

## Phylogenetics of Stipeae (Poaceae: Pooideae) Based on Plastid and Nuclear DNA Sequences

Konstantin Romaschenko,<sup>1</sup> Paul M. Peterson,<sup>2</sup> Robert J. Soreng,<sup>2</sup>  
Núria Garcia-Jacas,<sup>3</sup> and Alfonso Susanna<sup>3</sup>

<sup>1</sup>M. G. Kholodny Institute of Botany,  
Tereshchenkivska 2, 01601 Kiev, Ukraine

<sup>2</sup>Smithsonian Institution, Department of Botany MRC-166,  
National Museum of Natural History, P.O. Box 37012, Washington,  
District of Columbia 20013-7012 USA.

<sup>3</sup>Laboratory of Molecular Systematics, Botanic Institute of Barcelona  
(CSIC-ICUB), Pg. del Migdia, s.n., E08038 Barcelona, Spain

*Author for correspondence (kromaschenko@gmail.com)*

**Abstract**—The Stipeae tribe is a group of 400–600 grass species of worldwide distribution that are currently placed in 21 genera. The ‘needlegrasses’ are characterized by having single-flowered spikelets and stout, terminally-awned lemmas. We conducted a molecular phylogenetic study of the Stipeae (including all genera except *Anemanthele*) using a total of 94 species (nine species were used as outgroups) based on five plastid DNA regions (*trnK-5’matK*, *matK*, *trnH<sup>GUG</sup>-psbA*, *trnL5’-trnF*, and *ndhF*) and a single nuclear DNA region (ITS). Our parsimony analysis of DNA sequences supports: the monophyly of the Stipeae including *Macrochloa* as sister to all other Stipeae; the removal of *Lorenzochloa erectifolia* from *Ortachne* since it does not align with *Ortachne* but with some species of *Anatherostipa*; *Ptilagrostis* as occurring in two separate clades; and *Oryzopsis asperifolia* as a monotypic genus. *Achnatherum* and *Piptatherum* as currently circumscribed are polyphyletic and we provide good support to split the former into four groups and the latter into three groups. *Achnatherum* s.s., *Aciachne*, *Amelichloa*, *Austrostipa*, *Hesperostipa*, *Jarava* s.s., *Ortachne*, *Pappostipa*, *Piptatherum* s.s., *Piptochaetium*, *Ptilagrostis* s.s., *Stipa* s.s., and *Trikeriaia* are all well supported as monophyletic genera. *Stipa capensis* and *S. parviflora* both of African distribution are apparently misplaced and are not closely related to other species of *Stipa* s.s. but are allied to achnatheroids. Based on molecular evolution of plastid sequences and lemma epidermal pattern we present a stepwise model for the

evolution of the Stipeae with two initial deep bifurcations followed by two further bifurcations that are geographically consistent. The segregation of new groups or clades tentatively called: *Eriocoma*, *Nasselloid*, 'Neotrinia', 'Piptatheropsis', and *Timouria*, require further study.

**Keywords**—Classification, grasses, ITS, phylogeny, plastid DNA, taxonomy.

The tribe Stipeae Dumort. consists of temperate, cool-season ( $C_3$ ) grasses that are widespread, mostly tussock-forming grasses, of predominantly open grasslands in temperate and warm temperate regions. They are characterized by having single-flowered spikelets without rachilla extensions, terminally-awned lemmas where the awn is the result of fusion between the central and two lateral vascular traces, florets with three, rarely two, linear lodicules that are slightly indurate at maturity, usually with the lemma concealing the palea (if the palea is exposed when the floret is closed, then the palea is coriaceous), and small-sized chromosomes with a base number  $x = 10-12$  (Tzvelev 1989). The tribe comprises approximately 400 to 600 species; the number depending on how finely the Asian taxa are divided. The Stipeae are placed in subfamily Pooideae (Grass Phylogeny Working Group (GPWG) 2001). Within Pooideae the Stipeae is an early diverging lineage that arose after the separation of the Brachyelytreae Ohwi, Lygeae J. Presl, and Nardeae W.D.J. Koch (Davis and Soreng 2007; Soreng et al. 2007) from the remainder of tribes.

There are 21 accepted genera of Stipeae (Soreng et al. 2003, 2008) – *Achnatherum* P. Beauv., *Aciachne* Benth., *Ampelodesmos* Link, *Amelichloa* Arriaga and Barkworth, *Anemanthele* Veldkamp, *Austrostipa* S.W.L. Jacobs and J. Everett, *Celtica* F.M. Vázquez and Barkworth, *Hesperostipa* (M.K. Elias) Barkworth, *Jarava* Ruiz and Pav., *Macrochloa* Kunth, *Nassella* (Trin.), *Ortachne* Nees ex Steud., *Orthoraphium* Nees, *Oryzopsis* Michx., *Pappostipa* (Speg.) Romasch., P.M. Peterson and Soreng, *Piptatherum* P. Beauv., *Piptochaetium* J. Presl, *Psammochloa* Hitchc., *Ptilagrostis* Griseb., *Stipa* L., and *Trikeriaia* Bor. The above 20 genera, minus *Ampelodesmos* (which when included in Stipeae is placed in subtribe Ampelodesminae Conert), represent 'core Stipeae'. In addition, subtribe Duthieinae Pilg. ex Potztl is sometimes placed in or lumped into Aveneae Dumort., Stipeae, or Phaenospermateae Renvoize and Clayton, including in its broadest sense: *Anisopogon* R.Br., *Danthoniastrum* (Holub) Holub, *Megelachne* Steud., *Metcalfia* Conert, *Podophorus* Phil., *Pseudodanthonia* Bor and C.E. Hubb., *Sinochasea* Keng, *Duthiea* Hack. and *Stephanachne* Keng (Clayton and Renvoize 1986; Soreng et al. 2003, 2008; Wu and Phillips 2006).

Two monotypic genera, *Timouria* Roshev. and *Lorenzochloa* Reeder and C. Reeder, are currently attributed to *Achnatherum* and *Ortachne*, respectively (Clayton and Renvoize 1986; Wu and Phillips 2006). Generic boundaries among the genera in the Stipeae are problematic, especially among those named in the 18<sup>th</sup> and 19<sup>th</sup> centuries. Delimitation among these led many 20<sup>th</sup> century agros-

tologists to adopt a broad concept of the genus *Stipa* to encompass all of the currently accepted genera except *Oryzopsis*, *Aciachne*, and *Piptochaetium* in the New World (Spegazzini 1901; Hitchcock 1935, 1951) and *Piptatherum* in the Old World (Freitag 1975, 1985). New studies were performed to describe new genera, emend generic limits, or present novel relationships (Parodi 1947, 1960; Rojas 1997; Barkworth 1983, 1990, 1993; Jacobs and Everett 1996; Peñailillo 1996, 2002, 2003; 2005; Torres 1997; Barkworth and Torres 2001; Cialdella and Giussani 2002; Vázquez and Barkworth 2004; Arriaga and Barkworth 2006; Cialdella et al. 2007; Romaschenko et al. 2008).

Phylogenetic inferences for Stipeae are scarce. Based on the morphological features the most comprehensive review was made by Tzvelev (1977) where phylogenetic weight was assigned to such characters as shape of the lemma and callus, and development of awn indumentum. Tzvelev (1977) reported 16 genera of Stipeae including such genera as *Eriocoma* Nutt. (currently attributed to *Achnatherum*), *Parodiella* Reeder and C. Reeder ( $\equiv$  *Lorenzochloa* which is commonly placed in *Ortachne*), *Stephanachne* (as *S. nigrescens* Keng), *Pappagrostis* Roshev. [as *Stephanachne pappophorea* (Hack.) Keng], and *Streptachne* R.Br. (= *Aristida*). Tzvelev's suggested there were two major lineages: 1) *Stipa* s. s. (with long lanceolate lemmas, awns having strongly developed indumentum, and sharp calluses) and; 2) *Piptatherum* (characterized by short lemmas, short, hairless, caducous awns and blunt calluses). The *Piptatherum* lineage was thought to have originated from more primitive *Achnatherum*-like species, whereas *Ptilagrostis* and *A. chinense* (Hitchc.) Tzvel. were considered to be intermediate taxa between *Achnatherum* and *Stipa* s. s., and *Achnatherum* and *Piptatherum*, respectively. *Piptochaetium* and *Nassella* were considered to be close relatives and *Eriocoma* was thought to be a New World vicariant of *Piptatherum*.

Thomasson (1978, 1980, 1981, 1982, 1985) was first to document the phylogenetic importance of the lemma epidermal pattern in the Stipeae. Barkworth and Everett (1987) used this information extensively and pointed out that *Stipa* and *Piptatherum* have elongated lemma epidermal cells with sinuous lateral walls and that *Achnatherum* and *Austrostipa* have short lemma epidermal cells with slightly sinuous to straight lateral walls. Barkworth and Everett (1987) followed Tzvelev (1977) and used the shape of the lemma and callus and development of awn indumentums and therefore postulated a similar phylogenetic scheme. *Hesperostipa* and the 'Obtusa group' of Parodi (1946; = *Anatherostipa*), and *Nassella* and *Piptochaetium*, were thought to be two pairs of closely related genera. In a series of morphological studies (Barkworth 1990, 1993; Barkworth and Torres 2001; Cialdella and Guissani 2002; Thomasson 1978, 1982) and a phylogenetic study with a molecular analysis (Jacobs et al. 2000), it was suggested that *Nassella* and *Piptochaetium* shared a most recent common ancestor [i.e., to be sister genera]. In more recent molecular phylogenetic analyses it was suggested that the *Piptatherum*/*Oryzopsis* complex along with *Stipa* s. s. and *Hesperostipa* were among early diverging lineages; *Austrostipa* was shown as a member of a derived

clade (Jacobs et al. 2007), and *Nassella* was more closely related to *Jarava* than to *Piptochaetium* (Cialdella et al. 2007).

