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Molecular phylogenetic analysis resolves *Trisetum* (Poaceae: Pooideae: Koeleriinae) polyphyletic: Evidence for a new genus, *Sibirotrisetum* and resurrection of *Acrospelion*

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Abstract—To investigate the evolutionary relationships among the species of *Trisetum* and other members of subtribe Koeleriinae a phylogeny based on DNA sequences from four gene regions (ITS, *rpl32-trnL* spacer, *rps16-trnK* spacer, and *rps16* intron) is presented. The analyses, including type species of all genera in Koeleriinae (*Acrospelion, Avellinia, Cinnagrostis, Gaudinia, Koeleria, Leptophyllochloa, Limnodea, Peyritschia, Rostraria, Sphenopholis, Trisetaria, Trisetopsis, Trisetum*), along with three outgroups, confirms previous indications of extensive polyphyly of *Trisetum.* Here we focus on the monophyletic *Trisetum* sect. *Sibirica* clade that we interpret here as a distinct genus, *Sibirotrisetum* gen. nov. We

include a description of *Sibirotrisetum* with the following seven new combinations: *Sibirotrisetum aeneum, S. bifidum, S. henryi, S. scitulum, S. sibiricum, S. sibiricum* subsp. *litorale*, and *S. turcicum*; and a single new combination in *Acrospelion*: *A. distichophyllum*. *Trisetum* s.s. is limited to 1, 2 or 3 species pending further study.

Keywords *Acrospelion*, Aveneae, grasses, molecular systematics, Poeae, *Sibirotrisetum*, Taxonomy, *Trisetum*.

1 INTRODUCTION

Trisetum Pers. [Poaceae (R. Br.) Barnhart: subfamily Pooideae Benth.], the yellow oatgrasses [type = *Trisetum flavescens* (L.) Pers.] traditionally comprises approximately 70 species inhabiting temperate and cold regions, mainly in the northern hemisphere, but also in South America, Australia, and New Zealand (Clayton & Renvoize, 1986; Barberá et al., 2017a, 2017b, 2018a). The morphological characters defining the genus are the perennial habit, 2 to 5-flowered spikelets, upper glume sub-equal or shorter than the spikelet, the bifid, usually dorsally-awned lemmas, gaping, scareous to hyaline paleas, usually glabrous ovaries, and soft, sometimes liquid endosperm. These characteristics along with typical core Pooideae seed, seedling, leaf, lodicule states and base chromosome number of x = 7 (large), led agrostologists to classify *Trisetum* in the narrow subtribe Koeleriinae Asch. & Graebn. with *Koeleria* Pers. (June grasses) [Tzvelev, 1976; Quintanar et al., 2007, 2010; Saarela et al., 2017], or alternatively within a sensu lato subtribe Aveninae J. Presl with *Avena* L. (oats) [Maire, 1953; Clayton & Renvoize, 1986; Grebenstein et al., 1998; Soreng & Davis, 2000; Soreng et al., 2003, 2007, 2015, 2017; Döring et al., 2007; Schneider et al., 2009; Saarela et al., 2010; Wölk & Röser, 2014, 2017].

 Trisetum is critical from an evolutionary and taxonomic point of view since there is a series of related genera (summarized below) some that may have branched from

within *Trisetum*. Its key role as an ancestral lineage for these taxa has been eluded to by different authors (e.g., Mosulishvili, 1991; Quintanar et al., 2010), notably by Clayton & Renvoize (1986) who recognized a "*Trisetum* group," encompassing genera that have traditionally shaped the Koeleriinae in having keeled lemmas and gaping paleas, and generally smaller spikelets relative to Aveninae sensu stricto. In recent decades since the pioneering survey of Soreng & Davis (1998, 2000), several teams have made considerable progress exploring the phylogeny and systematics of *Trisetum* and relatives (Quintanar et al., 2007; Saarela et al., 2010, 2017; Wölk & Röser, 2017). Subtribe Koeleriinae (ca. 250 species) currently consists of the world-wide distributed perennials *Trisetum* and *Koeleria*; the Irano-Turanian-Mediterranean annuals *Trisetaria* Forssk., *Rostraria* Trin., *Avellinia* Parl., and *Gaudinia* P. Beauv.; the American perennials *Graphephorum* Desv., *Leptophyllochloa* C.E. Calderón, *Peyritschia* E. Fourn., and *Sphenopholis* Scribn.; the American annual *Limnodea* L.H. Dewey; a substantially large set of Central and South American species classified either in *Calamagrostis* Adans. or in *Deyeuxia* Clarion ex P. Beauv.; and the recently described *Trisetopsis* Röser & A. Wölk (Barberá et al., 2018b). *Trisetum* s.l. turned out to be polyphyletic as first suggested by Soreng & Davis (2000) and Quintanar et al. (2007).

Recent infrageneric classifications of *Trisetum* by Barberá et al. (2017a, 2017b, 2018a) accepted four sections. *Trisetum* sect. *Acrospelion* (Besser) Pfeiff. (seven species) and *T.* sect. *Sibirica* (Chrtek) Barberá (six species) are mainly distributed in the Old World (Barberá et al., 2017a, 2017b); and *T.* sect. *Trisetaera* Asch. & Graebn. (±20 species) and *T.* sect. *Trisetum* (14 species) are distributed worldwide (Finot et al., 2004, 2005a, 2005b; Finot, 2010; Barberá et al., 2018a). The Mexican and Central American *T.* subg. *Deschampsioidea* (Louis-Marie) Finot (7 species) and several

isolated species such as *T. angustum* Swallen, *T. pringlei* (Scribn. ex Beal) Hitchc., and *T. filifolium* Scribn. ex Beal systems (Finot et al., 2004, 2005a, 2005b), are unplaced in current sectional or generic treatments (Barberá et al., 2017a, 2017b, 2018a).

