Phylogenetic Analysis of Subtribe Alopecurinae *sensu lato* (Poaceae)

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Table of Contents

Abstract

Subtribe Alopecurinae (Poeae, Poaceae) *sensu lato*'s seven genera share interesting morphological similarities (dense spicate panicles and one-flowered spikelets) that were widely thought to have a common origin. However, recent molecular evidence for three of the genera has suggested that the subtribe may be polyphyletic. To test this, five DNA regions were sequenced and analyzed using phylogenetic methods. Results confirm that Alopecurinae s.l. as presently treated is polyphyletic and should be dissolved. Additionally, the genus *Cornucopiae* may be just another *Alopecurus*. *Limnas* and *Pseudophleum* are not closely allied to *Alopecurus* or each other, and are even further from *Phleum*. *Phleum* is a distinct lineage that is not closely allied to any other included Alopecurinae genus. Evidence for revising infrageneric classifications of *Alopecurus* and *Phleum* is presented, as is evidence for separating *A. magellanicus* into two or more subspecies.

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List of Tables

List of Figures

List of Appendices

INTRODUCTION

The grass family (Poaceae Barnhart) is the second largest monocot family and the fifth largest plant family encompassing approximately 10 000 species and 700 genera (Clayton and Renvoize, 1986; Caetano-Anolles, 2005; Perreta *et al*., 2009). These genera have further been combined into subtribes, tribes, supertribes, and subfamilies, all varying widely in their morphology and physiology but sharing a distinctive inflorescence structure called the spikelet (Kellogg, 2001; Perreta *et al*., 2009). The fruiting structure (known as the grain or caryopsis) that grasses produce is unique amongst angiosperms and has great agricultural value (Kellogg, 2001; Caetano-Anolles, 2005). Members of this family also occupy a very wide range of ecological niches worldwide and are present on all continents including Antarctica (Souto *et al*., 2006). Despite their potential for use in urban landscaping, as food or as fodder, grasses can also be quite pervasive weeds, generating resistance to widely used agricultural herbicides (Mohamed *et al*., 2012). It is therefore important to gain insight into the various characteristics of and relationships in this family in order to be able to make informed taxonomic decisions and improve natural resource management planning.

Poaceae is composed of 12 separate subfamilies, of which Pooideae Benth. is the largest having 13-14 tribes and one third of all grass species circumscribed within it (Clayton & Renvoize, 1986; Soreng & Davis, 1998; GPWG, 2001). Poeae R.Br. is the largest tribe within Pooideae and contains 22 subtribes according to recent classifications (Soreng *et al*., 2007; Quintanar *et al*., 2007; Gillespie *et al*., 2008). Recent molecular studies include a well supported major clade within this tribe: PPAM (subtribes Puccinelliinae, Poinae, Alopecurinae, Miliinae; Soreng *et al*., 2007). In studies that focus on PPAM, the PAM (Poinae, Alopecurinae, Miliinae) clade is well supported (Quintanar *et al*., 2007; Gillespie *et al*., 2010). Additionally, subtribe

Puccinellinae and PAM usually have strong support as sister clades, while Alopecurinae and Poinae genera are intermixed within PAM (Doring *et al*., 2007; Gillespie *et al*., 2007; Soreng *et al*., 2007; Quintanar *et al*., 2007; Gillespie *et al*., 2008; Gillespie *et al*., 2010). The genus *Cinna* L. (previously subtribe Cinninae) is now also placed in Poinae (Soreng *et al*., 2003; Gillespie *et al*., 2008).

The genus *Limnodea* Dewey has endured a long standing disagreement as to its placement. It is briefly investigated in this thesis in order to finally determine whether it is a part of the PPAM clade (Gillespie *et al*., 2008) or not. *Limnodea* contains one species that is distributed throughout southern United States and northern Mexico. Clayton and Renvoize (1986) placed it near *Cinna* L. which is now known to be in PPAM, while Soreng *et al* (2003) recognized it in subtribe Aveninae, as a member of an informal group with an Aveneae chloroplast type.

Subtribe Alopecurinae Dumort. *sensu lato*, on which the following research is based, includes approximately 60 species, which function as native pasture and cultivated fodder plants, as well as prevalent weeds. Both the distribution and diversity of subtribe Alopecurinae's currently considered seven genera (*Alopecurus* L., *Beckmannia* Host., *Cornucopiae* L., *Limnas* Trin., *Phleum* L., *Pseudophleum* Dogan, *Rhizocephalus* Boiss.) is centered on the Mediterranean, but some species can be found globally including all over Asia, Africa, Australia and North/ South America (Clayton & Renvoize, 1986). Alopecurinae genera are known for having an interesting set of morphological similarities that are not often encountered in grasses: dense spicate panicles and one-flowered spikelets (Soreng & Davis, 2000; Soreng *et al*., 2007; Gillespie *et al*., 2007, Gillespie *et al*., 2010). As a result of this morphological similarity and the absence of molecular data on the subtribe, the genera of Alopecurinae have been grouped together in the past.

Recent molecular advances have brought around many changes in the circumscription of tribe Poeae members and as a result subtribe Alopecurinae as well (Soreng *et al*., 2007). Previous classification attempts were based mostly on spikelet and floret characteristics that are now thought to not be truly representative of evolutionary relationships in the grasses (Walters and Keil, 1996). Modern classification based on molecular data is thought to better represent phylogenetic relationships, but it is unfortunately not as easy to use for identification (Walters and Keil, 1996; GPWG, 2001). In 1976, Tzvelev grouped all current Alopecurinae members into the tribe Phleeae Dumort. The genera *Alopecurus* and *Limnas* were placed in subtribe Alopecurinae, while subtribe Phleinae Benth. consisted of *Phleum*, *Pseudophleum* and *Rhizochephalus*. *Beckmannia* was treated in its own subtribe, Beckmanninae Nevski (see table 5). Clayton and Renvoize (1986) placed all current Alopecurinae s.l. members (as well as others which are now considered in different subtribes) together in Aveneae Dumort., Alopecurinae. Due to differing inflorescence types, Alopecurinae were originally thought to be distant from subtribe Poinae (Tzvelev, 1976; Clayton & Renvoize, 1986), but recent molecular data have proved otherwise (Gillespie *et al*., 2008, 2010; Schneider *et al*., 2009; Hoffmann *et al*., 2013). Now Alopecurinae s.l. is treated as a single subtribe in Poeae, Alopecurinae (Soreng *et al*, 2003; Soreng *et al*, 2007). However, recent molecular analyses including three Alopecurinae species (*Alopecurus*, *Beckmannia*, *Phleum*) have shown *Phleum* to be a distinct lineage warranting its own subtribe -Phleinae (Gillespie *et al*., 2010). Since *Pseudophleum* and *Rhizocephalus* were thought to be closely related to *Phleum* on the basis of morphological analyses (Dogan, 1982; Dogan, 1988; Dogan & Us, 1995; no molecular data available), they were also placed in subtribe

Phleinae. All other sampled Alopecurinae members (*Alopecurus*, *Beckmannia*) were shown to be intermixed with members of the closely related subtribe Poinae. Although historically treated in its own subtribe, no support from molecular data exists for separating *Beckmannia* from *Alopecurus* (Gillespie *et al*., 2010). Overall subtribe Alopecurinae is polyphyletic and its circumscription in tribe Poeae needs to be revised (Gillespie *et al*., 2010).

This thesis attempts to evaluate the placement of Alopecurinae genera in relation to each other and in relation to related subtribes. It also seeks to compare previous infrageneric groupings (based on numerical taxonomy) in the two largest genera, *Alopecurus* and *Phleum* with molecular results from the present research.

The thesis also explores the case of *Alopercurus magellanicus* Lam. (1791), commonly known as the alpine/ boreal foxtail that occurs in wet mountain slopes and meadows throughout non-tropical North and South America. It is differentiated from other *Alopecurus* species by having ovate to elliptic spikelets with 2 mm anthers (Dogan, 1999) and also by glumes that are pilose all over the outer surface (Tsvelev, 1976). Depending on the location in which it was described, *Alopecurus magellanicus* has been given many names including *A. borealis* Trin., *A. alpinus* Sm., *A. stejnegeri* Vasey, and *A. glaucus* Less. (among others). Although these are all now treated as synonyms of *A. magellanicus* (Soreng *et al*., 2003), their previously recognized subspecies have not been officially transferred to A. *magellanicus* (subsp. *glaucus*, *borealis*, *stejnegeri*) as the complex is pending further study. In 1976 Tsvelev divided *A. alpinus* (an illegitimate species name, predated by a different species *A. alpinus* Villars, 1786) into four subspecies: *stejnegeri* Vasey, *alpinus* Smith, *glaucus* Less., and *pseudobrachystachyus* Ovcz. Tszelev hypothesized that *A. alpinus* subsp. *pseudobrachystachyus* was a product of introgressive hybridization between *A. alpinus* s.l. and *A. pratensis* s.l. In 1999 Dogan divided *A.*

borealis (in which he included *A. magellanicus* as a synonym) into 2 subspecies: *borealis* and *glaucus*, but did not consider any South American samples. He separated them based on an ovoid (*borealis*) versus a broadly cylindrical (*glaucus*) panicle and an awn that is exerted up to 1 mm in *borealis* and more than 2 mm in *glaucus*. This thesis examines various samples of *Alopecurus magellanicus* from North and South America in order to determine whether infraspecific classification is warranted.

The following subsections of the introduction present an overview of the current and past classifications, along with habits and distributions of Alopecurinae s.l. genera (see Appendix A for photos). This thesis is presented as a single study with an all-encompassing methodology and results and discussion sections that are arranged in order of general to more specific issues dealing with subtribe Alopecurinae.

***Alopecurus* L.**

The largest Alopecurinae genus *Alopecurus* L. (the foxtail genus) was first described in 1753 and is known for having the characteristic appearance for this subtribe: a spike-like panicle inflorescence with one-flowered, laterally compressed spikelets (Dogan, 1999; Shenglian and Phillips, 2006). It is separated from other Alopecurinae genera by its membranous ligule, glabrous or hairy sheaths and a rachilla that disarticulates below the glumes (Dogan, 1999). It is comprised of 40-50 annual, biennial and perennial species of economically important fodder plants including many alpine species which serve as valuable pasture grasses (Dogan, 1988). For this reason many *Alopecurus* species have become naturalized in areas that they are not native to. Species of *Alopecurus* are distributed throughout most non-tropical regions of the northern and southern hemispheres (Shenglian and Phillips, 2006), and their centre of diversity lies in southwest Asia.

Many previous attempts have been made to construct infrageneric groupings for this genus (e.g. Tzvelev, 1976; Dogan, 1991) with most not being truly representative due to the selection of too few characters for comparison. The most recent was by Dogan (1997), and included a numerical taxonomy analysis on 34 different character states within each species (29 at the time) of *Alopecurus*. As a result, the genus is presently divided into three different sections: section *Alopecurus*, section *Alopecurium* and section *Colobachne*. Section *Alopecurus* consists of both annuals and perennials that have ovoid to cylindrical panicles with each branch bearing one to ten spikelets. Florets have acute glumes that are equal or longer than the lemma and connate in the lower half. The palea is absent. Section *Alopecurium* also consists of either annuals or perennials and has cylindrical to oblong panicles and branches with one to five spikelets. Florets have acute to obtuse glumes (connate in the lower 1/8- 4/5) that are longer than or equaling the lemma and being coriaceous to membranous. The palea is once again absent. The third and final section, *Colobachne* consists of perennials with broadly elliptical or cylindrical panicles and branches bearing one to six spikelets. The glumes have an aristate point, are longer than the lemma (connate up to 1/3 of their length in the lower part) and are membranous or coriaceous-membranous. The palea of species in this section is most often present and very rarely absent.

Recent molecular studies which include *Alopecurus* (Doring *et al*., 2007; Gillespie *et al*., 2007; Soreng *et al*., 2007; Quintanar *et al*., 2007; Gillespie *et al*., 2008; Gillespie *et al*., 2010) always place it within the PPAM (Puccinelliinae, Poinae, Alopecurinae, Miliinae) and/or PAM (excluding Puccinelliinae) clade, not closely allied to any other sampled Alopecurinae genera (only *Beckmannia* and *Phleum* thus far).

Phleum **L.**

Originally described in 1753, the timothy genus *Phleum* L. is the second largest in subtribe Alopecurinae s.l. *Phleum* species can be either annual or perennial with flat leaf blades and a spike-like panicle inflorescence, having hermaphroditic, laterally compressed spikelets with a single floret (Britton & Brown, 1913; Dogan, 1991). It is distinguished from other Alopecurinae by its short panicle branches that are often fused with the rachis (Tsvelev, 1976). Its species inhabit temperate zones of both hemispheres, but its distribution is centred on the Mediterranean (Dogan & Us, 1995). The genus comprises many economically important pasture species and common weeds, and all perennial species are considered to be valuable fodder plants (Cenci *et al*., 1984; Dogan, 1991). For mainly this reason species of *Phleum* have been able to spread greatly beyond their native range, all over North America.

Depending on its circumscription, *Phleum* includes 15 to 20 species which are divided into three to five sections (Tsvelev, 1976; Dogan, 1991; Dogan & Us, 1995; Scholz, 1999). Tsvelev (1976) placed *Phleum* in tribe Phleeae Dum. alongside the genera *Beckmannia*, *Rhizocephalus*, *Limnas*, and *Alopecurus*. In Tzvelev's treatment, *Phleum* and *Rhizocephalus* were placed in subtribe Phleinae Benth. Dogan and Us (1995) circumscribed *Phleum* in tribe Phleeae similar to Tsvelev, but also included the newly described genus *Pseudophleum* (Dogan, 1982). More recently *Phleum* has been included within subtribe Alopecurinae (tribe Poeae) and placed close to the largest Poeae subtribe, Poinae (Soreng *et al*., 2007). However, recent molecular studies show strong support for separating *Phleum* from *Beckmannia* and especially from *Alopecurus* (Soreng *et al*., 2007; Quintanar *et al*., 2007; Gillespie *et al*., 2008). As a result *Phleum* is now once again treated in a distinct subtribe, Phleinae, alongside *Pseudophleum* and *Rhizocephalus* (Gillespie *et al*., 2010).

The most recent infrageneric classification of *Phleum* was published by Dogan & Us (1995). *Phleum* was divided into five separate sections: section *Phleum*, section *Chilochloa*, section *Achnodon*, section *Maillea*, and section *Echinata*. Section *Phleum* comprises two perennial species with cylindrical panicles and oblong to lanceolate spikelets. Their glumes are wingless, lemmas are three- to seven-veined and florets have three stamens. Section *Chilochloa* has five species which are also all perennials with oblong to cylindrical panicles. The spikelets are most often oblong with wingless glumes, three- to seven-veined lemmas and florets with three stamens. Section *Achnodon* consists of six annual species with ovate to cylindrical panicles and branches that are free from the main axis. The spikelets in this section are cuneate to elliptic and florets have three stamens. The glumes are wingless and the lemmas are three- to seven-veined. The final two sections, *Maillea* and *Echinata* each contain only a single annual species. Section *Maillea* has an ovate panicle with branches that are adnate to the main axis. The spikelets are ovate and the glumes have a wing. The lemma has a single vein and is glabrous, and florets have two stamens. Section *Echinata* has a spiciform panicle with branches adnate to the main axis and oblong spikelets and glumes. The lemma has three to seven veins and is ciliate.

***Limnas* Trin.**

Containing only two perennial species (both of which are used as pasture plants), *Limnas* Trinius is characterized as being very similar to *Alopecurus* by having a moderately dense to contracted panicle inflorescence with one-flowered spikelets, a palea 1/4 length of the lemma, two stamens, and lodicules present (Clayton & Renvoie, 1986). The pedicel in *Limnas* is longer (Tsvelev, 1976; Clayton & Renvoize, 1986; Clayton *et al*., 2006; Hoffmann *et al*., 2013). The genus is distributed from Central Asia to northeast Siberia and is usually found in open woods and on stony slopes (Clayton & Renvoize, 1986; Watson & Dallwitz, 1992). A molecular analysis by Doring, 2009 –unpublished, based on the plastid gene *matK* placed *Limnas* within a clade that includes other Alopecurinae (*Alopecurus*, *Beckmannia*, *Cornucopiae*, *Rhizocephalus*) and Poinae members, but excludes *Phleum*. In their 2013 study, Hoffmann *et al.* found based on ITS sequences that *Limnas* may have played a role as a second parent in the origin of the Poinae genus *Dupontia*.

Beckmannia **Host**

The genus *Beckmannia* Host, more commonly known as American slough grass, has endured a long history of taxonomic disagreement. Since its discovery in 1805, it has been assigned (based on morphology) to tribes Phalarideae, Chloriideae, Beckmanniinae, and finally Aveneae (subtribe Alopecurinae) (Tsvelev, 1976; Clayton & Renvoize, 1986), suggesting that a molecular investigation of this genus and its relatives is needed. Soreng *et al*., (2007) placed *Beckmannia* with all other sampled Alopecurinae s.l. members in Alopecurinae, Poeae, but provided support for potentially separating *Beckmannia*, *Alopecurus* and *Phleum* from each other. Since then, molecular studies including *Phleum* and/or *Alopecurus* and *Beckmannia* have not been able to show strong support for *Beckmannia* as a distinct lineage, tentatively leaving it within subtribe Alopecurinae (Gillespie *et al*., 2008; Xu *et al*., 2009; Gillespie *et al*., 2010).

Beckmannia currently comprises two perennial species which are distributed throughout meadows and damp places of temperate regions in the Northern hemisphere (Rao *et al*., 2008). *Beckmannia eruciformis* is distributed in Europe and Asia, while *Beckmannia syzygachne* is found in North America and Asia. They are characterized by their orbicular one- (sometimes two-) flowered spikelets, somewhat one-sided panicles and long spicate branches with a trigonal rachis that is scabrous along ridges (Tsvelev,1976). Species of *Beckmannia* are believed to have a great deal in common with *Phleum* in terms of morphology (Clayton & Renvoize, 1986).

Cornucopiae **L.**

The genus *Cornucopiae* L. consists of two annual species which are distributed in ditches and wet places throughout the eastern Mediterranean and Iraq. They are characterized by a capitate panicle inflorescence with one-flowered solitary spikelets, subtended by inflated sheaths (Clayton & Renvoize, 1986). According to Trinius (1840) and Post (1888), *Cornucopiae* species were once treated as members of *Alopecurus*. A 2011 study by Sciandrello and Tomaselli noted that *Cornucopiae* is a relatively rare genus and that one of its species (*C. cucullatum*) should be given endangered status under IUCN due to its limited geographic range. Doring (2009 - unpublished)'s plastid analysis placed *Cornucopiae* with all other sampled Alopecurinae genera (and some Poinae), but excluding *Phleum*. A close relationship with *Alopecurus* was not evident.