The relationships between the Stipeae and the Phaenospermateae-Duthieinae-*Anisopogon* complex were tested in a *matK* (Döring et al. 2007) and ITS analysis (Romaschenko et al. 2007). In the latter analysis the poorly supported clade including *Anisopogon*, *Danthoniastrum*, *Duthiea*, *Phaenosperma*, and *Sinobasea* was recovered as sister clade to the Stipeae. Since the representatives of Meliceae Link ex Endl. and Diarrheneae (Ohwi) C.S. Campb. were not included in Romaschenko et al. (2007) analysis, no conclusion was suggested regarding their phylogenetic relationships. In Döring et al. (2007) a trichotomy was formed among members of the Meliceae, Stipeae, and a clade with *Anisopogon*, *Danthoniastrum*, *Duthiea*, *Phaenosperma*, and *Sinobasea*.

The main objective of the present paper is to provide a phylogenetic hypothesis for all the currently accepted genera within the core Stipeae using plastid (*trnK-5'matK*, *matK*, *trnH<sup>GUG</sup>-psbA*, *trnL5'-trnF*, and *ndbF*) and nuclear ribosomal DNA ITS sequences. In addition, we hope to elucidate generic boundaries within the Stipeae and resolve questions regarding these lineages within the tribe.

## Materials and Methods

**Taxon Sampling**—Samples were chosen to represent the taxonomic diversity in the Stipeae. The sample set consists of 94 accessions/species (Appendix 1) representing all accepted genera (except *Anemantbele*) in the Stipeae (Soreng et al. 2003) as well as major intergeneric groups within the polyphyletic genera detected in our previous study (Romaschenko et al. 2008). We sampled evenly from both American and Eurasian groups of *Achnatherum* and *Piptatherum*, and included the type species for all groups. We included South American: *Aciachne*, *Lorenzochloa* (currently included in *Ortachne*), and *Ortachne*; North American *Oryzopsis*; Asian *Trikeriaia*, *Psammochloa*, and *Timouria* (as part of *Achnatherum*); monotypic Mediterranean: *Ampelodesmos*, *Celtica*, and *Macrochloa*; and Eurasian: *Stipa*. We also included the genera *Anisopogon*, *Duthiea*, *Phaenosperma*, *Sinobasea*, and *Danthoniastrum*, some of which were occasionally attributed to or thought to be related to the Stipeae (Avdulov 1931; Tzvelev 1977; Wu and Phillips 2006). In order to outline the limits of the tribe and its relationships to other tribes we included *Brylkinia caudata* (Brylkinieae Tateoka) and *Diarrhena obovata* (Diarrheneae). *Brachyelytrum erectum* (Brachyelytreae Ohwi) and *Nardus stricta* (Lygeaeae J. Presl) were chosen as outgroups based on previous studies of the *Pooideae* (Hilu et al. 1999; Soreng and Davis 2000; GPWG 2001; Davis and Soreng 2007; Soreng et al. 2007).

**DNA Extraction, Amplification, and Sequencing**—Leaf tissue was disrupted and homogenized using Qiagen TissueLyser, and DNA was isolated using a BioSprint 96 DNA Plant Kit (Qiagen, Valencia, California, USA). PCR amplifications were performed in MJ Research or PE 9700 thermal cyclers. Genomic

DNA was combined with 1x reaction buffer (200 mM Tris-HCl, 500 mM NH<sub>4</sub>) [Bioline Biolase Taunton, Madison, USA] without Mg<sup>++</sup>, 2 mM MgCl<sub>2</sub>, 200 mM dNTP's, 1.5µl of Taq polymerase (Bioline Biolase Taunton, Madison, USA), 40 pmol/µl each of forward and reverse primers.

The entire nuclear ribosomal Internal Transcribed Spacer (ITS) region was amplified using primers ITS4 (White et al. 1990) and ITS5A (Stanford et al. 2000) with the following thermocycler settings: initial denaturation step of 4 min at 95°C, followed 35 cycles at 94°C for 30 seconds, 50–56°C for 30 seconds, 72°C for 1 min 30 seconds, and a final extension of 10 min at 72°C.

Five chloroplast DNA regions were sequenced: *trnK-5'matK*, *matK*, *trnH*<sup>GUG</sup>, *psbA*, *trnL5'-trnF*, and the terminal portion of *ndhF* gene. The *trnK-5'matK* portion of *trnK* intron and *5'matK* were amplified separately and this significantly raised the efficiency of amplification. The major part of *trnK-5'matK* (~ 580 pb) was easily amplified using the forward primer *trnK3914F* (Johnson and Soltis 1995) and reverse primer *trnK660SR* (Romaschenko et al. 2008). The set of primers *trnK660SF* and *matK1412SR* (Romaschenko et al. 2008) were used to amplify ~ 560 pb of the 5'-end of *matK*. The *trnH*<sup>GUG</sup>-*psbA* intergenic spacer was amplified with primers *trnH*<sup>GUG</sup> (Tate and Simpson 2003) and *psbA* (Sang et al. 1997). The *trnL-trnF* region (which included a portion of 3'*trnL* intron, the 3'*trnL* exon, and the *trnL-trnF* intergenic spacer) was amplified using primers 5'*trnL*<sup>UAA</sup>(f) and *trnF*<sup>GAA</sup>(c) (Taberlet et al. 1991). Using GeneBank sequences of *ndhF* gene for *Oryza*, *Lolium* and *Triticum* (~ 2230 pb) we designed the set of primers to amplify and sequence the most variable 3' portion of *ndhF* gene. The primer *ndhF1311F*- 5'ACTGCAGGATTAAGTTCGTT'3 was used as forward and primer *ndhF2091R*- 5'GACCCACTCCATTGGTAATTC'3 as reverse to amplify approximately 780 bp of *ndhF* gene. The labelling numbers correspond to the position of the primer according to *Oryza*'s non-aligned sequence of this gene from its 5' end. These primers are positioned close to the 1318R and 2110R primers described by Olmstead and Sweere (1994). The sequence length between 700 and 800 bp was chosen to fit the general condition for routine amplification of all the chloroplast regions used in this study. The region was sequenced using only the forward primer.

The amplification parameters for all chloroplast regions were: 95°C, 4 min; 35 cycles of 94°C for 40 seconds, 51–56°C for 40 seconds, 72°C for 1 min 40 seconds; 72°C for 10 min. PCR products were cleaned with ExoSAP-IT (USB, Cleveland, Ohio, USA). DNA sequencing was performed with BigDye Terminator Cycle Sequencing v.3.1 (PE Applied Biosystems, Foster City, CA, USA) according to the following parameters: 80°C, 5 min; 25 or 30 cycles of 95°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 min. Sequenced products were analyzed on an ABI PRISM 3730 DNA Analyzer 7900HT (ABI).

**Phylogenetic Analyses**— Sequences were aligned manually using BioEdit v.7.0.5.3 (Hall 1999) and are available upon request from KR. Indels and regions where the alignment was considered ambiguous were excluded from analyses.

	<i>trnK- matK</i>	<i>matK</i>	<i>trnH- psbA</i>	<i>trnL- trnF</i>	<i>ndhF</i>	Combined plastid data	ITS
Sequence bp length (aligned)	587	554	662	753	782	3338	639
Sequence bp length (unaligned, average)	564	554	573	649	782	3122	587
Number of taxa	94	93	93	88	90	94	94
Proportion of excluded characters (%)	8.7	–	10.3	10.4	–	5.9	10.3
Proportion of sequenced taxa per region (%)	100	98.9	98.9	93.6	95.7	–	100
Number of parsimony informative characters	46	46	43	59	133	327	197
Number of PICs per sequence (%)	7.8	8.3	6.5	7.8	17	–	30.8
Tree length	92	86	94	123	330	807	845
Consistency index (CI)	0.57	0.69	0.58	0.65	0.55	0.57	0.36
Retention index (RI)	0.87	0.90	0.89	0.87	0.85	0.84	0.76

**Table 1.** Summary of *trnK-matK*, *matK*, *trnH-psbA*, *trnL-trnF*, *ndhF* and ITS regions used in this study.

The amount of excluded data for each region is presented in Table 1. No data were excluded from *matK* and *ndhF*. All gaps were treated as missing data.

We used maximum parsimony and Bayesian analysis to infer phylogeny. Parsimony analysis was performed using PAUP v. 4.obio (Swofford 2000) and PAUPRat v.1b (Sikes and Lewis 2001), which implements the Parsimony Ratchet of Nixon (1999). Parsimony searches were carried out for individual regions, combined plastid, and nuclear regions. In searching for the shortest tree, the heuristic method was used and the tree bisection-reconnection (TBR) branch swapping algorithm was chosen. Character states were specified as unordered and unweighted. PAUPRat searches were set for generating 1001 most parsimonious trees as recommended by the program's developers. All of the most parsimonious trees in our analyses were of the same length. These data sets were used to yield the majority-rule consensus trees. Bootstrap support (BS) for branches in the parsimonious trees was calculated through 'fast-bootstrap' mode which excluded branch swapping. The number of random taxon addition replicates was set at 2,000,000. Branches with bootstrap support values of 90–100% were considered to be relatively strongly supported, 70–89% moderately supported, and 50–69% weakly supported (Mason-Gamer and Kellogg 1997). Branches with 50% or less bootstrap support values were not reported.

Bayesian posterior probabilities were estimated using MrBayes v.3.01 (Huelsenbeck and Ronquist 2001; Ronquist et al. 2005) and the best-available substitution models were selected using MrModeltest 1.1b (Nylander 2002). The symmetrical model with gamma-distributed rate variation across sites

(SYM+I+G) was selected by hierarchical likelihood ratio tests (hLRT) and Akaike information criterion (AIC) for the ITS data set; the plastid data set, MrModeltest indicated that TVMef was the preferred model. Bayesian analysis was initiated with random starting trees with sampling frequency of chains set to every 100<sup>th</sup> iteration. Burn-in function was set at the proportion of one fourth for all runs. Internal nodes with posterior probabilities (PP) 0.95–1.00 were considered statistically significant.

