the first analysis, plus four that individually differed from various taxa in the first analysis only in being scored "missing" for one or more informative characters. This analysis, based on 15 terminals, also was conducted using *ie**. The third analysis included all terminals in the second analysis, plus 12 (i.e., all 27 accessions) that were identical in all informative characters to terminals in the first and second analyses. This analysis was based upon too many individuals for *ie** to be used, so it was conducted using the command *mhennig** followed by *bb**; the analysis was repeated 100 times using different random taxon-entry sequences generated with the *spin* command of *Dada* (Nixon, 1993).
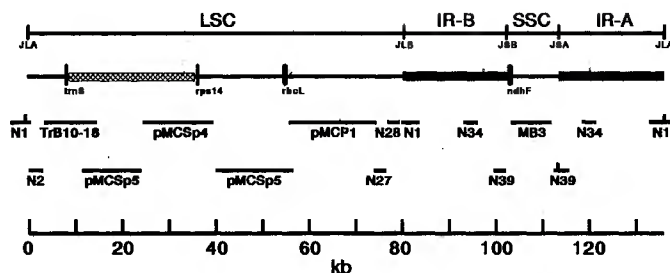

Fig. 1. Map of the chloroplast genome of *Oryza sativa*, reflecting orientation and nucleotide enumeration (in scale bar) in published sequence (Hiratsuka et al., 1989). Chloroplast genomes of grass genera in the present study are colinear with that of *Oryza* (see text). Locations of large and small single-copy regions (LSC, SSC), inverted-repeat regions (IR-A, IR-B), and junctions between these four regions (JLA, JLB, JSA, JSB) are indicated at top. The circular genome is displayed linearly with break at JLA (point 0). Cross-hatching in LSC demarcates region in which chloroplast genomes of grasses differ from those of most other plant families by presence of three inversions. Positions of four genes are shown, including two (*trn*S and *rps*14) that flank the inversion region. Also shown are regions probed by clones of *Pennisetum americanum* (pMC), *Nicotiana tabacum* (N), *Triticum aestivum* (Tr), and *Vigna radiata* (MB) used in the present analysis. Vertical bars on some clones indicate that they extend across a junction: N1 extends across JLA but not JLB; N39 extends across JSA but not JSB.

Clados (Nixon, 1991) was used to optimize characters on cladograms and to generate printed cladograms.

RESULTS

Observed restriction fragment length polymorphisms were interpreted as reflecting 66 restriction site polymorphisms, 34 of which are cladistically informative (Tables 2, 3). In the matrix of 27 taxa scored for 66 characters, 13 cells (0.7%) were scored as missing because of unobserved fragments or fragment patterns not unambiguously scorable for restriction sites scored for other taxa. Eleven of the cells scored as missing occurred among the 34 informative characters, so 1.2% of the data for informative characters are missing values.

Among the 27 terminals in the analysis are 11 unique combinations of cladistically informative characters, including seven unique individuals and four groups of accessions identical for all nonmissing informative characters. Two of these groups are assemblages of several accessions of *Puccinellia* (Tables 1–3): individuals 10, 12, 13, 16, 19, and 25 (group I); and 7, 8, 9, 14, 15, 17, 23, 24, 26, and 27 (group II). Each of these groups is composed predominantly of individuals scored for all informative characters, and each also includes two individuals with missing values for informative characters (individuals 10 and 19 in group I; individuals 9 and 24 in group II; Table 3). Groups I and II differ from each other in one informative character (number 63), with the apomorphic state (relative to the outgroup accession, *Sesleria*) present in group II; every accession in the data set has a nonmissing score for this character. Both groups I and II include accessions from both North America and Eurasia, and each includes at least one accession assigned on the basis of morphology to *P. lucida* Fern. & Weath., *P. poecilantha* (C. Koch) Grossh., and *P. nuttalliana* (J. A. Schultes) A. S. Hitchc. Each group also includes an accession of isozyme species 1 of *P. nuttalliana* (Davis and Manos, 1991).

