

**Molecular Phylogeny of *Poa L. sensu lato*
(Poaceae) with a Focus on West Asian Species**

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Abstract

Poa L., is known as a highly diverse cosmopolitan genus with taxonomic difficulties that includes unknown species and species with uncertain affinities mainly in West Asia and North Africa. *Poa* also exhibits a close relationship with two West Asian genera, *Eremopoa* Roshev. and *Oreopoa* H. Scholz & Parolly. This study was conducted to: 1) fill the gap of information on the affinities between *Poa* species with an emphasis on West Asian *Poa*; 2) revise and evaluate the accuracy of traditional infrageneric classification of West Asian *Poa*; and 3) clarify the relationship between *Poa* and two allied genera of Poaceae Barnhart, *Eremopoa* and *Oreopoa*. DNA molecular evidence from present phylogenetic analyses of West Asian species of *Poa*, *Eremopoa* and *Oreopoa*, resulted in some great findings as follow: I) *Poa caucasica* Trin., which is currently assigned to subsection *Nivicolae* of section *Poa* from subgenus *Poa* resolved as a unique new distinct lineage within *Poa*. II), New treatments are suggested for *Poa densa* Troitsky, *Poa masenderana* Freyn & Sint., *Poa cenisia* All., *Poa psychrophila* Boiss. & Heldr. and *Poa lipskyi*. III) Three unclassified species of *Poa pseudobulbosa*, *Poa diversifolia* and *Poa aitchisonii* are assigned here to subgenus *Poa* and supersection *Poa*. IV), The present molecular evidence supports inclusion of *Eremopoa* in *Poa* and confirms reduction of *Eremopoa* to a level of subgenus of *Poa*. V) Present phylogenetic analyses also indicate that monotypic genus *Oreopoa* H. Scholz & Parolly is part of *Poa*. These findings require an urgent modification in subgeneric and sectional classification of the genus *Poa*.

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1 Introduction

1.1 General Introduction

Among widespread grasses, *Poa* (Poaceae Barnhart) has been pointed out as a genus with the lack of satisfactory infrageneric classification (Clayton & Renvoize 1986). There is no worldwide monograph for this huge and extensively diverse genus. Exploring the uncertain evolutionary relationships within West Asian *Poa*, which are poorly investigated and include unclassified *Poa* species, as well as the affinity between *Poa* and its two closely allied genera *Eremopoa* and *Oreopoa* have been the main interests of this thesis. Since local floristic studies have not been able to ascertain the infrageneric evolutionary relationship in *Poa*, the present investigation used informative molecular characteristics to address the unknown phylogenetic relationships of West Asian species. Also, some areas for further studies have been highlighted in this research.

1.2 Poaceae, Significance, Evolution, Intrafamilial Classification

The Gramineae (grass family) or Poaceae Barnhart (1895) is the fourth largest plant family and the second largest family of monocotyledonous flowering plants (Peterson 2013). The grass family is ecologically and socioeconomically important: The family provided a major portion (3/4) of human diets (Guala 2002) and the main sources of forage for domestic animals; Grasslands support diverse invertebrate and vertebrate communities (Hilu 1985); Grasses have also many other economically important uses in bedding, roofing, fences, walls, flooring; Grasses are considered as important elements of the landscape, and also important elements in the development and stabilization of

soil (Shantz 1954). Shantz (1954) estimated that grasslands compose 20% of the vegetation cover of the earth's surface (as cited in Kellogg 2001), while Peterson recently (2013) estimated that grasslands occupy 31 – 43% of the earth's surface due to their ecological dominance.

Three evolutionary groups comprising Bambusoideae, Pooideae and a group including all other grasses were determined to be the Poaceae division on the basis of the cladistic analysis (Kellogg & Campbell 1987; Davis & Soreng 1993; Kurt 1999) and taxonomic data (Clayton & Renvoize 1986; Watson & Dallwitz 1992; Kurt 1999). The tropical forest-edge species that were recognized as the origin of Bambusoideae and thus Poaceae adapted three various developmental routes and the species of these paths evolved to survive in three distinct habitats (Clayton & Renvoize 1986; Stebbins 1987; Renvoize & Clayton 1992; Kurt 1999). The first evolutionary group (Bambusoid) settled in tropical woods, the second group (Poooids) moved into cool-temperate habitats, and the third group (Panicoideae/Arundinoideae/Chloridoideae) developed adaptations to survived in warm season grasslands and savannahs. (Renvoize & Clayton 1992).

In general, some essential evolutionary changes in grasses that enabled them to get radiant to open areas (Clayton 1981; Renvoize & Clayton 1992) and “even benefit from grazing pressure, fire, and drought” (Kurt 1999, p. 10) are: 1) diminution in the floral size and initial numbers of flower segments, 2) choosing abiotic cross pollination strategy and use wind as a functional vector, and 3) the efficient adjustment in both morphology and physiology (Stebbins 1981; Clayton 1981; Connor 1981; Coughenour 1985) as cited in (Kurt 1999).

The estimate of the number of genera belonging to Poaceae has been increased over time from approximately 600 – 700 (Clayton & Renvoize 1986; Watson & Dallwitz

1992) to more than 800 genera (Bouchenak-Khelladi *et al.* 2008; Peterson 2013). There was an agreement on 10,000 as the approximate number of species for the family (Clayton & Renvoize 1986; Watson & Dallwitz 1992; Bouchenak-Khelladi *et al.* 2008) before Peterson (2013) reported 11,000 species for Poaceae.

The Grass Phylogeny Working Group II (GPWG II 2001) provided a classification of 12 subfamilies and Peterson (2013) accepted 50 tribes and 81 subtribes for these 12 subfamilies for the family.

The base chromosome number across Pooideae tribe Poeae is “ $x = 7$ ” (Stebbins 1956), which is known only for two other tribes: Triticeae and Bromeae. According to the most recent studies (Gillespie *et al.* 2008), subtribe Poinae consists of 15 genera and more than 550 species distributed worldwide, mostly throughout cool temperate regions and arctic areas.

Recent plastid analyses determined two clades with a strong support within tribe Poeae. The first clade (PAM clade) consists of subtribes Poinae (including *Poa*), Alopecurinae and Miliinae. This clade and its sister clade including subtribe Puccinelliinae (P clade) form the second clade (PPAM clade) (Gillespie *et al.* 2007, 2008). Based on the molecular phylogenetic analyses of PPAM clade, Gillespie *et al.* (2010) recognized subtribe Phleinae as a distinct lineage and modified the classification of the PPAM.

All investigated genera in this study belong to five subtribes including Alopecurinae Dumort., Phleinae Benth., Miliinae Dumort., Puccinelliinae Soreng & Davis and Poinae Dumort. The Genus *Poa* L., which is the main subject of this study, belongs to the subtribe Poinae.

1.3 *Poa* L.

Poa L. (1753) is the largest genus of Poaceae (Soreng *et al.* 2010). *Poa* is a Greek name for grasses or fodder (Watson & Dallwitz 1992), but the genus also has several common names in different areas. In Europe and Asia, it is called meadow-grass. In North America, the genus is known as bluegrass and sometimes spear grass. Tussock (Edgar 1986; Soreng *et al.* 2009) is the common name for *Poa* in New Zealand and Australia. *Poa* has an important role in soil stabilization, sport fields, lawns and forage (Tzvelev 1976; Zhu *et al.* 2006).

Poa species are found from sea level (e.g., *P. macrantha* Vasey native to British Colombia) to altitudes approximately 4300 – 4450 m (e.g. *P. chamaeclinis* Pilg. in Bolivia, Peru & Mexico) (Soreng & Peterson 2012). *Poa* species also have a huge range of heat tolerance and adapt to arctic zones like *P. abbreviata* R. Br. in Ellesmere Island (Gillespie & Boles 2001) to high temperatures around 45 °C in locations around the Lout Desert in Iran (Bor 1970). Some species of *Poa* are reported from areas with high annual average humidity indicating that *Poa species* are highly compatible with moisture. The species in central Iran mostly occur in humidity close to zero.

In late Tertiary, a climate change (the world wide temperature reduction) and distribution of the largely cold-acclimated taxa of *Poa* group occurred. These two events caused “the retreat of forests” and the further advent of cold adapted pasturelands first in the northern and later in some southern hemisphere’s regions. The cold resistance (primarily obtained within the diversification of the subfamily Pooideae) is one of the main ecological key adaptation during the Pliocene/Pleistocene, the time of distribution of the *Poa* group (Hoffmann 2013).

“Features such as intercalary meristems, drought tolerance, vegetative reproduction, and dispersal mechanisms played important roles in the extensive Tertiary diversification of *Poa* in cooler climates” (Clark 2004 p. 509). During the rapid and global diversifications of *Poa* some lineages were distributed to the regions with the different ecological circumstances and are spreading actively (Hoffmann 2013).

1.3.1 Morphology of *Poa*

Significant morphological characters of the genus *Poa* are provided here as described in published floristic studies (Roshev. 1934; Parsa 1950; Bor & Guest 1968; Bor 1960, 1970; Tutin 1980; Tzvelev 1976; Cope 1982; Edmondson 1980, 1985; Soreng *et al.* 2003; Zhu *et al.* 2006; Soreng 2007; Soreng *et al.* 2009; Davidse *et al.* 2009):

Poa is an annual or perennial herb. Most *Poa* species are monoecious but a few dioecious species exist. The hollow culm is erect. The inflorescence is a lax or contracted panicle with 1 – 9 branches per node. The spikelet is laterally compressed and comprises 2 – 8 (rarely 10) bisexual, female or rarely male flowers. Glumes are unequal or sub-equal, usually glabrous and strongly keeled in most species. The upper glume has 3 – 5 veins and usually is 1/2 – 1/3 of the length of the spikelet. The lower glume has 1 – 3 veins and the length is usually more than 1/3 of the length of the spikelet. The typical lemma in the genus is awnless (except in *P. flabellata* Lam. Raspail) with a blunt apex laterally compressed and usually is distinctly keeled in the back with 5 – 7 veins. Callus can be glabrous or rarely with a line of hairs around the base of the lemma, but the presence of a separate tuft of rough hair (web) on callous is known only in the genus *Poa*. The palea has smooth and glabrous margin and is almost always shorter than lemma (sub- equal to the lemma or infrequently 2/3 length of lemma). Palea with

distinctly separated green keels is usually scabrid or smooth sometimes pilose to villous.

Poa species are distinguished from each other by employing informative morphological characters such as presence or absence of rhizome, anther length, presence or absence of web or tuft of hairs on the callus, pubescence of lemma body and palea keels, scabridity of panicle branches and length of ligules.

1.3.2 Distribution of *Poa* Species

Poa species are cosmopolitan C₃ grasses and distributed in both the northern and southern hemisphere at different altitudes, and at latitudes ranging from arctic to temperate regions and extending to most subtropical and tropical mountains (Giussani 2000). They are not present in tropical and subtropical lowlands (GPWG II 2012).

Based on a preliminary study of plastid DNA of a small sample size and estimation of biogeographical events, Soreng (1990) grouped *Poa* species in the following phylogenetic groups: 1) a group of *Poa* species that is originated in Eurasia, 2) two groups in South America including 62 species, 3) one group in New Zealand, Australia and Malaysia and 4) a small group of *Poa* species that colonized North America.

Nearly 35% of the total number of species of *Poa* (175 species and 57 lower taxa and four hybrids) are reported from the New World (Soreng *et al.* 2003). These species belong to two subgenera, 18 sections and four subsections (Soreng *et al.* 2003).

Forty three species of *Poa* are native to Australia (Sharp & Simon 2002; Jacobs *et al.* 2008; Walsh *et al.* 2009). Thirty two native species of *Poa* occur in New Zealand and one endemic species is shared between Australia and New Zealand (Edgar 1986; Edgar & Connor 2000).

Thirty five native species are reported for Malesia (Veldkamp 1994). Of several

African *Poa* species *P. binata* and *P. leptoclada* are native to South Africa and some native species also are reported from mountains in Kenya and Tanzania.

The polyploid species distributed in all continents. Of 13 sections of *Poa*, in which diploids are known, almost all species are centered in, or restricted to western Eurasia, with three following species indigenous to North America (Soreng *et al.* 2007, 2010): *P. pseudoabbreviata* Roshev. of subgenus *Stenopoa* section *Abbreviatae*, which has a Beringian distribution; two other species endemic to North America *P. lettermanii* Vasey of subgenus *Stenopoa* section *Abbreviatae* and *P. occidentalis* Vasey belonging to the subgenus *Poa* section *Homalopoa*.

Since the focus of this thesis is on West Asian *Poa*, they will be described in more detail in section 1.3.5.

1.3.3 Diversity, Traditional Infrageneric Classification and Molecular Phylogeny of *Poa* Species

A high speciation rate resulted in a rapid diversification in *Poa* (Hoffmann *et al.* 2013). There are approximately 1304 described species in *Poa* (Soreng *et al.* 2014). Recently estimates based on many synonymy events reduced the number of described *Poa* species to 500+ accepted species (Soreng 1990; Soreng *et al.* 2007; Gillespie *et al.* 2007; Soreng *et al.* 2010).

A large number of *Poa* species share a high degree of morphological similarity. In addition, *Poa* exhibits extensive polyploidy and hybridization so *Poa* is considered as a taxonomically difficult genus (Gillespie & Soreng 2005; Soreng *et al.* 2010).

Due to the large number of *Poa* species most local floras developed a sectional classification or subgeneric arrangements. *Flora Orientalis* (Boiss. 1884) assigned two sections for 29 species of *Poa*: section *Eupoa* Griseb. with five-nerved lemma and

section *Psilantha* K. Koch. with three-nerved lemma. *Flora Iranica* (Bor 1970) classified 40 species of *Poa* in 11 groups including *Bulbosae* subgenus *Ochlopoa*, *Pratenses*, *Glabratae*, *Alpinae*, *Paucidentatae*, *Triviales*, *Steriles*, *Nemorales*, *Setulosae* and *Palustres* but there is no identification key for these groups. Cope (1982) reported 33 species of *Poa* for Pakistan but did not categorize them in subgenera or sections. *Flora Europaea* (Tutin 1980) categorized *Poa* species in 17 sections, but no subgeneric arrangement. The Grasses of USSR (Tzvelev 1976), Catalogue of New World Grasses (Soreng *et al.* 2003) and Flora of North America (Soreng 2007) sorted *Poa* species in two subgenera *Arctopoa* and *Poa*, and gave a sectional arrangement. Zhu *et al.* (2006) employed some morphological traits including margin of lemma, glumes, surface, presence or absence of rhizomes and bulbs scabridity of palea keels and the number of veins of lower glumes and defined five of six subgenera with subordinate sections for the flora of China. Table 1 provides a brief history of infrageneric classification of *Poa* (subgeneric and sectional) on the basis of floristic studies from 1884 – 1990 and molecular studies from 1990 to 2014 (Boiss. 1884; Bor 1970; Tzvelev 1976; Soreng & Davis 2000; Soreng *et al.* 2003; Gillespie & Soreng 2005; Zhu *et al.* 2006; Gillespie *et al.* 2007, 2008, 2009; Soreng *et al.* 2010; and the most recent treatment Soreng *et al.* 2014). In 1990 Soreng conducted the first cladistic analysis on plastid DNA restriction sites of *Poa* and constructed a cladogram including five major groups supporting *Dioicopoa* is a distinct clade and distinguishing three subgenera for *Poa* including *Arctopoa* (Griseb.) Prob. *Dioicopoa* (E. Desv.) J.R. Edm. and *Poa*. Phylogenetic study on cpDNA restriction sites of *Poa* (Gillespie & Soreng 2005) supported five major clades including: 1) ArcSyl, *Poa* subgenus *Arctopoa* sections *Arctopoa* and *Aphydris*, and *Poa* subgenus *Poa* sect. *Sylvestres*; 2) BAPO, *Poa* subgenus *Poa* sections

(*Bolbophorum* | *Alpinae*) (*Parodiochloa* | *Ochlopoa*); 3) SPOSTA, *Poa* subg. *Poa* sections (*Secundae* (*Pandemos* (*Orienos* | *Stenopoa* | *Tichopoa* | *Abbreviatae*))); 4) PoM, *Poa* subgenus *Poa* sections (*Poa* | *Macropoa*); 5) HAMBADD, *Poa* subgenus *Poa* sections (*Homalopoa*, *Acutifoliae*, *Brizoides*, *Madropoa*, *Austrofestuca*, *Dasypoa*, *Dioicopoa*, and informal groups “Punapoa” and “Australopoa”). The arrangement for the divergence of these clades is: ArcSyl (BAPO (SPOSTA ((PoM) (HAMBADD))).

The phylogram resulting from maximum parsimony analysis on plastid DNA region of *trnTLF* (Gillespie *et al.* 2007) supported removing the subgenus *Andinae* from *Poa* and raising that to the level of genus *Nicoraepoa* Soreng & L. J. Gillespie. The species belonging subgenus *Poa* formed two clades that were recognized as two supersections *Poa* and *Homalopoa* (Dumort.) Soreng & L. J. Gillespie (Gillespie *et al.* 2007). Two genera *Austrofestuca* (Tzvelev) E.B. Alexeev (Gillespie *et al.* 2007) and *Parodiochloa* (C.E. Hubb.) Soreng & L. J. Gillespie were included in *Poa* as sections respectively within subgenus *Poa* supersection *Homalopoa* and subgenus *Ochlopoa* (Gillespie *et al.* 2005). Also monotypic genus *Anthochloa* Nees and Meyen was included in *Poa* as a member of the subgenus *Poa*.

The subgenus *Arctopoa* and the section *Sylvestres* form the basal clade in all phylogenetic trees based on cpDNA (Gillespie & Soreng 2005; Gillespie *et al.* 2007, 2008, 2010). This indicates that they have similar cpDNA, but the subgenus *Arctopoa* and the section *Sylvestres* have dissimilar morphology. Subgenus *Arctopoa* is placed outside of the *Poa* clade in phylogenetic trees based on nuclear (ITS) data (Gillespie *et al.* 2008). This significant evidence can justify that a new subgenus is required for *Sylvestres* members and subgenus *Arctopoa* should be excluded from *Poa* and be treated as a genus of ancient hybrid origin (Gillespie *et al.* 2008).

In phylogenetic trees of Australian *Poa* from analyses of cpDNA (*trn*TLF), nrDNA (ETS and ITS) and combined cpDNA (*trn*TLF) and nrDNA (Gillespie *et al.* 2009) the placement of *Poa* subgenera *Sylvestres*, *Ochlopoa*, *Stenopoa* and *Poa* was congruent with the trees from all previous published studies (Gillespie & Soreng 2005; Gillespie *et al.* 2007, 2008). Two species, *Saxipoa* Soreng L.J. Gillespie & S.W.L. Jacobs and *Sylvipoa* Soreng L.J. Gillespie & S.W.L. Jacobs, previously treated as *P. queenslandica* C.E. Hubbard and *P. saxicola* R. Br., were not resolved in the *Poa* clade. Instead, they were resolved with the members of other genera of *Poinae* clade (including *Cinna* and *Alopecurus* respectively from subtribes Cinninae and Alopecurinae) (Gillespie *et al.* 2009). So, they were removed from *Poa* as two new monotypic genera.