The partition homogeneity test (incongruence length difference, ILD; Farris et al. 1995) implemented in PAUP v. 4.ob10 (Swofford 2000) was carried out to verify the congruence of plastid and nuclear datasets. The *P*-value (adjusted at <0.01) was scored after a 1000 replication run for two established partitions excluding uninformative characters and using heuristic search and a random addition of sequences.

## Results

The total dataset (94 accessions/taxa with no duplicates) comprised 3977 aligned nucleotide positions; 3338 represented plastid data and 639 represented nuclear ribosomal ITS data (Appendix 1, Table 1). The rate of amplification for all regions was consistently high (>90%) even for material from herbarium specimens. The *trnL-F* region had the lowest rate of amplification (88 sequenced taxa out of 94 or 93.6% of the dataset). A similar proportion achieved for the newly sequenced *ndhF* region (90 sequenced taxa or 95.7% of the dataset) reflects the lack of variability in the DNA template for four specimens of *Nassella*. In the *trnL-F*, *trnH-psbA*, and ITS regions 10.3–10.4% of the data were excluded from analysis because of the presence of many indels and consequent ambiguity during alignment. Only 8.7% of the data were excluded from the *trnK-5'matK* intron, which is lowest figure among non-coding regions. No data were excluded from *matK* and *ndhF* gene encoding regions. Therefore the overall rate of excluded data for the combined plastid dataset remained comparatively low (5.9%). The number of parsimony informative characters (PICs) was much greater in the *ndhF* region (133) than all other plastid regions (43–59%). The number of PICs detected for the entire plastid data set (327) greatly exceeds the number of PICs for the ITS data set (197). The density of PICs per sequence length was much higher in ITS (30.8%); nearly double that found in *ndhF* region (17%) and significantly higher than for other plastid regions (6.5–8.3%).

Even though the combined plastid data set was larger than the ITS data set in sequence length and number of PICs, the combined plastid maximum parsimony tree was 38 steps shorter than the ITS maximum parsimony tree. In all separate and combined plastid analyses the values of CI (0.55–0.69) and RI (0.84–0.90), were higher than in the ITS analysis (CI=0.36; RI=0.76; see table 1). The partition homogeneity test (incongruence length difference or ILD) for plastid and nuclear data sets yielded a *P* value <0.002, indicating significant

incongruence between the two topologies, and as a consequence we did not combine the data.

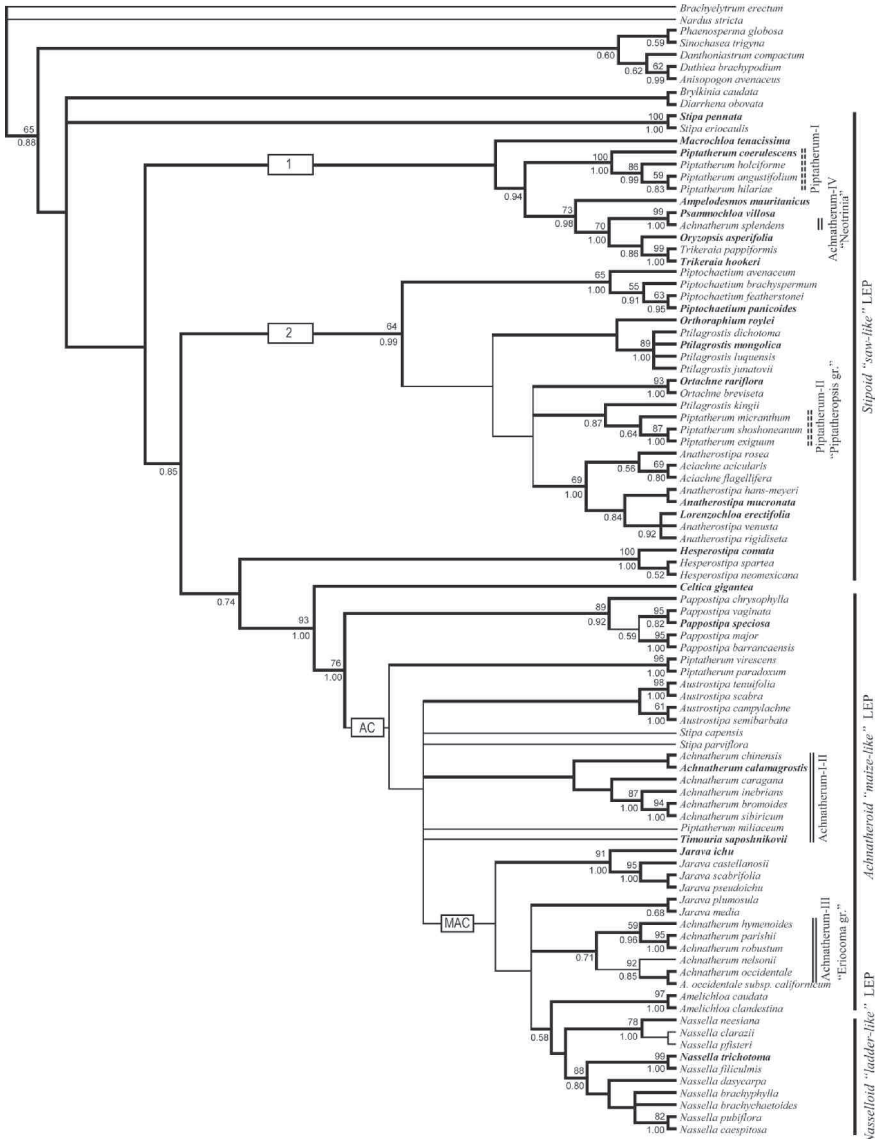
**ITS Analysis**—The ITS phylogenetic tree is poorly resolved with no or poor support for many nodes (Fig. 1). The tree does not support the monophyly of the Stipeae. With *Brachyelytrum* (Brachyelytreae) and *Nardus* (Nardeae) as outgroups, the first bifurcation in the subfamily Pooideae has BS of 65, and PP of 0.88. The branch including *Phaenosperma* (Phaenospermateae) and *Simochasea* (Duthieinae) as sister to *Danthoniastrum* (Duthieinae), *Duthiea* (Duthieinae), and *Anisopogon* (Duthieinae, see Soreng et al. 2008) has a PP of 0.60 but no BS. Sister to this is an unsupported trichotomy of: *Brylkinia* (Brylkinieae) and *Diarrhena* (Diarrheneae) grouped together; a strongly supported clade of *Stipa* s. s., [includes *Stipa pennata* (the type of the genus) and *S. eriocalis*; BS=100, PP=1.00], and; an unsupported clade of all the remaining Stipeae taxa in this study, and no elements of other tribes.

One of the branches at the next bifurcation, clade 1 (see Fig. 1), has no support. In this *Macrochloa tenacissima* is sister to a clade with PP of 0.94. One strongly supported subclade (*Piptatherum* I; BS=100, PP=1.00) encompasses one subset of Asian *Piptatherum* s. s. (including type of the genus, *P. coerulescens*). *Piptatherum* I is sister to a moderately supported (BS=73, PP=0.98) clade of a very diverse group of small and monotypic genera. *Ampelodesmos mauritanicus* (the only species in Stipeae with a spikelet with more than one floret) is a sister taxon to a moderately supported clade (BS=70, PP=1.00), that splits into two subclades: A—*Psammochloa villosa* and *Achnatherum splendens* (BS=99, PP=1.00), and; B—a clade with PP of 0.86 including *Oryzopsis asperifolia* (the only American species in clade 1), as sister to *Trikeriaia hookeri* (= type of the genus) and *T. pappiformis* (BS=99, PP=1.00).

The sister clade to clade 1 (PP=0.85) splits into two weakly supported clades: with clade 2 (BS=64, PP=0.99; see Fig. 1), as sister to the remaining taxa. Within clade 2, the *Piptochaetium* clade is weakly supported (BS=65; PP=1.00) as the sister to the remaining subclades that are only partially resolved in the strict consensus tree. Terminal clades within clade 2 include: 1) — An Asian lineage with *Orthoraphium* as sister to a moderately supported *Ptilagrostis* s. s. clade (BS=89, PP=1.00; including the type of the genus *P. mongolica*); 2) — A strongly supported *Ortachne* s. s. clade (BS=93, PP=1.00) with *O. breviseta* and *O. rariflora*; 3) — an unsupported *Piptatherum* II, ‘*Piptatheropsis* group’ clade (PP=0.87), including *Ptilagrostis kingii* and New World *Piptatherum*; and 4) — a weakly supported clade of *Aciachne*, *Anatherostipa*, and *Lorenzochloa* (BS=69, PP=1.00).

The sister to clade 2 (PP=0.74), includes *Hesperostipa* (strongly supported (BS=100, PP=1.00), as sister to the remaining taxa (BS=93, PP=1.00). The next split places *Celtica gigantea* as sister to the remaining taxa (BS=76, PP=1.00). This is followed by a split between a moderately supported *Pappostipa* clade (BS=89, PP=0.92), as sister to an unsupported Achnatheroid Clade (AC). Structure within AC is poorly resolved, but includes: 1—a strongly supported clade of two species





**Fig. 1.** Majority-rule consensus tree of 1001 equally most parsimonious trees based on maximum parsimony analysis of sequence data from nuclear rDNA ITS region. Branches in bold are also in the strict consensus tree. Numbers above branches correspond to bootstrap (BS) values; numbers below branches correspond to Bayesian posterior probability (PP) values; AC=Achnatheroid Clade; LEP = lemma epidermal pattern; MAC=Major American Clade; *Piptatherum* I (Asian); *Piptatherum* II, '*Piptatheropsis* group' (New World); *Achnatherum* I-II [joint clade of Asian *Achnatherum* I and *Achnatherum* II, '*Timouria* group' resolved in the plastid analysis]; *Achnatherum* III, '*Eriocoma* group' (New World); *Achnatherum* IV, '*Neotrinia*' (Eurasian); Nasselloid 'ladder-like' LEP (lemma epidermal pattern); achnatheroid 'maize-like' LEP; stipoid 'saw-like' LEP; the type species of each genus is in bold; clades labelled 1 and 2 are discussed in text.

