





## Research Article

# Biogeography, timing, and life-history traits in the PPAM clade: Coleanthinae (syn. Puccinelliinae), Poinae, Alopecurinae superclade, Miliinae, and Avenulinae and Phleinae (Poaceae, Pooideae, Poeae)

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**Abstract** We conducted a biogeographic analysis of the PPAM clade of Poeae Plastid DNA Group 2, which includes 12 subtribes of  $C_3$  grasses. One hundred and eighty-four species sampled represent 42 of 43 accepted genera and taxonomic diversity in large genera. We analyzed plastid sequences of *matK*, *trnC-rpoB*, and *trnT-trnL-trnF* using BEAST to produce a dated tree and MrBayes to produce a Bayesian tree, on which we ran Bayesian-Binary-Markov-Chain analyses on a worldwide biogeographic data set of 12 areas. PPAM split in southwestern Asia into subtribe Coleanthinae and PAM clades in the Early Miocene. PAM diversified rapidly in the Middle Miocene in southwestern Asia into four monogeneric lineages, Avenulinae, Phleinae, Miliinae, Poinae, and the Alopecurinae superclade (seven subtribes with 27 genera). In the Late Miocene, Pliocene, and mostly Pleistocene, the latter four lineages diversified and dispersed across Eurasia and established in North America. Dispersals to the southern hemisphere occurred in the Pliocene and Pleistocene. Annuals occur in 15 Mediterranean and southwestern Asia genera, but in few genera in other regions. Beyond phylogenetically isolated annual species dating to the Miocene, all other annuals evolved in the Pliocene and Pleistocene. Cold tolerance is high among perennial species, many occurring in the alpine, nine genera ranging into the Arctic. We suggest that alpine and subalpine habitats were ancestral. High tolerance of saline and alkaline conditions arose between the Pliocene and Pleistocene in Coleanthinae, Alopecurinae, Poinae, Hookerochloinae, Beckmanniinae, and *Arctopoa*. Combinations are proposed for *Cornucopiae alopecuroides* in *Alopecurus* and for *Paracolpodium colchicum* in *Hyalopodium*. A nothogenus  $\times$  *Catanellia* is proposed for *Catabrosa*  $\times$  *Puccinellia*.

**Key words:** dispersal, grasses, molecular clock, Neogene, taxonomy, worldwide.

## 1 Introduction

### 1.1 Previous phylogenetic studies

“PPAM” is shorthand for the clade including subtribes Puccinelliinae, Poinae, Alopecurinae s.l., and Miliinae (Gillespie et al., 2008), while the “PAM” clade refers to the latter three. These are cool season grasses of tribe Poeae. They include lawn and pasture grasses, such as meadow foxtail (*Alopecurus pratensis* L.), timothy (*Phleum pratense* L.), and Kentucky bluegrass (*Poa pratensis* L.), many valuable forage and land stabilization species, and alkali grasses. They occur on all continents in temperate deciduous, coniferous and boreal forests, arctic and alpine tundra, arid to mesic steppe, saltmarshes, and alkali wetlands, almost always where cool to frigid winters combine with winter precipitation. Subtribe Puccinelliinae is now correctly called Coleanthinae due to the

addition of *Coleanthus* (Soreng et al., 2015b). At the time of coining PPAM, Gillespie et al. (2008) considered several smaller tribes to be part of Alopecurinae s.l. (Beckmanniinae, Phleinae, Cinninae, along with miscellaneous unplaced genera). Since then, five other studies have investigated the phylogeny of PPAM in detail (Gillespie et al., 2009, 2010, 2022; Soreng et al., 2015a; Tkach et al., 2020). The unplaced genera were subsequently recognized in clades and subtribes as sampling and sequencing expanded (Brizochloinae, Dupontiinae, Hookerochloinae, Ventenatinae) (Soreng et al., 2017; Tkach et al., 2020; Gillespie et al., 2022). Alopecurinae, in this broad sense, with *Phleum* removed, is called the Alopecurinae superclade (Gillespie et al., 2022, and here). The placement of *Avenula pubescens* (*Avenula* is now monotypic after removal of all other species to *Helictochloa* [Romero Zarco, 2011]) within this assemblage was evident,

but initially considered to represent some reticulation event (Soreng & Davis, 2000). Although we now accept it in PPAM, its sister relationship has remained ambiguous (Soreng et al., 2017; Tkach et al., 2020).

There is now no doubt concerning the split of the PPAM clade from the rest of the tribe (e.g., Gillespie et al., 2022; Soreng et al., 2015a, 2015b, 2017; Saarela et al., 2015, 2018; Orton et al., 2019, 2021). Tkach et al. (2020) went a step further in confining tribe Poeae s.s. to just PPAM. However, because nuclear ribosomal DNA (nrDNA) evidence leaves seven other Poeae s.l. subtribes in limbo between their proposed resurrection of Aveneae, Festuceae and narrowed circumscription of Poeae (see below), Gillespie et al. (2022) consider their proposal unsatisfactory and retain Poeae in the broader sense (Soreng et al., 2015b, 2017, 2022). The PPAM clade is distinct in both plastid and nrDNA analyses, yet there remains lingering doubt among agrostologists and taxonomists in general (e.g., Stace, 2020) about what might be found in the nuclear genome, beyond nrDNA, which might provide a deeper reticulate history between PPAM and other clades of Poeae s.l. PPAM currently (here and elsewhere in this JSE issue) contains 12 subtribes, 43 accepted genera, and ca. 870 species (Table 1). One additional genus, *Cinnastrum* (Gillespie et al., 2022), and two new genera proposed by Tkach et al. (2020) were recognized since the classification in Soreng et al. (2017).

Previous molecular phylogenetic analyses of subfamily Pooideae are discussed by Soreng (in Gallaher et al., 2022). Poeae sensu Soreng et al. (2017), here equivalent to supertribe Poodae, is the largest tribe within the subfamily. More than 2650 species, about 1/3 of the Poaceae total, are encompassed in 142 genera distributed in 34 subtribes. All the genera are clearly apportioned to two distinct and strongly supported plastid DNA clades (e.g., Soreng & Davis, 1998; Döring, 2009; Saarela et al., 2015, 2017, whole plastomes; Tkach et al., 2020; Orton et al., 2021, whole plastomes), Poeae “chloroplast” or Plastid DNA Group 1 (Aveneae type) and Group 2 (Poeae type) as named and applied by Soreng et al. (2003, 2007, 2015b, 2017, 2022). As often noted (beginning with Soreng et al., 1990), these plastid DNA groups do not correspond well to tribes Aveneae and Poeae of pre-molecular classifications (e.g., Clayton & Renvoize, 1986; Watson & Dallwitz, 1992). Soreng et al. (2017) recognized three distinct internal plastid DNA groups within Poeae that are consistent with nrDNA clades, as super-subtribes (Agrostidinae, Loliodinae, and Poodinae [=PAM clade]). In plastid analyses by Tkach et al. (2020), the most broadly sampled to date across Poodae, a subset of subtribes with Group 2 plastid types (Airinae, Antinoriinae, Aristaveninae, Helictochloinae, Holcinae, Scolochloinae, and Sesleriinae; called the AAAHHSS subtribes here) align (Airinae aside) within a poorly supported clade including Loliodinae (comprised of subtribes Ammochloinae, Cynosurinae, Dactylidinae, Loliinae, and Parapholiinae). This clade, Airinae, and PPAM resolved in a strongly supported Group 2 clade, but in a trichotomy. Results of other studies are similar, but often with Airinae included in a clade with Loliodinae + AAAHHSS subtribes (Quintanar Sánchez et al., 2007; Saarela et al., 2010). Loliodinae plus the AAAHHSS subtribes here is called “Loliodinae+”.

Although PPAM is well supported in plastid and nrDNA analyses and Loliodinae is well supported in nrDNA trees, the plastid Loliodinae+ so far as sampled by different authors, is rearranged in analyses of nrDNA (Grebstein et al., 1998; Quintanar Sánchez et al., 2007; Saarela et al., 2010, 2017; Tkach et al., 2020). When nrDNA was examined by Tkach et al. (2020), the AAAHHSS subtribes partially collapsed into a polytomy including Plastid DNA Group 1, Loliodinae, and PPAM clades (except for the placement of Antinoriinae, which nested in Loliodinae). Subsets of AAAHHSS subtribes were not resolved, except for Airinae + Holcinae, which was well supported as the sister to Plastid DNA Group 1 genera and subtribes, and Scolochloinae and Sesleriinae were separately nested among Group 1 subtribes. Other nrDNA analyses of Poeae (Grebstein et al., 1998; Quintanar Sánchez et al., 2007; Saarela et al., 2010, 2017) found similar arrangements of AAAHHSS subtribes, these variously arranged in a polytomy or grading to all genera of Group 1 genera, also with Scolochloinae and Sesleriinae intercalated within Group 1. Notably, all genera with Group 1 plastid DNAs remain in Group 1 in nrDNA analyses. As Tkach et al. (2020) noted, this discrepancy is highly suggestive of one or several past reticulation events between Group 1 and Group 2. However, PPAM was always resolved as monophyletic and within Group 1.

Several studies have attempted to date the major clades of Poaceae subfamily Pooideae using fossil priors and relaxed molecular clock algorithms (see Gallaher et al., 2022, for review). However, few such studies have provided dates within major lineages of Poeae DNA Group 2 genera (Inda et al., 2008; Minaya et al., 2017, for Loliinae; Pimentel et al., 2017; Schubert et al., 2019b). Three studies have attempted to put some dates on *Poa* (Hoffmann et al., 2013; Birch et al., 2014; Giussani et al., 2016). In addition to extensive coverage in *Poa*, Hoffmann et al. (2013) analyzed ITS of representatives of 35 other PPAM.

## 1.2 Early biogeographic studies

Hartley (1961) evaluated the climatic parameters affecting the distribution of the genus *Poa* and concluded that the proportion of *Poa* species in the grass flora drops off sharply where the average daily mid-summer (July) isotherms exceeded 24 °C in North America. This correlation was looser elsewhere in the world. Hartley (1973) then evaluated geography in Pooideae (the genera included were concordant with the modern subfamily placements, with few exceptions), with tribes Stipeae and Triticeae roughly current in circumscription, but Poeae and Aveneae genera (comprising Poeae as recognized here) were intermixed much as in Clayton & Renvoize (1986), and he again determined that the proportion of Pooideae in floras declined under high average daily summer temperatures. Hartley basically discovered the C<sub>3</sub>/C<sub>4</sub> boundary where reduced photorespiration gave C<sub>4</sub> grasses advantage over C<sub>3</sub> grasses under higher summer temperatures.

A series of papers addressed the biogeographic distribution of Poaceae with chorology (Clayton, 1975; Clayton & Cope, 1980; Cross, 1980) based on the developing, late-pre-molecular, “modern-synthesis” classification of Clayton & Renvoize (1986). Although these were very broad-brush papers often focused on numbers of all species in areas

Table 1 PPAM classification

Subtribe	Genus	No. spp.	Area (Native geographic range)	Annuals	Habitat
Coleanthinae	<i>Catabrosa</i> P. Beauv.	7	Northern Hemisphere, with disjunctions in Africa and South America	Annuals	Non-calcareous to sub-saline or sub-alkaline wetlands at all elevations
Coleanthinae	<i>Catabrosella</i> (Tzvelev) Tzvelev	6	Southwestern Asia		Non-calcareous to calcareous substrates uplands and alpine
Coleanthinae	<i>Coleanthus</i> Seidl	1	Northern Eurasia, and northwestern North America	Annual	Ephemeral brackish, sub-saline, or alkaline wetlands
Coleanthinae	<i>Colpodium</i> Trin.	9	Africa, Southwestern Asia		Non-calcareous to calcareous upland substrates uplands and alpine
Coleanthinae	<i>Hyalopoa</i> (Tzvelev) Tzvelev	6	Southwestern Asia (Caucasus and Pontic Mts.)		Non-calcareous alpine
Coleanthinae	<i>Hyalopodium</i> Röser & Tkach	2	Southwestern Asia		Non-calcareous alpine
Coleanthinae	<i>Paracolpodium</i> (Tzvelev) Tzvelev s.s.	6	Southwestern Asia (Central Asia only)		Calcareous alpine
Coleanthinae	<i>Phippsia</i> (Trin.) R. Br.	2(3?)	Eurasia, North America, (South America?)		Sub-saline or alkaline wetlands, mainly arctic, some alpine
Coleanthinae	<i>Puccinellia</i> Parl.	116	Worldwide	Few annuals	Saline and alkaline wetlands, coasts, steppe, alpine, and arctic
Coleanthinae	<i>Sclerochloa</i> Stapf	3	Eurasia	Annuals	Ephemeral sub-saline or alkaline wetlands
Coleanthinae	Zingeria P.A. Smirn. (= <i>Colpodium</i> )	(5)	Southwestern Asia	Annuals	Ephemeral niches of steppe and forest openings
Avenulinae	<i>Avena</i> (Dumort.) Dumort.	1	Eurasia		Mesic steppe
Phleinae	<i>Phleum</i> L.	16	Eurasian with one worldwide perennial	Several annuals	Steppe and alpine to arctic
Miliinae	<i>Milium</i> L.	6	Northern Eurasia, with disjunct populations in eastern North America	3 annuals	Forests to low alpine, and steppe
Poinae	<i>Poa</i> L.	570	Worldwide	ca. 40 annuals	Non-calcareous or less often calcareous substrates, temperate to boreal forests, coastal dunes, steppe, wetlands (rarely subsaline), alpine, and arctic
Alopecurinae *	<i>Alopecurus</i> L.	44	Worldwide	Several annuals	Steppe, alpine and arctic, and brackish wetlands
Alopecurinae *	<i>Comucopiae</i> L. (= <i>Alopecurus</i> )	(2)	Southwestern Asia	Annuals	Ephemerals low steppe wetlands
Alopecurinae *	<i>Limnas</i> Trin.	3	Northeastern Asia		Alpine and arctic
Beckmanniinae *	<i>Beckmannia</i> Host	2	Eurasia, North America		Brackish to sub-saline and alkaline wetlands, coasts, and steppe
Beckmanniinae *	<i>Pholiurus</i> Host ex Trin.	1	Eastern Europe	Annual	Ephemeral brackish to sub-saline and alkaline steppe wetlands
Beckmanniinae *	<i>Pseudophleum</i> Doğan	2	Southwestern Asia	Annuals	Ephemeral steppe wetlands

Continued

Table 1 Continued

Subtribe	Genus	No. spp.	Area (Native geographic range)	Annuals	Habitat
Beckmanniinae *	<b>Rhizocephalus</b> Boiss.	1	Southwestern Asia	Annual	Ephemeral saline steppe wetlands
Brizochloinae *	<b>Brizochloa</b> V. Jirásek & Chrtek	1	Southeastern Europe, Southwestern Asia (Balkan lowlands, Anatolia to Iran)	Annual	Steppe
Cinninae *	<b>Aniselytron</b> Merr.	2	Southeastern Asia		Highland forests
Cinninae *	<b>Cinna</b> L.	4	Eurasia, North America		Temperate to boreal forests
Cinninae *	<b>Cinnastrum</b> L.	1	Central Mexico to northern South America		Subtropical highland forests
Cinninae *	<b>Cyathopus</b> Stapf	1	Eastern Himalaya (Sikkim to Yunnan)		Subtropical highland forests
Cinninae *	<b>Simplicia</b> Kirk	3	New Zealand		Mesic to seasonally dry forests
Dupontinae *	<b>Arctohyalopoa</b> Röser & Tkach	5	Northeastern Asia (Buryatia to Yakutia/Sakha Republic)		Alpine and arctic
Dupontinae *	<b>Arctophila</b> (Rupr.) Rupr. ex Andersson	1	Eurasia, North America		Boreal to arctic wetlands
Dupontinae *	<b>Dupontia</b> R. Br.	1	Eurasia, North America		Arctic wetlands
Dupontinae *	<b>Dupontopsis</b> Soreng, L.J. Gillespie & Koba	1	Japan (Hokkaido and northern-most Honshu)		Alpine tundra, often on serpentine
Hookerochloinae *	<b>Arctagrostis</b> Griseb.	2	Eurasia, North America		Boreal to arctic wetlands
Hookerochloinae *	<b>Hookerochloa</b> E.B. Alexeev	2	Southeastern Australia		Forest wetlands
Hookerochloinae *	<b>Nicoraepoa</b> Soreng & L.J. Gillespie	7	Patagonian South America		Brackish to hypersaline and alkaline wetlands, coasts to alpine
Hookerochloinae *	<b>Saxipoa</b> Soreng, L.J. Gillespie & S.W.L. Jacobs	1	Southeastern Australia		Non-calcareous alpine
Hookerochloinae *	<b>Sylvipoa</b> Soreng, L.J. Gillespie & S.W.L. Jacobs	1	Eastern Australia		Mesic subtropical forests
Ventenatinae *	<b>Apera</b> Adans.	5	Eurasia [except Eastern and Northern Asia]	Annuals	Mesic temperate steppe and forest
Ventenatinae *	<b>Bellardiocloa</b> Chiov.	5	Southern Europe, Southwestern Asia (Spain to Iran)		Alpine and subalpine
Ventenatinae *	<b>Gaudinopsis</b> (Boiss.) Eig	5	Southeastern Europe to Southwestern Asia	Annuals	Non-calcareous and calcareous steppe and dry forest
Ventenatinae *	<b>Nephelochloa</b> Boiss.	1	Southwestern Asia (eastern Anatolia)	Annuals	Steppe, endemic to an unknown substrate
Ventenatinae *	<b>Parvotrisetum</b> Chrtek	1	Northeastern Mediterranean	Annual	Mediterranean lowlands
Ventenatinae *	<b>Pilgerochloa</b> Eig. (= <i>Ventenata</i> )	(2)	Southwestern Asia (Anatolia and Levant)	Annuals	Non-calcareous and calcareous steppe and dry forest
Ventenatinae *	<b>Ventenata</b> Koeler	3	Southern Europe, Southwestern Asia	Annuals	Mediterranean lowlands
incertae sedis	<b>Agrostopoa</b> Davidse, Soreng & P.M. Peterson	3	Colombia		Páramo, superpáramo
incertae sedis, reticulate	<b>Arctopoa</b> (Griseb.) Prob.	8	Central and eastern Asia, northern North America		Boreal to arctic sub-saline or alkaline wetlands

\*, supersubtribe Alopecurinae. Genera not in bold are synonyms; their species numbers are in parentheses and tallied under the accepted genus.

(Clayton & Cope, 1980) or genera in areas (Clayton, 1975), they do provide evidence of Pooideae generic and species diversity being by far the highest in Eurasia, with peak diversity in the Mediterranean and southwestern Asia, and declining diversity in more northern areas.

### 1.3 Previous biogeographic studies of Poeae using DNA

Few studies have focused explicitly on Poeae biogeography, and even fewer on PPAM genera. Strömberg (2011) correlated grassland evolution around the World with climate shifts from the Late Cretaceous to present. Pimentel et al. (2017) and Schubert et al. (2019b) correlated climate shifts through the Tertiary period with phylogeny in Pooideae. Inda et al. (2008) and Minaya et al. (2017) explored the biogeographic history of the Loliinae clade. Soreng (1990) and Hoffmann et al. (2013) focused on PPAM and the genus *Poa*, and Birch et al. (2014) and Giussani et al. (2016) discussed biogeography within the large *P. supersect.* *Homalopoa* clade.

### 1.4 Goals of the present study

Our study presents a plastid phylogenetic tree, a dated tree, and a formal biogeographic analysis for the PPAM clade in a robust framework of taxonomic diversity. We combine these results to illustrate areas and timing of origins and dispersals of the subtribes and genera. In addition, we explore dispersals and disjunctions and some life-history traits associated with the geographic spread and geological ages.

## 2 Material and Methods

### 2.1 Taxon sample

We used a DNA data set of 220 taxa, including 36 outgroups, to root the PPAM clade (Appendix I). The outgroups included 2 Brachypodieae, 1 Littledaleae, 1 Bromaeae, 3 Triticeae, and 29 Poeae species covering 17 subtribes. The PPAM ingroup

included all currently accepted genera (Table 1), except *Agrostopoa* (for which, we only have preliminary ITS data that need to be confirmed).

We include 184 of the ca. 870 species in PPAM. For the large PPAM genera (*Alopecurus*, *Phleum*, *Poa*, *Puccinellia*), to work with a data set of reasonable size for analyses and to focus on the major PPAM clades and subtribes, we included only a subset of our sequenced samples selected to represent the phylogenetic diversity in those genera. Representatives of *Poa* (40 of ca. 570 species), *Puccinellia* (37 of 116), *Alopecurus* (25 of 44), and *Phleum* (6 of 16) were chosen from our larger sequence data set to represent their major clades as far as we have DNA evidence of them. For the other genera, we included single representatives of all species completed for the gene regions studied. Exceptions were made for *Milium effusum*, in which two samples representing groups with divergent sequences were included and coded for different regions, and for *Alopecurus magellanicus* subsp. *glauca* due to a later reidentification. Voucher information and country of origin are provided in Appendix I for each sample (or a literature reference for GenBank outgroup samples).