The monotypic genus *Tovarochloa* was treated as the member of *Poa* (Soreng *et al.* 2003).

In phylogenetic analyses of nuclear DNA (ITS) and plastid DNA (*trn*TLF) regions genera *Dissanthelium* and monotypic *Tovarochloa* species were nested within the broadly defined monophyletic genus *Poa* (Refulio-Rodríguez *et al.* 2012). Refulio-Rodríguez *et al.* (2012) suggested inclusion of two additional sections, *Dissanthelium* and *Tovarochloa*, in *Poa* subgenus *Poa* supersection *Homalopoa* and made two essential combinations for the sectional classification.

A new major clade is positioned between *Poa* clades including subgenera *Sylvestres*, *Ochlopoa*, *Stenopoa* and *Poa* in phylogenetic studies (Gillespie *et al.* 2007, 2008, 2009, 2010; Refulio-Rodríguez *et al.* 2012). This clade that was designated as *Eremopoa* clade will be fully described in 1.3.3.

The base chromosome number in *Poa* like other members of the tribe *Poeae* is $n=7$ (Stebbins 1956; Soreng *et al.* 2007), although 91 percent of *Poa* species with

chromosome counts available are polyploid (Soreng *et al.* 2010). *Poa* is a genus with a high ploidy levels and the chromosome counts of “ $2n=14 - 266$ ” has been reported for *Poa* (Hultén 1942; Hitchcock 1951; Marsh 1952; Tutin 1952; Munz 1959; Bowden 1961; Tzvelev 1976; Hiesey & Nobs 1982; Kellogg 1985; Soreng 1985; Duckert-Henroid & Favarger 1987; Soreng 1991^a, 1991^b, 1998; Soreng & Keil 2003; Gillespie & Soreng 2005; Zhu *et al.* 2006; Gillespie *et al.* 2007).

Polyploidy has the main role in speciation (Madlung 2012) and much of polyploidy in *Poa* is due to allopolyploidy (Stebbins 1950; Darmency & Gasquez 1997; Brysting *et al.* 2000, 2004; Patterson *et al.* 2005; Soreng *et al.* 2010). Also apomixis which is asexual reproduction without fertilization (Winkler 1908) commonly occurs in some subgenera such as *Poa* and *Stenopoa* (Soreng 1990; Zhu *et al.* 2006).

Most *Poa* species are polyploid. Thirty species (Thirteen to 15% of the diploid have polyploid populations too. Only 9% of *Poa* species are just diploids (Soreng *et al.* 2010). There are no reported diploid species for the subgenus *Sylvestres* (V.L. Marsh *ex* Soreng) Soreng & L.J. Gillespie (Soreng *et al.* 2010), while the following sections with diploid species belong to the other four known subgenera: 1) *Alpinae* (Hegetschweiler *ex* Nyman) Stapf, *Arenariae* (Hegetschweiler *ex* Nyman) Stapf and *Micrantherae* Stapf from subgenus *Ochlopoa* (Aschers. & Graebn.) Hylander, 2) *Homalopoa* Dumort. *Macropoa* F., Hermann *ex* Tzvelev and *Nivicolae* (Roshev.) Tzvel. belonging to subgenus *Poa*, and 3) *Abbreviatae* Nannf. *ex* Tzvel. *Kolymenses* Prob. *Oreinos* (Aschers. *et* Graebn.) Jiras *Pandemos* Aschers. & Graebn. and *Tichopoa* (Aschers. *et* Graebn.) Maire of subgenus *Stenopoa* (K. Koch) Stapf.

1.3.4 Biogeography of *Poa*

Poa was hypothesized to have a Eurasian center of origin and radiation of the sectional groups on the basis of two lines of evidence: 1) the greatest number of sections or groups of *Poa* occur in Eurasia (Soreng 1990); 2) Eurasia was thought to be the center of geographical distribution of basal lineages of *Poa* (Soreng 1990) and most of Poinae genera (Gillespie *et al.* 2009). However, in all phylogenetic trees resulted from the recent molecular analyses (Gillespie *et al.* 2005, 2007, 2008, 2009, 2010; Soreng *et al.* 2010) subgenus *Sylvestres* has been the basal clade. This hypothesis was supported by floristic studies, since Takhtajan (1986) clearly suggested a Eurasian origin for temperate *Poa* and spreading towards the Nearctic regions, the southern hemisphere continents. On the basis of these studies, Inda *et al.* (2008) postulated a biogeographical scenario of the origin (described in next paragraph) and expansions of grasses and stated that Soreng (1990) suggested biogeographical events for *Poa* based on cpDNA phylogenetic analyses and came up with the same scenario in *Poa*, so this scenario could be the general rule for all members of the subfamily Pooideae.

According to Inda *et al.* (2008 p. 948) grasses diverged 60.2 ± 5.2 million years ago, Triticeae separated from Aveneae – Poeae $\sim 20.9 \pm 4.9$ million years ago. They estimated that the divergence and dispersal-vicariance of the broad-leaved and fine-leaved Loliinae took place ~ 13 million ago in the Miocene, in West Asia (Mediterranean) and then split towards Central and East Asia in a first polyploid radiation in northern hemisphere. Then they dispersed in North America (New World) $\sim 3.8 - 10$ million years ago in their secondary polyploid dispersal in northern hemisphere. They migrated and scattered in South America and in tertiary polyploid radiations in the

southern hemisphere. At the same time, several spreading events occurred between two continents South America and New Zealand Inda *et al.* (2008).

Based on McLoughlin's assessment (2001) indicating that a continental segregation took place ~37 million years ago and Australia and South America separated from Antarctica, Gillespie *et al.* (2009 p. 425) claimed that “distributions of subtribe Poinae taxa in Australasia resulted from long-distance dispersal, and not vicariance”.

The molecular phylogenetic analyses that Gillespie *et al.* (2009) performed on *trnTLF*, ETS and ITS DNA sequence data of Australian species of subtribe Poinae, indicated that *Poa* and other genera of Poinae scattered into Australia in different dispersal events. This study also estimated three and two spreading events for *Poa* respectively into New Zealand and New Guinea (Gillespie *et al.* 2009). Inda *et al.* (2008) indicated that several dispersal events took place from south west of Europe and north west of Africa 2.5 – 3.6 million years ago in the Pliocene. They assumed that the lineages with high polyploidy, which settled in recently deglaciated regions in both hemispheres, originated 1 – 2 million years ago in the Pleistocene. Hoffmann *et al.* (2013) assessed that diversifying of *Poa* alliance dates back 2.5 – 0.23 million years ago.

The phylogenetic research by Soreng (1990) resulted in the theory of the radiation of *Poa* species. Seven of 11 sections including diploid species are centered in western Eurasia (Soreng 1990). The other four studied sections including *Oreinos* and *Abbreviata* with diploid species are apomicts, Arctic distributed and are centered in Alaska (Soreng 1990). Species of the section *Poa* originated in northeastern Asia and share morphological similarities with species basically from New World. These two groups are sisters in cpDNA tree (Soreng 1990). Also section *Homalopoa* with diploid species centered in Europe is morphologically identical to some North American species

belonging to the sections of *Diversipoa*, *Dasyppoa*, *Homalopoa*, *Madropoa*, *Dioicopoa* and *Nervosae*, and the group *Punapoa* (Soreng 1990). In short, supersections *Homalopoa* and *Poa* diversified in their origin Eurasia, had a primary link to North America, and a secondary diversification back to Eurasia (Soreng 1990).

1.3.5 West Asian *Poa* Species and Allied Genera

Poa occurs in all West Asian countries including Armenia, Azerbaijan, Bahrain, Cyprus, Georgia, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Nakhchivan Autonomous Republic, Oman, Palestine and Sinai, Qatar, Saudi Arabia, Syria, Turkey, United Arab Emirates and Yemen. Among west Asian countries Iran and Turkey are rich in terms of diversity of *Poa* species.

Taxonomic studies on West Asian *Poa* species have been mostly restricted to floristic investigations based on morphological characteristics. The available floras provided useful information on diversity of *Poa* species, their distribution pattern and sporadic chromosome accounts. The floristic treatments of West Asian *Poa* species in these floras sometimes date back to 50 years ago or more and there has been no comprehensive revision for the species, thus no monograph for the genus. One of the reasons that the identification keys resulting from floristic studies are not always accurate and efficient is due to possible phenotypic plasticity problems. Also because morphological characteristics are not truly representative of evolutionary relationships in the grasses, there is a significant lack of information on the affinity between species from Asia, Middle East and North Africa and the classification attempts based on morphological characteristics fail to show the evolutionary relationships in such a huge genus.

By realizing the ancestral relationship within species, we can address the origin of species, investigate the evolutionary changes, approach to developmental changes associated with speciation and finally save endangered species.

Of 68 West Asian *Poa* species, 64 belong to four subgenera (Tzvelev 1976) congruent with the current subgeneric classification (Zhu *et al.* 2006), while Bor (1970) assigned other four species to a groups with the species that currently belong to several subgenera and sections and they have not been a subject of a taxonomical study after (See Appendix).

1.4 *Eremopoa* Roshev.

Eremopoa from the Greek *Eremos* (solitary, lonely) (Quattrocchi 2006) is a herbaceous grass. Also the Greek *Eremos* (a desert uninhabited area) and *Poa* (grass fodder) pointing to habitat is mentioned for *Eremopoa* (Watson & Dallwitz 1992). The genus is called Siberian grass.

Eremopoa Roshev. is a small genus belonging to subfamily Pooideae tribe Poeae and subtribe Poinae Roshevitz (1934) applied the name *Eremopoa* to *Poa persica* and introduced the genus *Eremopoa* with six species (described in part 1.3.3) to the world for the first time.

1.4.1 Morphology of *Eremopoa*

Significant morphological characters of the genus *Eremopoa* are provided here as described in published floristic studies by Roshevitz (1934), Parsa (1950), Bor (1960, 1970), Bor & Guest (1968), Tzvelev (1976), Mobayen (1980), Tutin (1980), Scholz (1981), Cope (1982), Miller (1985), Clayton & Renvoize (1986), Soreng *et al.* (2003), Zhu *et al.* (2006), Darbyshire (2007), Rahmanian & Rahiminejad (2012) and Rahmanian

et al. (2014) as follows:

Eremopoa is an annual solitary and tufted herb with the slender, hollow and erect culm. The ribbed leaf sheath, which is longer than adjacent culm internodes is closed only at base. Leaves are non-auriculate and linear. Leaf upper surface is glabrous and smooth rarely scabrous, but leaf lower surface is scabrid. Leaf blade is flat or rolled and has an acuminate apex. The inflorescence is lax or rarely compressed panicle with 3 – 10 scabrous branches per node. It is delicate elliptic or narrowly-broadly ovate and comprises 2 – 6 florets within spikelets. The unequal glumes are shorter than floret, are ovate to lanceolate and have acute or rounded apex and membranous margin. Upper glume is narrowly ovate and has three veins and is $>2/5$ of lemma, while the lower glume is lanceolate and 1(3) veined and is $2/7 - 2/3$ lemma. Lemma is lanceolate to oblong membranous glabrous or slightly hairy round on top and slightly keeled at the base parts of the five veins that it has. The marginal veins are pilose. The apex is acuminate or mucronate with a mucro up to 0.5 mm. The short callus is glabrous. Palea is equal to lemma or shorter than lemma. Palea is two keeled (See Figures 1 – 6).

1.4.2 Distribution of *Eremopoa* Species

Distribution of *Eremopoa* species suggests a Eurasian origin for *Eremopoa* followed by primary diversification and dispersal to the areas where currently *Eremopoa* occurs:

- ❖ West Asia: Iran (Bor 1970), Turkey (Miller 1985), Iraq (Bor & Guest 1968), Armenia (Bor 1970), Azerbaijan (Bor 1970; Tzvelev 1976), Syria, Palestine & Sinai (Post 1896), Lebanon (Mouterde 1966; Bou Dagher-Kharrat M. 2013) and Russia (Tzvelev 1976)

- ❖ South Asia: Pakistan and India (Bor 1960; Cope 1982)
- ❖ Central Asian highlands: Afghanistan (Podlech 2012), Mongolia (Rilke & Najmi 2011), Uzbekistan, Tajikistan & Kyrgyzstan (Bor 1970) and Turkmenistan & Kazakhstan (Bor 1970; Tzvelev 1976)
- ❖ Europe: Mediterranean area and Belgium (Tutin 1980)
- ❖ North East Africa: Egypt. Just two species occur in the tropics and is not recorded in entire distribution Egypt. (Boulos 2009)
- ❖ In North America it is just cultivated in Manitoba, Canada (Darbyshire 2007)
- ❖ East Asia: (One species as a synonym of *Poa diaphora*) Himalayas China (Zhu *et al.* 2006)

1.4.3 Diversity, Traditional Infrageneric Classification and Molecular Phylogeny of *Eremopoa* Species

For the first time Boissier (1884) classified *Poa* species in two sections: section *Psilantha* K. Koch. comprising *Eremopoa* species with 3-nerved glumes and section *Eupoa* Griseb. including other *Poa* species with 5-nerved glumes. Hackel (1887) defined three sections for *Poa*: *Dioicopoa* comprising American species, *Pseudopoa* including *Eremopoa* species, and *Eupoa* consisting of the rest of *Poa* species. Stapf (1897) followed Boissier's classification, but modified the taxonomical level of this arrangement and defined two subgenera for *Poa*: subgenus *Pseudopoa* K. Koch. consisting of *Eremopoa* species with 3-nerved glumes (often very faintly) and subgenus *Eupoa* Griseb. including the rest of *Poa* species with 5-nerved glumes (two lateral ones are sometimes obscure).

Roshevitz (1934) treated some species of *Poa* as a new genus *Eremopoa* since

these species differed from *Poa* L. on the basis of the diagnostic characteristics of lemma glumes and life style. In his taxonomic treatment for the Flora of USSR, Roshevitz (1934) included six species in this new genus. All of these species were previously treated as lower taxa of the genera *Aira* L., *Glyceria* Nutt., *Festuca* L., *Nephelochloa* Boiss. and *Poa*. These six species include *Eremopoa altaica* (Trin.) Roshev., *E. bellula* (Regel) Roshev., *E. multiradiata* (Trautv.) Roshev., *E. oxyglumis* (Boiss.) Roshev., *E. persica* (Trin.) Roshev. and *E. songarica* (Schrenk) Roshev.. He included *E. nephelochloides* to the genus based on the samples collected from Iran in 1945 by K ie.

Parsa (1950) reported five species of *Eremopoa* for Iran including two new species of *E. arundinacea* (L.) Roshev. and *E. nephelochloides* Roshev. along with three previously reported species including *E. oxyglumis* (Boiss.) Roshev., *E. persica* (Trin.) Roshev. and *E. songarica* (Schrenk) Roshev. No one followed the treatment of *E. arundinacea* (L.) Roshev. *Eremopoa bellula* was reported for Iran and *E. persica* and *E. songarica* were treated as two varieties of *E. persica*: *E. persica* var. *persica* and *E. persica* var. *songarica* (Schrenk) Bor in Flora Iranica and flora of Iraq (Bor & Guest 1968; Bor 1970) and by Rahmanian & Rahiminejad (2012). Mobayen (1980) mentioned only one species *E. persica* for Iran and treated the other taxa as synonyms of *E. persica*. Scholz (1981) added a new species *E. medica* H. Scholz to *Eremopoa* occurring in Iran.

Tzvelev (1966) treated *E. songarica* and *E. oxyglumis* as two subspecies of *Poa altaica*. He also reduced *E. multiradiata* as a subspecies of *Poa persica* in 1976. Overall he reported five taxa for Russia Federation (1976). Tutin (1980) reported only one species *E. songarica* for Europe.

Only two species *E. altaica* and *E. persica* have been described in Flora of

Pakistan (Cope 1982). Miller (1985) reported five species for Turkey. Catalogue of New World Grasses (Soreng *et al.* 2003) and Flora of North America (Darbyshire 2007) counted one species of the genus *Eremopoa*. Flora of China (Zhu *et al.* 2006) reunited these two genera and reduced *Eremopoa* to the level of subgenus of *Poa* subgenus *Pseudopoa* (K. Koch) Stapf.

Recent morphological study along with cytological study including chromosome counts of diploid and triploid accessions of Iranian *E. persica* $2n = 14$ and 21 and tetraploid samples of *E. songarica* $2n = 28$ by Rahmanian & Rahiminejad 2012 reduced *E. songarica* to the level of variety under *E. persica* based on a few distinguishing but somewhat overlapping characters as follows (the characters in brackets correspond to *E. songarica*): panicle branches 7 – 13 per node (3 – 14 per node), length of ligule 4 mm (1 – 1.5 mm), color and number of florets in spikelet 3 – 6 (15) greenish purple florets (1 – 2 (3) green florets), height 10 – 60 cm (10 – 30 cm), leaf blade shape wide linear with acute tip (narrow linear with long acuminate tip) and leaf width 2 – 4 mm (0.5 – 2 mm). The authors did not agree on treatment of *E. songarica* as *E. altaica* subsp. *songarica* but they accepted *E. songarica* as the variety of *E. persica* (Rahmanian & Rahiminejad 2012). In the most recent taxonomic revision of *Eremopoa* species of Iran Rahmanian *et al.* (2014) provided the most effective diagnostic characters among Iranian *Eremopoa* taxa as: “the number of spikelets florets, the width of leaf blade, sterility of the lowest 1 – 2 whorls of panicle, the number of branches per panicle’s whorl, the habit of plant and apex of lemma” (Rahmanian *et al.* 2014, Pp. 9 – 10). Based on these characters they recognized four species and three varieties for Iranian *Eremopoa* and provided an identification key to separate these taxa as follows: *E. nephelochloides*, *E. bellula*, *E. medica*, *E. persica* var. *oxyglumis*, *E. persica* var. *persica* and *E. persica* var. *songarica*.

Synonymy events between lower taxa of *Eremopoa* species and also between *Eremopoa* and the species of other relevant genera have been followed by the lead of Roshevitz (1934, 1945), Tzvelev (1976), Scholz (1981) and Miller (1985) to assign eight species for the genus *Eremopoa* from 12 described species (Clayton & Renvoize 1986). Also two lower taxa are determined for *E. altaica* subspecies *oxyglumis* (Boiss.) Tzvelev possessing spikelets with more than three florets and leaves greater than 2 mm broad and subspecies *songarica* (Schrenk) Tzvelev bearing 1 – 3 florets in each spikelet and leaves less than 2 mm broad.

Table 2 shows the infrageneric taxonomy of the genus *Eremopoa* based on the taxonomy of Soreng *et al.* (2014) which is followed in this study, and the geographical distribution of the *Eremopoa* species based on the local floras.

The chromosome numbers of *Eremopoa* species are reported as $2n=7, 14, 28, 42$ and 48 by Bor (1970), Tzvelev (1976) Miller (1985) and Zhu *et al.* (2006) suggesting possible hybridization and polyploidy in the genus.

