








## Research Article

# Phylogeny and biogeography of *Calamagrostis* (Poaceae: Pooideae: Poeae: Agrostidinae), description of a new genus, *Condilorachia* (Calothecinae), and expansion of *Greeneochloa* and *Pentapogon* (Echinopogoninae)

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**Abstract** To investigate the evolutionary relationships and biogeographical history among the species of *Calamagrostis* and other members of subtribes Agrostidinae, Calothecinae, Echinopogoninae, and Paramochoilinae, we generated a phylogeny based on DNA sequences from one nuclear ribosomal (ITS) and three plastid regions (*rpl32-trnL* spacer, *rps16-trnK* spacer, and *rps16* intron). Based on our phylogeny, we identified seven species groups (clades) within *Calamagrostis*: the Meridionalis group comprises two species from Central and South America, the Americana group comprises species from North America, the *Deyeuxia* and *Epigeios* groups comprise species from Eurasia, the Orientalis group comprises species from East Asia, the *Purpurea* group comprises species from Eurasia and North America, and the *Calamagrostis* group comprises species from Eurasia and North America. We hypothesize that *Calamagrostis* originated in North America with the primary split of the Meridionalis group, followed by split between the autochthonous Americana group and two future Eurasian branches encompassing all the remaining groups, which possibly dispersed into Eurasia independently. The molecular data suggest that hybridization and genomic introgression played a prominent role in the evolutionary history of *Calamagrostis*. We propose a new genus, *Condilorachia*, segregated from *Trisetum* s.l., with three species from South America for which we propose new combinations: *Condilorachia bulbosa*, *Condilorachia brasiliensis*, and *Condilorachia juergensii*; a new combination in *Greeneochloa*, *Greeneochloa expansa*; and the subsumption of *Dichelachne* into *Pentapogon* with 20 new combinations: *Pentapogon avenoides*, *Pentapogon brassii*, *Pentapogon chaseianus*, *Pentapogon crinita*, *Pentapogon densus*, *Pentapogon frigidus*, *Pentapogon gunnianus*, *Pentapogon hirtella*, *Pentapogon inaequiglumis*, *Pentapogon lautumia*, *Pentapogon micrantha*, *Pentapogon parva*, *Pentapogon quadrisetus*, *Pentapogon rara*, *Pentapogon robusta*, *Pentapogon scaberulus*, *Pentapogon sclerophyllus*, *Pentapogon suizanensis*, *Pentapogon sieberiana*, and *Pentapogon validus*. We provide a diagnosis, description, and a key to the species of *Condilorachia*.

**Key words:** *Calamagrostis*, *Dichelachne*, grasses, *Greeneochloa*, ITS, molecular systematics, *Pentapogon*, plastid sequences, taxonomy.

## 1 Introduction

Pooideae is one of three grass subfamilies comprising the BOP clade (Bambusoideae Luerss., Oryzoideae Kunth ex Beilschm., and Pooideae Benth.) and the largest of the 12 subfamilies in the Poaceae Barnhart, with 4124 species in 219 genera (Soreng et al., 2022a). Economically important species

in the subfamily include crops in genera *Triticum* L. (wheat), *Hordeum* L. (barley), and *Avena* L. (oats); numerous turfgrasses in genera such as *Agrostis* L., *Festuca* L., *Lolium* L., and *Poa* L.; and important pasture and wild forage grasses in genera such as *Alopecurus* L., *Dactylis* L., *Elymus* L., and *Phleum* L. Major lineages within the Pooideae have previously been identified (Macfarlane & Watson, 1980, 1982; Soreng

et al., 1990, 2007; Catalán et al., 2004; Davis & Soreng, 2007; Quintanar et al., 2007; Schneider et al., 2009, 2012; Saarela et al., 2010, 2015, 2017; Romaschenko et al., 2012; Barberá et al., 2019a, 2019b; Peterson et al., 2019a, 2019b, 2020; Sylvester et al., 2020; da Silva et al., 2022) and classified as supertribes, tribes, subtribes, and other subfamilial taxa (e.g., Clayton & Renvoize, 1986; Watson & Dallwitz, 1992 onwards; Grass Phylogeny Working Group, 2001; Soreng et al., 2003, 2007). Recent classifications recognize 15 (Soreng et al., 2017, 2022) and 10 tribes (Kellogg, 2015) in Pooideae. Of these, the diverse tribe Poeae R. Br. includes ca. 2693 species in 134 genera distributed in cool-temperate, Mediterranean, and Arctic climates (Soreng et al., 2022b).

Phylogenetic analyses of plastid DNA have identified two major clades in Poeae (Soreng & Davis, 2000; Döring et al., 2007; Quintanar et al., 2007; Schneider et al., 2009; Grass Phylogeny Working Group II, 2012; Saarela et al., 2015, 2018; Orton et al., 2021). Soreng & Davis (2000) described these clades as “taxa with Aveneae-type plastid DNA” and “taxa with Poeae-type plastid DNA” because most taxa in each clade were traditionally recognized in tribes Aveneae or Poeae s.str. These clades have since been referred to informally in various ways, often labeled “1” or “2,” with “1” always referring to the clade with Aveneae-type plastid DNA and “2” always referring to the clade with Poeae-type DNA. We refer to these two clades as “Poeae chloroplast group 1” and “Poeae chloroplast group 2” (Soreng et al., 2003; Schneider et al., 2009; Saarela et al., 2015; Soreng et al., 2015a, 2017).

Our investigation includes genera classified into four closely related subtribes in Poeae chloroplast group 1 (crown age of 25.28 My; Gallaher et al., 2022) that form super-subtribe Agrostidodinae Soreng. Subtribe Calothecinae Soreng consists of nine genera and at least two South American species of *Trisetum* Pers. s.l.: *Boldrinia* L.N. Silva, *Calotheca* Desv., *Chascolytrum* Desv., *Erianthecium* Parodi, *Lombardochloa* Roseng., *Microbriza* Parodi ex Nicora & Rúgolo, *Poidium* Nees, *Rhombolytrum* Link (syn.—*Gymnache* Parodi), *Rosengurttia* L.N. Silva, *Trisetum brasiliense* Louis-Marie, *T. bulbosum* Hitchc., and possibly *T. juergensii* Hack (Da Silva et al., 2022; Soreng et al., 2022a). Subtribe Paramochloinae L.N. Silva consists of two genera: *Laegaardia* P.M. Peterson, Soreng, Romasch. & Barberá and *Paramochloa* P. M. Peterson, Soreng, Romasch. & Barberá (Peterson et al., 2019a; Da Silva et al., 2022). Subtribe Echinopogoninae Soreng (Soreng et al., 2017) consisted of five genera: *Echinopogon* P. Beauv., *Pentapogon* R. Br., *Relchela* Steud., *Ancistragrostis* S. T. Blake, and *Dichelachne* Endl., revised to include four genera (Soreng et al., 2022a). Subtribe Agrostidinae Fr. consists of nine genera: *Agrostis* (syn.—*Bromidium* Nees & Meyen), *Agrostula* P.M. Peterson, Soreng, Romasch. & Sylvester, *Alpagrostis* P.M. Peterson, Soreng, Romasch. & Sylvester, *Calamagrostis* Adans. p.p. (syn.—*Ammophila* Host, *Deyeuxia* Clarion ex P. Beauv.), *Gastridium* P. Beauv., *Lachnagrostis* Trin., *Podagrostis* (Griseb.) Scribn. & Merr., *Polypogon* Desf., and *Triplachne* Link (Saarela et al., 2017; Soreng et al., 2017; Peterson et al., 2020).

The Agrostidinae is characterized by single-flowered spikelets and consists of ca. 412 species (Soreng et al., 2022a). Agrostidinae includes the diverse, ecologically important, and taxonomically difficult genera *Agrostis*

(ca. 200 species) and *Calamagrostis* (ca. 130 species) (Soreng et al., 2022a). Gallaher et al. (2022), in a biogeographical study of the grasses, found the subtribe to have a stem and crown date of 12.58 and 11.35 Ma, respectively. Historically, the major unresolved taxonomic problems in Agrostidinae were included in the circumscriptions of *Calamagrostis* and *Deyeuxia* (Clayton & Renvoize, 1986; Simon, 2014; Clayton et al., 2016; Soreng et al., 2003; Peterson & Saarela, 2012). The most comprehensive phylogenetic study of *Calamagrostis* and *Deyeuxia* to date was conducted by Saarela et al. (2017), who sampled 52 species of these genera in the context of a broader phylogenetic study. Among their key findings, Saarela et al. (2017) found that the types of *Deyeuxia* and *Calamagrostis* are closely related, supporting classification of the name *Deyeuxia* as a synonym of *Calamagrostis* (Kellogg, 2015; Soreng et al., 2015a, 2017, 2022a), that many American *Deyeuxia* species are part of subtribe Aveninae J. Presl, and that species classified in *Deyeuxia* sect. *Stylagrostis* (Mez) Rúgolo & Villav. are nested within *Deschampsia* P. Beauv. Accordingly, reassessment and reclassification of species once placed in *Deyeuxia* have begun. For example, Saarela et al. (2017) transferred species of *Deyeuxia* sect. *Stylagrostis* to *Deschampsia* and Peterson et al. (2019a) transferred most of the remaining American species of *Deyeuxia* into *Cinnagrostis* Griseb. (subtribe Aveninae).

Using plastid (*rpl32-trnL*, *rps16-trnK*, and *rps16* intron) and nuclear ITS nrDNA sequence markers, we present a new phylogenetic analysis of 114 species in 20 genera of tribe Poeae, emphasizing relationships within *Calamagrostis*, of which we include 73 species. An estimated 130 species are still considered to belong to the genus after removing those excluded based on DNA studies or related to the latter by morphology (Soreng et al., 2022a). A distinctive feature of the current analysis is the discriminant use of ITS nrDNA data. Notwithstanding the importance of ITS sequence data in understanding relationships among species, a number of molecular genetic processes impact the use of ITS sequences in ways that may mislead phylogenetic inference. Common complications in any phylogenetic inference are ancient or recent duplication events and intra- or interarray homogenization events. These phenomena can potentially, separately and collectively, confound a phylogenetic reconstruction (Alvarez & Wendel, 2003). We believe that this condition is especially prominent in *Calamagrostis*, which, to some extent, has prevented clear and meaningful phylogenetic reconstructions of the genus (Saarela et al., 2017). Significant progress in the phylogenetic reconstruction of *Calamagrostis* is made in our paper by selectively removing confounding ITS sequences and their subsequent testing against the “core” data set representing a resolved phylogeny. The rationale behind this procedure is to identify the main evolutionary lineages within the genus and untangle the complex evolutionary history of the species. This approach provided valuable insights into the origins, biogeography, and relationships among the species.

In addition, we discuss the biogeography of the principal genera examined in our study, propose changes to the classification of three South American species of *Trisetum* Pers., propose subsumption of *Dichelachne* and *Sclerodeyeuxia* (Stapf) Pilg. into *Pentapogon*, with new combinations for

20 species from Australasia, and provide a new combination for a species of *Calamagrostis* from Hawaii.

## 2 Material and Methods

### 2.1 Taxon sampling

Our sampling consists of 220 samples, representing 114 species (74 species from *Calamagrostis* as currently recognized) and 20 genera. A complete list of taxa including authorities, voucher information, and GenBank numbers can be found in Appendix S1. In most cases, we extracted DNA from silica dried material collected by the authors. The remaining samples were taken from specimens in the United States National Herbarium (US) and the Real Jardín Botánico (MA).

It is necessary to include the types of genera and other higher taxa when conducting molecular studies and revising taxonomic classifications based on phylogenetic results so that names of higher taxa can be correctly applied to lineages. The following species included in our analyses are the types of their respective genus: *Agrostis canina* L. (conserved name), *Agrostula truncatula* (Parl.) P.M. Peterson, Soreng, Romasch. & Sylvester, *Alpagrostis alpina* (Scop.) P.M. Peterson, Soreng, Romasch. & Sylvester, *Calamagrostis canescens* (Weber) Roth, *Chascolytrum subaristatum* (Lam.) Desv., *Deyeuxia pyramidalis* (Host.) Veldkamp [= *Calamagrostis arundinacea* (L.) Roth], *Dichelachne micrantha* (Cav.) Domin (heterotypic synonym of the type, *Dichelachne montana* Endl.), *Echinopogon ovatus* (G. Forst.) P. Beauv., *Gastridium ventricosum* (Gouan) Schinz & Thell., *Greeneochloa coarctata* (Eaton) P.M. Peterson, Soreng, Romasch. & Barberá, *Laegaardia ecuadoriensis* (Laegaard) P.M. Peterson, Soreng, Romasch. & Barberá, *Paramochloa effusa* (Kunth) P.M. Peterson, Soreng, Romasch. & Barberá, *Pentapogon quadrifidus* (Labill.) Baill., *Podagrostis aequivalvis* Trin., *Relchela panicoides* Steud., and *Triplachne nitens* (Guss.) Link.