### 2.2 Biogeographic areas

We identified 12 geographical areas, defined in Table 2, that, to the best of our knowledge, represented all regions of endemism around the world for PPAM taxa (see the map in Fig. 3), without overly subdividing them. Taxa of the PPAM clade are primarily temperate, favoring cool to frigid growing seasons, but are found scattered widely on mountains across the tropics and subtropics. For this reason, we defined areas broadly, rather than trying to delimit the actual distribution of PPAM on the map. Each species was coded for one (A to L) or more biogeographic areas in a.csv file for import into the biogeographic program (Table S1, text version). Area C, southwestern Asia, roughly corresponds to the Irano-Turanian Region of Takhtajan (1986), but we cut it

**Table 2** Biogeographic areas coded for RASP BBM MCMC analysis of PPAM species distributions, with definitions for each area

Distribution areas	Code	Definitions of areas for PPAM
Eastern Asia	A	Eastern Asia, east of high central Asian Mts. eastward through China and Japan, including the Himalayas (except western most ranges), south of 40° N latitude, with the alpine of northern Japan scored as A & B. Precipitation mainly monsoonal
Northern Asia	B	Northern Asia from the Urals eastward north of 40° N latitude
Southwestern Asia	C	Southwestern Asia including the Irano-Turanian region and central Asian steppe and mountains south of 40° N latitude. The high Caucasus Mountains are included here. Precipitation mainly in winter, with limited monsoonal influence
Northern Europe	D	Northern Europe, separated from northern Asia by the Ural Mts., and the Mediterranean by mountain chains and cold winters
Mediterranean	E	Coastal and adjacent lowlands around the Mediterranean Sea, including in northern Africa, with mild wet winters and dry hot summers
Africa	F	Other than the Mediterranean Region, mountains
Australia	G	
New Zealand	H	
North America	I	Including northern Mexico
Central America	J	Central America north to 26° N in Mexico
South America	K	Andes (Páramo and Puna) to Pampas and Patagonian regions of Argentina and Chile
Subantarctic Islands	L	Small islands in southern Atlantic and Indian Oceans (none in southern Pacific)

off at 40° N. Although our mapped region is broader in covering the Arabian Peninsula (Fig. 3), none of the species in this study occur there. Our Mediterranean Area is more coincident with Takhtajan's Mediterranean Region. We divided Takhtajan's Circumboreal Region into three areas. Northern Eurasia was separated into northern Asia and northern Europe divided along the Ural Mountains as there is a cohort of Asian species that do not make it to Europe and vice versa. On the other hand, we include Takhtajan's Caucasian Province in our southwestern Asia area due to consideration of genera and species of grasses shared with trans-Irano-Turanian Region mountain ranges that are absent from the European part of his Circumboreal Region. North America was treated as one area as we were more interested in intercontinental disjunctions than in subdivisions there. Eastern Asia was also broadly defined as including both monsoonal southern Asia and eastern Asia south of 40° N since there are relatively few PPAM genera and species there due to monsoonal climate with dry winters. We tried separating northern from southern South America, but finally united these because separating them for our data set was not instructive.

### 2.3 DNA data set

We sequenced three plastid markers (*matK*, *trnC-rpoB*, and *trnT-trnL-trnF* [TLF]) following the protocols outlined in Gillespie et al. (2008, 2022) and Soreng et al. (2015a). We assembled, edited, aligned, and concatenated sequences using Geneious ver. 8.1.9 (<http://www.geneious.com>). The MAFFT ver. 7.017 plugin (Katoh & Standley, 2013) was used for alignment, followed by manual adjustment using a similarity criterion. A 113 bp section near the middle of *trnT-trnL* was deleted from the final alignment since it was difficult to align (positions 681-791 in TLF). Two areas (61 bp, 59 bp) in the *trnT-trnL* sequence of *Bromus vulgaris* sequence were not alignable and were replaced with "?".

Most of the sequence data of Poaceae taxa are from our collections and were generated at the Canadian Museum of Nature. This applies to all PPAM data, except for three GenBank sequences of *Arctohyalopoa* and *Limnas* (data from Tkach et al., 2020). Sequences of Triticodae, one Brachypodieae, and some Poaceae outgroup samples come from whole-plastome sequences obtained from GenBank (Ogihara et al., 2002; Diekmann et al., 2008; Cahoon et al., 2010; Hand et al., 2013; Middleton et al., 2014; Saarela et al., 2015; Orton et al., 2019; Pischl et al., 2020).

### 2.4 Molecular analyses

For the phylogenetic and dated analyses, we used plastid sequences only. This allowed us to use as complete a taxon data set as possible for reconstructing areas. Nuclear DNA (nrDNA) provides some different phylogenetic placements, in part due to events we believe represent reticulation, and in part due to low variation and homoplasy in the separate data sets (see Gillespie et al., 2022, for comparisons of plastid and nrDNA trees, and combined analysis for a nearly congruent data set). Also, most previous dating analyses in Pooideae and Poaceae are based on plastid data, thus providing dates for secondary calibration (nrDNA rates of change often vary from plastid rates).

### 2.5 Bayes phylogenetic tree

Bayesian Markov chain Monte Carlo analyses were conducted in MrBayes ver. 3.2.6 (Ronquist et al., 2012). Optimal models of molecular evolution for individual markers were determined using the Akaike information criterion (AIC; Akaike 1974) conducted through likelihood searches in jModeltest with default settings (Darriba et al., 2012). Models were set at GTR+ $\Gamma$  for *matK* and TLF, and GTR+I+ $\Gamma$  for *trnC-rpoB* partitions based on the AIC scores and the models allowed in MrBayes. Two independent runs of four chained searches were performed for five million generations (analyses were stopped when the split frequency of 0.005 was closely approached), sampling every 500 generations, with default parameters. A 25% burn-in was implemented before summarizing a 50% majority rule consensus tree and calculating Bayesian posterior probabilities (pp). Trees were viewed in FigTree v1.4.0 (Rambaut, 2006+).

### 2.6 Beast dated tree

Gallaher et al. (2022) provided fossil calibrated molecular clock data for clades, tribes, and many subtribes in Poaceae. Applicable dates for Brachypodieae, Triticodae, Poodae (Poeae), Poaceae Plastid DNA Groups 1 and 2, and Lolioidinae+, along with standard deviations estimated from highest posterior density (HPD) values, were used to calibrate our Beast analysis (Drummond et al., 2012). One stem date was used to define the base of the tree (Poodae+Triticodae/Brachypodieae); the six other dates used were crown dates (Table 3, dates indicated by \*).

Molecular dating analyses were performed under a relaxed molecular clock using Bayesian methods in BEAST v1.10.4 (Drummond et al., 2012). XML files were prepared in BEAUTI v1.10.4 as a partitioned three-marker data set with independently estimated models of nucleotide evolution. We used two uncorrelated lognormal relaxed clock models separated into coding (*MatK*) and noncoding (*trnC-rpoB*, TLF) partitions. We used a random starting tree and set the process of speciation to Birth-Death (Gernhard, 2008). We used normal-distribution priors to constrain the stem of the Poodae+Triticodae clade to 44.8 mya (standard deviation = 2.4) and set dates for crowns of six clades: Poodae+Triticodae, Triticodae, Poodae, Poaceae Plastid DNA Groups 1 and 2, and Lolioidinae+ (Table 3). We performed four independent MCMC runs for 10 million generations, sampling every 1000 generations. Runs were assessed for convergence and ESS > 200 using TRACER, and then combined in LOGCOMBINER v1.10.4 after excluding the first million generations as burn-in. A maximum clade credibility tree with mean ages was summarized in TREEANNOTATOR v1.10.4 with a pp limit of 0.95.

Dates reported as geological ages follow the GSA Geologic Time Scale v. 5.0 (Walker et al., 2018).

### 2.7 Biogeographic analysis

We used the Reconstructed Ancestral State in Phylogenies (RASP) program (Yu et al., 2019 [manual]; Ali et al., 2021) to assess the ancestral geographic areas (states) using the Bayesian Binary Markov Chain method (BBM MCMC, BBM hereafter) (Ronquist & Huelsenbeck, 2003). Within RASP, we uploaded a Beast dated tree and a Bayesian majority rule

**Table 3** Age estimates for stem and crown nodes of PPAM major clades and outgroups

	Stem pp	Stem mean	Stem 95% HPD [sd]	Crown pp	Crown mean	Crown 95% HPD [sd]
<b>Brachypodieae</b>	–	<b>44.8</b>	<b>40.09–49.26</b>	<b>1</b>	<b>8.87</b>	<b>5.42–12.33</b>
<b>Poodeae + Triticodae</b>	–	<b>44.8</b>	<b>40.09–49.26</b>	<b>1</b>	<b>42.86</b>	<b>39.67–45.75</b>
Gallaher et al. (2022)		44.8*	40.09–49.26 [2.4]		40.78*	36.45–45.29 [2.23]
<b>Triticodae</b>	<b>1</b>	<b>42.86</b>	<b>39.67–45.75</b>	<b>1</b>	<b>18.67</b>	<b>13.99–24.18</b>
Gallaher et al. (2022)		40.78	36.45–45.29		23.02*	19.99–26.74 [1.9]
<b>Poodeae/Poeae</b>	<b>1</b>	<b>42.86</b>	<b>39.67–45.75</b>	<b>1</b>	<b>33.68</b>	<b>30.82–36.29</b>
Gallaher et al. (2022)		40.78	36.45–45.29		36.24*	32.02–40.60 [2.15]
<b>Poeae, Plastid DNA Group 1 (Aveneae type)</b>	<b>1</b>	<b>33.68</b>	<b>30.82–36.29</b>	<b>1</b>	<b>17.9</b>	<b>15.27–20.35</b>
Gallaher et al. (2022)		36.24	32.02–40.60		25.28*	22.26–27.83 [1.4]
<b>Poeae, Plastid DNA Group 2 (Poeae type)</b>	<b>1</b>	<b>33.68</b>	<b>30.82–36.29</b>	<b>1</b>	<b>24.19</b>	<b>21.46–26.66</b>
Gallaher et al. (2022)		36.24	32.02–40.60		29.84*	26.16–32.93 [1.9]
<b>Loliodinae + plastid clade</b>	<b>1</b>	<b>24.19</b>	<b>21.46–26.66</b>	<b>0.99</b>	<b>22.84</b>	<b>20.09–25.42</b>
Gallaher et al. (2022)		–	–		25.79*	22.17–28.96 [1.7]
<b>PPAM</b>	<b>1</b>	<b>24.19</b>	<b>21.46–26.66</b>	<b>1</b>	<b>21.81</b>	<b>19.12–24.34</b>
Gallaher et al. (2022)		29.84	26.16–32.93		26.65	23.31–30.55
Giussani et al. (2016) <sup>1</sup>		–	18.1–29.9		–	–
Minaya et al. (2017)		27.76	–		23.43	–
<b>Coleanthinae</b>	<b>1</b>	<b>21.81</b>	<b>19.12–24.34</b>	<b>1</b>	<b>11.31</b>	<b>9.08–13.41</b>
Gallaher et al. (2022)		26.65	23.31–30.55		17.99	14.45–21.75
<b>Colpodium</b>	<b>1</b>	<b>11.31</b>	<b>9.08–13.41</b>	<b>1</b>	<b>4.81</b>	<b>3.3–6.19</b>
Hoffmann et al. (2013) <sup>2</sup>		–	–		2.15/1.52	0.81–3.37
<b>Coleanthinae excl. Colpodium</b>		<b>11.31</b>	<b>9.08–13.41</b>		<b>8.25</b>	<b>6.43–9.94</b>
<b>Catabrosella + Catabrosa</b>	<b>1</b>	<b>8.25</b>	<b>6.43–9.94</b>	<b>1</b>	<b>5.58</b>	<b>3.78–7.15</b>
<b>Catabrosella</b>	<b>1</b>	<b>5.58</b>	<b>3.78–7.15</b>	<b>1</b>	<b>0.62</b>	<b>0.09–1.00</b>
<b>Catabrosa (one sample)</b>	<b>1</b>	<b>5.58</b>	<b>3.78–7.15</b>		–	–
<b>Phippisia + Coleanthus</b>	<b>0.61</b>	<b>7.23</b>	–	<b>1</b>	<b>1.14</b>	<b>0.37–2.05</b>
<b>Phippisia</b>	<b>1</b>	<b>1.14</b>	<b>0.37–2.05</b>	<b>0.96</b>	<b>0.67</b>	<b>0.12–1.07</b>
Hoffmann et al. (2013)		–	–		0.77/0.23	0.28–2.10
<b>Coleanthus (monotypic)</b>	<b>1</b>	<b>1.14</b>	<b>0.37–2.05</b>		–	–
<b>Puccinellia + Sclerochloa</b>	<b>0.61</b>	<b>7.23</b>	–	<b>1</b>	<b>3.37</b>	<b>2.39–4.12</b>
<b>Puccinellia</b>	<b>0.61</b>	<b>7.23</b>	–	<b>1</b>	<b>3.37</b>	<b>2.39–4.12</b>
Birch et al. (2014)		–	–		2.2/3.6 <sup>3</sup>	–
Hoffmann et al. (2013)		–	–		1.44/2.27	0.64–2.29
<b>PAM (Poodinae)</b>	<b>1</b>	<b>21.81</b>	<b>19.12–24.34</b>	<b>1</b>	<b>17.21</b>	<b>14.98–19.24</b>
Giussani et al. (2016)		–	–		–	13.5–23.1
<b>Poa<sup>4</sup></b>	<b>1</b>	<b>17.21</b>	<b>14.98–19.24</b>	<b>1</b>	<b>14.73</b>	<b>12.64–16.57</b>
Gallaher et al. (2022)		5.73	2.92–9.78		–	–
Giussani et al. (2016)		–	12.1–21.2		–	9.9–17.6
Hoffmann et al. (2013) [excluding Poa I clade]		–	–		8.8/10.9 <sup>5</sup>	–
<b>P. subg. Sylvestres<sup>4</sup></b>	<b>1</b>	<b>14.73</b>	<b>11.06–18.99</b>	<b>1</b>	<b>3.79</b>	<b>1.55–8.20</b>
Giussani et al. (2016)		–	9.9–17.6		–	–
<b>P. sect. Micrantherae (syn. sect. Ochlopoa)</b>	<b>1</b>	<b>8.75</b>	<b>7.04–10.31</b>	<b>1</b>	<b>5.4</b>	<b>3.91–6.68</b>
Hoffmann et al. (2013) [Poa I]		–	–		1.91	0.92–3.12
<b>P. Parodiochloa clade</b>	<b>1</b>	<b>8.75</b>	<b>7.04–10.31</b>	<b>1</b>	<b>4.37</b>	<b>2.82–5.79</b>
Hoffmann et al. (2013) [Poa II]		–	–		0.63/0.47	0.30–1.15
<b>P. sect. Alpinae</b>	<b>1</b>	<b>10.92</b>	<b>9.04–12.58</b>	<b>1</b>	<b>0.99</b>	<b>0.34–1.48</b>
Hoffmann et al. (2013) [Poa III]		–	–		1.12/0.64	0.54–1.76
<b>P. sect. Stenopoa</b>	<b>0.65</b>	<b>5.27</b>	–	<b>1</b>	<b>2.89</b>	<b>1.84–3.66</b>
Hoffmann et al. (2013) [Poa IV]		–	–		0.44/0.76	0.22–0.69
<b>P. subg. Poa</b>	<b>1</b>	<b>8.95</b>	<b>6.3–12.15</b>	<b>1</b>	<b>5.06</b>	<b>2.51–8.14</b>
Giussani et al. (2016)		–	6.8–12.4		–	4.8–9.7
<b>P. supersect. Poa</b>	<b>1</b>	<b>5.06</b>	<b>2.51–8.14</b>	<b>1</b>	<b>2.52</b>	<b>1.58–3.29</b>
Giussani et al. (2016)		–	4.8–9.7		–	0.9–6.2
<b>P. supersect. Homalopoa</b>	<b>1</b>	<b>5.06</b>	<b>2.51–8.14</b>	<b>1</b>	<b>2.38</b>	<b>1.14–4.60</b>
Giussani et al. (2016)		–	4.8–9.7		–	4.2–8.4

Continued

Table 3 Continued

	Stem pp	Stem mean	Stem 95% HPD [sd]	Crown pp	Crown mean	Crown 95% HPD [sd]
<b>Milium</b>	<b>0.88</b>	<b>16.39</b>	–	<b>1</b>	<b>12.37</b>	<b>10.25–14.26</b>
Gallaher et al. (2022)		10.87	6.31–15.15		–	–
Giussani et al. (2016)		–	9.1–19.3		–	–
Hoffmann et al. (2013)		–	–		2.70/0.29	1.34–3.25
<b>Avenula (monotypic)</b>	<b>0.55</b>	<b>14.78</b>	–		–	–
<b>Phleum</b>	<b>0.55</b>	<b>14.78</b>	–	<b>1</b>	<b>4.31</b>	<b>2.2–7.54</b>
Gallaher et al. (2022)		14.21	11.24–17.42		–	–
Giussani et al. (2016)		–	9.1–19.3		–	–
Hoffmann et al. (2013)		–	–		2.46/0.23	0.80–5.71
<b>Superclade Alopecurinae</b>	<b>0.39</b>	<b>15.57</b>	–	<b>1</b>	<b>12.68</b>	<b>10.66–14.45</b>
Giussani et al. (2016)		–	13.5–23.1		–	6.6–16.7
<b>Alopecurinae + Ventenatinae</b>	<b>1</b>	<b>12.68</b>	<b>10.66–14.45</b>	<b>1</b>	<b>11.46</b>	<b>9.58–13.19</b>
<b>Alopecurinae</b>	<b>1</b>	<b>11.46</b>	<b>8.21–16.86</b>	<b>1</b>	<b>10.37</b>	<b>8.51–11.93</b>
Gallaher et al. (2022)		10.5	8.49–12.79		–	–
Hoffmann et al. (2013) <sup>6</sup>		–	–		2.85/1.81	1.49–5.10
<b>Alopecurus</b>	<b>1</b>	<b>11.46</b>	<b>8.21–16.86</b>	<b>1</b>	<b>10.37</b>	<b>8.51–11.93</b>
<b>Limnas</b>	<b>0.86</b>	<b>6.74</b>	–	<b>1</b>	<b>2.42</b>	<b>0.52–4.64</b>
<b>Cornucopiae (=Alopecurus )</b>	<b>1</b>	<b>3.58</b>	<b>2.56–4.43</b>		–	–
<b>Ventenatinae</b>	<b>1</b>	<b>11.46</b>	<b>8.21–16.86</b>	<b>1</b>	<b>9.89</b>	<b>8.13–11.5</b>
Gallaher et al. (2022)		10.5	8.49–12.79		9.17	6.67–11.37
<b>Gaudinopsis (1 sample)</b>	<b>1</b>	<b>3.83</b>	<b>2.59–4.91</b>		–	–
<b>Ventenata (includes Pilgerochloa )</b>	<b>1</b>	<b>3.38</b>	<b>2.59–4.91</b>	<b>0.38</b>	<b>3.55</b>	–
<b>Parvotrisetum (monotypic)</b>	<b>0.99</b>	<b>8.63</b>	<b>6.86–10.26</b>		–	–
<b>Bellardiochloa + Apera + Nephelochloa</b>	<b>0.99</b>	<b>8.63</b>	<b>6.86–10.26</b>	<b>1</b>	<b>5.79</b>	<b>4.15–7.19</b>
Hoffmann et al. (2013)					2.41/0.64	0.72–6.98
<b>Bellardiochloa</b>	<b>1</b>	<b>5.79</b>	<b>4.15–7.19</b>	<b>1</b>	<b>2.80</b>	<b>1.70–3.73</b>
<b>Apera + Nephelochloa</b>	<b>1</b>	<b>5.79</b>	<b>4.15–7.19</b>	<b>1</b>	<b>1.89</b>	<b>1.06–2.55</b>
<b>Beckmannia + Pholiurus</b>	<b>0.62</b>	<b>9.57</b>	–	<b>1</b>	<b>1.88</b>	<b>0.85–3.20</b>
Hoffmann et al. (2013)					1.90/0.29	0.31–3.83
<b>Beckmannia</b>	<b>1</b>	<b>1.88</b>	<b>0.85–3.20</b>	<b>1</b>	<b>0.86</b>	<b>0.26–1.59</b>
<b>Dupontiinae</b>	<b>0.68</b>	<b>3.11</b>	–	<b>0.79</b>	<b>1.24</b>	–
<b>Arctophila + Dupontia</b>	<b>0.79</b>	<b>1.24</b>	–	<b>0.98</b>	<b>0.25</b>	<b>0–0.39</b>
<b>Hookerochloinae + Cinninae p.p.</b>	<b>0.9</b>	<b>7.18</b>	–	<b>0.79</b>	<b>3.66</b>	–
<b>Cinnastrum (monotypic)</b>	<b>0.92</b>	<b>2.92</b>	–		–	–
<b>Hookerochloinae + Simplicia</b>	<b>0.79</b>	<b>3.66</b>	–	<b>0.73</b>	<b>3.08</b>	–
Hoffmann et al. (2013) <sup>7</sup>					0.83/1.22	0.50–1.13
<b>Arctagrostis</b>	<b>0.73</b>	<b>3.08</b>	–	<b>0.79</b>	<b>1.64</b>	–
<b>Sylvipoa (monotypic)</b>	<b>0.07</b>	<b>2.05</b>	–		–	–
<b>Nicoraepoa<sup>8</sup></b>	<b>0.07</b>	<b>2.05</b>	–	<b>1</b>	<b>1.39</b>	<b>0.67–1.89</b>
<b>Simplicia</b>	<b>0.21</b>	<b>1.95</b>	–	<b>1</b>	<b>0.89</b>	<b>0.31–1.3</b>
<b>Saxipoa (monotypic)</b>	<b>0.95</b>	<b>1.19</b>	–		–	–
<b>Hookerochloa</b>	<b>0.95</b>	<b>1.19</b>	–	<b>0.35</b>	<b>0.89</b>	–

Major clades and dates from this study are in bold, with dates from other studies given below. Mean age (My), 95% highest posterior probability (HPD) confidence limits (not given for nodes with < 95% support in this study), and node posterior probability support (pp). Dates from Gallaher et al. (2022) marked with an asterisk (\*) were used to calibrate our Beast analysis, with the standard deviation [sd] estimated from the 95% HPD values. HPD, highest posterior density. <sup>1</sup>Missing Coleanthinae. <sup>2</sup>Hoffmann et al. (2013) provided three dates based on different secondary calibration dates and methods of analysis based on nrDNA ITS data. Two dates are given here, both based on a basal node age of 25 Mya: the first from the uncorrelated relaxed clock analysis and the second from the penalized likelihood analysis. The 95% HDP values are from the uncorrelated relaxed clock analysis. <sup>3</sup>Two mean dates based on different secondary calibrates dates. <sup>4</sup>Includes the hybrid genus *Arctopoa*. <sup>5</sup>Two dates from the text of Hoffmann et al. (2013) not included in their table, both from penalized likelihood analyses, based on basal node ages of 20 and 25 Mya, respectively. <sup>6</sup>Includes the reticulate subtribe Dupontiinae. <sup>7</sup>Hoffmann et al.'s (2013) “*Arctagrostis*” clade, equivalent to Hookerochloinae. <sup>8</sup>Excludes hybrid species *Nicoraepoa pugionifolia*.



consensus tree in separate runs, along with an areas of distribution file for all 220 taxa. After running “Check Status” separately on our dated and consensus trees, we opted to run the BBM program. With the dated tree, we were able to run exploratory BBM and DEC (Dispersal-Extinction-Cladogenesis; Ree & Smith, 2008) analyses. For the Bayesian consensus tree, which has some polytomies, we ran only BBM.

