DIVERSITY, PHYLOGENY, AND EVOLUTION IN THE MONOCOTYLEDONS
EDITED BY SEBERG, PETERSEN, BARFOD & DAVIS
AARHUS UNIVERSITY PRESS, DENMARK, 2010

Phylogeny and Reticulation in *Poa* Based on Plastid *trnTLF* and nrITS Sequences with Attention to Diploids

Robert J. Soreng,[1] Roger D. Bull,[2] and Lynn J. Gillespie[2]

[1]Smithsonian Institution, Department of Botany MRC-166, National Museum of Natural History, P.O. Box 37012, Washington, District of Columbia 20013-7012 U.S.A.

[2]Canadian Museum of Nature, Research Division, P.O. Box 3443, Station D. Ottawa, ON, K1P 6P4, Canada

Author for correspondence (sorengr@si.edu)

Abstract—*Poa* is the largest grass genus, with over 500 species, most of which are polyploid. Only 13 to 15% of species with chromosome counts have diploid populations. We conducted two phylogenetic analyses of combined chloroplast *trnT-trnL-trnF* (*TLF*) and nuclear ribosomal ITS sequence data for 42 outgroups and 47 samples of 30 species of *Poa*. One analysis focused on *Poa* taxa with diploid populations, and a second analysis included six more polyploid, and three possible hybrid taxa for which the data were not combined. The results are mostly congruent with results in published studies and provide further support for the recent classification of *Poa* into five serially diverging subgenera: *Sylvestres*, *Ochlopoa*, *Pseudopoa*, *Stenopoa*, and *Poa*. Incongruent placements of separate plastid and ITS sequences of *P. abbreviata* and *P. annua* are attributed to reticulate evolution, and of *P. trivialis* to long branch attraction. A genome notation system is introduced to simplify and facilitate characterization of plastid and ITS contributions in each accession and higher-level grouping resolved, and to identify the parental contributions in the possible hybrids. For example, we provide new evidence that a tetraploid, *Poa annua* (M^1M^2), is derived from hybridization between two diploids, *P. infirma* (M^1M^1) and *P. supina* (M^2M^2), where large capitals represent plastid and small capitals represent ITS genomes of *P.* subg. *Ochlopoa* sect. *Micrantherae* (Mm), and superscript numbers represent the genetically divergent genomes of the parental species within the section.

Keywords—DNA, genomes, hybridization, phylogeny, *Poa*, *Poa annua*, Poaceae, polyploidy.

Poa L. is known for its high frequency of polyploidy and large number of species (Stebbins 1950; Clayton and Renvoize 1986; Soreng 1990; Gillespie and Soreng 2005). The base chromosome number in *Poa* is $x = 7$, as it is across tribe Poeae s.l. (sens. Soreng et al. 2007) and the related tribes Triticeae and Bromeae, with few exceptions (Stebbins 1956). Among more than 5000 reported chromosome counts in *Poa* (Darlington and Wylie 1955; Cave 1959, 1965; Bowden 1961; Ornduff 1967-1969; Hair 1968; Fedorov 1969; Moore 1970-1974, 1977, 1982; Löve and Löve 1975; Goldblatt 1981, 1984, 1985, and on-line; Probatova 1985; Tateoka 1985; Duckert-Henriod and Favarger 1987; Dawson 2000; Soreng 2005), chromosome numbers are known for some 226 taxa of *Poa* accepted by us in at least 184 species, among the approximately 500 species we accept in the genus. Of *Poa* species for which chromosome counts are available, 91% include polyploids. We estimate that between 13 and 15% of the species have diploid populations, and that only nine percent are known only as diploids.

Given the high degree of polyploidy in the genus, the expectation is that much of it is due to allopolyploidy (Stebbins 1950). However, few studies have directly addressed allopolyploid origins in *Poa* species (Darmency and Gasquez 1997; Brysting et al. 2000, 2004; Patterson et al. 2005). Several studies have examined the phylogeny of the genus using plastid DNA data, with sampling dominated by polyploid elements (Soreng 1990; Gillespie and Boles 2001; Gillespie and Soreng 2005; Gillespie et al. 2007). Two studies, both with a preponderance of polyploid taxa, examined separate phylogenetic analyses of plastid and ITS data sets, but did not combine these data due to doubts about their compatibility (Brysting et al. 2004; Gillespie et al. 2008), and Nosov and Rodionov (2008) presented an ITS analysis of *Poa*. Gillespie et al. 2009, 2010 added ETS (a second nrDNA sequence) to the studies of subtribe Poinae and *Poa*, and combined the plastid and nrDNA data.

Might we detect different phylogenetic relationships among the diploid elements of *Poa* analyzed alone, as opposed to intermixed among samples dominated by polyploids? Might the ITS and plastid data for diploids in *Poa*, or diploids and polyploids, be combinable for some subset of taxa? And would we resolve topologies similar to those we had seen in previous studies? Gillespie et al. (2008) presented data for a subset of 42 species of *Poa* for which most plastid and ITS data were consistent. A few taxa were placed outside the genus by one genome, and within *Poa* with the other, leading the authors, for example, to accept *Arctopoa* as a genus of presumably ancient hybrid origin rather than as a subgenus within *Poa*. In exploratory analyses with many additional samples of *Poa*, Gillespie et al. (unpublished data) discovered multiple instances of incongruent plastid and ITS placements within *Poa*.

The 30 known species of *Poa* with diploid chromosome counts are distributed within 13 sections, in four of the five subgenera currently accepted by Gillespie et al. (2008) (number of diploid species is listed after each section name; see Table 1 for authorships of infrageneric taxa included in the present study):

(1) *P.* subg. *Ochlopoa* sects. *Alpinae* (6), *Arenariae* Stapf (5), *Micrantherae* (2);
(2) *P.* subg. *Poa* sects. *Homalopoa* (4), *Macropoa* (2), *Nivicolae* (1);
(3) *P.* subg. and sect. *Pseudopoa* (2);
(4) *P.* subg. *Stenopoa* sects. *Abbreviatae* (2), *Kolymenses* Prob. (1), *Oreinos* (1), *Pandemos* (1), *Tichopoa* Asch. & Graebn. (1).

No diploids are known from the fifth subgenus, *Sylvestres*. Owing to morphological differences correlated with plastid and/or ITS placements outside of *Poa*, two subgenera formerly accepted within *Poa* (Soreng et al. 2003) have been removed: *P.* subg. *Andinae* Nicora was elevated to genus *Nicoraepoa* Soreng & L.J. Gillespie, and *P.* subg. *Arctopoa* (Griseb.) Tzvelev sects. *Arctopoa* (Griseb.) Tzvelev and *Aphydris* (Griseb.) Tzvelev were accepted as sections of genus *Arctopoa* (Griseb.) Prob. (Soreng and Gillespie 2007; Gillespie et al. 2008). Diploids from *Poa* sects. *Nivicolae*, *Kolymenses*, *Nanopoa*, and *Tichopoa* have yet to be included in published DNA studies, and only a small analysis was published with a diploid, *P. dolosa*, of sect. *Oreinos* (Stoneberg-Holt et al. 2004). Sections *Kolymenses* and *Nivicolae* s.s. are placed in subgenera only by morphological characterizations, since no members, or no certain members (as in the case of *Nivicolae* s.l. in which *P. ircutica* and *P. veresczaginii* have been included [Tzvelev 1976], or excluded [Olonova 1990], from the section), have been included in DNA phylogenetic analyses. Placement of the monotypic *P.* sect. *Nanopoa* in our five-subgenus system has not been evaluated in previous DNA analyses.

Diploids in the 13 *Poa* sections with diploids are concentrated in Europe (Edmondson 1980; Moore 1982). One *Poa* section with diploids is centered in Beringia (*Abbreviatae*), one is centered in central and southwestern Asia (*Pseudopoa*), and two are endemic to far eastern Russia (*Nivicolae* s.s., and *Kolymenses*). *Poa* sect. *Homalopoa* s.s. includes diploids distributed across temperate forests of Eurasia and one in the southern Rocky Mountains. Only three *Poa* diploids are known outside of Eurasia, two that are endemic to North America (in *Abbreviatae* and *Homalopoa*) and one that is amphiberingian (in *Abbreviatae*) (*P. lettermanii*, *P. occidentalis* Vasey, and *P. pseudoabbreviata*, respectively). Of the 30 *Poa* taxa with diploid counts, ten of these also have higher ploidy levels. Three of these (*P. bulbosa*, *P. badensis*, and *P. diaphora*) have one or a few diploid counts and more polyploid counts, and the other seven have one or two higher polyploid counts (*P. cephalonica* H. Scholz, *P. molinerii*, *P. occidentalis*, *P. sinaica* Steud., *P. sibirica*, *P. supina*, *P. trivialis*). There may well be other undiscovered diploids. For example, there are several endemic species with polyploid chromosome counts from Africa (8), South America (10), SE Asia (7), Malesia (4), and Australia (3), but only 10 to 40% of the species in those areas have been cytologically examined. However, in floras outside of Europe and Russia that are cytologically well known, no *Poa* diploids have been found in Japan (Tateoka 1985), or New Zealand (Hair 1968), and only three are known in North America (Soreng 2007).

Given the potential for polyploid species to have more than one ITS type, and the requirement for cloning or other sensitive techniques to discover multiple copies (Bailey et al. 2003), and existing evidence (Gillespie et al. 2009, and unpublished) for distinct paralogous or hybrid copies of ITS in some polyploid accessions of *Poa* (i.e., copies that do not coincide with any phylogeny of plastid lineages), we have conducted an analysis primarily with diploid taxa. In a second analysis we also include a few polyploids (mainly tetraploids) from selected sections that are unrepresented by diploids, to determine their placements by plastid and ITS data. In order to investigate the possible hybrid origins of these polyploids, we include one diploid (*P. trivialis*) and two polyploid taxa (*P. annua* and *P. abbreviata*) that in exploratory analyses are placed in radically different positions in plastid and ITS trees. The latter three taxa have been included in most of our published plastid analyses. We present new ITS data for these and several other diploid taxa of *Poa* here.