Rhizocephalus **Boiss.**

First described in 1844, the genus *Rhizocephalus* Boissier is characterized as being similar to *Phleum*, differing mainly by its protruding, terminally mucronate lemma (Dogan, 1988). It is a dwarf annual with capitate panicles that are shorter than the basal leaves, and are subtended by an inflated sheath. It exists in arid environments of the eastern Mediterranean and Iran (Clayton & Renvoize, 1986), and little is known regarding its dispersal patterns. *Rhizocephalus* is currently placed within subtribe Phleinae alongside *Phleum* and *Pseudophleum* (Gillespie *et al*., 2010). Doring (2009 -unpublished)'s analysis placed *Rhizocephalus* within a clade with Alopecurinae genera *Alopecurus*, *Beckmannia*, *Limnas*, and *Cornucopiae* (as well as with some Poinae genera). It was shown to be more closely related to all other Alopecurinae than to *Phleum*.

Pseudophleum Dogan

Pseudophleum Dogan can be characterized as being an annual with unequal glumes, a keeled lemma, a spiciform panicle, and convolute basal leaves. It is endemic to the eastern Mediterranean/ Iran area (centred on Turkey) and prefers arid environments (Dogan, 1988; Clayton &Renvoize, 1986). This genus was previously treated as a member of *Phleum* under *Phleum gibbum* Boiss., but was shown to be a separate monotypic genus within subtribe Alopecurinae (Dogan, 1982; 1988). It is thought to be closely related to *Phleum* and *Rhizocephalus*, differentiated micromorphologically by the structure of its cell wall (Dogan, 1988). Numerical taxonomy studies by Dogan and Us (1995) determined that *Pseudophleum* was more morphologically similar (thus potentially more closely related) to *Rhizocephalus* than to *Phleum*. Recently *Pseudophleum* has been placed together with these two genera in the subtribe Phleinae (Gillespie *et al*., 2010). No molecular data existed on this genus prior to this thesis.

Objectives

The hypothesis driving this thesis is that subtribe Alopecurinae as currently circumscribed is polyphyletic. To test this, the relationships between all seven Alopecurinae genera were evaluated as well as the relationships of Alopecurinae to other closely related subtribes (Poinae, Miliinae, Puccinelliinae). Additionally, this thesis set out to test the congruency of present molecular findings to previous morphometric ones (for the two largest Alopecurinae genera *Alopecurus* and *Phleum*). The final objective of this thesis is to determine whether infraspecific classification is warrented in *Alopecurus magellanicus*.

METHODOLOGY

Molecular Markers

Since multiple loci are more powerful than a single locus at discriminating between different taxa (Schaal *et al*., 1988; Kress & Erickson, 2007; Fazekas *et al*., 2008), a combination of two nuclear and three plastid regions was chosen for analysis. The two nuclear regions include the ribosomal internal and external transcribed spacers (ITS, ETS), while the three plastid regions are: *trn*TLF (*trn*T-*trn*L spacer, *trn*L intron, *trn*L-*trn*F spacer), intergenic spacer *rpo*B-*trn*C and *mat*K (maturase K- region used for plant barcoding; CBOL, 2009). From here on, *trn*TLF and *rpo*B-*trn*C will be referred to as TF and rpoB respectively. All above DNA regions have been successfully used before in resolving grass phylogenies. See Table 1 for primers used to amplify and sequence each region.

Taxon Sampling

Sampling for this thesis was designed to capture the taxonomic diversity within subtribe Alopecurinae. This was accomplished primarily by obtaining specimens from various herbaria (CAN, US, MO, NSW, BH), but also by collecting fresh material in the field. During the summer of 2011, a two and a half week field excursion was undertaken in Turkey where Alopecurinae taxa endemic to the region (as well as other taxa) were collected. To accurately place Alopecurinae members, representatives of closely related subtribes Poinae, Miliinae and Puccinelliinae were also sampled. Samples were identified with the assistance of experts in the field (Robert Soreng, Lynn Gillespie, Musa Dogan, Evren Cabi) primarily using the Flora of Turkey (Edmonson, 1985), Grasses of the Soviet Union (Tzvelev, 1976), and Dogan's treatment of *Alopecurus* (Dogan, 1999). Outgroup taxa were selected such that they were closely related to the ingroups, but less closely than any of the ingroups were to each other. For the plastid and combined analyses, species of subtribe Puccinelliinae were chosen for this role based on the results of previous analyses (Gillespie *et al*., 2008; Gillespie *et al*., 2010). Different outgroups were chosen for the nuclear analysis in order to have the ability to properly place the genus *Limnodea*. As per Quintanar *et al*. (2007); Gillespie *et al*. (2008); and Gillespie *et al*. (2010), the outgroups chosen were: *Helictotrichon* sempervirens (Vill.) Pilg. (Aveneae, Aveninae), *Festuca baffinensis* Polunin (Poeae, Loliinae), *Molineriella laevis* (Brot.) Rouy (Poeae, Airinae) and *Deschampsia brevifolia* R. Br. (Poeae, Airinae). All sequences were either presented for the first time in this thesis or derived from previous published studies (Gillespie *et al*., 2008; Gillespie *et al*., 2010; Hoffmann *et al*., 2013). For a list of samples and voucher specimens used in all analyses please see Appendix B.

Each data matrix is made up of different samples, although most are consistent throughout all matrices. The combined nuclear data matrix (ETS+ITS) excludes all GenBank sequences that were only available for the ITS region as well as samples sequenced in-house that were only present for one region. It also excludes *Alopecurus creticus* and *A. rendlei*, since they were differently positioned in the ETS versus the ITS trees. The combined nuclear and plastid analysis (ETS+ITS+TF+rpoB+matK) excludes all taxa that are known or suspected hybrids (*Arctopoa*, *Alopecurus stejnegeri*), as well as all taxa that are missing a sequence for one or more region. All nuclear matrices (ETS, ITS, ETS+ITS) contain two Airinae, two Aveninae, and one Loliinae member. The nuclear ETS data matrix is made up of 166 sequences which include 112 Alopecurinae, 33 Poinae, three Miliinae, and 13 Puccinelliinae. The nuclear ITS data matrix is made up of 168 sequences of which 114 are Alopecurinae, 33 Poinae, three Miliinae, and 13 Puccinellinae. The combined nuclear ETS+ITS data matrix consists of 159 sequences, 105 of which are Alopecurinae, 33 Poinae, three Miliinae, and 13 Puccinelliinae. The plastid dataset

(TF+rpoB+matK) contains 132 sequences of which 88 are Alopecurinae, 31 are Poinae, three are Miliinae, and ten are Puccinelliinae. Finally, the combined dataset (ETS+ITS+rpoB+matK) contains 117 sequences of which 77 are Alopecurinae, 28 are Poinae, three are Miliinae, and nine are Puccinelliinae.

DNA Extraction, Amplification and Sequencing

As per Alexander *et al.* (2007), genomic DNA was extracted from relevant herbarium and silica gel specimens. Since the variable quality of herbarium vouchers has the ability to affect the DNA retrieved and the resulting molecular analyses (Drabkova *et al*., 2002), care was taken to ensure that when available, only the darkest green tissues were selected for extraction. For each sample, approximately 20mg of tissue was ground in g-tubes with two beads each in a BeadBeater (BioSpec Products). Warm incubation buffer (404 µL - made up of 320 µL plant homogenization buffer, 80µL plant lysis buffer and 4µL 100 mg/mL RNase A) was added to each powdered sample and each tube was vortexed and incubated at 65°C for half an hour. Potassium acetate (130 µL) was added to each tube and incubated at -20°C for five minutes. Tubes were then centrifuged at 18407 x g for ten minutes and the liquid supernatant was separated from the precipitate. Plant binding buffer (600 µL) was added to each tube and incubated at room temperature for five minutes. The mixture was then transferred to spin columns over collection tubes and centrifuged at 9391 x g for one minute. Ethanol (1000 µL, 70%) was added to each column in two installments and then centrifuged. THE (tris half EDTA; 150 µL, 65°C) buffer was added to the silica membrane of each column and incubated for five minutes. To elute the DNA, spin columns were centrifuged at 21130 x g for one minute. For old or degraded samples, the elution step was performed twice (using 75µL of THE buffer each time) to maximize yield. Extraction products were run on a 1.25% agarose gel using 4 µL of DNA extract and 2µL of bromophenol blue (BPB) loading to check for extraction success.

Each nuclear and plastid region was amplified and sequenced separately using the primers listed in Table 1. Recipes and thermocycler settings used for each DNA region are found in Table 2. Upon PCR completion, a 1.25% agarose gel with 2 µL of PCR product and 4µL of BPB was run to check for reaction success. Successful PCR products were purified to remove excess dNTPs (using 1µL Exonuclease I and 10 µL Shrimp Alkaline Phosphatase) and incubated in the thermocycler at 37°C for 30 minutes and then at 90°C for ten minutes. Purified products were sequenced using BigDye cycle sequencing and purified again, this time of all ddNTPs. Each purified plate was then loaded onto the Applied Biosystems (ABI) 3130xl automated sequencer and read.

Phylogenetic Analyses

Individual contigs from ABI runs were assembled using Sequencher version 4.9 and checked visually (GeneCodes, Michigan). Completed sequences were aligned using MUSCLE (Edgar, 2004), visually double-checked, and concatenated in Geneious version 6.0.6 (Drummond *et al*., 2011). Incongruence length difference (ILD; Farris, 1995) tests for each set of regions in each data matrix were conducted with 10,000 replicates using PAUP v4.0b10 (Swofford, 2002). Although the results of these tests were taken into account, incongrunence was primarily assessed by comparing maximum parsimony trees of each individual region sequenced to each other one. This was done due to the ILD test's predisposition to fail when presented with a large amount of phylogenetic noise (Darlu & Lecointre, 2001).

Maximum Parsimony

The computation of the most parsimonious tree assumes that the least amount of evolutionary change occurred in order to explain the present data (Fitch, 1971). Five separate matrices were analyzed using maximum parsimony in PAUP version 4.0b10. Heuristic searches were performed with a simple addition of taxa, the MULTREES option turned on, and a maximum number of 100,000 trees retained. A strict consensus tree was generated from the 100,000 trees. This was done for each of the three nuclear analyses (ETS, ITS, ETS+ITS), for the combined plastid (TF+rpoB+matK) analysis and for the overall combined (ETS+ITS+TF+rpoB+matK) analysis. Clade support was determined using bootstrap (BS) analysis (Felsenstein, 1985) with 10,000 heuristic search repetitions and the MULTREES option turned off (DeBry & Olmstead, 2000). As in Starr *et al* (2004), BS support was described as: strong (95-100), very good (85-94), good (75-84), moderate (65-74), weak (55-64), and very weak (less than 55). Separate trees for each individual region included in the plastid and combined data matrices were generated with a strict consensus of 20,000 trees.

Bayesian

Inferences of phylogeny through Bayesian analysis are based on posterior probabilities (phylogenetic trees and a model of evolution) and are calculated using Bayes theorem (Huelsenbeck *et al*., 2001). Bayesian analyses were performed on concatenated nuclear, concatenated plastid and combined concatenated alignments using MrBayes version 3.2.1 (Huelsenbeck *et al*., 2001). Best fit models were determined using the Akaike Information Criterion (AIC) in MrModeltest version 2 (Nylander, 2004; see table 3). Nuclear and plastid analyses were run for a total of 6,000,000 generations with two independent runs (nruns=2), four simultaneous chains (nchains=4) and a sampling frequency of 100 (samplefreq=100). A total of

40,000 and 60,000 trees were discarded as the initial burn-in for nuclear and plastid analyses respectively. This was determined by generating a plot of generation versus probability and seeing where on the plot a trend is no longer visible. The combined analysis was run with identical parameters as above with exception of being run for 8,000,000 generations and discarding 45,000 trees. Majority rule phylograms of 50% with posterior probability scores as a measure of support were constructed from the remaining trees.

RESULTS

Maximum parsimony strict consensus trees with bootstrap (BS) support for the separate nuclear ETS, ITS and combined ETS+ITS analyses are presented in Figures 1, 2 and 3 respectively. Strict consensus trees for the combined plastid (TF+rpoB+matK) and overall combined (ETS+ITS+TF+rpoB+matK) analyses are in Figures 5 and 7 respectively. The Bayesian ETS+ITS analysis phylogram is found in Figure 4, while the TF+rpoB+matK and ETS+ITS+TF+rpoB+matK Bayesian trees are found in Figures 6 and 8 respectively. Relevant statistics and branch support values for all parsimony and Bayesian analyses are presented in Tables 3 and 4 respectively.

Nuclear Analyses

ETS

The parsimony analysis of the nuclear ETS dataset resulted in a strict consensus tree with well supported internal branches but a weakly supported backbone (Figure 1). The PPAM clade is very weakly supported with a 54% BS. The Alopecurinae-Poinae minus *Phleum* and *Poa* (AP-PP) clade is moderately supported (68% BS), while the Puccinelliinae clade is weakly supported (58% BS) and the Miliinae (in this analysis, consisting of three species of *Milium* L.) clade is strongly supported (100% BS).

Overall, the genus *Alopecurus* does not form a distinct clade and includes both Poinae and other Alopecurinae genera. *Alopecurus myosuroides* Huds. is very weakly allied to *A. glacialis* K. Koch, *A. textilis* Boiss. and *A. ponticus* K. Koch (99% BS for latter three), and that whole group resolves very weakly with the genus *Beckmannia* (100% BS for genus). With the exception of *A. myosuroides*, *A. glacialis*, *A. textilis* and *A. ponticus*, the rest of the *Alopecurus* taxa plus *Cornucopiae* resolve as a very well (86% BS) supported clade. The genera *Arctophila* (Rupr.) Rupr. ex Andersson, *Dupontia* R. Br. (77% BS) and *Limnas* (94% BS) resolve as very weak allies to this *Alopecurus* clade. *Pseudophleum* is at the base of the AP-PP clade, but its relationships are uncertain. The genus *Phleum* is strongly supported (100% BS) as a clade, and allied with moderate support (72% BS) to another very well supported genus, *Poa* (93% BS). *Limnodea* resolves with the outgroup taxa *Helictotrichon* Besser, *Festuca* L. and *Molineriella* Rouy.

As described above, the largest genus *Alopecurus* does not resolve as a distinct clade. *Alopecurus pratensis* L. and *A. arundinaceus* Poir. resolve together with strong support (98% BS), but with unresolved relationships between each other. They are both very weakly (<50% BS) supported sisters to the weakly supported clade (62% BS) including *Cornucopiae*, *A. creticus* Trin. and *A. rendlei* Eig. The above mentioned clade resolves (with very weak support) with *A. japonicas* Steud., *A. aequalis* Sobol., *A. hitchcockii* Parodi, and *A. saccatus* Vasey (94% BS), which are strongly supported (99% BS) sisters to *A. stejnegeri* (synonym of *A. magellanicus*). *A. geniculatus* L. and *A. gerardii* (All.) Vill. are strongly supported (99% BS) as being together but with unresolved relationships. They are included in a moderately supported clade (74% BS) with *A. lanatus* Sibth. & Sm., *A. davisii* Bor and *A. vaginatus* (Willd.) Pall. ex Kunth (86% BS). All taxa mentioned so far in this paragraph form a weakly supported (56% BS) clade. However, the addition of *Alopecurus magellanicus* to this clade is very well supported (86% BS). In fact, *Alopecurus magellanicus* as a clade by itself is strongly supported (98% BS), with North American specimens as a distinct group (62% BS), separated from South American specimens. The remaining *Alopecurus* taxa are separated from those mentioned above by *Arctophila*, *Dupontia* and *Limnas*.

The second largest genus *Phleum* resolves as a well supported distinct clade (100% BS) that is sister (72% BS) to *Poa* L. *Phleum subulatum* (Savi) Asch. & Graebn. is well supported (82% BS) as sister to *Phleum exaratum* Hochst. ex Griseb. and *P. boissieri* Bornm. (100% BS; unresolved amongst each other). Including *P. pratense* L. and *P. alpinum* L. to the above taxa is strongly supported (96% BS for both). *Phleum montanum* K. Koch and *P. phleoides* (L.) H. Karst. are strongly supported as being together (96% BS), but are unresolved within the *Phleum* clade. *Phleum paniculatum* Huds. is also within the *Phleum* clade but its position is unresolved.

ITS

The parsimony analysis of the nuclear ITS data matrix resulted in a largely unresolved tree with only a few well-supported major clades (Figure 2). The PPAM clade is well supported with BS support of 83%, while the AP-PP clade is not resolved. Unlike in the ETS analysis, subtribe Puccinelliinae resolves at the top of a clade (BS<50%) which includes some Alopecurinae and some Poinae members. Subtribe Miliinae resolves as a very weakly supported sister to *Poa*.

With the exception of *Phleum montanum* and *P. phleoides* (68% BS; unresolved within PPAM in strict consensus tree, form a clade with all other *Phleum* in bootstrap tree 52% BS), *Phleum* is a very well supported (93% BS) distinct lineage whose relationships are uncertain. *Alopecurus* does not resolve as a distinct clade and is interspersed with other Alopecurinae, Poinae and Puccinelliinae genera (relationships unresolved). *Beckmannia* is positioned as sister to subtribe Puccinelliinae with very weak (50% BS support), while all *Limnas* taxa are unresolved within the PPAM clade. The two *Cornucopiae* species (94% BS) are sister to *Alopecurus arundinaceus* with weak bootstrap support (63% BS). *Pseudophleum* is unresolved within PPAM, while *Limnodea* is outside of PPAM. *Limnodea* is allied to *Helictotrichon* with very good support (90% BS).

Alopecurus does not form a distinct lineage and instead is interspersed throughout the PPAM clade. No major relationships between *Alopecurus* species and Poinae/ Puccinelliinae members are well supported. *Alopecurus geniculatus*, *A gerardii* and *A. rendlei* form a strongly supported clade (97% BS), as do *A. glacialis*, *A. textilis* and *A. ponticus* (98% BS). These two form weakly supported sister clades. *Alopecurus arundinaceus*, *Cornucopiae*, *A. lanatus*, *A. davisii*, *A. vaginatus*, and *A. creticus* all form a clade, but with very weak support (BS<50%). In this, *A. arundinaceus* and *Cornucopiae* form a clade (63% BS), but the rest of the taxa are unresolved. *Alopecurus japonicus*, *A. aequalis*, *A. saccatus*, *A. carolinianus* Walter, *A. hitchcockii*, and *A. stejnegeri* form a very well supported clade (94% BS) that is sister to *A. pratensis* with 94% BS. Both North and South American *Alopecurus magellanicus* are strongly (99% BS) supported together, with North American samples separated from South American with weak support (64% BS).