In exploring the data, we ran the BBM analyses with all 220 taxa. However, with such distant outgroups, which last shared ancestors with PPAM from the Eocene to Oligocene, and the outgroups distributed in all identified areas around the world, we were concerned about their impact on the probabilities and production of a lot of irrelevant results and areas combinations. Therefore, since all our trial runs with outgroups focused the area of origin for PPAM on southwestern Asia (highest probability), or that plus Mediterranean (a low probability in some runs), we tried setting all the outgroups to area sets of BCDEI or ACE. However, the results from both trials seemed biased, as ancestral areas still pointed to southwestern Asia. Therefore, for the final analysis, we followed the recommended procedure to remove outgroups (Yu et al., 2019) and let the program tell us where the probable ancestral area was. For this, we ran only BBM on the Bayesian majority rule consensus tree, and the results of that are reported below.

For our final BBM analyses, we ran one million cycles, starting with 10 chains and frequency of sampling 100, burn-in removing 5000 trees, at a temperature of 0.1. Maximum areas were set to 4, and null distribution was not allowed. We used the default models of estimated state frequencies (F81), and the equal Among-Site (area) rate variation. The estimated state frequencies improved results over the fixed option (JC).

The resulting trees from analyses were viewed within RASP with the Most Likely State (MLS) at each node (posted inside the circle), and with the proportionally probable states (shown as colors on the circle). Results, tree graphics, and color legends were exported as scalable vector graphics (svg) files for producing the final figures.

## 3 Results

### 3.1 DNA data set characteristics

The final data matrix included 220 samples and had an aligned length of 5807 characters, partitioned as follows: 791 *MatK*, 1970 *trnC-rpoB*, and 3046 TLF characters. TLF comprised *trnT-trnL* (1592), *trnL* including intron (904), and *trnL-trnF* (550 characters). Appendix I includes the GenBank accession numbers for the 615 sequences included in the analyses. Of these, 241 sequences are newly reported here for 83 taxa (GenBank numbers starting with OK in Appendix I). Samples were complete for all markers, except for three PPAM samples of two genera, *Arctohyalopoa* and *Limnas*, which are lacking *trnC-rpoB* and the *trnT-trnL* spacer of TLF (data from Tkach et al., 2020), and two outgroup samples, *Molineriella* and *Brachypodium distachyon*, which are missing *trnC-rpoB*. Overall, there were very little missing data in our concatenated alignment (<1%).

### 3.2 Phylogenetic analysis

The tree resulting from the Bayesian analysis of our plastid data set is shown in Fig. 1, with strongly supported branches (interpreted as  $pp \geq 0.95$ ) shown as solid lines. The PPAM clade is fully supported, as are its major clades, Coleanthinae, *Milium*, *Phleum*, *Poa* (including the hybrid genus *Arctopoa*), and superclade Alopecurinae (all  $pp = 1$ ). Coleanthinae is fully supported as sister to all other PPAM members (= PAM clade), whereas relationships among the remaining major clades are weakly or not supported. Within the Coleanthinae, *Sclerochloa* is in a polytomy with *Puccinellia* and the set is called the *Puccinellia-Sclerochloa* clade. The position of the monotypic genus *Avenula* as sister to *Phleum* is only weakly supported ( $pp = 0.76$ ).

Within superclade Alopecurinae, subtribes Alopecurinae and Ventenatinae are fully supported, and together form a fully supported clade. The genus *Cornucopiae* is nested within *Alopecurus* (see the Taxonomy section below for nomenclatural changes). Dupontiinae (reticulate in origin) is monophyletic, but only weakly supported ( $pp = 0.67$ ), whereas Beckmanniinae, Cinninae, and Hookerochloinae do not resolve as monophyletic in this analysis. Three of four genera of Beckmanniinae, *Beckmannia*, *Pholiurus*, and *Pseudophleum*, form a weakly supported clade ( $pp = 0.89$ ), with the fourth genus, *Rhizocephalus*, nearby but not resolved within any subtribe.

### 3.3 Dated analysis

The BEAST dated tree is presented in Fig. 2, with mean age estimates for all nodes (except weakly supported nodes < 2 mya), and 95% highest posterior density (HPD) confidence bars for all strongly supported nodes ( $pp \geq 0.95$ ). The six nodes used for secondary calibration of the tree are indicated by arrows. Table 3 presents stem and crown dates estimated here for PPAM and its major clades. All clades described below are strongly supported ( $pp \geq 0.95$ ), unless indicated otherwise.

The PPAM clade is estimated to have diverged from other Poeae (specifically the Lolioidinae+ plastid clade) in the Late Oligocene (24.19 mya), and the crown of PPAM is estimated to have diversified in the Early Miocene (21.81 mya). The crown of Coleanthinae was dated slightly younger than the Middle-Late Miocene boundary (11.31 mya), with the clade of all taxa excluding *Colpodium* in the Late Miocene (8.25 mya) and the *Puccinellia-Sclerochloa* clade in the Pliocene (3.37 mya). The crown of supersubtribe Poodinae (= PAM clade) is estimated to have diverged in the Early Miocene (17.21 mya). The crowns of *Poa* (14.73 mya), superclade Alopecurinae (12.68 mya), and *Milium* (12.37 mya) are dated to the Middle Miocene, and *Phleum* to the Early Pliocene (4.31 mya).

Within superclade Alopecurinae, subtribes Alopecurinae and Ventenatinae (crown 11.46 mya) are estimated to have diverged near the Middle-Late Miocene boundary, with the Alopecurinae (10.37 mya) and Ventenatinae (9.89 mya) crowns dated to the Late Miocene. Although the remaining subtribes form a clade in the dated analysis (vs. a grade below Alopecurinae + Ventenatinae in the Bayesian analysis, Fig. 1), confidence in the topology and support levels are insufficient for accurately estimating divergence times of both this clade ( $pp < 0.5$ ) and most of its subtribes.



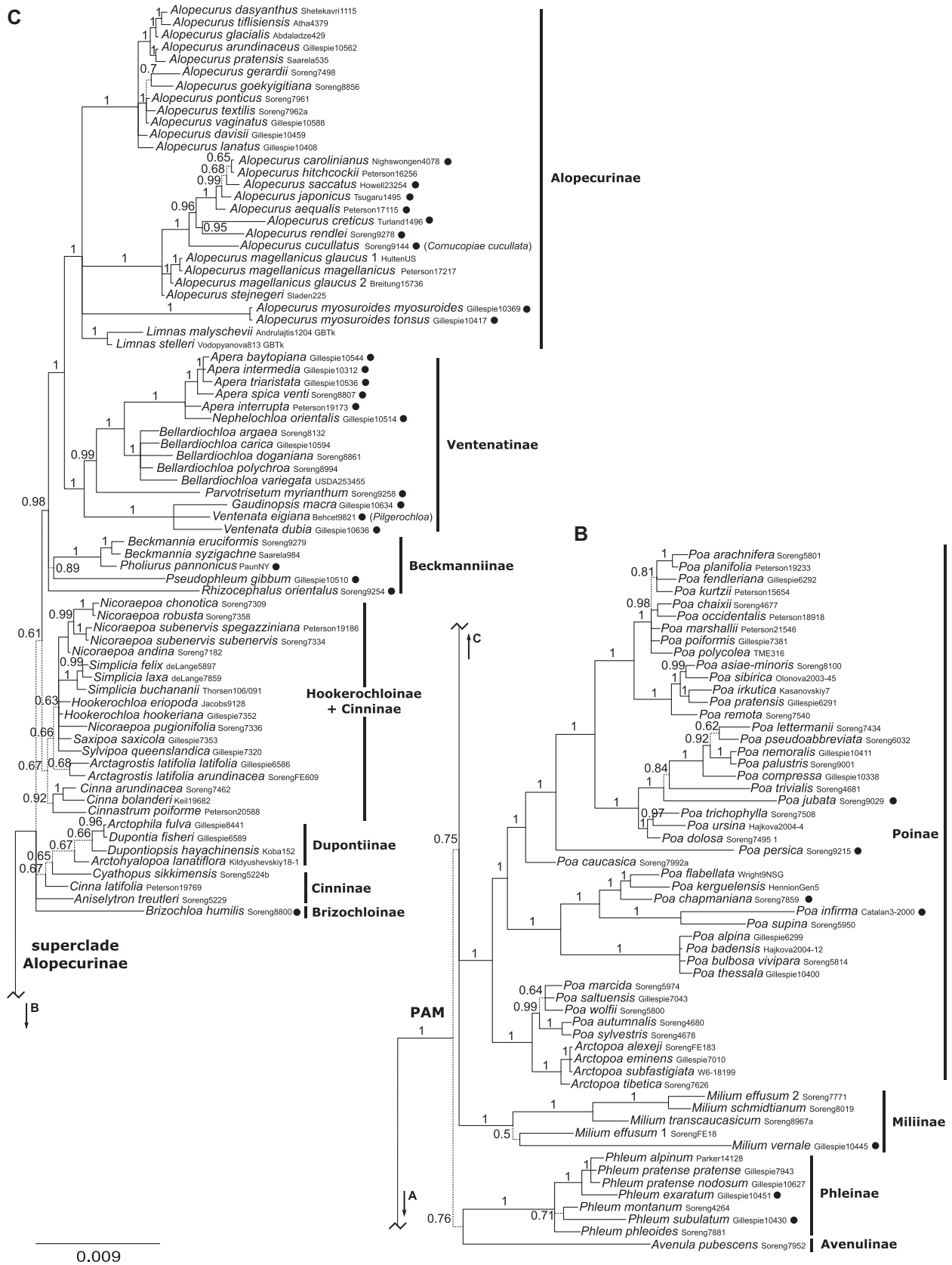


Fig. 1. Continued

Brizochloinae (*Brizochloa* monotypic; stem  $pp = 0.18$ ) diverged near the Miocene–Pliocene boundary (5.39 mya) (but note its different position on the tree in Fig. 1). The Dupontiinae crown ( $pp = 0.79$ ) is estimated at 1.24 mya in the late Pleistocene. Subtribes Beckmanniinae, Cinninae, and Hookerchloinae did not resolve as monophyletic. Beckmanniinae (excluding *Rhizocephalus*) stem and crown are dated to the Late Miocene (9.57 mya;  $pp = 0.52$ ), whereas *Rhizocephalus* (monotypic) diverged earlier in the latter part of the Middle Miocene (11.74 mya;  $pp = 0.34$ ). Crowns of Hookerchloinae plus three genera of Cinninae (*Cinna* s.p., *Cinnastrum*, and *Simplicia*) (3.66 mya;  $pp = 0.79$ ) and Hookerchloinae with *Simplicia* embedded (3.08 mya;  $pp = 0.73$ ) are dated to the Pliocene. Ages of other Alopecurinae clades and genera are shown in Table 3.

### 3.4 Biogeographic analysis

Figure 3 shows the PPAM topology from the RASP BBM MCMC analysis, with predicted proportional area distributions at each node with the Most-Likely-State (MLS) indicated inside the wheel-shaped circle. The letter coded areas are presented in Table 1 and mapped in Fig. 3. The colors for each single and compound area are shown in the legend (Fig. 3; black represents the total of single areas or area combinations with less than 5% probability each). Nodes are numbered in Table S2 and in the text below as in Fig. S1. The raw and summarized area data for each clade node can be found in Table S2, providing the MLS state, transitional routes to the child nodes, and the overall probability of the predicted routes, along with age, classification, ancestral area probabilities including all areas greater than 5%, and child node probabilities. Area codes, rather than area names, are listed in the results below, along with the probability of the MLS area, and probabilities of less likely states if their individual percentages were more than 10%.

Starting from the PPAM root node (node 314, Fig. S1), area C is ancestral (prob. 85%). From here onward, we report node numbers (as shown in Fig. S1) with their areas and area probabilities (as shown in Fig. 3, and listed in Table S2 part B) upward from the bottom of Fig. 3 to the top for each major clade as identified in Figs. 1, 3. (i) For Coleanthinae (node 313), with all 12 genera sampled here, area C is ancestral (prob. 94%). (ii) For PAM (node 282), area C is ancestral (prob. 48%) or BC (prob. 28%). (iii) For Phleinae + Avenulinae, *Phleum* + *Avena* (node 281), area BCDE is ancestral (prob. 82%). (iv) The next branch up (node 276) presents a polytomy of Miliinae, Poinae, and the Alopecurinae superclade, for which C is the ancestral area (prob. 70%) or BC (prob. 25%). (v) For Miliinae (node 275), *Milium* only, area C is ancestral (prob. 68%) or BC (prob. 23%). (vi) For Poinae (node 271), *Poa*,

area C is ancestral (prob. 57%), or BC (prob. 36%). Although all the major *Poa* clades are represented here, we plan to address biogeography within *Poa* in a future paper with many more taxa. (vii) The Alopecurinae superclade (node 236) originated in area C (prob. 76%), with a polytomy of *Brizochloa* (area C), *Aniselytron* (area A), and a clade (node 235) of *Cinna latifolia* > *Cyathopus* > Dupontiinae with basal area A (prob. 33%), AB (prob. 24%), or B (14% prob.). The fourth branch (node 230) in the polytomy represents a clade of the rest of the superclade, which originated in area C (prob. 91%). (viii) The next branching (from node 230) has a clade (node 229) with a basal area of C (41%) or I (36%). This is followed by a clade of *Cinnastrum* > *Cinna arundinacea* + *C. bolanderi* (node 228), ancestral area of I (prob. 72%), which is sister to the clade of Hookerchloinae + *Simplicia* (node 226), with an ancestral area of I (prob. 43%) or B (prob. 22%). After *Arctagrostis* (node 225, area BI, prob. 62%) diverged, ancestors dispersed (node 224) into southern hemisphere areas H, G, and L, possibly from area G (prob. 65%) or K (prob. 14%). (ix) The previous clade at node 229 is sister to a polytomy (node 217) of *Rhizocephalus*, *Pseudophleum* + *Beckmannia* + *Pholiurus* (the latter four all classified in Beckmanniinae), and Ventenatinae + Alopecurinae, with a basal area of C (prob. 94%). Beckmanniinae (excepting *Rhizocephalus*) (node 216) originated in area C (prob. 90%), where *Rhizocephalus* also occurs, and *Beckmannia* dispersed widely across Eurasia and into North America. The clade of subtribes Alopecurinae + Ventenatinae (node 213) has a basal area of C (prob. 71%) or CE (prob. 24%). Ventenatinae (node 212), with all seven genera included here, has a basal area of C (prob. 51%) or CE (prob. 42%), and has not dispersed out of these two areas. Alopecurinae, with both genera included here, shows a polytomy of *Limnas* (node 203) in area B (prob. 97%), and three clades of *Alopecurus* (nodes 202, 201, 191), branching, respectively, from areas CE (prob. 86%); C (prob. 32%), BC (prob. 26%), or B (prob. 16%); and C (prob. 92%). The *Alopecurus* BC clade (node 201) diversified and spread widely in Eurasia and the Western Hemisphere, while the others mostly stayed in their home areas. *Alopecurus cucullatus* (syn. *Cornucopiae cucullatum*) is nested within taxa of area C.

The BBM analysis also calculated the Dispersal, Vicariance, and/or Extinction events for each node (Table S2) and globally. Globally, there were 278 Dispersal events, 64 Vicariance events, and 1 Extinction event within and between areas, or 155, 38, and 1, respectively, between areas. Table S3 presents the BBM Dispersal and Speciation data. In order, the most dispersals between areas were from C (67.67), followed by D (18.33), B (16.83), I (15.5), A (7.58), E (6.08), G (3), K (2), and L (1), with no dispersals from F, H, or J. The most dispersals into areas were into I (25), followed by

**Fig. 1.** PPAM clade phylogeny based on Bayesian analysis of the plastid data set (*matK*, *trnC-rpoB*, *trnT-trnL-trnF*). PPAM subtribes and outgroup clades are labeled on the left and other major clades are labeled at nodes. Bayesian posterior probabilities (pp) are shown above branches; strongly supported branches ( $pp \geq 95\%$ ) are shown as solid lines and weakly supported branches ( $pp < 95\%$ ) are shown as dashed lines. Black dots after names indicate taxa with annual life cycles. **A**, Outgroups and PPAM subtribe Coleanthinae. **B**, PPAM subtribes Avenulinae, Miliinae, Phleinae, and Poinae. **C**, PPAM superclade Alopecurinae.



B (24), E (24), K (16), A (12), C (6), G (3), F and J (2), and H and L (1). The most dispersals within an area were in C (103), followed by I (45), B (32), E (21), D (20), K (10), A (6), G (6), H (2), and L (1). No dispersals occurred within area F or J. Numbers for speciation within an area are identical to numbers of dispersals within an area. Figure 4 maps PPAM dispersals between areas according to the RASP analysis, with line color showing the predicted direction and width proportional to frequency.

## 4 Discussion

### 4.1 Phylogeny

In this study, we focus on the major clades and subtribes of the PPAM clade based on plastid data. We will discuss relationships in more detail, including at the generic and species levels, and evidence for reticulation based on both plastid and nrDNA data in a future paper. Alopecurinae superclade subtribes Cinninae and Hookerochloinae were the focus of a recent study including concatenated plastid and nrDNA (ITS and ETS) data, where the subtribes were more resolved than presented here based on plastid only (Gillespie et al., 2022).

The PPAM clade is strongly supported as monophyletic, consistent with numerous other studies based on plastid and/or nrDNA data (e.g., Davis & Soreng, 2007; Gillespie et al., 2008, 2010, 2022; Quintanar Sánchez et al., 2007; Bouchenak-Khelladi et al., 2008, 2010; Schaefer et al., 2011; Schneider et al., 2011; Grass Phylogeny Working Group II, 2012; Soreng et al., 2007, 2015a; Tkach et al., 2020), single-copy nuclear genes (Minaya et al., 2013; Hochbach et al., 2015), and whole plastomes (Saarela et al., 2015, 2018; Duvall et al., 2020; Orton et al., 2019, 2021). Our present study is the most complete in terms of generic and species sampling, 42 of 43 accepted genera (Soreng et al., 2022) (with representatives of *Cornucopiae*, *Pilgerochloa*, and *Zingieria* included in the analysis, but treated as synonyms of *Alopecurus*, *Ventenata*, and *Colpodium*, respectively). *Agrostopoa* is missing because of lack of plastid DNA data, although a preliminary ITS sequence leads us to provisionally place it incertae sedis in the PAM clade (Soreng et al., 2022, classification in this issue).

Morphological characteristics that unite all the subtribes and genera of the morphologically diverse PPAM clade are not obvious from general aspect. Generally, beyond plesiomorphic Pooideae traits, they are of low to middle stature (infrequently over 1 m tall), have spikelets with lateral compression that are relatively short compared with other Pooideae tribes and some Poaeae genera (spikelets, excluding awns, in genera with multiple florets are usually less than 1 cm long, while single-flowered spikelets are less than 0.7 cm long), glumes often shorter than the florets, florets usually

without awns, usually with herbaceous paleas, lodicules 2 in number, free, more or less lanceolate, usually lobed, glabrous, and ovaries usually glabrous. Caryopses are generally short (less than 3 mm long) with apical styles (without a lobe), a hilum usually less than ¼ the grain in length, and only compound starch grains. However, many traits vary. Many genera have single florets, disarticulation is usually above the glumes but below the glumes in some genera, several genera have well-developed awns that are sometimes dorsal (Avenulinae, Ventenatinae, and Alopecurinae), other times subapical or apical, some have glumes longer than the basal floret, paleas are sometimes hyaline, or absent, some lack lodicules, and lipid is known to be present in the endosperm of many genera, but is absent in the few genera examined in Coleanthinae. However, the clade appears in DNA phylogenetic studies using plastid and/or nrDNA sequences almost without fail. Our analysis with 29 species from 17 outgroup subtribes, and all but one of the PPAM genera represented, cinches the case for the monophyly of this large clade.

Within the PPAM clade, subtribe Coleanthinae (10 genera, 159 species) is strongly supported as monophyletic and sister to the PAM clade, the latter corresponding to supersubtribe Poodinae (Soreng et al., 2017). PAM includes four main lineages: *Milium* (6 species), *Phleum* (16 species), *Poa* (ca. 570 species), and superclade Alopecurinae (27 accepted genera [*Cornucopiae* and *Pilgerochloa* are synonyms here of *Alopecurus* and *Ventenata*, respectively], 117 species). These results are consistent with previous analyses based on plastid data using fewer taxa (e.g., Gillespie et al., 2008, 2010; Soreng et al., 2007, 2015a; Tkach et al., 2020). The relationships among these four lineages are not well resolved and/or supported in this and previous plastid studies. A fifth lineage *Avenula* (monotypic) resolved within PAM, at least based on plastid data, but its position is also uncertain and never strongly supported (resolved with *Phleum* in this study, in a PAM polytomy in Tkach et al., 2020). *Avenula* is on a long branch and morphologically very different from *Phleum*. We presume that this clade has no basis (support was weak in the Bayesian analysis, Fig. 1). *Avenula*, historically treated within Aveneae, is morphologically unusual in PPAM anywhere it aligns (see Gillespie et al., 2022). Internal PPAM placement of *Avenula* using nrDNA has also been ambiguous (Tkach et al., 2020; Gillespie et al., unpublished). Investigation of its nuclear genome is needed. We treat *Agrostopoa* (not sampled here) and the reticulate genus *Arctopoa* as incertae sedis in PAM.

Among superclade Alopecurinae members, subtribes Alopecurinae and Ventenatinae each resolved as monophyletic and as sisters with strong support, Dupontinae with weak support, whereas other non-monospecific subtribes are not resolved. Poor resolution in these areas has been an

**Fig. 2.** Beast dated tree of the PPAM clade and outgroups. PPAM subtribes and outgroup clades are labeled on the left and other major clades are labeled at nodes. Mean age estimates in mya are given for each node (except for nodes <2 mya with  $pp < 95\%$ ); 95% HPD confidence bars are provided for all strongly supported nodes ( $pp \geq 0.95$ ). Arrows indicate nodes calibrated with dates from Gallaher et al. (2022) (see Table 3 for key clade dates). HPD, highest posterior density.