In the cpDNA phylogenetic tree of Gillespie *et al.* (2007) a major clade (*Eremopoa*) including one sample of the genus *Eremopoa* was placed between two clades of BAPO and SPOSTA respectively comprising the subgenera *Ochlopoa* and *Stenopoa*. This clade with the same sample of *Eremopoa* was positioned in the same place in the phylogenetic trees resulting from analyses of plastid DNA (*trnTLF*) (Gillespie *et al.* 2008), nuclear DNA (ITS) (Gillespie *et al.* 2009, 2010) and also combined plastid DNA (*trnTLF*) and nrDNA (Gillespie *et al.* 2009, 2010).

1.5 *Oreopoa* H. Scholz & Parolly

Oreopoa H. Scholz & Parolly is a monotypic genus of subtribe Poinae belonging

to tribe Poeae of subfamily Pooideae. *Oreopoa* was not determined as any other known genera. Also its affinity with other genera of Poaceae was not clear. Scholz (2003) claimed *Oreopoa* is distantly related to *Poa*. *Oreopoa* was created, described, mapped and introduced to the world as a new genus (Parolly & Scholz 2004).

Oreopoa anatolica is endemic to Turkey and recorded only from the Kizlar sivrisi (3086 m) at altitudes of about 2670 – 2700 m. It grows on limestone (Parolly & Scholz 2004). Parolly and Scholz (2004) suggested that *Oreopoa* must be listed as a critically endangered genus on the basis of IUCN Red List Categories (Ginsburg 2001). They infer that overgrazing and very limited area of occurrence put it at the risk of extinction despite the fact that this population includes more than 200 mature individuals with numerous seedlings and young plants.

Significant morphological characters of the genus *Oreopoa* are described here on the basis of the published studies of Parolly and Scholz (2004): *Oreopoa* is a perennial herb with the densely tufted caespitose and almost erect culm. The culm is also distinctly striate and completely glabrous. It is shining pale greyish green at base and the nodes are not clear. Leaves are mostly basal if there is one or rarely two stem leaves, then they have the same size as the basal leaves. The narrowly linear leaf blade is convolute, firm pale grey with a shade of green and an acuminate apex. The lax inflorescence is a erect pyramidal panicle with two branches per node. The panicle is slender and glabrous (scabrid below the spikelets). The spikelet comprises 3 – 5 (6) florets. 1 – 3 glabrous and dark purple spikelets loosely are spaced on each branch. Unequal elliptic glumes are green partly with purple spots and hyaline margins that are dentate in half top and acute apex. They are not keeled. Upper glume has three veins and lower glume has 1(3) veins. The lemma is rounded or very slightly keeled in the back and has 5 (7) veins. The

lemma is purple with creamy hyaline margins that are dentate above. Palea is scabrid in upper half. The floret has two 1.5 mm long, bi-lobed lodicules and three stamens.

1.6 Investigation in Morphological Characteristics and Affinities of *Poa*, *Eremopoa* and *Oreopoa*

1.6.1 *Poa* Versus *Eremopoa*

The hairy lemma is strongly keeled all along the length in *Poa*, but the glabrous lemma (rarely includes a line of hair around the base) is round on top and slightly keeled at the base of the veins in *Eremopoa*. In *Poa*, apex of lemma is obtuse or pointed, rarely acute and always awnless, while in *Eremopoa* is obtuse to tapering and sometimes with a very short cusp “if mucronate, with up to 0.5 mm mucro” (Zhu *et al.* 2006, p. 299).

Sharing a large number of identical morphological traits with *Poa* (See Table 3), *Eremopoa* shows affinities to *Poa* in particular to section *Micrantherae* Stapf of the subgenus *Ochlopoa*.

Eremopoa species can be separated from *Poa* species by presenting narrower glabrous lemmas, which sometimes are slightly pilose near the base. In *Eremopoa* panicle branches have fewer florets and palea keels are smooth and not scaly (Clayton & Renvoize 1986; Scholz 2003).

1.6.2 *Poa* Versus *Oreopoa*

Oreopoa shares three characteristic with *Poa* species of alpine and wind-swept places: densely tufted caespitose, leaf with xerophytic structure and short stature habit. Based on this evidence, *Poa* and *Oreopoa* are thought to have a close evolutionary relationship and were suggested to be a separate genus. “It may be a relic genus” (Parolly & Scholz 2004, p. 148).

Oreopoa is distinguished from *Poa* by two traits. *Oreopoa* has convolute leaf blades while leaf blades are flat or folded along the mid-vein in *Poa* (rarely loosely convolute, e.g. in *Poa pratensis*). In *Oreopoa*, the purple lemma is rounded or very weakly keeled in back with 5 (7) veins (Parolly & Scholz 2004), while *Poa* usually has a strongly keeled and five veins (Dore & McNeill 1980; Soreng 1998) (not in annual species like annua group which have a slightly keeled lemma with three distinct veins (Aiken *et al.* 2011)).

1.7 Objectives

1. Since some West Asian *Poa* species have not been assigned to any subgenus or section of *Poa*, the first goal of this study was to determine the possible subgenera and sections that these species belong to. This is done on the basis of morphological characteristics, which were obtained from previous traditional floristic studies and the strong molecular evidence that this study provides.
2. The second aim of this research is to expand nuclear and plastid phylogeny of *Poa* to clarify the relationship between species that have not been a part of molecular phylogenetic analyses (in particular West Asian *Poa* species) and the *Poa* species with certain phylogenetic positions. So, besides two commonly used nuclear spacers (ETS and ITS) and *trnT-trnL-trnF* (*trnTLF*, a plastid region), two informative plastid DNA markers of *rpoB-trnC* and *matK* (plastid gene) are utilized in order to construct phylogenetic trees with further resolution and support.
3. The forth goal is to provide an insight into the evolution of *Oreopoa*, an allied genus and clarify the relationship between this West Asian genus and *Poa* for the first time and explore the origin of these taxa.

4. One hypothesis driving this thesis is that *Eremopoa*, which is currently known as a West Asian genus closely related to *Poa*, might be part of *Poa*. So the last aim of this study is to test this and resolve the affinity between *Poa* and *Eremopoa*.

2 Methodology

In this research, classification of Tzvelev (1976) was primarily followed for the Asian and European species, while recent applied modifications in taxonomy of *Poa* by Gillespie & Soreng (2005), Gillespie *et al.* (2007, 2008), Soreng (2007), Soreng & Gillespie (2007), Soreng *et al.* (2009, 2010) and Soreng & Peterson (2012) were also applied here for choosing the taxa and the methods. For the North and South American species of *Poa* classification of Soreng (2007) was followed, whereas the modifications in subgeneric classification (Gillespie *et al.* 2007, 2008 & 2009; Soreng & Gillespie 2007; Soreng *et al.* 2009 & 2010; Soreng & Peterson 2012) were applied here. For Australian species classification of Gillespie *et al.* (2009) was followed.

2.1 Materials

The materials in this study included plant leaf material and previously extracted genomic DNA samples. Leaf samples were collected from the field, herbarium specimens and silica gel samples at the Canadian Museum of Nature. The utilized samples were mainly identified by *Poa* specialists Lynn Gillespie and Robert Soreng. Scientific names, taxonomic position, voucher accession, country of origin and GenBank number of the utilized samples including *Poa*, *Eremopoa*, *Oreopoa* and outgroups are provided in the Appendix.

2.1.1 *Poa* Samples

One hundred and thirty seven *Poa* samples including 94 known and one unknown species were utilized in this study. Ninety of these species belong to 22 sections and five subsections, and three species have not been classified to section in the genus *Poa*.

Poa samples were included from different geographical regions as follow:

Ten samples representing ten species from five sections were collected in Europe mostly by R.J. Soreng and S.D. Stoneberg-Holt; 26 samples representing 22 species from 13 sections were sampled in North America mostly sampled by L.J. Gillespie and R.J. Soreng; 18 samples representing 16 species from five sections were collected in South America mainly by P.M. Peterson and R.J. Soreng; 16 samples representing 15 species from three sections were sampled in Australia mostly by L.J. Gillespie; two samples representing two species from a section were sampled by F. Hennion from Sub-Antarctic Islands; eleven samples representing ten species from five sections were collected in East Asia mostly collected by J.F. Wendel and collaborators, and by Olovana; and four samples of four species from four sections were sampled in Central Asia by R.J. Soreng and collaborators.

Since many *Poa* species in West Asia have been yet to be studied for DNA, this research focused primarily on 50 West Asian samples of 19 species from five sections. Nineteen samples of 11 species from West Asia (Iran) including *P. aitchisonii* Boiss., *P. bactriana* Roshev. subsp. *glabriflora* (Roshev. ex Ovcz.) Tzvelev, *P. densa* Troitsky, *P. diversifolia* (Boiss. et Balansa) Hack. ex Boiss., *P. masenderana* Freyn & Sint., *P. pseudobulbosa* Bor., *P. psychrophila* Boiss. & Heldr., *P. sinaica* Steud., *P. sterilis* M. Bieb., *P. thessala* Boiss. et Orph., *P. versicolor* Besser subsp. *araratica* (Trautv.) Tzvelev and *P. versicolor* subsp. *reverdattoi* (Roshev.) Olovana et Zhu are new to molecular analyses and have been surveyed for the first time.

Materials from West Asia (Iran, Turkey and West Asian part of Russia) were obtained from different sources. During a field trip to Turkey Gillespie and collaborators in 2011 sampled 31 samples of nine species belonging to five known sections, and five samples of three species that have not been assigned to a section of *Poa* yet. These

specimens have been deposited in the Canadian Museum of Nature. Also a small joint project was developed with the Iranian Research Institute of Plant Protection (Herbarium of IRAN) and the Research Institute of Forests and Rangelands Herbarium (Herbarium of TARI). Among Iranian samples that we received, eleven samples of eight species from five sections and one sample with unknown section were used in the molecular phylogenetic study. Two samples of two species from two sections collected by

R.J. Soreng from the West Asian part of Russia also were included in the analyses. Twenty Iranian samples were taken from collections at the Smithsonian Institution US National Herbarium but none could be used in research because of low quality of DNA.

Fifty six samples for *trnT-trnL-trnF*, 137 samples for *rpoB-trnC*, 137 samples for *matK*, 60 samples for ITS and 80 samples for ETS are new to this study versus previous studies (Soreng 1990; Gillespie & Boles 2001; Gillespie & Soreng 2005; Davis & Soreng 2007; Gillespie *et al.* 2007, 2008, 2009 & 2010; Refulio-Rodriguez *et al.* 2012).

2.1.2 *Eremopoa* and *Oreopoa* Samples

In this study, ten samples from four species of the genus *Eremopoa* and two samples of the genus *Oreopoa* from West Asia (Iran and Turkey) were analyzed for the first time.

2.1.3 Outgroup Samples

Twenty four outgroup samples from 23 species of 15 genera were chosen as the representatives of five subtribes that form the PPAM clade including subtribes Phleinae (Gillespie *et al.* 2010), Puccinelliinae/Coleanthinae, Poinae, Alopecurinae, and Miliinae (Gillespie *et al.* 2007, 2008, 2009 & 2010). On the basis of the reported phylogenetic relationship between these subtribes in nuclear, plastid and combined nuclear and plastid

phylogenetic trees of previous studies (Soreng 1990; Gillespie & Boles 2001; Gillespie & Soreng 2005; Davis & Soreng 2007; Gillespie *et al.* 2007, 2008, 2009 & 2010; Refulio-Rodriguez *et al.* 2012) they were selected and included in the analyses.

Eight genera including *Apera* Adans., *Arctagrostis* Griseb., *Arctophila* (Rupr.) Rupr. *ex* Andersson, *Bellardiochloa* Chiov., *Dupontia* R. Br., *Hoocherchloa* E.B. Alekseev, *Nicoraepoa* Soreng & L.J. Gillespie and *Sylvipoa* Soreng, L.J. Gillespie & S.W.L. Jacobs were included as representatives of Poinae. The genus *Alopecurus* from Alopecurinae, *Phleum* L. from Phleinae and *Milium* L. as the only genus of Miliinae were utilized. *Catabrosa* P. Beauv., *Hyalopoa* (Tzvelev) Tzvelev, *Phippsia* (Trin.) R. Br. and *Puccinellia* Parl. were chosen from Puccinelliinae. All trees were rooted on the subtribe Puccinelliinae species based on its position in PPAM clade in previous molecular analyses (Gillespie *et al.* 2007, 2008 & 2009) that positioned the Puccinelliinae in the sister group (P clade) to PAM clade including the other subtribes.

Cladograms resulting from maximum parsimony analyses of combined plastid *trn*TLF and nrDNA sequences (Gillespie *et al.* 2009, 2010) and plastid *trn*TLF majority-rule consensus phylograms from the Bayesian analysis (Gillespie *et al.* 2010; Refulio-Rodriguez *et al.* 2012) also show the same relationship in the PPAM clade.

2.1.4 Extracted DNA

Previously extracted genomic DNA samples available in the Laboratory of Molecular Biodiversity at the Canadian Museum of Nature were utilized for 86 *Poa* and 24 outgroup samples.

2.2 Molecular Phylogenetic Analyses Utilizing DNA Sequence

Sixty eight samples in silica gel from previously collected specimens present at the

Canadian Museum of Nature were sent to the Biodiversity Institute of Ontario at Guelph University to be sequenced for the *matK* barcode locus as part of a Barcode Of Life Data project (Hebert P.D.N. *et al.* 2003).

2.2.1 DNA Extraction

Using an extraction protocol described by Alexander *et al.* (2007) and modified by Starr (2009), total genomic DNA was extracted from all new samples, including all Iranian and most Turkish samples.

Twenty mg of leaf tissue from each sample was placed in a closed g-tube to ensure zero cross-contamination between samples. Then leaf tissues were ground with small steel beads in a BioSpec Mini Beadbeater-96 (BioSpec, Oklahoma) for three minutes (this breaks over 90% of the plant cells in about 2 – 5 minutes of operation). The powdered leaf tissue was mixed with the warmed buffer consisting of 320 μ L of homogenization buffer¹, 4 μ L 100 mg/mL RNase A and 80 μ L lysis buffer². Disruption buffers break both cell membrane and nucleus membrane. After 15 seconds of vortex, the tubes were incubated on a shaking platform at 65° C for 30 minutes. Then 130 μ L of potassium acetate was added to the mixture, tubes were inverted a few times and incubated at –20° C in the freezer for five minutes. To purify the suspension and extract whole genomic DNA, samples were spun at 14,000 (18470 x g) rpm, using an Eppendorf 5424 centrifuge for ten minutes. The supernatant was removed and precipitate was transferred to a 1.5 μ L tube containing 600 μ L of plant binding buffer³ and incubated at room temperature for five minutes.

¹ Homogenization buffer consists of 0.1 m NaCl, 0.2 m Sucrose, 0.01 m EDTA, 0.03 m Tris-HCl pH 8.0.

² Lysis buffer is a mixture of 0.25 m EDTA, 0.5 m Tris-HCl pH 9.2 and 2.5% SDS.

³ Plant Binding Buffer (2M guanidine hydrochloride, 95% ethanol)

Six hundred and fifty μL of the mixture was transferred to a silica membrane spin column (Epoch Biolabs, Texas) and spun at 10,000 (9391 x g) rpm for a minute. The flow liquid that passed through was discarded and DNA was deposited. The last step was repeated for the remaining volume of mixture. To remove residual salts from deposited DNA that were bounded to the silica gel columns, the DNA was washed with 500 μL of 70% ethanol and spun at 14,000 rpm for 30 seconds. This elution was repeated once more with 500 μL of 70% ethanol, but spun at 14,000 rpm for three minutes. The spin columns were placed into the last set of tubes that were labeled with full sample information (including species name, extraction date and voucher number), and the flow liquid and the collection tubes were discarded. To complete purification, DNA in spin columns was eluted by adding 75 μL of pre-warmed 65 $^{\circ}$ C THE Buffer⁴ and incubating for five minutes. Then columns were spun at 15,000 rpm for a minute. The elution technique by THE buffer was done twice when extracting older samples or small amount of leaf tissue to increase DNA yield. The silica spin columns were discarded and the total genome DNA samples in the bottom of the tubes were checked for the extraction success. Four μL of each sample and 2 μL of BPB loading were run on a 1.25 % agarose gel. The DNA samples (full concentrated) were stored in the -80 $^{\circ}$ C freezer for long-term use. Diluted DNA samples in autoclaved ddH₂O (1:10) were prepared from full concentrated DNA samples and stored in the -20 $^{\circ}$ C freezer for short-term use (PCR reactions).

⁴ THE Buffer (10 mM Tris-HCl pH 8.0 and 0.5 mM EDTA)

Some leaf samples from herbarium collections were not well preserved (not completely green). Because of the degraded DNA, a small modification was made to the basic protocol. To have DNA with a good quality, more leaf tissue (up to two times) was used.

2.2.2 Amplification

2.2.2.1 DNA Regions and Primers

Three DNA markers were successfully used in the molecular studies of the tribe Poeae (Gillespie *et al.* 2008, 2010; Soreng *et al.* 2007). These markers also were used in phylogenetic investigations of the genus *Poa* (Gillespie & Boles 2001; Gillespie & Soreng 2005; Gillespie *et al.* 2007, 2009; Soreng *et al.* 2010). These regions were chosen for the molecular phylogeny analyses in this study. One of them was the plastid intergeneric spacer of trnTLF (trnT-trnL spacer, trnL intron, trnL-trnF spacer) and two were nuclear (ribosomal) markers including ETS (the External Transcribed Spacer) and ITS (the Internal Transcribed Spacer). Also two plastid regions not previously used in *Poa* studies including *matK* (maturase K, a regions used in plant barcoding CBOL, 2009) and *rpoB-trnC* (an intergeneric spacer) were chosen for this study to reconstruct the phylogenetic trees with better support and resolution. Likewise, by utilizing a combinations of DNA three datasets respectively including, nrDNA (ETS+ITS), cpDNA (*matK*+*rpoB-trnC*+trnTLF) and ARs ((All Regions (nrDNA+cpDNA)) more loci were obtained to evaluate the placement of *Poa* species because multiple loci are stronger than a single locus at taxa differentiation. Plastid and nuclear regions along with the primers utilized for PCR and sequencing are provided in Table 4.

2.2.2.2 Polymerase Chain Reaction (PCR)

For most DNA samples, a 15 μL PCR reaction volume with 1.5 μL 1:10 concentration genomic DNA was selected, while for some herbarium samples full concentrated DNA was tried or 50 μL reaction volume PCRs were attempted due to poor quality DNA instead.

The recipe for plastid DNA amplification contained 1.5 μL 1:10 concentration genomic DNA (20 mg), 0.12 μL (HS) *Taq* DNA Polymerase (5 U/ μL -BioShop Canada), 0.5 μL BSA [Bovine Serum Albumin (20 mg/mL-BioShop Canada INC.)], 0.0375 μL of each primer (10 μM - Alpha DNA, Montreal), 0.3 μL dNTPs (10 mM-Thermo Scientific), 1.2 μL MgCl_2 (25mM-BioShop Canada INC.), 1.5 μL 10X HS reaction buffer (BioShop Canada INC.), and 9.13 μL nuclease-free ddH₂O. Table 5 shows PCR programs and conditions for plastid regions.