Saarela et al. (2010) identified two major clades within Koeleriinae in their ITS phylogeny, referring to them as the "Old World *Trisetum* Alliance" and the "New World *Trisetum* Alliance." These two clades were later renamed "Koeleriinae clade A" and "Koeleriinae clade B", respectively (Saarela et al., 2017). Species of *T.* sect. *Acrospelion*, *T.* sect. *Trisetaera*, and Old World species of *T.* sect. *Trisetum* were resolved as part of "Koeleriinae clade A", together with species of *Koeleria*, *Trisetaria*, *Avellinia*, and *Gaudinia*; whereas *T.* subg. *Deschampsioidea* and New World species of *T.* sect. *Trisetum* were part of "Koeleriinae clade B" together with *Leptophyllochloa*, *Peyritschia*, *Sphenopholis*, *Limnodea*, *Trisetopsis*, and the Central and South American species of the *Calamagrostis*/*Deyeuxia* complex. However, relationships among *T.* sect. *Sibirica* and the "Koeleriinae clades A and B" in Saarela et al. (2017) were not resolved perhaps due to the small number of samples (the focus of the Saarela et al. analysis was on *Calamagrostis* s.l. and included only 17 of the 70 species of *Trisetum*). The North American *T. cernuum* and *Graphephorum* present discordant placements within the Koeleriinae in nuclear and plastid trees (Quintanar et al., 2007; Saarela et al., 2017; Wölk & Röser, 2017). To mitigate some of the problems of polyphyly within *Trisetum* noted above, and using our own unpublished results, Soreng et al. (2017) proposed to move the "*Trisetum spicatum* complex" (*T.* sect. *Trisetaera*) to *Koeleria*, as well as accepting generic status for *T.* sect. *Acrospelion*. A fuller solution is not readily apparent due to the complex taxonomic structure of *Trisetum*, particularly in the deep nodes (Barberá et al., in prep.).

Barberá et al. (2017b) included the following seven taxa in *Trisetum* sect.

Sibirica: *T. aeneum* (Hook. f.) R. R. Stewart, *T. bifidum* (Thunb.) Ohwi, *T. henryi*

Rendle, *T. scitulum* Bor ex Chrtek, *T. sibiricum* Rupr. subsp. *sibiricum*, *T. sibiricum*

subsp. *litorale* Rupr. ex Roshev., and *T. turcicum* Chrtek. The section ranges from

Eastern Europe, eastward to Alaska and Yukon Territory, Canada, with a center of

diversity in eastern Asia. The species are characterized in having goldish-brown

spikelets, lemmas with a callus glabrous or with short hairs, and glabrous ovaries.

Chrtek (1968) first differentiated this group within *T.* sect. *Trisetum* as series *Sibirica*

Chrtek, having recurved and nongeniculate awns, not clearly twisted below and

included *T. sibiricum*, the Central Asian *T. altaicum* Stephan ex Roshev., and the

Himalayan species *T. aeneum* and *T. micans* (Hook. f.) Bor. (the latter with some

doubts). Tzvelev (1976) in his treatment of *Trisetum* for the Soviet Union did not

discuss the series but included all ser. *Sibirica* taxa of USSR in *T.* sect. *Trisetum*.

Probatova (1979) and Veldkamp & van der Have (1983) recognized this assemblage

as *T.* subsect. *Sibirica* (Chrtek) Probat. including *T. sibiricum* and *T. turcicum* with

acute lemmas, a callus glabrous or with very short hairs, and short aristules on the

teeth of the lemma. The widespread *T. sibiricum* and the southeastern Asian *T.*

bifidum were sister to the remaining members of the Koeleriinae in the ITS and *matK*

trees of Saarela et al. (2017) and this topology was found in the ITS and the nuclear

gene *topo6* tree of Wölk & Röser (2017). However, in the plastid trees of these two

studies, *T. bifidum* and *T. sibiricum* were resolved in the large polytomy within the

Koeleriinae. Further efforts to effectively characterize and delimit *Trisetum* and

relatives are necessary (Saarela et al., 2017).

Evaluating relationships among genera in the Koeleriinae from morphology alone

is challenging since there are few synapomorphies, and when using molecules since

there is incongruence among markers for some individuals and sets of taxa. Past

reticulation and convergence events were evoked to explain the topological

incongruence among phylogenetic trees in the tribe Poeae R. Br. sensu lato (Soreng &

Davis, 2000; Quintanar et al., 2007, 2010; Gillespie et al., 2008). The objective of the

present study is to present a more focused phylogeny of *Trisetum* and relatives using

plastid (*rps16-trnK*, *rps16*, *rpl32-trnL*) and nuclear ribosomal (ITS) DNA regions

exposing the extensive polyphyly of *Trisetum* and showing that species of sect.

Sibiricum are phylogenetically isolated. We have increased the sampling of species in

this section, including six of seven recognized taxa (all except the Chinese species *T.

henryi*).