Phleum, with the exception of *P. montanum* and *P. phleoides,* resolves as a very well supported lineage (93% BS). *Phleum montanum* and *P. phleoides* are unresolved within PPAM in the strict consensus tree, but form a clade with the rest of *Phleum* taxa (52% BS) in the bootstrap tree. The *Phleum* clade shows *P. subulatum* as a sister (83% BS) to *P. exaratum* and *P. boissieri* (just as in the nuclear ETS analysis). That whole group is then allied to *P. crypsoides* (d'Urv.) Hack. (93% BS), and then to *P. pratense* (74% BS). *Phleum alpinum* and *P. paniculatum* are unresolved within the *Phleum* clade.

ETS & ITS combined

An ILD test run on the two nuclear regions indicated that there is significant incongruence between the datasets (p=0.001), thus cautioning their concatenation. Upon visually comparing the strict consensus tree of each individual region however, it is evident that no major conflicts exist in terms of the positioning of taxa. Any slight differences can be attributed to the lack of resolution in the ITS tree and a lack of backbone support in both trees. As such, the two regions were concatenated despite the significant ILD result. *Alopecurus creticus* and *A. rendlei* were removed from the combined nuclear analysis due to their different positions in the ETS versus the ITS tree.

Both parsimony and Bayesian analyses (Figures 3 and 4) of the combined nuclear dataset support the PPAM clade (98% BS, PP=1) and the AP-PP clade (74% BS, PP=1). In both analyses *Milium* resolves in a clade with *Poa* and *Phleum* (BS<50%, PP=0.94). The positioning of subtribe Puccinelliinae is not well supported, forming a trichotomy with *Phleum-Poa-Milium* and the APP clade in parsimony analysis, and as a sister to AP-PP in Bayesian. Nonetheless, Puccinelliinae as a distinct subtribe is strongly supported (99% BS, PP=1).

Just as in separate ETS and ITS analyses, the genus *Alopecurus* does not resolve as a clade. In parsimony analysis, the clade with all *Alopecurus* taxa (BS<50%) also includes *Cornucopiae* and *Beckmannia*. In Bayesian analysis however, the clade (PP=1) includes *Cornucopiae*, *Arctophila*, *Dupontia*, and *Limnas*. In both analyses *Cornucopiae* is shown as allied to *Alopecurus arundinaceus* (64% BS, PP=0.98). Additionally, in Bayesian analysis it is part of the *Alopecurus* subclade (PP=1; also including *Limnas*, *Arctophila*, *Dupontia*- as described above). In parsimony analysis, *Beckmannia* resolves with *Alopecurus myosuroides* with very weak bootstrap support. It is placed within AP-PP in a trichotomy with some Poinae genera and the *Alopecurus*-*Cornucopiae*-*Limnas*-*Arctophila*-*Dupontia* clade in Bayesian analysis. *Limnas* is included at the base of the *Alopecurus*-*Cornucopiae*-*Limnas*-*Arctophila*-*Dupontia* clade in Bayesian analysis. It is allied to *Arctophila* and *Dupontia* (PP=0.62), and that whole group resolves with *Alopecurus myosuroides* (PP=0.67). Parsimony analysis also shows *Limnas* to be allied with *Arctophila* and *Dupontia* with very good support (86% BS). This group is within the AP-PP clade but not closely allied to *Alopecurus myosuroides* as in Bayesian analysis. *Pseudophleum* resolves within AP-PP in both Bayesian and parsimony analyses. In parsimony analysis it is at the base of AP-PP, while in Bayesian it is weakly supported as sister to a large clade including all other Alopecurinae (except *Phleum*) and some Poinae genera. The genus *Phleum* is strongly supported in both types of analyses (100% BS, PP=1). It is allied to *Poa* (64% BS, PP=0.96), and the *Phleum-Poa* clade is sister to *Millium* (BS<50%, PP=0.94). Just as in separate ETS and ITS analyses, *Limnodea* is strongly supported as being allied to *Helictotrichon* (100% BS, PP=1) in the combined nuclear parsimony and Bayesian analyses.

As mentioned above, the largest genus *Alopecurus* does not resolve as a distinct clade in either analysis and always resolves in a clade that also includes other Alopecurinae and/or Poinae members. *Alopecurus myosuroides* is always near the base of this mixed clade, but allies with *Beckmannia* in the parsimony analysis (BS<50%) and with *Arctophila*, *Dupontia* and *Limnas* in the Bayesian analysis (PP=0.67). *Alopecurus glacialis*, *A. textilis* and *A. ponticus* resolve as a strongly supported clade in both analyses (100% BS, PP=1), and are allied to the remaining *Alopecurus* taxa and *Cornucopiae* (BS<50%, PP=0.82; excluding *A. myosuroides*). The remaining taxa (now excluding *A. myosuroides*, A. *textilis*, A. *glacialis*, and A. *ponticus*), form a very well supported clade (90% BS, PP=1). The relationships between them are better resolved in the Bayesian tree than in the parsimony. The grouping of *Alopecurus lanatus*, *A. davisii* and *A. vaginatus* is strongly supported (99% BS, PP=1), as is that of *A. geniculatus* and *A. gerardii* (100% BS, PP=1). The relationships of these two clades are unresolved in the parsimony analysis, but in Bayesian the clades resolve as sisters (PP=0.61). *Alopecurus arundinaceus* forms a clade with *Cornucopiae* (64% BS, PP=0.98), and specimens of *A. magellanicus* also form a strongly supported clade (100% BS, PP=1). North American specimens of *A. magellanicus* are separated from South American with very good support (86% BS, PP=1). A well supported subclade (82% BS, PP=1) including *Alopecurus japonicus*, *A. aequalis*, *A. saccatus*, *A. hitchcockii*, *A. stejnegeri*, and *A. pratensis* is included in a clade containing *Alopecurus*, some Alopecurinae and some Poinae). In this, *A. japonicus*, *aequalis*, *saccatus*, and *hitchcockii* are strongly supported together (98% BS, PP=1), but their relationships to each other remain unclear. The addition of *A. stejnegeri* to the above mentioned group is also strongly supported (100% BS, PP=1). This whole subclade is in turn sister to *A. pratensis* (82% BS, PP=1).

The relationships of *Phleum* taxa are identical in both parsimony and Bayesian trees. *Phleum* is strongly supported as a distinct genus (100% BS, PP=1). *Phleum subulatum* is strongly supported (100% BS, PP=1) as sister to *P. exaratum* and *P. boissieri* (100% BS, PP=1; unresolved between each other). That whole clade is then allied to *P. pratense* (100% BS, PP=1), *P. alpinum* (99% BS, PP=1) and *P. paniculatum* (90% BS, PP=0.99). *Phleum montanum* and *P. phleoides* are at the base of the *Phleum* clade with strong support for being allied (98% BS, PP=1) and also for being separated as distinct taxa.

Plastid Analysis

ILD tests run on every combination of the three plastid regions indicate that there is significant incongruence between TF and matK (p=0.0396) and rpoB and matK (p=0.0015). However no incongruence exists between TF and rpoB (p=0.0841). Visually examining strict consensus trees of each individual plastid region, it is evident that no major taxa placement differences exist. As such, all three plastid regions were concatenated and only the concatenated alignment was critically analysed using both Bayesian (Figure 6) and maximum parsimony (Figure 5) methods.

The PAM (Poinae-Alopecurinae-Miliinae) clade is supported in both parsimony and Bayesian analyses (100% BS, PP=1), as is the AP-PP clade (73% BS, PP=1). In parsimony analysis *Milium* is very weakly allied with *Phleum* (BS<50%), while in Bayesian it is unresolved at the base of PAM. In both analyses however it is strongly supported as a distinct subtribe (100% BS, PP=1). Similar to the combined nuclear analysis, *Phleum* once again forms its own clade (100% BS, PP=1) which is unresolved and is not part of AP-PP in the Bayesian analysis. Unlike in the nuclear analyses, the genus *Alopecurus* forms a distinct clade (75% BS, PP=1) within AP-PP. In both Bayesian and parsimony analyses, *Pseudophleum* and *Beckmannia* are near the base of the AP-PP clade, but their precise relationships are unclear.

The *Alopecurus* clade contains three major strongly supported subclades (one of which is *A. myosuroides*), but their relationships to each other are unresolved in the parsimony tree. In the Bayesian tree, *A. myosuroides* is sister to the two other subclades. At the top of the *Alopecurus* clade, the first well supported subclade (86% BS, PP=1) includes *A. arundinaceus*, *A. pratensis*, *A. glacialis*, and *A. textilis*. In this, only *A. glacialis* and *textilis* are resolved together (65% BS, PP=0.99), while all other taxa are unresolved. Including *A. gerardii*, *ponticus*, *tiflisiensis*, *vaginatus*, *davisii*, and *lanatus* to the above mentioned taxa is weakly supported (100% BS, PP=1). Note that *A. geniculaus* G2000 has recently benn re-identified as *A. gerardii*. The last subclade within the *Alopecurus* clade is weakly supported (<50% BS, PP=1) and has unresolved basal relationships in the parsimony analysis. It includes the *Alopecurus magellanicus* complex (including *A. stejnegeri*) as well as *A. saccatus*, *japonicus*, *hitchcockii*, *carolinianus*, *aequalis*, and *creticus*. *Alopecurus saccatus*, *japonicus*, *hitchcockii*, *carolinianus*, and *aequalis* form a strongly supported group (98% BS, PP=1) but are not well resolved amongst each other. *Alopecurus creticus* joins this group with strong support (99% BS, PP=1). Unlike in the nuclear analyses, the *Alopecurus magellanicus* samples do not divide up according to whether they are from North or from South America in the plastid analysis. Although all South American specimens do resolve together (86% BS, PP=1), that alliance also includes two North American samples. The rest of the North American samples are unresolved (three cluster together; 63% BS, PP=0.99), as is *A. stejnegeri*. *Alopecurus stejnegeri*'s position in the plastid anaylsis differs drastically from the nuclear, where it is allied to *A. japonicus*, *aequalis*, *saccatus*, and *hitchcockii*.

In the *Phleum* clade in both analyses, *P. pratense* and *P. alpinum* form a subclade (64% BS, PP=0.83), and they are sister to (99% BS, PP=1) *P. exaratum* and *P. boissieri* (unresolved between each other). *P. subulatum* is positioned below the above taxa with moderate support (69% BS, PP=0.98). *Phleum montanum* and *P. phleoides* are not well resolved at the base of the *Phleum* clade.

Combined Nuclear and Plastid Analysis

The combined nuclear and plastid analysis is based on a different dataset than both the plastid analysis and all three nuclear analyses. Potential or known hybrids (*Arctopoa* (Griseb.)

Prob., *Alopecurus stejnegeri*) as well as specimens with missing data for one or more region (see Appendix B- missing data not shown) were eliminated from the combined analysis. As expected from previous nuclear combined and plastid combined analyses, ILD tests run on every region combination in the nuclear and plastid combined dataset indicated that a few incongruencies do exist. The two nuclear regions are both incongruent with each other ($p=0.0001$) and also with every plastid region ($p<0.05$). The plastid regions on the other hand are all congruent with each other ($p>0.05$). Despite slight differences in the strict consensus trees of each region (particularly between the nuclear and plastid datasets), all regions were concatenated.

The combined (ETS+ITS+TF+rpoB+matK) analyses (Figures 7 and 8) provide better resolution -both in the backbone of the tree and in the internal branches- than do any of the nuclear or plastid analyses. The PAM clade is strongly supported (100% BS; PP=1) as is the AP-PP clade (91% BS, PP=1). *Milium* is one of three subclades forming a trichotomy at the base of PAM.

The genus *Alopecurus* forms its own clade (60% BS, PP=1) as does *Phleum* (100% BS, PP=1). In both parsimony and Bayesian analyses *Phleum* forms a clade with *Poa* (BS<50%, PP=0.84), which is unresolved within PAM in the parsimony analysis. *Beckmannia* and *Pseudophleum* are positioned within AP-PP and among Poinae members. *Beckmannia* resolves within a clade made up of all *Alopecurus* taxa and some members of subtribe Poinae (62% BS, PP=1). *Pseudophleum* is positioned below this clade, but is not well supported (BS<50%, PP=0.97).

As in plastid analyses, the *Alopecurus* clade is made up of three strongly supported subclades, one of which is *A. mysuroides*. *Alopecurus myosuroides* is sister (60% BS, PP=1) to the other two subclades (89% BS, PP=1 for the two subclade's position together) in both

Bayesian and parsimony analyses. In the first subclade (98% BS, PP=1), the following taxa are found: *Alopecurus pratensis*, *arundinaceus*, *gerardii*, *glacialis*, *textilis*, *ponticus*, *davisii*, *lanatus*, and *vaginatus*. In the second (100% BS, PP=1), the taxa found include *Alopecurus magellanicus*, *aequalis*, *hitchcockii*, *japonicus*, and *saccatus*. *Alopecurus arundinaceus* and *A. pratensis* in the first subclade resolve strongly together (99% BS, PP=1). Also nested within the first subclade is an unresolved grouping which includes *A. geniculatus* and *gerardii* (100% BS, PP=1), which are a weakly supported (53% BS, PP=0.57) sister to *A. glacialis*, *textilis* and *ponticus* (94% BS, PP=1). The last grouping in the first subclade includes *A. davisii*, *lanatus* and *vaginatus*, which are very well supported together (94% BS, PP=1) but are unresolved with other *Alopecurus* taxa. The second subclade includes a grouping of *Alopecurus magellanicus* (100% BS, PP=1; both North and South American samples) which is sister to A. *aequalis*, *hitchcockii*, *japonicus*, and *saccatus*. The latter four taxa are strongly supported together (100% BS, PP=1), but the relationships between them are all very weakly supported. South American specimens of *A. magellanicus* are well supported together (78% BS, PP=1), but North American specimens do not resolve together.

The relationships between *Phleum* taxa are identical in both parsimony and Bayesian analyses of the entire combined dataset. They are also very similar to the combined nuclear analyses. *Phleum subulatum* and *P. boissieri*/ *P. exaratum* form a well supported subclade at the top of the *Phleum* clade (79% BS, PP=0.91). *Phleum pratense* is positioned beneath with 75% BS, PP=0.91. Beneath that is *P. alpinum* (100% BS, PP=1) and then *P. phleoides* and *montanum*. *Phleum phleoides* and *montanum* are strongly supported together (99% BS, PP=1), and *P. phleoides* is also separate from *P. montanum* (90% BS, PP=1).

DISCUSSION

Major clades

PPAM, PAM

The PPAM clade (including subtribes Puccinelliinae, Poinae, Alopecurinae, and Miliinae) is present in all nuclear (ETS, ITS, ETS+ITS) strict consensus trees. Support for it is moderate in nuclear ETS and ITS separate analyses, but strong in the combined ETS+ITS analysis. This is consistent with previous ITS trees by Quintanar *et al*. (2007) and Gillespie *et al*. (2008), where support for PPAM was strong and very weak respectively. Combined ITS and ETS analyses by Gillespie *et al*. (2010) showed very good support for PPAM, similar to the present combined nuclear analysis.

Major structure differences of PPAM between ETS and ITS trees are mostly in the positioning of subtribe Puccinelliinae. Previous work (Doring *et al*., 2007; Gillespie *et al*., 2007; Quintanar *et al*., 2007; Soreng *et al*., 2007; Gillespie *et al*., 2008; Gillespie *et al*., 2010) provides strong support for Puccinelliinae as a sister clade to PAM (including subtribes Poinae, Alopecurinae and Miliinae). PAM is not supported in the ETS and ETS+ITS analyses presented here, and basal relationships are unresolved in both analyses. In some previous ITS analyses (Gillespie *et al*., 2008; Gillespie *et al*., 2010), as in the one here, there is no support for branching order of the subtribes. In Bayesian ETS+ITS analysis, Puccinelliinae is sister to AP-PP (Alopecurinae, Poinae minus *Phleum*, *Poa*) as opposed to PAM. These differing and somewhat strange positions for the subtribe could be a result of long branches combined with some sequence similarity. Despite this, Puccinelliinae always resolves as a distinct clade with varying support (BS=58-99).

Trees from plastid (TF+rpoB+matK) and combined (ETS+ITS+TF+rpoB+matK) analyses are based on the PPAM clade (using subtribe Puccinelliinae as the outgroup). In these analyses the PAM clade is strongly supported and is composed of four clades: AP-PP, *Phleum*, *Milium*, and *Poa*. The same can be found in previous nuclear and plastid analyses by Gillespie *et al*. (2010), even with fewer Poinae and Alopecurinae genera.

AP-PP

The AP-PP clade (Alopecurinae, Poinae minus *Phleum*, *Poa*) is present in all analyses performed with exception of the nuclear ITS analysis. In all of the trees it ranges from being moderately to very well supported. A similar clade but with fewer taxa can also be found in previous trees of Soreng *et al*. (2007), Gillespie *et al*. (2008) and Gillespie *et al*. (2010). These were based on a combination of morphological characters and/or nuclear and plastid sequence data. The strict consensus tree from the ITS analysis does not present the AP-PP clade. Subtribe branching order here is unsupported, with some Alopecurinae and most Poinae genera, as well as *Milium* collapsing into polytomies. A very poorly supported clade of Alopecurinae, Puccinelliinae and some Poinae is present in the ITS analysis. This has been shown before in the ITS analysis performed by Gillespie *et al*. (2008), where Puccinelliinae also did not resolve as sister to PAM. Despite this incongruence, all trees show that neither Poinae nor Alopecurinae are monophyletic.

Limnodea

Although no specific phylogenetic relationships are evident for the genus *Limnodea* based on the present analyses, it is clear that it does not resolve within PPAM. *Limnodea* was placed near *Cinna* -known to be part of PPAM- by Clayton and Renvoize in 1986, while Soreng *et al*. (2003) placed it in Aveninae, Avenae. A 2009 PhD dissertation by Doring (unpublished) also placed it in a clade with six unresolved Aveneae genera (based on plastid matK sequences). In the present ETS, ITS and ETS+ITS analyses *Limnodea* is always allied to *Helictotrichon*, another Aveninae, Avenae lineage, with very good to strong support. It is therefore suggested that this genus keep its most recent placement in tribe Avenae, subtribe Aveninae.

Subtribe Alopecurinae

Subtribe Alopecurinae as conceived by Clayton and Renvoize (1986) is very broad, with Agrostidinae genera forming the major lineage from which it branches. Alopecurinae's dorsal awns and long, scarious glumes were used to place it in tribe Aveneae, while the single flowered spikelets placed it in Alopecurinae s.l. In this, Alopecurinae genera (as discussed in this thesis) branch off as only a minor group. A more in depth view of the present Alopecurinae was put forth by Tzvelev (1976), where the genera were divided into three separate subtribes in tribe Phleeae (placed near Aveneae). They were all placed in this tribe based on their dense spicate panicles and one-flowered spikeletes. Soreng *et al* (2007) later placed Alopecurinae members into a single subtribe near subtribe Poinae. A recent study by Gillespie *et al*. (2010) removed *Phleum*, *Pseudophleum* and *Rhizocephalus* from subtribe Alopecurinae and placed them in subtribe Phleinae, with Alopecurinae s.str. now being made up of *Alopecurus*, *Beckmannia*, *Cornucopiae*, and *Limnas*.