Our taxon sample contains all North American species of *Calamagrostis*, except the recently described *C. utsutsuensis* Otting & B.L. Wilson (28 species), as well as 31% of the remaining South American (3 species) and 33% (37 species) of Eurasian species currently attributed to this genus (Peterson et al., 2019a). The group of Australian and Pacific *Calamagrostis* species, often treated within *Deyeuxia*, has only partial representation in our study (10 species, out of approximately 40 in Australia and New Zealand alone). We designed our study to characterize the relationships among species of *Calamagrostis* and relatives in the genera *Agrostis*, *Agrostula*, *Alpagrostis*, *Chascolytrum*, *Deyeuxia*, *Dichelachne*, *Echinopogon*, *Gastridium*, *Greeneochloa* P.M. Peterson, Soreng, Romasch. & Barberá, *Laegaardia*, *Paramochloa*, *Pentapogon*, *Podagrostis*, *Relchela*, and *Triplachne*. All of these genera have been found in a clade in previous molecular analyses and in our unpublished trees investigating a large number of species in *Agrostis*, *Calamagrostis*, *Cinnagrostis*, and *Koeleria* Pers. (Saarela et al., 2017; Barberá et al., 2019a, 2019b; Peterson et al., 2019a, 2020; da Silva et al., 2022), and are included in four of the six subtribes of tribe Poeae supersubtribe Agrostidodinae Soreng (Soreng et al., 2017, 2022a; Tkach et al., 2020). Previous analyses of *Polypogon* Desf. found members of the genus nested within

*Agrostis*, with incongruence between the plastid and nuclear ribosomal data (Saarela et al., 2017; Romaschenko et al., unpublished). We do not address this question here (i.e., *Polypogon* is not included in our sampling) since we only include a phylogenetically representative sample of species within *Agrostis*.

### 2.2 Phylogenetic analyses

All procedures related to the sequencing of the plastid and ITS regions were performed in the Laboratory of Analytical Biology at the Smithsonian Institution. Detailed methods for DNA extraction, amplification, and sequencing are described in Romaschenko et al. (2012) and Peterson et al. (2010a, 2010b, 2011, 2012, 2014, 2015a, 2015b, 2016). We used Geneious Prime v.2020.1.4 (Kearse et al., 2012) for contig assembly of bidirectional sequences and Muscle (Edgar, 2004) to align consensus sequences and adjust the final alignment. The Bayesian trees were constructed using MrBayes v3.2.7 (Huelsenbeck & Ronquist, 2001; Ronquist et al., 2012). All compatible branches were saved. The Maximum Likelihood parameters for each region were estimated using GARLI 2.0 (Zwickl, 2006) and were used as priors in Bayesian calculations (Table 1). The combined data set was split into four partitions containing the ITS, *rpl32-trnL*, *rps16-trnK*, and *rps16* intron sequences. In addition, we coded gaps in the plastid data set with FastGap 1.2 (Borchsenius, 2009) using the Simmons & Ochoterena (2000) method.

Bayesian analysis was initiated with random starting trees and was run for four million generations with the sampling frequency of trees set at the 100th iteration. Upon completion of the analyses, the variance of split sequences was less than 0.01 and the potential scale reduction factor was close or equal to 1.0, indicating convergence of the chains (Huelsenbeck & Ronquist, 2001). The search was also monitored using Tracer v1.7 (Rambaut et al., 2018). The effective sample size (ESS) value was greater than 100, and 25% of the sampled values were discarded. Posterior probabilities (PP) over 95% were considered as strong support. Maximum likelihood bootstrap analyses (Felsenstein, 1985) were performed using GARLI with 1000 repetitions. In PAUP\* v.5.0 (Swofford, 2000) was used to compute the majority rule consensus tree. Bootstrap (BS) values of 90–100% were interpreted as indicating strong support.

### 2.3 Assessment of incongruence and data combining strategy

Combining all congruent data (total evidence approach) provides better resolution of phylogenetic trees, strengthens support for the nodes, and maximizes the informativeness and explanatory power of the character data used in the analysis (Huelsenbeck & Cunningham, 1996). The plastid and ITS topologies resulting from Bayesian analysis were inspected for conflicting nodes with  $\geq 80\%$  BS support and/or PP  $\geq 0.95$ . If no supported incongruences were found, plastid and ITS sequences were combined and used in the overall phylogenetic analysis (Fig. 1). This analysis (combined plastid and ITS sequences) included a subset of the American and Eurasian species of *Calamagrostis*, the Australian and Pacific species of *Calamagrostis*, and representatives of

**Table 1** Characteristics of the four regions, *rpl32-trnL*, *rps16* intron, *rps16-trnK*, and ITS, and GAPpl and parameters used as priors in Bayesian analyses estimated using GARLI 2.0

	<i>rps16-trnK</i>	<i>rps16</i> intron	<i>rpl32-trnL</i>	Combined plastid sequences	ITS	Overall sequences	GAPpl
Total aligned characters	1044	1075	1031	3150	761	3911	77–
Number of sequences	122	110	220	452	214	666	224
Number of new sequences	95 (78%)	84 (77%)	189 (86%)	368 (81%)	184 (86%)	551 (83%)	
Likelihood score (-lnL)	1209.6	1351.5	1824.4		2235.3		
Number of substitution types	6	6	6		6		1
Model for among-site rate variation	Gamma	Gamma	Gamma		Gamma		Equal
Substitution rates	1.3541 1.8323 0.4689 0.9094 1.4504 1.0000	4.6729 2.2410 0.1818 0.0010 1.7115 1.0000	0.7960 0.6388 0.2890 0.2300 0.6642 1.0000	–	0.6553 2.2494 1.5750 0.0713 7.5761 1.0000		–
Character state frequencies	0.2907 0.1621 0.1586 0.3884	0.3529 0.1547 0.1870 0.3054	0.3801 0.1429 0.1312 0.3458	–	0.2243 0.2996 0.2921 0.1838		–
Proportion of invariable sites	0.5947	0.2192	0.6065	–	0.5533		–
Gamma shape parameter ( $\alpha$ )	0.8894	0.0500	0.8997	–	0.6453		–

nineteen other genera. *Phalaris arundinacea* L. (Phalaridinae Fr.), *Amphibromus scabrivalvis* (Trin.) Swallen (Torreyochloinae Soreng & J.I. Davis), and *Torreyochloa pauciflora* (J. Presl) G.L. Church (Torreyochloinae) were chosen as outgroups since they all lie outside of the Agrostidodinae Soreng, but inside the tribe Poeae R. Br. Chloroplast Group 1 (Aveneae type) (Saarela et al., 2017; Soreng et al., 2017, 2022a; Tkach et al., 2020).

The following 17 species of *Calamagrostis* were omitted from the overall combined analysis because they had incongruent signals between their plastid and ITS markers: *C. anthoxanthoides* (Munro ex Hook. f.) Regel, *C. arenaria* (L.) Roth, *C. baltica* Trin., *C. deschampsoides* Trin., *C. emodensis* Griseb., *C. gigas* Takeda, *C. gracilescens* (Blytt) Blytt, *C. hartmaniana* Fr., *C. holciformis* Jaub. & Spach, *C. holmii* Lange, *C. lahulensis* G. Singh, *C. macilenta* (Griseb.) Litv., *C. macrolepis* Litv., *C. obtusata* Trin., *C. perplexa* Scribn., *C. purpurascens* R. Br., and *C. rubescens* Buckley. When *Calamagrostis breviligulata* (Fernald) Saarela, *C. breweri* Thurb., and *C. muiriana* B.L. Wilson & Sami Gray were included in the overall combined analysis, support for Clades B and C collapsed. Therefore, we omitted these three species from the combined analysis based on the confounding effect of their ITS signal and included them, plus the 17 species with incongruent ITS and plastid data, in the taxon duplication analyses (see Figs. 3A–3D).

#### 2.4 Network analysis

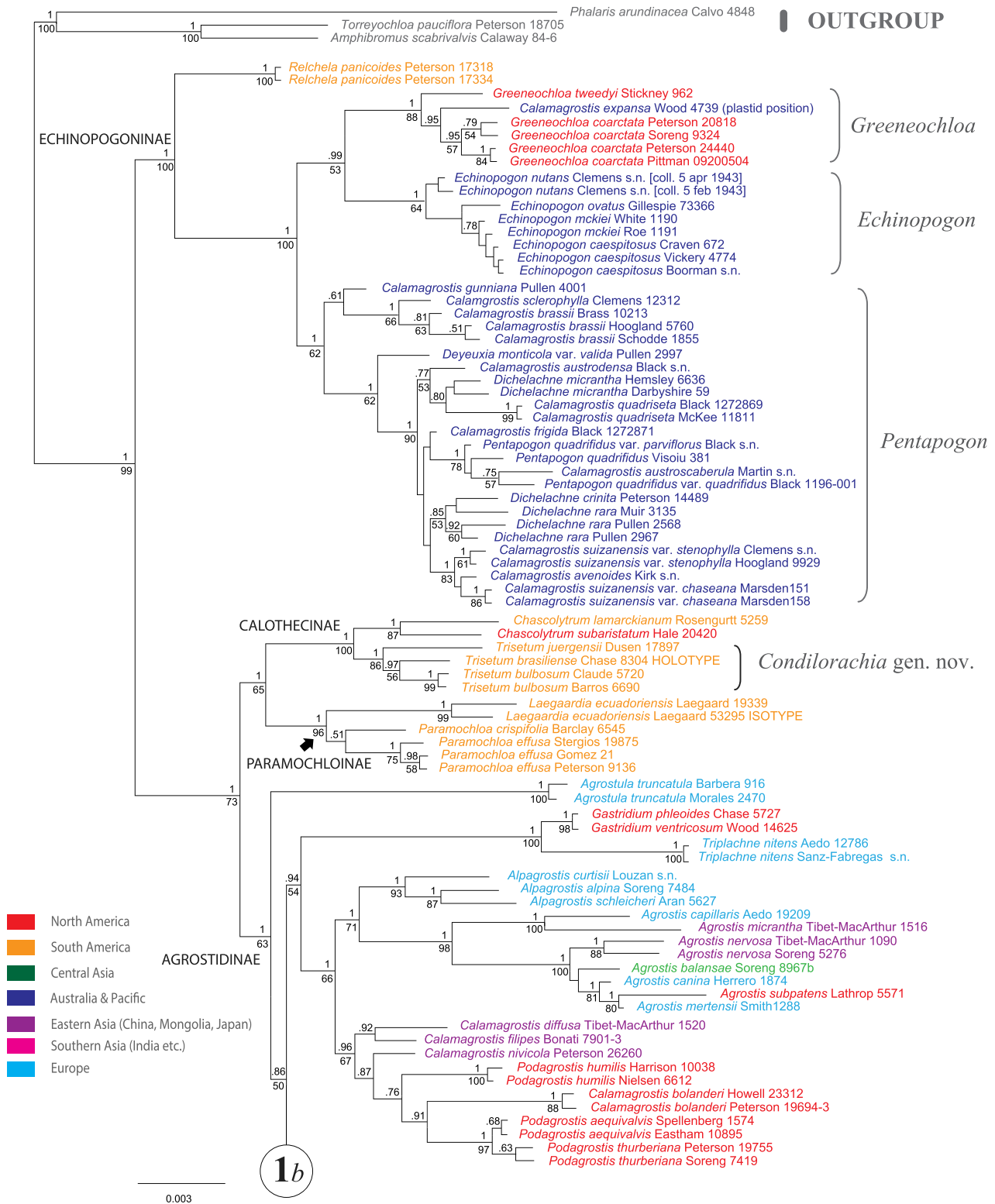
To investigate the relationships among the species of *Calamagrostis*, we constructed a phylogenetic network for

ITS (Fig. 2) using the neighbor-net algorithm (Bryant & Moulton, 2004) implemented in SplitsTree version 5.1.4 (Huson & Bryant, 2006). Uncorrected p-distances were used to weight the splits. Samples with identical sequences were removed from our analysis for a clearer presentation. We use the phylogenetic network to detect putative cases of ancient hybridization among well-defined lineages with supported conflicting positions. The outgroup set comprises *Gastridium phleoides* (Nees & Meyen) C.E. Hubb., *Agrostis canina*, and *Agrostis mertensii* Trin.

#### 2.5 Taxon duplication analysis

A taxon duplication analysis (Pirie et al., 2008; Pelsner et al., 2010; Peterson et al., 2015a, 2016, 2020, 2022) was performed on the full set (Appendix S1) of the American and Eurasian species of *Calamagrostis*, including the taxa/specimens with incongruent plastid and ITS data. In the latter case, the plastid and ITS data were used separately, testing their alternative placements against phylogenetic grouping and tree topology obtained in the *Calamagrostis* core analysis. The outgroup set included *Gastridium phleoides*, *Agrostis canina*, and *Agrostis mertensii*.

Where conflicting topologies were found, the data sets for inconsistently placed taxa were duplicated in the matrix (see Figs. 3A–3D). One set of the taxon was represented by the corresponding plastid sequences and the other taxon set by only ITS sequences. The remaining positions for the truncated data sets were then coded as missing data. We used this “taxon duplication” approach to resolve our phylogenetic tree, thereby minimizing the diffusing effects



**Fig. 1. A, B,** Bayesian tree inferred from combined plastid (*rpl32-trnL*, *rps16-trnK*, and *rps16 intron*) and ITS sequences. Numbers above branches show posterior probabilities, and numbers below branches show bootstrap values; color indicates the geographic origin of the specimens (see legend); and vertical bars indicate new, emended, or provisional taxonomic units (“groups”). *Calamagrostis* is represented by the core set excluding specimens with detected incongruence of ITS and plastid data.