Materials and Methods

Taxa and Collections—The taxon sample includes 28 species of *Poa*. Forty-two outgroups from within tribe Poeae s.l. also were used, 28 of them from subtribes Puccinelliinae, Poinae, Alopecurinae, and Miliinae (**PPAM** clade; Gillespie et al. 2008), including 15 samples from subtribe Poinae (including subtribe Cinninae). The tree was rooted with *Brachypodium distachyon* of tribe Brachypodieae. Taxon names, infrageneric classification, collection citations, reported chromosome numbers for the taxa, country of origin, and GenBank accession numbers for *Poa* are reported in Table 2. Plastid and ITS sequences from 29 samples were included from 16 species of *Poa* reported to have diploid populations (Table 2). For outgroup phylogenetic results using essentially the same outgroup taxa and DNA datasets see Gillespie et al. (2008, 2010). No attempt was made to verify the diploid status of these samples except *P. supina* accession 1 (Soreng 2005; but see Patterson et al. 2005). Although several of the species are known to be diploids from multiple chromosome counts, others have only one or two reported counts, and others have a few or multiple polyploid counts (Table 2).

DNA sequences—For protocols for generating and aligning the *trnT-trnL-trnF* (*TLF*) plastid and ITS1-5.8S-ITS2 nuclear ribosomal DNA sequences see Gillespie et al. (2008). One hundred and twenty-four sequences were generated at the Canadian Museum of Nature by LJG & RDB, 30 of which are new for this study. Thirty-four outgroup and 11 *Poa* sequences were generated by other labs as reported in GenBank. Insertion and deletion (indel) and inversion characters were not included in the present data matrix.

Analyses—The *TLF* and ITS datasets were merged into a single NEXUS format data matrix. The incongruence length difference test of Farris et al. (1995) was performed using the partition-homogeneity test of PAUP* 4.0b10 (Swofford

2002) to check for the extent of conflict between the *TLF* and ITS data partitions. Each taxon responsible for incongruence between the two partitions was subsequently treated as two separate ITS and *TLF* operational taxonomic units (OTUs).

Parsimony and bootstrap (BS) analyses were run as in Gillespie et al. (2008). Complete heuristic searches were performed in PAUP* for both analyses, with no maximum number of trees set in Analysis I (see below), and with a maximum number of trees set at 10,000 for Analysis II. Bootstrap analysis was performed with 1000 replicates, TBR swapping, and the 'MULTREES' setting on for Analysis I, and with 1000 replicates, 10 addition sequences per replicate, TBR swapping, and the 'MULTREES' setting turned off for Analysis II due to long search times (DeBry and Olmstead 2000). Following the suggestion of Starr et al. (2004), clade support was characterized as very poor (BS <55%), poor (BS 55-64%), moderate (BS 65-74%), good (BS 75-84%), very good (BS 85-94%), or strong (95-100% BS).

After exploratory parsimony analyses were run, two final parsimony analyses were conducted. **Analysis I** included 15 species of *Poa* known to have diploid populations (two samples are represented only by plastid data, but in one case a second sample of the same species was included, with both plastid and ITS data), plus four representative tetraploid species of *P.* sect. *Sylvestres*. Although no diploids are known in *Sylvestres*, these species are included in Analysis I because of their consistent and significant placement in previous analyses. In plastid analyses *P.* sect. *Sylvestres* was consistently resolved, with *Arctopoa* (Soreng 1990; Gillespie and Soreng 2005; Gillespie et al. 2007, 2008), as the sister to the remainder of *Poa*. In an ITS analysis sect. *Sylvestres* was also sister to the remainder of *Poa*, but *Arctopoa* was placed outside the genus (Gillespie et al. 2008). **Analysis II** included all the taxa in Analysis I, plus three species which were placed in conflicting positions in exploratory *TLF* and ITS trees, and eight samples/accessions of seven additional species which had consistent placements between *TLF* and ITS trees in previously published analyses (Gillespie et al. 2007, 2008). The latter seven taxa were included to add some depth to the sample in Analysis II, and to narrow down the affinities of the three inconsistently placed taxa. Six of the seven are tetraploids, while *P. flabellata* has tetraploid and hexaploid counts, and chromosome numbers are unknown for *P. porsildii*. In Analysis II, to avoid problems with taxa with incongruent plastid and ITS data, the data for the three taxa with conflicting placements were treated as separate lines of plastid and ITS data. For these three species, plastid data are included for one sample of *P. abbreviata*, two of *P. annua*, and two of *P. trivialis*, and ITS data are included for three samples of *P. abbreviata*, three of *P. annua*, and six of *P. trivialis*. For all merged samples except *P. ligulata*, the plastid and ITS data are from the same collection.

Taxon	2*n*=	Subgenus	Section	Genomes / Lineages in *Poa*	Country of Origin	[1]*Voucher*, herbarium, citation, replicate no.	*TLF* GenBank	ITS GenBank
Poa abbreviata R. Br.	(28?), 42	*Stenopoa*	*Abbreviatae*	SS	Canada, Nunavut	1. *Gillespie & Chatenoud 5957* CAN	DQ353996	GQ324481
Poa abbreviata R. Br.	(28?), 42	*Stenopoa*	*Abbreviatae*	*SH	Canada, Nunavut	2. *Gillespie 5818* CAN		GQ324480
Poa abbreviata R. Br.	(28?), 42	*Stenopoa*	*Abbreviatae*	*SH	Canada, Nunavut	3. *Gillespie 5816* CAN		AY237835
Poa alsodes A. Gray	not known	*Sylvestres*	*Sylvestres*	YY	Canada, Quebec	*Gillespie 6467* CAN	DQ353981	EU792374
Poa annua L.	28	*Ochlopoa*	*Micrantherae*	M[1]M[2]	China, Yunnan	2. *Soreng 7456* US	EU792452	GQ324485
Poa annua L.	28	*Ochlopoa*	*Micrantherae*	M[1]M[2]	Canada, Ontario	1. *Gillespie 6284* CAN	DQ353983	EU792386
Poa annua L.	28	*Poa*	*Ochlopoa*	__M[2]	[Argentina]	3. Corach et al. unpubl.	__	AF521901
Poa autumnalis Elliott	28	*Sylvestres*	*Sylvestres*	YY	USA, Maryland	*Soreng 4680* US	DQ353979	EU792379
Poa badensis Haenke ex Willd.	(14), 18-21, 28	*Ochlopoa*	*Bolbophorum*	AA	Bulgaria	*Hajkova 2004-12* US, *SH2004-12*	GQ324402	GQ324490
Poa chaixii Vill.	14	*Poa* (supersect. *Homalopoa*)	*Homalopoa s.s.*	HH	Russia	*Soreng 4677* US	EU854590	EU792404
Poa diaphora Trin.	14, 28, 42	*Pseudopoa*	*Pseudopoa*	EE	Turkey	*Soreng & Güney 4165* US	DQ353987, DQ353988	EU792400
Poa dolosa Boiss. & Heldr.	14	*Stenopoa*	*Orienos*	NN	Bulgaria	1. *Stoneberg SH18* US	GQ324413	GQ324503
Poa dolosa Boiss. & Heldr.	14	*Stenopoa*	*Orienos*	NN	Greece	2. *Soreng et al. 7495-1* US	GQ324414	GQ324502
Poa flabellata (Lam.) Raspail	28, 42	*Ochlopoa*	*Parodiochloa*	RR	South Georgia Islands	2. *Wright 9NSG* (seed vouchered US)	EU792453	EU792381

Poa flabellata (Lam.) Raspail	28, 42	*Ochlopoa*	*Parodiochloa*	RR	Falkland Islands	1. *Wright 4NCD* (seed vouchered US)	DQ353982	EU792380
Poa hybrida Gaudin	14	*Poa* (supersect. *Homalopoa*)	*Homalopoa*	H__	Greece	PI 249765, Patterson et al. 2005	AY589130	__
Poa infirma Kunth	14	*Ochlopoa*	*Micrantherae*	M^{I}M^{I}	Spain	1. Torrecilla and Catalán 2002	DQ367407, AF488773	AF488773
Poa infirma Kunth	14	*Ochlopoa*	*Micrantherae*	M^{I}M^{I}	Spain	2. *Catalán 3-2000-2* UZ	GQ324427	AF393012
Poa ircutica Roshev.	28	*Poa* (supersect. *Poa*)	*Nivicolae?*	PH	Russia, Siberia	*Kasanovskiy 2002-7* CAN	DQ354007	EU792402
Poa lettermanii Vasey	14	*Stenopoa*	*Abbreviatae*	SS	USA, Colorado	*Soreng & Soreng 7434* US	GQ324431	GQ324521
Poa ligulata Boiss.	14	*Ochlopoa*	*Alpinae*	AA	Morocco (*TLF*), Spain (ITS)	PI 517033, Patterson et al. 2005 (*TLF*), 166095 JACA (ITS)	AY589134	GQ324522
Poa macrantha Vasey	28	*Poa* (supersect. *Homalopoa*)	*Madropoa*	HH	USA, Oregon	*Soreng 5861* US	DQ354028	EU792407
Poa media (L.) Cav.	14	*Ochlopoa*	*Alpinae*	NN	Bulgaria	1. *Stoneberg SH17* US	GQ324437	GQ324527
Poa media (L.) Cav.	14	*Ochlopoa*	*Alpinae*	NN	Bulgaria	2. *Hajkova et al. 2004-11* US	GQ324436	GQ324526
Poa molinerii Balb.	14, 28	*Ochlopoa*	*Alpinae*	AA	Slovak Republic	*Stoneberg SH13* CAN	DQ354036, DQ354037, AY504639	EU792389
Poa nervosa (Hook.) Vasey	28	*Poa* (supersect. *Homalopoa*)	*Madropoa*	HH	USA, Oregon	*Soreng 5849* US	DQ354025	EU792405
Poa porsildii Gjaerev.	not known	*Poa* (supersect. *Homalopoa*)	*Madropoa*	HH	USA, Alaska	*Soreng & Soreng 6147-1* US	DQ354024	GQ324538