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TABLE 2.   Chloroplast DNA restriction fragment length polymorphisms observed among 27 accessions of *Puccinellia* and related genera (cf. Table
1). Polymorphisms are categorized by restriction enzyme and by probe or probes with which they are observed (cf. Fig. 1). *Nicotiana* clones
listed in parentheses for *Bam*H I, *Bgl* II, and *Hin*d III provide more precise locations for fragments that have been mapped (see Materials and
Methods). Each polymorphism is described as a transformation from the state observed in *Sesleria insularis* (accession 1) to the state observed
in one or more other accessions, except for multicharacter transformations (e.g., characters 7–8) and characters in which the state in *Sesleria*
is unknown. For multicharacter transformations, plesiomorphic states that do not occur in *Sesleria* and apomorphic states not directly observed
in one or more other accessions (because of further transformation, as described in successive characters) are underlined: and reference is made
in the accession column [in brackets] to the related character or characters.

Enzyme and character number	Change in fragment phenotype (kb)	Probe(s)	Accessions with apomorphic state
*Bam*H I			
1.	4.9-> 3.5 + 1.45	N2, TrB10-18	2–3
2.	4.9-> 3.2 + 1.75	N2, TrB10-18	11
3.	4.9-> 3.4 + 1.50	N2, TrB10-18	4
4.	3.5-> 2.8 + 0.70	pMCSp6, pMCSp4 (N8, N9)	4–27
5.	6.7-> 4.4 + 2.3	pMCSp4 (N6, N7)	4
6.	3.3-> 2.0 + 1.3	pMCSp4 (N13)	2–27
7.	9.4 + 6.4-> 17	pMCSp5 (N19)	5–27 [8]
8.	17-> 11.2 + 4.2	pMCSp5 (N19)	5–6, 11, 18, 21–22 [7]
9.	4.4 + 0.7-> 5.1	pMCP1 (N22, N23)	2–27 [10]
10.	5.1-> 2.5 + 2.3	pMCP1 (N22, N23)	14 [9]
11.	4.6-> 2.4 + 2.2	pMCP1 (N26)	2–27
12.	8.0-> 5.5 + 2.5	N27, N28	2–3
13.	1.5 + 0.2-> 1.65	N1 (N31)	4–27
Bgl II			
14, 15.	3.4 + 1.3 + 2.14-> 6.9	N1, N2, TrB10-18	2–3
16.	1.3-> 0.8 + 0.6	TrB10-18	18
17.	15-> 12 + 3.3	pMCSp6	2–3
18.	15-> 8.0 + 7.0	pMCSp6 (N8)	5–6
19.	1.6 + 1.3-> 2.9	pMCSp4 (N7)	2–27
20.	2.5 + 0.9-> 3.4	pMCSp4 (N6)	2–27
21.	3.8-> 2.8 + 0.8	pMCSp5 (N15)	11, 21
22.	3.8 + 1.8-> 5.6	pMCSp5 (N15)	3
23.	5.3-> 1.5 + 4.1	pMCSp5 (N19, N20)	2–27 [24]
24.	1.5-> 0.8 + 0.64	pMCSp5 (N19)	2–3 [23]
25, 26.	1.75 + 0.5 + 0.2-> 2.4	pMCP1 (N21, N22)	2–27 [27]
27.	7.6 + 2.4-> 9.5	pMCP1 (N22, N23)	5–6 [25–26]
28, 29.	3.4-> 2.9 + 0.55 + 0.15	N39	2–27 [30]
30.	0.55-> 0.28 + 0.25	N39	4 [28–29]
Dra I			
31, 32.	3.8 + 1.9 + 0.8-> 6.6	TrB10-18	8
33.	1.9-> 1.4 + 0.4	TrB10-18	4
34, 35.	0.9 + 0.8-> 1.5 + 0.2	TrB10-18	4
36.	3.2-> 2.2 + 0.95	pMCSp6	2–27 [37]
37.	2.2 + 0.3-> 2.5	pMCSp6	2–3 [36]
38.	0.4 + 0.34-> 0.65	pMCSp6	2
39.	2.8 + 1.45-> 4.5	pMCSp4	11
40.	2.8-> 2.5 + 0.35	pMCSp5	2–3
41.	1.1 + 0.6-> 1.7	pMCSp5	2–3
42.	1.9-> 1.0 + 0.8	pMCP1	11
43.	2.5-> 1.6 + 0.9	N27	2–3, 22
44.	0.45-> 0.25 + 0.20	N27	5–27
45.	7.2 + 1.1-> 8.2	N28	2–3, 20
46.	2.7 + 1.3-> 4.0	MB3	2–6 [47]
47.	4.0 + 1.0-> 4.5	MB3	2–3, 5–6 [46, 48]
48.	4.5 + 1.7-> 6.6	MB3	2–3 [47]
Hae III			
49.	0.8 + 0.4-> 1.2	TrB10-18	2–27
50.	1.5-> 1.0 + 0.4	TrB10-18	11
51.	1.5 + 0.7-> 2.2	TrB10-18	21
52.	2.0-> 1.7 + 0.35	pMCSp6	2–27
53.	1.7-> 1.1 + 0.7	pMCSp5	4–5, 7–27
54.	2.6-> 1.5 + 1.1	pMCSp5	7–27
55, 56.	6.0-> 2.7 + 2.2 + 1.1	N27, N28	5–27 [57]
57.	2.7-> 2.0 + 0.75	N27, N28	5–6 [55, 56]
58.	1.65-> 1.05 + 0.65	MB3	2–3
59.	2.2-> 1.4 + 0.6	MB3	5–27
60.	1.85 + 0.6-> 2.4	MB3	4, 22