The PCR recipe for the nuclear regions were the same as for plastid reactions, but the minor adjustments for nuclear DNA are presented as follows: 0.5 μL BSA (20 mg/mL) was replaced with 3 μL Betaine (Sigma Aldrich), 1.2 μL MgCl_2 was reduced to 0.9 μL , nuclease-free ddH₂O was decreased to 5.79 μL , and 0.75 μL DMSO (Sigma Aldrich) was added to the recipe. Table 6 shows Recipe and PCR programs and conditions for nuclear regions. Reaction success was verified by running 2 μL of PCR products on a 1.25% agarose gel.

2.2.3 Sequencing

Successful PCR products were purified using a modified ExoSAP protocol (Canadian Museum of Nature's DNA Lab Manual) as follows: 0.1 μL of EXO (Exonuclease I-Affymetrix) and 0.9 μL SAP (Shrimp Alkaline Phosphatase (Affimetrix) were added to 9 μL of successful PCR products, incubating at 37^o C for 30 minutes,

followed by 90° C for ten minutes in a thermocycler. After purification, the PCR products were sequenced using BigDye v3.1 (Life Technologies Corporation, Carlsbad, California). The sequences were purified according to a sodium acetate/ethanol procedure (Applied Biosystems) and then were loaded onto an automated sequencer (3130xl Genetic Analyser (ABI Applied Biosystem)) and read.

2.2.4 DNA Matrices

To assemble two individual strands from ABI runs for each region and to edit the assembled contigs, Sequencher version 4.10.1 (Gene Codes, Corporation, Ann Arbor, Michigan, USA) was used. Using MUSCLE version 3.3 (Edgar 2004) complete DNA sequences were aligned. The final alignments were manually adjusted by eye and concatenated into three datasets using Geneious version 6.0.6 (Drummond *et al.* 2011).

The generated alignments of DNA sequences for nuclear regions of ETS and ITS and plastid region of *trnTLF* were added to the alignments including sequences for these regions from previous studies (Gillespie *et al.* 2007, 2008, 2009, 2010; Soreng *et al.* 2007, 2010), and used for the analyses.

Using PAUP v4.0b10 (Swofford 2002), a separate parsimony analysis for each region was performed. (See 2.5.5.). Since multiple loci are better than a locus in taxa differentiation, an attempt was made to combine data matrices. but before that PAUP was used for Incongruence Length Difference (ILD; Farris 1995) tests with 100 replicates. This test was performed for nuclear regions (ETS+ITS) and chloroplast regions of *matK+rpoB-trnC*, *matK+trnTLF*, *rpoB-trnC+trnTLF* and *matK+rpoB-trnC+trnTLF*.

ETS and ITS sequences subsequently were concatenated and an analysis was performed on the concatenated matrix of nuclear DNA sequences. The same process was done for combined plastid DNA regions of *matK*, *trnTLF* and *rpoB-trnC* matrices. It should be noted that *Poa curtifolia* with missing data for *rpoB-trnC* region, and *Poa fibrifera* and *Poa ursina* with missing data for *matK* region were eliminated from the combined analysis of cpDNA.

To combine nrDNA and cpDNA sequences and analyze the combined matrices as a single concatenated dataset, visual adjustment was conducted and the following species with incongruent position in nrDNA and cpDNA analyses and potential hybrid origins were removed from the combined analysis: *Oreopoa*, *P. khasiana*, *P.*

sikkimensis, *P. macrocalyx*, *P. smirnowii*, *P. lipskyi*, *P. abbreviata* and *P. psychrophila*

2. The incongruence length difference (ILD) tests was performed for the combination of nrDNA and cpDNA sequences before and after the elimination.

All new sequences for *Poa* species and outgroup samples will be submitted to GenBank. The available GenBank numbers are indicated in the Appendix.

2.2.5 Maximum Parsimony Analysis

First, phylogenetic trees were constructed based on the individual DNA alignment for each region. Next, the nrDNA, cpDNA and the combined nuclear and plastid DNA (ARs) alignments were analyzed to build phylogenetic trees.

A heuristic search in PAUP version 4.0b10 (Swofford *et al.* 2002) was carried out under following criteria: Simple addition of taxa was chosen; MULTREES option turned on; and A strict consensus tree was generated from the maximum number of 100,000 retained trees for all matrices (five individual DNA datasets of ETS, ITS, *matK*, *rpoB-trnC* & *trnTLF* DNA regions and three combined datasets nrDNA matrix “ETS+ITS”, cpDNA dataset “*matK+trnTLF+rpoB-trnC*” and a combined data matrix of cpDNA+nrDNA).

To assess support for the branches of the maximum parsimony trees, bootstrap (BS) analyses (Felsenstein, 1985) were performed using PAUP version 4.0b10 (Swofford *et al.* 2002) with 10,000 heuristic search repetitions and with the MULTREES option off (DeBry & Plmstead 2000). These bootstrap values were applied on the strict consensus tree resulted from maximum parsimony analysis.

Scores (bootstrap values = BS) between 95 – 100%, 85 – 94%, 75 – 84%, 65 – 74%, and 55 – 64%, were considered strong, very good, good, moderate, and weak, respectively. The branches with bootstrap values less than 55% were very weak (Starr *et al.* 2004). The Bootstrap values lower than 50% assumed no support for the branch.

3 Results

The phylogenetic trees of maximum parsimony analysis (Figures 7 –10, 12 – 14, 16) were the result of analyses performed on separate regions of ETS, ITS, *matK*, *rpoB-trnC* and *trnTLF*, and on combined regions of nrDNA, cpDNA and ARs (nrDNA+cpDNA).

The continent of origin for ingroup taxa can be simply explored with assistance of the following signs in all trees:

◇Australia ●Central Asia ■East Asia *Europe
✚North America ✕South America ❖Subantarctic Islands △West Asia

In present phylogenetic trees tribes that outgroup species are assigned to are also labeled as: Alopecurinae = ALO, Poineae = POI, Puccinelliinae = PUC, Miliinae = MIL and Phleinae = PHL.

It should be also noted that in this study, a main clade does not necessarily include a large number of species. It is assigned to define a clade consisting of the genus (e.g. *Eremopoa* or/and *Oreopoa*), a subgenus of *Poa* (e.g. *Sylvestres*) or section of *Poa* (e.g. *Pandemos*, *Parodiochloa*, etc.). The “major clade” is defined here as a clade with a great number of species with different taxonomic positions (e.g. a clade including all ingroup taxa).

To fully understand labels of clades in present phylogenetic trees, here I describe a special feature, which stands in all phylogenetic trees, “N clade”. Species of the section *Bolbophorum* are divided in three parts on the basis of their positions. The section *Bolbophorum* [part 1] is allied to the other species of the subgenus *Ochlopoa* and includes *P. bactriana* subsp. *glabriflora*, *P. badensis*, *P. bulbosa* (three samples), *P.*

ligulata, *P. sinaica* (from Iran), *P. thessala* and *P. timoleontis*. The section *Bolbophorum* [part 2] consists of *Poa bulbosa* (three samples), *P. dolosa*, *P. sinaica* (from Turkey) and *P. ursina*. This part is equivalent to “N clade” in previous study (Gillespie *et al.* 2009). *Poa densa* belongs to section *Bolbophorum* but in all present analyses is nested within species of subgenus *Poa*.

Three species, *P. aitchisonii*, *P. diversifolia* and *P. pseudobulbosa*, have not been assigned to a section and here after will be mentioned as unclassified group where each species is discussed individually. *Poa psychrophila* has not been assigned to a section either, but since it is currently treated as a synonym of the *P. cenisia* All. and two samples of *P. psychrophila* have different placement in present nuclear and plastid analyses, it will be fully discussed in 4.4.5.

In Table 7 summary of relevant statistics of maximum parsimony analyses are provided for individual regions, combined datasets of nrDNA, cpDNA and a combined dataset of nrDNA and cpDNA.

3.1 Plastid Analyses

Four phylogenetic trees resulted from analysis on individual nuclear regions and a combination of plastid regions dataset.

3.1.1 *trn*TLF (*trn*T-*trn*L-*trn*F)

Overall, the clades on the strict consensus tree resulting from maximum parsimony analysis of *trn*TLF are acceptably supported and largely resolved in terms of the placement of sections in main clades of the relevant subgenera, none of the subgenera are monophyletic (Figure 7).

Two ingroup genera *Eremopoa*, *Oreopoa* and four subgenera of *Poa* form seven

main clades on the tree as follows: **I**) A strongly supported clade (98% BS) including species of the subgenus *Ochlopoa* (excluding *Poa densa* of section *Bolbophorum* and “N Clade”); **II**) *Eremopoa* clade (100% BS); **III**) A weakly supported clade (64% BS) consisting of “N Clade”, two samples of *P. psychrophila* and *P. cenisia* (both from supersection *Poa* section *Poa* subsection *Cenisiae*), and section *Secundae* of the subgenus *Stenopoa*; **IV**) A main clade comprising subgenus *Stenopoa* (excluding *Secundae*) and *P. lipskyi* from section *Malacanthae* of the supersection *Poa* has a strong support of 100% BS; **V**) The supersection *Poa* (excluding *P. cenisia*, *P. caucasica* and *P. lipskyi*), *P. remota* and *P. masenderana* from subgenus *Poa*, section *Homalopoa* and unclassified group form a main clade with a very good support of 93% BS; **VI**) *Oreopoa* species form a main clade with strong support of 95% BS; **VII**) The species of the supersection *Homalopoa* (excluding *P. remota* and *P. masenderana*) form a main clade with weak support of 59% BS, with a huge polytomy and uncertain relationship among members.

Subgenus *Sylvestres* is not monophyletic. *Sylvestres* members are sisters with *P. caucasica*, clade I and a major clade including clades II, III, IV, V, VI and VII. *Poa caucasica* is collapsed with clade I and a major clade including clades II, III, VI, V, VI and VII in the very well supported clade (86% BS). The clade II is sister to a strongly supported major clade (97% BS) including clades III, IV, V, VI and VII, with a strong support of 96% BS. A major clade with a very good support (93% BS) includes two clades III and IV. This clade separates from the strongly supported major clade (100% BS) consisting of main clades V, VI and VII. Two clades VI and VII are sisters with a moderate support (73% BS). The clade that they form separates from the clade V with a strong support of 100% BS.

3.1.2 *rpoB-trnC*

The maximum parsimony analysis on *rpoB-trnC* region resulted in the strict consensus tree (Figure 8). The ingroup clade is well supported (84% BS).

In general, the major clades do not have high support, while main subclades and internal branches have acceptable support and form seven main clades as follows: **I)** *Sylvestres* species form a strongly supported main clade (98% BS); **II)** *Bolbophorum* [part 1], *Alpinae*, *Micrantherae* and *Parodiochloa* from the subgenus *Ochlopoa* including are placed in a main clade with a very well support of 93% BS; **III)** *Eremopoa* species form a main clade with a strong support of 100% BS; **IV)** The subgenus *Stenopoa*, *P. cenisia*, *P. lipskyi*, two samples of *P. psychrophila* and “N Clade” form a main clade, which is strongly supported (96% BS); **V)** A main clade of *Oreopoa* is strongly supported (99% BS); **VII)** The subgenus *Poa* (excluding *P. cenisia*, *P. lipskyi* and *P. caucasica*), *P. masenderana*, *P. remota*, *P. densa* and unclassified group form a main clade with a very good support of 93% BS.

A clade is formed by two supersections *Homalopoa* and *Poa*, and *Oreopoa* with a weak support of 59% BS.

In *rpoB-trnC* tree: 1) Unlike *trnTLF* tree the species of “N Clade” do not form a clade with the section *Secundae*, they are collapsed into a polytomy with the members of the subgenus *Stenopoa*; 2) Supersection *Poa* forms a clade inside the clade of supersection *Homalopoa*, while in *trnTLF* tree they are separated; 3) *Poa remota* is sister to the other species of the clade VII very well supported (93% BS); 4) *Poa caucasica* is collapsed in a polytomy with clades III, VI and VII with a very weak support of 52% BS.

3.1.3 *matK*

The strict consensus tree resulted from maximum parsimony analysis of *matK* is unresolved to a large extent, even the affinities between outgroup and ingroup taxa are not clear due to a huge polytomy on the tree (Figure 7) The outgroups (excluding Puccinelliinae members) are collapsed into a polytomy with ingroup clades, with a very good support of 93% BS.

Briefly, six following main clades of *Poa* species with polytomies inside are determined in the tree: **I**) *Micrantherae* and *Parodiochloa* form a main clade with a very good support of 86% BS; **II**) *Eremopoa* members form a main clade with a strong support of 99% BS; **III**) *Bolbophorum* [part 1] and *Alpinae* are strongly supported (100% BS) in their main clade; **IV**) Subgenus *Stenopoa*, *P. lipskyi*, *P. cenisia*, “N Clade”, and two samples of *P. psychrophila* form a main clade with a very good support of 83% BS.; **V**) *Oreopoa*, Subgenus *Poa* (excluding *P. caucasica*, *P. lipskyi* and *P. cenisia*), *P. densa* and unclassified group form a moderately supported main clade (66% BS) with a huge polytomy inside; **VI**) The well supported clade of *Oreopoa* (80% BS) is nested within the subgenus *Poa*.

Sylvestres species do not form a distinct clade as in *rpoB-trnC* and not positioned in the base of the tree as in *trnTLF*. They are collapsed into a polytomy with *P. caucasica*, *Stenopoa*, subgenus *Poa* (excluding *P. caucasica*), *P. densa*, “N Clade”, unclassified group and *Oreopoa* in a major clade with no support.

The species of “N Clade” do not form a clade with the section *Secundae* like *trnTLF*, they are collapsed into a polytomy with species of the subgenus *Stenopoa*, *P. psychrophila*, *P. cenisia* and *P. lipskyi*.

Unlike *trnTLF* and *rpoB-trnC* trees: 1) *Ochlopoa* (excluding “N Clade”) form two clades respectively including a clade comprising *Parodiochloa* and *Micrantherae* and a clade including *Bolbophorum* [part 1] and *Alpinae*; 2) *Eremopoa* is not sister to a major clade comprising subgenera *Stenopoa* and *Poa*, “N Clade” and *Oreopoa*; 3) Supersections of the subgenus *Poa* are not resolved and form a huge polytomy with *Oreopoa*; 4) *Poa caucasica* is collapsed with the *Sylvestres* members.

3.1.4 Combined Plastid Analysis (cpDNA Analysis)

Comparing the strict consensus trees of *matK*, *rpoB-trnC* and *trnTLF* trees visually, main clades branching orders look incongruent in *matK* and *rpoB-trnC*, and in *matK* and *trnTLF*, but since the bootstrap support for these branches are low or there is no support for them, no incongruence was determined in branching orders and the position of subgenera within plastid trees. The ILD tests, which was run on three possible combinations of plastid regions, indicates that the test is significant and there is incongruence between *trnTLF* and *matK*, and between *matK* and *rpoB-trnC* with p values of 0.0432 and 0.002. This might be attributed to a great lack of resolution and huge polytomies in *matK* tree. As expected, *rpoB-trnC* and *trnTLF* were congruent (The test was not significant; p=0.081). Based on this evidence, maximum parsimony analysis was performed on a single data matrix from combination of three regions, which resulted in a phylogenetic tree with stronger support for many clades than the support in individual trees obtained from *matK*, *rpoB-trnC* and *trnTLF* dataset.

The strict consensus tree resulted from maximum parsimony analysis of cpDNA are consistent with the recent infrageneric grouping of *Poa* (Soreng *et al.* 2010) (Figure 10). In this tree, sections are largely placed in the main clades of the pertaining

subgenera, but still only *Eremopoa* and the subgenus *Sylvestres* are monophyletic.

Concisely, eight main clades including genera *Poa*, *Eremopoa* and *Oreopoa* are easily distinguished as follows: **I**) The species of *Sylvestres* form a strongly supported clade (100% BS); **II**) *Ochlopoa* (excluding *Bolbophorum* [part 2], *Nanopoa* and *P. densa*) is strongly supported (100% BS) as a main clade; **III**) *Eremopoa* species form a main clade with a strong support of 100% BS; **IV**) A main clade including “N Clade”, *Secundae*, *P. cenisia* and two samples of *P. psychrophila* has a weak to moderate support of 65% BS; **V**) The subgenus *Stenopoa* (excluding *Secundae*) and *P. lipskyi* from a very well supported main clade (92% BS); **VI**) A main clade comprising supersection *Poa* (excluding *P. lipskyi*, *P. cenisia* and *P. caucasica*), *P. densa*, unclassified group, *P. masenderana* and *P. remota* has a strong support of 100% BS; **VII**) A main clade of *Oreopoa* is strongly supported (100% BS); **VIII**) There is no support for the main clade including supersection *Homalopoa* (excluding *P. masenderana* and *P. remota*).

The clade *Sylvestres* forms a major clade with the other ingroup taxa with a strong support of 95% BS. The clade II is sister to a major clade including clades III – VIII with a very well support of 92% BS. In this major clade *P. caucasica* is sister to the other species of the clade with a good support of 82 % BS. *Eremopoa* (clade III) and the major clade comprising sections IV – VIII form the other major clade with a strong support of 97% BS. Two sister clades IV and V that form a strongly supported clade (100% BS). A major clade consisting of three clades VI, VII and VIII has the strong support of 100% BS. A major clade consisting of VI – VIII is sister to a major clade including IV and V with a strong support of 100% BS.

The position of *Poa caucasica*, *Eremopoa*, *Ochlopoa* (excluding “N Clade”) is

consistent with *rpoB-trnC* tree, while the placement of “N Clade” (III), *Stenopoa* (IV), *Oreopoa* (VI) and supersections *Poa* (V) and *Homalopoa* (VII) is consistent with *trnTLF* tree. *Sylvestres* species are positioned at the base of the tree as in *rpoB-trnC* and *trnTLF* trees.

3.2 Single Plastid DNA Region versus Several Plastid DNA Regions

As Figure 11 shows, in a comparison between *trnTLF* and cpDNA analyses, 16 clades (highlighted with red circles) are determined to have greater support in cpDNA tree. Of these clades, six clades include small parts of a section (s), while other ten clades consist of a subgenus or subgenera or section (s) as follows: 1) ingroup taxa; 2) ingroup taxa (excluding subgenus *Sylvestres*); 3) supersection *Poa*; 4) subgenus *Stenopoa* (excluding *Secundae*) + (“N Clade” + *Secundae* + (*P. psychrophila* + *P. cenisia*)); 5) subgenus *Ochlopoa* (excluding “N clade”); 6) *Tichopoa*; 7) (subgenus *Poa* + *Oreopoa*) + (subgenus *Stenopoa* (excluding *Secundae*)+ (“N Clade” + *Secundae* + (*P. psychrophila* + *P. cenisia*))); 8) *Eremopoa* + (subgenus *Poa* + *Oreopoa*) + (subgenus *Stenopoa* (excluding *Secundae*) + (“N Clade” + *Secundae* + (*P. psychrophila* + *P. cenisia*))); 9) *Parodiochloa* + *Micrantherae*; 10) *Oreopoa*. *Eremopoa* and all subgenera of *Poa* are better resolved in cpDNA tree than in *trnTLF* analysis, although the resolution in subgenus *Ochlopoa* is not considerable (Figure 11).