Taxon	2*n*=	Subgenus	Section	Genomes / Lineages in *Poa*	Country of Origin	[1]*Voucher*, herbarium, citation, replicate no.	*TLF* GenBank	ITS GenBank
Poa pseudoabbreviata Roshev.	14	*Stenopoa*	*Abbreviatae*	Ss	USA, Alaska	*Soreng & Soreng 6032-1* US	DQ353997	EU792398
Poa reflexa Vasey & Scribn.	28	*Poa* (supersect. *Homalopoa*)	*Homalopoa s.l.*	HH	USA, Colorado	*Soreng 7422* US	GQ324450	GQ324543
Poa remota Forselles	14	*Poa* (supersect. *Homalopoa*)	*Homalopoa s.s.*	PH	Czech Republic	1. *Stoneberg PB3-A* US	GQ324451	GQ324544
Poa remota Forselles	14	*Poa* (supersect. *Homalopoa*)	*Homalopoa s.s.*	PH	Kyrgyz Republic	2. *Soreng et al. 7540* US	GQ324452	GQ324545
Poa saltuensis Fernald & Wiegand	28	*Sylvestres*	*Sylvestres*	YY	Canada, Ontario	*Gillespie 7043* CAN	EU792451	EU792378
Poa sibirica Roshev. subsp. *sibirica*	14	*Poa* (supersect. *Poa*)	*Macropoa*	PH	Russia, Siberia	1. *Olonova 2002-1* CAN	DQ354044, DQ354045	EU792401
Poa sibirica Roshev. subsp. *sibirica*	14	*Poa* (supersect. *Poa*)	*Macropoa*	PH	Russia, Siberia	2. *Olonova 45* CAN	GQ324455	GQ324547
Poa stuckertii (Hack.) Parodi	28	*Poa* (supersect. *Homalopoa*)	*Dioicopoa*	HH	Chile	*Soreng & Soreng 7132* US	DQ354022	EU792414
Poa supina Schrad.	14, (28?)	*Ochlopoa*	*Micrantherae*	M^2M^2	USA (cult., introduced)	1. *Soreng & Cayouette 5950-2* US	DQ353984	EU792387
Poa supina Schrad.	14, (28?)	*Ochlopoa*	*Micrantherae*	M^2__	Morocco	2. PI 517033 UTC, Patterson et al. 2005	AY589147	__
Poa sylvestris A. Gray	28	*Sylvestres*	*Sylvestres*	YY	USA, Maryland	*Soreng 4678-3* US	DQ353980	EU792375
Poa trichophylla Heldr. & Sart. ex Boiss.	14	incertae sedis	*Nanopoa*	NN	Greece	*Soreng et al. 7508* US	GQ324461	GQ324554
Poa trivialis L.	14, (28?)	[11]incertae sedis	*Pandemos*	VV	USA, Maryland (cult., introduced)	1. *Soreng 4681-1* US	GQ324462	GQ324555

Poa trivialis L.	14, (28?)	incertae sedis	*Pandemos*	—^{V}	[cult.]	2. Gaut et al. 2000	—	AF171184
Poa trivialis L.	14, (28?)	incertae sedis	*Pandemos*	—^{V}	[cult.]	3. Gaut et al. 2000	—	AF171185
Poa trivialis L.	14, (28?)	incertae sedis	*Pandemos*	—^{V}	[cult.]	4. Gaut et al. 2000	—	AF171186
Poa trivialis L.	14, (28?)	incertae sedis	*Pandemos*	—^{V}	[France]	5. Charmet et al. 1997	—	AJ240161
Poa trivialis L.	14, (28?)	incertae sedis	*Pandemos*	^{V}—	Turkey	6. PI 204484 UTC, Patterson et al. 2005	AY589148	—
Poa trivialis L.	14, (28?)	incertae sedis	*Pandemos*	—^{V}	Spain	7. López-Rodríguez 01090, Catalán et al. 2004	—	AF532932
Poa wolfii Scribn.	28	*Sylvestres*	*Sylvestres*	^{Y}Y	USA, Missouri	*Soreng 5800* US	DQ354032, DQ354033	EU792377

Table 2. Collections of *Poa* examined for plastid and ITS DNA, chromosome number from the literature, infrageneric classification, plastid and ITS genomes, country of origin (and in some cases, smaller political units within countries), vouchers, and GenBank accession numbers. The infrageneric classification follows Gillespie et al. (2008). Chromosome numbers given in parentheses are infrequent numbers in taxa with multiple counts, or of questionable identification. Genome letter and superscript designations are described in the Results section of the paper. * = plastid genome designations based on results from a restriction-site study (Gillespie and Soreng 2005). Species replicates are numbered as in Figs. 1 and 2 in the Voucher column. Outgroup data are reported in Gillespie et al. (2008).

[I] Voucher notes: Field collections are in italic, followed by herbarium of deposition (Holmgren et. al. 1990). PI = U.S.D.A. Plant Introduction Station numbering, some of which are vouchered at UTC. Where the field collection is not cited, additional voucher information can be found in publications cited and GenBank.

[II] Formerly classified in *P.* subg. *Stenopoa*.

Results

Data Characteristics—Characteristics of the sequences were reported in Gillespie et al. (2008), where many of the same taxa were included. The whole plastid *TLF* and ITS dataset comprised 3214 aligned nucleotide positions after exclusion of 20 large and medium-sized gaps. The *TLF* data partition comprised 1995 positions, the ITS 635 positions. Percent missing data for the dataset was <0.1% for *TLF* and <0.5% for ITS, with data missing primarily from outgroup taxa (*trnT-trnL* spacer was missing for *Sclerochloa dura*). For the combined data set 442 positions were parsimony informative (PI) and 371 parsimony uninformative (PU) in Analysis I, and 465 were PI, and 369 were PU in Analysis II All OTUs included both ITS and *TLF* data, except for three species with incongruent plastid and ITS data, and *P. hybrida* and *P. supina* accession 1 for which no ITS data were available.

Exploratory analyses of separate plastid and ITS data resolved all of the *Poa* diploid taxa (except *P. trivialis*) and all of the *Poa* polyploid taxa included in this study (except *P. abbreviata* and *P. annua*) in the same cladistic relationships in separate plastid and ITS trees (trees not shown). Partition Homogeneity tests showed that our plastid and ITS data partitions were incompatible for matrices that included all taxa included in both Analyses I and II datasets (P = 0.01). However, for taxa included here from the PPAM clade (Gillespie et al. 2008), which includes *Poa*, the data partitions were not significantly incompatible (Analysis I dataset: P = 0.55; Analysis II dataset: P = 0.57), when the plastid and ITS data for *Poa abbreviata*, *P. annua*, and *P. trivialis* were subdivided as separate OTUs in Analysis II. Since our primary focus was the *Poa* clade (within PPAM), we feel that combining the plastid and ITS data for the entire dataset for Analyses I and II is justified, except for the above three species. When *TLF* and ITS data for *Poa abbreviata*, *P. annua*, and *P. trivialis* were combined, *TLF* and ITS data partitions were significantly incompatible for PPAM taxa (P=0.01).

Lineage Names—To facilitate discussions of genomic constitutions of lineages, taxa, and possible hybrids we introduce a simplified notation for identifying generalized major lineages in *Poa* (Table 1). Capital letters represent plastid genomes. Small capital letters represent ITS types/lineages. An ITS type is presumably a proxy for the nuclear genome in diploid taxa, but may or may not

Table 1. *Poa* classification and plastid genome and ITS lineage designations for sections in the present study, and clade acronym equivalents from previous studies. In the Genomes/Lineages column: capital letters = plastid; small caps = ITS; superscripts = subsets within plastid genomes or ITS lineages. For further explanation see Results and Table 2. Formerly, acronyms derived from section name initials were used for major clade names (Gillespie and Soreng 2005; Gillespie et al. 2007, 2008); the infrageneric classification based on the major clades follows Gillespie et al. (2008). Sections with diploid taxa, and plastid and ITS types for diploid representatives, are in bold.