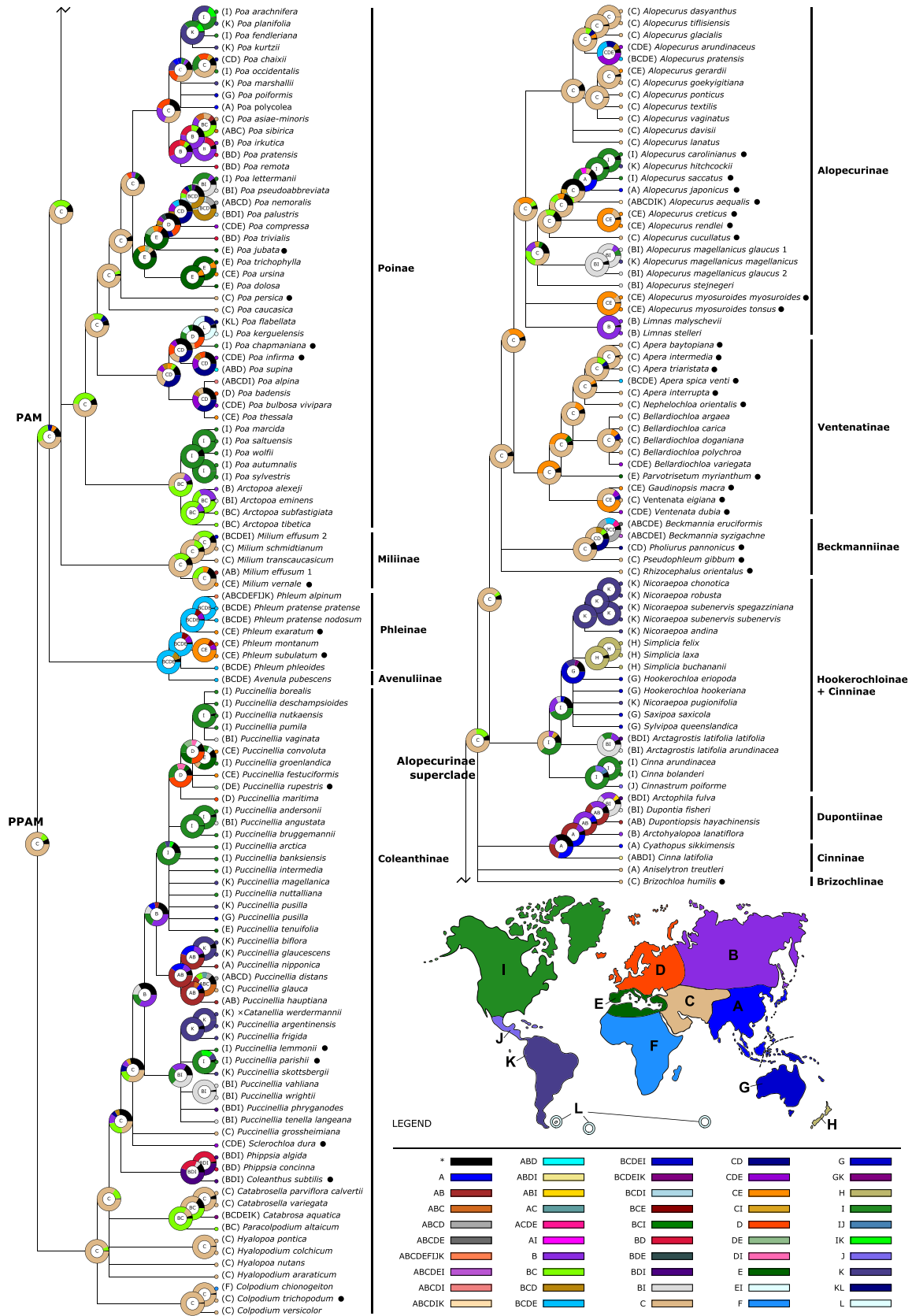


Fig. 3. Continued

issue in all previous plastid analyses of this group. Tkach et al. (2020), the previous study most complete at the generic level (all superclade Alopecurinae (“ABCV clade”) genera sampled except *Pseudophleum* and the newly recognized *Cinnastrum* [Gillespie et al., 2022]), resolved only one of the superclade Alopecurinae subtribes, Ventenatinae, in their plastid analysis. Two of the subtribes, Cinninae and Hookerochloinae, did not resolve as monophyletic here in our plastid analyses for various reasons, including low sequence diversity and homoplasious states in these two subtribes, but they were resolved as sister clades using nrDNA combined with plastid data (Gillespie et al., 2022). Hookerochloinae was strongly supported (“HSAQN” clade) using ITS + ETS data (Gillespie et al., 2009, 2022; Soreng et al., 2015a; Tkach et al., 2020), or ITS alone (Hoffmann et al., 2013). Neighboring long branches for certain annuals (all from southwestern Asia) may also have contributed to the lack of resolution at the base of the superclade (*Brizochloa*), and further up in the tree (*Rhizocephalus* and *Pseudophleum* of *Beckmanniinae*; and *Alopecurus myosuroides*) (Fig. 1C). New DNA data for *Pseudophleum* place it in superclade Alopecurinae, weakly supported as sister to *Beckmannia* + *Pholiurus*, supporting Soreng et al.’s (2017) placement of the genus in subtribe Beckmanniinae.

Several PPAM taxa included in our analyses are known to be reticulate in origin or have extra nrDNA copies from introgression. *Arctopoa* is nested within *Poa* in this and other plastid analyses, but in nrDNA studies, it resolves within the Alopecurinae superclade, thus exposing its reticulate origin (e.g., Gillespie et al., 2008, 2009, 2010, 2022; Soreng et al., 2015a; Tkach et al., 2020). The reticulate origin of the DAD clade of Soreng et al. (2015a), now four genera (including *Arctohyalopoa* described in Tkach et al., 2020), all hexaploids, was postulated by Soreng et al. (2015a) and discussed and named Dupontiinae by Gillespie et al. (2022). It is postulated that its plastid parent (Fig. 1) comes from Cinninae, while its nrDNA parent is in Alopecurinae and postulated to be an ancestor of *Limnas* (Soreng et al., 2015a; Gillespie et al., 2022), all within the supersubtribe. *Aniselytron* has also been suggested to be a product of introgression or of reticulate origin based on the presence of two different copies of ITS, one matching plastid and ETS placements, and one nested within the *Poa* sect. *Sylvestres* clade (Gillespie et al., 2008, 2010) (also discussed in Tkach et al. [2020] and Gillespie et al. [2022], where only the first copy was analyzed). Within the subtribe Coleanthinae, incongruent placements in plastid (Fig. 1) and nrDNA analyses indicate that *xCatanelia werdermannii* (syn. *Catabrosa werdermannii*) (Gnutikov et al., 2020; Gillespie et al., 2008, 2009; see Section 4.6), *Hyalopodium araraticum* (Tkach et al., 2020), and *H. colchicum* (syn. *Paracolpodium colchicum*) (this study and Gillespie, unpublished data; see Section 4.6) are also of reticulate origin.

#### 4.2 Dated tree

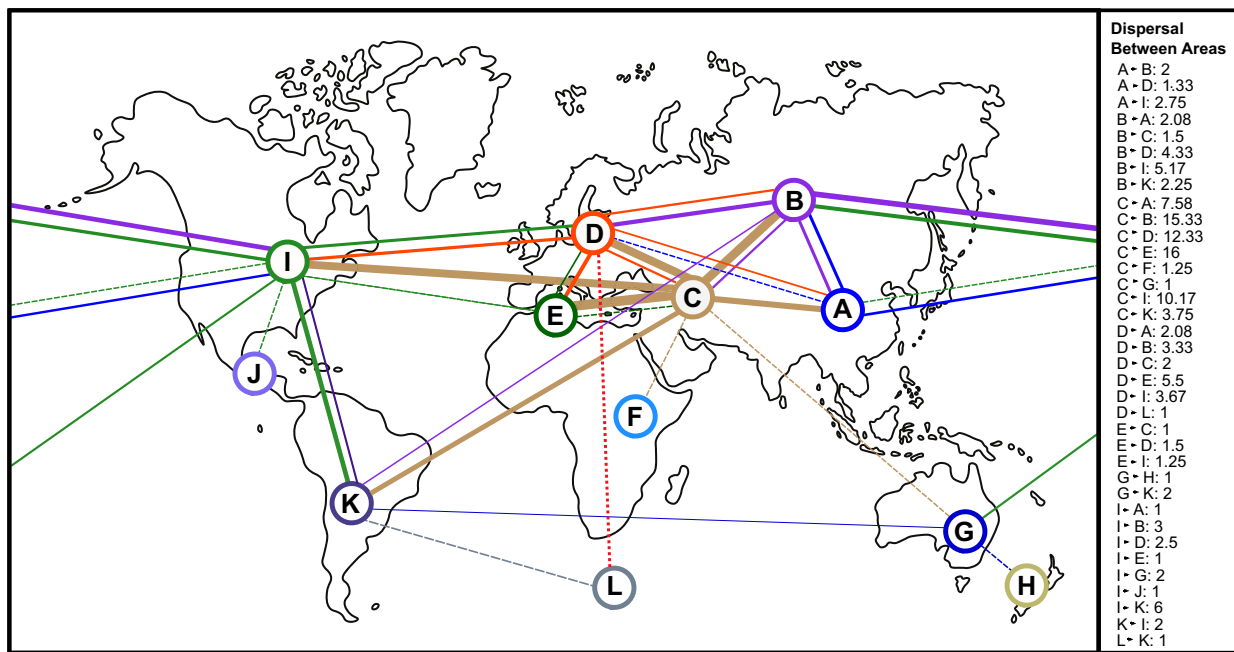
Few studies to date have estimated stem or crown ages of the PPAM clade. Here, we estimate the divergence of PPAM and the Loliodinae+ plastid lineage in the Late Oligocene (stems 24.19 mya) and the beginning of diversification of extant PPAM in the Early Miocene (crown 21.81 mya) (Fig. 2), ages that are generally consistent with previous studies using many fewer PPAM genera (Table 3). Pimentel et al. (2017) also estimated a Late Oligocene stem age and a very Early Miocene crown age (no specific dates given). Giussani et al. (2016), in their study, focused on the American *Poa* subg. *Poa* supersect. *Homalopoa* based on plastid and nrDNA data estimated a PPAM stem range consistent with our dates (HPD 18.1–29.2 mya). Gallaher et al. (2022) estimated somewhat older ages with both stem (29.84 mya) and crown ages (26.65 mya) in the Late Oligocene; our dates are younger, even though we used one stem date and six crown dates for major outgroup clades from that study for calibrating our tree. Minaya et al. (2017), in their study, focused on Loliinae, using both plastid and ITS data, and estimated PPAM ages (stem 27.76 mya, Late Oligocene; crown 23.43 mya, Oligocene/Miocene boundary) intermediate between those of Gallaher et al. (2022) and our dates based on plastid only.

Within PPAM, our dated tree (Fig. 2) differs somewhat from the Bayesian tree (Fig. 1) in both the degree of resolution and the topology. The Bayesian tree includes multiple polytomies (e.g., trichotomy of *Milium*, *Poa*, and superclade Alopecurinae), whereas in the dated tree, these polytomies are fully resolved, but with low support. The trees also differ in topology in some areas that are weakly supported in both trees. Notable differences include the branching order of PAM major clades and the positions within superclade Alopecurinae of *Brizochloa* and Beckmanniinae. This lack of resolution and low support result from a combination of factors, including low sequence divergence of some taxa, long branch lengths of some annuals, and conflicting data due to homoplasy or other factors. Tree topology in these areas of weak support on the dated tree may not represent the best estimate of phylogenetic relationships, and ages should be viewed with caution. For this reason, HPD confidence bars are provided only for strongly supported ( $pp \geq 0.95$ ) nodes on the dated tree (Fig. 2).

Within PPAM, we estimate that the first diversification event was the divergence of Coleanthinae and PAM lineages in the Early Miocene (stems 21.81 mya). The PAM clade then rapidly diversified into major lineages starting in the late part of the Early Miocene (Fig. 3, Table 3). The oldest stem ages in the PAM clade are *Poa* (stem 17.05 mya), followed by *Milium* (stem 16.38 mya), *Phleum* + *Avenula*, and superclade Alopecurinae (both stems 15.67 mya). However, none of these nodes are strongly supported, and the branching order of the five lineages differs from the Bayesian tree (where

**Fig. 3.** Reconstructed Area States of PPAM taxa from RASP BBM MCMC analysis plotted on the Bayesian consensus tree (outgroups not shown). Color-coded areas on circles represent proportional area probabilities for each node, with the most likely state in the center. The Areas Color Legend covers all discovered combinations, except for areas with individual probabilities less than 5%, which are collectively coded as black\*. The map outlines the 12 primary areas. PPAM subtribes are labeled on the left and other major clades are labeled at nodes. Black dots after names indicate taxa with annual life cycles.





**Fig. 4.** PPAM dispersals between biogeographic areas calculated by RASP BBM MCMC analysis. The color of each line corresponds to the most probable area of origin of the dispersal event; the width of the line corresponds to the number of dispersals in that direction, and dashed lines represent single events. Calculated numbers of dispersals for each line are listed to the right of the map (no line was plotted if the calculated dispersal events were less than 1; see also Table S3).

*Phleum* + *Avenula* diverges first, followed by a polytomy of the other three). All we can say with confidence is that separation of PAM into these lineages occurred very rapidly in the latter part of the Early Miocene and earliest Middle Miocene. Extant *Poa*, *Milium*, and the Alopecurinae superclade each radiated from the Middle Miocene (crowns 14.73, 12.37, 12.68 mya), whereas extant *Phleum* diversified considerably later in the Early Pliocene (4.31 mya).

Two previous studies focused on ages for major clades within PPAM. Giussani et al. (2016) provided broad date ranges, slightly earlier for the PAM crown (HPD 13.5–23.1 mya, but note that its sister Coleanthinae was not represented) and slightly later for the crowns of *Poa* (HPD 9.9–17.6 mya) and the Alopecurinae superclade (HPD 6.6–16.7 mya) than in our analysis. Hoffmann et al. (2013) included representatives of 40 PPAM genera (35 currently recognized) in their analysis of the ages of *Poa* and related genera using nrDNA ITS sequences. They found no support for any backbone clades within PPAM or any sister relationships among subtribes, but did resolve several subtribes and some smaller clades. They used two calibrations for PPAM, 20 and 25 mya, and for the 25 mya date, provided separate Penalized Likelihood and Uncorrelated Relaxed Clock date results (Hoffmann et al., 2013, fig. 1, table 1; see Table 3). In their text, they mainly discussed their Penalized Likelihood crown dates. Their resulting wide ranges of dates sometimes fall within our estimated date ranges, but most are significantly younger (Table 3). Their oldest dates for the crowns of Coleanthinae (ca. 5 mya, estimated from their Fig. 1), *Poa* (10.9 mya), *Milium* (2.7 mya), and *Phleum* (2.46 mya) are considerably younger than ours

(Table 3), but consistent for a late diversification in *Phleum*. Superclade Alopecurinae was not monophyletic in their ITS tree, but instead formed a paraphyletic group at the base of PPAM, with the stem dates of major internal lineages estimated at 20–25 Mya (based on their fig. 1), much older than our date for the superclade. Their very different ages are in part due to a backbone topology that differs from all other PPAM studies, both plastid and nuclear (e.g., Gillespie et al., 2008, 2009, 2010, 2022, and here; Soreng et al., 2015a; Giussani et al., 2016; Tkach et al., 2020), which may be the result of phylogenetically remote and/or insufficient out-group selection, less phylogenetic diversity sampled within subtribes (except Poinae/*Poa*), and limitations of using only ITS sequences.

We estimate that extant Coleanthinae separated into major lineages (crown 11.31 mya), later than the PAM clade (17.21 mya), starting in the mid-Miocene with the split of *Colpodium* from the rest. The latter appear to have diversified rapidly in the Late Miocene into multiple lineages, including *Catabrosella* + *Catabrosa*, *Phippsia* + *Coleanthus*, and *Puccinellia*-*Sclerochloa*. *Hyalopoa*, *Hyalopodium* (plastid parent), and *Paracolpodium* s.s. also originated then, either as a clade (Fig. 2) or independently (Figs. 1, 3). *Puccinellia*, the second largest genus in PPAM after *Poa*, originated relatively recently in the Late Pliocene (crown 3.37 mya) and diversified mostly in the Pleistocene, in sharp contrast to the much earlier origin and diversification of *Poa*, a difference also noted by Hoffmann et al. (2013). *Coleanthus* and *Phippsia* were estimated to also have separated very recently in the Late Pleistocene, as found by Hoffmann et al. (2013). Gallaher et al. (2022) provided an earlier age than ours for the crown

of Coleanthinae (17.99 mya, vs. 11.31 mya), contrasting again with Hoffmann et al.'s (2013) much later age (ca. 5 mya). Birch et al. (2014) provided an oldest crown date for *Puccinellia* (3.6 mya), consistent with our estimate for the *Puccinellia*–*Sclerochloa* clade in the Late Pliocene (3.37 mya). Hoffmann et al.'s (2013) crown dates for clades of *Colpodium*, *Phippsia*, and *Puccinellia* are comparable with ours or younger (Table 3).

Within the Alopecurinae superclade, subtribes Alopecurinae and Ventenatinae split (stems 11.46 mya) (10.5 mya stems in Gallaher et al., 2022) and diversified (crowns 10.37, 9.89 mya) in the Late Miocene. We date the genus *Alopecurus* to 10.37 mya, with *A. cucullata* (syn. *Cornucopiae cucullatum*) arising within it much later in the Pliocene (3.58 mya). Although the tree suggests that *Limnas* also arose within *Alopecurus* (6.74 mya), this has weak support and we believe that it is incorrect, its position likely affected by the long branch of the annual species, *A. myosuroides*. We suggest that *Limnas* is sister to *Alopecurus* with an origin likely in the Early part of the Late Miocene, which would be consistent with its position in the Bayesian tree (Fig. 1) and morphology (e.g., panicles less dense with elongated branches, paleas present and larger, lodicules present and well developed). The remaining superclade Alopecurinae subtribes were mostly insufficiently resolved and/or supported in the dated tree to allow estimation of ages with any confidence (also, the topology is inconsistent with the Bayesian tree, Fig. 1). Dupontiinae and Hookerochloinae (in this analysis including *Simplicia*) appear to have originated in the mid-Pliocene, whereas Beckmanniinae may have evolved much earlier in the Miocene. Hookerochloinae genera date from the Late Pliocene and Pleistocene. Hoffmann et al. (2013) provided some dates applicable within the superclade Alopecurinae. Although their dates for basal nodes (estimated based on their fig. 1) within the superclade are suspect (as described above), ages given for one well-supported subclade, *Beckmannia* + *Pholiurus*, are consistent with ours (Table 3), whereas their Pleistocene ages for the *Bellardiachloa* + *Apera* + *Nephelochloa* clade and Hookerochloinae are considerably younger than ours. They also provide crown dates for *Alopecurus* + *Limnas* + Dupontiinae (1.81–2.85 mya), which, although not directly comparable due to the reticulate nature of Dupontiinae, are considerably younger than our crown age estimate for *Alopecurus* + *Limnas* (10.37 mya).

*Poa* is currently divided into seven subgenera (Soreng et al., 2020) and 36 sections, and subg. *Poa* is divided into supersections *Poa* and *Homalopoa* (Gillespie et al., 2007). Our dates for the *P.* subg. *Poa* extant radiation starting in the Early Pliocene (5.06 mya) and that of supersect. *Homalopoa* in the Early Pleistocene (2.2 mya) overlap with but are somewhat later than the estimates given in Giussani et al. (2016) (Table 3). Birch et al. (2014), in their study of Australasian *Poa*, provided evidence for diversification of the Australasian supersect. *Homalopoa* sect. *Brizoides* in the Late Pliocene or Early Pleistocene (2.5 or 3.9 mya, depending on the secondary calibration dates used); the younger date is consistent with our crown age estimate for the encompassing supersect. *Homalopoa*. Hoffmann et al. (2013) provided dates for eight subclades within *Poa*. Their older of three crown age estimates for *P.* sect. *Brizoides* (2.56 mya;

“*Poa* VIII”) is consistent with our age for the encompassing supersect. *Homalopoa* (2.38 mya), but their other dates are younger than ours for comparable clades (Table 3). In a future paper, we will include many more of our *Poa* sequences and this will likely push our dates into deeper time.

#### 4.3 Biogeography

The PPAM clade (stem 24.19 mya; crown 21.81 mya) is decidedly southwestern Asian in origin. The Early Miocene date for PPAM correlates well with phytolith evidence of expansion of C<sub>3</sub> grasslands in the Early Miocene of western Eurasia, whereas C<sub>3</sub> grasslands expanded somewhat earlier in China, Central and North America, and southern South America (Strömberg, 2011).

Within Coleanthinae (crown 11.31 mya), the crown and the basal clades reside in southwestern Asia. *Colpodium* dispersed to Africa and *Hyalopoa* dispersed to the western edge of the Tibetan plateau. *Catabrosa aquatica* dispersed widely in the northern hemisphere and jumped the Western Hemisphere tropics to southern South America. In *Puccinellia*, some interesting patterns are apparent (Figs. 2, 3) regarding adaptation to salt tolerance between the Late Miocene (stem) and Early Pleistocene (crown) in the *Puccinellia*–*Sclerochloa* clade. See Life-History-Traits below for further discussion of salt tolerance.

The PAM clade (stem 21.81, crown 17.21 mya) is the sister to Coleanthinae. The most likely area here is southwestern Asia, but combinations of southwestern Asia with northern Asia, the Mediterranean, and northern Europe regions have significant probabilities (totaling 38% of the probability as opposed to the 47% probability of southwestern Asia). In the Late Miocene, Pliocene, but mostly in the Pleistocene, the major PAM lineages, except for *Avenula* (monotypic), diversified and dispersed across Eurasia and established in North America.

The first clade to diverge in PAM is *Phleum* + *Avenula*. This clade is unsupported (Fig. 1), but the two genera originated in Eurasia outside of eastern Asia. *Avenula* has one species that is widespread in Eurasia. *Phleum* (stem 14.78 mya, crown 4.31 mya) has 16 species, with each known major clade within the genus represented here. The perennials produced annuals in the southwestern Asia and Mediterranean regions, and the perennial *Phleum alpinum* dispersed widely around the world, reaching Africa, and spreading to North America and southern South America stepping along alpine corridors.

The sister clade to *Phleum* + *Avenula* is again decidedly southwestern Asian (62% probability), with northern Asia in combination with southwestern Asia having 27% probability. This clade begins with a trichotomy of *Milium*, *Poa*, and the Alopecurinae superclade. *Milium*, with three perennial and one (to several; Probatova, 1977, 2000) annual species, is decidedly southwestern Asian in origin, with a low probability of southwestern Asia with northern Asia (BC).