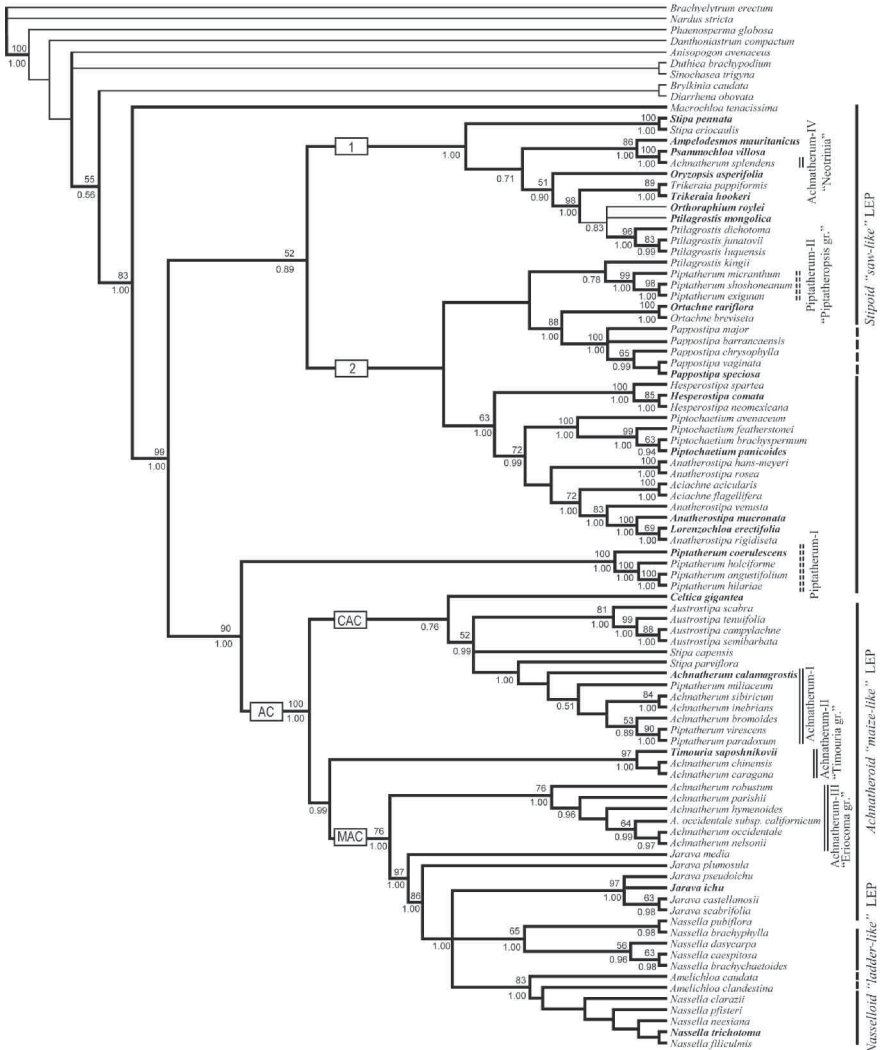
of Old World *Piptatherum*, 2—an unsupported *Austrostipa* clade, 3—an unsupported Old World *Achnatherum* clade (*Achnatherum* I–II), and; 4—an unsupported Major American Clade (**MAC**).

MAC includes a strongly supported *Jarava* s. s. clade (BS=91, PP=1.00) of four species excluding *J. media* and *J. plumosula*, both of which are members of a polytomy that includes a clade of North American *Achnatherum* species (*Achnatherum* III or ‘*Eriocoma* group’, PP=0.71), and an unsupported clade of *Nassella* and *Amelichloa* (PP=0.58). *Amelichloa* (BS=97, PP=1.00) is sister to *Nassella*, which has some internal support.

**Combined Plastid Analysis**—The combined plastid phylogenetic tree indicates moderate support (BS=83, PP=1.00) for a monophyletic Stipeae (Fig. 2) that excludes other representatives of Pooideae tribes and subtribes (Phaenospermateae, Duthieinae, Brylkinieae and Diarrheneae). *Macrochloa tenacissima* is supported as sister to a clade of the remaining taxa which has strong support (BS=99, PP=1.00). In the next bifurcation a joint clade (BS=52, PP=0.89) consists of clade 1 (PP=1.00) and clade 2 (unsupported) that are sister to strongly supported clade (BS=90, PP=1.00) [Fig. 2]. Clade 1 is, except for *Oryzopsis asperifolia*, strictly Eurasian. It includes a strongly supported *Stipa* clade (BS=100, PP=1.00) that is sister to the remaining members (PP=0.71). The next split includes a moderately supported clade (BS=86, PP=1.00) containing *Ampelodesmos mauritanicus*, *Psammochloa villosa*, and *Achnatherum splendens* (*Achnatherum* IV ‘*Neotrinia*’). The later two species (*P. villosa* and *A. splendens*) form a strongly supported (BS=100, PP=1.00) terminal clade. Sister to the previous clade is a weakly supported clade (BS=51, PP=0.90) that includes *Oryzopsis asperifolia* as sister to a strongly supported clade (BS=98, PP=1.00) of *Trikeriaia*, *Orthoraphium*, and *Ptilagrostis*. A moderately supported clade (BS=89, PP=1.00) of *Trikeriaia* species is sister to poorly supported trichotomy (PP=0.83) that includes *Orthoraphium roylei*, *Ptilagrostis mongolica*, and a strongly supported clade (BS=96, PP=1.00) with three species of *Ptilagrostis*.

Clade 2 contains only New World elements and bifurcates into two clades: 1—an unsupported subclade that includes *Ptilagrostis kingii* as sister to a strongly supported clade of North American species of *Piptatherum* II, the ‘*Piptatheropsis*’ group (BS=99, PP=1.00); this subclade is sister to a moderately supported South American clade (BS=88, PP=1.0) containing *Ortachne* s. s. and *Pappostipa*, within which is resolved a strongly supported *Ortachne* clade (BS=100, PP=1.00) as sister to a strongly supported (BS=100, PP=1.00) *Pappostipa* clade; 2—a weakly supported clade (BS=63, PP=1.00) clade that includes a strongly supported *Hesperostipa* clade (BS=100, PP=1.00) that is sister to a moderately supported clade with a strongly supported subclade (BS=100, PP=1.00) of *Piptochaetium* as sister to an unsupported subclade that includes a non-monophyletic *Anatherostipa*, within which are nested *Lorenzochloa*, and a strongly supported clade (BS=100, PP=1.00) of *Aciachne*.

Sister to clades 1 and 2 (as labelled in Fig. 2) is a strongly supported clade (BS=90, PP=1.00) with the remaining taxa. A strongly supported clade (BS=100,



**Fig. 2.** Majority-rule consensus tree of 1001 equally most parsimonious trees based on maximum parsimony analysis of sequence data from chloroplast DNA *trnK-matK*, *matK*, *trnH-psbA*, *trnL-F*, and *ndhF* regions. Branches in bold are found in the strict consensus tree. Numbers above branches correspond to bootstrap (BS) values; numbers below branches correspond to Bayesian posterior probability (PP) values; AC=Achnatheroid Clade; CAC=Core Achnatheroid Clade; LEP = lemma epidermal pattern; MAC=Major American Clade; *Piptatherum* I (Asian); *Piptatherum* II, '*Piptatheropsis* group' (New World); *Achnatherum* I (Asian); *Achnatherum* II, '*Timouria* group' (Asian); *Achnatherum* III, '*Eriocoma* group' (New World); *Achnatherum* IV, '*Neotrinia*' (Eurasian); Nasselloid-2 ladder-like LEP (lemma epidermal pattern); achnatheroid 'maize-like' LEP; stipoid 'saw-like' LEP; the type species of each genus is in bold; clades labelled 1 and 2 are discussed in text.

PP=1.00) of Eurasian *Piptatherum* s. s. (*Piptatherum* I) is sister to a strongly supported (BS=100, PP=1.00) American Clade (AC). AC bifurcates into the Core Achnatheroid Clade (CAC; without BS, PP=0.76), and a supported sister clade (PP=0.99). CAC includes Eurasian, Afro-Mediterranean, and Australian taxa with *Celtica* as sister to a clade of the remaining taxa (BS=52, PP=0.99). Terminally within CAC, there is moderate support (BS=81, PP=1.00) for a clade of *Austrostipa*, a grade of Afro-Mediterranean *Stipa* species, and an unsupported *Achnatherum* I subclade that includes the type of the genus, *A. calamagrostis* and three species of Old World *Piptatherum*. Thus the Old World *Piptatherum* species (including the type of the genus *P. coerulescens*) are in two distinct clades, one resolves as sister to AC, and the New World *Piptatherum* species are in a third clade. Species of *Achnatherum* are scattered in four clades.

The MAC clade is moderately supported (BS=76, PP=1.00) and sister to this is a strongly supported clade (BS=97, PP=1.00) of Asian *Achnatherum caragana*, *A. chinensis*, and *Timouria saposhnikovii* (*Achnatherum* II 'Timouria group'). Within MAC one branch leads to another moderately supported clade (BS=76, PP=1.00) of New World *Achnatherum* (*Achnatherum* III 'Eriocoma group'); this is sister to a strongly supported clade (BS=97, PP=1.00) that includes *Nassella* and *Amelichloa* and a para- or polyphyletic *Jarava*. Within the *Amelichloa*-*Jarava*-*Nassella* trichotomy (PP=1.00) there is a weakly supported clade (BS=65, PP=1.00) of *Nassella* with *N. brachychaetoides*, *N. brachyphylla*, *N. caespitosa*, *N. dasycarpa*, and *N. pubiflora*; a moderately supported *Amelichloa*-*Nassella* clade (BS=83, PP=1.00) with *Amelichloa caudata*, *A. clandestina*, *N. clarazii*, *N. filiculmis*, *N. neesiana*, *N. pfisteri*, and *N. trichotoma* (= type of the genus); and a single strongly supported *Jarava* s. s. clade (BS=97, PP=1.00) with *Jarava castellanosi*, *J. ichu* (= type of the genus), *J. pseudoichu*, and *J. scabrifolia*.