Subgenus, Supersection	Sections and informal groups	Genomes / Lineages	Former Acronym
1) *P.* subg. *Sylvestres* (Soreng) Soreng & L.J. Gillespie.		YY	*Syl clade
	Sylvestres Soreng	YY	
2) *P.* subg. *Ochlopoa* (Asch. & Graebn.) Hyl.		OO	BAPO clade
	Alpinae (Nyman) Stapf	AA, NN	
	Parodiochloa (C. E. Hubb.) Soreng & L. J. Gillespie	RR	
	Micrantherae Stapf (syn.: *P.* sect. *Ochlopoa* Asch. & Graebn.)	MM	
3) *P.* subg. *Pseudopoa* (K. Koch.) Stapf		EE	*Pseudopoa* lineage (syn.: *Eremopoa*)
	Pseudopoa (K. Koch.) Hack.	EE	
4) *P.* subg. *Stenopoa* (Dumort.) Soreng & L.J. Gillespie		SS	SPOSTA clade
	Nanopoa J. R. Edm.	NN	
	Pandemos Asch. & Graebn.	VV	
	Oreinos Asch. & Graebn.	NN, **SS	****OSTA clade
	Abbreviatae Tzvelev	SS, SH	OSTA clade
5) *P.* subg. *Poa*.		PH	
P. subg. *Poa* supersect. *Poa*		PH	PoM clade
	Macropoa F. Herm. ex Tzvelev	PH	
	Nivicolae (Roshev.) Prob.	***PH	
	Poa	PH	
P. subg. *Poa* supersect. *Homalopoa* (Dumort.) Soreng & L.J. Gillespie		HH (PH)	HAMBADD clade
	Dioicopoa E. Desv.	HH	
	Homalopoa Dumort.	HH (PH)	
	Madropoa Soreng	HH	

* The clade was called ArcSyl (Gillespie and Soreng 2005; Gillespie et al. 2007), before *Arctopoa* was removed from the genus (Gillespie et al. 2008).
** No polyploid taxa from *P.* sect. *Oreinos* are included in the present study, but its type species, *Poa laxa* s.l. or s.s., was included in Gillespie et al. (2007, 2008; see Discussion).
*** Our postulated representative of *Nivicolae* is a polyploid species of uncertain sectional relationship to the diploid type species (see Discussion).
**** The OSTA plastid clade includes four sections (*Oreinos*, *Stenopoa* Dumort., *Tichopoa*, and *Abbreviatae*) and is a subset of the SPOSTA clade, which also includes sects. *Secundae* Soreng, and *Pandemos*.

be derived from the same nuclear genome or genomes present in polyploids. Also, the unusual characteristics of ITS sequence evolution could scramble phylogenetic signal to an extent that ITS no longer effectively reflects the phylogeny of the nuclear genome it was derived from (Álvarez and Wendel 2003). In cases where the ITS type is suspected to be derived from wide hybridization (i.e., more than one ITS lineage is or was likely present at some point in the taxon under consideration) we append an asterisk to the ITS type. The logic and necessity of a shorthand genomic and lineage naming system will become more apparent in future papers with many additional species, sections of the genus, and hybrids, but similar systems are widely employed by geneticists for whole genomes (e.g., Dewey 1984). In selecting which plastid genomes or nrDNA lineages to name by letters we focus on the taxonomic subgenera and sections of the genus, or if the plastid genome or ITS lineage is relatively homogeneous between or among sections then one letter may be applied to several sections. If and when additional independent nuclear DNA markers are employed these may be appended to the nuclear DNA side of these notations.

At the subgenus – major clade – level, the following letters are used: Yy = *Sylvestres*; Oo = *Ochlopoa*; Ee = *Pseudopoa* (formerly the genus *Eremopoa*); Ph or Hh = *Poa* / *Homolopoa*; Ss = *Stenopoa*. Where there are diverse groups represented within the above five major clades additional letters are applied: Aa = *Alpinae*; Mm = *Micrantherae*; Rr = *Parodiochloa*; Hh = *Poa* supersect. *Homalopoa* (with several sections, but where phylogenetically independent ITS types are not supported between supersects. *Poa* and *Homalopoa* by analysis of ITS data alone); Nn = sect. *Nanopoa*, *P. dolosa* and *P. media* (the latter two species are classified in different sections of the genus but are little differentiated from sect. *Nanopoa*); Ss = *Stenopoa-Tichopoa-Oreinos-Abbreviatae*. Superscript numbers are added as needed to represent sublineages within each of the more general genome types. As examples from the five subgenera, *Poa sylvestris* is Yy; *P. ligulata* is Aa and *P. infirma* is M^1M^1; *P. diaphora* is Ee; *P. chaixii* is Hh; *P. trichophylla* is Nn; and *P. pseudoabbreviata* is Ss. For *P. trivialis* (and thus *P.* sect. *Pandemos*), under evaluation as a possible hybrid between elements from two major clades, the genome constitution is represented as Vv because it is only remotely associated with other genomes in either plastid or nrDNA analyses. See Table 2 for plastid genome and ITS lineage designations for each sample. This system allows us to readily identify the genetic constitution of any sample without referring to the section name or cumbersome acronyms previously applied to *Poa* clades when dealing with the plastid genome alone (Gillespie and Soreng 2005; Gillespie et al. 2007). In Figs. 1 and 2, for Analyses I and II, the subgenera – major clades – are identified on the far right by the plastid capital letters as Y clade, O clade, E lineage, P clade, N clade and S clade.

Parsimony Analyses—For this paper we report structure and BS support only for *Poa* and clades within *Poa* (see Gillespie et al. [2010] for discussion of and support for PPAM clades outside of *Poa*).

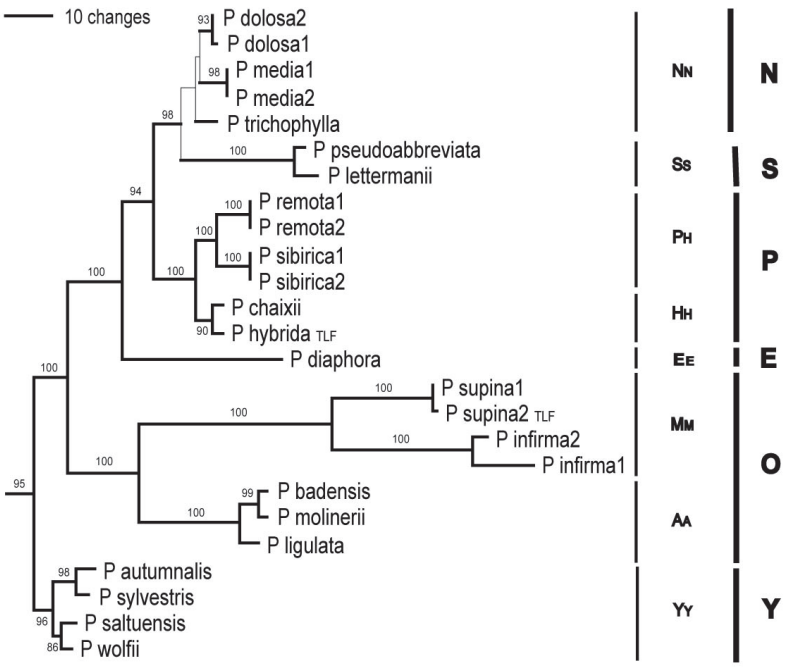

Fig. 1. Cladogram with summary of the strict consensus tree for *Poa* elements resulting from parsimony analysis of the plastid *TLF* and nuclear ITS data matrix in Analysis I (length 2013 steps; CI excluding uninformative characters = 0.461; RI 0.769). Outgroups are not displayed (see Gillespie et al. [2008, 2010] for discussion of those). Analysis I includes *P*. subg. *Sylvestres* tetraploids. All other *Poa* samples are from species with diploid populations. The strict consensus tree is indicated by bold lines on one of 59 most parsimonious trees. Bootstrap values of 50% or higher are given on the branches. Major clades are indicated on the far right, and major subclades just to the left of those with their genomic constitutions (see Tables 1 and 2), where: A = *Alpinae*; E = *Pseudopoa*; H = *Homalopoa*; M = *Micrantherae*; N = *Nanopoa*; O = *Ochlopoa*; P = *Poa*; S = *Stenopoa*; Y = *Sylvestres*. For genomes, capital letters represent plastid lineages, and small capitals represent ITS lineages. Two samples have *TLF* data only as noted, and the *P. ligulata* entry is a composite of two collections.

Analysis I: Number of most parsimonious trees (MPTs) = 59; Length (L) of MPTs = 2013 steps; Consistency Index (CI) = 0.606, CI excluding uninformative characters = 0.461; Retention Index (RI) = 0.769. A cladogram illustrating one of the most parsimonious trees and lineages detected in the strict consensus tree (SCT; shown as bold branches) is depicted in Fig. 1.

Poa was resolved as monophyletic (BS = 95). Within *Poa*, BS support was between 90 and 100 for all but one of 22 clades, and 18 of these had BS support of