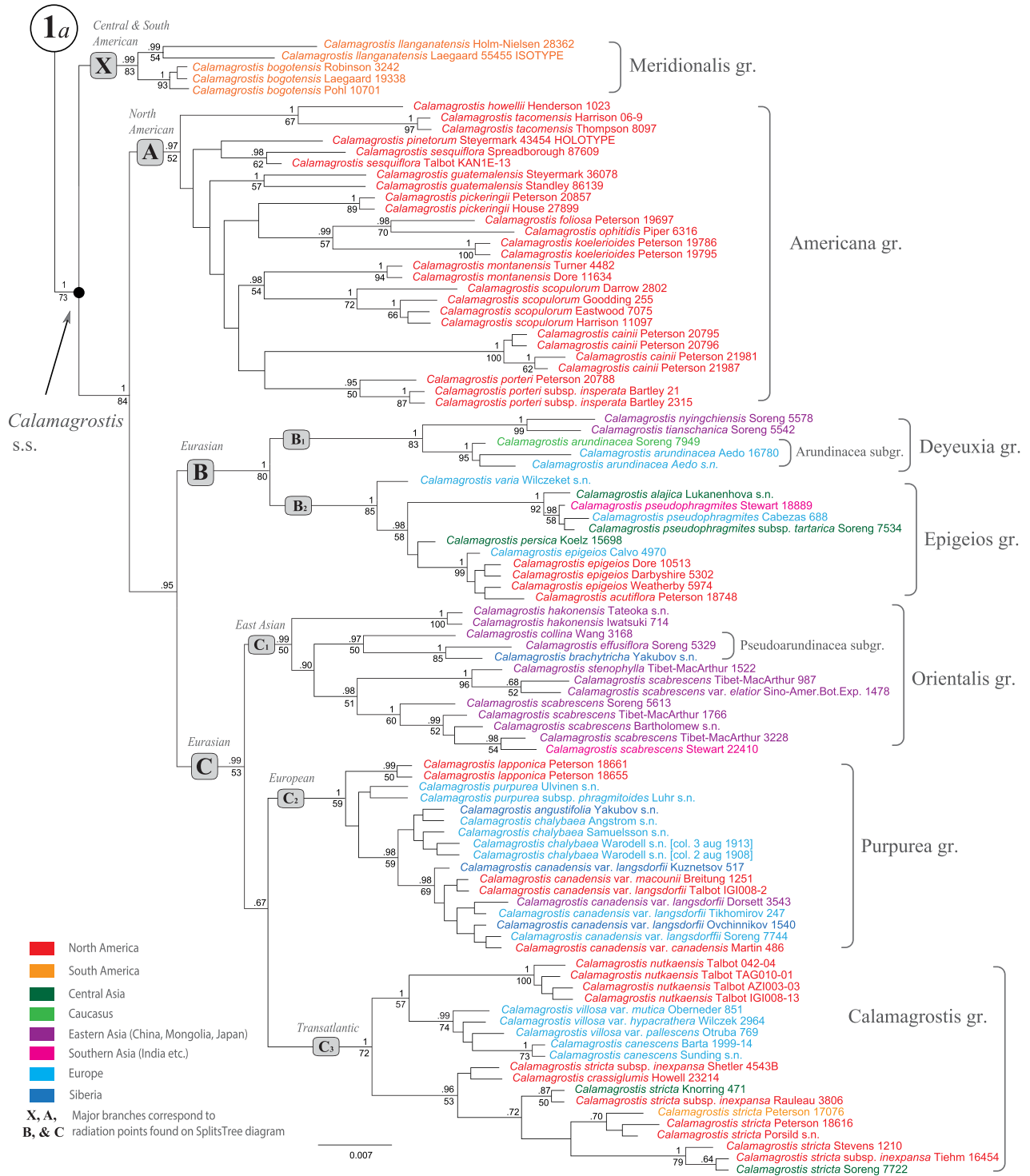
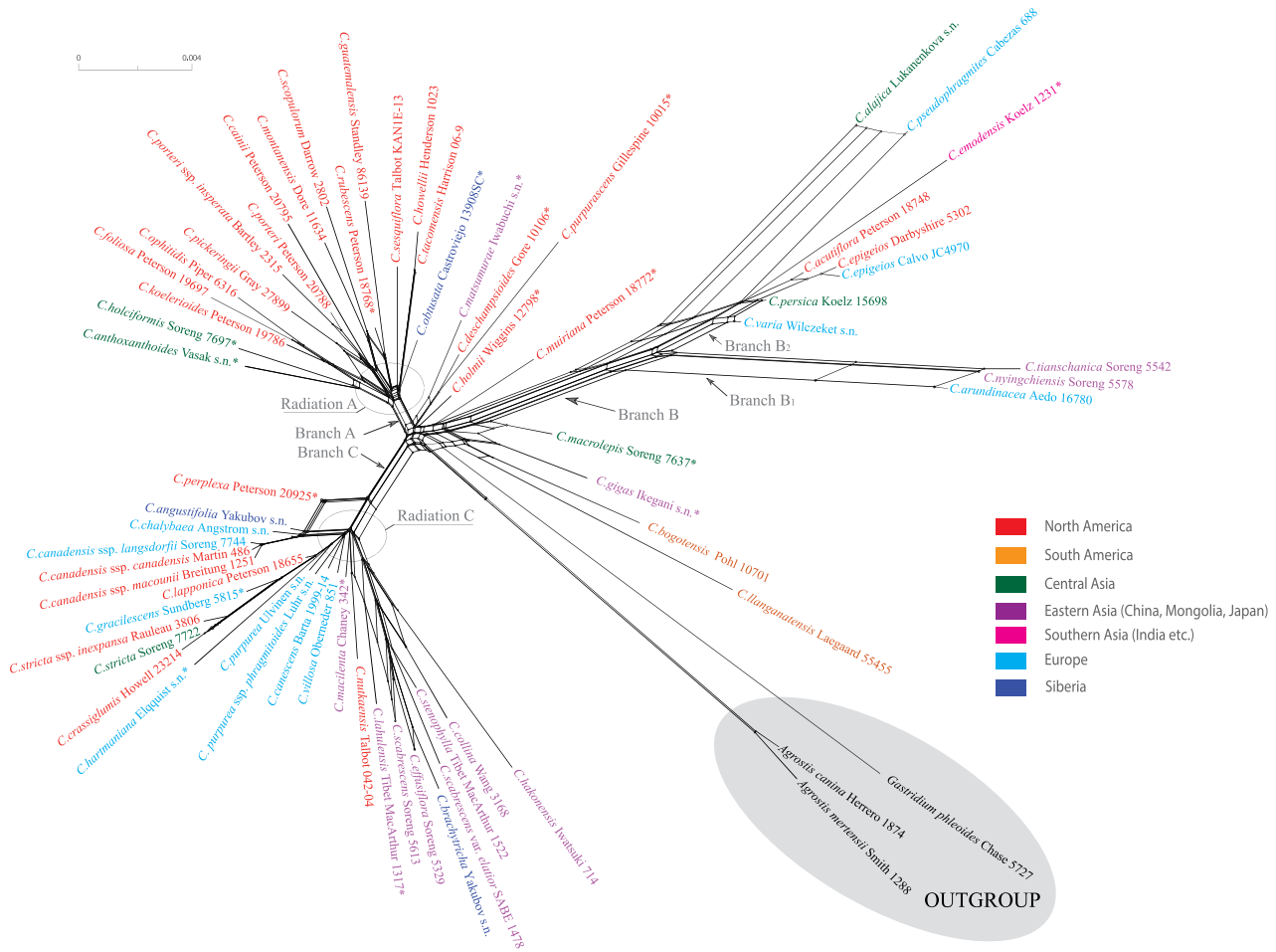


Fig. 1. Continued

of taxa with strongly supported incongruence between the plastid and ITS data or taxa having confounding ITS sequences (multiple alignments) and to represent their alternative placements in relation to the remaining phylogenetic groups among which relationships are congruent. This allowed us to hypothesize multiple origins and elucidate

complex evolutionary histories within phylogenetic groups. We inserted the 17 species with incongruent plastid and ITS signals and the three species with confounding ITS sequences into four abbreviated cladograms to elucidate their alignment with the remaining core of *Calamagrostis*.



**Fig. 2.** Neighbor-net phylogenetic network of the *Calamagrostis* s.s. nuclear ITS data set. Color indicates the origin of the specimens (see legend); asterisk (\*) indicates taxa not included in the overall combined tree of Fig. 1; arrows indicate branches corresponding to clades recovered in overall combined analysis; and circles outline detected radiation points.

### 3 Results

#### 3.1 Phylogenetic analyses

Eighty-three percent (551/666) of the sequences in our study are newly reported here (and in GenBank), and 17% (115) are existing GenBank accessions (Appendix S1) generated in an earlier study (Peterson et al., 2020). A total of 24% of the sequences in our data set are missing. Total aligned characters for individual regions and other parameters are shown in Table 1.

#### 3.2 Phylogenetic tree (congruent species)

The Bayesian tree from combined plastid (*rpl32-trnL*, *rps16-trnK*, and *rps16*) and ITS regions (Fig. 1A) is well resolved and many major clades are strongly supported. The clade corresponding to Echinopogoninae is maximally supported and includes clades corresponding to *Relchela*, *Greeneochloa*, and *Echinopogon* and a lineage we refer to as the *Pentapogon* clade. The *Greeneochloa* (PP = 1, BS = 88) plus *Echinopogon* (PP = 1, BS = 64) clades are sister (PP = 0.99; BS = 53) to the *Pentapogon* (PP = 1, BS = 62) clade that includes nine species of *Calamagrostis*, three species of *Dichelachne* [*D. crinita* (L. f.) Hook. f., *D. micrantha*, and *D. rara* (R. Br.) Vickery], and

one species of *Deyeuxia*. The *Greeneochloa* clade includes the Hawaiian *Calamagrostis expansa* (Munro ex Hillebr.) Hitchc. ( $\equiv$  *Deyeuxia expansa* Munro ex Hillebr.) and the two recognized *Greeneochloa* species.

The clade corresponding to Calothecinae is weakly supported (PP = 1, BS = 65). It comprises a lineage corresponding to *Chascolytrum* (PP = 1, BS = 87), with two species, sister to three species of *Trisetum* (*T. brasiliense*, *T. bulbosum*, and *T. juergensii* Hack.), which we call the *Condiliorachia*, gen. nov. clade (PP = 1, BS = 86). The *Chascolytrum* plus *Condiliorachia* clade is sister to a strongly supported clade comprising *Paramochloa* plus *Laegaardia* (PP = 1, BS = 96; *Paramochloinae*).

The Calothecinae–*Paramochloinae* clade is sister to a large, weakly supported clade corresponding to Agrostidinae (PP = 1, BS = 63). Within the latter clade, *Agrostula* (PP = 1, BS = 100) is sister to the remaining species of Agrostidinae. Among these remaining species, a weakly supported clade (PP = 0.94, BS = 54) includes three main lineages. One lineage is maximally supported and comprises two species of *Gastridium* (PP = 1, BS = 98) and *Triplachne nitens* (Guss.) Link. A second lineage, which is sister to *Gastridium* plus *Triplachne*, comprises two subclades: one comprising

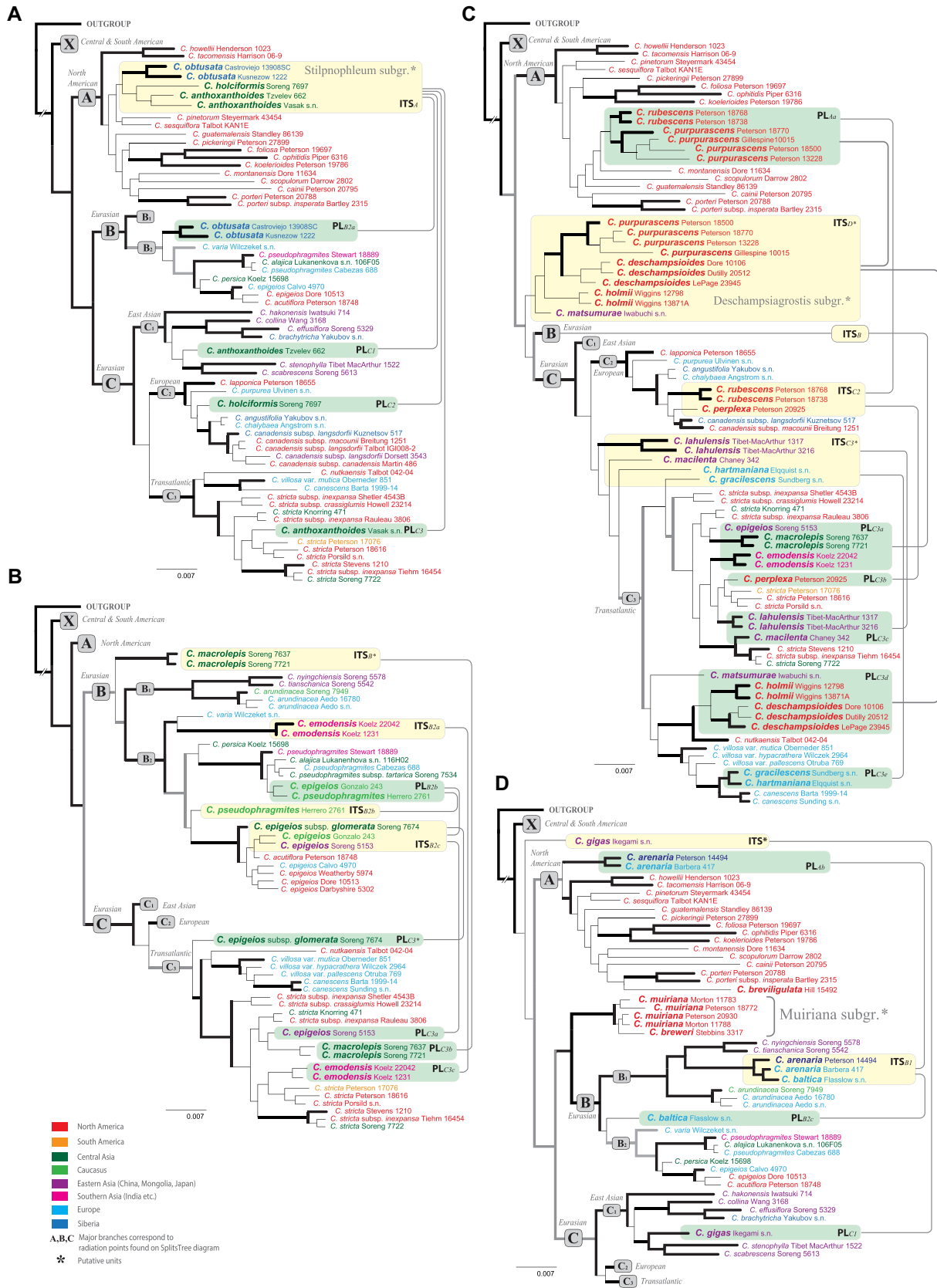


Fig. 3. Continued



*Alpargrostis* (PP = 1, BS = 93) and the other *Agrostis* (PP = 1, BS = 98); *Alpargrostis* and *Agrostis* are sister groups (PP = 1, BS = 73), and this clade is sister to a weakly supported clade of three East Asian *Calamagrostis* species [*C. diffusa* (Keng) Keng f., *C. filipes* (Keng) P.C. Kuo & S.L. Lu ex J.L. Yang, and *C. nivicola* (Hook. f.) Hand.-Mazz.], the Californian endemic *C. bolanderi* Thurb., and three *Podagrostis* species (PP = 0.76).