## Discussion

There are many obvious similarities and differences between the ITS and plastid trees (Figs. 1-2), especially between Stipeae clades 1 and 2. Rather than belabor these point by point, we will state from the outset that we have much more confidence in the often well-supported relationships detected by the large plastid data set, than in those resolved by the smaller more homoplasious ITS dataset, in which clades are usually poorly supported. Our discussion will focus primarily on relationships supported by the plastid data.

The cladograms presented in this paper are polarized in a way that is consistent with the distribution of lemma epidermal characteristics (LEP) as labeled in Figs. 1-2. In Fig.2 outgroup species such as *Anisopogon avenaceus*, *Brylkinia caudata*, *Diarrhena obovata*, *Duthiea brachypodium*, and *Sinochasea trigyna*, and species in clades 1 and 2, plus *Macrochloa tenacissima* and *Piptatherum* I clade have a 'saw-like' lemma pattern. This lemma pattern is characterized as having epidermal fundamental cells (long-cells) that often not regularly alternate with

short-cells (generally as silica/cork-cell pairs, or solitary single silica-cells), the long-cells being generally longer than the silica-cells; long-cell side walls that are sinuate, dentate, or lobate; and silica-bodies within the silica-cells that are oval to oblong-rectangular and slightly unequal. Species in the Achnatheroid Clade (AC) [Figs. 1-2, excluding *Celtica gigantea*] have an achnatheroid 'maize-like' lemma epidermal pattern characterized in which short fundamental cells regularly alternate with silica-cells that are about the same length or longer than the fundamental cells; side walls that are straight or slightly uneven; and silica-bodies that are short-rectangular, often equal (Romaschenko et al. 2008). A modification of the 'maize-like' lemma epidermal pattern is found only in species of *Nassella* and this is termed nasseloid 'ladder-like.' In it, the short-cells lack silica and are indistinguishable from the very short fundamental cells.

The monophyly of Stipeae including *Macrochloa tenacissima* as sister to all other Stipeae is moderately supported by the plastid analysis. Lack of support for the majority of crown nodes in the ITS tree (Fig. 1) is caused by the high level of homoplasy in the data; consequently, the ITS data does not support a monophyletic Stipeae. Some agrostologists have suggested that genera in Duthieinae belong in the Stipeae (Soreng et al. 2003; Wu and Phillips 2006). However, the elements of the clade (Fig. 1) or grade (Fig. 2), of *Phaenosperma*, *Danthoniastrum*, *Anisopogon*, and *Duthiea-Sinochasea* (the latter four considered to be members of subtribe Duthieinae), were not placed within or as a sister group to Stipeae. The *Brylkinia-Diarrhena* clade is sister to Stipeae in the plastid tree (Fig. 2), or in a polytomy with two clades of Stipeae in the ITS tree (Fig. 1). This basal grade of Pooideae genera was recovered in another phylogenetic study of Pooideae (Davis and Soreng 2007).

Both ITS and plastid trees demonstrate the polyphyly of *Achnatherum* where species are found in four separate clades, *Piptatherum* where species are found in three separate clades, and for treating *Stipa* in a very narrow sense (Romaschenko et al. in prep.). Barkworth (1993, 2006) interpreted *Achnatherum* as the largest and most widespread genus in the Stipeae. As compared to the well developed anemochoric (*Stipa* s. s.) or endozochoric (*Piptatherum* s. lat.) morphological characteristics of some putatively related genera, *Achnatherum* was thought to be relatively unspecialized and primitive. This reasoning explains why in previous phylogenetic inferences based on morphological studies (Tzvelev 1977; Barkworth and Everett 1987) the origins of such genera as *Stipa* (through *Ptilagrostis*) and *Piptatherum* (through *Piptatherum* sect. *Virescentia* Roshev. ex Freitag) were considered to be derived from the *Achnatherum* lineage. Our molecular analysis revealed the polyphyletic nature of *Achnatherum* and *Piptatherum* and that these genera are not closely related to *Stipa* s.s. Our phylogenetic inferences provide support for splitting *Achnatherum* into four groups (see Fig. 2) characterized as follows: 1) *Achnatherum* I (s. s. with Asian distribution) with a 'maize-like' lemma epidermal pattern, fusiform spikelets, lemmas without a ciliate crown, and persistent awns that are inconspicuously geniculate; 2) *Achnatherum* II,

'*Timouria* group' (Asian distribution) with a 'maize-like' epidermal pattern, comparatively short hairy lemmas that lack a ciliate crown, and caducous awns; 3) *Achnatherum* III, '*Eriocoma* group' (New World distribution) with a 'maize-like' epidermal pattern, fusiform or obovoid spikelets, lemmas often with a ciliate crown, persistent or caducous awns, and paleas often short; and 4) *Achnatherum* IV, '*Neotrinia*' (Eurasian distribution) with stipoid 'saw-like' lemma epidermal pattern.

In recent circumscriptions *Piptatherum* is treated as a northern hemisphere genus with approximately 30 species, six of which are American (Barkworth 2006). With the acceptance of a monotypic concept for *Oryzopsis* Michx. s. s. (Tzvelev 1977; Freitag 1975; Barkworth and Everett 1987), other American species formerly placed in *Oryzopsis* were transferred to *Piptatherum* or to (along with many American taxa that remained in *Stipa*) *Achnatherum* (Barkworth 1993; Dorn 2001; Soreng et al. 2003). Our analyses support splitting *Piptatherum* into three groups: 1) *Piptatherum* I (= *Piptatherum* s.s. of Eurasian distribution) with stipoid 'saw-like' lemma epidermal pattern, glumes longer than the floret with 3–7 veins, dorsally compressed florets, transversally elliptic foveola (disarticulation scar), lemma borders proximally set apart and not fused (exposing the base of the palea), and lemma coriaceous to cartilaginous with the awns caducous and straight; 2) *Piptatherum* II, '*Piptatheropsis*-group' (New World distribution) with stipoid 'saw-like' lemma epidermal pattern, dorsally compressed or terete florets, circular foveola, lemma borders basally fused into a small fleshy wart covering the base of the palea, and lemmas coriaceous to membranous with awns caducous, straight to once or twice-geniculate; and 3) subsuming some Old World *Piptatherum* (*P. miliaceum*, *P. paradoxum*, and *P. virescens* at a minimum) in *Achnatherum* I as discussed in the previous paragraph (see Fig. 2).

**Stepwise Model of Stipeae Evolution**—Molecular evolution of plastid sequences, lemma epidermal pattern, and geography suggests that the Stipeae have evolved generally in a stepwise fashion with two initial bifurcations followed by two further bifurcations. With the exception of two morphologically 'transitional' clades (*Achnatherum* II, '*Timouria*-group,' and *Piptatherum* I), the first bifurcation point splits Stipeae into two major lineages based on having either a stipoid 'saw-like' lemma pattern (clades 1 and 2 in Fig. 2) or an achnatheroid 'maize-like' lemma pattern (AC clade in Fig. 2). The second level of bifurcations occurs in each of the previous clades. In the stipoid lemma epidermal pattern grade, clade 1 (Fig. 2) comprises species with Eurasian distribution (excluding the North American *Oryzopsis asperifolia*), and clade 2 (Fig. 2) comprises species of New World distribution. In the achnatheroid lemma epidermal pattern grade, there is a split into a Core Achnatheroid Clade (CAC) that contains species of Eurasian, African, and Australian distribution, and a major American clade (MAC) that contains species solely of New World distribution.

**Clade 1: Eurasian Distribution**—This clade displays the greatest morphological diversity among the genera. The taxa in many cases represent relictual

and very specialized forms that are adapted to widely divergent habitats. It includes genera with well developed anemochoric florets (as seen in species of *Stipa*) and others with unspecialized dispersal mechanisms. The plants are Asian, drought tolerant, stout, and extremely fibrous as seen in *Achnatherum splendens*, and species of *Ampelodesmos*, *Psammochloa*, and *Trikeriaia*. The only exception here is the American monotypic genus *Oryzopsis* which is sister to *Trikeriaia* (Fig. 1) or sister to *Trikeriaia*–*Orthoraphium*–*Ptilagrostis* (Fig. 2). *Oryzopsis asperifolia* is a low growing, mesophytic, shade tolerant, boreal forest species. Despite morphological differences from species of the above genera, *Oryzopsis* shares the known chromosome number ( $2n=48$ ,  $x=12$ ) of this group (Bowden 1960). The lemma epidermal pattern of *O. asperifolia* is similar to *Trikeriaia pappiformis* in having long lobate sidewalls and round silica bodies that are not regularly paired with suberin-cells (cork-cells). The other species of *Trikeriaia* in our study (*T. hookeri*) has a lemma epidermal pattern similar to that of *Ptilagrostis* s. s. (i.e., excluding *P. kingii*), characterized by slightly elongated silica bodies that are fused in the middle. In our plastid tree (Fig. 2) *Ptilagrostis* s. s., *Orthoraphium*, and *Trikeriaia* form a strongly supported clade.

In addition to sharing stipoid ‘saw-like’ lemma epidermal characteristics, *Stipa* s. s. have moderately elongated, rectangle-shaped fundamental cells with thick, sinuate sidewalls; straight end walls, regularly alternating with silicified square-based hooks paired with short suberin cells. Another distinctive morphological feature of *Stipa* s. s. is the absence of protruding lemma lobes that are found in all other genera of Stipeae. In our analysis we included *Stipa pennata* L. [we have confirmed by examining the lectotype (L!) that *S. pennata* is synonymous with *S. joannis*]] and *S. eriocaulis*.