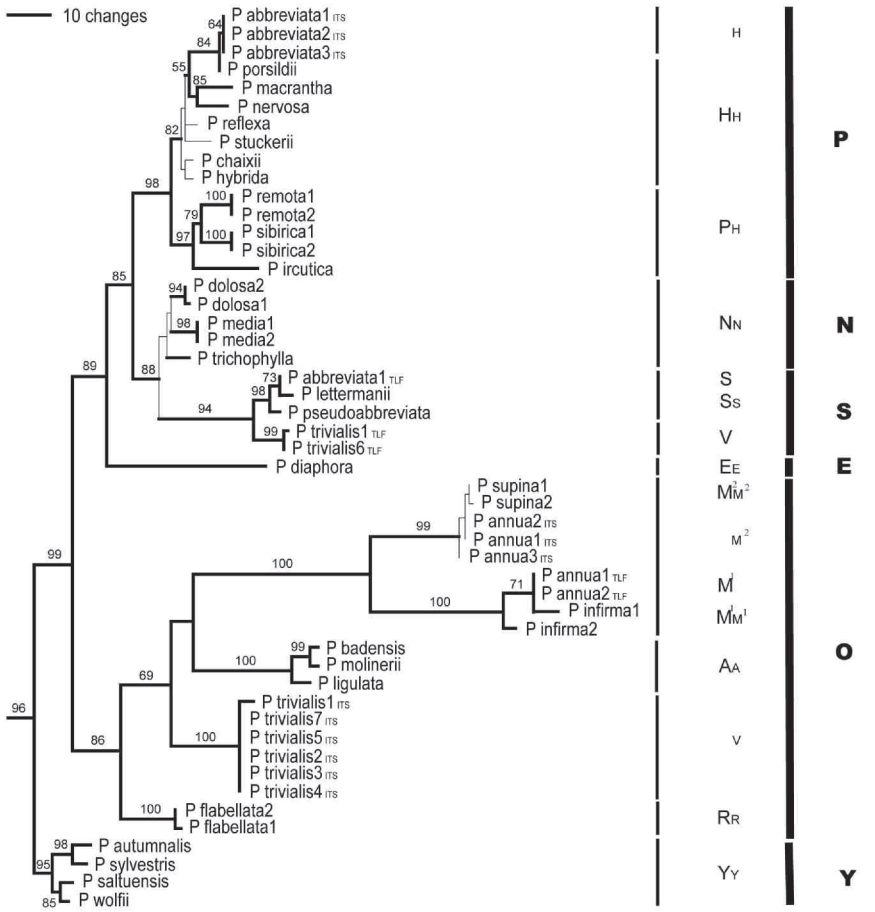

Fig. 2. Cladogram with summary of the strict consensus tree for *Poa* elements resulting from parsimony analysis of the plastid *TLF* and nuclear ITS data matrix in Analysis II (length 1752 steps; CI excluding uninformative characters = 0.485; RI 0.760). Outgroups are not displayed (see Gillespie et al. [2008, 2010] for discussion of those). Analysis II includes all taxa in Analysis I plus 11 additional taxa which are polyploid, except *P. porsildii* (ploidy unknown), and *P. trivialis* (diploid). The strict consensus tree is indicated by bold lines on one of 10,000 most parsimonious trees. Bootstrap values of 50% or higher are given on the branches. Major clades are indicated on the far right, and major subclades just to the left of those, with their genomic constitutions (see Tables 1 and 2), where: A = *Alpinae*; E = *Pseudopoa*; H = *Homalopoa*; M = *Micrantherae*; N = *Nanopoa*; O = *Ochlopoa*; P = *Poa*; R = *Parodiochloa*; S = *Stenopoa*; V = *Pandemos*; Y = *Sylvestres*. For genomes/lineages, capital letters represent plastid lineages, and small capitals represent ITS lineages. Incongruent placements of *P. abbreviata*, *P. annua* and *P. trivialis* samples are followed by ITS or *TLF* to indicate the data source for each sample. Two samples from Analysis I have *TLF* data only as noted in Fig. 1, and the *P. ligulata* entry is a composite of two collections.

95 to 100. The lowest BS support was 86, for the union of *P. saltuensis* and *P. wolfii* within *P.* subg. *Sylvestres*. In the SCT there was one polytomy within *Poa*, which included *P. dolosa*, *P. media*, *P. trichophylla*, and *P. lettermanii* plus *P. pseudoabbreviata*. There were no plastid characters to resolve this polytomy. *Poa lettermanii* and *P. pseudoabbreviata* (Ss) are united on a long branch (25 steps) from their most recent common ancestor with the previous three species: The other three species in this polytomy differ among themselves by only 10-12 steps, with only 1 ITS character separating them. There is limited differentiation among the latter three species, and repeated detection of a series of taxa with limited differentiation in a clade (Soreng 1990; Gillespie et al. 2007, in 2009 [plastid analysis]) or polytomous relationship (Gillespie and Soreng 2005) to the highly differentiated set of S clade taxa (**OSTA** acronym in our previous studies, see Table 1; Gillespie and Soreng 2005; Gillespie et al. 2007). Thus, we choose to designate this set of three species as Nn taxa, and the second pair as Ss even though Nn taxa did not form a separate clade in the SCT for the present study. As further justification, when ETS data are added to ITS data an n clade is resolved that is not a sistergroup to any clade containing elements with S type nrDNA (Gillespie et al. 2009).

Analysis II: Number of MPTs = 10,000; L of MPTs = 1752; CI = 0.606, CI excluding uninformative characters = 0.485; RI = 0.76. A cladogram illustrating one of the most parsimonious trees and lineages detected in the SCT (shown as bold branches) is given in Fig. 2.

Lower BS numbers in Analysis II, as compared to Analysis I, may partly result from missing data for the three taxa added to Analysis II whose samples were coded as separate plastid and ITS OTUs (i.e., in *P. abbreviata*, *P. annua*, and *P. trivialis*).

Poa was resolved as monophyletic (BS = 96). There were six polytomies within *Poa* in the SCT. Three were within taxa with subdivided plastid and ITS data. The other three were among subsets of taxa with, for the most part, limited nucleotide variation. Within *Poa*, BS support was between 55 and 100 for all 34 dichotomies in the SCT except those for relationships between *P. trivialis* (ITS sequence data only), and sects. *Micrantherae* and *Alpinae* (Mm Aa) (BS < 50). The major clades were resolved with very good to strong support: Y clade (BS =95), remainder of *Poa* (BS = 99), O clade (BS = 86), remainder of *Poa* (BS = 89), E lineage (includes just one sample, thus no BS number), remainder of *Poa* (BS = 85), P clade (BS = 98), S clade (BS = 88) (Fig. 2).

Of *Poa abbreviata*, *P. annua*, and *P. trivialis*, for which plastid and ITS data were divided as separate OTUs, their plastid OTUs were resolved in well differentiated clades from their ITS OTUs, and each OTU was placed with others of its own kind (Fig. 2). The *P. annua* plastid samples were separated from the ITS samples by three intervening nodes with BS support of 71, 99, and 100. The *P. abbreviata* samples were separated by nine intervening nodes with BS support, and good to strong BS support for six of them. The *P. trivialis* samples were separated by six nodes with BS support, and good to strong support for five of them.

Discussion

Analyses of Diploids—Phylogenetic analysis of diploids in isolation from polyploids provides us with an opportunity to explore the impact of polyploids in present and previous analyses (Kellogg et al. 1996; Doust et al. 2007). Among putative diploids, samples of *Poa chaixii*, *P. diaphora*, *P. molinerii*, *P. pseudoabbreviata*, *P. sibirica*, and *P. supina* were included here and in plastid and ITS analyses by Gillespie et al. (2008), with ETS also (Gillespie et al. 2009), and in some earlier plastid analyses (Stoneberg-Holt et al. 2004; Gillespie and Soreng 2005; Gillespie et al. 2007). *Poa hybrida*, *P. occidentalis*, and *P. pseudoabbreviata* were included in a restriction-site plastid analysis by Soreng (1990). *Poa trivialis* is a diploid included here only in Analysis II, but previously only in our plastid analyses (Soreng 1990; Gillespie and Soreng 2005; Gillespie et al. 2007), until Gillespie et al. (2009) added ETS data. Here we include plastid and/or ITS data for an additional nine species not in our previous analyses of DNA sequence data that are known to have diploid populations (*P. badensis*, *P. dolosa*, *P. hybrida*, *P. infirma*, *P. lettermanii*, *P. ligulata*, *P. media*, *P. remota*, and *P. trichophylla*), and ITS data for *P. trivialis* (see also Gillespie et al. 2009). Most of the diploid taxa included in previous analyses (e.g., Gillespie et al. 2008) are placed here, in Analyses I and II (and in separate plastid and ITS trees not shown), in phylogenetically consistent plastid and ITS relationships. *Poa trivialis* is an exception and was excluded from Analysis I because including it resulted in incompatible plastid and ITS datasets for PPAM; its plastid and ITS data were run as separate OTUs in Analysis II (see Reticulate Evolution below). The placements of the other newly added taxa with diploids are consistent for plastid and ITS data (separate trees not shown, but see Gillespie et al. 2009).

The positions of the taxa with diploid populations are sometimes consistent and sometimes not with respect to accepted taxonomic relationships resolved for diploid and polyploid consectional elements included in the present or previous studies (Gillespie and Soreng 2005; Gillespie et al. 2007, 2008). Within *P.* sect. *Micrantherae* (Mm), *P. infirma* is united in the clade with *P. supina*. Within *P.* sect. *Abbreviatae* (Ss for diploids), *P. lettermanii* was placed with *P. pseudoabbreviata*.

Within *P.* sect. *Alpinae*, *P. badensis*, and *P. ligulata* are united with *P. molinerii* (all Aa genotypes): However, *P. media* was placed in a position remote from the other elements of *P.* sect. *Alpinae* sampled here (see Tables 1 and 2), near *P. trichophylla* and *P. dolosa* (all Nn genotypes). This calls the monophyly of sect. *Alpinae* into question. The situation in sect. *Alpinae* is mirrored within *P. bulbosa* (sect. *Arenariae*), which has both Aa and Nn genotypes (see Gillespie et al. 2008). This disparity requires further study, but so far no samples with An nor Na (i.e., intermixed genotypes) have been detected.

Within *P.* subg. *Poa*, the supersects. *Poa* (Ph) and *Homalopoa* (Hh) are well to strongly supported sublineages that divide on plastid data only. They have

not been resolved as independent with ITS data (Gillespie et al. 2008, and unpublished), thus all members are designated as having H ITS genotypes, and most H genotypes have diverged little from their most recent common ancestor (Gillespie et al. 2008, 2009, and unpublished). We chose H instead of P for the ITS name because H represents the larger group and we suspect that *Homalopoa* taxa may have contributed the ITS type to the supersect. *Poa*. Among elements taxonomically placed in *P.* sect. *Homalopoa*, *P. hybrida* was united with *P. chaixii* (HH). In an earlier study (Soreng 1990, Fig. 2) a different accession of *P. hybrida* and one of *P. occidentalis* (also a diploid of sect. *Homalopoa* s.s.) aligned in a clade equivalent to supersect. *Homalopoa* (H). However, *P. remota*, traditionally placed in sect. *Homalopoa*, was aligned with *P. sibirica* of *P.* supersect. *Poa* sect. *Macropoa* (PH). This result held up in extended analyses (Gillespie et al. 2009) of plastid and nrDNA data, and suggests that the relationship of *P. remota* needs further study.