3.3 Nuclear Analyses

Three phylogenetic trees resulted from analysis on individual nuclear regions and a combination of nuclear regions dataset. Presence of a clade, “X clade” is a feature exclusively in phylogenetic trees of nuclear analysis. The “X clade” is equivalent to “X

clade” in previous studies (Gillespie *et al.* 2009). Here “X clade” includes *P. leptocoma* of the subgenus *Stenopoa* (section *Oreinos*), *P. khasiana* and *P. sikkimensis* of the subgenus *Poa* supersection section *Homalopoa* (section *Homalopoa*), and *Poa smirnowii* and *Poa macrocalyx* of the subgenus *Poa* Supersection *Poa* (section *Macropoa*).

3.3.1 ETS

In ETS strict consensus tree, among outgroups, *Phleum* presents a closer relationship with ingroup taxa (Figure 12). The clade *Phleum* form a clade with the strongly supported major clade (of 96% BS) of ingroups with a very weak support of 50% BS. The ingroup clade has a strong support of 96% BS.

The strict consensus tree from maximum parsimony analysis of the ETS data matrix is somewhat unresolved in terms of the position of the *Poa* sections in subgenera and none of subgenera are monophyletic (Figure 12).

Eleven main clades of *Poa* species are easily distinguished based on the position of subgenera and sections as follows: **I**) The clade of *Parodiochloa* has a strong support of 100% BS; **II**) The main clade of *Poa trivialis* samples of the *Pandemos* is strongly supported (100% BS); **III**) A main clade including *Alpinae* and *Bolbophorum* [part 1] has a strong support of 100% BS; **IV**) The subgenus *Stenopoa* (excluding *P. leptocoma*, *P. abbreviata* and *Pandemos*), *P. cenisia* and *P. psychrophila* 1 form a main clade with the support of 71% BS; **V**) The main clade of the subgenus *Sylvestres* has a good support of 83% BS; **VI**) The main clade of *Micrantherae* is strongly supported (100% BS); **VII**) Distinct main clade of *Eremopoa* has a strong support of 100% BS; **VIII**) A main clade consisting of *Oreopoa*, “N Clade” and *P. psychrophila* 2 is well supported

83% BS; **IX**) The “X Clade” as a main clade has a strong support of 96% BS; **X**) A main clade including *P. pratensis* (section *Poa*, subsection *Poa*) and *P. granitica* (section *Poa* subsection *Nivicolae*) has a weak to moderate support of 65% BS; **XI**) Supersection *Homalopoa* (excluding *P. khasiana* and *P. sikkimensis*), *P. densa*, *P. lipskyi*, *P. abbreviata*, *Macropoa* and *P. ircutica* of the section *Poa* subsection *Nivicolae*) and unclassified group form a main clade with no support.

Three clades II, III and IV are collapsed into a polytomy and form a strongly supported (95% BS) clade, which is sister to the clade I with a very good support of 90% BS. The clade IX and X form a clade that is collapsed into a polytomy with clades VIII and XI. The well supported clade (83% BS) comprising clades VII – XI is sister to the clade VI with the good support of 83% BS.

3.3.2 ITS

The strict consensus parsimony tree resulted from analysis on ITS data matrix is somewhat unresolved in terms of positioning of sections in subgenera and placement of subgenera on the tree (Figure 13). The members of subgenera with the exception of *Sylvestres* do not form distinct clades. The ingroups and subtribe Miliinae form a clade with a moderate support of 66% BS. So, the ingroup is not monophyletic since, the clade Miliinae is sister to the *Micrantherae* with no support.

Ten following main clades are identified based on the position of subgenera and sections of *Poa*: **I**) *Sylvestres* members form a weakly supported clade (56% BS); **II**) The clade including *Parodiochloa* species is very well supported (93% BS); **III**) The main clade comprising *Micrantherae* has the strong support of 100% BS; **IV**) The clade of *Pandemos* species is strongly supported (100% BS); **V**) The support for the main

clade including *Bolbophorum* [part 1] and *Alpinae* is strong (100% BS); **VI**) *Eremopoa* species are strongly supported (100% BS) in their main clade; **VII**) A not supported main clade includes the species of the subsection *Poa* and *Poa granitica*. This clade is the sister group to *Poa planifolia* from the section *Acutifoliae* of supersection *Homalopoa* with no support. **VIII**) The section *Brizoides* (excluding *P. fibrifera*) from supersection *Homalopoa* form a well supported main clade (83% BS). **IX**) The members of the “X Clade” form a main clade with the good of 79% BS. **X**) Subgenus *Stenopoa* (excluding *Pandemos*, *P. abbreviata* and *P. leptocoma*), *P. cenisia* and *P. psychrophila* 1 form a main clade with a strong support of 100% BS.

The main clades form three huge major clades with no support. The members of “N Clade” and *Oreopoa* are collapsed into a polytomy with IX and “X clades” in first major clade. This major clade is also collapsed into a polytomy with the clades VI, VII, VIII, *P. abbreviata*, *P. densa* and the other members of the subgenus *Poa* to form the second major clade. The third major clade is formed by the clades II, III, IV and V.

3.3.3 Combined Nuclear Analysis (nrDNA Analysis)

ETS and ITS strict consensus trees of individual regions were compared visually to justify concatenation of these datasets. A significant conflict has been found in the trees regarding the main clades branching order. Thus, the positions of *Micrantherae* clade and the members of the subgenera *Sylvestres* and *Stenopoa* (excluding *P. abbreviata*, *P. leptocoma* and *Pandemos*) were determined to be incongruent between ETS and ITS trees. Also running ILD test on combined nuclear regions represented a significant incongruence between two datasets (p=0.002).

An ILD test fails when: 1) Data sets have different evolutionary conditions; 2)

Trees have the same evolutionary patterns but are symmetric and asymmetric, and have topological differences; 3) Trees have very unequal branch lengths; 4) When informative characters are low (Darlou & Guillaume 2002). Here the incongruence can be attributed to 1) the topological differences of ETS and ITS trees; 2) short regions with low informative characters of ETS and ITS; and 3) based on (Dolphin *et al.* 2000; Lee 2001; Scotland & Steel 2015) the effects of abundant phylogenetic noises on the ILD tests result.

Given that incongruence between ETS and ITS data has been recorded previously for several diploid species of *Poa* (Gillespie *et al.* 2010), but considering that present strict consensus trees of ETS and ITS have similar clades with species belonging to the same section(s) and the level of support for the incongruent positions are low, the data matrix of these two regions were merged and analyzed as a single concatenated dataset. Combining ETS and ITS data resulted in a phylogenetic tree with stronger support for many clades (Figure 14) than the support in individual trees obtained from ETS (Figure 12) and ITS dataset (Figure 13).

Eleven main clades are easily determined in nrDNA tree based on the position of subgenera and sections as follows: **I)** *Sylvestres* species form a very well supported (90% BS) clade; **II), III)** and **IV)** Three main clades respectively including species of *Parodiochloa*, *Pandemos* and *Bolbophorum* [part 1] + *Alpinae* are strongly supported (100% BS); **V)** The clade of the subgenus *Stenopoa* (excluding *Pandemos*, *P. abbreviata* and *P. leptocoma*), *P. cenisia* and *P. psychrophila* 1 has a very strong support of 100% BS; **VI)** *Micrantherae* is not allied with the other members of the *Ochlopoa* and form a strongly supported clade (100% BS); **VII)** Members of *Eremopoa* form a distinct clade with a strong support of 100% BS. **VIII)** The “X Clade” is also a strongly supported

(100% BS) main clade; **IX**) In the very well supported main clade (85% BS) *Nanopoa* is sister to a clade including *Oreopoa*, *Bolbophorum* [part 2] and *P. psychrophila* 2; **X**) The members of the section *Brizoides* (including subsections *Australopoa* and *Austrofestuca*) form a main clade with no support; **XI**) The main clade including section *Poa* and *Poa granitica* are strongly supported (99% BS).

Here is the description for the main clades orders and the supports for the few major clades that they form: In the very well – strongly supported major clade of (95%). major clade *Sylvestres* clade is sister to a clade including all ingroup taxa (excluding *Sylvestres*). The clades II, III, IV and V form the first major clade with the good support of 83% BS. The clade II is sister to the clades III, IV and V. Two clades III and IV form a clade with the moderate support of 71% BS and are separated from the clade V with the strong support of 100% BS. The second major clade consists of two main clades, VIII and IX, with the good support of 76% BS. The third major clade includes subgenus *Poa* (excluding *P. caucasica*, *P. cenisia* and the ones in the “X Clade”), *P. densa*, *P. abbreviata* and the unclassified species with a moderate support of 69% BS. The second and third major clades are sisters with the good support of 80% BS. The clade VII form a clade with the clade including clade second and third major clades with the very good support of 90% BS. The clade VI is sister to the second and third major clades and the clade VII with no support. *Poa caucasica* is sister to a clade comprising all ingroup species excluding *Sylvestres* and *P. caucasica* with no support.

In short, nrDNA tree is more similar to ETS tree than the ITS tree. The placement of *Stenopoa* (excluding *Pandemos* and *Abbreviatae*) and *Micrantherae* in nrDNA tree is similar to ETS tree, while *Sylvestres* species form the basal clade as in ITS tree.

3.4 Nuclear Analyses Versus Plastid Analyses

The incongruence between present nuclear and plastid trees is attributed to following taxa with different placement in nuclear and plastid analyses (Figure 15): **I)** *Oreopoa* is placed between two supersections of the subgenus *Poa* in plastid trees while it is positioned within “N clade” species in nuclear trees; **II)** *Poa abbreviata* is allied with the other members of the subgenus *Stenopoa* in plastid trees, while it is nested within the species of the subgenus *Poa* in nuclear trees; **III)** *Poa lipskyi* is placed in *Stenopoa* clade in plastid trees, but in nuclear trees positioned within the species belonging to supersection *Poa* of the subgenus *Poa*; **IV)** section *Pandemos* is allied in *Stenopoa* clade in plastid trees, while it is sister to two sections of the subgenus *Ochlopoa*, *Bolbophorum* [Part 1] and *Alpinae* in nuclear trees; **V)** section *Secundae* is allied within species of “N Clade” in plastid trees, but is positioned in *Stenopoa* clade in nuclear trees; **VI)** *Poa cenisia* and *P. psychrophila* 1 are sisters in both nuclear and plastid analyses, but the clade that they form has different placement. In plastid trees this clade is placed in “N clade” and in nuclear trees is nested within *Stenopoa* clade; **VII)** “X clade” as a special feature of nrDNA tree is a strongly supported clade consisting of two species of *Malacanthae* (*P. macrocalyx* & *P. smirnowii*), two species of *Homalopoa* (*P. khasiana* & *P. sikkimensis*) and one species section *Oreinos* (*P. leptocoma*). “X Clade” is sister to “N clade” (including *Oreopoa*) with a good support. In plastid trees, these species are allied to the members of the sections that they belong to. Figure 15 shows the taxa with incongruent placement in cpDNA and nrDNA analyses. The species with checkmark in cpDNA tree formed the “X Clade” in nrDNA tree. Numbers I – IV show the other incongruent taxa in both trees.

3.5 Overall combined Nuclear and Plastid (ARs) Analyses

Several significant conflicts have been found in the strict consensus trees of nrDNA and cpDNA regarding the main clades branching order, where nrDNA and cpDNA trees were compared visually. Thus, the position of *Oreopoa* and several following species of *Poa* was determined to be incongruent between cpDNA and nrDNA and they were eliminated from the analysis: *Poa abbreviata*, *P. khasiana*, *P. leptocoma*, *P. lipskyi*, *P. macrocalyx*, *P. psychrophila* 2, *P. sikkimensis*, *P. smirnowii*. The ILD test was run on all possible combinations of nuclear and plastid datasets after eliminating the incongruent taxa. Both ETS and ITS were significantly incongruent with individual and combination of plastid regions (cpDNA) ($p < 0.05$). The maximum parsimony analyses performed on combined alignments of all regions as a single dataset, which resulted in a phylogenetic tree with stronger support than the support in individual trees obtained from the cpDNA and nrDNA datasets. This tree is called ARs tree (All Regions tree).

Overall, the strict consensus tree from maximum parsimony analysis of the ARs data matrix (nrDNA+cpDNA) is greatly resolved in terms of the position of the sections pertaining to a subgenus (Figure 16).

Seven main clades or lineages are easily distinguished on the basis of *Eremopoa* and four subgenera, two supersections and sections of *Poa* as follows:

- I)** The species of *Sylvestres* form a main clade with a strong support of 100% BS;
- II)** A main clade including *Ochlopoa* (excluding “N Clade” and *P. densa*) is strongly supported (97% BS);
- III)** *Eremopoa* species form a main clade with a strong support of 100% BS;
- IV)** N Clade is strongly supported (96% BS);
- V)** The subgenus *Stenopoa*, *P.*

cenisia and *P. psychrophila* 1 are strongly supported (89% BS) in their main clade; **VI**
The subgenus *Poa* (excluding *P. cenisia* and *P. caucasica*), *P. densa*, unclassified group,
P. masenderana and *P. remota* form a main clade with a strong support of 98% BS; **VII**
The species of the supersection of *Homalopoa* (excluding *P. remota* and *P.*
masenderana) are placed in a main clade of with a moderate support of (70% BS).

The clade I is sister to a other ingroup taxa (excluding *Sylvestres*) in a strongly supported clade (100% BS); The clade II is sister to a major clade including clades III, IV, V, VI, VII and *Poa caucasica* with the very good support of 87% BS; *Poa caucasica* forms a clade with the clades III – VII with the weak support of 62% BS; The clade III is allied to a clade including IV, V, VI and VII with the strong support of 97% BS; Two clades IV and V form a very well supported clade (89% BS); The clade comprising two clades VI and VII is moderately supported (76% BS); Two clades respectively including VI – VII and V – VI have a very good support of 91% BS.

4 Discussion

In short, this thesis with the focus on the evolutionary relationships between West Asian species of *Poa*, *Eremopoa* and *Oreopoa* resulted in: 1) changing the taxonomical level of the genus *Eremopoa* to subgenus *Pseudopoa* of *Poa*, 2) defining a phylogenetic position for some unclassified species, 3) finding a new lineage compatible with *Poa* subgenera (*P. caucasica*), and 4) improving the taxonomical position of some misclassified *Poa* species (*P. masenderana*, *P. densa* & *P. psychrophila*).

4.1 Subgenus *Pseudopoa* (*Eremopoa*)

Overtime, and in absence of the molecular data, the genus *Eremopoa* has been classified as three different taxa: 1) it was recognized as a separate genus (Roshev. 1934; Parsa 1950; Bor 1960 & 1970; Bor & Guest 1968; Nasir & Ali 1970; Tzvelev 1976; Mobayen 1980; Tutin *et al.* 1980; Scholz 1981; Cope 1983; Miller 1985; Clayton & Renvoize 1986; Soreng *et al.* 2003; Darbyshire 2007; Rahmanian & Rahiminejad 2012; Rahiminejad *et al.* 2014); 2) it was assigned to section *Psilantha* of *Poa* (Boiss 1881) or section *Pseudopoa* of *Poa* (Hackel 1887); and 3) it has been treated as the subgenus *Pseudopoa* of *Poa* (Stapf 1897; Zhu *et al.* 2006).

Poa and *Eremopoa* share a large number of similar morphological traits that overlap each other (See Table 3). These similarities led to the idea that *Eremopoa* morphologically fits within *Poa*. Also in recent molecular investigations on *Poa* and allied genera by Gillespie *et al.* (2007, 2008, 2009) and Soreng *et al.* (2010), the placement of one sample of *Eremopoa* (between clade BAPO including including subgenus *Ochlopoa*, and the clade SPOSTA-HAMBADD comprising subgenera of *Stenopoa* and *Poa*) clarified the close affinities of *Poa* and *Eremopoa*.

The clade of *Eremopoa* is positioned above *P. caucasica*, *Sylvestres* and *Ochlopoa* (excluding “N Clade” and *P. densa*) as sister to *Stenopoa*, *Poa* excluding *P. caucasica*, *Oreopoa*, “N Clade”, *P. densa* and unclassified species with strong support in *rpoB-trnC*, *trnTLF*, cpDNA and ARs trees. The placement of *Eremopoa* in *matK* tree is congruent with these trees, but the tree is not resolved and there is no support for the huge polytomy that *Eremopoa* clade is collapsed into. *Eremopoa* is sister to “N Clade”, “N Clade”, *Oreopoa*, *Poa* (excluding *P. caucasica* and *P. cenisia*), *P. abbreviata*, *Micrantherae* and with a high support in ETS and nrDNA trees.

Due to multiple multiple samples and species present molecular analyses provide greater evidence to ascertain the evolutionary relationship between *Eremopoa* and the genus *Poa* than previous studies (Gillespie *et al.* 2007, 2008, 2009 & 2010; Refulio *et al.* 2012; Soreng *et al.* 2010) since these studies were based on one sample of one species because these studies were based on one sample of one species. In all present analyses the position of the distinct strongly supported clade of *Eremopoa* is consistent with the previous molecular works on *trnTLF* (Gillespie *et al.* 2007, 2008, 2009; Refulio *et al.* 2012), ITS (Gillespie *et al.* 2008 & Refulio *et al.* 2012), nrDNA (Gillespie *et al.* 2008, 2009), ITS+ETS+ *trnTLF* (Gillespie *et al.* 2009, 2010) and ITS+*trnTLF* (Soreng *et al.* 2010) and contradict the treatment of *Eremopoa* as a separate genus. So, the present molecular analyses support inclusion of *Eremopoa* to *Poa* and indicate that the genus *Eremopoa* must be treated as a distinct lineage within *Poa*. In current molecular analyses the position of *Eremopoa* clade is compatible with the other subgenera of *Poa*. This is consistent with the phylogenetic analyses of Gillespie *et al.* (2007, 2008 & 2009). *Pseudopoa* is the earliest valid name that was published for *Eremopoa* as a subgenus of *Poa* (Stapf 1897). So, following “The Principle of Priority” (1991), from here on,

Eremopoa will be referred to as subgenus *Pseudopoa*.

Among eight currently accepted species for the subgenus *Pseudopoa*, ten samples of four species from Iran and Turkey (the richest area of the occurrence for the subgenus *Pseudopoa*) were included in present molecular analyses. *Eremopoa capillaris* form a clade with all or most samples of *E. altaica* with the strong support in all present analyses. Two species *medica* and *persica* also form a clade with a strong support of 96% BS in cpDNA and ARs trees.

4.2 *Oreopoa*

Oreopoa is an endemic grass to Turkey, which is recognized from *Poa* by its convolvulate leaf blade and purple, round, 5 (7)-veined lemma (Parolly & Scholz 2004). *Oreopoa* was included in molecular study for the first time. Two samples of *Oreopoa* are closely related to “N Clade” and *Poa psychrophila* 2 in nuclear trees. *Oreopoa* is sister to *P. ursina* and *Nanopoa* with a very good support in nrDNA tree and a weak support in ETS tree. The position of *Oreopoa* in ITS tree is consistent with ETS and nrDNA trees, while the tree is not resolved. In plastid trees *Oreopoa* shows a close affinity with subgenus *Poa*. *Oreopoa* is sister to main part of supersection *Homalopoa* with a moderate to low support in plastid trees. Since there is a significant incongruence between the placement of *Oreopoa* in nuclear and plastid analyses (between two supersections of the subgenus *Poa* in plastid trees and among “N clade” in nuclear trees), *Oreopoa* was eliminated from ARs analysis.