Molecular results from all analyses are consistent in showing that members of subtribe Alopecurinae do not resolve as a distinct clade and are instead always intermixed with members of subtribe Poinae. Therefore subtribe Alopecurinae is polyphyletic, as is potentially subtribe Poinae (since *Poa* is in a separate lineage). This supports previous molecular studies in which the three Alopecurinae genera sampled (*Alopecurus*, *Beckmannia*, *Phleum*) were always separated by Poinae genera (Gillespie *et al*., 2007; Quintanar *et al*., 2007; Gillespie *et al*., 2008; Gillespie *et al*., 2010). Doring (2009- unpublished) sequenced six of the seven Alopecurinae genera (*Alopecurus*, *Phleum*, *Beckmannia*, *Limnas*, *Cornucopiae*, *Rhizocephalus*) for the plastid region matK. This analysis resulted in a well supported clade containing genera of tribes Poeae and Aveneae. All sampled Alopecurinae genera with exception of *Phleum* formed a clade with members of subtribe Poinae. Their relationships -with the exception of *Alopecurus* which was loosely allied to *Poa*- were all unresolved. *Phleum* (including *Maillea crysoides*) formed a well supported clade outside of the aforementioned clade.

Pseudophleum and *Rhizocephalus*

As in all previous analyses including Alopecurinae genera (see above), present analyses confirm that of the seven Alopecurinae genera, six of them (excluding only *Phleum*) are more closely related to each other than any of them are to *Phleum*. These six form part of the AP-PP clade in all presented analyses except for in ITS, where members of PPAM collapsed into numerous polytomies. *Rhizocephalus* was not sampled for this thesis, but Doring (2009-unpublished) places it in a clade with all other Alopecurinae (minus *Phleum*, *Pseudophleum* not sampled) and some Poinae members (similar to the AP-PP clade here). In all present analyses (except ITS), *Pseudophleum* (presented for the first time in this thesis) is positioned at or near the base of AP-PP with very weak to moderate support. Previous assumptions have implied that *Phleum*, *Pseudophleum* and *Rhizocephalus* are most closely related to each other than to any other subtribe Alopecurinae members. According to studies by Dogan and Us (1995) however, *Pseudophleum* and *Rhizocephalus* are morphologically more similar to each other than they are to *Phleum*, perhaps suggesting that they are not as closely related to *Phleum* as thought. This distance from *Phleum* is confirmed by the present trees, at least in terms of *Pseudophleum*, but more work is needed to confirm a placement for *Rhizocephalus*.

Beckmannia

Based on its somewhat unusual morphology, *Beckmannia* was previously placed in its own subtribe (Beckmanniinae) in tribe Phleeae (Tzvelev, 1976). Previous phylogenetic analyses which have included *Beckmannia* are quite consistent in placing it with PPAM lineages (Quintanar *et al*., 2007; Gillespie *et al*., 2008; Xu *et al*., 2009; Doring, 2009- unpublished; Gillespie *et al*., 2010). An ITS analysis by Quintanar *et al*. (2007) placed it with *Alopecurus* and three Poinae genera (*Apera*, *Ventenata*, *Cinna*), though with unresolved relationships. The ITS analysis performed by Gillespie *et al*. (2008) places it as sister to Puccinelliinae in PPAM (BS<50), while the TF analysis places it among Poinae genera (unresolved) in PAM with strong support. In both ITS and TF trees of Xu *et al*. (2009) *Beckmannia* resolves in a clade with *Alopecurus*, allied to Poinae and Puccinelliinae genera. A recent study by Gillespie *et al*. (2010) places it at the base of AP-PP, loosely allied to *Bellardiochloa* and *Apera* in both nuclear (ITS+ETS) and combined (ITS+ETS+TF) analyses.

In present analyses *Beckmannia* almost always resolves within AP-PP, where it is separated from *Alopecurus* by Poinae genera (except in parsimony ITS, ETS, ETS+ITS analyses). In nuclear ETS and ETS+ITS analyses, it is allied to *Alopecurus myosuroides* (in ETS+ITS) and to the *A. myosuroides*, *A. glacialis*, *A. textilis* and *A. ponticus* complex (in ETS) with very low support. ITS analysis places it as sister to Puccinelliinae (BS<50), just as in Gillespie *et al*. (2008). In both plastid (TF+rpoB+matK) and combined (ETS+ITS+TF+rpoB+matK) MP analyses *Pseudophleum* is more resolved than *Beckmannia*. *Pseudophleum* and *Beckmannia* are equally resolved in Bayesian plastid and combined analyses. Overall the results suggest that there is no strong evidence for removing *Beckmannia* and *Pseudophleum* from Alopecurinae, but more constraint testing should be done.

Limnas

Historically *Limnas* has always been placed with *Alopecurus* and *Cornucopiae* in Alopecurinae (Tzvelev, 1976; Clayton & Renvoize, 1986), and has never been placed in its own subtribe as other Alopecurinae s.l. members. It is thought to be very similar morphologically to *Alopecurus*, and in some instances barely worth separating from it (Clayton & Renvoize, 1986). *Limnas* is not involved in any in-depth morphological studies, and has only recently been included in molecular phylogenetic analyses (Doring, 2009- unpublished; Hoffmann *et al*., 2013). Doring's matK analysis placed *Limnas* in a clade with all sampled Alopecurinae genera (except *Phleum*) as well as some Poinae. The relationships between all Alopecurinae lineages in this clade were unfortunately unresolved. Hoffmann *et al*.'s ITS analysis resolves a clade which includes *Limnas*, *Alopecurus*, *Arctophila*, and *Dupontia*. This is similar to a clade in the present ITS analysis (although BS<50), but *Limnas*, *Arctophila* and *Dupontia* are all unresolved within it. In all present ETS and ETS+ITS (no data for plastid regions) analyses, *Limnas* forms a very well supported clade as sister to *Arctophila* and *Dupontia* in AP-PP. This supports Hoffman *et al*. (2013)'s hypothesis that *Limnas* may be a second parent in the origin of *Dupontia* (with *Arctophila* being the first). Overall, the data suggest that *Limnas* is most closely related to Poinae genera *Arctophila* and *Dupontia* and therefore not to *Alopecurus* as previously thought. However, plastid data on *Limnas* is needed to confirm this.

Cornucopiae

Originally placed in Aveneae subtribe Alopecurinae by Clayton and Renvoize (1986) and Watson and Dallwitz (1992), *Cornucopiae*'s interesting combination of morphological characters (capitate panicle inflorescence with one-flowered spikelets, subtended by a bract) has led it to be distinguished as a unique lineage within Poeae subtribe Alopecurinae (Soreng *et al*., 2007). A matK parsimony tree by Doring (2009- unpublished) placed *Cornucopiae* in a clade with all sampled Alopecurinae (except *Phleum*) members and some Poinae. In all current analyses, *Cornucopiae* also resolves within the AP-PP clade (except in ITS), and is always embedded within *Alopecurus*. In the ETS+ITS analysis it is positioned within a subclade of *Alopecurus*, and is a weakly supported sister to *A. arundinaceus*. As supported by all present analyses, *Cornucopiae* may actually be just another *Alopecurus*. This is perhaps not too surprising considering that species of *Cornucopiae* (specifically *cucullatum* and *involucratum*) have been treated within *Alopecurus* (as *A. cornucopiae* and *A. involucratus* respectively) in the past (Trinius, 1840; Post, 1888). Based on the nuclear results in this study, it is recommended that species of *Cornucopiae* be considered as *Alopecurus* with their respective basionyms. More plastid data on the genus is needed to fully confirm this however.

Alopecurus

Alopecurus is the type genus for subtribe Alopecurinae. Throughout its history it has been placed in tribes Phleeae, Aveneae and finally in Poeae, near Poinae (Tzvelev, 1976; Clayton & Renvoize, 1986; Watson & Dallwitz, 1992). Recent molecular analyses including *Alopecurus* consistently place it within PPAM and separate from *Phleum* (Quintanar *et al*., 2007; Gillespie *et al*., 2008; Gillespie *et al*., 2010). More so, it is always shown to be more closely related to all other sampled Alopecurinae genera than to *Phleum*. Similar results are seen in the trees presented in this thesis. *Alopecurus* resolves as a genus in only four of the eight analyses performed (specifically in parsimony and Bayesian TF+rpoB+matK and ETS+ITS+TF+rpoB+matK), but resolves in AP-PP in all analyses except ITS (where the AP-PP clade is not present).

There are a few differences in clade structure of *Alopecurus* between the ETS and ITS trees. Many subclades are consistent, but how they are related to each other differs perhaps suggesting a historically hybrid origin. *Alopecurus creticus* and even more so *A. rendlei* have different position in the two nuclear trees. In the ETS tree *A. rendlei*, *A. creticus* and *Corcnucopiae* resolve together in a clade that is sister to *A. arundinaceus* and *A. pratensis*. The ITS tree shows *A. rendlei* in a clade with *A. geniculatus* and *A. gerardii*, while *A. creticus* is sister to a clade which includes *A. arundinaceus*, *Cornucopiae*, *A. lanatus*, *A. davisii*, and *A. vaginatus*. As a result of these differences, *A. creticus* and *A. rendlei* were removed from the combined ETS+ITS analysis, producing a nicely resolved tree (although *Alopecurus* still does not resolve as its own clade). Since the support for the above discussed differences is moderate, it could be that *Alopecurus* has a rather complicated history involving extensive hybridization.

An infrageneric classification of *Alopecurus* by Dogan (1999) divided the genus (29 species at the time) into three separate sections on the basis of morphological similarity: *Alopecurus*, *Alopecurium* and *Colobachne*. Over the span of all analyses, a total of 20 *Alopecurus* taxa were analyzed. Since in all nuclear analyses (ETS, ITS, ETS+ITS) the genus never forms a distinct clade, only plastid (TF+rpoB+matK) and combined (ETS+ITS+TF+rpoB+matK) analyses were compared to the previous infrageneric classification (see Figure 9). *Alopecurus* clade structure in all four trees (plastid and combined parsimony and Bayesian) is very similar if not identical. The only discrepancy is in the Bayesian plastid tree which places *A. lanatus* as allied to a clade which includes *A. pratensis*, *A. arundinaceus* (section *Alopecurus*), *A. glacialis* and *A. textilis* (section *Colobachne*). In all other trees, *A. lanatus* is either allied to *A. davisii* and *A. vaginatus* or is unresolved close to the above mentioned taxa. All four trees analyzed produce three major clades within *Alopecurus* (see Figure 9). The first includes members of sections *Alopecurus*, and *Colobachne* (*A. geniculatus* G2000 recently re-idetified to be *A. gerardii*- included in section *Colobachne*). The second is made up of sections *Alopecurium* and *Colobachne*, while the third is comprised of section *Alopecurus* and contains only one species: *A. myosuroides*. Discrepancies between previous infrageneric classifications and results from both plastid and combined molecular analyses follow. Firstly, section *Alopecurus* taxa do not form a clade in any of the trees. *Alopecurus pratensis* and *A. arundinaceus* form a clade at the top of the *Alopecurus* clade, but *A. myosuroides* is positioned at the base in the plastid parsimony analysis and is sister to the other two *Alopecurus* subclades in all other analyses. The second discrepancy revolves around section *Colobachne*'s *A. magellanicus*, and its strongly supported (in Figure 9B) resolution with members of section *Alopecurium*. Finally, section *Alopecurium* resolves as a strongly supported clade (note *A. geniculatus* G2000 has recently been re-identified as *A. gerardii* - please see Figure 9). Overall the present molecular results support a revision in infrageneric classification for the genus *Alopecurus*. However more taxon sampling is still needed to account for all variation within the genus.

Previously recognized *Alopecurus* species that have been synonimized under *A. magellanicus* occur all over the world in cool temperate climates. Since this taxon is so widespread, all subspecies of the previously recognized species have not yet been assigned as subspecies under *A. magellanicus*, pending further study. In the present nuclear trees, North and South American *A. magellanicus* are well delineated, with North American samples forming a well supported clade separate from South American samples. In the plastid trees South American samples are all in a clade (including one North American sample), while North American samples do not form a single clade with each other. Mostly unresolved relationships between

North American samples in the plastid analysis suggest that the spread of *A. magellanicus* occurred from North to South America. It appears warranted to divide *A. magellanicus* into at least two subspecies according to the present analyses: *Alopecurus magellanicus* subsp. *borealis* and/or subsp. *glaucus* for North American taxa and *A. magellanicus* subsp. *magellanicus* for South American taxa (Soreng *et al*., 2003).

Alopecurus stejnegeri is another synonym of *A. magellanicus* (Soreng *et al*., 2003), originally described from the Aleutian Islands. It ranges from the Far East Russian arctic to North America. Tzvelev (1976) recognized it as one of four subspecies of *A. alpinus* (also a synonym of *A. magellanicus*). In nuclear analyses, *A. stejnegeri* resolves in a strongly supported clade related to other *Alopecurus* taxa, and not at all to the *A. magellanicus* clade. In plastid analyses it is interspersed with North American *A. magellanicus*. The drastically different position of *A. stejnegeri* in the nuclear versus the plastid analyses suggests that it may be a historic hybrid. It has been widely suggested that hybridization has shaped the evolution of many Poeae and Poinae genera (Kellogg & Watson; 1993; Gillespie *et al*., 2008). Additionally many members of *Alopecurus* have been known to hybridize with other *Alopecurus* taxa (Tzvelev, 1976). It is therefore suggested that *A. stejnegeri* may be a case of reticulate evolution.

Phleum

Phleum is the second largest genus in Alopecurinae s.l. It was placed in Phleeae subtribe Phleinae by Tzvelev (1976) and in Aveneae subtribe Alopecurinae by Clayton and Renvoize (1986). More recently it has been treated in a distinct subtribe (Phleinae, Poeae) alongside *Rhizocephalus* and *Pseudophleum* (Gillespie *et al*., 2010). Recent molecular analyses which include *Phleum* always place it as its own distinct lineage, separate from all other (only *Alopecurus* and *Beckmannia* were looked at) Alopecurinae genera (Quintanar *et al*., 2007;

Gillespie *et al*., 2008; Gillespie *et al*., 2010). Additionally, *Phleum* has been shown to resolve close to or as sister to *Poa* and/or *Milium* (Quintanar *et al*., 2007; Doring *et al*., 2007; Gillespie *et al*., 2008; Schneider *et al*., 2009; Gillespie *et al*., 2010). The results presented in this thesis are similar to those discussed above, and show *Phleum* close to *Poa* and/or *Millium* and as a distinct lineage. They also contradict the placement of *Phleum* with genera *Pseudophleum* and *Rhizocephalus* (Dogan, 1995; Gillespie *et al*., 2010), which were originally combined based on morphology (and lacking molecular data until the present research). Doring (2009- unpublished) has shown in her matK parsimony analysis that *Rhizocephalus* is more closely related to all other Alopecurinae genera than it is to *Phleum*. The same is true for *Pseudophleum*'s relatedness to *Phleum* in all present analyses except in ITS. It is recommended here that both *Rhizocephalus* and *Pseudophleum* be treated as members of Alopecurinae instead of Phleinae. Still, more work on *Rhizocephalus* is warranted.

In all analyses (except in ITS) *Phleum* resolves as a strongly supported genus. In the ITS analysis a clade including *P. montanum* and *P. phleoides* is collapsed into a polytomy within PPAM, while all other *Phleum* taxa form a very well supported clade. In all ETS and ETS+ITS+TF+rpoB+matK analyses, *Phleum* forms a clade with *Poa*. *Phleum* forms a clade with both *Poa* and *Milium* in the ETS+ITS parsimony analysis (only with *Poa* in Bayesian), and with *Milium* in the TF+rpoB+matK analysis (only in parsimony, unresolved in Bayesian). The latter relationship between *Phleum* and *Milium* is most often encountered in phylogenetic analyses (Quintanar *et al*., 2007; Gillespie *et al*., 2008; Gillespie *et al*., 2010). However in Schneider *et al*. (2009), *Phleum* is part of a clade with Poinae members that is sister to both *Poa* and *Milium*.

Maillea is generally considered to be a synonym of the genus *Phleum*, and *Maillea crypsoides* a species of *Phleum* (*Phleum crypsoides*; WCSP, 2013). Doring (2009 -

unpublished)'s sample of *Maillea crypsoides* resolved in a clade with four other *Phleum* species with strong support. In the ITS analysis presented here, *P. crypsoides* is imbedded within the *Phleum* clade with strong support, thus strengthening the evidence for synonymization of the names.

In 1995 Dogan and Us revised the infrageneric classification of *Phleum* to include five sections: *Phleum*, *Chilochloa*, *Achnodon*, *Maillea*, and *Echinata*. The latter two sections only consist of one species each (*Phleum crypsoides* and *Phleum echinatum* respectively). Section *Maillea* is only present in the ITS tree and is positioned with strong support in between sections *Achnodon* and *Phleum*. Other than that, the positions of *Phleum* taxa are very similar between ETS and ITS trees, so the combined nuclear analysis (ETS+ITS) will only be discussed onwards. Both Bayesian and parsimony combined nuclear trees are identical and consist of five major strongly supported clades with strong support for their positions. For an outline of the previous infrageneric classification atop the current ETS+ITS, TF+rpoB+matK and ETS+ITS+TF+rpoB+matK molecular analyses please see Figure 10. The nuclear and combined analyses (Figure 10 A, C) consist of two monophyletic sections (composed of sections *Achnodon* and *Phleum*) and one paraphyletic one (Section *Chilochloa*). With exception of the position of *Phleum paniculatum*, Section *Achnodon* forms a clade in both nuclear and combined analyses. The position of *Phleum alpinum* differs between the nuclear and plastid tree, suggesting that hybridization may have played a role in its past. Overall it appears that a revision in infrageneric classification may be warranted, but more *Phleum* taxa should be sampled first.