*Poa* (stem 17.21 mya, crown 14.73 mya), with about 570 species, most likely originated in southwestern Asia or less likely that area plus northern Asia. The clade containing *P.* subg. *Sylvestres* (node 267) is the first clade to diverge within *Poa*. Today, this subgenus is confined to North America. The genus *Arctopoa*, as noted elsewhere (Gillespie et al., 2008, 2010, 2022; Soreng et al., 2015a), is reticulate

between some ancestor of *Poa* subg. *Sylvestres* (plastid parent, North America) and some basal ancestor (perhaps in Cinninae) of the Alopecurinae superclade (nrDNA parent, probably in eastern Asia or northeastern Asia), where nrDNA places it among the earliest diverging lineages. The sister clade of subg. *Sylvestres* has the same origin in southwestern Asia as the genus with some lesser combined probabilities with northern Asia or northern Europe. Within this lineage, subg. *Ochlopoa* is the first clade to diverge (Fig. 3), and is primarily southwestern Asia and northern Europe in origin. Along the backbone, the probable area for *Poa* remains high for southwestern Asia for subgenera *Caucasicae*, *Pseudopoa*, and the clade encompassing *Stenopoa* and *Poa*. Subgenus *Poa* (node 245, with P and H plastid types) diverges from its sister clade (node 254, with N, J, V, and S plastid types; Soreng et al., 2020). Both clades diversified extensively, subgenus *Poa* with over 400 species worldwide, and its sister plastid clade with nearly 100 species mostly in Eurasia. The probable origin of subg. *Poa* is split between southwestern Asia, northern Asia, and northern Europe, whereas its sister clade is decidedly Mediterranean (Baltic) in origin. Based on our limited sampling, supersect. *Homalopoa* appears southwestern Asian in origin, whereas supersect. *Poa* has its origin in northern Eurasia. Giussani et al. (2016) and Birch et al. (2014) discuss rapid diversification within *P.* subg. *Poa* supersect. *Homalopoa* (ca. 330 species; RJS estimate here) in the Americas and Australasia, respectively, beginning in the Late Miocene or Early Pliocene. A more complete biogeographic analysis of *Poa*, including many more species, is a project for the future. (For broader phylogenetic and geographical samplings within this genus, see: Gillespie et al., 2007, 2008, 2009, 2018; Refulio Rodríguez et al., 2012; Hoffmann et al., 2013; Birch et al., 2014; Soreng et al., 2015a, 2017, 2020; Giussani et al., 2016; Cabi et al., 2016, 2017; Nosov et al., 2019; and papers cited there in).

The Alopecurinae superclade (crown 12.68 mya), with 27 genera, most likely evolved in southwestern Asia. Several clades and single taxon lineages diverge next. Although Cinninae and Hookerchloinae are not resolved here (see Section 4.1), they are in other studies. The distributions of Cinninae and Hookerchloinae genera are unusual in PPAM. *Aniselytron* and *Cyathopus* are confined to upland forests in eastern Asia, *Hookerchloa*, *Saxipoa*, and *Sylvipoa* to eastern Australia, *Simplicia* to New Zealand, and *Cinnastrum* to Mexico and further south. *Cinna latifolia* is primarily distributed across North America (where the other two *Cinna* species occur) and northern Eurasia, where it just crosses into eastern Asia. *Arctagrostis* occurs across boreal and arctic northern Eurasia and North America. The crown of Cinninae p.p. and Hookerchloinae is suggested to be in North America (43%) or southwestern Asia (29%), where none of the taxa occur today, but the ancestors may have come from. After *Arctagrostis* diverged, the remaining genera, which align in a polytomy, are from Australia, New Zealand, and southern South America, with a suggested origin in Australia (63%) or less likely southern South America (16%). Putative parent lineages of Dupontiinae (see the Phylogeny discussion above) are centered in eastern Asia (*Cinna latifolia* and *Cyathopus*) or northern Asia (*Cinna latifolia* and *Limnas*).

The Ventenatinae + Alopecurinae clade (crown 11.46 mya) and Alopecurinae (crown 10.37 mya), with two genera, have the highest probabilities of area of origin in southwestern Asia, followed by a lower probability in the combined Mediterranean and southwestern Asia areas. Ventenatinae (crown 9.89 mya), with seven genera, has either a southwestern Asian or a southwestern Asian-Mediterranean origin and has not dispersed out of there. *Alopecurus*, with about 40 species, has speciated extensively and dispersed widely, reaching eastern Asia, across northern Europe and northern Asia to North America and southern South America. *Limnas*, which is confined mostly to mountains of northeastern Asia (also in subarctic lowlands), appears to represent a separate dispersal event stemming from southwestern Asia or that plus the Mediterranean.

#### 4.4 Dispersals and disjunctions

The PPAM clade and all its major internal clades origins are centered in Eurasia, with most arising in southwestern Asia. According to the RASP BBM analysis on the Bayesian tree, most dispersal events (Table S3, mapped in Fig. 4) occurred within Eurasia between contiguous areas, even if not contiguous habitats, from C to E (16), C to B (15.33), C to D (12.33), C to A (7.58), D to E (5.5), B to D (4.33), D to B (3.33), B to A (2.08), D to C (2), B to C (1.5), E to D (1.5), and E to C (1). Few dispersals between non-contiguous areas within Eurasia are reported (D to A [2.08] and A to D [1.33]). Some species occur across steppe or wetland habitats and dispersed widely across Eurasia (e.g., *Alopecurus aequalis*, *A. pratensis*, *Beckmannia* spp., *Catabrosa aquatica*, *Phleum phleoides*, *P. pratense*), some reaching North America or further lands. Many other species are confined to high mountains or other narrow habitats and are restricted to single areas. Only a few alpine species dispersed widely (*Phleum alpinum*, *Poa alpina*).

We could provide estimated stem or crown dates for the lineages that are widely dispersed, but actual dates of the dispersals and disjunctions may have occurred significantly later for taxa that occur in multiple areas. Such situations are better tested by samples from each of the areas where they occur. Some disjunctions detected by the program are in part due to taxa that inhabit multiple areas, or polytomies (present in the Bayesian tree, but not the Beast dated tree), and/or incomplete sampling particularly in large genera. Some disjunctions are unlikely, such as C to K (3.75), which probably reflected widespread species or polytomies including area I, with C as their ancestral area, or in another case C to G (i.e., *Poa poiformis* of area G), the ancestors of which probably came from or through area A (Australian *Poa* origins discussed by Gillespie et al., 2009).

Dispersals to areas disjunct from Eurasia (Fig. 4) occurred most frequently from Eurasia to North America: C to I (10.17), B to I (5.17), D to I (3.67), A to I (2.75), and E to I (1.25) (Table S3). These events mostly occurred in the Pleistocene (Table S2). Wide dispersals from North America were most frequently to Eurasia (7.5), mainly to northern Asia, but also occurred to southern South America (I to K, 6), and to Central America/Mexico (I to J, 1). Australia contributed two dispersals to South America. However, we have little confidence in this direction since it went from Patagonia to Australia in a separate BBM analysis run on the dated tree

(a trial analysis not included in the paper). Australia and New Zealand were entered only by *Poa*, *Puccinellia*, and an ancestor of Cinninae-Hookerochloinae. Most other BBM dispersal values were fractional (less than 1) and are not tabulated here.\*

Of the 97 dispersal events in PPAM between areas in our RASP BBM analysis (Tables S2, S3), 56 occurred in the Pleistocene, 21 in the Pliocene, 13 in the Late Miocene, and 6 in the Middle Miocene, and one in the Early Miocene (Table S2). Although stem dates of some early *Poa*, *Milium*, and early superclade Alopecurinae lineages predate 10 mya, all these are forest lineages and probably did not arrive in North America until the Late Pliocene or Pleistocene (Tables 3, S2).

Dispersals into North America occurred 23 times in this BBM analysis (see footnote \*). Of these, three dispersals occurred in the Late Miocene, two in Coleanthinae, and one in the early Alopecurinae superclade. In the Pliocene, there were three dispersals, once each in *Poa* subg. *Sylvestres*, *Puccinellia*, and the early Alopecurinae superclade (Cinninae p.p. + Dupontinae). The other 16 dispersals into North America occurred in the Pleistocene, one time from southern South America (*Poa*) and 15 times from Eurasia. Pleistocene dispersals out of North America occurred 15 times (six times solely from, and nine times including northern Asia); solely from North America including four times to southern South America (once in *Alopecurus* and *Poa*, and two times in *Puccinellia*), once to Australia (*Puccinellia*), and four other dispersals involved arctic and boreal range extensions of species to Asia or Europe of Coleanthinae. In the Pliocene, we found one dispersal south to Latin America (*Cinnastrum*), one spread from North America to northern Asia (*Arctagrostis*), and starting off a Hookerochloinae radiation in Australia, suggested as a possibility by Gillespie et al. (2007) (but note our doubt in the preceding paragraph regarding the route).

Dispersals into South America, apart from those solely originating from North America, occurred nine times, seven times from Eurasia, twice from Australia (giving rise to *Nicoraepoa* in the Pliocene, direction again questionable, and some lines of *Poa*), and once from the Subantarctic Islands (Fig. 1; Table S3). Two of these represent range extensions of widespread species that may have arrived in South America much after their stem ages (*Catabrosa aquatica*, *Phleum alpinum*).

Wen et al. (2016) used molecular data to analyze the ages of plant disjunctions between eastern Asia and North America. Most cool temperate disjunctions occurred in the last 10 mya continuing through the Pleistocene. About 2/3 of these came from Asia and 1/3 from North America. Only a few temperate disjunctions between Eurasia and North America predate this. This tallies well with our findings within PPAM of 18 arrivals from Asia (12 involving areas A

and/or B) and three from North America post Miocene, and only three arrivals from Asia in the Late Miocene.

#### 4.5 Life-history traits and climate shift hypotheses

Stepping back from all the centers of distribution and dispersals, we can consider some of the underlying life-history traits and their correlations with the above area patterns and ages.

Were Ancestral PPAM broad-bladed forest grasses? Was this the plesiomorphic state among PPAM lineages? PPAM broad-bladed forest grasses, defined here (more inclusively than in Gallaher et al., 2019) as having flat culm-leaf blades frequently exceeding 5 or more mm in width, and growing in forests, are scattered across the group. All extant Coleanthinae are open land species. The broadest bladed species in the analysis in this subtribe are *Colpodium chionogeton* and *Hyalopoa nutans*, with *Hyalopodium colchicum* rarely reaching 5 mm wide, and all are alpine wetland species. However, a lot could have happened in their ancestors between stem and crown dates of 21.81 and 11.31 mya. In Miliinae (crown 12.37 mya), *Milium effusum* is a strictly temperate forest grass with broad blades (to 15 mm wide); the other *Milium* species occur in more open spaces. Our evidence suggests that *M. effusum* populations diverged vicariantly in eastern and northeastern Asia from western Eurasia. *Poa* is a mixed bag, but all the species of the earliest diverging clade, *Poa* subg. *Sylvestres*, as the name suggests, are forest species and moderately broad-bladed, with three species having leaves reaching 5 to 8 mm wide. All confirmed members occur in North America today; however, nrDNA evidence suggests that some ancestor hybridized with *Aniselytron* in eastern Asia, perhaps in the eastern Himalaya (Gillespie et al., 2010, 2022). Other broad-bladed forest species in the study include *P. trivialis* and *P. occidentalis*, but other broad-bladed species grow in wetlands (often in forest glades and forest margins), such as *P. asiae-minoris*, *P. chaixii*, *P. palustris*, *P. persica*, *P. planifolia*, and *P. remota*. Cinninae genera (*Aniselytron*, *Cinna*, *Cinnastrum*, *Cyathopus*), and species are all flat-bladed and broad-bladed (max. 6 to 20 mm wide), except for *Simplicia* (max. 4 mm wide), and forest species, apart from *S. laxa* (which may have been more common in forested lands now mostly gone). The clade that includes all the Cinninae species dates to the Late Miocene (stem 9.57, crown 7.18 mya). Hookerochloinae arose in the Pliocene and among these genera, *Sylvipoa* (blades to 15 mm wide) lives in damp forests; *Nicoraepoa andina* s.l. (sensu Finot Saldías et al., 2018; treating *N. chonotica* as a subspecies) has broad blades (to 14 mm wide) and occurs in wetland forest openings to alpine and coastal meadows, while the other species of the genus, beyond *N. robusta* and *N. subnervis* (with broad blades, occurring in open, subsaline, wetland habitats), are xeromorphic species of saline habitats (e.g., *N. pugionifolia*); *Arctagrostis latifolia* subsp. *arundinacea* is broad-leaved (to 15 mm wide) and occurs in boreal forest wetlands. In Dupontinae, only *Arctophila* has broad leaves (to 5 [-10] mm wide) and it grows in shallow water in tundra and riparian boreal forests. In Ventenatinae, *Apera spica-venti* has broad blades (to 10 mm wide), and frequently grows in mesic forests and forest openings but also more open wetlands. In Alopecurinae, even the broadest-bladed species (to 14 mm wide) of

\*Cumulative fractional numbers from individual dispersal values of less than one calculated by the BBM analysis, and rounding to the nearest integers one or above, account for minor differences in numbers reported in the following paragraphs of this section. Numbers are also affected by polytomies and species with multiple areas.

*Alopecurus* typically occur in open habitats. The rest of the PPAM are typically narrow-bladed (blades 1 to 5 mm wide), whether in forested or open habitats. Therefore, Milinae, early *Poa*, and Cinninae could represent vestiges of ancient broad-bladed forest grasses in PPAM, while other broad-bladed PPAM are representative of open habitats, ecotones, or secondary forest incursions. We consider that the evidence is equivocal for a forest origin hypothesis for PPAM.

Another life-history trait of note is high cold tolerance. Sandve & Fjellheim (2010) and Schubert et al. (2019a) discussed high cold tolerance in Pooideae, and Hoffmann et al. (2013) wrote about this in relation to the biogeographic distribution of *Poa*. Hartley (1961) showed that *Poa* species could tolerate cold, but not long days with high temperatures combined with summer season precipitation. Although species of Pooideae are generally cold tolerant, many PPAM taxa are especially cold tolerant. Because PPAM's origin is in southwestern Asia, we suggest that alpine and subalpine habitats were likely ancestral. Many of the perennial species in PPAM occur above tree lines around the world, particularly alpine and tundra regions. All the subtribes, except Avenulinae, Beckmanniinae, Cinninae, and Miliinae, have species that occur in these cold regions. However, only a few of the genera have broached the exigencies of life in polar tundra regions, most notably *Alopecurus magellanicus* complex, *Arctophila*, *Dupontia*, *Arctohyalopoa*, *Limnas malyschevii* (subarctic), *Phippsia*, *Phleum alpinum*, *Poa* (various spp.), and *Puccinellia* (various spp.).

Tolerance of saline and alkaline conditions is noted for Coleanthinae above and explored here. This is the only subtribe of Poeae with lineages that have extensively adapted to high alkaline and/or saline conditions. Salt tolerances may well have developed along coastlines, but the expansion of arid saline and alkaline basins with mid-Tertiary increasing aridity and cold would provide much broader niches for tolerant lineages to fill. Within Coleanthinae, the ancestral lineages are in wet to seasonally dry alpine upland areas of southwestern Asia. The genus *Colpodium*, sister to the rest, is restricted to non-calcareous substrates. *Hyalopoa* and *Hyalopodium*, mostly unresolved at the base of the sister clade to *Colpodium*, are also restricted to non-calcareous. The other two lineages in this polytomy initially include at least some limited development of tolerance of higher pH. One is the *Paracolpodium* s.s.-*Catabrosella-Catabrosa* clade, and the other begins with divergence of *Phippsia* plus *Coleanthus*. Higher tolerances of saline and alkaline conditions initially developed in the *Coleanthus-Phippsia* clade (which dispersed to North America from northern Eurasia) and evolved further in the *Sclerochloa-Puccinellia* clade. *Puccinellia* speciated extensively (116 species) and dispersed to all areas, except New Zealand and the Subantarctic Islands. *Puccinellia* dispersed to South America at least four times, three times from North America and once from the combined northern Asia-eastern Asia area. These disjunctions were undoubtedly mediated by birds that frequent saline lakes, marshes, and sea and oceanic coasts where the species are distributed. Several PAM clade genera also have high levels of salt tolerances: *Arctopoa*, *Nicoraepoa* (hypersaline in some), *Rhizocephalus*, and some *Poa* (*P. flabellata* and *P. kerguelensis* in this study, but also others).

Another trait of note is the evolution of various annual genera and species in the PPAM clade (annuals indicated in Fig. 3). Annual species occur in 15 genera in the Mediterranean and southwestern Asia, whereas this habit evolved in few genera outside of these regions. Stem dates for the annuals are all under 10 mya (except *Milium vernale* and *Rhizocephalus*), but we cannot be sure when they became annuals. Beyond these two and other isolated annual species with stems dating to the Late Miocene (*Alopecurus myosuroides*, *Brizochloa*, *Parvotrisetum*, *Pseudophleum*, *Poa infirma*, *P. jubata*, *P. persica*), all the other 22 annuals in the study evolved in the Pliocene and Pleistocene. Crown dates help here, and where we have them for sets of species (e.g., *Apera* + *Nephelochloa* and *Alopecurus cucullatus* to *A. carolinianus*), they are under 4 mya, and often are under 1 mya. Most annuals in the analysis originated in the Mediterranean and southwestern Asian regions: *Apera* (all 5 spp.), *Alopecurus creticus*, *A. cucullatus*, *A. myosuroides*, *A. rendlei*, *Brizochloa*, *Colpodium trichopodium*, *Gaudinopsis*, *Milium vernale*, *Nephelochloa*, *Parvotrisetum*, *Phleum exaratum*, *P. subulatum*, *Ventenata eigiana*, *Pholiurus*, *Poa infirma*, *P. jubata*, *P. persica*, *Puccinellia rupestris*, *Rhizocephalus*, *Sclerochloa* (the later three in saline habitats), and *Ventenata*. Although we did not include all our sequenced annuals in the present analysis in *Phleum* (all confined to the same two areas), *Alopecurus*, and *Poa*, this area of concentration is remarkable as there actually are very few annuals in PPAM outside of these two regions. *Coleanthus* (stem date 1.14 mya) is native across northern Eurasia, reaching northwestern North America. *Alopecurus aequalis* (annual to short-lived perennial) has dispersed widely and its sister clade has produced three annuals (less than 1 mya) in eastern Asia (*A. japonicus*) and North America (*A. caroliniana* and *A. saccatus*) in wetlands. About 15 other annuals (not included in this analysis) of *Poa* supersection *Homalopoa* arose in eastern Asia, North America, South America, and Australia, some adapted to Mediterranean climates or Mediterranean-like niches, and all evolved post 2.3 mya. In the *Parodiochloa* clade (including sections *Diversipoa*, *Parodiochloa*, and *Tzvelevia*), *Poa chapmanniana*, of eastern North America, sister to *P. flabellata* + *P. kerguelensis* of the Subantarctic islands, diverged earlier (stem 4.37).

Dispersal mechanisms of course aid in increasing range sizes and moving between areas (Davidse, 1987; Linder et al., 2017). *Alopecurus* and *Phleum* have spikelets that disarticulate below the glumes (tardily in *Phleum*) with antrorse hairs on the glumes such that their diaspores could work their way into feathers (Vivian-Smith & Stiles, 1994). *Puccinellia* and other open wetland genera may readily adhere to birds' muddy feet (Vivian-Smith & Stiles, 1994). This is expected to be important for Beckmanniinae dispersal also. Callus hairs are also effective in aiding seed dispersal by birds and mammals, and this may explain the wide distribution of *Poa*. A key factor in the dispersal of *Poa* is the presence of long and tangled callus hairs, often confined to the dorsal side of the lemma callus. These and other floret hairs easily become entangled in feathers and fur (Davidse, 1987; Linder et al., 2017). Callus hairs are also probably effective for dispersal where they form a crown as in *Arctopoa*, many Coleanthinae, *Bellardiochloa*, Dupontiinae, *Nicoraepoa*, and some *Poa*. Rachilla hairs would also be

effective for dispersal as in *Bellardiochloa* and *Avenula*. Elongated callus hairs can also enhance wind-dispersal (Davidse, 1987). Hairs on floret bracts (present in some Coleanthinae, e.g., *Puccinellia*, and elaborated in many species of *Poa*), or on glumes in genera with disarticulation below the glumes (*Alopecurus* and *Phleum*), surely aid in dispersal.

Awns can be effective for dispersal (Davidse, 1987; Linder et al., 2017), but these are absent in Coleanthinae, Milliinae, Poinae, and negligible in Beckmanniinae, Hookerochloinae, Dupontiinae. Ventenatinae + Alopecurinae may be called the PPAM “awned clade”. Awns are present on lemmas in Ventenatinae annuals where they may be dorsal or subapical, but are negligible in *Bellardiochloa*, which is perennial. Awns are also inserted dorsally on lemmas in most Alopecurinae species.

#### 4.6 Taxonomy

In past studies we sampled two collections of *Catabrosa werdermannii* from wetlands above high altiplano salt lakes of central Argentina (Peterson et al. 1937) and Chile (Soreng & Soreng 7150). We examined nrDNA (ITS and ETS) of both collections in previous analyses (Gillespie et al., 2008, 2009, 2010; but note that Soreng & Soreng 7150 was incorrectly identified as *Catabrosa aquatica* at the time, the name was subsequently corrected in GenBank), and found them to be highly divergent from *Puccinellia* and in a clade with *C. aquatica* when a correctly identified sample of this species was included. Other authors have picked up some of our *C. werdermannii* data from GenBank for their analyses (Gnutikov et al., 2020 [GenBank number in ITS tree misnumbered]; Tkach et al., 2020), where it is well supported in *Catabrosa* (revised by Tzvelev, 2013; Punina et al., 2016) or with *C. aquatica*. Prior plastid DNA analyses (Gillespie et al., 2008, 2009) resolved these two *C. werdermannii* samples within the *Puccinellia*-*Sclerochloa* clade (Gillespie et al., 2008) or closely related and sister to *Puccinellia* when only one species of the latter was included (Gillespie et al., 2009) (authentic *C. aquatica* was not included in these analyses). In analyses of plastid data sets with ample species sampling in Coleanthinae (Fig. 1; Gillespie, unpublished; Gnutikov et al., 2020) *C. werdermannii* clearly is resolved within *Puccinellia*, with *P. argentinensis* or *P. frigida* the likely plastid parent (Fig. 1). As it is now obvious (first pointed out by Gnutikov et al., 2020, p. 265) that this species is derived from hybridization between *Catabrosa* (nrDNA parent) and *Puccinellia* (plastid parent), we propose a new hybrid genus name. The nrDNA parent is likely *C. aquatica* which is widespread, including in South America; the other species of *Catabrosa* (revised by Tzvelev, 2013; Punina et al., 2016) having restricted Eurasian distributions.