TABLE 2. Continued.

Enzyme and character number	Change in fragment phenotype (kb)	Probe(s)	Accessions with apomorphic state
Hind III			
61.	13.5-> 10.3 + 2.2	pMCSp6 (N13)	2–3
62.	13.5-> 12.5 + 1.2	pMCSp6 (N9)	7–8, 11–18, 20–23, 25–27
63.	2.8-> 1.8 + 0.9	pMCSp4 (N6)	7–9, 14–15, 17, 23–24, 26–27
64.	6.6 + 0.7-> 7.3	pMCSp5 (N17)	5–27
65.	9.5-> 6.0 + 3.5	pMCP1 (N19)	2–9, 11–27
66.	8.5-> 7.9 + 0.7	N28	3

Each of the other two groups of individuals with identical scores for informative characters consists of two accessions with nonmissing scores for every character: individuals 2 and 3 (the two accessions of *Catabrosa*, differing from each other in three uninformative characters); and individuals 11 and 21 (*Puccinellia lemmonii* [Vasey] Scribner and *P. parishii* A. S. Hitchc., differing from each other in five uninformative characters).

The first cladistic analysis, based on the seven unique individuals and one representative with no missing values from each of the four groups specified above, resolved three most-parsimonious trees. The second analysis, based on the 11 individuals used in the first analysis, plus the four from groups I and II with missing scores for informative characters, resolved eight most-parsimonious trees of length 40, consistency index 0.85 (Kluge and Farris, 1969), and retention index 0.85 (Farris, 1989; all three

numbers calculated exclusively on the basis of informative characters).

Removal of the four additional terminals representing groups I and II in the second analysis from the eight trees resolved by this analysis causes each tree to assume the identity of one of the three that were resolved by the first analysis. Thus, the eight trees resolved by the second analysis represent alternative positionings of the four additional terminals among those included in the first analysis, without altering relationships among the 11 original terminals. The additional topologies resolved by the second analysis are supported only by characters with missing values for the four additional taxa, and in all cases this support is absent under at least one optimization of these characters. The third analysis, based on the entire data set, also resolved eight most-parsimonious trees. As with the eight resolved by the second analysis, removal of the four terminals of groups I and II with missing values leaves

TABLE 3. Presence/absence of 66 polymorphic restriction sites among chloroplast genomes of 27 accessions of *Puccinellia* and related genera, arranged by restriction enzyme (cf. Tables 1, 2).