Within subgenus *Stenopoa*, among elements taxonomically placed in *P.* sect. *Oreinos*, *P. dolosa* (NN) was not placed in the Ss clade where polyploid consectional elements were placed in previous studies. *Poa fernaldiana*, *P. flexuosa*, and *P. laxa* (the former two sometimes treated as subspecies of *P. laxa*), appeared in previous plastid analyses with *P. pseudoabbreviata* and sects. *Stenopoa* and *Tichopoa* elements (all Ss genomes; ITS published for *P. fernaldiana*, but new for the other two; see Gillespie and Soreng 2005; Gillespie et al. 2007, 2008, 2009; Tables 1 and 2, Fig. 2). All Ss genome elements are resolved within a strongly supported but fairly undifferentiated plastid clade as sister to the V plastid genome of *Poa trivialis*. Thus, it appears that *P.* sect. *Oreinos*, as currently delimited, is nonmonophyletic. Whether or not *P. dolosa* was involved in the origin of the polyploid elements in the section requires further study.

This study, and Gillespie et al. (2009, 2010) are the first published analyses to include *P. trichophylla*, the sole member of *P.* sect. *Nanopoa*. *Poa trichophylla* aligned in a polytomy with *P. dolosa*, *P. media* and a clade of *P. lettermanii* plus *P. pseudoabbreviata*. DNA sequences of *Poa trichophylla*, *P. dolosa*, *and P. media* have diverged little from their most recent common ancestor (all NN genomes), whereas the most recent common ancestor of *P. lettermanii* and *P. pseudoabbreviata* (Ss genomes) had diverged greatly from the other elements in the SCT polytomy. Edmondson (1980) placed his sect. *Nanopoa* between sects. *Abbreviatae* and *Arenariae*. Although the phylogenetic picture appears to be quite complex, it appears that Edmondson's general placement was a reasonable first approximation given the present trees (however, see Gillespie et al. 2009).

Comparison of Trees in Analyses I and II—Trees based on combined *TLF* and ITS data (Figs. 1 and 2) are highly congruent for each of the major clades detected, and for relationships within these clades. Support for major clades was high in both trees. The tree in Fig. 1 is entirely congruent with all previous separate analyses of plastid and ITS data (analyzed separately from ETS data) for taxa that overlap between studies.

There were a few differences between the tree in Fig. 2 and the plastid trees in Gillespie and Soreng (2005, Figs. 1 and 2) and Gillespie et al. (2007, Fig. 1b, 2008, Fig. 1, and 2009 Fig. 1). The only significant difference from previous plastid studies is with the order of branching of *P.* sect. *Parodiochloa* as sister to the moderately supported clade of *Micrantherae* plus *Alpinae* in Analysis II (Fig. 2). In previous plastid analyses (Gillespie and Soreng 2005, Fig. 1; Gillespie et al. 2007, Fig. 2; 2008, Fig. 1) *Alpinae-Arenariae* was sister to the clade of *Parodiochloa* plus *Micrantherae* with moderate, strong, and very good support, respectively. The difference in branch order here reflects the pull of ITS data on *Parodiochloa* away from *Micrantherae* plus *Alpinae* in our Analysis II (Fig. 2). This pull is illustrated in the ITS analysis of Gillespie et al. (2008, Fig. 3; BS < 50 for each node) where *Parodiochloa* was resolved as sister to the rest of *Poa* excluding sections *Sylvestres*, and *Micrantherae* plus *Alpinae-Arenariae* (see also Gillespie et al. 2009 Fig. 2). Despite these differences in branching order, the Partition Homogeneity tests resolved our plastid and ITS data sets as compatible for genera of the PPAM clade. Additional study of this nexus is warranted, but, for now, we continue to include *Parodiochloa* and *Tzvelevia* within *P.* subg. *Ochlopoa* (*Poa* sect. *Tzvelevia* was included only in the Gillespie et al. [2008] plastid and ITS analyses and there it was resolved in a polytomy with *P.* sect. *Parodiochloa* elements).

Evaluation of Reticulate Evolution—In Analysis II, *P. abbreviata*, *P. annua*, and *P. trivialis* were included to demonstrate their conflicting placements in plastid and ITS trees. We have uncovered several other similar cases of highly conflicting plastid and ITS placements for species and species groups within *Poa*, which we will deal with in a subsequent paper. Three additional cases of conflicting placements were discovered between *Poa* and lineages outside of *Poa* (see introductory section, and Gillespie et al. 2008, 2010). Here we discuss evidence that the above three species are or are not hybrids in origin, and evidence of their parentage.

Poa annua: *Poa annua* was postulated by Nannfeldt (1935, 1937), and Tutin (1957), to be a stabilized tetraploid species derived from a cross between two diploid species, *P. infirma* and *P. supina*. *Poa annua* is intermediate between the putative parents in anther length, panicle form, spikelet density along branches, length of the distal rachilla internode, lemma pubescence, and vegetative mode of reproduction and longevity. Although extreme forms of *P. annua* are difficult to separate from either parent, the parents are quite distinct within the bounds of variation seen in *P.* sect. *Micrantherae*. Koshy (1968), however, concluded that the karyotypes of the putative parents did not support this parentage, and suggested the involvement of a third, possibly extinct, species. In contrast to karyological evidence, isozyme profiles in artificially produced sterile diploid hybrids between the two putative parents (produced by open pollination in a greenhouse), which looked like *P. annua*, showed additive properties of bands matching those found in *P. annua*, along with some additional bands not found

in *P. annua* (Darmency and Gasquez 1997). Furthermore, *P. annua* did not have isozyme bands that were absent in the putative parents. In plastid DNA analyses *P. annua* was consistently the sister taxon to *P. supina*, but these two species were separated from each other by rather long branches (Gillespie and Soreng 2005; Gillespie et al. 2007; see also Patterson et al. 2005). This is the first time *P. infirma* has been included in a phylogenetic analysis with the previous two taxa.

In Fig. 2, *Poa annua* plastid samples are sisters to *P. infirma*, and ITS samples are sisters to *P. supina*, with strong support in each case. In fact, the ITS sequences of *P. annua* differ by only 1 to 3 steps from *P. supina*. The plastid *TLF* sequences of *P. annua* are identical to one another, and align between two samples of *P. infirma* that differ from one another by 16 steps. *Poa infirma* and *P. supina TLF* and ITS genomes differ from their most recent common ancestor by a minimum of 31 and 22 steps, respectively, and from each other by 53-67 steps. Relative to other taxa in these data sets, this is a huge difference between presumed closely related species! It appears as though species of the section are evolving at an exceptionally high rate. Even sect. *Micrantherae* differs from its most recent common ancestor with sect. *Alpinae* by 41-42 steps. Collectively, the Mm elements are on the longest branch within *Poa* in the trees.

Thus, their genomes are designated as follows: $M^1M^1$for *P. infirma*, $M^2M^2$for *P. supina*, and $M^1M^2$for *P. annua*. The combination of artificially produced hybrids, isozyme data, and our DNA data, provides solid support for *P. annua* having a hybrid origin of the parentage proposed by Nannfeldt (1935, 1937) and Tutin (1957). We classify *P. annua* as a segmental allopolyploid (Stebbins 1950), as the parents are easily recognized as closely related, and distinguished subtly, though completely, by morphological and life-history traits. This suggests a different explanation for Koshy's result: that the *P. annua* karyotype differentiated after the original hybridization event.

Poa abbreviata: The origin of *Poa abbreviata*, as far as we know, has not been proposed by anyone. Twelve chromosome counts of $2n=42$ suggest that it is primarily hexaploid (one tetraploid count remains unverified, and one decaploid count represents an unrelated species). This species has not been suspected to be derived from wide parentage. It was consistently placed in a section with morphologically and ecogeographically similar diploids before molecular data became available (Tzvelev 1976; Probatova 1985; Soreng 1985), and by plastid data for nine or more collections (Soreng 1990; Gillespie et al. 1997, 2007; Gillespie and Boles 2001; Gillespie and Soreng 2005). However, it does have an ITS sequence like that of a species from a distantly related section.

Poa abbreviata aligns in the plastid S subclade with the two consectional diploids, *P. lettermanii* and *P. pseudoabbreviata* (both with Ss genomes), so it has an S plastid type (Fig. 2). It was moderately supported as more closely related to *P. lettermanii* than to *P. pseudoabbreviata*. However, whereas the placements of the diploid taxa are consistent between plastid and ITS trees (Ss genotypes),

the three *P. abbreviata* ITS samples that were tested aligned within *P.* subg. *Poa* supersect. *Homalopoa* (Hh genotypes). This placement was detected in a separate ITS study (Nosov and Rodionov 2008), and in a plastid and ITS study focused on the genus *Dupontia* (Brysting et al. 2004), using one of our *P. abbreviata* samples (*Gillespie 5816*). The ITS analysis places *P. abbreviata* as sister to *P. porsildii* (even in expanded analyses of taxa from supersect. *Homalopoa*, unpublished). ITS sequences of *P. abbreviata* and *P. porsildii* differ by 1 step, and *P. porsildii* is separated from its most recent common ancestor with any other taxon in Analysis II by 7 steps (5 *TLF* and 2 ITS steps, even in expanded analyses, Gillespie et al. unpublished). In Analysis II, there are nine supported clades between the plastid and ITS placements of *P. abbreviata*. Therefore, the genome constitution of *P. abbreviata* is designated as Sh. It is improbable that *P. abbreviata* could have retained, from an ancient polyploidization event, an ITS sequence that is paralogous but nearly identical to that of *P. porsildii*. Thus, we conclude that *P. abbreviata* is actually a modern hybrid in origin. *Poa porsildii* is morphologically isolated from *P. abbreviata* (Soreng 2007). It is endemic to the mountains around the Mackenzie Ice Free Corridor, which is the geographical center of *P.* sect. *Abbreviatae* taxa in North America, and as such it could have served as the H parent of the circumarctic, but mainly nearctic, *P. abbreviata*. Further study is needed to determine the extent of the genetic contribution of the H ITS donor to hexaploid *P. abbreviata*.