**Clade 2: New World Distribution**—This clade includes species of *Hesperostipa*, *Piptochaetium*, *Anatherostipa*, *Aciachne*, *Lorenzochloa*, *Ortachne*, and ‘*Piptatheropsis* group’, which all share the stipoid ‘saw-like’ lemma epidermal pattern (Fig. 2). The long suspected affinity of such genera as *Hesperostipa*, *Piptochaetium*, and *Anatherostipa*, inferred from similarities in palea structure and lemma epidermal pattern, has been confirmed for the first time in our plastid analysis based primarily on a sufficiently high number of PICs with a relatively low level of homoplasy (Table 1). *Pappostipa* is included in this lineage in the plastid tree (Fig. 2), however, these species do not share the same lemma epidermal pattern (Romaschenko et al. 2008). Likewise, *Hesperostipa* is included in this lineage only in the plastid tree (Fig. 2) but was excluded from this clade in our ITS tree and in previous analyses (Romaschenko et al. 2008).

*Ptilagrostis kingii* is sister to *Piptatherum* clade (*Piptatherum* II, ‘*Piptatheropsis* group’) in both of our trees and is apparently not related to other species that currently reside in *Ptilagrostis*. *Ptilagrostis kingii* was placed in *Oryzopsis* [*O. kingii* (Bolander) Beal] for many years before it was transferred to *Ptilagrostis* by Barkworth (1983). She based her decision on a number of features seen in other members of *Ptilagrostis*: caespitose habit, narrow acicular leaf blades, awn persist-

ence and indumentum (usually lacking, but sometimes weakly developed), and anthocyanic color. Barkworth (1983) acknowledged that all these characteristics were perhaps a result of convergent evolution, since *P. kingii* and other members of this genus all reside in alpine environments. In our view, the lemma epidermal pattern of *P. kingii* suggests a close relationship with *Piptatherum* (*Piptatherum* II, ‘*Piptatheropsis* group’) whose species lack square to angled suberin-cells that are regularly paired with silica-bodies (a pattern common in *Ptilagrostis* s. s.). *Ptilagrostis kingii* also differs from other species of *Ptilagrostis* in having weakly developed lemma lobes similar to those found in *Piptatherum* II, ‘*Piptatheropsis* group.’

Our molecular analyses indicate that *Lorenzochloa erectifolia* is part of an *Anatherostipa*-*Aciachne* clade (Figs 1-2). Based on spikelet morphology Clayton (1985) placed *L. erectifolia* in *Ortachne* [*O. erectifolia* (Swallen) Clayton] since all species have glumes shorter than the floret. Our plastid tree (Fig. 2) indicates the core species of *Anatherostipa* (*A. venusta*, the type species *A. mucronata*, and *A. rigidisetata*) form a clade with *Lorenzochloa*. A monophyletic *Aciachne* then is sister to the core *Anatherostipa* (including *Lorenzochloa*); and then sister to this, are two species of *Anatherostipa* (*A. hans-meyeri* and *A. rosea*) that, unlike the others, have a well developed pappus at the apex of the lemma. Thus *Anatherostipa* is paraphyletic (Fig. 2). More data are needed to ascertain whether this ‘pappus group’ of *Anatherostipa* represents a separate phylogenetic entity. Regardless, the circumscription of *Anatherostipa* should be revisited to include such characteristics as the short pungent awn and short glumes found in *L. erectifolia*. If this clade is recognized at the generic level, *Lorenzochloa* would be the correct name since it has nomenclatural priority over *Anatherostipa*.

**Core Achnatheroid Clade**—CAC is a major group of Austral-Eurasian-Mediterranean and African species that share the ‘maize-like’ lemma epidermal pattern (with the exception of *Celtica gigantea* and *Piptatherum miliaceum*). The latter two species have long lemma epidermal fundamental cells (extremely long in *Celtica*) with straight sidewalls and irregularly placed round silica bodies. The phylogenetic position of *Celtica* is unstable in our analyses. *Celtica* is found as sister (PP=0.76) to the remaining members of our CAC-clade in our plastid tree (Fig. 2) and in a grade between clades of *Hesperostipa* and *Pappostipa* in our ITS tree (Fig. 1). Two species with uncertain phylogenetic position that are assigned to CAC for the first time are: *Stipa parviflora* and *S. capensis*. Both of these species have the ‘maize-like’ lemma epidermal pattern, and in the majority of plastid trees *S. parviflora* was sister to the remaining taxa in the *Achnatherum* group, whereas *S. capensis* often was sister to the *Austrostipa* clade.

In all analyses *Austrostipa* [represented by *Austrostipa* subg. *Falcatae* S.W.L. Jacobs and J. Everett (*A. scabra* and *A. tenuifolia*) and *A.* subg. *Austrostipa* (*A. semibarbata* and *A. campylachne*)] was recovered as monophyletic. The morphologically distinctive *Austrostipa* subg. *Aulax* S.W.L. Jacobs and J. Everett and the monotypic New Zealand *Anemanthele* were not available for sampling at the



time this study was carried out. However, in Jacobs et al. (2007) ITS analysis, *Anemanthele* and *Austrostipa setacea* (R. Br.) S.W.L. Surrey and J. Everett were nested within their *Austrostipa* clade. Barkworth and Everett (1987) reported an unusual achnatheroid type of lemma epidermal pattern of fundamental cells with thin, slightly sinuate but elongated sidewalls for *Austrostipa* subg. *Aulax* species. Our analysis of the lemma epidermal pattern for *Anemanthele* (results not shown) suggests the typical achnatheroid pattern with very short fundamental cells. Therefore, *Austrostipa* appears as a relatively homogeneous and phylogenetically distinct group, sister to *Achnatherum* s. s. plus the two Afro-Mediterranean 'Stipas', in our plastid tree (Fig. 2).

Our ITS tree yielded an unsupported Asian *Achnatherum* clade (excluding *Timouria saposhnikovii*, which is usually placed within *Achnatherum*), and within this is a moderately supported clade (BS=87, PP=1.00) of *A. inebrians*, *A. bromoides*, and *A. sibiricum* (*Achnatherum* I in Fig. 1). Two representatives of *Piptatherum* sect. *Virescentia* (Roshev.) Freitag (*P. virescens* and *P. paradoxum*) form a strongly supported clade embedded within *Achnatherum* I (Fig. 2). Barkworth and Everett (1987) also noted the similarity in lemma epidermal pattern that suggests a possible close relationship between *Piptatherum virescens* and some species of *Achnatherum*. We agree with Barkworth and Everett (1987, p. 254) that "...features important to successful reproduction and establishment are poor indicators of phylogeny because they are likely to evolve independently in several different lines." The evolutionary significance of lemma and palea induration, often associated with small (short) florets, should be reassessed. Within the Achnatheroid Clade (AC) these features are indeed likely to have evolved independently in the different lineages, i.e., the small florets of *Achnatherum hymenoides* (*Achnatherum* III, 'Eriocoma group'), *Piptatherum paradoxum* and *P. virescens* (*Piptatherum* sect. *Virescentia* in *Achnatherum* I), and members of *Austrostipa* subg. *Aulax*. In order to incorporate *Piptatherum virescens* and *P. paradoxum* into *Achnatherum*, the taxonomic description of the genus should be emended to include such features as the coriaceous nature of the lemma and palea.

**Major American Clade**—MAC, as in our previous study (Romaschenko et al. 2008), encompasses taxa that are restricted to the New World, including such genera as: *Nassella*, *Amelichloa*, *Jarava* s. s. (excluding newly segregated genus *Pappostipa*), and the American clade of *Achnatherum* [*Achnatherum* III, 'Eriocoma group', named for *Eriocoma hymenoides* (Roem. and Schult.) Rydb., the type of that genus]. The lemma epidermal pattern is not homogeneous in MAC since *Achnatherum* III, 'Eriocoma group', *Jarava* s. s., and *Amelichloa* share the 'maize-like' pattern whereas all species of *Nassella* share the 'ladder-like' pattern where the fundamental cells are very short and short-cells lack silica; and these short-cells are indistinguishable from the fundamental cells. Presumably, the 'ladder-like' pattern is derived from the 'maize-like' pattern.

In our ITS tree (Fig. 1) we found a polytomy with five clades: unsupported *Nassella*, strongly supported *Amelichloa*, unsupported *Achnatherum* III 'Eriocoma

group', strongly supported *Jarava* s. s., and an unsupported clade of *Jarava media* and *J. plumosula* (the latter two belonging to *Stipa* subg. *Ptilostipa* Speg.; sensu Spegazzini 1901). In our plastid tree (Fig. 2) we have support for the monophyly of four clades: *Achnatherum* III 'Eriocoma group' (moderate), *Jarava* s. s. (strong), *Nassella*–*Amelichloa* (moderate), and *Nassella* (weak; *Stipa* subg. *Dasystipa* Speg. sensu Spegazzini 1901). Since *Nassella* is the largest genus ( $\pm 115$  species) in the Stipeae, we suspect the phylogenetic structure is complex and since this is a very small sample we are reluctant to infer definite conclusions until more samples are incorporated including the type of *Amelichloa*.

Sister to MAC in our plastid tree (Fig. 2) is a strongly supported clade, *Achnatherum* II, 'Timouria group' that includes: *A. caragana*, *A. chinensis*, and *Timouria saposhnikovii* (Fig. 2). Species in this clade have hairy lemmas with a caducous awn and the typical achnatheroid 'maize-like' lemma epidermal pattern. Although *Timouria* is usually classified within *Achnatherum* (Tzvelev 1976; Wu and Phillips 2006), we call this the 'Timouria-group' [Roshevits (1916) first described the genus (*Timouria*) and the type, *T. saposhnikovii*, is a member of this clade].