2 MATERIAL AND METHODS

2.1 Taxon sampling

We included 74 ingroup samples, representing 48 species and most of the genera (and

type species) within the subtribe Koeleriinae: *Avellinia* (1 species),

Calamagrostis/*Deyeuxia* s.l. (10 species), *Cinnagrostis* (1 species), *Gaudinia* (1

species), *Koeleria* (5 species), *Leptophyllochloa* (1 species), *Limnodea* (1 species),

Peyritschia (2 species), *Rostraria* (3 species), *Sphenopholis* (2 species), *Trisetaria* (4

species), *Trisetopsis* (2 species), and *Trisetum* s.l. (15 species). The monospecific

subtribe Lagurinae, and *Avena barbata* Pott ex Link and *Helictotrichon filifolium*

subsp. *velutinum* (Boiss.) Romero Zarco (Aveninae) were chosen as outgroups.

Acrospelion Besser is included in this analysis. *Graphephorum* was excluded because

it is reticulate between Koeleriinae A and B clades, and *Tzveleviochloa* was excluded

because is reticulate between Aveninae and Koeleriinae. Samples were collected in

silica or obtained from herbarium material from existing collections housed in the

United States National Herbarium (US) and the herbarium of the Royal Botanic

Garden of Madrid (MA), GOET (1 sample), K (1 sample), LE (7 samples), M (1 sample), MEXU (1 samples), MO (1 sample), and NY (1 sample) [herbarium acronyms follow Thiers 2018]. A complete list of taxa and voucher information can be found in Appendix 1.

2.2 DNA extraction, amplification, and sequencing

Most procedures were performed in the Laboratory of Analytical Biology (LAB) at the Smithsonian Institution, and some in the Laboratory of Molecular Systematic (LSM) at the Madrid Royal Botanic Garden. DNA isolation, amplification, and sequencing of *rpl32-trnL* spacer (small single-copy region), *rps16-trnK* spacer, *rps16* intron (large single-copy region), and ITS follows procedures outlined in Peterson et al. (2010a, 2010b). We specifically targeted three plastid regions that were highly variable in previous studies on chloridoid grasses (Peterson et al., 2010a, 2010b, 2011, 2012, 2014a, 2014b, 2014c, 2015a, 2015b).

2.3 Phylogenetic analyses

We used Geneious v.8.0.3 (Kearse et al., 2012) for contig assembly of bidirectional sequences of the four markers, and MUSCLE v.9.1.3 (Edgar, 2004) to align consensus sequences, and then adjust the final alignment manually.

   All the analyses were conducted on the CIPRES science Gateway (Miller et al., 2010). We applied maximum likelihood (ML) and Bayesian searches to infer overall phylogeny. The combined datasets were partitioned in accordance with the number of the markers used. We selected the models of molecular evolution for the cpDNA and nrDNA regions using Akaike´s information criterion, as implemented in MrModeltest v.2.3 (Nylander, 2004). The best fit models for the data partitions were SYM+G for each marker and the combined plastid and nuclear dataset, incorporating a gamma distribution for the combined plastid and ITS. ML analyses were performed using

RAxML-HPC2 on XSEDE (Stamatakis, 2014), assuming a GTR model (default model) using the rapid bootstrap algorithm option, and 1000 replicates for assessing branch support (BS). In all analyses, gaps were treated as missing data. Bootstrapping was automatically halted based on default criteria. Bootstrap (BS) values of 90%–100% were interpreted as strong support, 70%–89% as moderate, and 50%–69% as weak.

Bayesian analyses (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) were performed using Mr. Bayes v.3.2.6 (Ronquist et al., 2012). Two runs were executed each with eight Markov chain Monte Carlo (MCMC) chains for twenty million generations, sampling once per 1000 generations. The analysis was run until the value of the standard deviation of split sequences dropped below 0.01 and the potential scale reduction factor was close to or equal to 1.0. The fraction of the sampled values discarded as burn-in was set at 0.25. Posterior probabilities of 0.95–1.00 were considered to be strong support. Trees were visualized in FigTree v.1.4.3.

3 RESULTS

3.1 Phylogenetic analyses

Ninety-nine percent (283/284) of the sequences used in our study are newly reported here (Appendix 1). All of the sequences of *rpl32-trnL* (73), *rps16 intron* (68), and *rps16-trnK* (70) are newly reported; and 72 sequences of ITS (99%). Sequence alignment length for *rpl32-trnL* is 1589 bp, *rps16 intron* is 1075 bp, *rps16-trnK* is 1044 bp, and ITS is 799. Nine sequences or 0.03% (9/284) were missing.

3.2 Analysis of ITS sequences

The phylogenetic tree derived from ITS sequences (Fig. 1) strongly supports Lagurinae and Koeleriinae as sister (BS = 94, PP = 1.00) while the Koeleriinae clade is weakly supported (BS = 61, PP = 0.53). Koeleriinae includes a strongly supported

Trisetum sect. *Sibirica* (*Sibirotrisetum*) clade (BS = 99, PP = 1.00) sister to the remaining members of the subtribe.

Within *Trisetum* sect. *Sibirica*, one of the samples (*Tatli 5331*) of *Trisetum turcicum* is sister (BS = 97, PP = 1.00) to the remaining samples of the section. Two subclades occur, one moderate to strongly supported (BS = 70, PP = 0.98) containing *T. aeneum* (BS = 92, PP = 1.00) and *T. bifidum*, together with *T. scitulum* (BS = 89, PP = 1.00), and other strongly supported subclade (BS = 96, PP = 1.00) including *T. sibiricum* taxa (subspecies *sibiricum* and *litorale*). The other sample of *T. turcicum* (*Soreng 7950*) is a member of a trichotomy that includes the two subclades, above. The remaining species of Koeleriinae are separated into two clades, a strongly supported (BS = 97, PP = 1.00) "Koeleriinae clade A", and a moderately supported (BS = 79, PP = 0.92) "Koeleriinae clade B."