	Characters				
	*Bam*H 1	*Bgl* II	*Dra* I	*Hae* III	*Hind* III
Accessions	0000000001111 1234567890123	1111112222222223 4567890123456789 0	3333333334444444444 1234567890123456 78	455555555556 901234567890	666666 123456
1. *Sesleria insularis*	0000001010001	11000110100111000	11010011-010001---	101000000001	000100
2. *Catabrosa aquatica* A	1000011000111	00010000111001110	110101001100100000	001100000101	100110
3. *Catabrosa aquatica* B	1000011000111	00010000011001110	110101011100100000	001100000101	100111
4. *Phippsia algida*	0011111000100	11000000110001111	111011111010001011	001110000000	000110
5. *Sclerochloa dura* A	0001010100100	11001000110000110	110101111010011001	001110111011	000010
6. *Sclerochloa dura* B	0001010100100	11001000110000110	110101111010011001	001100111011	000010
7. *Puccinellia distans*	0001010000100	11000000110001110	110101111010011111	001111110011	011010
8. *Puccinellia fasciculata*	0001010000100	11000000110001110	000101111010011111	001111110011	011010
9. *Puccinellia festuciformis*	0001010000100	11000000110001110	110101111010011111	001111110011	--1010
10. *Puccinellia howellii*	0001010000100	11000000110001110	110101111010011111	001111110011	--00-0
11. *Puccinellia lemmonii*	0101010100100	11000001110001110	110101110011011111	011111110011	010010
12. *Puccinellia limosa*	0001010000100	11000000110001110	110101111010011111	001111110011	010010
13. *Puccinellia lucida* A	0001010000100	11000000110001110	110101111010011111	001111110011	010010
14. *Puccinellia lucida* B	0001010001100	11000000110001110	110101111010011111	001111110011	011010
15. *Puccinellia nuttalliana* 1 A	0001010000100	11000000110001110	110101111010011111	001111110011	011010
16. *Puccinellia nuttalliana* 1 B	0001010000100	11000000110001110	110101111010011111	001111110011	010010
17. *Puccinellia nuttalliana* 2	0001010000100	11000000110001110	110101111010011111	001111110011	011010
18. *Puccinellia nuttalliana* 3	0001010100100	11100000110001110	110101111010011111	001111110011	010010
19. *Puccinellia nuttalliana* 5	0001010000100	11000000110001110	110101111010011111	001111110011	--0010
20. *Puccinellia nuttalliana* 6	0001010000100	11000000110001110	110101111010010111	001111110011	010010
21. *Puccinellia parishii*	0001010100100	11000001110001110	110101111010011111	000111110011	010010
22. *Puccinellia phryganodes*	0001010100100	11000000110001110	110101111010111111	001111110010	010010
23. *Puccinellia poecilantha* A	0001010000100	11000000110001110	110101111010011111	001111110011	011010
24. *Puccinellia poecilantha* B	0001010000100	11000000110001110	110101111010011111	001111110011	--1010
25. *Puccinellia poecilantha* C	0001010000100	11000000110001110	110101111010011111	001111110011	010010
26. *Puccinellia pumila* A	0001010000100	11000000110001110	110101111010011111	001111110011	011010
27. *Puccinellia pumila* B	0001010000100	11000000110001110	110101111010011111	001111110011	011010

only three trees, in this case also including multichotomies among the various terminals within groups I and II that are identical and that have no missing values.

One of the most-parsimonious trees is depicted in Fig. 2, annotated to show the structure of the strict consensus tree. All trees exhibit a basal trichotomy among *Sesleria* (the outgroup), a monophyletic *Catabrosa*, and a third group comprising *Phippsia*, a monophyletic *Sclerochloa*, and a monophyletic *Puccinellia* (Fig. 2). All trees resolve *Sclerochloa* and *Puccinellia* as sister taxa, with *Phippsia* the sister group of this pair. Two monophyletic groupings are resolved within *Puccinellia* by all trees, each supported by a single nonhomoplasious character: *Puccinellia lemmonii* plus *P. parishii*; and group II. Differences between the topologies (apart from those that place taxa with missing values at different locations relative to each other and to taxa that are otherwise identical to them, solely on the basis of the characters with missing values) are restricted to variation among two cladistic groupings within *Puccinellia*: the grouping of *P. lemmonii* and *P. parishii* is sometimes placed within a larger clade that also includes *P. nuttalliana* isozyme species 3 and *P. phryganodes* (Trin.) Scribner & Merr. (Fig. 2); and group II is sometimes placed within a larger clade that also includes all accessions of group I plus *P. nuttalliana* isozyme species 6.

DISCUSSION

Catabrosa, Sclerochloa, and *Puccinellia,* all three of which are represented by two or more accessions, are separately resolved as monophyletic; the solitary accession of *Phippsia* is not resolved as nested among the accessions of any of these three genera. Thus, to the extent that phylogenetic relationships among the species in the sample are congruent with cladistic relationships among the chloroplast genomes that were sampled (cf. Neigel and Avise, 1986; Pamilo and Nei, 1988; Harris and Ingram, 1991; Doyle, 1992), the results are specifically inconsistent with the derivation of *Phippsia* from among the species of *Puccinellia*.