Poa trivialis: *Poa trivialis* is a morphologically isolated species from Europe and western Asia and northern Africa. It has been maintained in a separate section for over a hundred years (sect. *Pandemos* Asch. & Graebn. [syn. *Coenopoa* Hyl.]: Ascherson and Graebner 1898-1902; Hylander 1953; Tzvelev 1976; Edmondson 1980; Soreng 1998, 2007; Zhu et al. 2006). The section is currently classified within subg. *Stenopoa* (Zhu et al. 2006; Soreng 2007). More than 80 chromosome counts indicate that it is diploid. Although two tetraploid counts have been reported in the literature, one of these was for a separate subspecies (subsp. *sylvicola* [Guss.] H. Lindb.). We are not aware of any suggestion in the literature that *P. trivialis* had a hybrid origin. In a genome analysis by Patterson et al. (2005), *P. trivialis* behaved as a diploid with no inter- or intra-locus variation, in contrast to the multiple paralogues detected in several high polyploid taxa. Those authors placed *P. trivialis*, in separate plastid and nuclear gene (thioredoxin-like protein) analyses, in a position consistent with it having an Ss genome, but a second nuclear gene (CDX504) placed *P. trivialis* in a position somewhat remote from other elements that we would interpret as having Ss or Oo major genotypes.

In Analysis II, *Poa trivialis* plastid samples aligned in the S clade, and ITS samples aligned in the O clade (Fig. 2). There are seven intervening branches between the plastid and ITS placements of *P. trivialis*, all but one of them with good to strong BS support. Neither the plastid nor the ITS genome of *P. trivialis* matches any other generalized genome that has been identified.

The plastid genome consistently places *P. trivialis* as sister to elements with Ss genomes (Soreng 1990; Gillespie and Soreng 2005; and here with strong BS support), and the independence of these sister groups has very good (Gillespie and Soreng 2005) to strong BS support (here). The ITS sequences of *P. trivialis* place the species well within the O clade with moderate and good BS support (Fig. 2), and these differ from those that occur in any most recent common ancestor among Oo genome sections (Aa, Mm, or Rr) by 21-28 bps. Therefore, we designate *P. trivialis* as having its own generalized genome, Vv. Our plastid and ITS data suggest that it may have arisen as a diploid hybrid derived from elements in the S and O clades. However, the addition of ETS data and the results of a combined plastid and nrDNA analysis suggest an alternative possibility (Gillespie et al. 2009). Long branch attractions (Felsenstein 1978) in the nexus between the O, E, P, N and S clades may have resulted in a spurious association of N and S clades as sister to the P clade. Gillespie et al. (2009) provide new evidence (very good to strong BS support) that Ss and Vv genomes may be sister groups, and that the N clade is more closely related to the P clade than to the S clade: (Y(O((SV)(E(NP))))). Further study is needed to confirm the real topology in this nexus. For now the section *Pandemos* is retained in subg. *Stenopoa*, as previously proposed (Table 1), and *P. trivialis* is not accepted as a hybrid.

Conclusions—Phylogenetic analysis of combined plastid *TLF* and ITS DNA sequences for *Poa* diploids in isolation from most polyploids revealed a well supported phylogenetic structure in the genus. This structure was consistent with that resolved in previous analyses with few diploids and numerous polyploids using separate or combined plastid and ITS data. Based on strongly supported differences in their placements by plastid and ITS data, two polyploid taxa were shown to have hybrid origins. Our data point to a precise parentage for *P. annua* (2*n*=28), and a likely parentage for *P. abbreviata* (2*n*=42). The placements of a diploid taxon with statistically incongruent plastid and ITS data, *P. trivialis*, are more likely attributable to long branch attraction, resulting from remote origins and/or rapid rates of evolution, than to a hybrid origin. A notational system for identifying *Poa* plastid genomes and ITS lineages is introduced to replace the previously applied clade acronym system. This simplified and facilitated discussions of major and minor genome types in individuals, species, higher groupings, and genomic parentage within hybrids and lineages possibly derived from hybridization.

acknowledgements. We gratefully acknowledge the following people for providing leaf samples: Marina Olonova, Sierra Stoneberg-Holt, Pilar Catalán, Stoney Wright. Field work by Soreng was partially funded by the USDA and the Smithsonian. The molecular and field research by Gillespie was funded by grants from the Canadian Museum of Nature.

Literature Cited

Álvarez, I. and J. F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417-434.

Ascherson, P. and P. Graebner. 1898-1902. *Synopsis der Mitteleuropäischen Flora. 2(1). Familie 22. Grámina*. Leipzig: W. Engelmann.

Bailey, C. D., T. G. Carr, S. A. Harris, and C. E. Hughes. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. *Molecular Phylogenetics and Evolution* 29: 435-455.

Bowden, W. M. 1961. Chromosome numbers and taxonomic notes on northern grasses. IV. Tribe Festuceae: *Poa* and *Puccinellia*. *Canadian Journal of Botany* 39: 123-138.

Brysting, A. K., A. Holst-Jensen, and I. Leitch. 2000. Genomic origin and organization of the hybrid *Poa jemtlandica* (Poaceae) verified by genomic in situ hybridization and chloroplast DNA sequences. *Annals of Botany* 85: 439-445.

Brysting, A. K., M. F. Fay, I. J. Leitch, and S. G. Aiken. 2004. One or more species in the arctic grass genus *Dupontia*? – a contribution to the Panarctic Flora project. *Taxon* 53: 365-382.

Catalán, P., P. Torrecilla, J. Á. López Rodríguez, and R. G. Olmstead. 2004. Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and *trnL-F* sequences. *Molecular Phylogenetics and Evolution* 31: 517-541.

Cave, M. S. (ed.). 1959. *Index to Plant Chromosome Numbers for 1956-1957*. 1(1-2 and Supplement Prior to 1956). Berkeley, California: California Botanical Society.

Cave, M. S. (ed.). 1965. *Index to Plant Chromosome Numbers for 1958-1964*. 1(3-4), 2(5-9). Chapel Hill, North Carolina, U.S.A.: University of North Carolina Press.

Charmet, G., C. Ravel, and F. Balfourier. 1997. Phylogenetic analysis in the *Festuca-Lolium* complex using molecular markers and ITS rDNA. *Theoretical and Applied Genetics* 94: 1038-1046.

Clayton, W. D. and S. A. Renvoize. 1986. *Genera Graminum. Grasses of the world*. London: Her Majesty's Stationery Office.

Darlington, C. D. and A. P. Wylie (eds). 1955. *Chromosome atlas of flowering plants*. London: George Allen and Unwin.

Darmency, H. and J. Gasquez. 1997. Spontaneous hybridization of the putative ancestors of the allotetraploid *Poa annua*. *New Phytologist* 136: 497-501

Dawson, M. I. 2000. Index of chromosome numbers of indigenous New Zealand spermatophytes. *New Zealand Journal of Botany* 38: 47-150.

DeBry, R. W. and R. G. Olmstead. 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. *Systematic Biology* 49: 171-179.

Dewey, D. R. 1984. The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. Pp. 209-279 in *Gene manipulation in plant improvement*, ed. J. P. Gustafson. New York: Plenum Publishing Corporation.

Doust, A. N., A. M. Penly, S. W. L. Jacobs, and E. A. Kellogg. 2007. Congruence, conflict, and polyploidization shown by nuclear and chloroplast markers in the monophyletic 'bristle clade' (Paniceae, Panicoideae, Poaceae). *Systematic Botany* 32: 531-544.

Duckert-Henriod, M. M. and C. Favarger. 1987. Contribution à la cytotaxonomie et à la cytogéographie des *Poa* de la Suisse, index des nombres chromosomiques des

Spermatophytes de la Suisse: II. Poaceae, genere *Poa*. *Mémoires de al Société Helvétique des Sciences Naturelles* 100.

Edmondson, J. R. 1980. *Poa* L. Pp. 159-167 in *Flora Europaea*, vol. 5, eds. T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb. Cambridge, UK: Cambridge University Press.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1995. Constructing a significance test for incongruence. *Systematic Biology* 44: 570-572.

Fedorov, A. A. (ed.). 1969. *Chromosome numbers of flowering plants*. Leningrad: Academy of Natural Sciences of the USSR.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. *Systematic Zoology* 27: 401-410.

Gaut, B. S., L. P. Tredway, C. Kubik, R. L. Gaut, and W. Meyer. 2000. Phylogenetic relationships and genetic diversity among members of the *Festuca-Lolium* complex (Poaceae) based on ITS sequence data. *Plant Systematics and Evolution* 224: 33-53.

Gillespie, L. J. and R. Boles. 2001. Phylogenetic relationships and infraspecific variation in Canadian Arctic *Poa* based on chloroplast DNA restriction site data. *Canadian Journal of Botany* 79: 679-701.

Gillespie, L. J. and R. J. Soreng. 2005. A phylogenetic analysis of the bluegrass genus *Poa* L. (Poaceae) based on cpDNA restriction site data. *Systematic Botany* 30: 84-105.

Gillespie, L. J., L. L. Consaul, and S. G. Aiken. 1997. Hybridization and the origin of the arctic grass *Poa hartzii* (Poaceae): evidence from morphology and chloroplast DNA restriction site data. *Canadian Journal of Botany* 75: 1978-1997.

Gillespie, L. J., A. Archambault, and R. J. Soreng. 2007. Phylogeny of *Poa* (Poaceae) based on *trnT-trnF* sequence data: major clades and basal relationships. *Aliso* 23: 420-434.

Gillespie, L. J., R. J. Soreng, R. D. Bull, S. W. L. Jacobs, and N. F. Refulio-Rodriguez. 2008. Phylogenetic relationships in subtribe Poinae (Poaceae, Poeae) based on nuclear ITS and plastid *trnT-trnL-trnF* sequences. *Botany* 86: 938-967.