**Summary**—The phylogenetic structure of Stipeae is complex. Yet our hypothesis for the evolution within Stipeae is represented by a simple scheme consisting of two serial cladogenic events: the first split of the plastid tree separates the tribe into two major lineages based on having a stipoid 'saw-like' or achnatheroid 'maize-like' lemma epidermal patterns. Each of these major groups splits into two clades that are biogeographically restricted to the Old World (clade 1) and the New World (clade 2). This general scheme was not supported by our ITS tree because of increasing levels of homoplasy, but has reasonable support in our plastid tree when considered along with morphology and geography.

Some morphological trends in the evolution of the Stipeae are clarified, but some traditional interpretations are cast into doubt. Many characteristics commonly used for phylogenetic inferences in the tribe, e.g., length and shape of lemma and callus, and persistence of the awn and development of the awn indumentum proved to be of little phylogenetic significance, since these characters appear to have evolved independently in different lineages. Short spikelets, coriaceous florets, and lemmas with caducous awns, all characteristics initially attributed to *Piptatherum*, have evolved in many of the major clades within the Stipeae. Based on our current phylogenetic hypothesis *Oryzopsis* is monotypic; *Achnatherum* should be split into four groups and *Piptatherum* into three groups; and *Ptilagrostis* into at least two groups. Segregation of new groups or clades tentatively called: *Eriocoma*, 'Neotrinia', 'Piptatheropsis', and *Timouria*, require further studies with denser sampling in order to provide phylogenetic background for delimitation and subsequent nomenclatural emendation. Examples of apparent taxonomic misplacement are: *Stipa capensis* and *S. parviflora*; they are apparently not closely related to other species of *Stipa* s. s., but are allied to the achnatheroids. *Piptatherum miliaceum*, *P. paradoxum*, and *P. virescens* apparently

are allied to *Achnatherum* s. s.; *Lorenzochloa erectifolia* does not align with *Ortachne* but with some species of *Anatherostipa*; and *Achnatherum splendens* ('*Neotrinia*') seems most closely related to *Psammochloa*.

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## Appendix 1

Taxa and collections (herbaria as abbreviated in Holmgren et al. 1990) sampled, their GenBank accession numbers indicated in order for *trnK-matK*, *MatK*, *trnH-psbA*, *trnL-trnF*, *ndhF*, and ITS. All new sequence data is in bold, an asterisk (\*) is given for a missing sequence, and pound sign (#) is given for a partially incomplete sequence.

\* \* \*

*Achnatherum bromoides* (L.) P. Beauv., UKR, *Romaschenko 439* & *Didukh* (KW), GU254905, GU254708, GU254845, \*, GU254734, GU254624; *Achnatherum calamagrostis* (L.) P. Beauv., ESP, *Pyke 164* (US), GU254899, GU254698, GU254851, GU254940, GU254743, GU254638; *Achnatherum caragana* (Trin.) Nevski, KAZ, *Goloskokov s.n.* (US), GU254915, GU254690, GU254852, GU254973, GU254732, GU254631; *Achnatherum chinense* (Hitchc.) Tzvelev, CHN, *Petrov s.n.* (LE), GU254900, GU254691, GU254860, \*, GU254735, GU254630; *Achnatherum hymenoides* (Roem. & Schult.) Barkworth, USA, *Saarela 205* (UBC), GU254894, GU254710, GU254844, GU254972, GU254733, GU254632; *Achnatherum inebrians* (Hance) Keng ex Tzvelev, CHN, *Soreng 5393* (US), GU254903, GU254695, GU254847, GU254990, GU254750, GU254626; *Achnatherum nelsonii* (Scribn.) Barkworth, CAN, *Saarela 593* (UBC), GU254895, GU254713, GU254854, GU254971, GU254761, GU254633; *Achnatherum occidentale* (Thurb. ex S. Watson) Barkworth, CAN,

*Saarela 594*, *Sears & Maze* (UBC), GU254896, EU489171, EU489238, EU489312, GU254740, EU489090; *Achnatherum occidentale* subsp. *californicum* (Merr. & Burtt Davy) Barkworth, USA, *Howell 36354* (US), EU489383, GU254714, GU254855, GU254970, GU254754, GU254634; *Achnatherum parishii* (Vasey) Barkworth, USA, *Roos 4895 & Roos* (US), EU489385, GU254716, EU489240, EU489314, GU254756, EU489092; *Achnatherum robustum* (Vasey) Barkworth, MEX, *Peterson 10583 & Annable* (US), GU254906, GU254715, GU254856, GU254969, GU254755, GU254635; *Achnatherum sibiricum* (L.) Keng ex Tzvelev, CHN, *Soreng 5104* (US), GU254904, GU254696, GU254846,, GU254741, GU254610; *Achnatherum splendens* (Trin.) Nevski, CHN, *Soreng 5121* (US), GU254913, GU254687, GU254818, GU254951, GU254787, GU254668; *Aciachne acicularis* Laegaard,, *Peterson 13931 & Refugio Rodriguez* (US), GU254930, GU254673, GU254865, GU254988, GU254806, GU254625; *Aciachne flagellifera* Lægaard, ECU, *Laegaard 19436* (AAU), GU254893, GU254672, GU254877, GU254987, GU254805, GU254654; *Amelichloa caudata* (Trin.) Arriaga & Barkworth, ARG, *Peterson 11398 & Annable* (US), EU489388, EU489175, EU489241, EU489317, GU254764, EU489095; *Amelichloa clandestina* (Hack.) Arriaga & Barkworth, USA, *Barkworth 5103* (US), GU254898, GU254717, GU254853, GU254968, GU254765, GU254636; *Ampelodesmos mauritanicus* (Poir.) T. Durand & Schinz, ESP, *Pyke 702* (BC), GU254911, GU254722, GU254832, GU254955, GU254797, GU254667; *Anatherostipa hans-meyeri* (Pilg.) Peñailillo, PER, *Peterson 20645, Soreng & Romaschenko* (US), EU489391, EU489177, EU489244, EU489319, GU254804, EU489098; *Anatherostipa mucronata* (Griseb.) F. Rojas, ARG, *Peterson 19551* (US), *Soreng, Salariato & Panizza*, GU254887, GU254697, GU254861, GU254985, GU254803, GU254611; *Anatherostipa rigidisetata* (Pilg.) Peñailillo, BOL, *Beck s.n.* (LPB), GU254890, GU254699, GU254869, GU254984, GU254809, GU254612; *Anatherostipa rosea* (Hitcch.) Peñailillo, ECU, *Laegaard 10864* (AAU), GU254889, GU254671, GU254867, GU254986, GU254795, GU254652; *Anatherostipa venusta* (Phil.) Peñailillo, CHL, *Pfister 9394* (US), GU254885, GU254680, GU254870, GU254983, GU254801, GU254613; *Anisopogon avenaceus* R. Br., AUS, *Soreng 5905* (US), GU254933, GU254682, GU254823, GU254946, GU254769, GU254657; *Austrostipa campylachne* (Nees) S.W.L. Jacobs & J. Everett, AUS, *Peterson 14267, Soreng, Rosenberg & MacFarlane* (US), GU254902, GU254694, GU254848, GU254975, GU254737, GU254627; *Austrostipa scabra* (Lindl.) S.W.L. Jacobs & J. Everett, AUS, *Peterson 14442, Soreng & Rosenberg* (US), EU489395, EU489181, EU489248, EU489323, GU254738, EU489102; *Austrostipa semibarbata* (R. Br.) S.W.L. Jacobs & J. Everett, AUS, *Symon 13439* (US), GU254901, GU254693, GU254849, GU254974, GU254736, GU254628; *Austrostipa tenuifolia* (Steud.) S.W.L. Jacobs & J. Everett, AUS, *Peterson 14248, Soreng & Macfarlane* (US), EU489397, EU489183, EU489250, EU489325, GU254739, EU489104; *Brachyelytrum erectum* (Schreb.) P. Beauv., USA, *Soreng 7440* (US), EU489398, EU489184, EU489251, EU489326, GU254790, EU489105; *Brylkinia caudata* (Munro) F.Schmidt, CHN, *Ping s.n.* (US), GU254914, GU254725, GU254835, GU254957, GU254780, GU254647; *Celtica gigantea* (Link) F. M. Vázquez & Barkworth, ESP, *Pyke 705* (BC), GU254919, GU254726, GU254843, GU254961, GU254775,