The analysis supports the recognition of nested sets of genera as follows: *Puccinellia* + *Sclerochloa, Puccinellia* + *Sclerochloa* + *Phippsia,* and if *Catabrosa* is interpreted as more closely related than *Sesleria* to these genera, a third grouping of *Puccinellia* + *Sclerochloa* + *Phippsia* + *Catabrosa.* If the nothogenus ×*Pucciphippsia* is temporarily removed from consideration, because its origin represents a clear departure from hierarchic descent (Funk, 1985; Kellogg, 1989), the available evidence concerning phylogenetic relationships in this complex is consistent with the conventional recognition of *Puccinellia, Phippsia, Sclerochloa,* and *Catabrosa* as separate genera. If cladistic relationships among the chloroplast genomes examined in the present study are indicative of relationships among the sampled species, and with other unsampled species within these genera, a genus that included all species usually assigned to *Phippsia* and *Puccinellia* would be monophyletic only if it also included *Sclerochloa*. Because *Sclerochloa* (1812) has nomenclatural priority over both *Phippsia* (1823) and *Puccinellia* (1848), recognition of a single genus that combines the three (plus ×*Pucciphippsia,* its constituent species then regarded as nothospecies) would require many new nomenclatural com-

binations in *Sclerochloa.* Because the continued recognition of *Sclerochloa, Puccinellia,* and *Phippsia* (plus ×*Pucciphippsia* to accommodate sterile hybrids between the latter two) satisfies the demands of monophyly (except for the clearly delimited nothogenus), while obviating the need for major nomenclatural adjustments, we endorse this alternative.

Although the present analysis is decisive concerning relationships among genera, only two groupings within *Puccinellia* are resolved, each supported by just one character. The sister group relationship detected between *P. lemmonii* and *P. parishii* (Fig. 2) should not be overemphasized, as it could not be more weakly supported and still be resolved; yet it provides an interesting, if tentative, perspective on relationships within *Puccinellia*. These two taxa, the only diploid species of *Puccinellia* in temperate North America, are distinct from each other in morphology and isozyme profile, and the known reservoir of isozyme variation of *P. parishii,* as surveyed across 19 loci, includes just two alleles that have not been observed in *P. lemmonii* (Davis and Goldman, in press). The resolution of a clade that includes only these two species suggests that they have not contributed a chloroplast genome to any of the many polyploid species of *Puccinellia* in North America (possible exceptions being *P. phryganodes* and *P. nuttalliana* isozyme species 3). The direct implication is that most temperate North American polyploid species of *Puccinellia* are descended, at least in part, from diploid taxa other than these two. Additional sampling may help to determine whether the ancestry of these polyploids involves as yet unsampled populations of *P. lemmonii* or *P. parishii* with different plastid types, or diploid taxa from other regions. Alternatively, the plastid types found in the polyploids may have been derived from North American diploid taxa that are now extinct, or from plastid lineages once present in *P. lemmonii* or *P. parishii* and now absent among diploids.