Within "Koeleriinae clade A", *Trisetum glaciale* is sister to the remaining species, which are included in an unsupported polytomy containing three clades. One of these includes two samples *Trisetum distichophyllum* (BS = 88, PP = 1.00). Another strongly supported clade (BS = 100, PP = 1.00) contains the type species of *Trisetaria* (*T. linearis*) that is sister to a weakly supported clade (BS = 51, PP = 0.53) comprising a strongly supported clade (BS = 91, PP = 0.99) of *Trisetum gracile* and *T. flavescens* (type) sister to a weakly supported clade (BS = 59, PP = 0.53) containing two species of *Rostraria* (*R. litorea* and *R. pumila*). The third strongly supported clade (BS = 91, PP = 1.00) of this polytomy contains a polytomy of four subclades: (1) a strongly supported clade (BS = 92, PP = 0.99) containing *Trisetaria ovata* sister with weak support (BS = 55, PP = 0.53) to *Rostraria cristata*, *Gaudinia fragilis*, and *Trisetaria dufourei−Trisetaria loeflingiana* (BS = 83, PP = 0.99); (2) *Avellinia festucoides*; (3) a weak to moderately supported clade (BS = 69, PP = 0.94)

of *Koeleria vallesiana*, *K. pyramidata*, and *K. crassipes*; and (4) a moderately supported clade (BS = 83, PP = 0.93) of *Koeleria capensis* sister with moderate support (BS = 86, PP = 1.00) to *Trisetum montanum*, *T. spicatum* subsp. *spicatum,* and *T. spicatum* subsp. *virescens* (BS = 88, PP = 1.00), sister to *Koeleria vurilochensis* and *Trisetum rosei* (BS = 75, PP = 0.64).

"Koeleriinae clade B" contains a monophyletic *Sphenopholis* (BS = 77, PP = 1.00) that is sister to the remaining species that form a polytomy. Within this polytomy, the species are organized in several subclades that correspond to: (1) a strongly supported subclade (BS = 93, PP = 1.00) of *Calamagrostis tolucensis−Trisetum virletii*; (2) two samples of *Leptophyllochloa micrathera*; (3) a weak supported clade (PP = 0.57) of *Limnodea arkansana*, *Calamagrostis vulcanica*, and *Trisetum palmeri*; (4) *Calamagrostis erectifolia*; (5) a moderately supported subclade (BS = 80, PP = 0.87) of *Peyritschia*; (6) a weakly supported subclade (BS = 61, PP = 0.85) of *Trisetopsis milanjiana* and *T. elongata*; (7) *Trisetum durangense*, *Deyeuxia rigescens*, *Deyeuxia minima*, and *Calamagrostis pringlei* form a weak subclade (PP = 0.56); and (8) a strongly supported clade (BS = 91, PP = 0.96) of several species of *Deyeuxia* (*Deyeuxia rigida*, *D. tarmensis*, *D. violacea*, and *D. polygama* subsp. *filifolia*) and *Cinnagrostis polygama*.

3.3 Analysis of combined plastid sequences

The phylogenetic tree based on combined plastid sequences (Fig. 2) supports Lagurinae and Koeleriinae as sister (BS = 93, PP = 0.75), while the Lagurinae and Koeleriinae are each strongly supported as separate clades (BS = 100, PP = 1.00). Koeleriinae includes two strongly supported clades corresponding to "Koeleriinae clade A" (BS = 93, PP = 1.00) and "Koeleriinae clade B" (BS = 97, PP = 1.00).

The "Koeleriinae clade A" is organized into three subclades, two strongly supported (BS = 96−100, PP = 1.00) and one weakly supported (BS = 69, PP = 0.80). One strongly supported subclade (BS = 96, PP = 1.00) contains *Trisetum distichophyllum* and *T. glaciale*. A second weakly supported subclade (BS = 69, PP = 0.80) contains two samples of *Avellinia festucoides* sister to a strongly supported clade (BS = 100, PP = 1.00) of *Trisetaria linearis* (type) as sister to a strongly supported clade (BS = 90, PP = 0.85) with two subclades: (1) a moderately supported clade (BS = 87, PP = 1.00) with *Trisetum gracile* as sister to *T. flavescens*; (2) a strongly supported clade (BS = 100, PP = 1.00) with *Rostraria pumila*, *R. litorea*, and *R. cristata* (type). The third strongly supported subclade (BS = 100, PP = 1.00) in the "Koeleriinae clade A" includes two strongly supported clades: (1) a polytomy with three subclades that include: *Gaudinia fragilis*, *Trisetaria ovata*, and a strongly supported clade (BS = 91, PP = 0.90) including *Trisetaria loeflingiana* and *Trisetaria dufourei*; (2) clade (BS = 96, PP = 1.00) with two local clades containing: (a) a moderately supported subclade (BS = 80, PP = 0.95) of *Koeleria capensis* and *K. pyramidata* (type); (b) a strongly supported clade (BS = 99, PP = 1.00) with a moderately supported clade (BS = 77, PP = 0.98) of *Koeleria crassipes*−*K. vallesiana*, sister to a strongly supported clade (BS = 90, PP = 1.00) of *Trisetum montanum*, *T. spicatum* subsp. *spicatum*, *T. spicatum* subsp. *virescens*, *Koeleria vurilochensis*, and *Trisetum rosei*.