Originally, *Oreopoa* as a new genus has been placed in tribe Poeae (sensu Clayton & Renvoize 1986) by Parolly and Scholz (2004), but present molecular analyses distinguish *Oreopoa* as synonym of *Poa*. The present molecular data suggest that

Oreopoa is a hybrid between subgenus *Poa* and “N Clade” because it is placed as a unique lineage within subgenus *Poa* in cpDNA analysis and is positioned in nrDNA analysis with high support in both trees. The *Oreopoa* samples in this study came from the same mountain, and not from the type locality, so to verify where *Oreopoa* belongs in *Poa*, further molecular analyses needed on the samples from several populations and in particular on the type locality samples.

4.3 *Poa* Phylogeny

4.3.2 Subgenus *Sylvestres*

Subgenus *Sylvestres* is a group of shady woodland species that is endemic to North America. First, it was described as a section under subgenus *Poa* (Soreng 1998). Soreng *et al.* assigned *Sylvestres* as a section to subgenus *Arctopoa* (2003), but its sister group *Arctopoa* was removed from the genus *Poa* by Gillespie *et al.* (2007) and *Sylvestres* was treated as the section belonging to subgenus *Poa*. Soreng and Gillespie (2008) increased the taxonomic level of *Sylvestres* to subgenus by the evidence that they provided in molecular phylogenetic analyses.

In present phylogeny analyses of cpDNA, nrDNA and ARs the species of *Sylvestres* form the basal clade. The position of *Sylvestres* in these trees is consistent with previous molecular studies (Gillespie & Boles 2001; Gillespie & Soreng 2005; Gillespie *et al.* 2007, 2008, 2009, 2010; Refulio *et al.* 2012).

4.3.2 Subgenus *Ochlopoa*

Ochlopoa species (excluding *P. densa*) do not form a distinct clade in none of present phylogenetic analyses. *Poa densa* Troitsky is placed within species of the subgenus *Poa* in all analyses and will be fully discussed in section 4.4.2.

First part of the subgenus “N Clade” is common in all present phylogenetic analysis. Second part the subgenus is monophyletic in cpDNA and ARs trees. In nrDNA analysis *Micrantherae* and *Parodiochloa* are separated from the second part. *Micrantherae* is placed far from the second part, however there is no support for this separation. *Parodiochloa* is also sister to a clade including to a clade including *Stenopoa* and *Ochlopoa* (excluding “N Clade” and *Micrantherae*). Previous molecular studies on *trnTLF* Gillespie & Boles (2001), Gillespie & Soreng (2005), Gillespie *et al.* (2007, 2008, 2009) and Refulio *et al.* (2012) are consistent with present molecular analyses in terms of the position of “N clade” and other part of the subgenus.

Two species of the section *Bolbophorum* including *P. bactriana* Roshev. subsp. *glabriflora* (Roshev.) Tzvelev, and *P. timoleontis* Heldr. *ex* Boiss. have not been a part of molecular studies before. *Poa timoleontis*, *P. bactriana* and *P. sinaica* were grouped with *P. glabriflora* and a few other species by Bor (1970). Tzvelev (1976) also assigned them to subgenus *Poa*, section *Poa* and subsection *Bulbosae* and treated *P. glabriflora* as subspecies of *P. bactriana*. In present analyses they are allied to other species of *Bolbophorum* [part 1].

4.3.3 Subgenus *Stenopoa*

Apart from *P. abbreviata* and *P. leptocoma*, the species of the subgenus *Stenopoa* form two clades in nuclear trees including a clade of *Pandemos* and a clade comprising the rest of the subgenus *Stenopoa* species, *P. cenisia* and *P. psychrophila* 1. Two sections of *Tichopoa* and *Pandemos* are resolved in all present phylogenetic trees, while three species of *Oreinos* and two sections of *Secundae* form two clades and section *Stenopoa* is remained unresolved in all analyses. *Poa abbreviata* and *P. leptocoma* with

unstable position in nuclear and plastid trees are nested within subgenus *Poa* and will be discussed later respectively in sections 4.4.9 and 4.4.10. The section *Pandemos* also will be discussed in detail in section 4.4.8 because of its different placement in nuclear and plastid trees. *Pandemos* is placed between the sections of *Ochlopoa* with a strong support in ETS and with a moderate support in nrDNA tree.

In plastid trees species of the subgenus *Stenopoa* are closely related to *Poa cenisia*, *Poa lipskyi* and “N Clade”. In *trnTLF* and cpDNA *Poa lipskyi* are placed within main part of the subgenus, and *Secundae* is sister to two samples of *Poa psychrophila*, “N Clade”. In ARs tree, subgenus *Stenopoa* forms a distinct clade with *Poa cenisia* and *Poa psychrophila* 1. This clade is sister to “N Clade”.

The species of the section *Stenopoa* including *P. albertii* Regel, *P. attenuata* Trin., *P. sterilis* M. Bieb., *P. versicolor* Besser subsp. *araratica* (Trautv.) Tzvelev and *P. versicolor* Besser subsp. *reverdattoi* (Roshev.) Olonova & G.H. Zhu, are new to molecular study and are positioned within the other species of the subgenus *Stenopoa*.

Poa albertii was determined as a synonym of *P. attenuata*, under subsection *Stenopoa* and section *Stenopoa* by Tzvelev (1976). Zhu *et al.* (2003) treated these two taxa as separate species under subgenus *Poa* and section *Stenopoa*. In all present phylogenetic trees these two species are placed with the species of the subgenus *Stenopoa*, although in *matK* tree they are less resolved.

Both species of *P. sterilis* and *P. versicolor* are placed under subsection *Stenopoa* and section *Stenopoa* by Tzvelev (1976). In all present analyses these two species are positioned among species of the subgenus *Stenopoa*.

Overall, there is a close affinity between sections of two subgenera *Stenopoa* and *Ochlopoa* in all present analyses: In ARs tree “N Clade” is sister to *Stenopoa*; In cpDNA

tree *Stenopoa* excluding *Secundae* is sister to “N Clade” and *Secundae*; and in nrDNA tree *Stenopoa* excluding *Pandemos*, *P. leptocoma* and *P. abbreviata* is sister to *Bolbophorum* [Part 1] and *Alpinae*.

This close relationship is fully consistent with previous molecular studies on *trnTLF* (Gillespie *et al.* 2005, 2007, 2008, 2009), ITS (Gillespie *et al.* 2008), nrDNA (Gillespie *et al.* 2008, 2009), ITS+ETS+*trnTLF* (Gillespie *et al.* 2009, 2010) and ITS+*trnTLF* (Soreng *et al.* 2010).

4.3.4 Subgenus *Poa* (supersections *Poa* and *Homalopoa*)

Subgenus *Poa* is the largest subgenus of the *Poa*. Both supersections of the subgenus *Poa* are sisters and mostly include their relevant species and are placed on the top of the cpDNA and ARs trees with a strong support. The species of these two supersections are not resolved in nrDNA trees.

Present study provided similar position and support for two supersections of *Poa* and *Homalopoa*, sections as in previous *trnTLF* analysis (Gillespie *et al.* 2007, 2008, 2009). In ARs, cpDNA and nrDNA trees section *Brizoides* with Australian species are well resolved, while species belonging to group *Punapoa* greatly resolve together in ARs tree. The section *Poa* is well resolved in nrDNA, cpDNA and ARs and two species of *P. caucasica* and *P. cenisia* will be discussed in detailed in respectively in sections 4.4.4 and 4.4.1.

Among the species of subgenus *Poa* that are new to molecular analysis, *P. planifolia* Trin. from *Acutifoliae*; *P. glaberrima* Tovar, *P. linerifolia* Refulio and *P. marshallii* Tovar from group *Punapoa*; *P. huancavelicae* Tovar, *P. khasiana* Stapf, *P. mairei* Hack., *P. mulleri* Swallen, *P. paucifolia* Keng ex Shan Chen, *P. reflexa* Vasey &

Scribn., *P. remota* Forselles and *P. sunbisinii* Soreng & G.H. Zhu from section *Homalopoa* are placed within the relevant supersection (*Homalopoa*) and subgenus *Poa*. *Poa bucharica* Roshev. and *Poa longifolia* Trin. from *Macropoa* are also placed within the subgenus *Poa* and supersection *Poa* that they have been assigned to by traditional classification. It seems that *Poa masenderana* Freyn & Sint. of the section *Homalopoa* as a new species for the molecular analysis better fits to supersection *Poa* and this will be discussed in section 4.4.3.

Also three *Poa* species, which are included in molecular study for the first time, *P. caucasica* Trin. from section *Poa* (subsection *Nivicolae*), *P. cenisia* All. from section *Poa* (subsection *Cenisiae*) and *P. lipskyi* Roshev. subsp. *lipskyi* of *Malacanthae* represent unstable position in nuclear and plastid trees and will be discussed respectively in sections 4.4.1, 4.4.4 and 4.4.6.

4.4 Misplaced Species; Misclassification or Hybridization and Reticulate Evolution

The position of ten following species in present analyses contradicted what traditional classifications have provided and this can be attributed to misclassification.

4.4.1 *Poa caucasica*, A New Distinct Lineage

Poa caucasica (Trin. 1830) occurs in Caucasus in a very restricted area, where Caucasus mountains are shared between Azerbaijan, Turkey, Georgia, Armenia and Russian Federation. Tzvelev (1976) classified this West Asian species under subsection *Nivicolae* of section *Poa* from subgenus *Poa* with three East Asian species including *Poa veresczaginii* Tzvelev, *Poa ircutica* and *Poa shumushuensis* Ohwi on the basis of few morphological characteristics that these species share. The species of *Nivicolae* are distinguished by creeping underground shoots and a thin base of the aerial shoot. The

lemma is pilose along veins in basal part. The callus has a small tuft of long sinuate hair. Glumes also have a small tuft of long sinuate hair and mostly are membranous (Tzvelev 1976).

In present molecular analyses of ARs and cpDNA trees *Poa caucasica* is sister to ingroup excluding *Sylvestres* and *Ochlopoa* (excluding “N Clade”). It has the same position in nrDNA tree but there is no support for this.

The results of all molecular analyses contradict the placement of *Poa caucasica* within subsection *Nivicolae* or even within the subgenus *Poa*. Indeed, the new taxonomical position which present analyses provide for *Poa caucasica* indicates it should be treated as a unique lineage within genus *Poa*. To explore the definite relation between *Poa caucasica* and *Poa* subgenera, more *Poa caucasica* samples and samples from *Poa vereschaginii* Tzvelev, *Poa ircutica* (Soreng *et al.* 2010) and *Poa shumushuensis* Ohwi of *Nivicolae* species should be analyzed.

4.4.2 *Poa densa*

Poa densa was classified in section *Poa* subsection *Bulbosae* (equivalent to the current section *Bolbophorum*) by Tzvelev (1976) on the basis of some morphological characteristics such as smooth panicle branches, pilose lemma, viviparous spikelet and the sheaths, which are closed over 1/4 – 2/3 of their length from the base. In molecular study by Rodionov *et al.* (2010), *Poa densa* was placed in a clade with two groups of highly polyploid species and tetraploid species of subsection *Australopoa* (supersection *Homalopoa* section *Brizoides*) from Australia in nuclear analysis with no support as was reported by Rodionov *et al.* (2010), as well as molecular analyses of Gillespie *et al.* (Unpublished). The present molecular evidence demonstrates that this species does not

belong to *Ochlopoa* but represents a close relation with the species of the supersection *Poa* in *rpoB-trnC*, *trnTLF* and this species is strongly supported in a clade with diploid species of *Macropoa* in ARs tree. Most probably *Poa densa* is not a hybrid species, since it is a diploid species closely related to two diploid species of *Poa sibirica* and *Poa bucharica* from section *Macropoa* in present study. Here a new treatment for this species as a member of the supersection *Poa* is suggested. To verify the exact section that *Poa densa* belongs to, more samples of this species and more samples from supersection *Poa* in particular section *Macropoa* should be included in molecular analysis.

4.4.3 *Poa masenderana*

Poa masenderana has been classified conflictingly over the time. It was assigned to series *Palustres* by Roshevitz (1932) equivalent to subgenus *Stenopoa* section *Stenopoa* in current classification (Zhu *et al.* 2006; Soreng 2007). Bor (1970) classified *P. masenderana* in the group *Alpinae* along with *P. alpina* (currently assigned to the section *Alpinae*), *P. tibetica* (currently assigned to the genus *Arctopoa* and section *Aphydris*), *P. falconeri*, *P. pagophila*, *P. stapfiana* Bor and *P. polycolea* (currently assigned to section *Homalopoa*), and *P. Koelzii* and *P. roemeri* (currently assigned to section *Stenopoa* and subsection *Stenopoa*). *Poa masenderana* was assigned to subgenus *Poa* and section *Homalopoa* by Tzvelev (1976). *Poa masenderana* has not been a part of molecular study before. In present molecular analyses two samples from this West Asian species are closely allied to the species of the supersection *Poa* species in plastid and ARs tree, while in nuclear trees they form a clade which is collapsed into a polytomy with species of subgenus *Poa* section *Macropoa* and *P. aitchisonii* and *P. chaixii*. *Poa*

masenderana better fits to supersection *Poa*.

4.4.4 *Poa cenisia*

Poa cenisia, which occurs in Europe and Turkey was described by Allioni (1789) for the first time. Tzvelev (1976) treated *P. cenisia* as a synonym of *Poa deylii* Chrtek et Jiras. under subsection *Poa*, section *Poa* and subgenus *Poa*. Czerepanov (1981) also treated this species as a synonym of *Poa deylii*, while *Poa deylii* itself was treated as *Poa granitica* Braun-Blanq. subsp. *disparilis* (Nyár.) Nyár. by Edmondson J.R. (1980). Soreng et al. (2003) determined *Poa cenisia* var. *virens* Griseb. a synonym for *Poa glauca* Vahl from section *Stenopoa*.

Overall, *P. cenisia* is positioned far from subgenus *Poa* in all nuclear, plastid and ARs trees. It is nested within subgenus *Stenopoa* clade in nuclear trees and especially with a strong support in nrDNA tree. In plastid trees *Poa cenisia* is nested within “N Clade” in a strongly supported major clade with the species of the subgenus *Stenopoa*. The position of *P. cenisia* is identical in nrDNA and ARs trees as sister to *P. psychrophila* 1 with a high support. *Poa cenisia* represents the closest relationship to section *Secundae* than other sections of *Stenopoa*. A new classification should be proposed for *P. cenisia* as a member of the subgenus *Stenopoa*.

4.4.5 *Poa psychrophila*

Poa psychrophila Boiss. & Heldr. (1854) has been considered as a synonym of *P. cenisia* by Scholz (1991), and Cabi & Doğan (2012). Only a few DNA sequence data such as the sequence of psbA (a plastid coding locus for a polypeptide, which is involved in electron transport through photosystem II) are available for this species but they are not parts of a published study. So this species was a part of molecular

phylogenetic studies for the first time.

Two samples of *Poa psychrophila* (*P. psychrophila* 1 & *P. psychrophila* 2) in present molecular analyses are positioned far from each other in nuclear trees.

Poa psychrophila 1 is sister to *P. cenisia* in with strong support in cpDNA, nrDNA and ARs trees. The position of the clade that *P. psychrophila* 1 and *P. cenisia* form is different in nuclear and plastid trees. In nuclear trees it is positioned within of subgenus *Stenopoa* (excluding *Pandemos*, *Poa abbreviata* and *Poa leptocoma*), while it is placed in a clade including “N Clade” and *Secundae*, which is sister clade to subgenus *Stenopoa* (excluding *Secundae*) in plastid trees. *Poa psychrophila* 1 is highly supported in both of these positions. The placement of *Poa psychrophila* 1 in present phylogenetic trees is consistent with the synonymy event by Scholz (1991), and Cabi & Doğan (2012), considering *Poa psychrophila* a synonym of *Poa cenisia*. So *Poa psychrophila* 1 should be classified as a member of subgenus *Stenopoa*.

Poa psychrophila 2 is not sister to *P. cenisia*, but this sample is placed in a clade including *P. psychrophila* 1 and other species of “N Clade” in plastid trees. In nuclear trees *Poa psychrophila* 2 is sister to *P. ursina* with a very good support in nrDNA and ITS trees. These two species are placed in a clade with *Oreopoa* and the species of “N Clade” species. *Poa psychrophila* 2 belongs to “N Clade” and might be related to *Bolbophorum* [Part 2].

4.4.6 *Poa lipskyi* subsp. *lipskyi*

Poa lipskyi is another new species for molecular analysis. This species is distributed in East Asia and Central Asia. It was assigned to the subgenus *Poa* section *Poa* subsection *Poa* (Tzvelev 1976). *Poa lipskyi* was assigned to section *Poa* subsection

Cenisiae (Zhu *et al.* 2006) and was assigned to section *Malacanthae* (unpublished reference). *Poa lipskyi* is nested within subgenus *Poa* in all nuclear trees, while it is allied to species of the subgenus *Stenopoa* in all plastid trees. There is a possibility that this species is a hybrid between a member of *Malacanthae* and a member of sections *Stenopoa* or *Oreinos* species if it is not a diploid. To test this, first a chromosome counting and additional molecular analyses with species morphologically close to it such as *P. nitidespiculata* Bor. and *P. mairei* Hack. should be conducted. Also there is a need to look at more samples of the species, to determine if only this sample is a hybrid or the species.

4.4.7 *Poa remota*

Poa remota is a diploid species distributed in east Asia, Central Asia and Europe. This diploid species was assigned to section *Homalopoa* (Tzvelev 1976; Edmondson 1980) and to section *Poa* subsection *Poa* (Zhu *et al.* 2006). In plastid and ARs trees *Poa remota* is sister to species of the supersection *Poa*, while in nuclear this species is nested within species of subgenus *Poa*. This is consistent with previous analyses (Gillespie *et al.* 2009, 2010; Soreng *et al.* 2010) that determined the same relationship between *P. remota* and section *Poa* in nuclear trees. Also this is consistent with plastid analyses by Gillespie *et al.* (2009, 2010) and Soreng *et al.* (2010). *Poa remota* has a closer relationship to supersection *Poa* than supersection *Homalopoa*.

4.4.8 *Poa trivialis*

Poa trivialis is distributed in Europe, Asia, North and South America and Australia. This diploid species has been maintained in a section *Pandemos* Asch. & Graebn. (1898 – 1902) by Hylander (1953), Tzvelev (1976), Edmondson (1980), Soreng

(1998, 2007) and Zhu *et al.* (2006), and was assigned to series *Triviales* Roshev. (Roshev. 1934; Bor 1970) on the basis of the lemma characteristics as follows: The lemma invariably has distinct nerves and is almost fully glabrous, if not then with villous nerves and is very slightly webbed at the base. Tzvelev (1976) assigned *Poa trivialis* to subgenus *Poa* section *Coenopoa* Hyl. (syn. of the section *Pandemos* Asch. & Graebn.) according to lemma with tuft of long sinuate hair on callus.