The second grouping consistently resolved within *Puccinellia* (group II; Fig. 2) is incongruent with the delimitation of three morphological species, including *P. nuttalliana,* and with the more narrowly delimited isozyme species 1 within *P. nuttalliana* (Davis and Manos, 1991). This grouping also is inconsistent with the recognition of separate North American and Eurasian species complexes within *Puccinellia,* even if species believed to have been introduced to North America from Eurasia (e.g., *P. distans* [L.] Parl. and *P. fasciculata* [Torrey] E. P. Bicknell) are removed from consideration. Like the groupings of *P. lemmonii* with *P. parishii,* support for group II is based on a single character. It is possible that further sampling with additional restriction enzymes will result in a different resolution more in line with biogeographic and taxonomic groupings, but the structure that has been resolved cannot be dismissed at this time. *Puccinellia* is widely recognized as a "difficult" genus. While some investigators have recognized numerous species (e.g., Sørensen, 1968; Tsvelev, 1983), others have suggested that the observed diversity represents the existence of weakly differentiated and inbred populations among which few species are sharply delimited (e.g., Hitchcock, 1969; Welsh, 1974). Complicating this situation is the ever present possibility that the plastid phylogeny is inconsistent with the species phylogeny. With this caveat, it does appear that
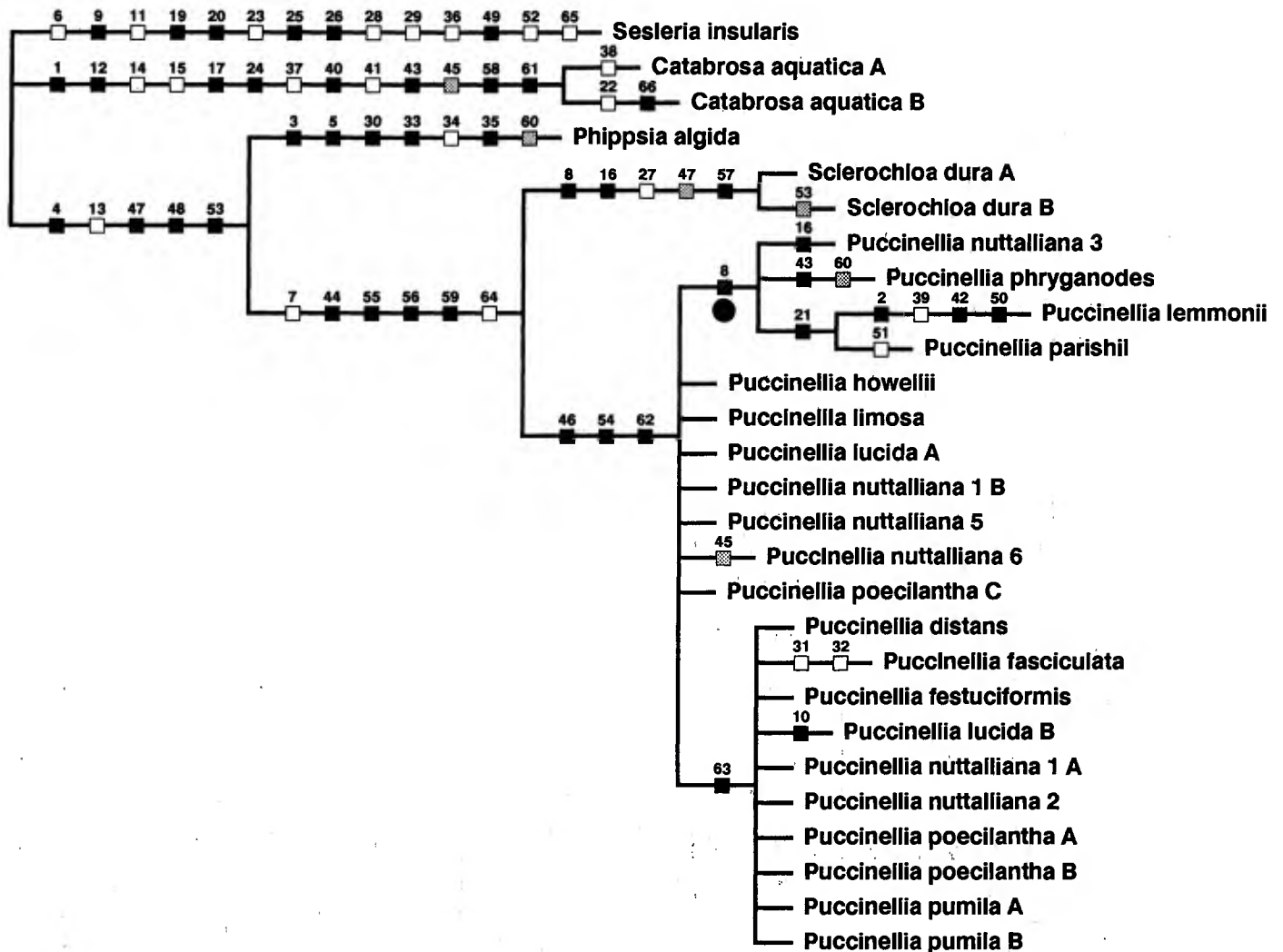
Fig. 2. One of three unambiguously supported (see text) most-parsimonious cladograms depicting relationships among chloroplast genomes of 27 accessions of *Puccinellia* and related genera, as determined from variation in 34 cladistically informative restriction site polymorphisms; 32 uninformative restriction sites also are depicted (cf. Tables 1–3). Site gains for nonhomoplasious characters are depicted with black bars, losses with white bars; gains for homoplasious characters are depicted with dark shading, losses with light shading. Filled circle marks clade that is absent in strict consensus tree.

if additional data substantiate the present resolution, or one much like it, major portions of the genus, distributed widely across both northern continents, might best be regarded as one or a few complexes of barely differentiated species, or perhaps as a mosaic of polymorphisms within which any species delimitation would be artificial.

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