Conclusions and future work

Results presented in this thesis agree with and enhance many previous studies focused on delineating the relationships between tribe Poeae's PPAM (Puccinelliinae, Poinae, Alopecurine, Miliinae) clade. The presence of the AP-PP (Alopecurinae, Poinae minus *Phleum*, *Poa*) clade and the general intermixing of subtribe Alopecurinae and Poinae members implies that both subtribes are polyphyletic. As a result, it appears that subtribe Alopecurinae's unique morphological characters evolved many times over the history of Poeae. Based on presented data, the infrageneric classifications of the two largest Alopecurinae genera (*Alopecurus* and *Phleum*) need to be revised. In order to achieve this however, more taxon sampling within *Alopecurus* and especially within *Phleum* is needed. Present and previous results agree that the genus *Phleum* is a distinct lineage with potential alliances to *Poa* and/or *Milium*, separate from all other Alopecurinae members. Both *Beckmannia* and *Pseudophleum* are positioned within the AP-PP clade, but their relationships are unclear. *Pseudophleum*'s position away from *Phleum* indicates that these two genera are not closely related, as previously assumed based on similarities in morphology. The genus *Limnas* is also positioned in the AP-PP clade, as sister to Poinae genera *Arctophila* and *Dupontia* with strong support. The position of *Limnas* implies that it is not closely related to *Alopecurus* as was also previously indicated by morphology, although plastid data is needed to confirm this. *Cornucopiae* taxa, thought to be distinct from *Alopecurus* in their morphology may actually be part of *Alopecurus* based on present results. Once again however, plastid data is needed. Current results also suggest dividing *Alopecurus magellanicus* into at least two subspecies: *A. magellanicus* subsp. *borealis* and/or *glaucus* and *Alopecurus magellanicus* subsp. *magellanicus*. Overall it is suggested that subtribe Alopecurinae s.l. as presently circumscribed should be dissolved. Based on the molecular results, a new subtribe Alopecurinae should be formed (see figure 11) and should include all Poinae minus *Poa* as well as all Alopecurinae minus *Phleum*. The new subtribe Poinae would then consist of the single genus *Poa*. The genus *Phleum* should continue to be treated in its own subtribe Phleinae, and Milium in its own subtribe Miliinae.

Future work on subtribe Alopecurinae and related subtribes should include greater taxa sampling (both Poinae and Alopecurinae) and the sequencing of additional DNA regions. Special effort should be taken to include more *Phleum* and *Alopecurus* taxa in order to be able to make accurate infrageneric classifications. Samples of *Alopecurus magellanicus* across its distribution range should be analyzed to circumscribe them as appropriate subspecies. Recently collected plant material is needed for both *Cornucopiae* and *Limnas* in order to be able to conduct plastid analyses with these genera. Finally, data on *Rhizocephlaus* is urgently needed in order to reconstruct relationships between all Alopecurinae genera.

Tables and Figures

Table 1. Primers used for PCR and sequencing reactions of various nuclear and plastid DNA regions and their respective references

DNA region	Primer	Sequence	Reference
ITS	AB102	TAGAATTCCCCGGTTCGCTCGCCGTTC	Douzery *et al*., 1999
	KRC	GCACGCGCGCTACACTGA	Torrecilla & Catalan, 2002
ETS	18S-R	AGACAAGCATATGACTACTGGCAGG	Starr *et al*., 2004
	RETS4-F	TGGCTACGCGAGCGCATGAG	Gillespie *et al*., 2010
trnT-trnL-trnF	TA3	GCCGACTATCGGATTTGAACCG	Travis Columbus[a]
	c	CGAAATCGGTAGACGCTACG	Taberlet *et al*., 1991
	b	TCTACCGATTTCGCCATATC	Taberlet *et al*., 1991
	d	GGGGATAGAGGGACTTGAAC	Taberlet *et al*., 1991
	f	ATTTGAACTGGTGACACGAG	Taberlet *et al*., 1991
rpoB-trnC	rpoB	CKACAAAAYCCYTCRAATTG	Shaw *et al*., 2005
	trnCgcaR	CACCCRGATTYGAACTGGGG	Shaw *et al*., 2005
matK	390f	CGATCTATTCATTCAATATTTC	Cuenoud *et al*., 2002
	1326r	TCTAGCACACGAAAGTCGAAGT	Cuenoud *et al*., 2002

[a] Rancho Sanata Ana Botanic Garden

Table 2. PCR recipes and thermocycler settings used for the amplification of various DNA regions. The TF region was amplified using two sets of primers.

ETS PCR recipe		**Thermocycler settings**			
Reagent	Volume (µL)	PCR step	Temperature (°C)	Time (s)	No. of cycles
DNA water	3.86	Initial	95	120	
10x HS buffer	1	Denaturing	95	45	
$MgCl_2$ (25mM)	0.6	Annealing	58	60	
dNTPs (10mM)	0.3	Extension	72	120	
Primer A(10µM)	0.35	Final	72	300	
Primer B (10µM)	0.35				30
DMSO	0.5				
Betaine	2				
HS Taq (5U/µL)	0.04				
Template DNA	1				
ITS PCR recipe					
Reagent	Volume (µL)		Temperature (°C)	Time (s)	
DNA water	3.86	Initial	95	120	
10x HS buffer	1	Denaturing	95	45	
$MgCl_2$ (25mM)	0.6	Annealing	58	45	
dNTPs (10mM)	0.3	Extension	72	90	
Primer A(10µM)	0.35	Final	72	300	
Primer B (10µM)	0.35				34
DMSO	0.5				
Betaine	2				
HS Taq (5U/µL)	0.04				
Template DNA	1				
TF PCR recipe					
Reagent	Volume (µL)		Temperature (°C)	Time (s)	
DNA water	9.13	Initial	95	300	
10x HS buffer	1.5	Denaturing	95	45	
$MgCl_2$ (25mM)	1.2	Annealing	60-54	60	
dNTPs (10mM)	0.3	Extension	72	120	
Primer A,B(10µM)	0.375				15
Primer C,D (10µM)	0.375	Denaturing	95	45	
BSA (20mg/mL)	0.5	Annealing	54	60	
NEB Taq (5U/µL)	0.12	Extension	72	120	
Template DNA	1.5				20
		Final	72	300	
rpoB PCR recipe					
Reagent	Volume (µL)		Temperature (°C)	Time (s)	
DNA water	5.82	Initial	95	300	
10x HS buffer	1	Denaturing	95	45	
$MgCl_2$ (25mM)	0.6	Annealing	60-48	60	
dNTPs (10mM)	0.3	Extension	72	120	
Primer A(10µM)	0.35				15
Primer B (10µM)	0.35	Denaturing	95	60	
BSA (20mg/mL)	0.5	Annealing	48	120	
HS Taq (5U/µL)	0.08	Extension	72	300	
Template DNA	1				20
		Final	72	300	

matK PCR recipe

Reagent	Volume (µL)		Temperature (°C)	Time (s)	
DNA water	5.82	Initial	94	60	
10x HS buffer	1	Denaturing	94	30	
$MgCl_2$ (25mM)	0.6	Annealing	52	20	
dNTPs (10mM)	0.3	Extension	72	50	
Primer A(10µM)	0.35	Final	72	300	
Primer B (10µM)	0.35				35
DMSO	0.5				
HS Taq (5U/µL)	0.08				
Template DNA	1				

Table 3. Summary statistics pertaining to maximum parsimony and Bayesian analyses of nuclear, plastid and combined data matrices. Analyses with an asterisk represent a strict consensus of 20,000 trees as opposed to 100,000 for all others (presented in Appendix B and C). Consistency indices (CI) presented in brackets exclude parsimony uninformative characters. RI: retention index.

	Analysis	Model[a]	No. of samples	No. of characters	No. of parsimony informative characters	Tree Length	CI	RI
Nuclear analysis	ETS	GTR+G	166	1172	304	1125	0.513 (0.4601)	0.8825
	ITS	GTR+I+G	168	621	205	774	0.4767 (0.4304)	0.8747
	ETS/ITS	-	159	1793	500	1872	0.4920 (0.4474)	0.8762
Plastid analysis	TF*	GTR+I+G	132	2308	204	438	0.7831 (0.7139)	0.9545
	rpoB*	GTR+G	132	1452	177	380	0.7632 (0.6980)	0.9502
	matK*	GTR+I+G	132	785	75	193	0.7409 (0.6454)	0.9311
	TF\rpoB\matK	-	132	4545	456	1039	0.7469 (0.6708)	0.9431
Combined analysis	ETS*	GTR+G	117	730	235	761	0.5690 (0.5155)	0.8994
	ITS*	GTR+I+G	117	622	173	531	0.5744 (0.5150)	0.8979
	TF*	GTR+I+G	117	2251	198	402	0.7985 (0.7379)	0.9559
	rpoB*	GTR+I+G	117	1367	172	336	0.7589 (0.7076)	0.9499
	matK*	HKY+I+G	117	786	72	164	0.7866 (0.7107)	0.9451
	ETS\ITS\TF\rpoB\matK	-	117	5756	850	2298	0.6279 (0.5624)	0.9106

[a] Best fit model as determined by the Akaike Information Criterion in MrModeltest. Only used for Bayesian analyses

Table 4. Bootstrap and Bayesian support values (BS/PP) for major clades in nuclear, plastid and combined analyses. Only parsimony analyses were performed for ETS and ITS. No outgroup external to PPAM was used for plastid and combined analyses, thus no support value is presented. Dashes indicate that either the clade is not present or support is below 50/0.5.

Analysis		**Major clades**				
		PPAM	PAM	AP-PP	*Alopecurus*	*Phleum*
Nuclear analyses	ETS	54	-	68	-	100
	ITS	83	-	-	-	-
	ETS/ITS	98/1	-	74/1	-	100/1
Plastid analysis	TF/rpoB/matK	N/A	100/1	73/1	75/1	100/1
Combined analysis	ETS/ITS/TF/rpoB/matK	N/A	100/1	91/1	60/1	100/1

Table 5. List of Alopecurinae genera with previous tribal and subtribal classifications (by Tzvelev, 1976- morphological; Clayton and Renvoize, 1986- morphological; Soreng *et al*., 2007- morphological/molecular; Gillespie *et al*., 2010- molecular) as well as present proposed treatment. Classifications are presented as tribe/ subtribe. Dashes indicate missing data and asterisks indicate a change in present treatment from the most recent classification.

Genus	**Tzvelev**	**Clayton & Renvoize**	**Soreng *et al***	**Gillespie *et al***	**Proposed treatment**
Alopecurus	Phleeae/ Alopecurinae	Aveneae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Alopecurinae
Phleum	Phleeae/ Phleinae	Aveneae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Phleinae	Poeae/ Phleinae
Pseudophleum	-	Aveneae/ Alopecurinae[b]	Poeae/ Alopecurinae	Poeae/ Phleinae	Poeae/ Alopecurinae*
Rhizocephalus	Phleeae/ Phleinae	Aveneae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Phleinae	Poeae/ Alopecurinae*
Beckmannia	Phleeae/ Beckmanniinae	Aveneae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Alopecurinae
Limnas	Phleeae/ Alopecurinae	Aveneae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Alopecurinae
Cornucopiae	-	Aveneae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Alopecurinae	Poeae/ Alopecurinae[a]

[a] Proposed treatment as part of *Alopecurus*

[b] Considered a synonym of *Phleum*

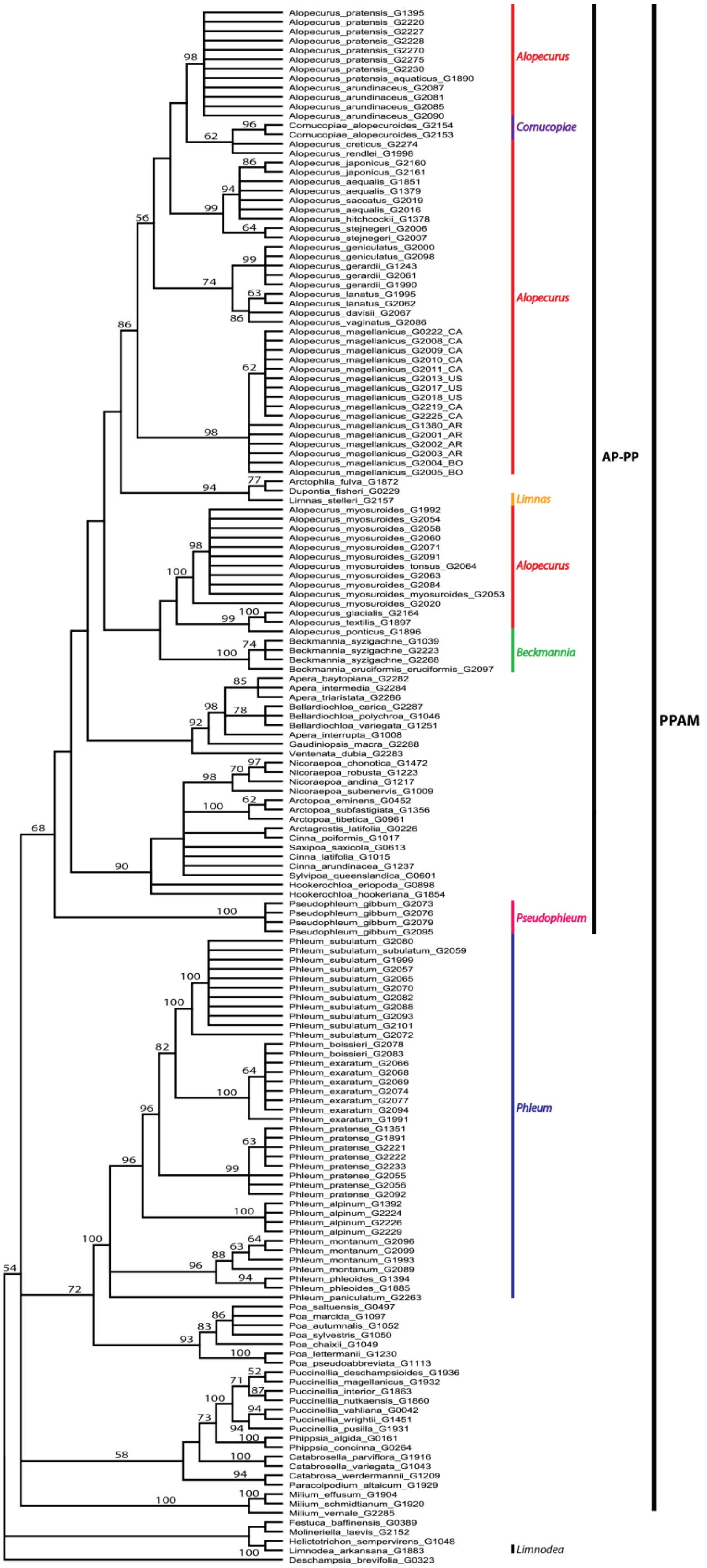

Figure 1. Strict consensus of 100,000 trees from maximum parsimony analysis of nuclear DNA region ETS. Bootstrap values >50 are indicated above branches. Important taxa and major clades are labelled.

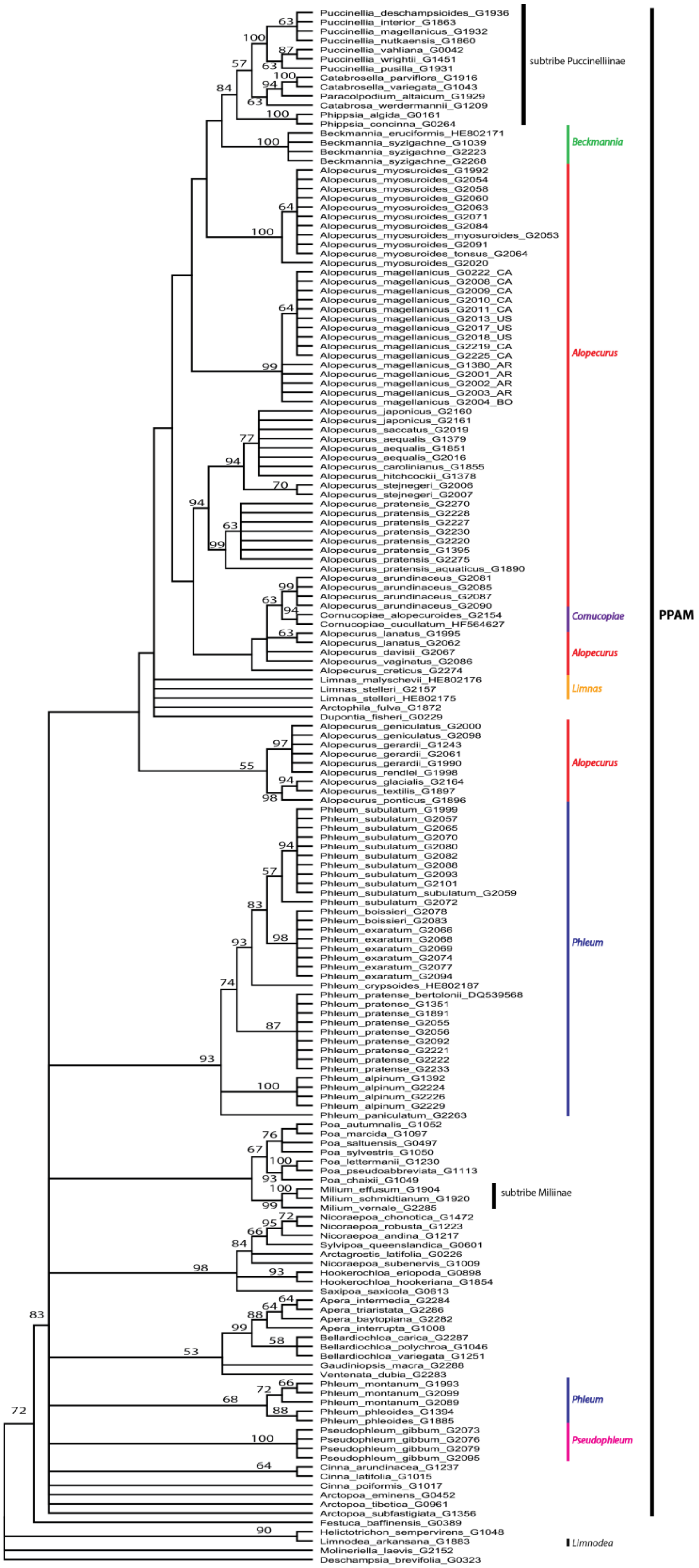

Figure 2. Strict consensus of 100,000 trees from maximum parsimony analysis of nuclear DNA region ITS. Bootstrap values >50 are indicated above branches. Important taxa and major clades are labelled.