In present molecular analyses *Poa trivialis* is allied to other species of subgenus *Stenopoa* in plastid and ARs trees with the high strong support, although in nuclear trees it is positioned with *Ochlopoa*, while it does not originally belong to *Ochlopoa*. This relation is strongly supported in ETS tree and moderately supported in nrDNA tree. This is consistent with previous molecular work (Gillespie *et al.* 2009). Soreng *et al.* (2010) attributed the incongruent situation of *Poa trivialis* in plastid and nuclear analyses more likely to long branch attraction, resulting from remote origins and/or rapid rates of evolution, than to a hybrid origin.

4.4.9 *Poa abbreviata*

Poa abbreviata, the hexaploid species is distributed in North America and Arctic areas of Russia and East Asia. It was assigned to the section *Abbreviatae* Hyl. (Tzvelev 1976; Soreng 2007). In this study *Poa abbreviata* was eliminated from ARs analysis because the its position is significantly incongruent between the nuclear and plastid trees. This species is allied to other species of the subgenus *Stenopoa* with a very good support in cpDNA trees. In all nuclear trees *Poa abbreviata* is placed with species of subgenus *Poa*. This is consistent with previous molecular studies by Soreng *et al.* (2010) that suggested the incongruent placements are attributed to reticulate evolution and *Poa*

abbreviata is a hybrid.

4.4.10 *Poa leptocoma*

Poa leptocoma was described by Trinius (1830). This hexaploid species is distributed in North America, Arctic areas of Russia and East Asia. It was assigned to series *Pratenses* as a synonym of *Penicillata* Roshev. (Roshev. 1934) and was treated as member of *Oreinos* (Tzvelev 1976; Soreng 2007). In present study this species is excluded from ARs analyses since its placement conflicted in nuclear and plastid analysis. In all present plastid analyses *Poa leptocoma* is placed in a clade with species of the subgenus *Stenopoa* section *Oreinos* *Poa laxa* subsp. *fernaldiana* and section *Abbreviatae* *Poa abbreviata*, or is placed close to these species in a polytomy with other species of the subgenus *Stenopoa*. In all nuclear trees *Poa leptocoma* is strongly supported as a new member of the “X clade” with a strong support along with two species of *Malacanthae* and two species of *Homalopoa*.

4.5 Unclassified Species

The following species have never been assigned to a section or otherwise are placed within a recent accepted subgeneric classification.

4.5.1 *Poa aitchisonii*

The perennial species *P. aitchisonii* was described for Pakistan for the first time. This species, which is native to Pakistan was classified with other three species of *P. annua* (currently assigned to *Micrantherae* of *Ochlopoa*), *P. pratensis* (currently assigned to section *Poa* of supersection *Poa*) and *Poa trivialis* (currently assigned to section *Pandemos*) by Boissiere (1984) based on its 5-nerved lemma with eminent lateral veins, which is hairy on keels and margins. *Poa aitchisonii* occurs in East Asia

(Liou 2003), Central Asia (Bor 1970; Cope 1982; Rajbhandari 1991; Liou 2003) and West Asia (Bor 1970; Rajbhandari 1991; Liou 2003) and there is no record of molecular study on this species. This is the only time its relationships has been discussed.

In present of ARs, cpDNA trees, *P. aitchisonii* is sister to *P. longifolia* (*Macropoa*) with very good and good supports in the clade of supersection *Poa*. So the present analyses indicate that *Poa aitchisonii* should be assigned to subgenus *Poa* of supersection *Poa*. The present analyses of ARs, cpDNA regions also suggest the inclusion of *Poa aitchisonii* to *Macropoa*. To explore the definite taxonomical (sectional) position of this species, the combination of morphological and molecular data of more samples of *P. aitchisonii* and related species from subgenus *Poa* should be included to phylogenetic analyses.

4.5.2 *Poa diversifolia*

Hackel (1884) described the perennial species *P. diversifolia* for the first time from Afghanistan Valley, and this species was grouped with four species on the basis of its five-nerved glabrous lemma with prominent lateral nerves by Boissiere (1884). Of these species, *P. longifolia* and *P. pariadrica* Boiss. (as a synonym of *P. longifolia* (Edmondson 1985; Cabi & Doğan 2012) are currently treated as members of the section *Poa* of the supersection *Poa*, while other two species *P. hybrida* and *P. chaixii* are currently assigned to section *Homalopoa* of supersection *Homalopoa* (Gillespie *et al.* 2008, 2010; Soreng *et al.* 2010).

Poa diversifolia grows in Lebanon and Turkey (Hackel 1884; Edmondson 1985). In present nuclear analyses *P. diversifolia* is nested within the species of the subgenus

Poa in a clade with no support and. The position of *P. diversifolia* within supersection *Poa* is strongly supported in cpDNA and ARs tree and very. So the analyses suggest the inclusion of *P. diversifolia* in supersection *Poa*.

4.5.3 *Poa pseudobulbosa*

Poa pseudobulbosa Bor is a perennial, alpine and densely caespitose with no rhizome. Based on the information from two samples collected in 1947, Bor (1972), Davis *et al.* (1985) and Clayton *et al.* (2002) reported a narrowly restricted geographic range of approximately 185 km² in central Taurus Mountains in south of Turkey. However, Eren *et al.* (2006) reported another collection in 2004 from west of Taurus Mountains. There are only three populations of the species that grow in three isolated sites (Romand-Monnier & Bachman 2013 in <URL: <http://www.iucnredlist.org/details/44392549/0>). This species was listed in the IUCN Red List Categories (2013) as a “Near Threatened” species.

The results of the present analyses portend a close relation with subgenus *Poa* and in particular with supersection *Poa*. *Poa pseudobulbosa* is sister to a clade of subgenus *Poa* and *Oreopoa* with moderate support in nrDNA. In plastid analyses *P. pseudobulbosa* is sister to supersection *Poa* and shows a closer affinity to *Poa* than supersection *Homalopoa*. The strong support for this relation in cpDNA and ARs trees is a good indication that *Poa pseudobulbosa* belongs to supersection *Poa*.

4.6 Future Work Direction

Some areas of taxonomic ambiguity, and some areas with unfinished investigations that require further studies are highlighted here:

- ❖ Since in phylogenetic works, samples are required to cover all distribution areas of

the taxa, there is an essential need of a great sampling in West Asia and Africa, where many *Poa* species have not been studied yet and interesting unknown taxa can definitely be met.

- ❖ Future review on molecular phylogeny of the genus *Poa* should provide additional sequenced DNA regions, combine new data matrices with the available molecular datasets to have phylogenetic trees with the better resolution and higher supports.
- ❖ *Poa caucasica* is now recognized as its own lineage; however, its evolutionary relationships have yet to be thoroughly studied. Fresh plant material of *Poa caucasica* across its distribution range is also required for phylogenetic analyses to reconstruct the relationship between this species and other *Poa* species.
- ❖ Species of the subgenus *Ochlopoa* are not monophyletic and there is always a misplaced complex group of *Nanopoa* and *Bolbophorum* (“N Clade”). This complex forms a clade with “X Clade” in nuclear trees and have a close relationship with subgenus *Stenopoa* in plastid trees. More work is needed to explore the of evolution for this group.
- ❖ As *Eremopoa* is decreased to the rank of subgenus of *Poa* (*Pseudopoa*), so the next attempt should be made on classification of *Pseudopoa* species.
- ❖ An effort should be taken for a greater sampling of *Oreopoa* from the type locality and inclusion of *Oreopoa* to phylogenetic analyses in order to explore the exact taxonomical position of *Oreopoa* (assign that to possible subgenus and section within *Poa* and define a scientific name for that).

Table 1. The brief history of the infrageneric classification of *Poa* from 1884 to 2014 (Application of subgenera, sections and subsections is on the basis of floristic and molecular studies). The codes for the used signs are: → No change was made; — Subgenus not defined; ♦ Supersection *Homalopoa*; ◇ Supersection *Poa*; ★ Subsection; + Inclusion of a genus as the section of *Poa*; ○ A group which has not been assigned to a section yet; **Bold** Introduced for the first time; ⊙ Accepted as a group (equivalent to section), where subgenus was not defined; 🌿 Morphological studies and 🧬 Molecular studies.

Subgen. & Supersect.	Boiss. 1884	Stapf 1897	Bor 1970	Tzvelev. 1976 (1983)	Soreng & Davis 2000	Soreng et al. 2003
<i>Andinae</i>					Monotypic subgenus	→
<i>Arctopoa</i>				<i>Arctopoa</i> <i>Aphydris</i>	→	→
<i>Poa</i>				<i>Abbreviatae</i> <i>Coenopoa</i> <i>Homalopoa</i> <i>Macropoa</i> <i>Ochlopoa</i> <i>Oreinos</i> <i>Poa</i> ★ <i>Caespitosae</i> ★ <i>Bulbosae</i> ★ <i>Poa</i> ★ <i>Nivicolae</i> <i>Stenopoa</i> ★ <i>Stenopoa</i> ★ <i>Tichopoa</i>	<i>Abbreviatae</i> <i>Acutifoliae</i> <i>Alpinae</i> <i>Daysypoa</i> <i>Dioicopoa</i> <i>Homalopoa</i> <i>Madropoa</i> <i>Ochlopoa</i> <i>Oreinos</i> <i>Parodiochloa</i> <i>Plicatae</i> <i>Secundae</i> <i>Stenopoa</i> <i>Sylvestres</i> <i>Tichopoa</i>	<i>Madropoa</i> ★ <i>Epiles</i> ★ <i>Madropoa</i> <i>Monandropoa</i> <i>Pandemos</i> <i>Parodiochloa</i> (<i>Tropicos</i>) <i>Poa</i> <i>Secundae</i> ★ <i>Halophyte</i> ★ <i>Secundae</i>
◇ supersect. <i>Homalopoa</i>			⊙ <i>Glabratae</i>			
♦ supersect. <i>Poa</i>			⊙ <i>Pretense</i>			
*species Group			<i>Poa compressa</i>			
	Section <i>Eupoa</i> (including all <i>Poa</i> species)	Subgenus <i>Eupoa</i> (including All <i>Poa</i> species)				
			⊙ <i>Nemorales</i>			
			⊙ <i>Palustres</i>			
<i>Stenopoa</i>			⊙ <i>Paucidentata</i>	—	—	—
			⊙ <i>Steriles</i>			
			⊙ <i>Triviales</i>			
<i>Ochlopoa</i>			⊙ <i>Bolbosae</i>			
			⊙ <i>Ochlopoa</i>	—	—	—
<i>Sylvestres</i>						
<i>Pseudopoa</i>	Section <i>Psilantha</i>	Subgenus <i>Pseudopoa</i>	Genus EREMOPOA	Genus EREMOPOA	—	Genus EREMOPOA

Table 1. Continued.








Gillespie & Soreng, 2005	Zhu <i>et al.</i> 2006	Gillespie <i>et al.</i> 2007	Gillespie <i>et al.</i> 2008	Gillespie & Soreng	Soreng <i>et al.</i> 2010	Soreng <i>et al.</i> 2014
						
→	→	→ as genus NICORAEPOA	→	→	→	→ Genus NICORAEPOA
→	→	<i>Arctopoa</i> <i>Aphydris</i> <i>Sylvestres</i>	→ as genus ARCTOPOA	→	→	→ Genus ARCTOPOA
		◆ <i>Acutifoliae</i> ◆ <i>Anthochloa</i> ◆ <i>Austrofestuca</i> ◆ <i>Brizoides</i> ◆ <i>Dasympoa</i> ◆ <i>Dioicopoa</i> ◆ <i>Homalopoa</i> ◆ <i>Madropoa</i> ※ <i>Australopoa</i> ※ <i>Punapoa</i> ◇ <i>Leptophyllae</i> ◇ <i>Macropoa</i> ◇ <i>Nivicola</i> ◇ <i>Poa</i>				◇ <i>Malacanthae</i> ◇ <i>Poa</i> ◇ ★ <i>Cenisiae</i> ◇ ★ <i>Nivicolae</i>
<i>Bolbophorum</i> <i>Pauciflorae</i>	<i>Macropoa</i> <i>Poa</i> ★ <i>Cenisiae</i> ★ <i>Poa</i> ★ <i>Nivicolae</i>		→	→	→	
—	<i>Abbreviatae</i> <i>Oreinos</i> <i>Pandemos</i> <i>Secundae</i> <i>Stenopoa</i> <i>Tichopoa</i> <i>Alpinae</i> <i>Arenariae</i> <i>Micrantherae</i> (<i>Ochlopoa</i>)	→	→	→	→	<i>Abbreviatae</i> <i>Oreinos</i> <i>Pandemos</i> <i>Secundae</i> <i>Stenopoa</i> <i>Tichopoa</i>
—		<i>Bolbophorum</i> <i>Parodiochloa</i>	→	→	→	<i>Bolbophorum</i> <i>Nanopoa</i> <i>Tzvelevia</i>
—			<i>Sylvestres</i>	→	→	<i>Sylvestres</i>
Genus EREMOPOA	Subgenus <i>Pseudopoa</i>	→	→	→	→	<i>Pseudopoa</i>

Table 2. The infrageneric taxonomy of the genus *Eremopoa* (Soreng *et al.* 2014 in URL: <http://www.tropicos.org> The abbreviations used in this table are *Gl.*, *Glyceria*; *E.*, *Eremopoa*; *P.*, *Poa*.

Taxa	Synonyms	Geographical Distribution and Reference
<i>E. altaica</i> Roshev. subsp. <i>altaica</i> (1934) 2n=28 & 42	<i>Aira altaica</i> Trin. (1835)	
	<i>Catabrosella songarica</i> (Tzvelev) Czrep. (1981)	
	<i>E. altaica</i> (Trin.) Roshev.	Armenia (Bor 1970)
	subsp. <i>songarica</i> (Schrenk) Tzvelev (1966)	Azerbaijan (Bor 1970; Tzvelev 1976)
	<i>E. bellula</i> (Regel) Roshev. (1934)	China (Bor 1970)
	<i>E. glareosa</i> Gamajun. ex Pavlov (1964)	Europe (Tutin 1980)
	<i>E. persica</i> (Trin.) Roshev.	Iraq (Bor 1970)
	var. <i>songarica</i> (Schrenk) Bor (1960)	Iran (Parsa 1950; Bor 1970)
	<i>E. songarica</i> (Schrenk) Roshev. (1934)	Kazakhstan (Bor 1970; Tzvelev 1976)
	<i>Festuca bellula</i> Regel (1881)	Kyrgyzstan (Bor 1970)
	<i>Festuca persica</i> (Trin.) K. Koch. (1848)	Mongolia (Pilke & Najmi 2011)
	<i>Gl. songarica</i> Schrenk ex Fisch & C.A. Mey (1841)	North America (Soreng 2003; Darbyshire 2007)
	<i>Nephelochloa altaica</i> (Trin.) Griseb. (1852)	Pakistan (Bor 1960; Cope 1982)
	<i>Nephelochloa persica</i> (Trin.) Griseb. (1852)	Grasses of the Soviet Union (Tzvelev 1976)
	var. <i>songarica</i> (Schrenk) Griseb. (1852)	Tajikistan (Bor 1970)
	<i>Nephelochloa songarica</i> (Schrenk) Griseb. (1852)	Turkey (Miller 1985; Cabi & Doğan 2012)
<i>P. diaphora</i> Trin. (1836) subsp. <i>diaphora</i>	Turkmenistan (Bor 1970; Tzvelev 1976)	
<i>P. paradoxa</i> Karavaev et Kir. (1841)		
<i>P. persica</i> Trin. (1830)		
var. <i>songarica</i> (Schrenk) Stapf (1897)		
<i>P. songarica</i> (Schrenk) Boiss. (1884)		
<i>P. subtilis</i> Kar. & Kir. (1842)		
var. <i>songarica</i> (Schrenk) Stapf (1897)		
<i>P. songarica</i> (Schrenk) Boiss. (1884)		
<i>P. subtilis</i> Kar. & Kir. (1842)		
<i>E. altaica</i> Roshev. subsp.	<i>E. oxyglumis</i> (Boiss.) Roshev. (1934)	Grasses of the Soviet Union (Tzvelev 1976)
	<i>E. persica</i> (Trin.) Roshev.	Iran (Parsa 1950; Bor 1970)
	var. <i>oxyglumis</i> (Boiss.) Grossh. (1939)	Turkey (Tzvelev 1976)
<i>Oxyglumis</i> Tzvelev (1966)	<i>P. diaphora</i> Trin (1836)	Turkmenistan (Tzvelev 1976)
subsp. <i>oxyglumis</i>	Soreng & G.H. Zhu (2004)	
<i>E. attalica</i> H. Scholz. (1981)		Iran, Turkey (Miller 1986)
<i>E. capillaris</i> R.R. Miller (1985)		Turkey (Miller 1985)
<i>E. medica</i> H. Scholz (1981)		Iran (Scholz 1981)
<i>E. mardinensis</i> R.R. Mill. (1985)		Turkey (Miller 1986)

Table 2. Continued.

Taxa	Synonyms	Geographical Distribution and Reference
<i>E. nephelochloides</i> Roshev. (1945)		Iran (Roshev. 1945; Parsa 1950)
<i>E. persica</i> Roshev. (1934) $2n=14$	<i>Festuca heptantha</i> K. Koch (1848) <i>Festuca persica</i> (Trin.) K. Koch (1848) <i>Festuca polygama</i> K. Koch (1848) <i>Nephelochloa persica</i> (Trin.) Griseb. (1852) <i>Poa persica</i> Trin. (1830) <i>Poa parvula</i> Roem & Schult. (1817)	Afghanistan (Podlech 2012) Azerbaijan (Bor 1970) Egypt–Sinai (Bor 1960; Post 1896) Iraq (Bor & Guest 1968; Bor 1970) Iran (Parsa 1950; Bor 1970) Lebanon (Mourterde P. 1966; URL: http://www.lebanon-flora.org 2013) Pakistan (Bor 1960 & Cope 1982) Russian Federation–Western Siberia (Tzvelev, 1976) Syria (Bor 1960; Post 1896) Turkey (Miller 1986; Tzvelev 1976) Turkmenistan (Bor 1970)

Table 3. Morphological characteristics in *Eremopoa* and *Poa* .