×*Catanellia* L.J. Gillespie & Soreng, **nothogen. nov.** = *Catabrosa* P.Beauv. × *Puccinellia* Parl. Species ×*Catanellia werdermannii* (Pilg.) L.J.Gillespie & Soreng, **comb. nov.** Basionym: *Phippsia werdermannii* Pilg., Notizbl. Bot. Gart. Berlin-Dahlem 10(97): 759. 1929. Syn. *Catabrosa werdermannii* (Pilg.) Nicora & Rúgolo. 1981.

*Hyalopodium colchicum* (syn. *Paracolpodium colchicum*) resolves closer to *Hyalopoa* and *Hyalopodium*, than it does to *P. altaicum* (type species) in our plastid tree. Like *Hyalopodium araraticum* (Tkach et al., 2020), *H. colchicum* is

strongly supported in our nrDNA analyses (Gillespie et al., unpublished) as related to *Colpodium* s.s. *Hyalopodium* was postulated to be an ancient hybrid between the *Colpodium* s.s. clade (nrDNA parentage) and some ancestor of the larger sister clade (Tkach et al., 2020). Thus we transfer *Paracolpodium colchicum* to *Hyalopodium*. *Paracolpodium* s.s. then resides in central Asia, in the eastern mountain alpine of our southwestern Asia area, whereas *Hyalopodium* occurs further west in southwestern Asian. *Hyalopodium* in this sense has sheaths closed  $\frac{1}{2}$ – $\frac{3}{4}$  their length, versus closed  $\frac{1}{4}$ – $\frac{1}{3}$  their length in *Paracolpodium* s.s., and a crown of hairs on the lemma callus, whereas *Paracolpodium* s.s. has glabrous calluses.

***Hyalopodium colchicum*** (Albov.) L.J.Gillespie & Soreng, **comb. nov.** Basionym: *Catabrosa colchica* Albov, Prodr. Fl. Colchic. 259. 1895. Syn. *Colpodium colchicum* (Albov) Woronow ex Grossh. 1928; *Paracolpodium colchicum* (Albov.) Tzvelev. 1966.

The type species of the genus *Cornucopiae*, as noted in the results and shown in Figs. 1–3, is nested within *Alopecurus*. Although we only included the type species here, we have DNA data on both accepted species and obtain the same result with a fuller sample of *Alopecurus* species (Gillespie et al., in prep.). Accordingly, we sink *Cornucopiae* in *Alopecurus* here, as already proposed in antiquity. The primary distinction of *Cornucopiae* was its highly contracted inflorescence axis.

*Alopecurus* L., Sp. Pl. 1: 60. 1753. Type species: *A. pratensis* L. Syn. *Cornucopiae* L., Sp. Pl. 1: 54. 1753. Type species: *Cornucopiae cucullatum* L.

***Alopecurus alopecuroides*** (L.) L.J.Gillespie, Cabi & Soreng, **comb. nov.** Basionym: *Cornucopiae alopecuroides* L., Syst. Nat. (ed. 12) 2: 85. 1767.

*Alopecurus cucullatus* (L.) Raspail, Ann. Sci. Nat. (Paris) 5: 294. 1825. Basionym *Cornucopiae cucullatum* L., Sp. Pl. 1: 54. 1753. Syn. *Alopecurus cornucopiae* Trin., nom. illeg. superfl., Mém. Acad. Imp. Sci. Saint-Petersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat. 4: 46. 1840.

## 5 Conclusions

The Bayesian Binary Markov Chain analysis as initiated within RASP performed well and provided many insights into the origin and diversification of the diverse PPAM clade. Running BBM analysis on the BEAST dated tree gave some biogeographic results that were inconsistent with the BBM analysis on the Bayesian consensus tree, and we decided to use the latter rather than the former so as not to force unsupported conclusions. The major conclusion is that PPAM evolved from a stem event in the Late Oligocene and a crown in the Early Miocene, and diversified in and spread out of southwestern Asia across Eurasia, and to all corners of the globe in several lineages. Many lineages have Pliocene and Pleistocene crown ages, but a few radiated in the Middle to Late Miocene. Long branches in our analyses for many annual species may be a result of their mode of evolution rather than deep ages, and the effects on dating and phylogenetic analyses need further investigation. Lineages of the PPAM clade produced many species adapted to alpine and then arctic climates, and to calcareous and then strongly alkaline and saline habitats, and

generated many annual species and genera in the Mediterranean and southwestern Asia.

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## References

- Akaike H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19(6): 716–723.
- Ali SS, Yu Y, Pfosser M, Wetschnig W. 2021. Inferences of phylogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian MCMC analysis implemented in RASP (Reconstructed Ancestral State in Phylogenies). *Annals of Botany* 109: 95–107.
- Birch JL, Cantrill DJ, Walsh NG, Murphy DJ. 2014. Phylogenetic investigation and divergence dating of *Poa* (Poaceae, tribe Poeae) in the Australasian region. *Botanical Journal of the Linnean Society* 175: 523–552.
- Bouchenak-Khelladi Y, Salamin N, Savolainen V, Forest F, van der Bank M, Chase MW, Hodkinson TR. 2008. Large multi-gene phylogenetic trees of the grasses (Poaceae): Progress towards complete tribal and generic level sampling. *Molecular Phylogenetics and Evolution* 47: 488–505.
- Bouchenak-Khelladi Y, Savolainen V, Hodkinson TR. 2010. Diversification of the grasses (Poaceae): A phylogenetic approach to reveal macro-evolutionary patterns. In: Seberg O, Petersen G, Barfod AS, Davis JI eds. *Diversity, phylogeny, and evolution in the monocotyledons*. Aarhus: Aarhus University Press. 451–474.
- Cabi E, Soreng RJ, Gillespie LJ. 2017. Taxonomy of *Poa jubata* and a new section of the genus (Poaceae). *Turkish Journal of Botany* 41: 404–415, f. 1–4.
- Cabi E, Soreng RJ, Gillespie LJ, Amiri N. 2016. *Poa densa* (Poaceae), an overlooked Turkish steppe grass, and the evolution of bulbs in *Poa*. *Willdenowia* 46(2): 201–211.
- Cahoon AB, Sharpe RM, Mysayphonh C, Thompson EJ, Ward AD, Lin A. 2010. The complete chloroplast genome of tall fescue (*Lolium arundinaceum*; Poaceae) and comparison of whole plastomes from the family Poaceae. *American Journal of Botany* 97: 49–58.
- Clayton WD. 1975. Chorology of the genera of Gramineae. *Kew Bulletin* 30(1): 111–132.
- Clayton WD, Cope TA. 1980. The chorology of Old World species of Gramineae. *Kew Bulletin* 35: 135–171.
- Clayton WD, Renvoize SA. 1986. Genera Graminum. Grasses of the World. *Kew Bulletin, Additional Series* 13: 1–389.
- Cross RA. 1980. Distribution of sub-families of Gramineae in the Old World. *Kew Bulletin* 35: 279–289.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature, Methods* 9: 772.
- Davidse G. 1987 [1988]. Chapter 14: Fruit dispersal in the Poaceae. In: Soderstrom TR, Hilu KW, Campbell CS, Barkworth ME eds. *Grass systematics and evolution*. Washington, DC: Smithsonian Institution Press. 14: 143–155.
- Davis JI, Soreng RJ. 2007. A preliminary phylogenetic analysis of the grass subfamily Pooideae (Poaceae), with attention to structural features of the plastid and nuclear genomes, including an intron loss in GBSSI. *Aliso* 23: 335–348.
- Diekmann K, Hodkinson TR, Fricke E, Barth S. 2008. An optimized chloroplast DNA extraction protocol for grasses (Poaceae) proves suitable for whole plastid genome sequencing and SNP detection. *PLoS ONE* 3(7): E2813.
- Döring E. 2009. *Molekulare Phylogenie der Hafer-Gräser (Poaceae: Pooideae: Aveneae)*. Dissertation. Halle-Wittenberg: Martin-Luther-Universität. 1–86. <https://d-nb.info/1024859843/34>
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.
- Duvall MR, Burke SV, Clark DC. 2020. Plastome phylogenomics of Poaceae: Alternate topologies depend on alignment gaps. *Botanical Journal of the Linnean Society* 192(1): 9–20.
- Finot Saldías VL, Soreng RJ, Giussani LM, Muñoz RG. 2018. A multivariate morphometric delimitation of species boundaries in the South American genus *Nicoraepoa* (Poaceae: Pooideae: Poeae). *Plant Systematics and Evolution* 304(5): 679–697.
- Gallaher TJ, Adams DC, Attigala L, Burke SV, Craine JM, Duvall MR, Klahs PC, Sherratt E, Wysocki WP, Clark LG. 2019. Leaf shape and size track habitat transitions across forest–grassland boundaries in the grass family (Poaceae). *Evolution* 73–5: 927–946.
- Gallaher TJ, Peterson PM, Soreng RJ, Zuloaga FO, Li DZ, Clark LG, Tyrrell CD, Welker AD, Kellogg EA, Teisher JK. 2022. Grasses through space and time: An overview of the biogeographical and macroevolutionary history of Poaceae. *Journal of Systematics and Evolution* 60: 522–569.
- Gernhard T. 2008. The conditioned reconstructed process. *Journal of Theoretical Biology* 253(4): 769–778.
- Gillespie LJ, Archambault A, Soreng RJ. 2007. Phylogeny of *Poa* (Poaceae) based on *trnT-trnF* sequence data: Major clades and basal relationships. *Aliso* 23: 420–434.
- Gillespie LJ, Soreng RJ, Bull RD, de Lange PJ, Smitsen RD. 2022. Morphological and phylogenetic evidence for subtribe Cinninae and two new subtribes, Hookerochloinae and Dupontiinae (Poaceae tribe Poeae PPAM clade). *Taxon* 71(1): 52–84.
- Gillespie LJ, Soreng RJ, Bull RD, Jacobs SWL, Refulio-Rodríguez NF. 2008. Phylogenetic relationships in subtribe Poinae (Poaceae, Poeae) based on nuclear ITS and plastid *trnT-trnL-trnF* sequences. *Botany* 86: 938–967.
- Gillespie LJ, Soreng RJ, Cabi E, Amiri N. 2018. Phylogeny and taxonomic synopsis of *Poa* subgenus *Pseudopoa* (including *Eremopoa* and *Lindbergella*) (Poaceae, Poeae, Poinae). *PhytoKeys* 111: 69–101.
- Gillespie LJ, Soreng RJ, Jacobs SWL. 2009. Phylogenetic relationships of Australian *Poa* (Poaceae: Poinae), including molecular evidence for two new genera, *Saxipoa* and *Sylvipoa*. *Australian Systematic Botany* 22(6): 413–436.
- Gillespie LJ, Soreng RJ, Paradis M, Bull RD. 2010. Phylogeny and reticulation in subtribe Poinae and related subtribes (Poaceae) based on nrITS, ETS, and trn TLF data. In: Seberg O, Petersen G,



- Barfod AS, Davis JI eds. *Diversity, phylogeny, and evolution in the monocotyledons*. Aarhus: Aarhus University Press. 589–618
- Giussani LM, Gillespie LJ, Scataglini MA, Negritto MA, Antón AM, Soreng RJ. 2016. Breeding system diversification and evolution in American *Poa* supersect. *Homalopoa* (Poaceae: Pooeae: Poinae). *Annals of Botany* 118(2): 281–303.
- Grebenstein B, Röser M, Sauer W, Hemleben V. 1998. Molecular phylogenetics relationships in Aveneae (Poaceae) species and other grasses as inferred from ITS1 and ITS2 rDNA sequences. *Plant Systematics and Evolution* 213: 233–250.
- Gnutikov AA, Nosov NN, Punina EO, Probatova NS, Rodionov AV. 2020. On the placement of *Coleanthus subtilis* and the subtribe Coleanthinae within Poaceae by new molecular phylogenetic data. *Phytotaxa* 468(3): 243–274.
- Grass Phylogeny Working Group II (Aliscioni S, Bell HL, Besnard G, Christin P-A, Columbus JT, Duvall MR, Edwards EJ, Giussani L, Hasenstab-Lehman K, Hilu KW, Hodkinson TR, Ingram AL, Kellogg EA, Mashayekhi S, Morrone O, Osborne CP, Salamin N, Schaefer H, Spriggs E, Smith SA, Zuloaga FO). 2012 [2011]. New grass phylogeny resolves deep evolutionary relationships and discovers C4 origins. *New Phytologist* 193(2): 304–312.
- Hand ML, Spangenberg G, Forster JW, Cogan NOI. 2013. Plastome sequence determination and comparative analysis for members of the *Lolium-Festuca* grass species complex. *G3 Genes/Genomes/Genetics* 3(4): 607–616.
- Hartley W. 1961. Studies on the origin, evolution, and distribution of the Gramineae. IV. The genus *Poa* L. *Australian Journal of Botany* 9: 152–161.
- Hartley W. 1973. Studies on the origin, evolution, and distribution of the Gramineae. V. The subfamily Festucoideae. *Australian Journal of Botany* 21: 201–234.
- Hochbach A, Schneider J, Röser M. 2015. A multi-locus analysis of phylogenetic relationships within grass subfamily Pooideae (Poaceae) inferred from sequences of nuclear single copy gene regions compared with plastid DNA. *Molecular Phylogenetics and Evolution* 87: 14–27.
- Hoffmann MH, Schneider J, Hase P, Röser M. 2013. Rapid and recent world-wide diversification of bluegrasses (*Poa*, Poaceae) and related genera. *PLoS ONE* 8(3): 1–9.
- Inda LA, Segarra-Moragues JG, Müller J, Peterson PM, Catalán P. 2008. Dated historical biogeography of the temperate Loliinae (Poaceae, Pooideae) grasses in the northern and southern hemispheres. *Molecular Phylogenetics and Evolution* 46(3): 932–957.
- Katoh K, Standley D. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780.
- Linder HP, Lehmann CER, Archibald S, Osbourne CP, Richardson MD. 2017. Global grass (Poaceae) success underpinned by traits facilitating colonization, persistence and habitat transformation. *Biological Reviews (Cambridge)* 93(2): 1125–1144.
- Middleton CP, Senerchia N, Stein N, Akhunov ED, Keller B, Wicker T, Kilian B. 2014. Sequencing of chloroplast genomes from wheat, barley, rye and their relatives provides a detailed insight into the evolution of the Triticeae tribe. *PLoS ONE* 9(3): E85761.
- Minaya M, Hackel J, Namaganda M, Brochmann C, Vorontsova MS. 2017. Contrasting dispersal histories of broad- and fine-leaved temperate Loliinae grasses: Range expansion, founder events, and the roles of distance and barriers. *Journal of Biogeography* 44(on-line): 1–14.
- Minaya M, Pimentel M, Mason-Gamer RJ, Catalán P. 2013. Distribution and evolutionary dynamics of Stowaway Miniature Inverted Repeat Transposable Elements (MITEs) in grasses. *Molecular Phylogenetics and Evolution* 68: 106–118.
- Nosov NN, Tikhomirov VN, Machs EM, Rodionov AV. 2019. On polyphyly of the former section *Ochlopoa* and the hybridogenic section *Acroleuca* (*Poa*, Poaceae): Insights from molecular phylogenetic analyses. *Nordic Journal of Botany* 37(3): 1–16.
- Ogihara Y, Isono K, Kojima T, Endo A, Hanaoka M, Shiina T, Terachi T, Utsugi S, Murata M, Mori N, Takumi S, Ikeo K, Gojobori T, Murai R, Murai K, Matsuoka Y, Ohnishi Y, Tajiri H, Tsunewaki K. 2002. Structural features of a wheat plastome as revealed by complete sequencing of chloroplast DNA. *Molecular Genetics and Genomics* 266(5): 740–746.
- Orton LM, Barberá P, Nissenbaum MP, Peterson PM, Quintanar Sánchez A, Soreng RJ. 2021. A 313 plastome phylogenomic analysis of Pooideae: Exploring relationships among the largest subfamily of grasses. *Molecular Phylogenetics and Evolution* 159(2121): 1–14.
- Orton LM, Burke SV, Duvall MR. 2019. Plastome phylogenomics and characterization of rare genomic changes as taxonomic markers in plastome groups 1 and 2 Pooeae (Pooideae; Poaceae). *PeerJ* 7: e6959.
- Pimentel M, Escudero M, Sahuquillo E, Minaya M, Catalán P. 2017. Are diversification rates and chromosome evolution in the temperate grasses (Pooideae) associated with major environmental changes in the Oligocene-Miocene? *PeerJ* 5: e3815.
- Pischi PH, Burke SV, Bach EM, Duvall MR. 2020. Plastome phylogenomics and phylogenetic diversity of endangered and threatened grassland species (Poaceae) in a North American tallgrass prairie. *Ecology and Evolution* 10(14): 7602–7615.
- Probatova NS. 1977. De speciebus generis *Milium* L. (Poaceae) notullae systematicae. *Novosti Sistematiki Vysshchikh Rastenii* 14: 6–14.
- Probatova NS. 2000. Caryotaxonomy of the genus *Milium* L. and some related genera of grasses (Poaceae). *Komarovskie Čtenija (Vladivostok)* 46: 105–146.
- Punina EO, Nosov NN, Myakoshina YuA, Gnutikov AA, Shmakov AI, Olonova MV, Rodionov AV. 2016. New octoploid *Catabrosa* (Poaceae) species from Altai. *Kew Bulletin* 71: 35.
- Quintanar Sánchez A, Castroviejo Bolívar S, Catalán P. 2007. Phylogeny of the tribe Aveneae (Pooideae, Poaceae) inferred from plastid trnT-F and nuclear ITS sequences. *American Journal of Botany* 94(9): 1554–1569.
- Rambaut A. 2006+. FigTree, tree figure drawing tool, version 1.4.3. Institute of Evolutionary Biology. Available from <http://tree.bio.ed.ac.uk/> [accessed January 2020].
- Ree RH, Smith SA. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* 57(1): 4–14.
- Refulio Rodríguez N, Columbus JT, Gillespie LJ, Peterson PM, Soreng RJ. 2012. Molecular phylogeny of *Dissanthelium* (Poaceae: Pooideae) and its taxonomic implications. *Systematic Botany* 37(1): 122–133.
- Romero Zarco C. 2011. *Helictochloa* Romero Zarco (Poaceae), a new genus of oat grass. *Candollea* 66(1): 87–103.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542.
- Saarela JM, Bull RD, Paradis M, Ebata SN, Peterson PM, Soreng RJ, Paszko B. 2017. Molecular phylogenetics of cool-season grasses