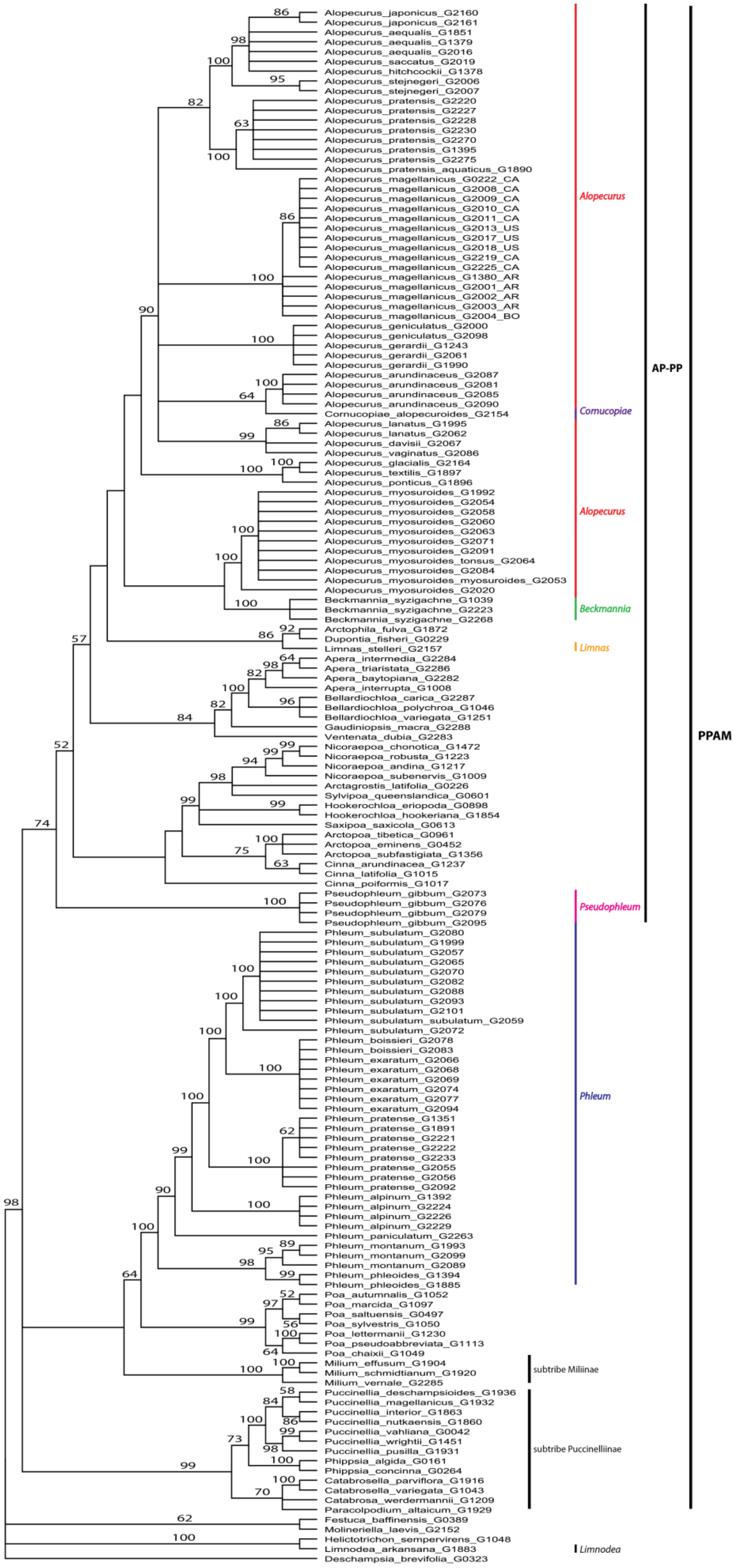

Figure 3. Strict consensus of 100,000 trees from maximum parsimony analysis of combined nuclear DNA regions ETS and ITS. Bootstrap values >50 are indicated above branches. Important taxa and major clades are labelled.

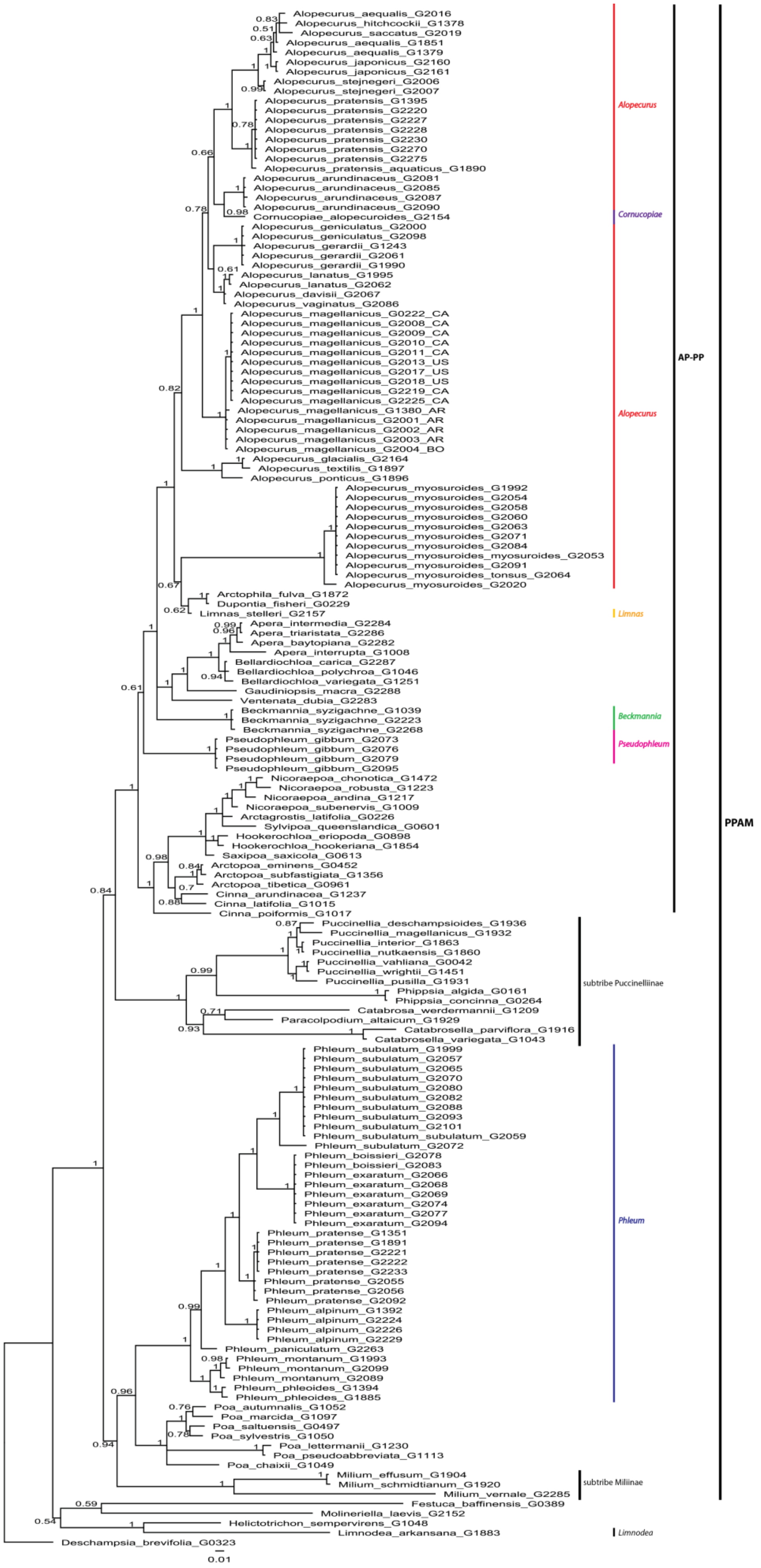

Figure 4. Phylogram from Bayesian analysis of combined nuclear DNA regions ETS and ITS. Posterior probability values >0.5 are indicated above branches. Important taxa and major clades are labelled..

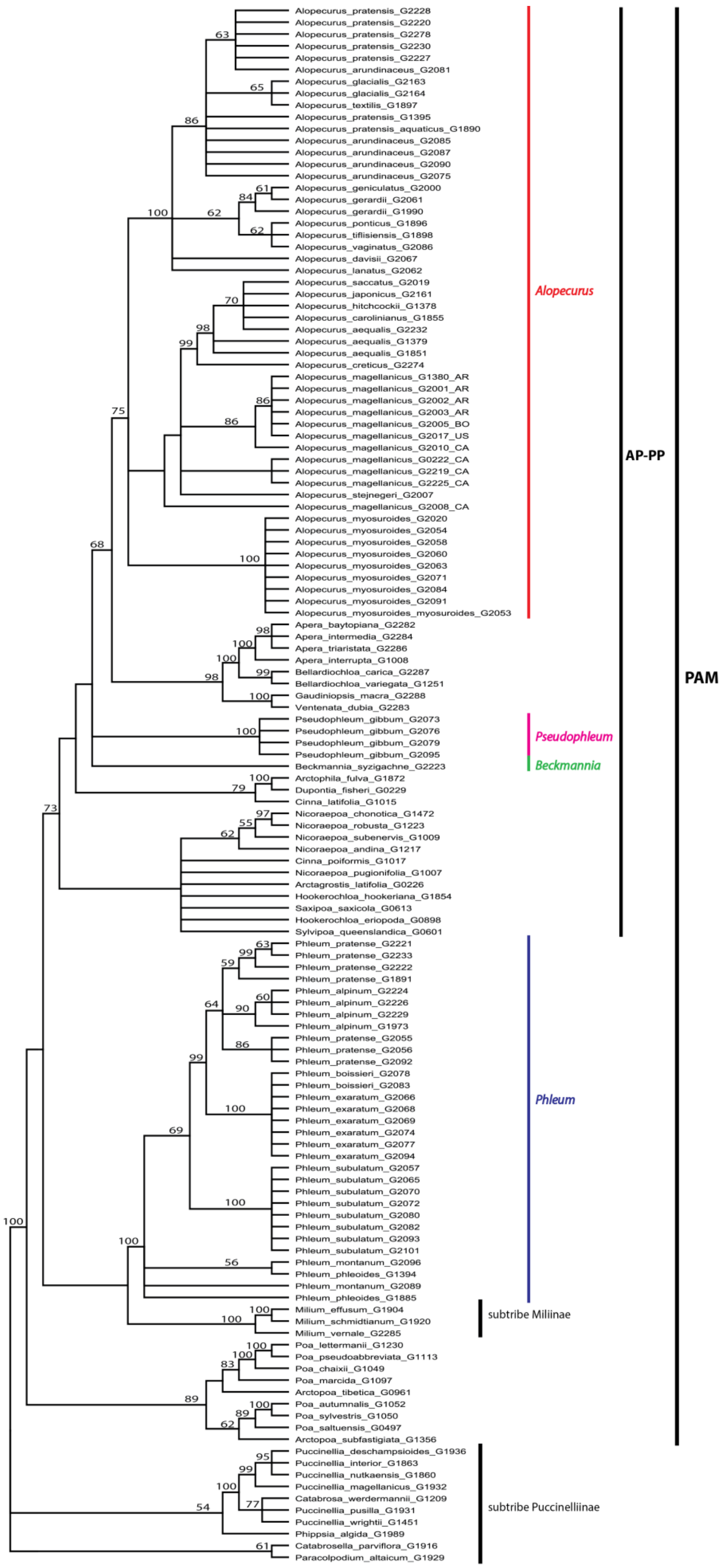

Figure 5. Strict consensus of 100,000 trees from maximum parsimony analysis of combined plastid DNA regions TF, rpoB and matK. Bootstrap values >50 are indicated above branches. Important taxa and major clades are labelled.

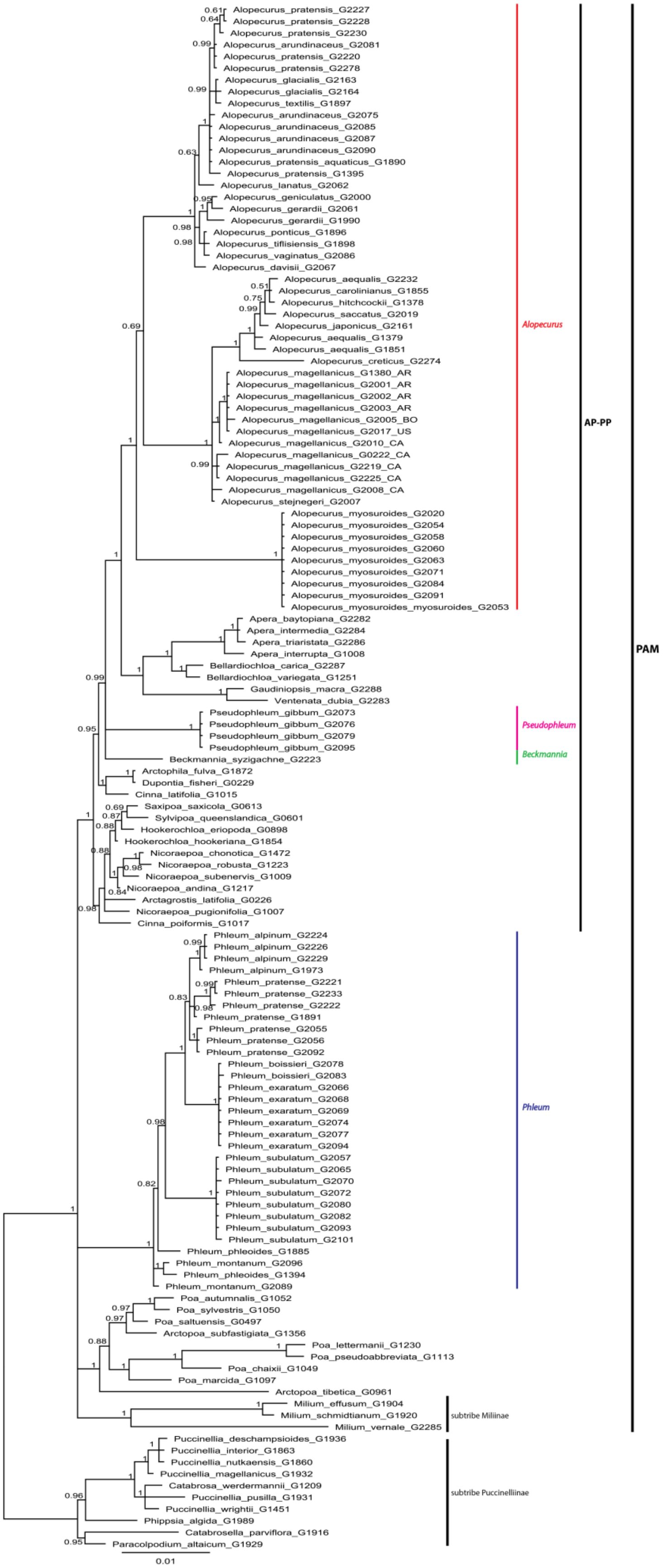

Figure 6. Phylogram from Bayesian analysis of combined plastid DNA regions TF, rpoB and matK. Posterior probability values >0.5 are indicated above branches. Important taxa and major clades are labelled.

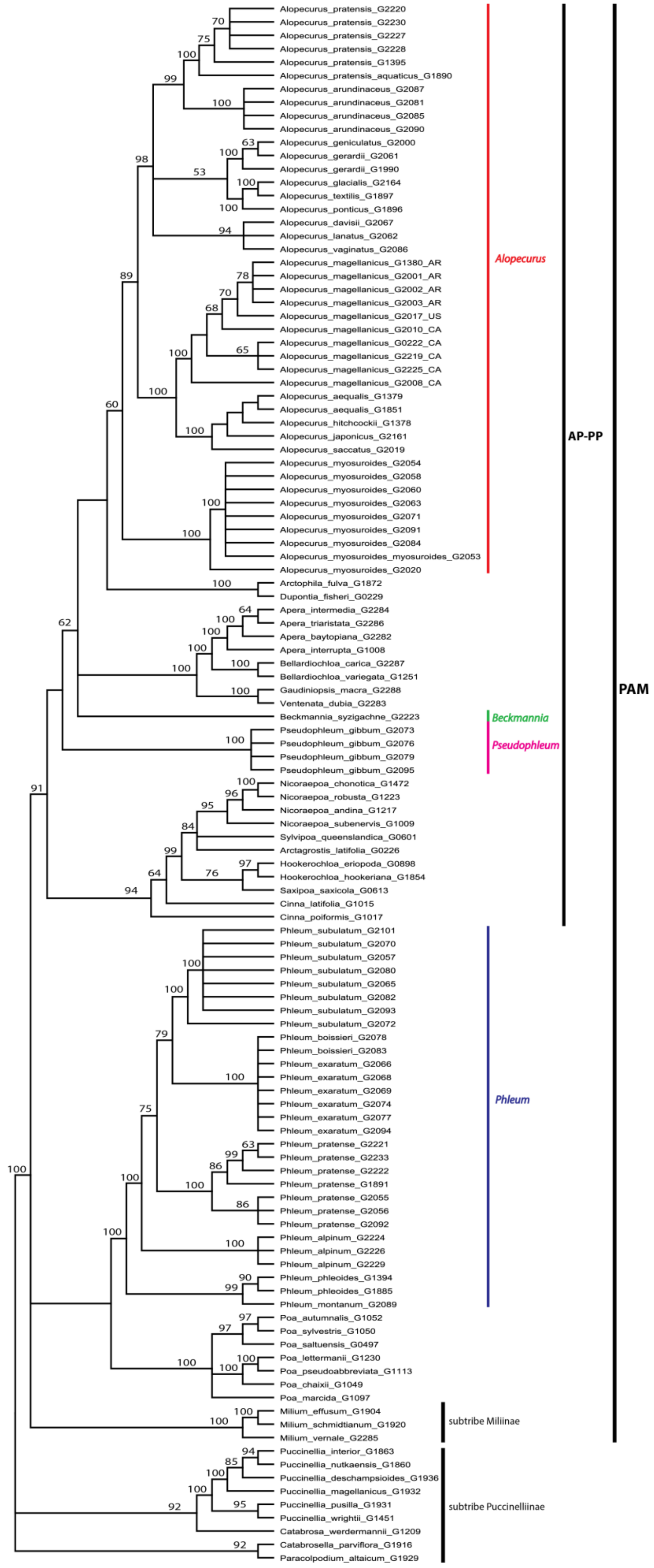

Figure 7. Strict consensus of 100,000 trees from maximum parsimony analysis of combined nuclear and plastid DNA regions ETS, ITS, TF, rpoB, and matK. Bootstrap values >50 are indicated above branches. Important taxa and major clades are labelled.