GU254642; *Danthoniastrum compactum* (Boiss. & Heldr.) Holub, GRC, Soreng 7520-1 (US), GU254907, GU254720, GU254836, GU254958, GU254779, GU254646; *Diarrhena obovata* (Gleason) Brandenburg, USA, Soreng 7439 (US), GU254922, GU254730, GU254834, GU254956, GU254783, GU254669; *Duthiea brachypodium* (P.Candargi) Keng & Keng f., CHN, Soreng 5358 (US), GU254934, GU254683, GU254819, GU254947, GU254793, GU254656; *Hesperostipa comata* (Trin. & Rupr.) Barkworth, CAN, Saarela 595, Sears & Maze (UBC), EU489399, EU489185, EU489252, EU489327, GU254812, EU489106; *Hesperostipa neomexicana* (Thurb.) Barkworth, MEX, Peterson 18934 & Valdes-Reyna (US), EU489400, EU489186, GU254840, EU489328, GU254808, EU489107; *Hesperostipa spartea* (Trin.) Barkworth, USA, Holmes 214 (US), EU489401, EU489187, EU489253, EU489329, GU254745, EU489108; *Jarava castellanosii* (F.A. Roig) Peñailillo, ARG, Peterson 10336 & Annable (US), EU489405, EU489191, EU489256, EU489333, GU254770, EU489112; *Jarava ichu* Ruiz & Pav., PER, Peterson 20745, Soreng & Romaschenko (US), EU489415, EU489202, EU489267, EU489344, GU254763, EU489124; *Jarava media* (Speg.) Peñailillo, ARG, Peterson 19337, Soreng, Salariato & Panizza (US), EU489419, EU489205, EU489272, EU489347, GU254758, EU489129; *Jarava plumosula* (Nees ex Steud.) F. Rojas, PER, Peterson 20471, Soreng & Romaschenko (US), EU489422, EU489207, EU489275, EU489350, GU254757, EU489133; *Jarava pseudoichu* (Caro) F. Rojas, PER, Peterson, Soreng & Romaschenko (US), EU489424, EU489209, EU489277, EU489352, GU254762, EU489135; *Jarava scabrifolia* (Torres) Peñailillo, ARG, Peterson 11712 & Annable (US), EU489425, EU489210, EU489278, EU489353, GU254760, EU489136; *Lorenzochloa erectifolia* (Swallen) Reeder & C. Reeder, BOL, Peterson 12632, Annable, Laegaard & Soreng (US), GU254884, GU254706, GU254871, GU254982, GU254800, GU254614; *Macrochloa tenacissima* (Loefl. ex L.) Kunth, ESP, Pyke 701 (BC), GU254912, GU254723, GU254833, GU254978, GU254782, GU254648; *Nardus stricta* L., KGZ, Soreng 7497 (US), EU489432, EU489217, EU489285, EU489360, GU254791, EU489143; *Nassella brachychaetoides* (Speg.) Barkworth, BOL, Peterson 11748 & Annable (US), EU489433, EU489218, EU489286, EU489361, \*, EU489144; *Nassella brachyphylla* (Hitcch.) Barkworth, BOL, Peterson 20631, Soreng & Romaschenko (US), EU489434, EU489219, EU489287, EU489362, \*, EU489145; *Nassella caespitosa* Griseb., ARG, Peterson 19540, Soreng, Salariato & Panizza (US), EU489435, EU489220, EU489288, EU489363, \*, EU489146; *Nassella clarazii* (Ball) Barkworth, ARG, Peterson 11651 & Annable (US), EU489436, EU489221, EU489289, EU489364, GU254766, EU489147; *Nassella dasycarpa* (Hitcch.) Torres, ARG, Peterson 10344 & Annable (US), EU489437, EU489222, EU489290, EU489365, \*, EU489148; *Nassella filiculmis* (Delile) Barkworth, CHL, Soreng 7009 (US), EU489439, EU489224, EU489292, EU489367, GU254768, EU489150; *Nassella neesiana* (Trin. & Rupr.) Barkworth, ARG, Peterson 10258 & Annable (US), EU489444, EU489228, EU489297, EU489371, GU254767, EU489155; *Nassella pfisteri* (Matthai) Barkworth, CHL, Soreng 7017a (US), EU489446, EU489229, EU489299, EU489372, \*, EU489157; *Nassella pubiflora* (Trin. & Rupr.) E. Desv., ARG, Peterson 11618 & Annable (US), EU489447, EU489230, EU489300,

EU489373, \*, EU489158; *Nassella trichotoma* (Nees) Hack. ex Arechav., ARG, *Peterson 11506* & *Annable* (US), EU489451, EU489232, EU489305, EU489376, GU254742, EU489164; *Ortachne breviseta* Hitch., ARG, *Laegaard 12802* (AAU), GU254924, GU254674, GU254868, GU254989, GU254810, GU254666; *Ortachne rariflora* (Hook. f.) Hughes, CHL, *Mariz 924* (CONC), GU254925, GU254675, GU254864, GU254952, GU254771, GU254665; *Orthoraphium roylei* Nees, CHN, *Soreng 5261* (US), GU254881, GU254703, GU254858, GU254979, GU254753, GU254617; *Oryzopsis asperifolia* Michx., CAN, *Saarela 384* (UBC), GU254908, GU254686, GU254821, GU254964, GU254788, GU254653; *Pappostipa barrancaensis* (F.A. Roig) Romasch., ARG, *Peterson 11371* & *Annable* (US), EU489404, EU489190, EU489255, EU489332, \*, EU489111; *Pappostipa chrysophylla* (E. Desv.) Romasch., ARG, *Peterson 19220*, *Soreng Salariato* & *Panizza* (US), EU489407, EU489193, EU489258, EU489335, GU254781, EU489114; *Pappostipa major* (Speg.) Romasch., CHL, *Soreng 7222* (US), EU489427, EU489212, EU489280, EU489355, GU254798, EU489138; *Pappostipa speciosa* (Trin. & Rupr.) Romasch., ARG, *Peterson 11549* & *Annable* (US), EU489426, EU489211, EU489279, EU489354, GU254772, EU489137; *Pappostipa vaginata* (Phil.) Romasch., ARG, *Peterson 11744* & *Annable* (US), EU489431, EU489216, EU489284, EU489359, GU254816, EU489142; *Phaenosperma globosa* Munro ex Benth., CHN, *Soreng 5325* (US), GU254935, GU254684, GU254948, GU254792, GU254655; *Piptatherum angustifolium* (Munro ex Regel) Roshev., TJK, *Nepli 144* (LE), GU254923, GU254707, GU254825, GU254944, GU254811, GU254659; *Piptatherum coerulescens* (Desf.) P. Beauv., GRC, *Soreng 3765* (US), GU254936, GU254685, GU254822, GU254949, GU254789, GU254639; *Piptatherum exiguum* (Thurb.) Dorn, USA, *Reveal 1073* (KW), GU254927, GU254677, GU254859, GU254976, GU254752, GU254663; *Piptatherum hilariae* Pazij, TJK, *Potaliev 343* (LE), GU254929, GU254679, GU254828, GU254943, GU254807, GU254660; *Piptatherum holciforme* (M. Bieb.) Roemer & Schultes, UKR, *Didukh 1203* (KW), GU254931, GU254670, GU254829, GU254945, GU254817, GU254658; *Piptatherum micranthum* (Trin. & Rupr.) Barkworth, CAN, *Peterson 18437*, *Saarela* & *Smith* (US), GU254926, GU254676, GU254866, GU254950, GU254815, GU254664; *Piptatherum miliaceum* (L.) Coss., JOR, *Gillett 16094* (US), GU254918, GU254729, GU254838, GU254960, GU254776, GU254643; *Piptatherum paradoxum* (L.) P. Beauv., ESP, *Pyke 831* (BC), GU254916, GU254727, GU254862, GU254977, GU254744, GU254622; *Piptatherum shoshoneanum* (Curto & Douglass M. Hend.) P.M. Peterson & Soreng, USA, *Eno 13* (US), GU254928, GU254688, GU254841, GU254941, GU254814, GU254662; *Piptatherum virescens* (Trin.) Boiss., UKR, *Romaschenko 445* & *Didukh* (KW), GU254917, GU254728, GU254837, GU254959, GU254777, GU254644; *Piptochaetium avenaceum* (L.) Parodi, USA, *Soreng 7739* & *Romaschenko* (US), GU254883, GU254705, GU254872, GU254981, GU254799, GU254615; *Piptochaetium brachyspermum* (Speg.) Parodi, ARG, *Peterson 11252* & *Annable* (US), EU489452, EU489233, EU489306, EU489377, GU254802, EU489165; *Piptochaetium featherstonei* (Hitchc.) Tovar, ARG, *Peterson 10314* & *Annable* (US), GU254882, GU254704, GU254873, GU254980, GU254796, GU254616; *Piptochaetium panicoides* (Lam.) E. Desv., CHL, *Soreng 7011* (US), EU489453,

EU489234, EU489307, EU489378, GU254794, EU489166; *Psammochloa villosa* (Trin.) Bor, MNG, *Safronova 952* (LE), GU254892, GU254678, GU254820, GU254939, GU254786, GU254651; *Ptilagrostis dichotoma* Keng ex Tzvelev, CHN, *Soreng 5647*, *Peterson & Hang* (US), GU254880, GU254702, GU254874, GU254965, GU254749, GU254618; *Ptilagrostis junatovii* Grubov, RUS, *Kotukhov s.n.* (LE), GU254879, GU254701, GU254842, \*, GU254748, GU254619; *Ptilagrostis kingii* (Bol.) Barkworth, USA, *Peirson 10819* (US), GU254937, GU254712, GU254827, GU254942, GU254813, GU254661; *Ptilagrostis luquensis* P.M. Peterson, *Soreng & Z.L. Wu*, CHN, *Soreng 5383* (US), GU254878, GU254700, GU254875, \*, GU254747, GU254620; *Ptilagrostis mongholica* (Turcz. ex Trin.) Griseb., KGZ, *Koloskov s.n.* (LE), GU254886, GU254689, GU254876, GU254938, GU254746, GU254621; *Sinochasea trigyna* Keng, CHN, *Soreng 5644* (US), GU254932, GU254681, GU254824, \*, GU254778, GU254645; *Stipa capensis* Thunb., ESP, *Pyke 703* (BC), GU254921, GU254724, GU254826, GU254963, GU254773, GU254640; *Stipa eriocalis* Borbás, FRA, *Romaschenko 314*, *Romo & Hidalgo* (BC), GU254888, GU254709, GU254863, GU254966, GU254751, GU254623; *Stipa parviflora* Desf., ESP, *Romaschenko 74 & Romo* (US), GU254920, GU254719, GU254839, GU254962, GU254774, GU254641; *Stipa pennata* L., UKR, *Romaschenko 466* (BC), GU254891, GU254718, GU254857, GU254967, GU254759, GU254637; *Timouria saposhnikovii* Roshev., CHN, *Soreng 5475* (US), GU254897, GU254692, GU254850, \*, GU254731, GU254629; *Trikeria hookeri* (Stapf) Bor, IND, *Koelz 2328* (US), GU254909, GU254711, GU254830, GU254953, GU254785, GU254650; *Trikeria pappiformis* (Keng) P.C. Kuo & S.L.Lu, CHN, *Soreng 5653* (US), GU254910, GU254721, GU254831, GU254954, GU254784, GU254649.