*Calamagrostis* s.s. (PP = 1, BS = 73) comprises seven major clades (Fig. 1B), which we refer to using letter codes A–G (for clarity), and the following informal names: the Meridionalis, Americana, Deyeuxia, Epigeios, Orientalis, Purpurea, and *Calamagrostis* groups. The Meridionalis group (Clade X, PP = 0.99, BS = 83) is sister to the remaining species in the genus and includes two species native to northern South America and Central America. The Americana group (Clade A, PP = 0.97, BS = 52) is the next successive sister to the remaining species in the genus. It consists of 12 species that occur in North and Central America and one species (*C. sesquiflora*) that is north temperate, extending to Asia. The remaining species of *Calamagrostis* form two major clades: B (PP = 1, BS = 80) and C (PP = 0.99, BS = 53). Clade B comprises two major lineages: the Deyeuxia group (Clade B<sub>1</sub>, PP = 1, BS = 83), which consists of three species, and the Epigeios group (Clade B<sub>2</sub>, PP = 1 BS = 85), which consists of six species. Clade C (PP = 0.99, BS = 53) comprises three major subclades. Clade C<sub>1</sub>, the Orientalis group (PP = 0.99, BS = 50), comprises six species primarily from East Asia; Clade C<sub>2</sub> (PP = 1, BS = 59), the Purpurea group, comprises five species from Europe and North America. Clade C<sub>3</sub> (PP = 1, BS = 73), the *Calamagrostis* group, comprises five species primarily occurring in the northern hemisphere, although *C. stricta* (Timm) Koeler extends into temperate South America.

### 3.3 Network analysis

The ITS network (Fig. 2) clearly depicts a radiative pattern for *Calamagrostis* Clade A (North American) even with the addition of *C. anthoxanthoides* and *C. holciformis* from Central Asia, and *C. obtusata* from Siberia (three species

with incongruent plastid/ITS signals mentioned earlier). Likewise, Clade C (Eurasian) has a radiative pattern with the addition of *Calamagrostis gracilescens* and *C. hartmaniana* from Europe, and *C. macilentata* and *C. lahulensis* from Eastern Asia. *Calamagrostis perplexa* from North America is shown splitting off early in the derivation of branch C just below the radiation event. Near the junction of branches A and B, *C. deschampsioides*, *C. holmii*, and *C. purpurascens* from North American, and *C. matsumurae* from Eastern Asia split off. Near the base of branch B (Eurasian), *Calamagrostis gigas* from Eastern Asia and *C. macrolepis* from Central Asia branch off, while *C. emodensis* from Southern Asia splits off of branch B<sub>2</sub> just below *C. epigeios* (L.) Roth.

### 3.4 Phylogenetic trees (including incongruent species with taxon duplication)

Based on ITS markers, *Calamagrostis obtusata* plus *C. holciformis* and *C. anthoxanthoides* form a weakly supported clade within the Americana group (Clade A; Fig. 3A). Based on plastid markers, *C. obtusata* is part of Clade B<sub>2</sub> (the Epigeios group), *C. holciformis* is part of Clade C<sub>3</sub> (the Purpurea group), one accession of *C. anthoxanthoides* is part of Clade C<sub>1</sub> (the Orientalis group), and the other accession of *C. anthoxanthoides* is part of Clade C<sub>3</sub> (the *Calamagrostis* group).

Based on ITS markers, *Calamagrostis macrolepis* from Central Asia is sister to Clades B<sub>1</sub> and B<sub>2</sub> while the plastid markers place it in Clade (Fig. 3B). *Calamagrostis epigeios* is not monophyletic in the plastid trees. Based on plastid markers, *C. epigeios* (Gonzalo 243) is part of Clade B<sub>2</sub>, in a strongly supported clade including four accessions of *C. pseudophragmites* (Haller f.) Koeler and *C. alajica* Litv., and *C. epigeios* ssp. *glomerata* (Boiss. & Buhse) Tzvelev from Central Asia (Soreng 7674) is sister to Clade C<sub>3</sub>. Based on ITS markers, these two samples are part of a strongly supported clade within Clade B<sub>2</sub> that includes all other accessions of *C. epigeios* and *C. acutiflora* (Schrad.) DC. in the Clade B<sub>2</sub>. Based on ITS markers, two accessions of *C. emodensis* form a strongly supported clade sister to the *C. varia* Host at the base of Clade B<sub>2</sub>, while the plastid signal places the pair

**Fig. 3.** Phylogenetic schemes inferred from Bayesian analyses of *rpl32-trnL*, *rps16-trnK*, and *rps16* intron and ITS data representing the multiple origins of the species of *Calamagrostis* with uncertain phylogenetic position (in **bold**). Thick black branches indicate posterior probabilities 0.95–1 found for the similar phylogenetic groupings in the *Calamagrostis* core set analysis shown in Fig. 1B. Thick gray branches indicate reduced probability values (<0.95) as compared to *Calamagrostis* core analysis due to the introduction of new data. “**ITS**” and “**PL**” represent ITS and plastid sets, respectively, sampled separately for the tested taxa. Labeling of major phylogenetic groups and branches is the same in Fig. 1B. **A**, A-clade (Americana group) resident, and putative ITS concerting subgroup *Stilpnophleum*\* (**ITS<sub>A</sub>**) represent four different plastid lineages of Eurasian origin (**PL<sub>B2a</sub>**, **PL<sub>C1</sub>**, **PL<sub>C2</sub>**, and **PL<sub>C3</sub>**) nested within the groups Epigeios and *Calamagrostis* (Clades B and C). (See the full set of specimens in Appendix S1, set 3a). **B**, Complete and incomplete introgression in the Epigeios group. **ITS<sub>B\*,B2-a,b,c</sub>** represents Epigeios ITS concerted lineages reflecting different stages of putative genomic introgression. The Epigeios group (Clade B) relates to several plastid lineages (**PL<sub>C\*,C3a-c</sub>**) originated within the *Calamagrostis* group (Clade C<sub>3</sub>) (see the full set of specimens in Appendix S1, set 3b). **C**, The putative ITS concerted/confounding group *Deschampsia*grostis\* (**ITS<sub>D\*</sub>**) represents two different plastid lineages of Eurasian and American origin nested in Clades A (Americana group) and C<sub>3</sub> (*Calamagrostis* group). (**PL<sub>A</sub>**, **PL<sub>C3d</sub>**). The *Calamagrostis* group (Clade C<sub>3</sub>) includes five plastid donating lineages (**PL<sub>C3a-e</sub>**) and putative peripheral ITS confounding lineage **ITS<sub>C3\*</sub>**. The Purpurea group (Clade C<sub>2</sub>) encompasses the ITS concerted lineage **ITS<sub>C2</sub>** (see the full set of specimens in Appendix S1, set 3c). **D**, Phylogenetic affinities of *Calamagrostis arenaria*, *Calamagrostis berviligulata*, and *Calamagrostis baltica*; Californian endemic *Calamagrostis muiriana* and *Calamagrostis breweri*; and Asian *Calamagrostis gigas* (see the full set of specimens in Appendix S1, set 3d).

within Clade C<sub>3</sub> in a strongly supported clade including *C. stricta*, one accession of *C. epigeios*, *C. macrolepis*, and *C. emodensis* (Fig. 3B).

Based on plastid markers, *Calamagrostis rubescens* Buckley and *C. purpurascens* are sister groups within Clade A (Fig. 3C). Based on ITS markers, *C. purpurascens* is sister to *C. deschampsoides*, and this lineage is part of a weakly supported clade also including *C. holmii* and *C. matsumurae*. This four-taxon clade is novel (i.e., it is not present in Fig. 1) and sister to the lineage comprising Clades B and C. Based on ITS markers, two accessions of *C. rubescens* together align in Clade C<sub>2</sub>. Based on plastid markers, *C. holmii* and *C. deschampsoides* form a clade sister to *C. nutkaensis* (J. Presl) Steud. in Clade C<sub>3</sub>; *C. matsumurae* also is aligned in Clade C<sub>3</sub>. *Calamagrostis perplexa* aligns in Clade C<sub>2</sub> based on ITS markers; it is part of a strongly supported clade also including *C. angustifolia*, *C. chalybaea*, *C. rubescens*, and *C. canadensis*. It is part of Clade C<sub>3</sub> based on plastid markers, and weakly supported as sister to a subset of *C. stricta* accessions. Within Clade C<sub>3</sub>, *C. gracilescens*, *C. hartmaniana*, *C. lahulensis*, and *C. macilenta* (Griseb.) Litv. (Eastern Asia) change their alignments (Fig. 3C). Based on ITS markers *C. lahulensis*, *C. macilenta*, and *C. hartmaniana* are successive sisters (but weakly supported) to Clade C<sub>3</sub>, and *C. gracilescens* is sister to *C. stricta*. Based on plastid markers, *C. lahulensis* and *C. macilenta* are embedded within Clade C<sub>3</sub> in the *C. stricta* clade, whereas *C. hartmaniana* is sister to *C. gracilescens* within a strongly supported clade also including *C. villosa*.

Based on ITS data (Fig. 3D), *C. gigas* is a sister group to the rest of *Calamagrostis* excluding Clade X (the Meridionalis group), while the plastid markers place it in the C<sub>1</sub> clade, where it is strongly supported as the sister group to *C. stenophylla* Hand-Mazz. plus *C. scabrescens* Griseb. Based on plastid markers, the two accessions of *C. arenaria* are part of Clade A and sister to the remaining members in Clade A, while based on ITS markers, they form a strongly supported clade with *C. baltica* in Clade B<sub>1</sub>. In the plastid tree, the single *C. baltica* accession is weakly supported as sister to Clade B<sub>2</sub>. *C. breviligulata* belongs to the Americana group based on the strong support. A clade comprising the North American species *C. breweri* and *C. muriana*, which we refer to as the putative *Muiriana* subgroup, is strongly supported as sister to Clade B (Fig. 3D).

## 4 Discussion

### 4.1 Phylogeny

Our study was focused on four subtribes in Poeae chloroplast Group 1 that form supersubtribe Agrostodinae. The strongly supported sister group relationships between Calothecinae and Paramochloinae, Agrostidinae and Calothecinae–Paramochloinae, and Echinopogoninae and Agrostidinae–Calothecinae–Paramochloinae are consistent with the results of previous phylogenetic studies (Saarela et al., 2017; da Silva et al., 2022). Tkach et al. (2020) reported similar results, without Paramochloinae representatives, but including Agrostodinae subtribes Brizinae and Hypseo-chloinae, which are basal to the four subtribes represented in our analysis.

#### 4.1.1 Echinopogoninae

In our analyses, the clade corresponding to Echinopogoninae is maximally supported. However, our results indicate that

Echinopogoninae, in its current circumscription, is paraphyletic. In addition to *Echinopogon*, *Greeneochloa*, *Pentapogon* [syn. (proposed here)—*Ancistragostis*, *Dichelachne*, and *Sclerodeyeuxia*], and *Relchela*, the genera included in the subtribe by Soreng et al. (2022a), multiple species classified as *Calamagrostis* or *Deyeuxia* from Australia and New Zealand are part of the lineage (van Royen, 1979).

Based on the current sampling, *Echinopogon* is weakly supported as a monophyletic group. The genus is distributed in Australia, New Guinea, and New Zealand and characterized by having a perennial habit, spikelets with a rachilla extension, glumes as long as or longer than the flower, their apices obtuse to attenuate, callus of the flower pubescent, lemma apex entire or with two teeth or lobes, awned from the apex or the abaxial side, and the awn stiff; the lobes also awned in some species (Kellogg, 2015). We sampled four of the seven species currently recognized within *Echinopogon* (Soreng et al., 2017; POWO, 2021), whereas previous phylogenetic studies sampled only one or two species of the genus (e.g., Soreng et al., 2007; Grass Phylogeny Working Group II, 2012; Persson & Rudin, 2016; Saarela et al., 2017; Tkach et al., 2020; da Silva et al., 2022). The three species that we did not sample (*E. cheelii* C.E. Hubb., *E. intermedius* C.E. Hubb., *E. phleoides* C.E. Hubb.) have not yet been included in a phylogenetic analysis.