Gillespie, L. J., R. J. Soreng, and S. W. L. Jacobs (2009) Phylogenetic relationships of Australian *Poa* (Poaceae: Poinae), molecular evidence for two new genera, *Saxipoa* and *Sylvipoa*. *Australian Systematic Botany* 22: 413–436.

Gillespie, L. J., R. J. Soreng, M. Paradis, and R. D. Bull. 2010. Phylogeny and Reticulation in Subtribe Poinae and Related Subtribes (Poaceae) Based on nrITS, ETS, and trnTLF data. Pp. 589-617 in *Diversity, Phylogeny, and Evolution – Proceedings of the Fourth International Conference on the Comparative Biology of the Monocotyledons and the Fifth International Symposium on Grass Systematics and Evolution*, eds. O. Seberg, G. Petersen, A. S. Barfod, and J. I. Davis. Aarhus, Aarhus University Press.

Goldblatt, P. (ed.). 1981. Index to plant chromosome numbers for 1975-1978. *Monographs in Systematic Botany*, *Missouri Botanical Garden* 5. St. Louis: Missouri Botanical Garden.

Goldblatt, P. (ed.). 1984. Index to plant chromosome numbers for 1979-1981. *Monographs in Systematic Botany*, *Missouri Botanical Garden* 8. St. Louis: Missouri Botanical Garden.

Goldblatt, P. (ed.). 1985. Index to plant chromosome numbers for 1982-1983. *Monographs in Systematic Botany*, *Missouri Botanical Garden* 13. St. Louis: Missouri Botanical Garden.

Goldblatt, P. (ed.). *Index to plant chromosome numbers records from 1984 forward*, mobot. mobot.org/W3T/Search/vast.html.

Hair, J. B. 1968. Contributions to a chromosome atlas of the New Zealand. *New Zealand Journal of Botany* 6: 267-276.

Holmgren, P. K, N. H. Holmgren, and L. C. Barnett. 1990. *Index Herbariorum. 1. the herbaria of the world*, ed. 8. Bronx: New York Botanical Garden.

Hylander, N. 1953. Taxa et nomina nova in opere meo: Nordisk Kärlväxtflora I. *Botaniska Notiser* 1953: 352-259.

Kellogg, E. A., R. Appels, and R. J. Mason-Gamer. 1996. When genes tell different stories: the diploid genera of Triticeae. *Systematic Botany* 21: 321-347.

Koshy, T. K. 1968. Evolutionary origin of *Poa annua* L. in the light of karyotypic studies. *Canadian Journal of Genetics and Cytology* 10: 112-118.

Löve, Á, and D. Löve. 1975. *Cytotaxonomical atlas of the Arctic flora*. Vaduz: J. Cramer.

Moore, D. M (ed.). 1982. *Flora Europaea check-list and chromosome index*. Cambridge, U.K.: Cambridge University Press.

Moore, R. J. (ed.). 1970. Index to plant chromosome numbers for 1968. *Regnum Vegetabile* 68: 1-115.

Moore, R. J. (ed.). 1971. Index to plant chromosome numbers for 1969. *Regnum Vegetabile* 77: 1-112.

Moore, R. J. (ed.). 1972. Index to plant chromosome numbers for 1970. *Regnum Vegetabile* 84: 1-134.

Moore, R. J. (ed.). 1973. Index to plant chromosome numbers for 1967-1971. *Regnum Vegetabile* 90: 1-539.

Moore, R. J. (ed.). 1974. Index to plant chromosome numbers for 1972. *Regnum Vegetabile* 91: 1-108.

Moore, R. J. (ed.). 1977. Index to plant chromosome numbers for 1973/74. *Regnum Vegetabile* 96: 1-257.

Nannfeldt, J. A. 1935. *Poa supina* Schrad. i Sverige och dess hittills förbisedda hybrid med *P. annua* L. *Botaniska Notiser* 1935: 1-16.

Nannfeldt, J. A. 1937. The chromosome numbers of *Poa* sect. *Ochlopoa* A. & Gr. and their taxonomical significance. *Botaniska Notiser* 1937: 238-254.

Nosov, N. N., and A. V. Rodionov. 2008. Molecular phylogenetic study of relationships between members of the genus *Poa*. *Botanicheskii Zhurnal* 93: 1919-1935.

Olonova, M. V. 1990. *Poa* L. Pp. 163-186 in *Flora Siberia*, vol. 2. *Poaceae (Gramineae)*, eds. L. I. Malyschev and G. A. Peschkova. Novosibirsk, Russia: Nauka. [English translation: 2001. Enfield, New Hampshire, U.S.A.: Science Publishers Incorporated.]

Ornduff, R. (ed.). 1967. Index to plant chromosome numbers for 1965. *Regnum Vegetabile* 50: 1-129.

Ornduff, R. (ed.). 1968. Index to plant chromosome numbers for 1966. *Regnum Vegetabile* 55: 1-126.

Ornduff, R. (ed.). 1969. Index to plant chromosome numbers for 1967. *Regnum Vegetabile* 59: 1-129.

Patterson, J. T., S. R. Larson, and P. G. Johnson. 2005. Genome relationships in polyploid *Poa pratensis* and other *Poa* species inferred from phylogenetic analysis of nuclear and chloroplast DNA sequences. *Genome* 48: 76-87.

Probatova, N. S. 1985. *Poa* L. Pp. 263-303 in *Sosudistyle rasteniya Sovetskogo dal'nego vostoka*, vol. 1., ed. S. S. Kharkevich, vol. ed. N. N. Tzvelev. Leningrad, Russia: Nauka. [English translation: 2003. *Vascular plants of the Russian Far East*, vol. 1. Enfield, New Hampshire, U.S.A.: Enfield]

Soreng, R. J. 1985. *Poa* L. in New Mexico, with a key to middle and southern Rocky Mountain species (Poaceae). *Great Basin Naturalist* 45: 395-422.

Soreng, R. J. 1990. Chloroplast DNA phylogenetics and biogeography in a reticulating group: study in *Poa* (Poaceae). *American Journal Botany* 77: 1383-1400.

Soreng, R. J. 1998. An infrageneric classification for *Poa* in North America, and other notes on sections, species, and subspecies of *Poa*, *Puccinellia*, and *Dissanthelium* (Poaceae: Poeae). *Novon* 8: 187-202.

Soreng, R. J. 2005. Miscellaneous chromosome number reports for *Poa* L. (Poaceae) in North America. *Sida* 21: 2195-2203.

Soreng, R. J. 2007. *Poa* L. Pp. 486-601 in *Flora of North America*, *vol. 24*, *Magnoliophyta: Commelinideae (in part)*; *Poaceae*, *part 1*, eds. Flora of North America Editorial Committee; vol. eds. M. E. Barkworth, K. M. Capels, S. L. Long, and M. B. Piep. New York: Oxford University Press.

Soreng, R. J. and L. J. Gillespie. 2007. *Nicoraepoa* (Poaceae, Poeae), a new South American genus based on *Poa* subgenus *Andinae*, and emendation of *Poa* section *Parodiochloa* of the sub-Antarctic islands. *Annals of the Missouri Botanical Garden* 94: 821-849.

Soreng, R. J., L. M. Giussani, and M. Negritto. 2003. *Poa* L. Pp. 505–580 in *Catalogue of New World grasses (Poaceae): IV. Subfamily Pooideae*, eds. R. J. Soreng, P. M. Peterson, G. Davidse, E. J. Judziewicz, F. O. Zuloaga, T. S. Filgueiras, and O. Morrone. *Contributions from the United States National Herbarium* 48: 1–730.

Soreng, R. J., J. I. Davis, and M. A. Voionmaa. 2007. A phylogenetic analysis of Poaceae tribe Poeae sensu lato based on morphological characters and sequence data from three plastid-encoded genes: evidence for reticulation, and a new classification for the tribe. *Kew Bulletin* 62: 425-454.

Starr, J. R., S. A. Harris, and D. A. Simpson. 2004. Phylogeny of the unispicate taxa in Cyperaceae tribe Cariceae I: Generic relationships and evolutionary scenarios. *Systematic Botany* 29: 528-544

Stebbins, G. L. 1950. *Variation and evolution in plants*. New York: Columbia University Press.

Stebbins, G. L. 1956. Cytogenetics and evolution of the grass family. *American Journal of Botany* 43: 890-905.

Stoneberg-Holt, S. D., L. Horová, and P. Bureš. 2004. Indel patterns of the plastid DNA *trnL-trnF* region within the genus *Poa* (Poaceae). *Journal of Plant Research* 1117: 393-407.

Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4b10. Sunderland: Sinauer.

Tateoka, T. 1985. Chromosome numbers and their taxonomic implications in the genus *Poa* of Japan. *Botanical Magazine (Tokyo)* 98: 413-437.

Torrecilla, P. and P. Catalán. 2002. Phylogeny of broad-leaved and fine-leaved *Festuca* lineages (Poaceae) based on nuclear ITS sequences. *Systematic Botany* 27: 241-251.

Tutin, T. G. 1957. A contribution to the experimental taxonomy of *Poa annua* L. *Watsonia* 4: 1-10.

Tzvelev, N. N. 1976. *Zlaki SSSR*. Leningrad: Nauka Publishers. [English translation: 1983. *Grasses of the Soviet Union*, vol. 1 and 2. New Delhi: Amerind Publishing Company].

Zhu, G.-H., L. Liu, R. J. Soreng, and M. V. Olonova. 2006. *Poa* L. Pp. 257-309 in *Flora of China*. *vol. 22*, *Poaceae*, eds. Flora of China Editorial Committee. Saint Louis, Missouri, U.S.A.: Missouri Botanical Garden Press.