"Koeleriinae clade B" includes a strongly supported clade of *Trisetum* sect. *Sibirica* (*Sibirotrisetum*) [BS = 100, PP = 1.00] sister to the remaining species of "Koeleriinae clade B" (BS = 100, PP = 1.00).

Sibirotrisetum contains a polytomy that includes: (1) a poorly supported (PP = 0.94) clade with a moderately supported clade (BS = 76, PP = 1.00) of *T. aeneum*, *T.*

bifidum, and *T. scitulum*, and another with weakly supported clade (BS = 66, PP = 0.99) of three samples of *Trisetum sibiricum* subsp. *sibiricum*; (2) a strongly supported clade (BS = 96, PP = 1.00) with three samples of *Trisetum sibiricum* subsp. *sibiricum* (BS = 86, PP = 0.99); (3) one sample each of *Trisetum sibiricum* subsp. *sibiricum;* (4) on sample of *T. sibiricum* subsp. *litorale*; (5) a weakly supported clade (BS = 66, PP = 0.91) with two samples of *Trisetum turcicum*.

In the second large "Koeleriinae clade B" clade there is a polytomy with three clades: (1) two samples of *Limnodea arkansana* (BS = 99, PP = 1.00); (2) with *Sphenopholis obtusata* and *S. intermedia* (BS = 97, PP = 1.00); (3) a weakly supported clade (BS = 69, PP = 0.97) with two subclades. One weakly supported subclade includes: (1) *Calamagrostis tolucensis*, *Calamagrostis vulcanica*, a strongly supported clade with *Peyritschia koelerioides* and *P. pringlei* (BS = 95, PP = 1.00) sister to a weakly supported clade (BS = 63, PP = 1.00) of *Calamagrostis pringlei*, *Trisetum durangense* (BS = 66, PP = 0.78), and to *Calamagrostis erectifolia*, *Trisetum palmeri*, and *Trisetum virletii* (BS = 59, PP = 0.63). The other strongly supported subclade (BS = 98, PP = 1.00) contains: two samples of *Leptophyllochloa micrathera* sister to *Trisetopsis elongata* and *Trisetopsis milanjiana,* a weakly supported clade (BS = 66, PP = 1.00) of *Deyeuxia rigida* sister to *Deyeuxia minima* and *Deyeuxia rigescens* (BS = 70, PP = 0.99), and (3) unsupported members *Deyeuxia tarmensis*, *Cinnagrostis polygama*, *Deyeuxia polygama* subsp. *filifolia*, and *Deyeuxia violacea*.

3.4 Analysis of combined plastid and ITS sequences. — The overall topology of the combined phylogram (Fig. 3) is similar to that of the plastid-derived tree, even in the terminal branches. As in the plastid phylogram, *Trisetum* sect. *Sibirica* (*Sibirotrisetum*) is included in "Koeleriinae clade B" and sister to the remaining

members. Some notable minor differences with the plastid phylogeny occur in "Koeleriinae clade A", where *Avellinia festucoides* clade is sister (with weak support, BS = 66, PP = 0.8) to a *Trisetaria ovata*, *Gaudinia fragilis*, *Trisetaria dufourei*, and *T. loeflingiana* clade plus a clade of *Trisetum spicatum*, *T. rosei*, *T. montanum*, and the *Koeleria* species clade. Another difference with the combined plastid tree is placement of *Trisetum* sect. *Sibirica* in "Koeleriinae clade B". One sample of *Trisetum turcicum* (*Tatli 5331*) is sister to the remaining species of this clade, also found in the ITS-derived phylogram.

There are two main differences with the ITS and combined plastid trees. In "Koeleriinae clade A", *Trisetaria linearis* is sister (with strong support, BS = 99, PP = 1.00) to *Rostraria cristata* (only plastid accessions), *R. pumila*, and *R. litorea*. While in "Koeleriinae clade B", *Limnodea arkansana*, *Sphenopholis intermedia*, and *S. obtusata* form a clade (weak supported, BS = 61, PP = 0.69) sister to the remaining species of "Koeleriinae clade B" instead *Limnodea* aligning among those.

4 DISCUSSION

Our analyses show that *Trisetum* s.l., as traditionally circumscribed, is polyphyletic with representative species distributed in multiple clades that include the other genera of Koeleriinae (see Figs. 1, 2, 3, names in blue and red text). The species align in both Koeleriinae A (with *Acrospelion*, *Avellinia*, *Gaudinia*, *Koeleria*, *Rostraria*, *Trisetaria* *s.s.* and *s.l., Trisetum s.s.*), and Koeleriinae B (with *Cinnagrostis, Leptophyllochloa, Limnodea, Peyritschia, Sphenopholis,* and *Trisetopsis*). This is the first study to include the type species of all these genera (see Fig. 3 with labeled types). Our subdivision of the Koeleriinae into clades A and B is in agreement with Saarela et al. (2017) who employed a different set of plastid markers to confirm these two clades. Given this extensive polyphyly, if *Trisetum* monophyly is to be maintained, the genus