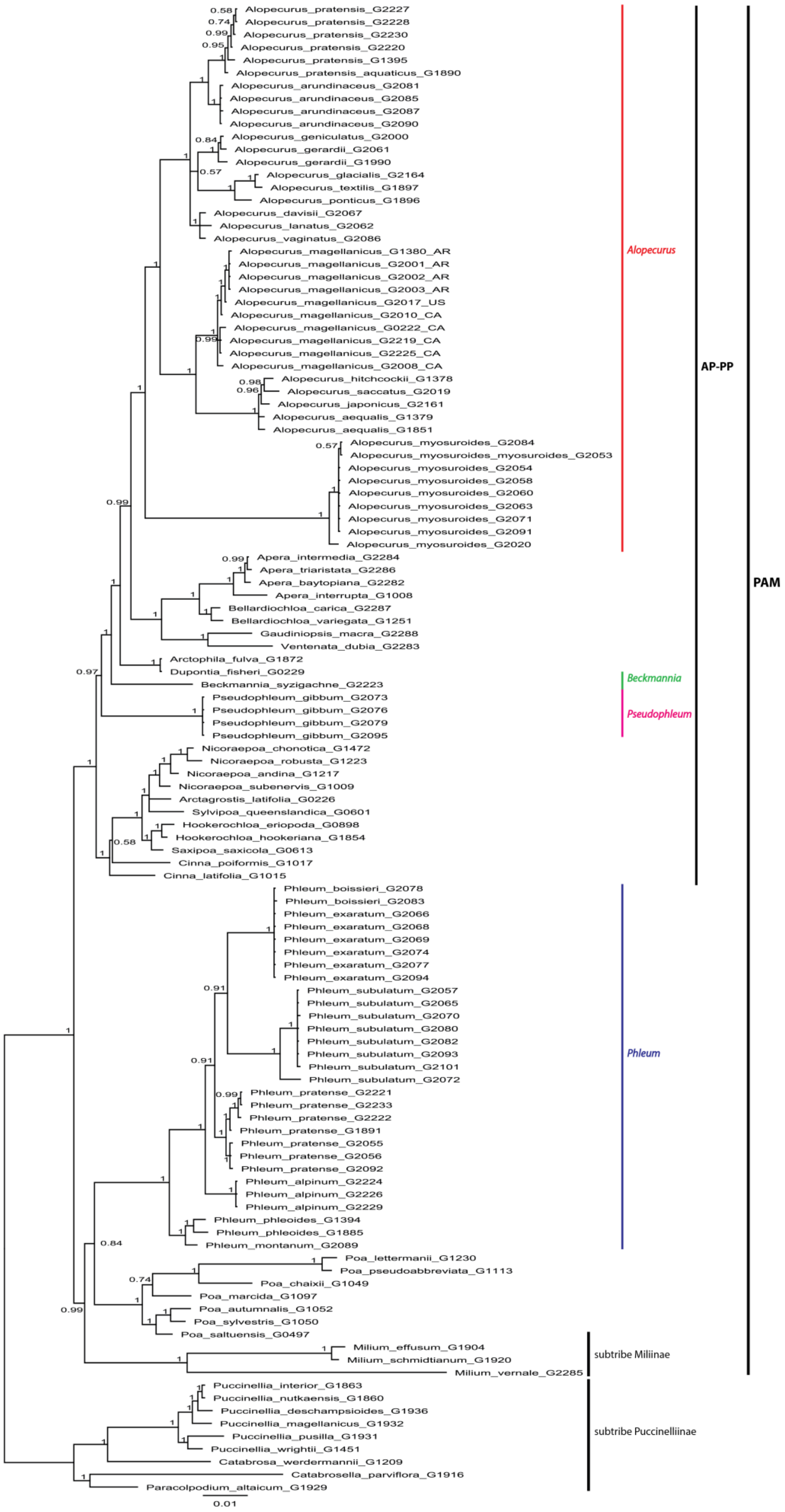

Figure 8. Phylogram from Bayesian analysis of combined nuclear and plastid DNA regions ETS, ITS, TF, rpoB, and matK. Posterior probability values >0.5 are indicated above branches. Important taxa and major clades are labelled.

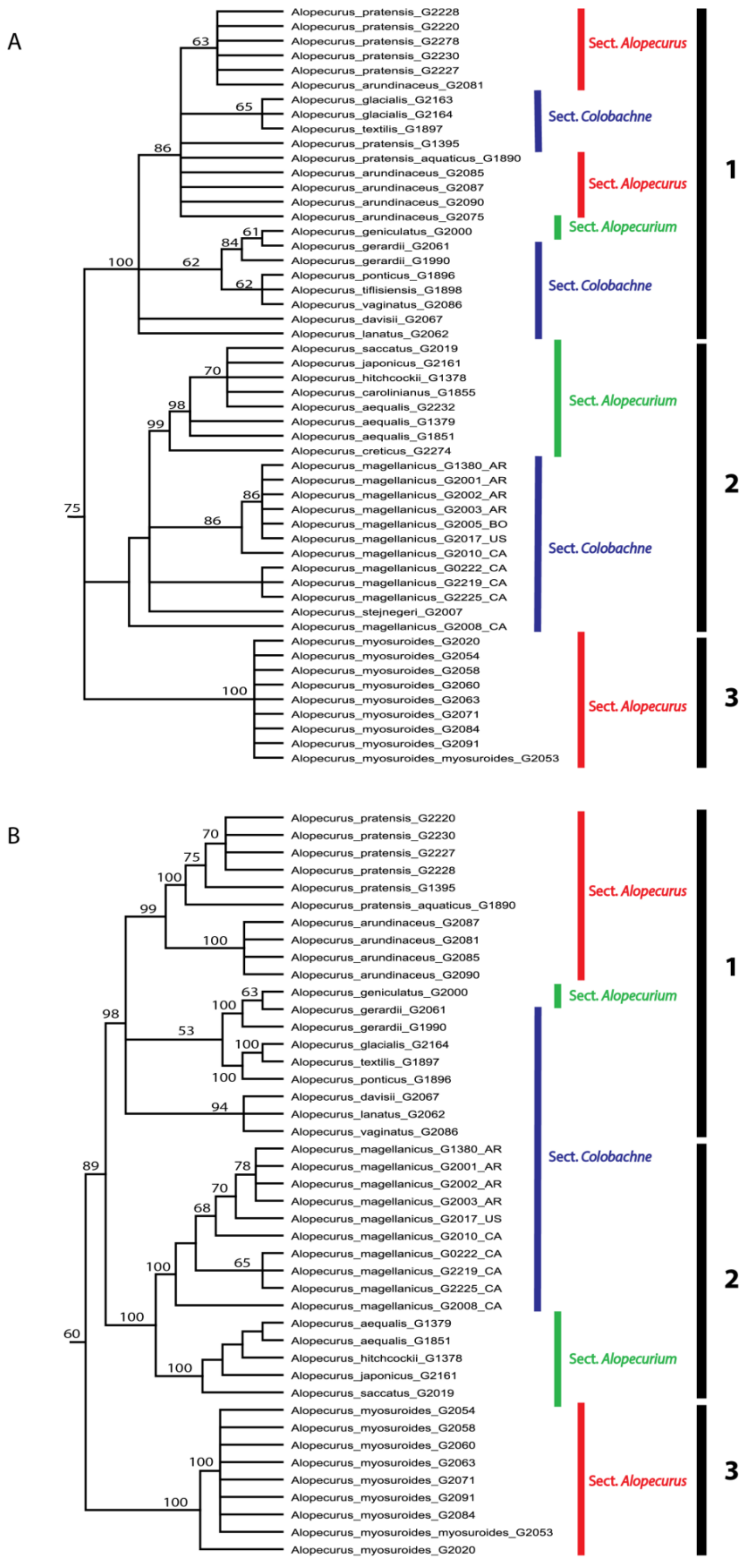

Figure 9. Close-up of *Alopecurus* clade from maximum parsimony plastid (TF+rpoB+matK ; panel A) and combined (ETS+ITS+TF+rpoB+matK; panel B) analyses showing infrageneric classification based on Dogan (1999) and proposed revisions.

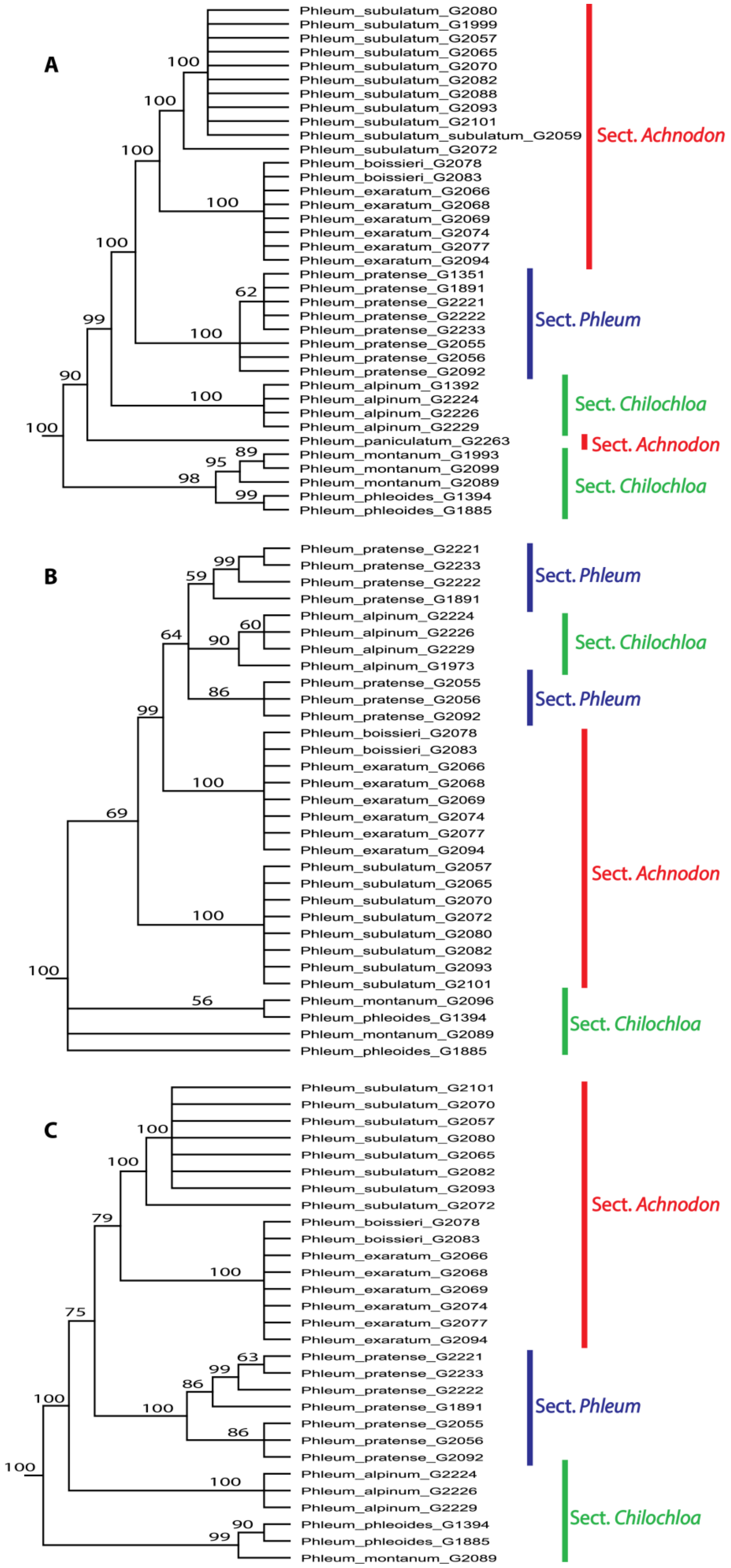

Figure 10. Close-up of *Phleum* clade from maximum parsimony nuclear ETS+ITS (panel A), plastid TF+rpoB+matK (panel B), and combined ETS+ITS+TF+rpoB+matK (panel C) analyses showing infrageneric classification based on Dogan and Us (1995).

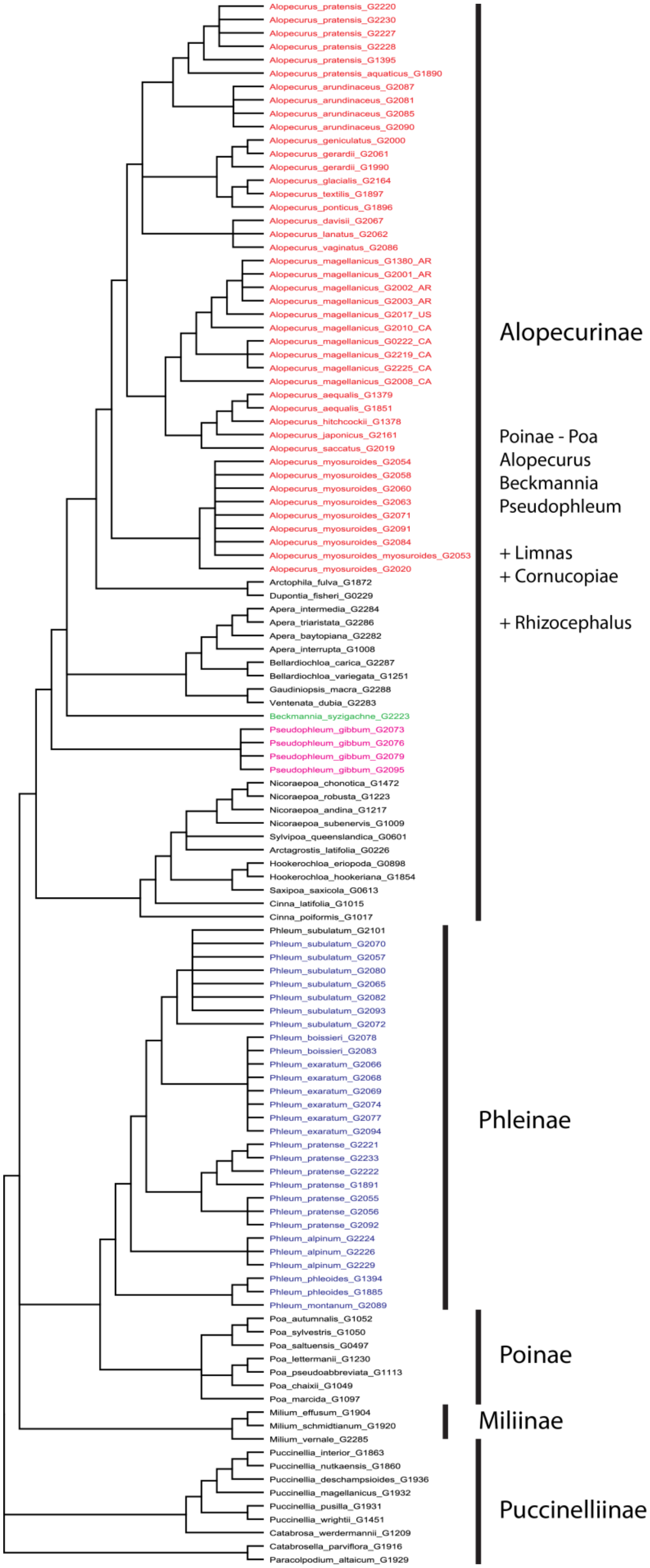

Figure 11. Overview of newly proposed subtribal organization including subtribes Alopecurinae, Phleinae, Poinae, Miliinae, and Puccinelliinae.

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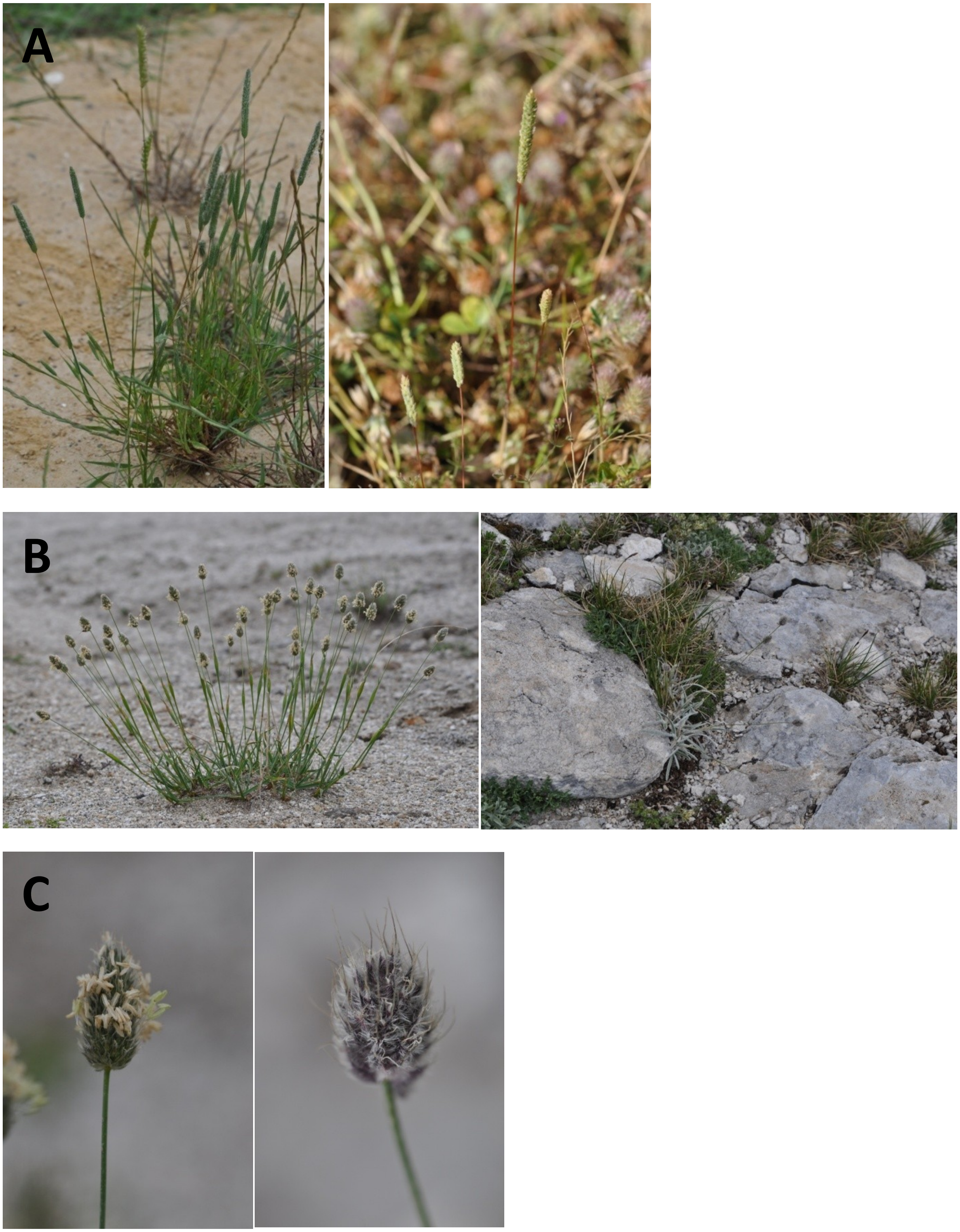
Appendix A. Photographs of some Alopecurinae genera found in Turkey. Panel A: *Phleum subulatum* (left), *Pseudophleum gibbum* (right). Panel B: Habit of *Alopecurus gerardii* (left) and *Alopecurus lanatus* (right). Panel C: Inflorescence of *Alopecurus gerardii* (left) and *Alopecurus lanatus* (right).



Appendix B. Specimens of all subtribes and outgroups used in DNA analysis. All specimens that were included in an analysis with a particular region, and for which a GenBank number is not yet present, will be submitted to GenBank for each of those regions. Voucher includes: collector, collection number, herbarium.

