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, Limmodea, Peyritschia, Rostraria, Sphenopholis, Trisetaria, Trisetopsis, Trisetum), along with three outgroups, confirm previous indications of extensive polyphyly of Trisetum. We focus on the monophyletic Trisetum sect. Sibrica 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 one, two or three species, pending further study.

Key words: 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 and in South America, Australia, and New Zealand (Clayton & Renvoize, 1986; Barberá et al., 2017a, 2017b, 2018a). The morphological characteristics defining the genus are perennial habit, two to five-flowered spikelets, upper glume sub-equal or shorter than the spikelet, the bifid, usually dorsally-awned lemmas, gaping, scariosus 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 et al., 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 of which may have branched from within Trisetum. Its key role as an ancestral lineage for these taxa has been alluded 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 worldwide 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., Leptophylochloa C.E. Calderón, Peyritschia E. Fourn., and Sphenopholis Scribn.; the American annual Linnmodea 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. Acropelion (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. Trisetaria 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. Deschampsioideae (Louis-Marie) Finot (7 species) and several isolated species such as T. angustum Swallen, T. pringieei (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 internal transcribed spacer (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. Acropelion, T. sect. Trisetaria, 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. Deschampsioideae and New World species of T. sect. Trisetum were part of “Koeleriinae clade B” together with Leptophylochloa, Peyritschia, Sphenopholis, Linnmodea, 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 Trin. 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. Trisetaria) to Koeleria, as well as accepting generic status for T. sect. Acropelion. 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 the series Sibirica Chrtek, having recurved and nongenulate 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 arilostes 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 top6 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 Pooideae. 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. Sibirica 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), Leptophylochloa (1 species), Linnmodea (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. Acropelion Besser is included in this analysis. Graphephorum was excluded because it is reticulate between Koeleriinae A and B clades, and Tzvelevichloa was excluded because it 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...
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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 follow the 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 adjusted 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 the overall phylogeny. The combined datasets were partitioned in accordance with the number of 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 generalized time-reversible (GTR) model (default) using the rapid bootstrap algorithm option, and 1000 replicates for assessing branch support. 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 probability (PP) 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 S1). All of the sequences of rpl32-trnL (73), rps16 intron (68), and rps16-trnK (70) are newly reported; and 72 sequences of ITS (99%) are newly reported. The 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 the 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 = 79, 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 clade (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 glacie is sister to the remaining species, which are included in an unsupporply containing three clades. One of these includes two samples of 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. pulimia). The third strongly supported clade (BS = 91, PP = 1.00) of this polytomy contains a polytomy of four subclades: (i) 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); (ii) Avellinia festucoides; (iii) a weak to moderately supported clade (BS = 69, PP = 0.94) of Koeleria vallesiana, K. pyramididata, and K. crusipes; and (iv) a moderately supported clade (BS = 83, PP = 0.95) 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 virillochensis and Trisetum rosei (BS = 75, PP = 0.64).

“Koeleriinae clade B” contains a monophyletic Sphenophilos (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: (i) a strongly supported subclade (BS = 93, PP = 1.00) of Calamagrostis toluensis—Trisetum virile; (ii) two samples of Leptophylochloa micrathena; (iii) a weak supported clade (PP = 0.57) of Limnodra arkansana, Calamagrostis vulcanica, and Trisetum palmeri; (iv) Calamagrostis erectifolia; (v) a moderately supported subclade (BS = 80, PP = 0.87) of Peyritschia; (vi) a weakly supported
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 = 1.00), 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. glaciare. A second weakly supported clade (BS = 87, PP = 0.80) contains two samples of Avellinia festuoides 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: (i) a moderately supported clade (BS = 87,
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Fig. 2. Maximum-likelihood tree inferred from combined plastid (rpl32-trnl, rps6 intron, and rps6-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.
T. sibiricum subsp. litorale; (v) 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: (i) two samples of Limnodea arkansana (BS = 99, PP = 1.00); (ii) with Sphenopholis obtusata and S. intermedia (BS = 97, PP = 1.00); (iii) a weakly supported clade (BS = 69, PP = 0.97) with two subclades. One weakly supported subclade includes (i) 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 = 0.99) of Calamagrostis pringlei, Trisetum durangense (BS = 66, PP = 0.78), and to Calamagrostis erecifolia, Trisetum palmeri, and Trisetum virletii (BS = 59, PP = 0.63). The other strongly supported clade (BS = 98, PP = 1.00) contains two samples of Leptophylochloa microthera 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 (iii) 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. 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 dufouriei, 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 the placement of Trisetum sect. Sibirica in “Koeleriinae clade B.” One sample of Trisetum turicum (Tatlı 5331) is sister to the remaining species of this clade, also found in the ITS-derived phylogram.

There are two main differences between 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 of 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. 1–3, 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, Leptophylochloa, Limnodea, Peyritzchia, 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. flavesens, 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 has shed new light on the problem (Saarela et al., 2017; Barberá et al., 2018b and in prep.). For instance, we now have data indicating that most of the Central and South American Deyeuxia or Calamagrostis s.l. belong to Koeleriinae clade B.

All species of Sibirotisetum 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 sisters with a weak support to the entire Koeleriinae. Previous molecular studies (Saarela et al., 2017; Wölky & 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 turicum, 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 Tatlı 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.

4.1779

5 Taxonomy

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. comb. nov.
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Prominent, obliquely angled dorsally, smooth, glabrous or twisted, rarely geniculate; callus is short, obscure or -1 narrowly elliptic or elliptic to oblanceolate, sometimes acute; lower glumes (0.7 microns to 1 millimeter long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes acute; lower glumes (0.7 – 1.5 millimeters long, narrowly elliptic to oblanceolate, sometimes ac...
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Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12533/suppinfo:

Appendix S1 List of specimens sampled. Taxon name, country and next division, voucher (collector, number, and where the specimen is housed), and GenBank accession number for DNA sequences ITS, rps16-trnK, rps16 intron, and rpl32-trnL; a dash (–) indicates missing data; *asterisk indicates the sequences was previously published in GenBank.