- in the subtribes Agrostidinae, Anthoxanthinae, Aveninae, Brizinae, Calothecinae, Koeleriinae and Phalaridinae (Poaceae, Pooideae, Poeae, Poeae chloroplast group 1). *PhytoKeys* 87: 1–139.
- Saarela JM, Burke SV, Wysocki WP, Barrett MD, Clark LG, Craine JM, Peterson PM, Soreng RJ, Vorontsova MS, Duvall MR. 2018. A 250 plastome phylogeny of the grass family (Poaceae): Topological support under different data partitions. *PeerJ* 6: e4299.
- Saarela JM, Liu Q, Peterson PM, Soreng RJ, Paszko B. 2010. Phylogenetics of the grass 'Aveneae-type plastid DNA clade'. In: Seberg O, Petersen G, Barfod AS, Davis JI eds. *Diversity, Phylogeny, and Evolution in the Monocotyledons*. Aarhus: Aarhus University Press. 557–588.
- Saarela JM, Wysocki WP, Barrett cf, Soreng RJ, Davis JI, Clark LG, Kelchner SA, Pires JC, Edger PP, Mayfield-Jones DR, Duvall MR. 2015. Plastid phylogenomics of the cool-season grass subfamily: Clarification of relationships among early-diverging tribes. *AoB Plants* 2015: 1–27.
- Sandve SR, Fjellheim S. 2010. Did gene family expansions during the Eocene–Oligocene boundary climate cooling play a role in Pooideae adaptation to cool climates? *Molecular Ecology* 19: 2075–2088.
- Schaefer H, Hardy OJ, Silva L, Barraclough TG, Savolainen V. 2011. Testing Darwin's naturalization hypothesis in the Azores. *Ecology Letters* 2011(14): 389–396.
- Schneider J, Winterfeld G, Hoffmann M, Röser M. 2011. Duthieae, a new tribe of grasses (Poaceae) identified among the early diverging lineages of subfamily Pooideae: molecular phylogenetics, morphological delineation, cytogenetics and biogeography. *Systematics and Biodiversity* 9: 27–44.
- Schubert M, Grønbold L, Sandve SR, Hvidsten TR, Fjellheim S. 2019a. Evolution of cold acclimation and its role in niche transition in the temperate grass subfamily Pooideae. *Plant Physiology (Lancaster)* 180: 404–419.
- Schubert M, Marcussen T, Sanchez Meseguer A, Fjellheim S. 2019b. The grass subfamily Pooideae: Cretaceous–Paleocene origin and climate-driven Cenozoic diversification. *Global Ecology and Biogeography* 28(8): 1168–1182.
- Soreng RJ. 1990. Chloroplast-DNA phylogenetics and biogeography in a reticulating group: Study in *Poa*. *American Journal of Botany* 77(11): 1383–1400.
- Soreng RJ, Davis JI. 1998. Phylogenetics and character evolution in the grass family (Poaceae): Simultaneous analysis of morphological and chloroplast DNA restriction site character sets. *The Botanical Review (Lancaster)* 64(1): 1–85.
- Soreng RJ, Davis JI. 2000. Phylogenetic structure in Poaceae subfamily Pooideae as inferred from molecular and morphological characters: Misclassification versus reticulation. In: Jacobs SWL, Everett J eds. *Grasses: Systematics and evolution*. Melbourne: CSIRO. 61–74.
- Soreng RJ, Davis JI, Doyle JJ. 1990. A phylogenetic analysis of chloroplast DNA restriction site variation in Poaceae subfam. Pooideae. *Plant Systematics and Evolution* 172: 83–97.
- Soreng RJ, Davis JI, Voionmaa MA. 2007. A phylogenetic analysis of Poaceae tribe Poeae sensu lato based on morphological characters and sequence data from three plastid-encoded genes: Evidence for reticulation, and a new classification for the tribe. *Kew Bulletin* 62(3): 425–454.
- Soreng RJ, Gillespie LJ, Consaul LL. 2017. Taxonomy of the *Poa laxa* group, including two new taxa from Arctic Canada and Greenland, and Oregon, and a re-examination of *P.* sect. *Oreinos* (Poaceae). *Nordic Journal of Botany* 35: 513–538.
- Soreng RJ, Gillespie LJ, Koba H, Boudko E, Bull RD. 2015a. Molecular and morphological evidence for a new grass genus, *Dupontlopsis* (Poaceae tribe Poeae subtribe Poinae s.l.), endemic to alpine Japan, and implications for the reticulate origin of *Dupontia* and *Arctophila* within Poinae s.l. *Journal of Systematics and Evolution* 53(2): 138–162.
- Soreng RJ, Olonova MV, Probatova NS, Gillespie LJ. 2020. Breeding systems and phylogeny in *Poa* L., with special attention to northeast Asia: The problem of *Poa shumushuensis* Ohwi and sect. *Nivicolae* (Poaceae). *Journal of Systematics and Evolution* 58(6): 1031–1058.
- Soreng RJ, Peterson PM, Davidse G, Judziewicz EJ, Zuloaga FO, Filgueiras TS, Morrone O. 2003. Catalogue of New World Grasses (Poaceae): IV. Subfamily Pooideae. *Contributions from the United States National Herbarium* 48: 1–730.
- Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Teisher JK, Clark LG, Barberá P, Gillespie LJ, Zuloaga FO. 2017. A worldwide phylogenetic classification of the Poaceae (Gramineae) II: An update and a comparison of two 2015 classifications. *Journal of Systematics and Evolution* 55(4): 259–290.
- Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Zuloaga FO, Judziewicz EJ, Filgueiras TS, Davis JI, Morrone O. 2015b. A worldwide phylogenetic classification of the Poaceae (Gramineae). *Journal of Systematics and Evolution* 53(2): 117–137.
- Soreng RJ, Peterson PM, Zuloaga FO, Romaschenko K, Clark LG, Teisher JK, Gillespie LJ, Barberá P, Welker CAD, Kellogg EA, Li DZ, Davidse G. 2022. A worldwide phylogenetic classification of the Poaceae (Gramineae) III: An update. *Journal of Systematics and Evolution* 60: 476–521.
- Stace CA. 2020. Molecules under the microscope. *Plant Review* 2020: 1–5. <https://www.rhs.org.uk/about-the-rhs/pdfs/publications/the-plant-review/articles/molecules-under-the-microscope.pdf>
- Strömberg CAE. 2011. Evolution of grasses and grassland ecosystems. *Annual Review of Earth and Planetary Sciences* 39: 517–544.
- Takhtajan A. 1986. *Floristic regions of the world*. Berkeley, CA: University of California Press. 522.
- Tkach N, Schneider J, Döring E, Wölk A, Hochbach A, Nissen J, Winterfeld G, Meyer S, Gabriel J, Hoffmann MH, Röser M. 2020. Phylogenetic lineages and the role of hybridization as driving force of evolution in grass supertribe Pooideae. *Taxon* 69(2): 1–44.
- Tzvelev NN. 2013. Notes on some genera of the family Poaceae. *Novosti Sistematiki Vysshchikh Rastenii* 44: 26–48.
- Vivian-Smith G, Stiles EW. 1994. Dispersal of salt-marsh seeds on the feet and feathers of waterfowl. *Wetlands* 14: 316–319.
- Walker JD, Gelsman JW, Bowring SA, Babcock LE. (compilers). 2018. *Geological Times Scale v. 5.0*. Geological Society of America. <https://www.geosociety.org/documents/gsa/timescale/timescl.pdf>
- Watson L, Dallwitz MJ. 1992. *The grass genera of the world*. Cambridge: C.A.B. International. 1038.
- Wen J, Nie Z-L, Ickert-Bond SM. 2016. Intercontinental disjunctions between eastern Asia and western North America in vascular plants highlight the biogeographic importance of the Bering land bridge from late Cretaceous to Neogene. *Journal of Systematics and Evolution* 54: 469–490.
- Yu Y, Blair C, Harris AJ, He XJ. 2019. A rough guide to RASP 4.2 (07/24/2019). Available from <https://sourceforge.net/projects/rasp2/> [accessed April 2021].

## Supplementary Material

The following supplementary material is available online for this article at <http://onlinelibrary.wiley.com/doi/10.1111/jse.12811/supinfo>:

**Fig. S1.** Reconstructed Area States from the RASP BBM MCMC analysis plotted on the PPAM Bayesian consensus tree. Color-coded areas of origin on circles/wheels represent the most likely state at each node, with node numbers as in Table S2. The Areas Color Legend covers all discovered combinations. Areas with probabilities less than 5% are coded as black\*.

**Table S1.** PPAM species exemplar, laboratory sequence number, and vouchers, with coded areas, A through L as explained in Table 3, identified for each taxon in the RASP BBM MCMC analysis.

**Table S2.** Part A: Node numbers and data table from RASP BBM MCMC biogeographic analysis of the PPAM clade. Dispersal, vicariance, and extinction events calculated for each node and event routes for transitions to and from each node along with the overall probabilities for those routes, and age and classification. Areas codes A through L are explained in Table 3. Event Route codes are: “->” = route step; “^” separates different areas or combinations of areas; “|” separates states at child nodes. Part B: Node numbers and detailed data table from RASP BBM MCMC biogeographic analysis of the PPAM clade. The Ancestral Area(s) and Child Nodes probability(s) including all areas > 5%, Child Taxon (or Taxa), in addition to dispersal, vicariance, and extinction events calculated for each node, along with event routes for transitions to and from each node and probabilities for those routes. Area codes A through L are explained in Table 3. Event Route codes are: “->” = route step; “^” separates different areas of combinations of areas; and “|” separates states at child nodes.

**Table S3.** Dispersal and Speciation data tables from RASP BBM MCMC analysis (see Fig. 4 in text). Area codes A through L are explained in Table 3. Dispersal directions are: “->”. The number and direction of dispersals are recorded and mapped in Fig. 4 only if the calculated value is 1 or greater (bold).

**Appendix I.** PPAM and outgroup taxa with GenBank accession numbers used in this study. Taxon, subtribe, country of origin, voucher information (and literature reference for plastome sequences), GenBank accession numbers (new data start with OK) ordered *trnT-trnL-trnF*, *matK*, and *trnC-rpoB*, or a single number for plastome sequences. En-dashes (–) indicate missing sequences.

**INGROUP. Poeae cp clade 1, PPAM clade.** *Alopecurus aequalis* Sobol, Alopecurinae, Argentina, Peterson et al. 1715 (US), KM524037, KM523821, KM523929. *Alopecurus arundinaceus* Poir., Alopecurinae, Turkey, Gillespie et al. 10562 (CAN), KM524038, KM523822, KM523930. *Alopecurus carolinianus* Walter, Alopecurinae, USA, Oklahoma, Nighswongen 4078 (NY), OK247924, OK247781, OK247853. *Alopecurus creticus* Trin., Alopecurinae, Crete, Turland 1496 (MO), OK247925, OK247782, OK247854. *Alopecurus cucullatus* (L.) Raspail (syn. *Cornucopiae cucullatum* L.), Alopecurinae, Turkey, Soreng 9144 (US), OK247947, OK247806, OK247878. *Alopecurus dasyanthus* Trautv., Alopecurinae, Georgia, Shetekavri 1115 (NY), OK247926, OK247783, OK247855. *Alopecurus davisii* Bor,

Alopecurinae, Turkey, Gillespie et al. 10459-1 (CAN), KM524039, KM523823, KM523931. *Alopecurus gerardi* (All.) Vill., Alopecurinae, Greece, Soreng et al. 7498 (US), KY363366, KY363375, KY363379. *Alopecurus glacialis* K. Koch, Alopecurinae, Georgia, Abdaladze et al. 429 (MO), KM524041, KM523825.2, KM523933. *Alopecurus goekyigitiana* Cabi & Soreng, Alopecurinae, Turkey, Soreng et al. 8856 (US), KY363368, KY363377, KY363381. *Alopecurus hitchcockii* Parodi, Alopecurinae, Peru, Peterson et al. 16256 (US), KM524043, KM523827, KM523935. *Alopecurus japonicus* Steud., Alopecurinae, Japan, Tsugaru et al. 1495 (MO), KM524044, KM523828, KM523936. *Alopecurus lanatus* Sibth. & Sm., Alopecurinae, Turkey, Gillespie et al. 10408-1 (CAN), KM524045, KM523829, KM523937. *Alopecurus magellanicus* Lam. subsp. *borealis* ined., Alopecurinae, Canada, Alberta, Breitung 15736 (US), KM524042, KM523826, KM523934. *Alopecurus magellanicus* Lam. subsp. *glaucus* (Less.) Valdés & H. Scholz, Alopecurinae, USA, Alaska, Hultén s.n. (US 2523446), OK247927, OK247784, OK247856. *Alopecurus magellanicus* Lam. subsp. *magellanicus*, Alopecurinae, Argentina, Peterson et al. 17217 (US), KM524046, KM523830, KM523938. *Alopecurus myosuroides* Huds. var. *myosuroides*, Alopecurinae, Turkey, Gillespie et al. 10369 (CAN), KM524047, KM523831, KM523939. *Alopecurus myosuroides* Huds. var. *tonsus* (Blanche ex Boiss.) R.R. Mill, Alopecurinae, Turkey, Gillespie et al. 10417 (CAN), OK247928, OK247785, OK247857. *Alopecurus ponticus* K. Koch, Alopecurinae, Russia, Kabardino-Balkariya, Soreng et al. 7961 (US), KM524049, KM523833, KM523941. *Alopecurus pratensis* L., Alopecurinae, Canada, British Columbia, Saarela & Sears 535 (CAN), KM524050, KM523834, KM523942. *Alopecurus rendlei* Eig, Alopecurinae, Turkey, Soreng & Kaya 9278 (US), OK247929, OK247786, OK247858. *Alopecurus saccatus* Vasey, Alopecurinae, USA, California, Howell 23254 (US), KM524051, KM523835, KM523943. *Alopecurus stejnegeri* Vasey, Alopecurinae, USA, Alaska, Sladen 225 (US), KM524052, KM523836, KM523944. *Alopecurus textilis* Boiss. subsp. *textilis*, Alopecurinae, Russia, Kabardino-Balkariya, Soreng et al. 7962b (US), OK247930, OK247787, OK247859. *Alopecurus tiftsiensis* (G. Westb.) Grossh., Alopecurinae, Georgia, Atha 4379 (NY), OK247931, OK247788, OK247860. *Alopecurus vaginatus* (Willd.) Pall. ex Kunth, Alopecurinae, Turkey, Gillespie et al. 10588-1 (CAN), KM524054, KM523838, KM523946. *Aniselytron treutleri* (Kunze) Soják, Cinninae, China, Yunnan, Soreng et al. 5229 (US), GQ324395, KM523839, KM523947. *Apera baytopiana* Doğan, Ventenatinae, Turkey, Gillespie et al. 10544-1 (CAN), KM524055, KM523840, KM523949. *Apera intermedia* Hack., Ventenatinae, Turkey, Gillespie et al. 10312 (CAN), KM524056, KM523841, KM523950. *Apera interrupta* (L.) P. Beauv., Ventenatinae, Argentina, Peterson et al. 19173 (US), EU792439, KM523842, KM523951. *Apera spica-venti* (L.) P. Beauv., Ventenatinae, Turkey, Soreng et al. 8807 (US), OK247932, OK247789, OK247861. *Apera triaristata* Doğan, Ventenatinae, Turkey, Gillespie et al. 10536 (CAN), KM524057, KM523843, KM523952. *Arctagrostis latifolia* (R. Br.) Griseb. subsp. *arundinacea* (Trin.) Tzvelev, Hookerochloinae, Russia, Magadan, Soreng & Olonova FE609 (US), OK247933, OK247790, OK247862. *Arctagrostis latifolia* (R. Br.) Griseb. subsp. *latifolia*, Hookerochloinae, Canada, Nunavut, Gillespie et al. 6586 (CAN), DQ353969, KM523924, KM523954. *Arctohyalopoa lanatiflora* (Roshev.) Röser & Tkach, Dupontinae, Russia, Yakutia, Kildyushevskiy 18/1 (LE), LR606633 (LF),

- LR606833, -. *Arctophila fulva* (Trin.) Rupr., Dupontiinae, Canada, Nunavut, Gillespie et al. 8441 (CAN), KM524058, KM523846, KM523956. *Arctopoa alexeji* (Sofieva & Vorosch.) Prob., Poinae x Cinninae, Russia, Sakhalin, Soreng & Olonova FE183 (US), OK247934, OK247791, OK247863. *Arctopoa eminens* (J. Presl) Prob., Poinae x Cinninae, Canada, Labrador, Gillespie 7010-2 (CAN), EU792446, KM523848, KM523958. *Arctopoa subfastigiata* (Trin.) Prob., Poinae x Cinninae, Mongolia, W6 18199, W94096 (GRIN ID#), EU792448, KM523849, KM523959. *Arctopoa tibetica* (Stapf) Prob., Poinae x Cinninae, Kyrgyz Republic, Soreng et al. 7626 (US), EU792445, KM523850, KM523960. *Avenula pubescens* (Huds.) Dumort., Avenulinae, Russia, Karachyevo-Cherkessiya, Soreng 7952 (US), OK247935, OK247792, OK247864. *Beckmannia eruciformis* (L.) Host, Beckmanniinae, Turkey, Soreng 9279 (US), OK247936, OK247793, OK247865. *Beckmannia syzigachne* (Steud.) Fernald, Beckmanniinae, Canada, Manitoba, Saarela 984 (CAN), OK247937, OK247794, OK247866. *Bellardiachloa argaea* (Boiss. & Balansa) R.R. Mill, Ventenatinae, Turkey, Soreng et al. 8132 (US), OK247938, OK247795, OK247867. *Bellardiachloa carica* R.R. Mill, Ventenatinae, Turkey, Gillespie et al. 10594-1 (CAN), KM524060, KM523851, KM523961. *Bellardiachloa doganiana* Cabi & Soreng, Ventenatinae, Turkey, Soreng et al. 8861 (US), OK247939, OK247796, OK247868. *Bellardiachloa polychroa* (Trautv.) Roshev., Ventenatinae, Turkey, Soreng & Cabi 8994 (US), OK247940, OK247797, OK247869. *Bellardiachloa variegata* (Lam.) Kerguelen, Ventenatinae, Yugoslavia, USDA PIS 253455, DQ353970, OK247798, OK247870. *Brizochloa humilis* (M. Bieb.) Chrték & Hadač, Brizochloinae, Turkey, Soreng & Cabi 8800 (US) (grown from seed), OK247941, OK247799, OK247871. *Catabrosa aquatica* (L.) P. Beauv., Coleanthinae, Turkey, Gillespie et al. 10624 (CAN), KM524061, KM523853, KM523963.2. *Catabrosella parviflora* (Boiss. & Buhse) E.B. Alexeev ex R.R. Mill subsp. *calvertii* (Boiss.) E.B. Alexeev ex R.R. Mill, Coleanthinae, Turkey, Soreng & Cabi 9000 (US) (grown from seed), OK247942, OK247801, OK247873. *Catabrosella variegata* (Boiss.) Tzvelev subsp. *variegata*, Coleanthinae, Russia, Karachyevo-Cherkessiya, Soreng 8013a (US), OK247943, OK247802, OK247874. *×Catanellia werdermannii* (Pilg.) L.J. Gillespie & Soreng (syn. *Catabrosa werdermannii* (Pilg.) Nicora & Rúgolo), Coleanthinae, Argentina, Peterson et al. 19371 (US), EU792431, OK247800, OK247872. *Cinna arundinacea* L., Cinninae, USA, West Virginia, Soreng & Olonova 7462-2 (US), EU792436, MW251089, MW251062. *Cinna bolanderi* Scribn., Cinninae, USA, California, Keil 19682 (UC), MW274723, MW251091, MW251064. *Cinna latifolia* (Trevir. ex Göpp.) Griseb., Cinninae, USA, California, Peterson et al. 19769 (US), GQ324396, KM523855, KM523965. *Cinna poiformis* (Kunth.) Scribn. & Merr., Cinninae, Peru, Peterson 20588 (US), MW274724, MW251092, MW251065. *Coleanthus subtilis* (Tratt.) Seidel, Coleanthinae, Czech Republic, Bures s.n. (US), OK247944, OK247803, OK247875. *Colpodium chionogeiton* (Pilg.) Tzvelev, Coleanthinae, Tanzania, Peterson et al. 24369 (US), OK247945, OK247804, OK247876. *Colpodium trichopodium* (Boiss.) Röser & Tkach, Coleanthinae, Turkey, Soreng et al. 8840 (US), OK247946, OK247805, OK247877. *Colpodium versicolor* (Steven) Schmalh., Coleanthinae, Russia, Karachyevo-Cherkessiya, Soreng et al. 8012 (US), KM524063, KM523856, KM523966. *Cyathopus sikkimensis* Stapf, Cinninae, China, Soreng 5224b (US), MW274725, MW251093, MW251066. *Dupontia fisheri* R. Br., Dupontiinae, Canada, Nunavut, Gillespie et al. 6589 (CAN), DQ353967.2, KC474675, KM523969. *Dupontiopsis hayachinensis* (Koidz.) Soreng, L.J. Gillespie & Koba, Dupontiinae, Japan, Koba 152 (US), KM524066, KM523861, KM523973. *Gaudinopsis macra* (Steven ex M. Bieb.) Eig, Ventenatinae, Turkey, Gillespie et al. 10634 (CAN), KM524068, KM523863, KM523976. *Hookerochloa eriopoda* (Vickery) S.W.L. Jacobs, Hookerochloinae, Australia, Jacobs 9128 (NSW), EU792433, KM523866, KM523979. *Hookerochloa hookeriana* (F. Muell. ex Hook.f.) E.B. Alexeev, Hookerochloinae, Australia, Gillespie et al. 7352 (CAN), KM524069, KM523867, KM523980. *Hyalopoa nutans* (Stapf) E.B. Alexeev ex Cope, Coleanthinae, India, Kashmir, Stewart 6888 (US), OK247949, OK247810, OK247882. *Hyalopoa pontica* (Balansa) Tzvelev, Coleanthinae, Russia, Karachyevo-Cherkessiya, Soreng 8014 (US), KM524070, KM523868, KM523981. *Hyalopodium araraticum* (Lipsky) Röser & Tkach, Coleanthinae, Turkey, Soreng & Cabi 8990 (US), OK247950, OK247811, OK247883. *Hyalopodium colchicum* (Albov) L.J. Gillespie & Soreng (syn. *Paracolpodium colchicum* (Albov) Tzvelev), Coleanthinae, Russia, Kabardino-Balkariya, Soreng 7969 (US), OK247954, OK247816, OK247887. *Limnas malyshevii* O.D. Nikif., Alopecurinae, Russia, Krasnoyarsk, Andrulajitis 1204 (NS/NSK), LR606726 (LF), LR606924, -. *Limnas stelleri* Trin., Alopecurinae, Russia, Yakutia, Vodopyanova et al. 813 (NS/NSK), LR606727 (LF), LR606925, -. *Milium effusum* L., Miliinae, 1) Russia, Primorsky, Soreng & Olonova FE018 (US), OK247951, OK247812, OK247884; 2) Sweden, Soreng & Soreng 7771 (US), KM524072, KM523870, KM523983. *Milium schmidtianum* K. Koch, Miliinae, Russia, Karachyevo-Cherkessiya, Soreng 8019 (US), KM524073, KM523871, KM523984. *Milium transcasicum* Tzvelev, Miliinae, Turkey, Soreng & Cabi 8967a (US), OK247952, OK247813, OK247885. *Milium vernale* M. Bieb., Miliinae, Turkey, Gillespie et al. 10445 (CAN), KM524074, KM523872.2, KM523985. *Nephelochloa orientalis* Boiss., Ventenatinae, Turkey, Gillespie et al. 10514-2 (CAN), KM524075, KM523873, KM523986. *Nicoraepoa andina* (Trin.) Soreng & L.J. Gillespie, Hookerochloinae, Chile, Soreng & Soreng 7182 (US), DQ353971, KM523874, KM523987. *Nicoraepoa chonotica* (Phil.) Soreng & L.J. Gillespie, Hookerochloinae, Chile, Soreng & Soreng 7309 (US), DQ353974, KM523875, KM523988. *Nicoraepoa pugionifolia* (Speg.) Soreng & L.J. Gillespie, Hookerochloinae, Chile, Soreng & Soreng 7336 (US), DQ353973, OK247815, OK247886. *Nicoraepoa robusta* (Steud.) Soreng & L.J. Gillespie, Hookerochloinae, Chile, Soreng & Soreng 7358 (US), DQ353975.2, KM523876, KM523989. *Nicoraepoa subenervis* (Hack.) Soreng & L.J. Gillespie subsp. *spgazziniana* (Nicora) Soreng & L.J. Gillespie, Hookerochloinae, Argentina, Peterson et al. 19186 (US), EU792443, KM523877, KM523990. *Nicoraepoa subenervis* (Hack.) Soreng & L.J. Gillespie subsp. *subenervis*, Hookerochloinae, Chile, Soreng & Soreng 7334 (US), DQ353972, MW251099, MW251072. *Paracolpodium altaicum* (Trin.) Tzvelev, Coleanthinae, Russia, Altai, Olonova 06-20 (CAN), KM524076, KM523878, KM523991. *Parvotrisetum myrianthum* (Bertol.) Chrték, Ventenatinae, Turkey, Soreng 9258 (US), OK247955, OK247817, OK247888. *Phippsia algida* (Sol.) R. Br., Coleanthinae, USA, Alaska, Soreng 6199 (US), OK247956, OK247818, OK247889. *Phippsia concinna* (Th. Fr.) Lindeb., Coleanthinae, Svalbard, Eidesen PC2-1 (CAN), OK247957, OK247819, OK247890. *Phleum alpinum* L., Phleinae, USA, Alaska, Parker 14128 (MO), OK247958, OK247820, OK247891.