[a] included in ETS analysis
[b] included in ITS analysis
[c] included in ETS+ITS analyses
[d] included in TF+rpoB+matK analyses
[e] included in ETS+ITS+TF+rpoB+matK analyses

Taxon	DNA ID	Voucher	Country of origin	ETS GenBank No.	ITS GenBank No.	TF GenBank No.
Alopecurus aequalis [abcde]	G1379	Peterson et al. 17115 US	Argentina			
Alopecurus aequalis [abcde]	G1851	Soreng 7841 US	Norway			
Alopecurus aequalis [abc]	G2016	Schofield 1799 US	USA, Alaska			
Alopecurus aequalis [d]	G2232	07-Saarela 0988 CAN				
Alopecurus arundinaceus [d]	G2075	Gillespie et al. 10499 CAN	Turkey			
Alopecurus arundinaceus [abcde]	G2081	Gillespie et al. 10533 CAN	Turkey			
Alopecurus arundinaceus [abcde]	G2085	Gillespie et al. 10562 CAN	Turkey			
Alopecurus arundinaceus [abcde]	G2087	Gillespie et al. 10600 CAN	Turkey			
Alopecurus arundinaceus [abcde]	G2090	Gillespie et al. 10621 CAN	Turkey			
Alopecurus carolinianus [bd]	G1855	Rothfels et al. 2817 CAN	USA, North Carolina			
Alopecurus creticus [abd]	G2274	Turland 1496				
Alopecurus davisii [abcde]	G2067	Gillespie et al. 10459-1 CAN	Turkey			
Alopecurus geniculatus [abcde]	G2000	Soreng 3827 US	Greece			
Alopecurus geniculatus [abc]	G2098	Soreng 4043				
Alopecurus gerardii [abc]	G1243	Soreng et al. 7494 US	Greece	GQ324238	EU792344	EU792432
Alopecurus gerardii [abcde]	G2061	Gillespie et al. 10399-1 CAN	Turkey			

Taxon	DNA ID	Voucher	Country of origin	ETS GenBank No.	ITS GenBank No.	TF GenBank No.
Alopecurus gerardii [abcde]	G1990	Soreng 7498 US	Greece			
Alopecurus glacialis [d]	G2163	Atha & Siukaev 3319 MO	Georgia			
Alopecurus glacialis [abcde]	G2164	Abdaladze et al. 429 MO	Georgia			
Alopecurus hitchcockii [abcde]	G1378	Peterson et al. 16256 US	Peru			
Alopecurus japonicus [abc]	G2160	Tsugaru 32566 MO	Japan			
Alopecurus japonicus [abcde]	G2161	Tsugaru et al. 1495 MO	Japan			
Alopecurus lanatus [abc]	G1995	Soreng 4042 US	Turkey			
Alopecurus lanatus [abcde]	G2062	Gillespie et al. 10408-1 CAN	Turkey			
Alopecurus magellanicus [abcde]	G0222	Gillespie et al. 6576 CAN	Canada, Nunavut	GQ324237	EU792345	DQ353966
Alopecurus magellanicus [abcde]	G1380	Peterson et al. 17397 US	Argentina			
Alopecurus magellanicus [abcde]	G2001	Peterson 17217 US	Argentina			
Alopecurus magellanicus [abcde]	G2002	Peterson 19191 US	Argentina			
Alopecurus magellanicus [abcde]	G2003	Peterson 17502 US	Argentina			
Alopecurus magellanicus [abc]	G2004	Renvoize 4505 US	Bolivia			
Alopecurus magellanicus [ad]	G2005	Renvoize 4512 US	Bolivia			
Alopecurus magellanicus [abcde]	G2008	Moss 891 US	Canada, Alberta			
Alopecurus magellanicus [abc]	G2009	Dore 12164 US	Canada, Alberta			
Alopecurus magellanicus [abcde]	G2010	Breitung 15736 US	Canada, Alberta			
Alopecurus magellanicus [abc]	G2011	Dore & Breitung 12458 US	Canada, Alberta			
Alopecurus magellanicus [abc]	G2013	Swallen 6450 US	USA, Montana			
Alopecurus magellanicus [abcde]	G2017	Hulten s.n. July 11, 1961 US 2523446	USA, Alaska			

Taxon	DNA ID	Voucher	Country of origin	ETS GenBank No.	ITS GenBank No.	TF GenBank No.
Alopecurus magellanicus [abc]	G2018	Cantlon & Gillis 57-1566 US	USA, Alaska			
Alopecurus magellanicus [abcde]	G2219	Gillespie et al. 7568 CAN	Canada, Nunavut			
Alopecurus magellanicus [abcde]	G2225	Gillespie et al. 8403 CAN	Canada, Nunavut			
Alopecurus myosuroides [abc]	G1992	Soreng 4036 US	Turkey			
Alopecurus myosuroides [abcde]	G2020	Barta 2004-290 US	Germany			
Alopecurus myosuroides [abcde]	G2054	Gillespie et al. 10342 CAN	Turkey			
Alopecurus myosuroides [abcde]	G2058	Gillespie et al. 10369 CAN	Turkey			
Alopecurus myosuroides [abcde]	G2060	Gillespie et al. 10390 CAN	Turkey			
Alopecurus myosuroides [abcde]	G2063	Gillespie et al. 10408-1 CAN	Turkey			
Alopecurus myosuroides [abcde]	G2071	Gillespie et al. 10479 CAN	Turkey			
Alopecurus myosuroides [abcde]	G2084	Gillespie et al. 10561 CAN	Turkey			
Alopecurus myosuroides subsp. *myosuroides* [abcde]	G2053	Gillespie et al. 10313 CAN	Turkey			
Alopecurus myosuroides [abcde]	G2091	Gillespie et al. 10623 CAN	Turkey			
Alopecurus myosuroides subsp. *tonsus* [abc]	G2064	Gillespie et al. 10417 CAN	Turkey			
Alopecurus ponticus [abcde]	G1896	Soreng 7961 US	Russia, Cabardino-Balkaria			
Alopecurus pratensis subsp. *aquaticus* [abcde]	G1890	Soreng 7942 US	Russia			
Alopecurus pratensis [abcde]	G1395	Soreng et al. 7651b US	Kyrgystan			
Alopecurus pratensis [abcde]	G2220	Saarela 1288 CAN	Canada, British Columbia			
Alopecurus pratensis [abcde]	G2227	Saarela 844 CAN	Canada, British Columbia			

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Alopecurus pratensis [abcde]	G2228	Saarela 535 CAN	Canada, British Columbia			
Alopecurus pratensis [abcde]	G2230	07-JMS-0889 CAN	Canada, British Columbia			
Alopecurus pratensis [abc]	G2270	K.J. & A.K. 2006 MO				
Alopecurus pratensis [abc]	G2275	Bangerter 5026 MO				
Alopecurus pratensis [d]	G2278	Skvartsov & Kostina MO				
Alopecurus rendlei [ab]	G1998	Soreng 3800 US	Greece			
Alopecurus saccatus [abcde]	G2019	Howell 23254 US	USA, California			
Alopecurus stejnegeri [abc]	G2006	Beals 303 US	USA, Alaska			
Alopecurus stejnegeri [abcd]	G2007	Sladen 225 US	USA, Alaska			
Alopecurus textilis [abcde]	G1897	Soreng 7962a US	Russia, Cabardino-Balkaria			
Alopecurus tiflisiensis [d]	G1898	Soreng 7962b	Russia			
Alopecurus vaginatus [abcde]	G2086	Gillespie et al. 10588-1 CAN	Turkey			
Apera baytopiana [abcde]	G2282	Gillespie et al. 10312 CAN	Turkey			
Apera intermedia [abcde]	G2284	Gillespie et al. 10536 CAN	Turkey			
Apera interrupta [abcde]	G1008	Peterson et al. 19173 US	Argentina	GQ324242	EU792364	EU792439
Apera triaristata [abcde]	G2286	Gillespie et al. 10594 CAN	Turkey			
Arctagrostis latifolia [abcde]	G0226	Gillespie et al. 6586 CAN	Canada, Nunavut	GQ324245	EU792351	DQ353969
Arctophila fulva [abcde]	G1872	Peterson 18565 US	Canada, N.W.T.			
Arctopoa eminens [abc]	G0452	Gillespie 7010-2 CAN	Canada, Labrador	GQ324247	GQ324470	EU792446
Arctopoa subfastigiata [abcd]	G1356	W6 18199 W94096	Mongolia	GQ324251	EU792372	EU792448
Arctopoa tibetica [abcd]	G0961	Olonova 2003-07 CAN	Russia	GQ324252	EU792369	EU792444

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Beckmannia eruciformis subsp. *eruciformis*[a]	G2097	Soreng 3801 US				
Beckmannia eruciformis[b]					HE802171	
Beckmannia syzigachne[abc]	G1039	Soreng 3513 US	USA, Wyoming	GQ324255	EU792342	DQ353965
Beckmannia syzigachne[abcde]	G2223	07-JMS-1055 CAN	Canada, British Columbia			
Beckmannia syzigachne[abc]	G2268	Ricketson & Walter 222 MO				
Bellardiochloa carica[abcde]	G2287	Gillespie et al. 10634 CAN	Turkey			
Bellardiochloa polychroa[abc]	G1046	Soreng & Guney 4191 US	Turkey	GQ324256	EU792363	
Bellardiochloa variegata[abcde]	G1251	Soreng et al. 7519-1 US	Greece	GQ324257	EU792361	EU792438
Catabrosa werdermannii[abcde]	G1209	Soreng & Soreng 7150 US	Chile	GQ324258	EU792334	DQ353958
Catabrosella parviflora[abcde]	G1916	Soreng 8013a US	Russia, Adygea			
Catabrosella variegata[abc]	G1043	Soreng & Davis 4044 US	Turkey			
Cinna arundinacea[abc]	G1237	Soreng & Olonova 7462 US	USA, West Virginia	GQ324260	EU792343	EU792436
Cinna latifolia[abcde]	G1015	Peterson et al. 19769 US	USA, California	GQ324261	GQ324473	GQ324396
Cinna poiformis[abcde]	G1017	Peterson 20588 US	Peru			
Cornucopiae alopecuroides[a]	G2153	Samuelsson 4536 MO	Syria			
Cornucopiae alopecuroides[abc]	G2154	Samuelsson 4645 MO	Syria			
Cornucopiae cucullatum[b]					HF564627	
Deschampsia brevifolia[abc]	G0323	Gillespie & Consaul 6810b CAN	Canada, N.W.T.	GQ324262	EU792328	DQ353962
Dupontia fisheri[abcde]	G0229	Gillespie et al. 6589 CAN	Canada, Nunavut	GQ324266	EU792346	DQ353967
Festuca baffinensis[abc]	G0389	Gillespie & Consaul 6920 CAN	Canada, N.WT.	GQ324268	GQ324476	DQ353951
Gaudiniopsis macra[abcde]	G2288	Gillespie et al. 10636 CAN	Turkey			

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Helictotrichon sempervirens [abc]	G1048	Soreng 4622 US	USA, New York	GQ324269	EU792325	DQ353955
Hookerochloa eriopoda [abcde]	G0898	Jacobs 9128 NSW	Australia	GQ324270	EU792349	EU792433
Hookerochloa hookeriana [abcde]	G1854	Gillespie et al. 7352 CAN	Australia			
Limnas malyschevii [b]					HE802176	
Limnas stelleri [abc]	G2157	Kharkevich & Buch s.n. 6-July-1979 MO				
Limnas stelleri [b]					HE802175	
Limnodea arkansana [abc]	G1883	Soreng 7860 US	USA, Mississippi			
Milium effusum [abcde]	G1904	Soreng 7978 US	Russia, Cabardino-Balkaria			
Milium schmidtianum [abcde]	G1920	Soreng 8019 US	Russia			
Milium vernale [abcde]	G2285	Gillespie et al. 10544 CAN	Turkey			
Molineriella laevis [abc]	G2152	Soreng 3740a US	Spain			
Nicoraepoa andina [abcde]	G1217	Soreng & Soreng 7182 US	Chile	GQ324275	EU792354	DQ353971
Nicoraepoa chonotica [abcde]	G1472	Soreng & Soreng 7309 US	Chile	GQ324276	EU792355	DQ353974
Nicoraepoa pugionifolia [d]	G1007	Peterson et al. 17128 US	Argentina	GQ324277	GQ324478	GQ324398
Nicoraepoa robusta [abcde]	G1223	Soreng & Soreng 7358 US	Chile	GQ324279	EU792357	DQ353975
Nicoraepoa subenervis [abcde]	G1009	Peterson et al. 19186 US	Argentina	GQ324281	EU792358	EU792443
Paracolpodium altaicum [abcde]	G1929	Olonova 06-18	Russia, Altai			
Phippsia algida [abc]	G0161	Gillespie et al. 6253 CAN	Canada, Nunavut		EU792331	DQ353949
Phippsia algida [d]	G1989	Gillespie 6913-2 CAN	Canada			
Phippsia concinna [abc]	G0264	Gillespie et al. 6668-1 CAN	Canada, Nunavut			
Phleum alpinum [abc]	G1392	Soreng et al. 7290 US	Chile			

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Phleum alpinum [d]	G1973	Soreng 7165 US	Chile			
Phleum alpinum [abcde]	G2224	07-JMS-1224 CAN	Canada, British Columbia			
Phleum alpinum [abcde]	G2226	07-JMS-1234 CAN	Canada, British Columbia			
Phleum alpinum [abcde]	G2229	07-JMS-1323 CAN	Canada, British Columbia			
Phleum boissieri [abcde]	G2078	Gillespie et al. 10518 CAN	Turkey			
Phleum boissieri [abcde]	G2083	Gillespie et al. 10554 CAN	Turkey			
Phleum crypsoides [b]					HE802187	
Phleum exaratum [a]	G1991	Soreng et al 3847 US	Turkey			
Phleum exaratum [abcde]	G2066	Gillespie et al. 10451 CAN	Turkey			
Phleum exaratum [abcde]	G2068	Gillespie et al. 10462 CAN	Turkey			
Phleum exaratum [abcde]	G2069	Gillespie et al. 10466 CAN	Turkey			
Phleum exaratum [abcde]	G2074	Gillespie et al. 10498 CAN	Turkey			
Phleum exaratum [abcde]	G2077	Gillespie et al. 10511 CAN	Turkey			
Phleum exaratum [abcde]	G2094	Gillespie et al. 10630 CAN	Turkey			
Phleum montanum [abc]	G1993	Soreng 4075 US	Turkey			
Phleum montanum [abcde]	G2089	Gillespie et al. 10614-2 CAN	Turkey			
Phleum montanum [ad]	G2096	Gillespie et al. 10637-1 CAN	Turkey			
Phleum montanum [abc]	G2099	Soreng 4264 US	Turkey			
Phleum paniculatum [abc]	G2263	Q.L.Li 3 98-2-7 MO				
Phleum phleoides [abcde]	G1394	Soreng et al. 7576 US	Kyrgystan			

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Phleum phleoides [abcde]	G1885	Soreng 7881 US	Russia, Stavropol			
Phleum pratense subsp. *bertolonii* [b]					DQ539568	
Phleum pratense [abc]	G1351	Soreng 4293 BH	USA, New York	GQ324284	EU792341	DQ353964
Phleum pratense [abcde]	G1891	Soreng 7943 US	Russia			
Phleum pratense [abcde]	G2055	Gillespie et al. 10347 CAN	Turkey			
Phleum pratense [abcde]	G2056	Gillespie et al. 10362 CAN	Turkey			
Phleum pratense [abcde]	G2092	Gillespie et al. 10627 CAN	Turkey			
Phleum pratense [abcde]	G2221	07-JMS-1269 CAN	Canada, British Columbia			
Phleum pratense [abcde]	G2222	07-JMS-1045 CAN	Canada, British Columbia			
Phleum pratense [abcde]	G2233	07-JMS-1002 CAN	Canada, Manitoba			
Phleum subulatum [abc]	G1999	Soreng 3772 US	Greece, Crete			
Phleum subulatum [abcde]	G2057	Gillespie et al. 10367 CAN	Turkey			
Phleum subulatum [abcde]	G2065	Gillespie et al. 10430 CAN	Turkey			
Phleum subulatum [abcde]	G2070	Gillespie et al. 10478 CAN	Turkey			
Phleum subulatum [abcde]	G2072	Gillespie et al. 10486 CAN	Turkey			
Phleum subulatum [abcde]	G2080	Gillespie et al. 10531 CAN	Turkey			
Phleum subulatum [abcde]	G2082	Gillespie et al. 10539 CAN	Turkey			
Phleum subulatum [abc]	G2088	Gillespie et al. 10601 CAN	Turkey			
Phleum subulatum [abcde]	G2093	Gillespie et al. 10628 CAN	Turkey			
Phleum subulatum [abcde]	G2101	Soreng 3794				

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Phleum subulatum subsp. *subulatum* [abc]	G2059	Gillespie et al. 10377 CAN	Turkey			
Poa autumnalis [abcde]	G1052	Soreng 4680 US	USA, Maryland	GQ324294	EU792379	DQ353979
Poa chaixii [abcde]	G1049	Soreng 4677 US	Russia			
Poa lettermannii [abcde]	G1230	Soreng & Soreng 7434 US	USA, Colorado	GQ324345	GQ324521	GQ324431
Poa marcida [abcde]	G1097	Soreng & Soreng 5974 US	USA, Oregon			
Poa pseudoabbreviata [abcde]	G1113	Soreng & Soreng 6032-1 US	USA, Alaska	GQ324370	EU792398	DQ353997
Poa saltuensis [abcde]	G0497	Gillespie 7043 CAN	Canada, Ontario	GQ324374	EU792378	EU792451
Poa sylvestris [abcde]	G1050	Soreng 4678-3 US	USA, Mayland	GQ324384	EU792375	DQ353980
Pseudophleum gibbum [abcde]	G2073	Gillespie et al. 10497 CAN	Turkey			
Pseudophleum gibbum [abcde]	G2076	Gillespie et al. 10510 CAN	Turkey			
Pseudophleum gibbum [abcde]	G2079	Gillespie et al. 10519 CAN	Turkey			
Pseudophleum gibbum [abcde]	G2095	Gillespie et al. 10632 CAN	Turkey			
Puccinellia deschampsioides [abcde]	G1936	Elven SUP03-101/ 1-3 CAN	Canada, Yukon			
Puccinellia interior [abcde]	G1863	Gillespie & Consaul 6359 CAN	Canada, N.W.T.			
Puccinellia magellanicus [abcde]	G1932	Soreng 7361 US	Chile			
Puccinellia nutkaensis [abcde]	G1860	Soreng & Soreng 6322 US	USA, Alaska			
Puccinellia pusilla [abcde]	G1931	Soreng 7360 US	Chile			
Puccinellia vahliana [abc]	G0042	Gillespie 5808 CAN	Canada, Nunavut	GQ324285	EU792336	EU854591
Puccinellia wrightii [abcde]	G1451	Elven SUP03-9-1 CAN				
Saxipoa saxicola [abcde]	G0613	Gillespie et al. 7353-4 CAN	Australia	GQ324391	GQ324557	GQ324463

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Sylvipoa queenslandica [abcde]	G0601	Gillespie et al. 7320 CAN	Australia	GQ324393	GQ324559	GQ324466
Ventenata dubia [abcde]	G2283	Gillespie et al. 10445 CAN	Turkey			