will be limited to its type species, *T. flavescens*, and perhaps one or two others. If it is expanded at all beyond this, it would be supplanted in priority by the older name *Trisetaria*. Kellogg (2015), relying on earlier published DNA studies and sequences with more limited sampling within genera, proposed lumping most of the Koeleriinae into *Trisetaria,* while accepting *Graphephorum*, *Limnodea* and *Sphenopholis*, and not mentioning *Acrospelion*, *Cinnagrostis* or *Trisetopsis*. Deeper sampling in recent investigations have shed new light on the problem (Saarela et al., 2017; Barberá et al., 2018b and in prep.). For instance, we now have data indicating most of the Central and South American *Deyeuxia* or *Calamagrostis* s.l. belong in Koeleriinae clade B. The oldest available name for these species appears to be *Cinnagrostis*, a little known genus described by Grisebach (1874) for a single dichogamous species, *C. polygama* (type), initially thought by the author to be near *Cinna* L. and *Agrostis* L. Now that we have evidence of substantial phylogenetic structure within Koeleriinae we are breaking up *Trisetum* s.l. into smaller genera as proposed by Soreng et al. (2017), yet some new genera and substantial realignments of species are needed. We have sampled nearly all of the 70 species of *Trisetum* s.l. (Barberá et al., 2018b) and there is strong evidence of reticulation among several other lineages. In our study *Sibirotrisetum* appears as a phylogenetically isolated and strongly supported lineage. All species of *Sibirotrisetum* align in a strongly supported monophyletic clade sister to the remaining species of "Koeleriinae clade B" in the plastid (Fig. 2) and combined plastid/ITS (Fig. 3) trees whereas in the ITS-derived tree they are sister with weak support to the entire Koeleriinae. Previous molecular studies (Saarela et al., 2017; Wölk & Röser, 2017) published before the revision of the section by Barberá et al. (2017b), identified a lineage in the plastid and nuclear trees that included only *T. sibiricum* and *T. bifidum*, and their relationship within the Koeleriinae was

unresolved. We have increased the species sampling here to include six of the seven taxa of *T*. sect. *Sibirica* (all except the Chinese species *T. henryi*), and propose a new genus for this group.

Trisetum turcicum, a species often confused with *T. sibiricum* but clearly differentiated by its longer anthers and geniculate awns (Probatova, 1979; Barberá et al., 2017b), is a member of *T*. sect. *Sibirica*. However, in our ITS and combined plastid/ITS trees, the *Tatli 5331* sample (from Turkey) was sister to the remaining clade while the *Soreng 7950* sample (from the Caucasus) appeared in a polytomy. Further study of this species is needed.

Trisetum bifidum and *T. scitulum* appear closely related and are sympatric in their distribution. Both species have morphological similarities that include very lax panicles and paleas much shorter than the lemma. These two species formed a clade together with the Himalayan species *T. aeneum*, a species with short anthers.

To test our subspecific ranking we included seven samples from different Asian regions of *T. sibiricum*, the most polymorphic and widespread species of this section. All samples of this species form a clade in the ITS (strongly supported) and plastid/ITS trees. However, in the plastid tree, three samples of *T. sibiricum* subsp. *sibiricum* from Kyrgyzstan form a clade sister to *T. bifidum*, *T. scitulum*, and *T. aeneum* while the remaining samples of *T. sibiricum* subsp. *litorale* and *T. sibiricum* subsp. *sibiricum* from Mongolia, Russia, and Alaska form a polytomy. None of our topologies present support for a monophyletic origin of the two subspecies, perhaps further study using low copy nuclear genes might resolve their relationships.

Since *Avena distichophylla*, lectotype species designated by Pfeiffer (1871) for *Tristeum* sect. *Acrospelion*, has not yet been combined in *Acrospelion*, we make the new combination below. We previously accepted *Acrospelion* placing 14 species in

the genus (Soreng et al., 2017). More study is needed on this genus since the nuclear ITS marker is not congruent with the plastid signal, suggesting hybridization.

5 TAXOMOMY

Because our molecular analysis recognizes a monophyletic and morphologically cohesive *Trisetum* sect. *Sibirica*, isolated from *Trisetum* s.s., we elevate the section to generic rank and provide seven new combinations. We also provide a new combination in *Acrospelion*. The species preceded by an asterisk (*) was not included in our DNA analysis.

Acrospelion distichophyllum (Vill.) Barberá, **comb. nov.** ≡ *Avena distichophylla* Vill., Prosp. Hist. Pl. Dauphine 16, tab. 1, f. 3. 1779 ≡ *Avena disticha* Lam., Encycl. 1: 333. 1783, nom. superfl. ≡ *Trisetum distichophyllum* (Vill.) P. Beauv., Ess. Agrostogr. 88, 153. 1812 ≡ *Trisetaria distichophylla* (Vill.) Paunero, Anales Jard. Bot. Madrid 9: 514. 1950 — Type: FRANCE. Dauphiné, 45°0'N, 5°15'E, *D. Villars 1489* (lectotype, designated by P. Barberá et al., Syst. Bot. 42(4): 759. 2017: P-LAM-564210! [image P00564210!]).

Sibirotrisetum Barberá, Soreng, Romasch., Quintanar & P.M. Peterson, **stat. nom. & gen. nov.** ≡ *Trisetum* ser. *sibirica* Chrtek, Acta Univ. Carol., Biol. 1967: 107. 1968 ≡ *T.* subsect. *Sibirica* (Chrtek) Prob., Novosti Sist. Vyssh. Rast.15: 20. 1979 ≡ *T.* sect. *Sibirica* (Chrtek) Barberá, Syst. Bot. 42: 784. 2017b — Type: *Trisetum sibiricum* Rupr.

Diagnosis. The species of *Sibirotrisetum* differs from *Trisetum flavescens* (L.) P. Beauv. in having panicles with a glabrous rachis or slightly hairy on the upper part, golden-brown, rarely pale yellowish spikelets, a glabrous or with short hairs up to 0.7 mm long callus, and a recurved or basally slightly twisted and rarely geniculate dorsal awn.