The clade corresponding to *Greeneochloa* is moderately supported. Peterson et al. (2019) described the genus *Greeneochloa*, based on a subset of the data presented here, to accommodate two species distributed in the United States formerly recognized in *Calamagrostis* (*G. coarctata* and *G. tweedyi*). *Greeneochloa* differs from *Calamagrostis* in having hairy ovaries, extravaginal shoot innovations, flat leaf blades, and panicles contracted usually with ascending tightly appressed or slightly spreading branches. Recognition of the genus and its inclusion in Echinopogoninae are consistent with studies that sampled one of the two species (*G. coarctata*) (Saarela et al., 2017; da Silva et al., 2022). We find a third species, *Calamagrostis expansa*, which is endemic to the Hawaiian Islands (Wagner et al., 1990), to be part of this lineage in our combined plastid analyses. This placement of *C. expansa* is incongruent with the ITS position, which is unresolved due to a possible confounding phylogenetic signal (results not shown). Given the incongruence between the plastid and nrDNA data, this species could be an intergeneric hybrid. Thorough sampling using low-copy genes would be necessary to elucidate past events. Despite the incongruence, we (here) transfer *Calamagrostis expansa* into *Greeneochloa*. *Calamagrostis expansa* is morphologically similar to *G. coarctata* and *G. tweedyi* in having hairy ovaries, extravaginal shoot innovations, flat leaf blades, and panicles contracted usually with ascending tightly appressed or slightly spreading branches (Peterson et al., 2019a).

Relationships within Echinopogoninae are often strongly supported in our analysis. The sister group relationship between *Greeneochloa* and *Echinopogon* is consistent with the combined plastid and ITS + ETS tree in da Silva et al. (2022) and with the ITS + ETS tree in Saarela et al. (2017), and we find this clade to be sister to the clade comprising *Pentapogon*, *Dichelachne*, and multiple species of *Calamagrostis* and *Deyeuxia* from Australia and New Zealand. Placement of *Relchela* sister to the rest of the subtribe in

our analyses is consistent with the ITS + ETS tree in Saarela et al. (2017), but not with their plastid tree, in which *Greeneochloa coarctata* (as *Calamagrostis coarctata*) and a clade comprising *Echinopogon* and *Relchela* are strongly supported sister groups. Da Silva et al. (2022), by contrast, found *Dichelachne crinita*, the only species of the genus they sampled, to be sister to the rest of the clade (i.e., *Relchela* and *Echinopogon*—*Greeneochloa*) in their combined plastid—nrDNA (ITS + ETS) analyses. The *Dichelachne crinita* sample that we sequenced, Peterson 1449, is the sample that da Silva et al. (2022) sequenced. In da Silva et al.'s (2022) combined plastid trees, *D. crinita* is included in the clade corresponding to *Echinopogoninae*, whereas in their nrDNA tree, it is nested within *Agrostidinae*, sister to *Podagrostis aequivalvis*. In the context of the current results, we suspect that the data for one or both of the nrDNA regions (ITS, ETS) for this sample in da Silva et al. (2022) are contaminants, and the placement of *D. crinita* in their combined plastid and nrDNA analysis sister to the rest of the clade is due to the signal in the erroneous sequence(s).

None of the genera within the *Pentapogon* clade are monophyletic in our analyses. Placement of a subset of the sampled *Calamagrostis* species here (and one of *Deyeuxia*) indicates that *Calamagrostis* s.l. is still polyphyletic. *Dichelachne* is not monophyletic, as two of the three sampled species (*D. crinita* and *D. rara*) form a weakly supported clade and one (*D. micrantha*) is part of a weakly supported clade also including *Calamagrostis austrodensa* and *C. quadriseta*. The monotypic *Pentapogon* is not monophyletic because a sample identified as *Calamagrostis austroscaberula* is nested among samples of *P. quadrifidus*. Given the lack of resolution among taxa within this clade, it seems appropriate to recognize all species in a single genus. There are few morphological differences between *Dichelachne* and *Pentapogon*. The latter has 5-awned lemmas, whereas both genera have single-flowered spikelets with 5-veined, coriaceous lemmas with a central awn arising just below the apex to dorsally inserted, and 1-veined, membranous glumes (Veldkamp, 1974; Kodela et al., 2009; Simon et al., 2009). *Pentapogon*, proposed by Robert Brown (1810), is the oldest genus name available for this clade. Even though we included only three of the 10 species of *Dichelachne*, we feel confident that with additional species sampled from *Dichelachne* in subsequent DNA sequence-derived phylogenies, the topology would not change, since there are very little genetic differences separating these three species from the type of *Pentapogon* (*P. quadrifidus*). In our analysis, we did not include many species of Australasian *Calamagrostis*, formerly referred to as *Deyeuxia* (9/35 or 25%), also embedded in *Pentapogon*. We make new combinations in *Pentapogon* for these, even though a more complete survey of the species is needed (Weiller et al., 2009a, 2009b).

#### 4.1.2 Calothecinae

In our analyses, three species of South American *Trisetum* (*T. brasiliense*, *T. bulbosum*, *T. juergensii*) form a clade that is sister to *Chascolytrum*. By contrast, da Silva et al. (2022) found *T. brasiliense* and *T. bulbosum* (they did not sample *T. juergensii*) to form a weakly supported clade in their nuclear ribosomal trees (ITS and ETS), with relationships among this clade, *Lombardochloa*, and a clade comprising the rest of Calothecinae unresolved. In their combined nuclear

and plastid trees, however, they found *T. brasiliense* to be sister to *Poidium*, a genus we did not sample here, and *T. bulbosum* to be part of a polytomy with a clade comprising *Lombardochloa*, *T. brasiliense*, and *Poidium*, and a clade comprising the rest of Calothecinae. We describe a new genus, *Condilorachia*, to accommodate these three species of *Trisetum*. The new genus differs from *Chascolytrum* in having 2- or 3-flowered spikelets (verses 3–14-flowered in *Chascolytrum*), dorsally awned lemmas from the base or near the middle (apically mucicous or mucronate lemmas in *Chascolytrum*), and a hairy callus (glabrous in *Chascolytrum*). In addition, all three species are perennials, usually with tufted culm bases, contain spikelets with a rachilla extension, and have caryopses with adherent pericarps. Finot (2003) and Finot et al. (2005) accepted *Trisetum brasiliense* in *Deschampsia* while maintaining that *T. bulbosum* and *T. juergensii* are not species of *Helictotrichon* or *Deschampsia*, respectively. In the Flora Argentina, *Trisetum bulbosum* is accepted as occurring in the province of Buenos Aires and in Bio-Bio, Chile (Finot, 2012).

#### 4.1.3 Agrostidinae

**4.1.3.1 Podagrostis and relatives** We sampled four of the 10 currently recognized species of *Podagrostis*. Our analysis does not include any of the five species formerly recognized in *Agrostis* and recently transferred to *Podagrostis* by Sylvester et al. (2019, 2020) or newly described, based in part on the results of our unpublished molecular analyses. We find *Calamagrostis bolanderi* to be nested within *Podagrostis*, although relationships among *P. humilis*, *C. bolanderi*, and *P. thurberiana*–*P. aequivalvis* are unsupported. Previous studies indicate that *C. bolanderi* may be a hybrid between *Podagrostis* and *Calamagrostis* (Saarela et al., 2017; Sylvester et al., 2020; Peterson et al., 2020). Our results also indicate that the East Asian species *Calamagrostis diffusa*, *C. filipes*, and *C. nivicola* are closely related to *Podagrostis* and *C. bolanderi*, as all these species form a weakly supported clade. Saarela et al. (2017) sampled two of these *Calamagrostis* species and found a different result. Their plastid tree identified a clade including *C. diffusa* (as *Deyeuxia diffusa* Keng), *C. tripilifera* (as *D. tripilifera* (Hook. f.) Keng), *C. stenophylla* Hand.-Mazz. (as *D. mazzettii* Veldkamp), *C. nivicola* (*D. nivicola* Hook f.), and *Agrostis rosei* Scribn. & Merr. (= *Podagrostis rosei* (Scribn. & Merr.) Sylvester & Soreng), a species from the mountains of central and southern Mexico, that was weakly supported as sister to a large *Agrostis* + *Polypogon* clade, with *Podagrostis* + *C. bolanderi* recovered as sister to the clade comprising all the above-mentioned taxa. Their ITS + ETS tree identified the same clade of Chinese *Calamagrostis* species, but relationships between this clade and the one sampled species of *Podagrostis* were unresolved. Further study of *Calamagrostis diffusa*, *C. filipes*, and *C. nivicola*, the additional species that Saarela et al. (2017) found to be closely related to a subset of these, and the many morphologically similar species from East Asia that have not yet been sampled in a molecular analysis is needed, before deciding whether to include them within an expanded *Podagrostis* or a new genus.

Sister to *Calamagrostis* in our overall combined analysis is the clade comprising *Gastridium*–*Triplachne*, *Agrostis*–*Alpagrostis*, and a clade of *Podagrostis*, *Calamagrostis bolanderi*,

and three East Asian species of *Calamagrostis*. These lineages share a common ancestor with *Agrostula*, a new segregate genus distributed in France, Spain, Portugal, and Morocco found in cold temperate, often high-elevation, environments (Peterson et al., 2020). A clade comprising the South American subtribes Calothecinae and Paramochloinae (a topology consistent with previous phylogenetic results; Da Silva et al., 2022) and *Agrostula* from Europe (Peterson et al., 2010b) are the successive sister groups to the clade comprising *Gastridium*–*Triplachne*, *Agrostis*–*Alpagrostis*, *Podagrostis*–*Calamagrostis* p.p., and *Calamagrostis* s.s.

**4.1.3.2 *Calamagrostis* s.s.** Our analyses considerably advance understanding of the evolutionary history of *Calamagrostis* s.s. The lineage corresponding to the genus is moderately to strongly supported, and multiple lineages within the genus are identified. Inclusion of the two species formerly recognized in the genus *Ammophila* within the *Calamagrostis* lineage is consistent with the findings of Saarela et al. (2017), who proposed synonymizing *Ammophila* with *Calamagrostis*. Similar to Saarela et al. (2017), we find conflict between ITS and plastid data in *C. arenaria* (syn. *Ammophila arenaria*), no conflict between the ITS and plastid data partitions in *C. breviligulata* (syn. *A. breviligulata*), and that the two species are not sister taxa. Both of these species hybridize with other species of *Calamagrostis* in their native ranges (*C. x baltica* (Flüggé ex Schrad.) Trin., *C. x don-hensonii* (Reznicek & Judz.) Saarela). F1 hybrids between *C. arenaria* and *C. breviligulata*, the species native to cool temperate European and northeastern North American coasts and interior sand dunes, respectively, have recently been recorded along the coast of the Pacific Northwest in the USA, where both species are introduced and their ranges overlap (Mostow et al., 2021), despite being distantly related to one another.

The Meridionalis group is sister to the rest of the genus. Based on the current sampling, it contains two species distributed in Central and South America. *Calamagrostis llanganatensis* Laegaard is found only in Ecuador (Laegaard 1998), and *C. bogotensis* (Pilg.) Pilg. is endemic to Colombia, Costa Rica, Ecuador, Panamá, and Venezuela (POWO, 2021). *Calamagrostis carchiensis* Laegaard, native to Colombia and Ecuador (POWO, 2021), which we did not sample, likely is also part of this lineage, as Saarela et al. (2017) found *C. carchiensis* and *C. llanganatensis* to be sister taxa in both their ITS + ETS and plastid trees. Our informal name for the clade, Meridionalis group, reflects its southern distribution.

The Americana group—so named because all but two species that are part of the clade are distributed in North America—includes at least 14 species (*C. cainii*, *C. foliosa*, *C. howellii*, *C. koelerioides*, *C. guatemalensis*, *C. ophitidis*, *C. montanensis*, *C. pickeringii*, *C. pinetorum*, *C. porteri*, *C. rubescens*, *C. scopulorum*, *C. sesquiflora*, *C. tacomensis*) based on the current sampling. In their plastid analyses, Saarela et al. (2017) identified a clade comprising a similar set of species, but excluding *C. porteri* and *C. montanensis* and including *C. purpurascens*, *C. rubescens*, and *Ammophila breviligulata*; they did not sample *C. tacomensis* or *C. pinetorum*. Our plastid data also place *C. purpurascens* and *C. rubescens* within this clade, whereas these taxa have different affinities in our ITS tree.