- Phleum exaratum** Hochst. ex Griseb., Phleinae, Turkey, Gillespie et al. 10451 (CAN), KM524080, KM523882, KM523995. **Phleum montanum** K. Koch, Phleinae, Turkey, Soreng 4264 (US), OK247959, OK247821, OK247892. **Phleum phleoides** (L.) Karst., Phleinae, Russia, Stavropol, Soreng 7881 (US), KM524082, KM523884, KM523997. **Phleum pratense** L. subsp. **nodosum** (L.) Dumort., Phleinae, Turkey, Gillespie et al. 10627 (CAN), OK247960, OK247822, OK247893. **Phleum pratense** L. subsp. **pratense**, Phleinae, Russia, Stavropol, Soreng et al. 7943 (US), KM524084, KM523886, KM523999. **Phleum subulatum** (Savi) Asch. & Graebn., Phleinae, Turkey, Gillespie et al. 10430 (CAN), OK247961, OK247823, OK247894. **Pholiurus pannonicus** (Host) Trin., Beckmanniinae, Romania, Paun s.n. (NY), OK247962, OK247824, OK247895. **Poa alpina** L., Poinae, USA, Colorado, Gillespie 6299 (CAN), DQ353985.2, KM523888, KM524001. **Poa arachnifera** Torrey, Poinae, USA, Oklahoma, Soreng & Soreng 5801 (US), DQ354021, KY944619, KY987041. **Poa asiae-minoris** H. Scholz & Byfield, Poinae, Turkey, Soreng et al. 8100 (US), MH921394, MH921343, MH921368. **Poa autumnalis** Elliott, Poinae, USA, Maryland, Soreng 4680 (US), DQ353979, KM523889, KM524002. **Poa badensis** Haenke ex Willd., Poinae, Bulgaria, Hajkova et al. 2004-12 (US), GQ324402, KY378861, KY378827. **Poa bulbosa** L. subsp. **vivipara** (Koeler) Arcang., Poinae, USA, Nevada (introduced), Soreng & Soreng 5814 (US), GQ324404, MH921345, MH921370. **Poa caucasica** Trin., Poinae, Russia, Kabardino-Balkariya, Soreng 7992a (US), MT273358, MT273299, MT273329. **Poa chaixii** Vill., Poinae, USA (cult., from Novgorodv, Russia), Soreng 4677 (US), EU854590, KM523890, KM524003. **Poa chapmaniana** Scribn., Poinae, USA, Virginia, Soreng 7859 (US), MW274728, MW251100, MW251073. **Poa compressa** L., Poinae, Turkey, Gillespie et al. 10338 (CAN), MT273359, MT273300, MT273330. **Poa dolosa** Boiss. & Heldr., Poinae, Greece, Soreng et al. 7495-1 (US), GQ324414, KM523891, KM524004.2. **Poa fendleriana** (Steud.) Vasey, Poinae, USA, Colorado, Gillespie 6292 (CAN), DQ354027, KY378869, KY37883. **Poa flabellata** (Lam.) Raspail, Poinae, South Georgia Islands, Wright 9NSG (not vouchered, except by seed at US), EU792453, KM523892, KM524005. **Poa infirma** Kunth, Poinae, Spain, Catalan 3-2000 (UZ), GQ324427, KY378871, KY378837. **Poa irkutica** Roshev., Poinae, Russia, Irkutsk, Kasanovskiy 2002-7 (CAN), DQ354007.2, KY378872, KY378838. **Poa jubata** A. Kern., Poinae, Turkey, Soreng et al. 9029-2 (US), KY378814, KY378873, KY378839. **Poa kerguelensis** (Hook. f.) Steud, Poinae, Subantarctic Islands, Kerguelen Islands, Hennion Gen5 (P), EU792457, MT273304, MT273334. **Poa kurtzii** R.E. Fr., Poinae, Chile, Peterson & Soreng 15654 (US), DQ354018, KM523893, KM524006. **Poa lettermanii** Vasey, Poinae, USA, Colorado, Soreng & Soreng 7434 (US), GQ324431, KY944653, KY987075. **Poa marcida** Hitchc., Poinae, USA, Oregon, Soreng & Soreng 5974 (US), DQ353978, KM523894.2, KM524007. **Poa marshallii** Tovar, Poinae, Peru, Peterson et al. 21546 (US), KM524086, KM523895, KM524008. **Poa nemoralis** L., Poinae, Turkey, Gillespie et al. 10411 (CAN), KY987110, KY944655, KY987077. **Poa occidentalis** Vasey, Poinae, Mexico, Peterson & Valdes Rena 18918 (US), KU763514.2, KY378877, KY378843. **Poa palustris** L., Poinae, Turkey, Soreng 9001 (US), MW274729, MW251103, MW251076. **Poa persica** Trin. subsp. **persica**, Poinae, Turkey, Soreng & Cabi 9215 (US), KY378816, KY378879, KY378845. **Poa planifolia** Kuntze, Poinae, Argentina, Peterson et al. 19233 (US), KM524087, KM523896, KM524009. **Poa poiformis** (Labill.) Druce, Poinae, Australia, Gillespie et al. 7381 (CAN), GQ324445, KM523897, KM524010. **Poa polycolea** Stapf, Poinae, China, Yunnan, Wen et al. (TME)1316 (US), GQ324447, MT273312, MT273342. **Poa pratensis** L. subsp. **pratensis**, Poinae, USA, Colorado, Gillespie 6291 (CAN), DQ354010, KY944659, KY987082. **Poa pseudoabbreviata** Roshev., Poinae, USA, Alaska, Soreng & Soreng 6032-1 (US), DQ353997, KY378881, KY378847. **Poa remota** Forselles, Poinae, Kyrgyz Republic, Soreng et al. 7540 (US), GQ324452, KY378883, KY378849. **Poa saltuensis** Fernald & Wiegand, Poinae, Canada, Ontario, Gillespie 7043 (CAN), EU792451, KM523899, KM524012. **Poa sibirica** Roshev. subsp. **sibirica**, Poinae, Russia, Khakassia, Olonova 2003-45 (CAN), GQ324455, KY378885, KY378851. **Poa supina** Schrad., Poinae, USA (cult., from Europe), Soreng & Cayouette 5950-2 (US), DQ353984, KY378888, KY378854. **Poa sylvestris** A. Gray, Poinae, USA, Maryland, Soreng 4678-3 (US), DQ353980.2, MT273321, MT273351. **Poa thessala** Boiss. & Orph., Poinae, Turkey, Gillespie et al. 10400 (CAN), KM524088, KM523901, KM524014. **Poa trichophylla** Heldr. & Sart. ex Boiss., Poinae, Greece, Soreng et al. 7508 (US), GQ324461, KY378889, KY378855. **Poa trivialis** L. subsp. **trivialis**, Poinae, USA, Maryland (introduced), Soreng 4681-1 (US), GQ324462, KY378891, KY378857. **Poa ursina** Velen., Poinae, Bulgaria, Hajkova et al. 2004-4 (CAN), KM524089, KM523902, KM524015. **Poa wolfii** Scribn., Poinae, USA, Missouri, Soreng & Soreng 5800 (US), AH015556.2, KY378893, KY378859. **Pseudophleum gibbum** (Boiss.) Doğan, Beckmanniinae, Turkey, Gillespie et al. 10510 (CAN), OK247964, OK247826, OK247897. **Puccinellia andersonii** Swallen, Coleanthinae, Canada, Northwest Territories, Gillespie et al. 9656 (CAN), OK247965, OK247827, OK247898. **Puccinellia angustata** (R. Br.) E.L. Rand & Redfield, Coleanthinae, Canada, Northwest Territories, Consaul et al. 2849-1 (MTMG), OK247966, OK247828, OK247899. **Puccinellia arctica** (Hook.) Fernald & Weath., Coleanthinae, Canada, Nunavut, Gillespie & Consaul 6342 (CAN), KM524090, KM523903, KM524016. **Puccinellia argentinensis** (Hack.) Parodi, Coleanthinae, Argentina, Barros 041 (SI), OK247967, OK247829, OK247900. **Puccinellia banksiensis** Consaul, Coleanthinae, Canada, Nunavut, Gillespie et al. 8055 (CAN), OK247968, OK247830, OK247901. **Puccinellia biflora** (Steud.) Parodi, Coleanthinae, Chile, Soreng 7151 (US), OK247969, OK247831, OK247902. **Puccinellia borealis** Swallen, Coleanthinae, USA, Alaska, Soreng 6154-1 (US), OK247970, OK247832, OK247903. **Puccinellia bruggemannii** T.J. Sørensen, Coleanthinae, Canada, Northwest Territories, Consaul & Archambault 3144-7 (CAN), KM524092.2, KM523905, KM524018. **Puccinellia convoluta** (Hornem.) Fourr., Coleanthinae, Turkey, Gillespie et al. 10435 (CAN), OK247971, OK247833, OK247904. **Puccinellia deschampsoides** T.J. Sørensen, Coleanthinae, Canada, Yukon, Elven SUP03-101/1-3 (CAN), KM524093, KM523906, KM524019. **Puccinellia distans** (Jacq.) Parl., Coleanthinae, Turkey, Soreng 4085 (US), OK247972, OK247834, OK247905. **Puccinellia festuciformis** (Host) Parl., Coleanthinae, Spain, Acedo & Llamas 275 (LEB), OK247973, OK247835, OK247906. **Puccinellia frigida** I.M. Johnst., Coleanthinae, Chile, Peterson et al. 15488 (US), OK247974, OK247836, OK247907. **Puccinellia glauca** (Regel) V.I. Krecz., Coleanthinae, Kyrgyz Republic, Soreng 7536 (US), OK247975, OK247837, OK247908. **Puccinellia glaucescens** (Phil.) Parodi, Coleanthinae, Chile, Soreng & Soreng 7152 (US),

- DQ353960.2, KM523907, KM524020.2. *Puccinellia groenlandica* T.J. Sørensen, Coleanthinae, Greenland, *Legaard* 540 (CAN), OK247976, OK247838, OK247909. *Puccinellia grossheimiana* V.I. Krecz., Coleanthinae, Turkey, Soreng et al. 4103 (US), OK247977, OK247839, OK247910. *Puccinellia hauptiana* (Trin. ex V.I. Krecz.) Kitag., Coleanthinae, Russia, Siberia, *Olonova* 08-13 (CAN), OK247978, OK247840, OK247911. *Puccinellia intermedia* (Schur) Janch., Coleanthinae, Turkey, *Gillespie* et al. 10309 (CAN), OK247979, OK247841, OK247912. *Puccinellia lemmonii* (Vasey) Scribn., Coleanthinae, USA, Nevada, *Davis* 414 (BH), KM524095, KM523909, KM524022. *Puccinellia magellanica* (Hook. f.) Parodi, Coleanthinae, Chile, Soreng 7361 (US), KM524096, KM523910, KM524023. *Puccinellia maritima* (Huds.) Parl., Coleanthinae, Spain, *Acedo & Llamas* 269 (LEB), OK247980, OK247842, OK247913. *Puccinellia nipponica* Ohwi, Coleanthinae, Russia, Primorsky, Soreng & *Olonova* FE089 (US), OK247981, OK247843, OK247914. *Puccinellia nutkaensis* (J. Presl) Fernald & Weath., Coleanthinae, USA, Alaska, Soreng & Soreng 6322 (US), KM524097, KM523911, KM524024. *Puccinellia nuttalliana* (Schult.) Hitchc., Coleanthinae, Canada, Nunavut, *Gillespie* 6327 (CAN), OK247982, OK247844, OK247915. *Puccinellia parishii* Hitchc., Coleanthinae, USA, New Mexico, *Davis & Manos* 568 (BH), OK247983, OK247845, OK247916. *Puccinellia phryganodes* (Trin.) Scribn. & Merr., Coleanthinae, Canada, Northwest Territories, *Gillespie et al.* 9940 (CAN), KM524098, KC475593, KM524025.2. *Puccinellia pumila* (Vasey) Hitchc., Coleanthinae, Canada, Labrador, *Consaul* 2563-26 (CAN), OK247984, OK247846, OK247917. *Puccinellia pusilla* (Hack.) Parodi, Coleanthinae, Chile, Soreng 7331b (US), OK247985, OK247847, OK247918. *Puccinellia rupestris* (With.) Fernald & Weath., Coleanthinae, USA, Ohio (introduced), *Thompson* 07-199 (US), OK247986, OK247848, OK247919. *Puccinellia skottsbergii* (Pilg.) Parodi, Coleanthinae, Chile, Soreng 7360 (US), KM524099, KM523912, KM524026. *Puccinellia stricta* (Hook. f.) Blom, Coleanthinae, Australia, *Peterson et al.* 14544 (US), DQ353959.2, KM523913, KM524027. *Puccinellia tenella* (Lange) Holmb. subsp. *langeana* (Berlin) Tzvelev, Coleanthinae, USA, Alaska, Soreng 6206-1 (US), KM524100.2, KM523914, KM524028. *Puccinellia tenuifolia* (Boiss. & Reut.) H. Lindb., Coleanthinae, Spain, Soreng 3580 (US), OK247987, OK247849, OK247920. *Puccinellia vaginata* (Lange) Fernald & Weath., Coleanthinae, Greenland, *Porsild, M.P.* s.n. (CAN 315828), OK247988, OK247850, OK247921. *Puccinellia vahliana* (Leibm.) Scribn., Coleanthinae, Canada, Nunavut, *Gillespie* 5808 (CAN), EU854591, KM523915, KM524029. *Puccinellia wrightii* (Scribn. & Merr.) Tzvelev, Coleanthinae, USA, Alaska, *Elven SUP03-9-1* (O), KM524101, KM523916, KM524030. *Rhizocephalus orientalis* Boiss., Beckmanniinae, Turkey, Soreng 9254 (US), OK247989, OK247851, OK247922. *Saxipoa saxicola* (R. Br.) Soreng, L.J. Gillespie, & S.W.L. Jacobs, Hookerochloinae, Australia, *Gillespie et al.* 7353-4 (CAN), GQ324463, KM523917, KM524031. *Sclerochloa dura* (L.) P. Beauv., Coleanthinae, USA, Maryland, Soreng 8072 (US), KM524102, KM523918, KM524032. *Simplicia buchananii* (Zotov) Zotov, Hookerochloinae, New Zealand, *Thorsen* 106/09 (AK), MW274730, MW251106, MW251079. *Simplicia felix* de Lange, J.R. Rolfe, Smissen & Ogle, Hookerochloinae, New Zealand, *de Lange* 5897 (AK), MW274732, MW251108, MW251081. *Simplicia laxa* Kirk, Hookerochloinae, New Zealand, *de Lange* 7859 (AK), MW274735, MW251110, MW251083. *Sylvipoa queenslandica* (C.E. Hubb.) Soreng, L.J. Gillespie, & S.W.L. Jacobs, Hookerochloinae, Australia, *Gillespie et al.* 7320 (CAN), GQ324466, KM523921, KM524035. *Ventenata dubia* (Leers) Coss., Ventenatinae, Turkey, *Gillespie et al.* 10636-1 (CAN), KM524104, KM523922, KM524036. *Ventenata eigiana* (H. Scholz & Raus) Doğan (syn. *Pilgerochloa eigiana* H. Scholz & Raus), Ventenatinae, Turkey, *Behçet* 9821 (NAKU), OK247963, OK247825, OK247896.
- OUTGROUPS.** *Agrostis mertensii* Trin., Poaeae cp clade 1, Agrostidinae, USA, New Hampshire, *Gillespie* 6802 (CAN), DQ353956, KM523820, KM523928. *Anthoxanthum monticola* (Bigelow) Veldkamp subsp. *alpinum* (Sw. ex Willd.) Soreng, Poaeae cp clade 1, Phalaridinae, Canada, Northwest Territories, *Gillespie & Consaul* 6859 (CAN), DQ353953, KM523923, KM523948. *Avena sativa* L., Poaeae cp clade 1, Aveninae, Canada, British Columbia, *Saarela & Percy* 775 (CAN), *Saarela et al.* 2015, NC\_027468 (plastome). *Avenella flexuosa* (L.) Drejer., Poaeae cp clade 2, Airinae, unknown, *Hill* 29437 (ILLS), *Pischl et al.* 2020, MN944893 (plastome). *Brachypodium distachyon* (L.) Roem. & Schult., Brachypodieae, unknown, *Sancho et al.* unpubl., KU170609 (plastome). *Brachypodium sylvaticum* (Huds.) P. Beauv., Brachypodieae, unknown, *MatK: Hilu et al.* 1999, TLF: *Scharidl et al.* 2008, EU119362, AF164400, -. *Briza maxima* L., Poaeae cp clade 1, Brizinae, Canada, Alberta, *Saarela* 284 (CAN), *Saarela et al.* 2015, NC\_027471 (plastome). *Bromus vulgaris* (Hook.) Shear, Triticodae, Bromaeae, Canada, British Columbia, *Saarela et al.* 822 (CAN), *Saarela et al.* 2015, NC\_027472 (plastome). *Calamagrostis breviligulata* (Fernald) *Saarela*, Poaeae cp clade 1, Agrostidinae, USA, New York, *Peterson & Saarela* 20867 (CAN), *Saarela et al.* 2015, NC\_027465 (plastome). *Dactylis glomerata* L. subsp. *hispanica* Roth, Poaeae cp clade 2, Dactylidinae, Spain, Soreng 3682 (US), DQ353961, KM523857, KM523967. *Deschampsia cespitosa* (L.) P. Beauv. subsp. *cespitosa*, Poaeae cp clade 2, Airinae, Russia, Karacheyevo-Cherkessiya, Soreng et al. 8028 (US), OK247948, OK247807, OK247879. *Deschampsia cespitosa* (L.) P. Beauv. subsp. *septentrionalis* Chiapella, Poaeae cp clade 2, Airinae, Canada, Northwest Territories, *Gillespie & Consaul* 6810b (CAN), DQ353962.2, KM523858, KM523968. *Dichelachne crinita* (L.f.) Hook.f., Poaeae cp clade 1, Echinopogoninae, unknown, *W6* 22474, *Orton et al.* 2019, MH569080 (plastome). *Drymochloa sylvatica* (Pollich) Holub, Poaeae cp clade 2, Loliinae, unknown, IBERS BS4384, *Hand et al.* 2013, NC\_019648 (plastome). *Dryopoa dives* (F. Muell.) *Vickery*, Poaeae cp clade 2, Loliinae, Australia, *Jacobs* 9239 (NSW), EU792430, OK247808, OK247880. *Festuca baffinensis* Polunin, Poaeae cp clade 2, Loliinae, Canada, Northwest Territories, *Gillespie & Consaul* 6920 (CAN), AH015554.2, KC474800, KM523975. *Festuca ovina* L., Poaeae cp clade 2, Loliinae, unknown, IBERS BL2643, *Hand et al.* 2013, NC\_019649 (plastome). *Helictochloa aetolica* (Rech.f.) *Romero Zarco*, Poaeae cp clade 2, Airinae, Greece, Soreng et al. 7514 (US), EU792437, KM523864, KM523977. *Helictotrichon convolutum* (C. Presl) *Henrard*, Poaeae cp clade 1, Aveninae, Greece, Soreng 3803 (US), DQ353954, KM523865, KM523978. *Helictotrichon sempervirens* (Vill.) *Pilg.*, Poaeae cp clade 1, Aveninae, USA, New York (cult.), Soreng 4622 (US), DQ353955, OK247809, OK247881. *Holcus lanatus* L., Poaeae cp clade 2, Holcinae, unknown, USDA PI 659841, *Gallaher et al.* unpublished, NC\_036689 (plastome). *Hordeum jubatum* L.,

Triticodae, Triticeae, Canada, Yukon, Peterson et al. 18478 (CAN), Saarela et al. 2015, NC\_027476 (plastome). **Limnodea arkansana** (Nutt.) L.H. Dewar, Poaeae cp clade 1, Agrostidinae, USA, Mississippi, Soreng 7860 (US), MW274726, MW251096, MW251069. **Littledalea racemosa** Keng, Triticodae, Littledaleeae, unknown, L122, Su, unpubl., NC\_036350 (plastome). **Lolium arundinaceum** (Schreb.) Darbysh., Poaeae cp clade 2, Loliinae, unknown, Cahoon et al. 2010, NC\_011713.2 (plastome). **Lolium perenne** L., Poaeae cp clade 2, Loliinae, unknown, Diekmann et al. 2008, NC\_009950 (plastome). **Lolium pratense** (Huds.) Darbysh., Poaeae cp clade 2, Loliinae, unknown, USDA PI 310482, Hand et al. 2013, NC\_019650 (plastome). **Molineriella laevis** (Brot.) Rovy, Poaeae cp clade 2, Airinae, Spain, Soreng 3740a (US), OK247953, OK247814, -. **Phalaris arundinacea** L., Poaeae cp clade 1, Phalaridinae, Canada, British Colombia, Saarela et al. 973 (CAN), Saarela et al. 2015, NC\_027481 (plastome). **Scolochloa festuacea** (Willd.) Link, Poaeae cp clade 2, Scolochloinae, Sweden (cult.), Hjertson 381 (UPS V237224), KM524103, KM523919, KM524033. **Secale cereale** L., Triticodae, Triticeae, unknown, Middleton et al. 2014, NC\_021761 (plastome). **Sesleria insularis** Sommier, Poaeae cp clade 2, Sesleriinae, Yugoslavia, Soreng 3889 (BH) (=USDA 253719), DQ353957.2, KM523920, KM524034. **Sesleria tenerrima** (Fritsch.) Hayek, Poaeae cp clade 2, Sesleriinae, Greece, Soreng et al. 7501-1 (US), OK247990, OK247852, OK247923. **Torreyochloa pallida** (Torr.) G.L. Church, Poaeae cp clade 1, Torreyochloinae, Canada, British Colombia, Saarela & Percy 1110 (CAN), Saarela et al. 2015, NC\_027486 (plastome). **Trisetum cernuum** Trin. subsp. **canescens** (Buckley) Calder & Roy L. Taylor (= **Grapphephorum canescens** (Buckley) Röser & Tkach), Poaeae cp clade 1, Aveninae, Canada, British Colombia, Saarela et al. 876 (CAN), Saarela et al. 2015, NC\_027487 (plastome). **Triticum aestivum** L., Triticodae, Triticeae, unknown, Ogihara et al. 2002, AB042240.3 (plastome).