Description. Perennial herbs, habit subrhizomatous with slightly isolated shoots in loose tufts or strictly tufted. Culms 12–150 (–165) cm tall, straight, glabrous; internodes elongated and separated along the culms, rarely with short internodes concentrated below; extravaginal shoots from arising from the lower nodes, rarely from upper nodes. Leaf sheaths sometimes longer than the internodes, glabrous to hairy, usually ciliate, margins of uppermost fused (5–) 10 or more mm (2.5–66% the length). Collars centric. Ligules 0.4–3.5 mm long, membranous, glabrous or hairy abaxially, apex truncate, denticulate or lacinate usually with some hairs on the upper part. Leaf blades (1.2–) 2–29.5 (–34.5) cm long x 1.5–8 (–11) mm wide, linear, flat, non-rigid, smooth or adaxially sparsely scabrous, glabrous or loosely hairy, usually ciliolate, midrib rarely conspicuously marked, persistent. Panicles lax, sometimes dense, oblong to elliptic or lanceolate in outline, sometimes ovate, with glabrous rachis or slightly hairy on the upper part, branches smooth or lightly to moderately rarely densely scabrous distally. Spikelets (4.5–) 5.8–9.7 (–11.1) mm long, with (1−) 2–3 (−4) bisexual flowers, compressed laterally, pedicellate, disarticulating above the glumes, golden-brown, rarely pale yellowish; rachilla hairy. Glumes unequal, rarely subequal, back thinly herbaceous (at least of upper glume), scabrous from the middle to top of the midvein and along the margins, outer sides and margins scareous, apex acuminate or sometimes acute; lower glumes (0.7–) 2.8–7.2 mm long, narrowly to broadly lanceolate, rarely narrowly elliptic, 1-veined; upper glumes (3.7–) 4.6–8 (–8.7) mm long, narrowly elliptic or elliptic to oblanceolate, sometimes lanceolate, 3-veined. Lemmas (3.8–) 4.7–8.7 mm long, herbaceous to chartaceous with narrow scareous margins, elliptic to oblong or lanceolate, golden brown or brownish to yellowish, punctate to scabridulous with prickle hairs from middle to the upper part of the midvein, 3-veined, apex with two apical teeth ending in two aristules, rarely

without them; dorsal awn (3–) 4.7–15 mm long, inserted near the middle of the lemma, sometimes closer to the apex, recurved or basally slightly twisted, rarely geniculate; callus short, obscure or prominent, obliquely angled dorsally, smooth, glabrous or with short stiff hairs up to 0.7 mm long, scar orbicular to elliptical. Paleas ½ to as long as the lemma, hyaline, obscurely 2-keeled, keels scaberulous distally, apex toothed. Lodicules 2, membranous, free, glabrous, apex lobate with 2 or 3 lobes, the lobes sometimes irregular, rarely lacinate or denticulate. Stamens 3; anthers (0.6–) 1–4 mm long. Ovary glabrous. Caryopsis ca 2 mm long, narrowly elliptic to oblong in side view, laterally moderately compressed, not sulcate. Hilum narrowly elliptical, less than 1/5 the grain in length. Endosperm soft and liquid (with lipid).

Sibirotrisetum aeneum (Hook. f.) Barberá, **comb. nov.** ≡ *Avena aenea* Hook. f., Fl. Brit. India 7: 279. 1896 ≡ *Trisetum aureum* Nees ex Steud., Syn. Pl. Glumac. 1: 225. 1854, nom. illeg., non *Trisetum aureum* (Ten) Ten. 1820 ≡ *Trisetum aeneum* (Hook. f.) R.R. Stewart, Brittonia 5: 431. 1945 — Type: INDIA. Himachal Pradesh, Shimla distr., Buran Pass, Kedarkanta, 31°23'N 78°8'E, *J. F. Royle 94* (lectotype, designated by Barberá et al., 800. 2017b: LIV–1952.121.10949 [image!]; isolectotypes: LIV–1952.121.10948 [image!], LIV–1952.121.10950 [image!]).

Sibirotrisetum bifidum (Thunb.) Barberá, **comb. nov.** ≡ *Bromus bifidus* Thunb., Syst. Veg. ed. 14: 119. 1784 ≡ *Trisetum bifidum* (Thunb.) Ohwi, Bot. Mag. (Tokyo) 45: 191. 1931 — Type: JAPAN. Probably near Nagasaki, *C. P. Thunberg s.n.* (lectotype, designated by Veldkamp & Van der Have, 129. 1983: UPS-THUNB–002576 [image!]; isolectotypes: L–50296!, P–2255904!, S–13–31156!, S–13–31784!).

*_Sibirotrisetum henryi_ (Rendle) Barberá, **comb. nov.** ≡ _Trisetum henryi_ Rendle, J. Linn. Soc., Bot. 36: 400. 1904 — Type: CHINA. Hubei, Fang Xian, 32°05'N 110°35'E, 1885–1888, _A. Henry 6643_ (lectotype, designated by Barberá et al., 798. 2017b: K–808693!; isolectotypes: B–10_0279997!, BM–959387!, GH–24560!).

Sibirotrisetum scitulum (Bor ex Chrtek) Barberá, **comb. nov.** ≡ _Avena flavescens_ Hook. f. 1897, nom. illeg. hom., non _Trisetum flavescens_ L. 1753 ≡ _Trisetum scitulum_ Bor ex Chrtek, Acta Univ. Carol., Biol. 1967: 105. 1967— Type: INDIA. Sikkim, 27°20'N 88°37'E, _J. D. Hooker s.n._ (holotype: K–32269!; isotypes: GH!, K!, L–50297 [image!], P–2255859!, W–25045!).