Deeper relationships within the Americana lineage are unresolved, but several smaller clades are highly supported

by the current data set. We find *C. howellii* and *C. tacomensis* to be sister groups. *Calamagrostis tacomensis* is a recently described species known only from the Cascade Mountains of Washington, the Olympic Peninsula of Washington, and the Steens Mountains of southeast Oregon (Marr & Hebda, 2006); it was previously confused with *C. sesquiflora*, which is included in the Americana clade. Our molecular analyses support the distinctiveness of *C. tacomensis* and *C. sesquiflora* at the species level, given that they are not sister groups. *Calamagrostis howellii*, restricted to the Columbia River Gorge of Washington and Oregon, is morphologically similar to *C. tacomensis* and grows in similar habits in the same general area (northwestern United States), but at lower elevations (Marr & Hebda, 2006). Placement of *Calamagrostis pinetorum*, endemic to Guatemala, in the Americana clade is a novel result, as the species has not previously been included in a molecular study. This species and *C. guatemalensis*, endemic to southeastern Mexico and Guatemala, are the most southerly distributed taxa of the Americana clade based on the current taxon sampling. Their relationships to one another and to other species within the clade, however, are not statistically supported. *Calamagrostis ophitidis*, *C. foliosa*, and *C. koelerioides*—species restricted to the western United States—form a weakly to strongly supported clade within the Americana clade. The sister species *C. ophitidis* and *C. koelerioides* have similar distributions in California, but differ in their ecology: the former grows in coastal scrub, forest, rock outcrops, crevices, and cliffs in the Outer North Coast Ranges, Central Coast, and the latter grows in meadows, seeps, grassland, chaparral, and forest, generally on serpentine soils in San Francisco Bay Area California Floristic Provinces and the latter in the North Coast, Klamath Ranges, and Outer North Coast Ranges California Floristic Provinces (Peterson et al., 2012). *Calamagrostis koelerioides* is partly sympatric with *C. ophitidis* and *C. foliosa*, but has a wider distribution, extending further south and west in California and north to Washington, Idaho, Montana, and Wyoming (Peterson et al., 2012). We find *C. montanensis*, distributed in the continental interior from eastern British Columbia and adjacent Alberta, south to southern Wyoming, and east to Manitoba and western Minnesota (Marr et al., 2007), and *C. scopulorum*, distributed from western Montana and Wyoming south to Arizona and New Mexico (Marr et al., 2007), to be weakly supported as sister taxa. By contrast, Saarela et al. (2017) found *C. scopulorum* and *C. foliosa* to be sister taxa in their plastid tree, also with weak (parsimony bootstrap) support. *Calamagrostis cainii*, known from only a few high-elevation sites in Tennessee and North Carolina (Marr et al., 2007), *C. porteri*, distributed in the northeastern United States (Marr et al., 2007), and *C. pickeringii*, distributed in the northeastern United States and adjacent Canada, are the only eastern North American species that are part of the Americana clade based on our sampling. Their relationships to one another and to the other species in the lineage are unsupported.

The Orientalis group comprises five East Asian species based on our taxon sampling. Within the clade, *Calamagrostis collina* Franc. is sister to a lineage comprising *C. effusiflora* (Rendle) P.C. Kuo & S.L. Lu ex J.L. Yang and *C. brachytricha* Steud. We refer to this clade as the *Pseudoarundinacea* subgroup because each of these taxa from East Asia have

been classified as varieties, subspecies, or synonyms of *C. arundinacea* (Veldkamp, 1992; Lu et al., 2006; POWO, 2021). Our data indicate, however, that they are not conspecific with or closely related to *C. arundinacea*. We sampled three accessions of *C. arundinacea* s.s.: two from Europe and one from the Caucasus. We found them to be part of a distantly related clade that we refer to as the *Deyeuxia* group. Our results indicate that there is significant genetic differentiation among *C. arundinacea* s.s., *C. collina*, *C. effusiflora*, and *C. brachytricha*, whereas their classification history in *C. arundinacea* suggests that there is little morphological differentiation among them. Similarly, Saarela et al. (2017) found collections from Asia identified as *C. arundinacea* to be part of a clade with the Asian species *C. brachytricha* and *C. distantiflora* (sometimes treated as *C. arundinacea* subsp. *distantiflora* (Luchnik) Tzvelev) and distinct from European specimens of *C. arundinacea*. A more detailed study of this group is warranted to clarify its evolutionary history and taxonomy.

The other species in the *Orientalis* group, based on the current sampling, are *C. hakonenis*, *C. scabrescens*, and *C. stenophylla*. We find the single sample of *C. stenophylla*, native to China South-Central, New Guinea, and Philippines, to be nested within *C. scabrescens*, sister to *C. scabrescens* var. *elatiore*, a species native to China North-Central, China South-Central, East Himalaya, Myanmar, Nepal, Pakistan, Qinghai, Tibet, and West Himalaya (POWO, 2021). In light of these results, additional study of *C. stenophylla*, including sampling of multiple individuals, is needed to clarify its affinities and taxonomic status. The circumscription of *C. scabrescens* also warrants further scrutiny, given the considerable genetic diversity among the seven accessions of *C. scabrescens* in our analyses.

The clade that we refer to as the *Deyeuxia* group includes three species: *C. arundinacea* s.s. (syn. *Deyeuxia pyramidalis*, the type of *Deyeuxia*, hence the informal clade name), *C. nyingchiensis* P.C. Kuo & S.L. Lu (Paszko, endemic to the Sichuan, Xizang, and Yunnan provinces of China (Paszko, 2016), and *C. tianschanica* Rupr., distributed from Central Asia to China (Gansu) (POWO, 2021). The *Deyeuxia* group is sister to a clade we refer to as the *Epigeios* group, which includes *C. varia*, *C. alajica*, *C. pseudophragmites*, *C. persica* Boiss., *C. epigeios*, and *C. acutiflora* in our complete tree as well as *C. arenaria*, *C. baltica*, and *C. emodensis* based only on ITS data. *Calamagrostis varia*, widespread across Europe (POWO, 2021), is sister to the rest of the clade, and *Calamagrostis alajica*, distributed in Kazakhstan, Kyrgyzstan, and Tajikistan (POWO, 2021), and *C. pseudophragmites*, widely distributed across Eurasia, are closely related species. The species *C. acutiflora* is embedded within *C. epigeios*. The compositions of the *Deyeuxia* and *Epigeios* clades and relationships among their species here are generally consistent with the trees in Saarela et al. (2017). Their ITS + ETS tree identified a clade including these same species [although they did not sample *C. tianschanica*, *C. persica*, and *C. alajica*] plus *C. rivalis* (Torges) H. Scholz], which they found to be closely related to *C. pseudophragmites*, *Ammophila arenaria*, and *C. baltica* (Flüggé ex Schrad.) Trin. (as *×Calammophila baltica* (Flüggé ex Schrad.) Brand, nom. inval.). Their plastid analyses identified a clade with the same species composition plus *C. baltica* (as *×Calammophila baltica*), which is a hybrid of *Calamagrostis arenaria* (syn. *Ammophila arenaria*) and *C. epigeios*, and *C.*

*sichuanensis* J.L. Yang (as *Deyeuxia sichuanensis* (J.L. Yang) S.M. Phillips & W.L. Chen), which they found to be closely related to *C. nyingchiensis*.

In our overall phylogeny, the *Calamagrostis* group includes *C. canescens* (the type of the genus), *C. nutkaensis*, *C. villosa*, *C. crassiglumis*, and *C. stricta*. Affinities of the western North American *C. nutkaensis* in our analyses are incongruent with the ITS + ETS tree in Saarela et al. (2017), in which *C. nutkaensis* forms a clade with several Eurasian species (*C. brachytricha*, *C. distantiflora*, *C. arundinacea* p.p., *C. diffusa* (Keng) P.C. Kuo & S.L. Lu ex J.L. Yang (as *D. diffusa* Keng), *D. pulchella* [= *C. lahulensis* G. Sing] and *D. scabrescens* [= *C. scabrescens* Griseb.]). The close relationship between the morphologically similar European species *C. canescens* and *C. villosa* (Paszko 2011) in our trees is consistent with the topology of both the ITS + ETS and plastid trees in Saarela et al. (2017).

We have identified numerous instances of strong conflict between the nuclear and plastid data sets within *Calamagrostis*, as in previous studies (Saarela et al., 2017). Accordingly, probable hybridization and introgression appear to have occurred many times in *Calamagrostis*. For example, we see this with the *C. obtusata*–*C. holciformis*–*C. anthoxanthoides* clade. Based on ITS markers, these taxa align within the *Americana* group (Fig. 3A), whereas based on plastid data, each of these taxa are part of either the *Orientalis*, *Purpurea*, or *Calamagrostis* clades. Although coming from different plastid-derived clades, *C. anthoxanthoides* and *C. holciformis* are morphologically similar in having narrow, spikelike panicles, 4–6 mm long lemmas with sub-basal inserted awns, and 4-denticulate apices, whereas *C. obtusata* has loose, open panicles, and lemmas dissimilar to the others (Lu et al., 2006). We refer to *C. anthoxanthoides*, *C. holciformis*, and *C. obtusata* as the *Stilpnophleum* Nevski subgroup (see Figs. 2, 3A) as treated in Wasiljew (1960).

Based on ITS markers, the clade that we refer to as the *Deschampsia* subgroup, comprising *C. purpurascens*, *C. deschampsoides*, *C. holmii*, and *C. matsumurae*, is sister to lineage comprising Clades B and C. Based on plastid markers, *C. deschampsoides*, *C. holmii*, and *C. matsumurae* are part of the *Calamagrostis* group and *C. purpurascens* is part of the *Americana* group. The lineage probably has a complicated history involving hybridization and/or subsequent introgression with unknown members of other major lineages. *Calamagrostis purpurascens* is wide ranging from Alaska through Canada to Greenland and Newfoundland, then again to eastern and central arctic Siberia, while *C. deschampsoides* is also wide ranging on coastal dunes in North America through Alaska across the arctic to the Hudson Bay and extending to the arctic coast of Europe to Siberia and Japan (Marr et al., 2007). Consequently, opportunity for contact with many species of *Calamagrostis* is high. The topology of subgroup *Deschampsia* is consistent with the ITS + ETS tree in Saarela et al. (2017), in which *C. purpurascens*, *C. deschampsoides*, *C. stricta* subsp. *groenlandica* (Schrank) Á. Löve, and *C. lapponica* (Wahlenb.) Hartm. p.p. formed a clade. Saarela et al. (2017) treated *C. holmii* as a synonym of *C. stricta* subsp. *groenlandica*, and their samples of the latter taxon are from the range of *C. holmii*, as recognized by some authors, in northwestern North America. Given the current results and those of Saarela et al. (2017), it appears that *C.*

*holmii* should be recognized as a distinct taxon. However, further sampling of Arctic specimens identified as *C. stricta* subsp. *groenlandica* is needed to determine if this taxon is distinct from *C. holmii*.

*Calamagrostis perplexa* is another species of hybrid origin, as hypothesized based on morphological characteristics (Howard et al., 2009). As in Saarela et al. (2017), we find the species, based on study of the same accession, Peterson 2095, to be allied with the *Purpurea* group based on nuclear ribosomal data and the *Calamagrostis* group based on plastid data. *Calamagrostis lahulensis* also has a putative hybrid origin. Lu et al. (2006) indicated that *C. lahulensis* (as *Deyeuxia pulchella* Hook. f.) is a polymorphic species that intergrades with *C. scabrescens*. In our ITS-derived phylogenetic network (Fig. 2), both of these species are near each other along a reticulate branch, but in our cladogram based on plastid markers (Fig. 3C), *C. lahulensis* is clearly embedded within multiple accessions of *C. stricta* in the *Calamagrostis* group ( $C_3$ ). Morphologically, *C. lahulensis* and *C. scabrescens* share conspicuously penicillate rachillas (Lu et al., 2006; Paszko, 2012).

In western North America, the *Muiriana* subgroup (Fig. 3D) contains two species. Morphologically, *C. breweri* and *C. muiriana* are very similar but differ in chromosome number ( $2n=42$  and  $2n=28$ , respectively) and leaf blade width (0.9–1.7 and 0.2–0.4 mm, respectively) (Wilson & Gray, 2002; Marr et al., 2007; Peterson & Saarela, 2012). In addition, they have separate isozyme phenotypes and are geographically isolated, with populations of *C. muiriana* occurring south of Sonora Pass, California, and *C. breweri* occurring near Carson Pass north to Mount Hood, Oregon (Wilson & Gray, 2002; Marr et al., 2007; Peterson & Saarela, 2012). In our study a single accession of *C. breweri* is nested among four accessions of *C. muiriana* (Fig. 3D). However, only a single plastid marker (*rpl32-trnL*) was recovered from our sample of *C. breweri* and this probably limited our ability to interpret the phylogenetic resolution between these two species.

#### 4.2 Biogeography

Stepping back from the current analysis for global context, the order of divergence of the subtribes of supersubtribe Agrostidodinae is consistent between plastid DNA analyses, and their geographic areas are well known. Saarela et al. (2017) reported the branching order as (Brizinae (Echinopogoninae (Calothecinae + Agrostidinae {+Paramochloinae})). Tkach et al. (2020) reported this as (Hypseochoinae (Brizinae) (Echinopogoninae (Calothecinae + Agrostidinae)). Orton et al. (2021) reported (Brizinae (Echinopogoninae + Agrostidinae) [Calothecinae not sampled]). Gallaher et al. (2022) reported (Brizinae) (Echinopogoninae (Calothecinae + Agrostidinae)). In each of the previous studies, Anthoxanthinae is sister to Agrostidodina. Our study resolves (Echinopogoninae ((Calothecinae + Paramochloinae) (Agrostidinae)), as did Da Silva et al. (2022) with Brizinae as sister to these (no *Anthoxanthum* representatives in these studies). Within Agrostidodinae, Gallaher et al. (2022) estimated Brizinae to be the oldest clade (stem 20.13 Ma, crown 17.21 Ma), with younger dates for Echinopogoninae (stem 15.56 Ma, crown 9 Ma) and Agrostidinae (stem 12.58 Ma, crown 11.35 Ma), and Calothecinae (stem 12.58 Ma, crown not

applicable). Anthoxanthinae are monogeneric: The genus *Anthoxanthum* L. has 50 species worldwide and is well diversified on all continents, except Antarctica. Hypseochoinae (only sampled by Tkach et al., 2020) is monogeneric: *Hypseochoa* C.E. Hubb. has two rare species that are endemic to equatorial African mountains. Brizinae has two genera: *Airopsis* Desv., monotypic, is endemic to southwestern Europe and northwestern Africa, and *Briza* L. has three species endemic to northwest Africa and western Eurasia and one extending to the Himalayas. Echinopogoninae (4 genera, 59 species predicted) are mostly confined to temperate Australasia, with a few outliers on Pacific Islands and North America, and its earliest diverging lineage, the genus *Relchela*, is endemic to Patagonian South America. Calothecinae (10 genera, 27 species) and Paramochloinae (2 genera, 3 species) are confined to temperate Latin America, mostly south of 20°S, with a one species reaching Central America. Agrostidinae (9 genera, 412 species) are again worldwide.

Within Echinopogoninae, the clade comprising the expanded *Pentapogon*, with 20 species analyzed, including *Dichelachne*, *Calamagrostis* p.p. non typica, and *Deyeuxia* p.p. nontypica, is native to Australasia and the Pacific. This clade shares a common ancestor with the lineage comprising *Echinopogon* from Australasia and East Asia and *Greeneochloa* from North America and the Pacific (Hawaii). Most likely, the ancestral geographic range of these three genera is Australasia, although sister to these is *Relchela*, a monotypic genus known only from Argentina and Chile (Rúgolo, 2012).

Within subtribe Agrostidinae, the southwestern European–northwestern African genus *Agrostula* is the first genus to diverge, as sister to two major clades. One clade has (*Gastridium* + *Triplachne*) (Mediterranean), as sister to (*Alpagrostis* + *Agrostis*) and (*Podagrostis* + 3 species of *Calamagrostis*). The second major clade includes *Calamagrostis* s.s., and the biogeography of this genus is of key interest.

The latest summary indicates that *Calamagrostis* s.s. contains about 130 species worldwide, distributed principally in north temperate regions of Eurasia and North America, with a few species extending into South America (Gallaher et al., 2022; Soreng et al., 2022a). The Meridionalis group (sister to the remaining species of *Calamagrostis* s.s.) contains two species distributed in Central and South America, suggesting that the genus may have originated in the western hemisphere estimated at about 11.35 Ma (the age of the Agrostidinae where *Calamagrostis* is sister to the remaining genera in the subtribe; Gallaher et al., 2022); the tribe is sister to the Calothecinae, which are confined to South America. The Americana group is composed of species principally distributed in North America, although some species (e.g., *C. purpurascens* and *C. stricta*, based on plastid data only) extend to Europe and Asia, and *C. guatemalensis* Hitchc. and *C. pinetorum* Swallen are endemic to Guatemala and adjacent Mexico. The ancestor of this lineage likely had a North American ancestral range. The remaining five clades or groups within *Calamagrostis* likely share Eurasia as their ancestral range. The *Deyeuxia* and *Epigeios* groups (classically, distinct subgenera, sections, and sister groups, although here, *Deyeuxia* is much reduced in species) are composed of several species native to Eurasia. At least one species (*C. epigeios*) is introduced in North America and

another (*C. acutiflora* = *C. epigeios* × *arundinacea*) is widely planted as an ornamental beyond the native range of its parental species. The Orientalis group is composed of species from East Asia, which is likely its ancestral range. The Purpurea group includes species distributed in Eurasia and North America. Its ancestral range is probably Europe since it shares a common ancestor with the Orientalis group. The Calamagrostis group includes many transatlantic or wide-ranging species from Eurasia and North America.

#### 4.2.1 Biogeographical implications

Chloroplast capture, concerted evolution, and recurrent paternal backcrossing are considered the most probable processes resulting in the phylogenetic incongruences in *Calamagrostis*. Hybridization is very common in Poaceae, and its role in the process of speciation is significant (Peterson et al., 2010a, 2010b, 2014, 2015a, 2016; Saarela et al., 2010, 2017; Romaschenko et al., 2013; Soreng et al., 2015b, 2022b; Tkach et al., 2020; Winterfeld et al., 2009, 2014; Barberá et al., 2019b; Wölk & Röser, 2014, 2017). The conflicts in the phylogenetic placement of several taxa and entire groups in *Calamagrostis* between the plastid and nrDNA data (Figs. 3A–3D) may be explained by chloroplast capture, which probably occurred frequently among species with overlapping areas of distribution.

#### 4.2.2 Characteristics and distribution of the main lineages

Current data suggest that *Calamagrostis* probably originated in North America. This event was followed by two consecutive splits separating South American and Asian major lineages. For species that form the Americana group, it seems that North American plains and mountainous areas did not provide sufficient environmental isolation to support the regular or dichotomous-like process of evolutionary speciation. Our ITS SplitsTree diagram reveals a radiative pattern (Radiation A, Fig. 2) of diversification in the clade, and our combined phylogenetic tree has little to no deep resolution among lineages. Another theory is that we are witnessing the effect of genetic drift occurring as a result of dramatic events such as extreme cooling during the Pleistocene including southward expansion of the ice sheets. It might have produced a bottleneck where only one or a few close lineages survived and then subsequently radiated in more recent times.

Since we have few South American species in our study attributed to *Calamagrostis*, it is difficult to place much confidence in the placement (basal) of the Central and South American Meridionalis group (Clade X, Figs. 1b, 3) in our phylogeny. Recent molecular study (Peterson et al., 2019; da Silva et al., 2022) showed that the majority of South American species formerly treated within *Calamagrostis* should be attributed to the distantly related genera *Cinnagrostis* and *Peyritschia* E. Fourn. Excluding local derivatives (sometimes treated as synonyms) of widely distributed taxa such as *C. epigeios* (Epigeios group, Clade B<sub>1</sub>) and *C. stricta* (Calamagrostis group, Clade C<sub>3</sub>), and North American migrants such as *C. guatemalensis* (Americana group, Clade A), there are 10 or 12 South American species that have not yet been included in molecular studies (Peterson et al., 2019). The expectation is that phylogenetic analysis, including a full set of South American species, will perhaps elucidate the evolutionary history of this autochthonous group. The SplitsTree diagram indicated some basal

reticulation between the Meridionalis group and Eurasian branch B, which indicates some interaction of these lineages of *Calamagrostis* after their primary split in the process of centrifugal or latitudinal (north to south) expansions of the genus. The plausible assumption could be that this northward expansion of *Calamagrostis* resulted in its migration into Asia via Beringia, and further into Siberia, and finally Europe. In fact, branch B is the only part of the ITS SplitsTree diagram that shows an internally supported tree-like topology, which probably indicates a more or less regular (bifurcating) evolutionary process for this group. This is not the case for Eurasian branch C, which shows a rapid radiation event (Radiation C, Fig. 2) similar to that of branch A, representing the North American group Americana. We have only marginal support for the simultaneous separation of branches (Clades) B and C from branch (Clade) A (Figs. 1B, 2). However, based on this scenario, both Eurasian groups, which share a common ancestor, probably migrated into Asia independently and, at different times, were fully developed (speciated) by the time their interactions began. It is possible that Radiations A and C were triggered by the same or similar factors (e.g., sudden availability of variable environments to genetically impoverished lineages that survived a bottleneck event). However, unlike in Clade A, which presents a clear example of explosive radiation, Clade C culminates in a trichotomy (Fig. 1B, Clades C<sub>1,2,3</sub>), fewer branches showing somewhat regular bifurcation patterns. Characteristically, branch C<sub>1</sub> is represented by a predominantly Asian group (Orientalis), showing a somewhat latitudinal distribution (south and central East Asia to Siberia), while branches C<sub>2</sub> and C<sub>3</sub> are represented, respectively, by the circumboreal group Purpurea and the transatlantic (Europe–Americas) group Calamagrostis, which shows a wide longitudinal distribution. The Purpurea group contains circumboreal species such as *C. lapponica* and *C. canadensis*, and *C. purpurea* was originally described from Transbaikalia (Siberia). *Calamagrostis lapponica* ranges to the far east, whereas *C. chalybaea* Fr. extends to western Siberia. Tzvelev (1976) suggests that *C. nutkaensis* was found in Kamchatka based on a single specimen, presumably a range extension from the Pacific Northwest in North America.

#### 4.2.3 Evolutionary relationships between main lineages and appearance of intermediate phylogenetic groups

The identified subgroup Stilpnophleum (*C. anthoxanthoides*, *C. holciformis*, and *C. obtusata*) unites these Siberian and central Asian species within the North American Clade A (Fig. 3A) based on nuclear ITS data, while the chloroplast data suggest affinities with mostly Asian lineages (Fig. 3A, Clades B<sub>2</sub>, C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>). This is one of many ITS homogenization zones found on the phylogenetic tree within *Calamagrostis*. In the case of subgroup Stilpnophleum, the process of ITS homogenization via recurrent genetic exchange involved interaction among distant lineages from different continents. The paternal backcrossing had a prevailing effect since it provided sufficient amount of genetic transformation for phylogenetic analysis to place ITS sequences of *C. anthoxanthoides*, *C. holciformis*, and *C. obtusata* within the Americana group (presumably a paternal lineage) with some degree of support. We suggest that the contact zone may have included the Central and Northern parts of East Asia and the Western part of Beringia. After recession of the

American element, the plants that remain on the Asian territory have undergone further speciation and westward expansion. Incidentally, although the specimens of *C. anthoxanthoides* from Tajikistan and Kyrgyzstan have similar ITS sequences, they contain different plastid lineages from the autochthonous East Asian group *Orientalis* (PL<sub>C1</sub>) and the ubiquitous group *Calamagrostis* (PL<sub>C3</sub>), respectively. Almost certainly, the plastid lineage of the specimen from Kyrgyzstan originated within *C. stricta*. It provides additional evidence for the feasibility of ITS homogenization zones.

The evolutionary history of *C. epigeios* (Clade B) involves a different pattern of speciation and biogeographical scenario. In our analysis of seven specimens of *C. epigeios* (Fig. 3B), plus cultivated *C. acutiflora*, from East, Central, and Minor Asia, Europe, and North America, all show little variation among ITS sequences. All American specimens and European accessions of *C. epigeios* have plastid sequences consistent with the ITS position, while plastid lineages of Asian accessions probably originated within *C. stricta* (Clade C) and within sister taxon, *C. pseudophragmites* (Fig. 3B). The plastid lineages of two other Asian species *C. macrolepis* and *C. emodensis* also originated within *C. stricta*. According to ITS data, *C. macrolepis* and *C. emodensis* are not closely related to *C. epigeios*, despite sharing many morphological features among the three species. Paszko (2013) recently named *C. emodensis* var. *brevisetata* Hack. as a synonym of *C. macrolepis*, and Tzvelev (1976) treated it as a subspecies of *C. epigeios* (*C. epigeios* subsp. *macrolepis* Tzvelev). We hypothesize the following: *C. epigeios* has a European origin, and its westward expansion to North America is attributed to multiple introductions; this would account for negligible genetic differentiation between European and American accessions. The eastward expansion of *C. epigeios* often resulted in genomic introgression via recurrent backcrossing and chloroplast capture of distant groups of Asian *Calamagrostis*. Thus, Asian specimens of *C. epigeios* represent the cases of complete genomic introgression, while, possibly, *C. macrolepis*, *C. emodensis*, and some specimens of *C. pseudophragmites* represent cases of incomplete introgression. The tentative position of *C. macrolepis* within the Clade B reflects the confounded state of ITS sequences and/or early stages of genomic introgression.

Similar to the *Stilpnophleum* subgroup (Fig. 3A), the putative ITS *Deschampsia* subgroup (Fig. 3C) could have appeared as a result of interaction between the North American (*Americana* group Clade A) and Eurasian lineages of circumboreal and transatlantic distribution (*Calamagrostis* group C<sub>3</sub>). On the phylogenetic tree, the group represents the ITS confounding/homogenization zone (Fig. 3C). The ambiguous phylogenetic position of the *Deschampsia* ITS subgroup indicates early stages of introgression of the presumably *Americana*-type genome. Biogeographically, the group reflects the circumboreal pattern of the Eurasian progenitors. The overall topology of the phylogenetic tree and the location of paternal lineages suggest the following evolutionary scenario: similar to the *Stilpnophleum* subgroup, we assume primary westward expansion from North America of one to a few representatives of the *Americana* group (e.g., *C. sesquiflora* and *C. "proto-purpurascens"*); creation of the peri-Beringial contact zone of American and Eurasian lineages (i.e., representatives of the *C. stricta*

complex); recurrent paternal backcrossing and early withdrawal of the paternal (American) element resulting in incomplete genomic introgression (the paternal genome is not fixed); speciation of *C. purpurascens*, *C. deschampsoides*, and *C. holmii*; and subsequent later eastward expansion into North America, possibly along with the elements of current circumboreal and transatlantic groups (e.g., *C. canadensis*, *C. stricta*, etc.). In the case of *C. perplexa* and *C. rubescens* (Fig. 3C), the hybrid speciation event that possibly involves *C. canadensis*, as a paternal lineage, and *C. stricta* and an unidentified representative of the group *Americana*, as the respective maternal lineages, probably had taken place after the circumboreal and transatlantic groups colonized North America. Such a colonization might have included migration of *C. nutkaensis*. Incidentally, in the 19th century, *C. nutkaensis* was collected from coastal marsh areas of Kamchatka (Tzvelev & Probatova, 2019), which could have represented the remnants of the original population.