Sibirotrisetum sibiricum (Rupr.) Barberá, **comb. nov.** ≡ _Trisetum sibiricum_ Rupr., Beitr. Pflanzenk. Russ. Reiches 2: 65. 1845 ≡ _Trisetum flavescens_ var. _sibiricum_ (Rupr.) Ostenf. ex B. Fedtsch., Izv. Imp. Bot. Sada Petra Velikago 14 (Suppl. 2): 64. 1913 ≡ _Trisetum bifidum_ subsp. _sibiricum_ (Rupr.) T. Koyama, Grass. Jap. Neighb. Reg. 533. 1987 ≡ _Trisetaria sibirica_ (Rupr.) Banfi & Soldano, Atti Soc. Ital. Sci. Nat. Mus. Civico Storia Nat. Milano 135(2): 385. 1996 — Type: RUSSIA. Bashkiria [now Bashkortostan Republic], Belaja River, 55°53'N 53°36'E, _F. J. I. Ruprecht s.n._ (lectotype, designated by Tzvelev, 262. 1976: LE–01043584!).

Sibirotrisetum sibiricum subsp. **_litorale_** (Rupr. ex Roshev.) Barberá, **comb. nov.** ≡ _Trisetum sibiricum_ subsp. _litorale_ Rupr. ex Roshev., Izv. Glavn. Bot. Sada R. S. F. S. R. 21: 90. 1922 — Type: RUSSIA. Kanin Peninsula [Arkhangelsk Oblast], 67°27'N 47°32'E, 13–14 Aug, _F. J. I. Ruprecht s.n._ (lectotype, designated by Tzvelev, 262. 1976: LE–01043580!; isolectotype: LE–01043581).

Sibirotrisetum turcicum (Chrtek) Barberá, **comb. nov.** ≡ _Trisetum turcicum_ Chrtek, Bot. Not.119: 487. 1966 — Type: TURKEY. Lazistan, Djimil valley, 40°40'N

39°40'E, Aug 1866, *B. Balansa 1551* (holotype: PRC–454965 [image!]; isotypes: G–176299!, G–176300!, GH!, GOET–6970!, JE–18873!, K–808699!, LE–9412!, LE–9413!, P–3644578!, P–3645245!, P–3351081!, P–3351082!, P–3351137!, P–2248780!, P–2248781!, RO [image!], US–1064357!, W–1889–0033667!).

ACKNOWLEDGEMENTS

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Figures

Fig. 1. Maximum-likelihood tree inferred from nuclear ribosomal ITS sequence data.

Numbers above branches are bootstrap values; numbers below branches are posterior

probabilities; color blue indicates species of *Trisetum s.l.*; color red indicates species

now included in *Sibirotrisetum*; scale bar = 2% substitutions/site.
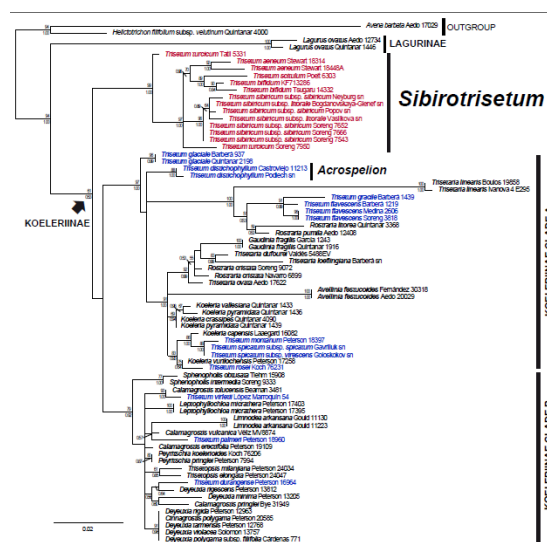
Fig. 2. Maximum-likelihood tree inferred from combined plastid (*rpl32-trnL*, *rps16 intron*, and *rps16-trnK*) sequences. Numbers above branches are bootstrap values; numbers below branches are posterior probabilities; color blue indicates species of *Trisetum s.l.*; color red indicates species now included in *Sibirotrisetum*; scale bar = 0.3% substitutions/site.
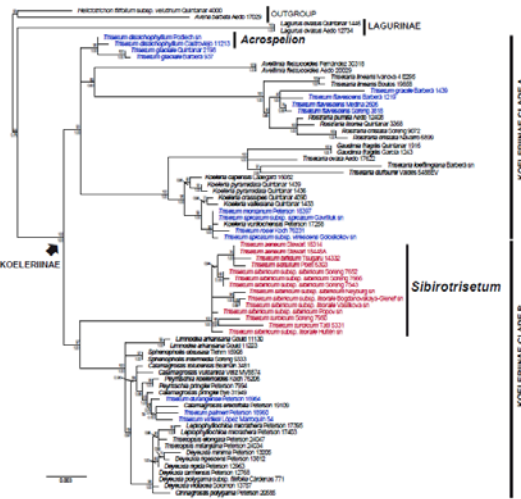

Fig. 3. Maximum-likelihood tree inferred from combined plastid (*rpl32-trnL*, *rps16 intron*, and *rps16-trnK*) and ITS sequences. Numbers above branches are bootstrap values; numbers below branches are posterior probabilities; color blue indicates species of *Trisetum s.l.*; color red indicates species now included in *Sibirotrisetum*; **T** indicates the type species of the genus; scale bar = 0.4% substitutions/site.