The *Calamagrostis* group (i.e., *C. stricta*, Clade C<sub>3</sub> in Figs. 3A–3C) “acts” as a major plastid donor group. Our data suggest that plastid types of this cosmopolitan group could be found within almost all phylogenetic lineages of Eurasian origin, including the *Stilpnophleum* and *Deschampsia* subgroups. This is in contrast to some other groups, such as *C. epigeios* or *C. canadensis*, which “act” as plastid capturing groups. It is possible to assume that the distribution of *C. stricta* in Eurasia is somehow receding, yielding to biogeographically more active groups.

The biogeographic history of Oregon-Californian endemics *C. muiriana* and *C. breweri* is ambiguous. Its phylogenetic position at the base of Eurasian Clade B suggests that these species probably represent a remnant of the ancient North American group that first dispersed into Asia via Beringia after the split with the autochthonous groups *Meridionalis* and *Americana*.

## 5 Taxonomy

**5.1 *Condilorachia*** P.M. Peterson, Romasch. & Soreng, **gen. nov.** Type: *Condilorachia brasiliensis* (Louis-Marie) P.M. Peterson, Romasch. & Soreng.

**Diagnosis:** *Condilorachia* differs from *Chascolytrum* Desv. s.s. (sensu da Silva et al., 2022) in having 2- or 3-flowered spikelets (verses 3–14-flowered in *Chascolytrum*), dorsally awned lemmas from the base or near the middle (apically muticous or mucronate lemmas in *Chascolytrum*), and a hairy callus (glabrous in *Chascolytrum*).

**Description:** Perennials, tufted. Culms 30–70 cm long, often bulbous at the base. Leaf sheaths glabrous, fibrous near the base; ligules 0.5–3 mm long, membranous, obtuse, sometimes lacerate; blades 1–25 cm long, flat, conduplicate, or involute to filiform, usually scaberulous, membranous, sometimes coriaceous and stiff. Inflorescence a panicle with pedicelled spikelets, linear, loosely flowered to somewhat compact or spiciform. Spikelets 2- or 3-flowered, oblong to lanceolate, laterally compressed with a rachilla extension; disarticulation above the glumes, the fertile florets falling free; rachilla internodes usually pubescent; callus pubescent; glumes 1- or 3-veined, persistent, longer, or shorter than the spikelet, membranous; lemmas 3- or 5-veined, cartilaginous,



sometimes membranous, dorsally awned from the base to nearly the middle, the awn geniculate with a twisted column, apex bifid; palea minute to as long as the lemma, 2-keeled, the keels ciliate; stamens 3, sometimes 1; lodicules 2, membranous. Caryopsis with an adherent pericarp.

**Distribution:** *Condilorachia* comprises three species found in Argentina, Brazil, and Chile. All three species are uncommon and known from very few historic and recent collections (Cabrera, 1970; Finot, 2012; GBIF, 2021).

**Etymology:** The generic name, *Condilorachia*, was chosen to emphasize that the three species have spikelets with a short cartilaginous rachis bearing florets.

### Key to the species of *Condilorachia*

- 1a. Fertile lemmas 8–10 mm long, 3-veined, principal lemma awns 12–17 mm long; glumes not exceeding the apex of the spikelet..... **C. bulbosa**
- 1b. Fertile lemmas 4.3–6.2 mm long, 5-veined, principal lemma awns 4–6 mm long; glumes exceeding apex of the florets..... 2
- 2a. Panicle densely flowered, spiciform; leaf blades 4–5 cm long; upper glumes 2.5 times as long as the adjacent fertile floret; ligules 2–3 mm long, not bilobed..... **C. juergensii**
- 2b. Panicle loosely flowered, bearing few spikelets; leaf blades generally 10–25 cm long; upper glumes 1.1 times as long as the adjacent fertile floret; ligules 0.5–1 mm long, bilobed..... **C. brasiliensis**

**5.2 *Condilorachia brasiliensis*** (Louis-Marie) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Trisetum brasiliense* Louis-Marie, *Rhodora* 30: 242. 1928 [1929] ≡ *Deschampsia brasiliensis* (Louis-Marie) Valencia, *Revista Argent. Agron.* 8: 128. 1941. Type: Brazil, Rio de Janeiro, Itatiaia, peaty soil among rocks above timberline, 2200–2400 m, 17 January 1925, A. Chase 8304 (holotype: US-1257235!; isotypes: BAA-fragment, GH-00024554 [image!], MO-2114543 [image!], NY-00604757 [image!], RB-00538835 [image!], US-1447199!, W-19300013187 [image!]).

***Condilorachia bulbosa*** (Hitchc.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Trisetum bulbosum* Hitchc., *J. Wash. Acad. Sci.* 17(9): 217, f. 2. 1927 ≡ *Helictotrichon bulbosum* (Hitchc.) Parodi, *Revista Argent. Agron.* 16: 211. 1949. Type: Chile, Concepción (San Pedro), 30 Oct 1926, C. Joseph 4607 (holotype: US-1297352!; isotype: BAA-fragment).

***Condilorachia juergensii*** (Hack.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Trisetum juergensii* Hack., *Verh. K.K. Zool.-Bot. Ges. Wien.* 65(2): 75–76. 1915 ≡ *Deschampsia juergensii* (Hack.) Valencia, *Revista Argent. Agron.* 8: 125. 1941. Type: Brazil, Rio Grande do Sul, Mun. Soledade, in campis siccis prope Fazenda S. Thome, 650 m, December 1910, C. Jürgens G289 (holotype: W-19160039326 [image!]; isotype: US-3413730 ex W!).

**5.3 *Greeneochloa expansa*** (Munro ex Hillebr.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Deyeuxia expansa*

Munro ex Hillebr., *Fl. Hawaiian Isl.* 519. 1888 ≡ *Calamagrostis expansa* (Munro ex Hillebr.) Hitchc., *Mem. Bernice Pauahi Bishop Mus.* 8(3): 149, f. 34. 1922. Type: USA, Hawaii, Maui Island, Maui Co., north bank of the crater of Haleakala, C. Wilkes s.n. (holotype: K000838377 [image!]; isotype: US-866012 fragm. ex GH!).

**5.4 *Pentapogon*** R.Br., *Prodr.* 173. 1810. Type: *Pentapogon billardieri* R. Br. = *Pentapogon quadrifidus* (Labill.) Baill. = *Dichelachne* Endl., *Prodr. Fl. Norfolk.* 20. 1833. Type: *Dichelachne montana* Endl. = *D. micrantha* (Cav.) Domin. = *Sclerodeyeuxia* (Stapf) Pilg., *Bot. Jahrb. Syst.* 74: 19. 1947. Type: *Sclerodeyeuxia sclerophylla* (Stapf) Pilg.

***Pentapogon avenoides*** (Hook.f.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Agrostis avenoides* Hook. f., *Handb. N. Zeal. Fl.* 330. 1864 ≡ *Deyeuxia avenoides* (Hook. f.) Buchanan, *Indig. Grass. N. Zeal.* (add. et corrig.) 11. 1880 ≡ *Calamagrostis avenoides* (Hook. f.) Cockayne, (*New Zealand Dep. Lands: Rep. Bot. Surv. Tongariro Natl. Park* 35. 1908.

***Pentapogon brassii*** (Hitchc.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Calamagrostis brassii* Hitchc., *Brittonia* 2(2): 116. 1936 ≡ *Deyeuxia brassii* (Hitchc.) Jansen, *Acta Bot. Neerl.* 1(3): 468. 1952.

***Pentapogon chaseianus*** (Bor) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Deyeuxia chaseana* Bor, *Brittonia* 14(1): 48, f. 1–2. 1962 ≡ *Deyeuxia stenophylla* var. *chaseana* (Bor) Veldkamp, *Alpine Fl. New Guinea* 2: 1132, t. 367. 1979 ≡ *Deyeuxia suizanensis* var. *chaseana* (Bor) Veldkamp, *Taiwania* 38(1–2): 2. 1993.

***Pentapogon crinitus*** (L. f.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Anthoxanthum crinitum* L. f., *Suppl. Pl.* 90. 1781 [1782] ≡ *Dichelachne crinita* (L. f.) Hook. f., *Fl. Nov.-Zel.* 1:293. 1853.

***Pentapogon densus*** (Benth.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Deyeuxia densa* Benth., *Fl. Austral.* 7: 582. 1878 ≡ *Calamagrostis austrodensa* Govaerts (non. *C. densa* Vasey), *World Checkl. Seed Pl.* 3(1): 9. 1999. (27 July 1999 epub).

***Pentapogon frigidus*** (F. Muell. ex Benth.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Deyeuxia frigida* F. Muell. ex Benth., *Fl. Austral.* 7: 583. 1878 ≡ *Calamagrostis frigida* (F. Muell. ex Benth.) Maiden & Betche, *Census New S. Wales Pl.* 21. 1916.

***Pentapogon gunnianus*** (Nees) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Echinopogon gunnianus* Nees, *London J. Bot.* 2: 413. 1843 ≡ *Agrostis gunniana* (Nees) F. Muell., *Pap. & Proc. Roy. Soc. Tasmania* 119. 1882 ≡ *Deyeuxia gunniana* (Nees) Benth., *Fl. Austral.* 7: 584. 1878 ≡ *Calamagrostis gunniana* (Nees) Reeder, *J. Arnold Arbor.* 31: 323. 1950.

***Pentapogon hirtellus*** (N.G. Walsh) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Dichelachne hirtella* N.G. Walsh, *Muelleria* 7: 454, 455, fig. 11–k. 1992.

***Pentapogon inaequiglumis*** (Hack. ex Cheeseman) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Dichelachne sciurea* var. *inaequiglumis* Hack. ex Cheeseman, Man. New Zealand Fl. 874. 1906 ≡ *Dichelachne inaequiglumis* (Hack. ex Cheeseman) Edgar & Connor, New Zealand J. Bot. 20(3): 307. 1982.

***Pentapogon lautumia*** (Edgar & Connor) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Dichelachne lautumia* Edgar & Connor, New Zealand J. Bot. 37: 67–68, f. 4. 1999.

***Pentapogon micranthus*** (Cav.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Stipa micrantha* Cav., Icon. 5: 42, 467, f.2. 1799 ≡ *Dichelachne micrantha* (Cav.) Domin, Biblioth. Bot. 85: 353. 1915 = *Dichelachne montana* Endl., Prodr. Fl. Norfolk. 20. 1833.

***Pentapogon parvus*** (B.K. Simon) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Dichelachne parva* B.K. Simon, Austrobaileya 1(5): 462. 1982.

***Pentapogon quadrisetus*** (Labill.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Avena quadriseta* Labill., Nov. Holl. Pl. 1: 25, t. 32. 1805 ≡ *Agrostis quadriseta* (Labill.) R. Br., Prodr. 1: 171. 1810 ≡ *Calamagrostis quadriseta* (Labill.) Spreng., Syst. Veg. ed. 16 [Sprengel] 1: 253 1825 [1824] ≡ *Deyeuxia quadriseta* Benth., Fl. Austral. 7: 581. 1878 ≡ *Bromidium quadrisetum* (Labill.) Nees, London J. Bot. 2: 416. 1843.

***Pentapogon rarus*** (R. Br.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Agrostis rara* R. Br., Prodr. 1: 171. 1810. ≡ *Dichelachne rara* (R. Br.) Vickery, Contr. New South Wales Natl. Herb. 1: 337. 1951.

***Pentapogon robustus*** (B.K. Simon) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Dichelachne robusta* B.K. Simon, Fl. Australia 44A: 386, 221. 2009.

***Pentapogon scaberulus*** (Vickery) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Deyeuxia scaberula* Vickery, Contr. New South Wales Natl. Herb. 1: 64. 1940 ≡ *Calamagrostis austroscaberula* Govaerts (non. *C. scaberula* Swallen), World Checkl. Seed Pl. 3(1): 9. 1999. (27 July 1999 epub).

***Pentapogon sclerophyllus*** (Stapf) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Deyeuxia sclerophylla* Stapf., Hooker's Icon. Pl. 27(1): t. 2605, 1–2. 1899 ≡ *Sclerodeyeuxia sclerophylla* (Stapf) Pilg., Bot. Jahrb. Syst. 74: 19. 1947 ≡ *Calamagrostis sclerophylla* (Stapf.) Hitchc., Brittonia 2(2): 117. 1936.

***Pentapogon sieberianus*** (Trin. & Rupr.) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: *Dichelachne sieberiana* Trin. & Rupr., Sp. Gram. Stipac. 2–3. 1842.

***Pentapogon suizanensis*** (Hayata) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Agrostis suizanensis* Hayata, Icon. Pl. Formosan. 7: 83–84, f. 50. 1918 ≡ *Calamagrostis suizanensis* (Hayata) Honda, Bot. Mag. (Tokyo) 40(476): 440,

444. 1926 ≡ *Deyeuxia suizanensis* (Hayata) Ohwi, J. Jap. Bot. 33(7): 211. 1958.

***Pentapogon validus*** (Vickery) P.M. Peterson, Romasch. & Soreng, **comb. nov.** Basionym: ≡ *Deyeuxia monticola* var. *valida* Vickery, Contr. New South Wales Natl. Herb. 1: 57. 1940 ≡ *Deyeuxia valida* (Vickery) C.M. Weiler, Fl. Australia 44A: 385. 2009.

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## Supplementary Material

**Appendix S1.** Taxon voucher (collector, number, and where the specimen is housed), country of origin, and GenBank accession for DNA sequences of *rps16-trnK*, *rps16* intron, *rpl32-trnL*, and ITS regions; **bold** indicates new accession; a dash (–) indicates missing data.