	<i>Eremopoa</i> Roshev.	<i>Poa</i> L.
Panicle	·With scabrous branches	·With scabrous or smooth branches
Spikelet	·Bearing 2–8 (10) bisexual flowers	· With (2) 3–6 (8) bisexual flowers
Rachilla	·Scabrous	· Mostly smooth, sometimes scabrous or short-pilose
	·Glabrous	· Glabrous (scabrous in base of veins)
	·Apex acute	· Apex acute or rounded
Glume	·Round in back or slightly keeled	· Strongly keeled (rarely smoothly keeled in <i>Micrantherae</i>)
	·Lower glume: ovate or elliptic, 1 veined	· Lower glume: lanceolate, one or three veined
	·Upper glume: ovate or elliptic, 3 veined	· Upper glume: ovate, 3–5 veined (Elliptic, 3 veined in <i>Micrantherae</i>)
	·Narrowly lanceolate	· Ovate or ovate-lanceolate
	·Glabrous (Slightly pilose near the base)	·Granulose or pubescent at base (Glabrous, slightly hairy at base in <i>Micrantherae</i>)
Lemma	·Rounded or rarely slightly keeled in back	· Strongly keeled all along the back
	·Apex truncate, obtuse, rarely with short cusp	· Apex obtuse or pointed, rarely acute, always without cusp
	·5 veined	· 5 (7) veined
Palea	·Almost glabrous	· Hairy along keels, often with short hair, Rarely only pilose
	·Obtuse	· Sharp apex, rarely obtuse
Life cycle	·Annual	Perennial (Annual in species of the subgenus <i>Ochlopoa</i>)
Height	· (5) 8–40 (60) cm	· (5) 10–120 (140) cm
	· Erect ephemeral	· With creeping underground or without & rather dense turf
Stem	· Scabrous	· Rarely Scabrous
Leaf	· Leaf sheaths closed only at base	· Leaf sheaths closed to varying degrees (even all along the length)
	· Glabrous and smooth or scabrous	· Smooth or scabrous (rarely in lower leaves short pilose)
Ligules	· Scabrous or sometimes smooth	· Glabrous or very short-pilose on dorsal surface & margins
	· Flat or loosely convolvulate	· Flat or lengthwise folded
Lamina	· Scabrous with spinules on both surfaces	· Glabrous or with scattered hair
	· (0.5) 0.8–2.5 (3.5) mm broad	· (0.6) 1–8 (12) mm broad

Table 4. Utilized primers for PCR and sequencing reactions of nuclear & plastid DNA of used specimens.

DNA region	Primer	Direction	Sequence	Reference
ETS	RETS4-F	F	AGACAAGCATATGACTACTGGCAGG	Gillespie <i>et al.</i> 2010
	18S-R	R	TGGCTACGCGAGCGCATGAG	Starr <i>et al.</i> 2003
ITS	AB102	F	TAGAATTCCCCGGTTCGCTCGCCGTTTC	Douzery <i>et al.</i> 1999
	KRC	R	GCACGCGCGCTACACTGA	Torreçilla & Catalán 2002
matK	390f	F	CGATCTATTCAATCAATATTTTC	Cuenoud <i>et al.</i> 2002
	1326r	R	TCTAGCACACGAAAGTCGAAGT	Cuenoud <i>et al.</i> 2002
rpoB-trnC	<i>trnC</i> ^{GCA} R	F	CACCCRGATTYGAACTGGGG	Shaw <i>et al.</i> 2005
	<i>rpoB</i>	R	CKACAAAAYCCYTCRAATTG	Shaw <i>et al.</i> 2005
trnT-trnL-trnF	TA3	F	GCCGACTATCGGATTTGAACCG	Columbus <i>et al.</i> 2007
	b	R	CGAAATCGGTAGACGCTACG	Taberlet <i>et al.</i> 1991
	c	F	TCTACCGATTTGCCATATC	Taberlet <i>et al.</i> 1991
	d	R	GGGATAGAGGGACTTGAAC	Taberlet <i>et al.</i> 1991
	f	R	ATTTGAACTGGTGACACGAG	Taberlet <i>et al.</i> 1991

Table 5. The profiles of PCR including the recipe and the thermocycler settings used for amplification of the plastid regions. The program of **LYNX_48_TD** was set up for amplification of *matK* and *rpoB-trnC* regions and the program of **LYNX_54_TD** was set up for amplification of *trnTLF* region. Both programs were set up for 35 cycles.

Process Step	Temp. (°C)	Time	# of Cycles
Initial	95	300s	
Denaturing	95	45s	
Annealing	60 – 48 for LYNX_48_TD	60s	15
	60 – 54 for LYNX_54_TD		
Extension	72	120s	
Denaturing	95	60s for LYNX_48_TD 45s for LYNX_48_TD	
Annealing	48 for LYNX_48_TD 54 for LYNX_54_TD	120s for LYNX_48_TD 60s for LYNX_54_TD	20
Extension	72	300s for LYNX_48_TD 120s for LYNX_54_TD	
Final		4 Hrs & 8 Min for LYNX_48_TD 2 Hrs & 27 Min for LYNX_54_TD	35

Table 6. The profiles of PCR including the recipe and the thermocycler settings used for amplification of the nuclear regions. For ETS region the program of ETS_583 was set up for 30 cycles and for the ITS region the program of ITS_58 was set up for 34 cycles.

Process Steps	Time	Temp. (°C)
Initial	60s for ETS_58	95
	120s for ITS_58	
Denaturing	45s	95
Annealing	45s	58
Extension	120s for ETS_58	72
	90s for ITS_58	
Final	2 Hrs & 17 Min for ETS_58	
	2 Hrs & 13 Min for ITS_58	

Table 7. Summary of relevant statistics for maximum parsimony analyses of individual datasets for ETS, ITS, *matK*, *rpoB-trnC*, *trnTLF*, nrDNA, cpDNA and ARs (nrDNA+cpDNA) regions. Total characters, parsimony informative characters for each dataset and the tree length are provided here. Consistency index (CI) presented in brackets excluded parsimony uninformative characters. RI is the retention index.

DNA Regions	Analysis and Number of Samples	Total Characters	Parsimony Informative Characters	% Parsimony Informative Characters	Tree Length	CI	RI
nrDNA	ETS 173	723	241	33.3%	867	0.5386 (4865)	0.8910
	ITS 173	650	186	28.7%	685	0.5036 (0.4472)	0.8623
	nrDNA 173	1373	427	31.1%	1584	0.5126 (0.4586)	0.8743
cpDNA	<i>matK</i> 171	698	79	11.3%	215	0.6386 (0.5629)	0.9340
	<i>rpoB-trnC</i> 172	1438	192	13.4%	418	0.7368 (0.6857)	0.9450
	TLF 173	2321	248	10.7%	577	0.7643 (0.6852)	0.9489
	cpDNA 170	4458	515	11.5%	1222	0.7046 (0.6328)	0.9370
ARs	nrDNA+cpDNA 160	5794	926	16%	2783	0.5799 (0.5143)	0.8970

Figure 1. Schematic drawings of *Eremopoa persica* subsp. *persica* and the illustrations of the morphological features as: a, node of culm; b, ligule; c1, leaf apex; d, upper surface of leaf; d2: lower surface of leaf; e, spikelet; f, upper glume; g, flower; h, lodicule; i, grain; j, lower glume; k, palea; l, lemma; m, branches of panicle in nodes; p, habit. (Adapted from Rahmanian S. *et al.* 2014).

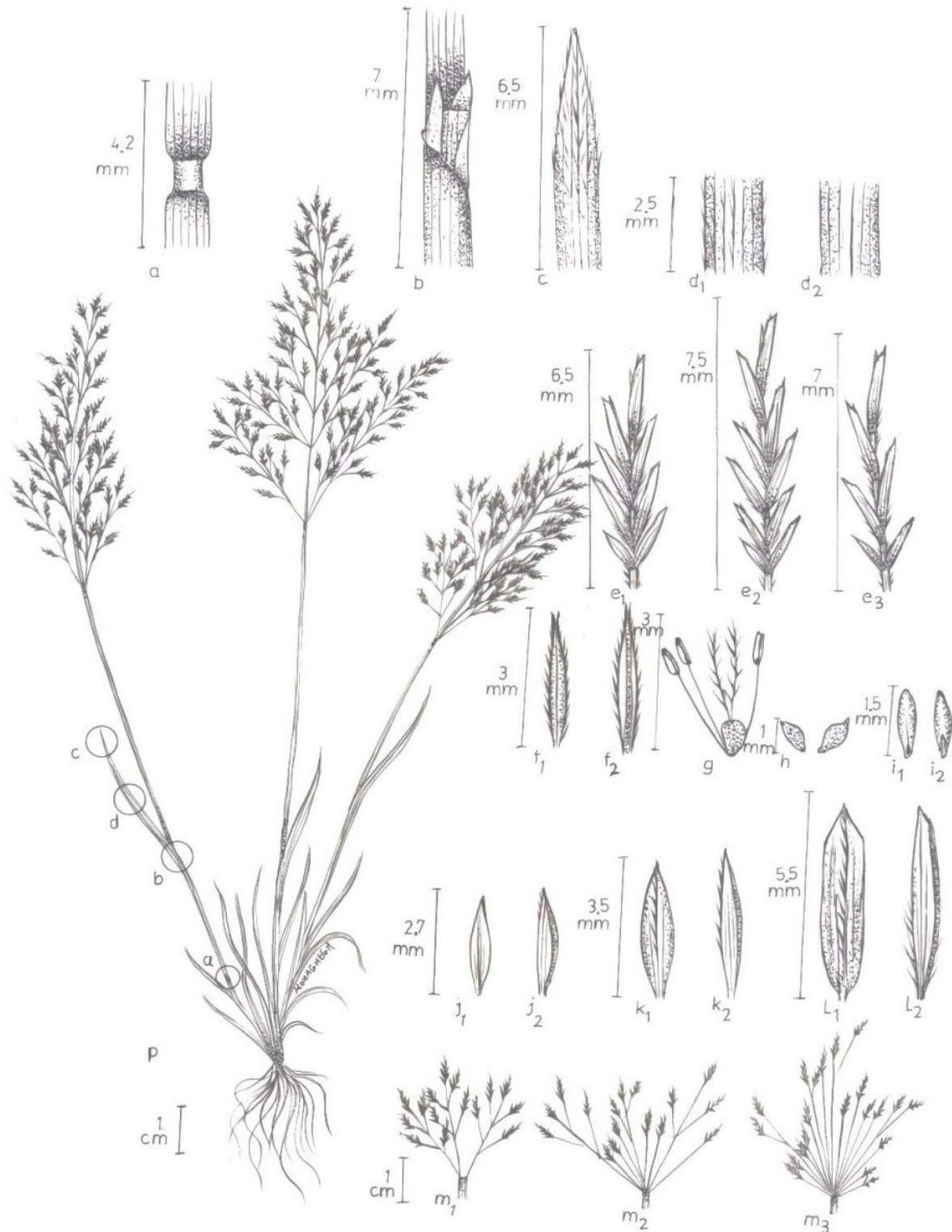


Figure 2. Schematic drawings of *Eremopoa. altaica* subsp. *songarica* in Iran and the illustration shows morphological features as: a node of culm; b ligule; c1 leaf apex; d upper surface of leaf; d2 lower surface of leaf; e flower; e1 upper glume; e2 lower glume; g lodicule; h grain; j lemma; k palea; m spikelet; n branches of panicle in nodes; p: habit. (Adapted from Rahmanian S. *et al.* 2014).

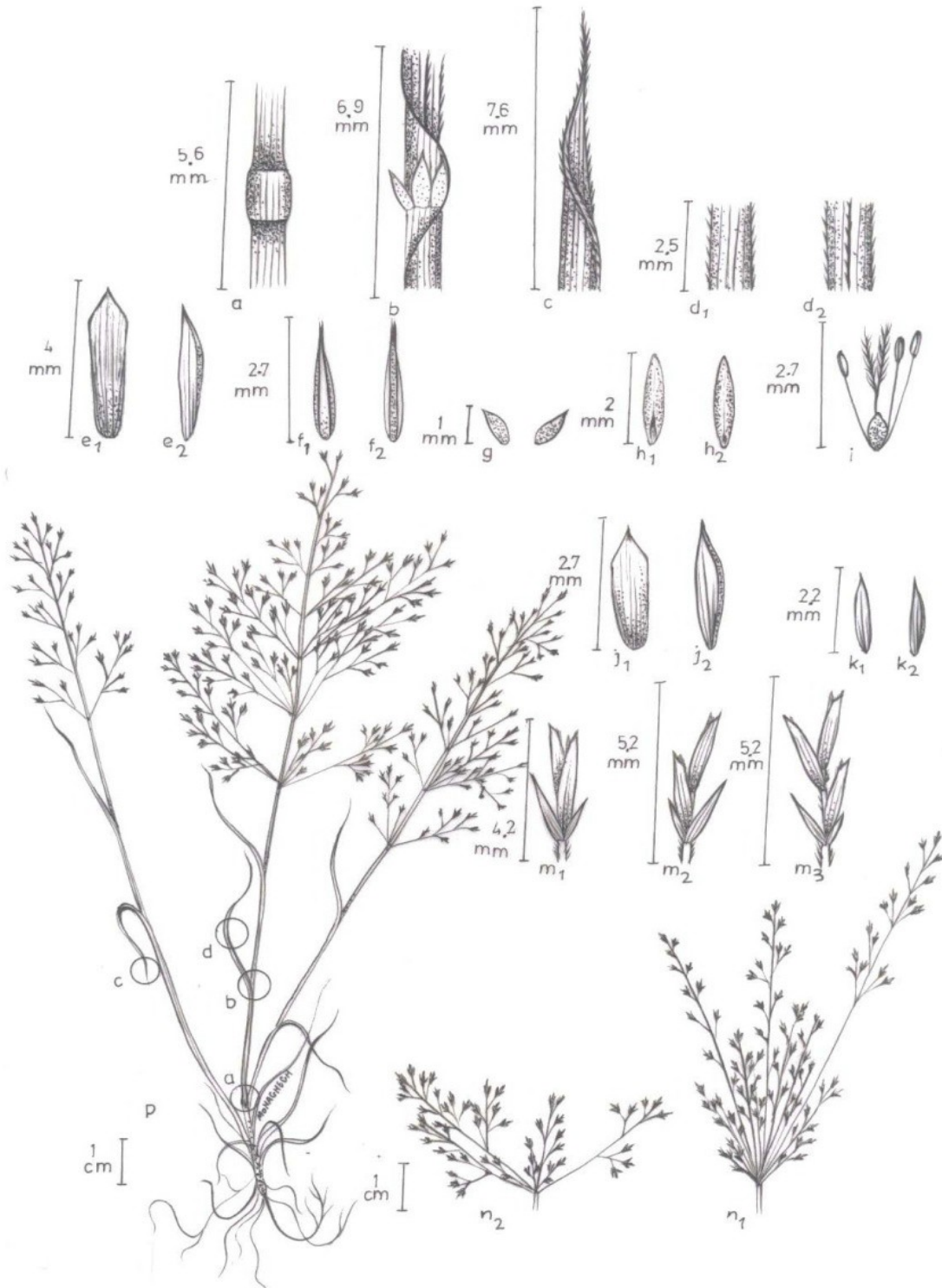


Figure 3. Schematic drawings of *Eremopoa altaica* subsp. *oxyglumis* in Iran and the illustration shows morphological features as: a node of culm; b ligule; c1 leaf apex; d upper surface of leaf; d2 lower surface of leaf; e flower; e1 upper glume; e2 lower glume; g lodicule; h grain; j lemma; k palea; m spikelet; p habit. (Adapted from Rahmanian S. et al. 2014).

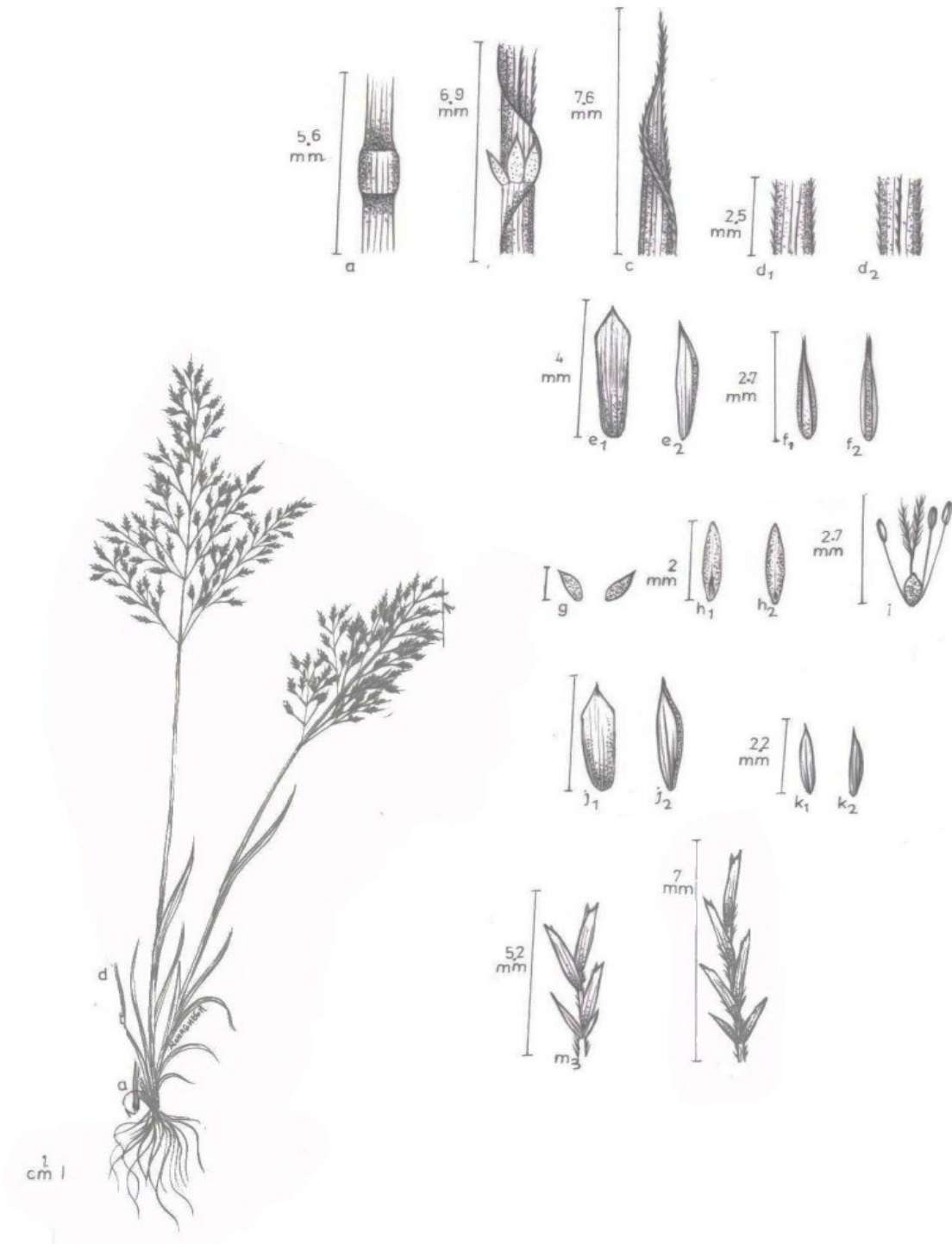


Figure 4. Schematic drawings of *Eremopoa altaica* subsp. *altaica* in Iran and the illustration shows morphological features as: a ligule; b leaf apex; c1 upper surface of leaf; c2 lower surface of leaf; d grain; e lodicule; f flower; g lemma; h palea; i upper glume; j lower glume; k spikelets; p habit. (Adapted from Rahmanian S. et al. 2014).

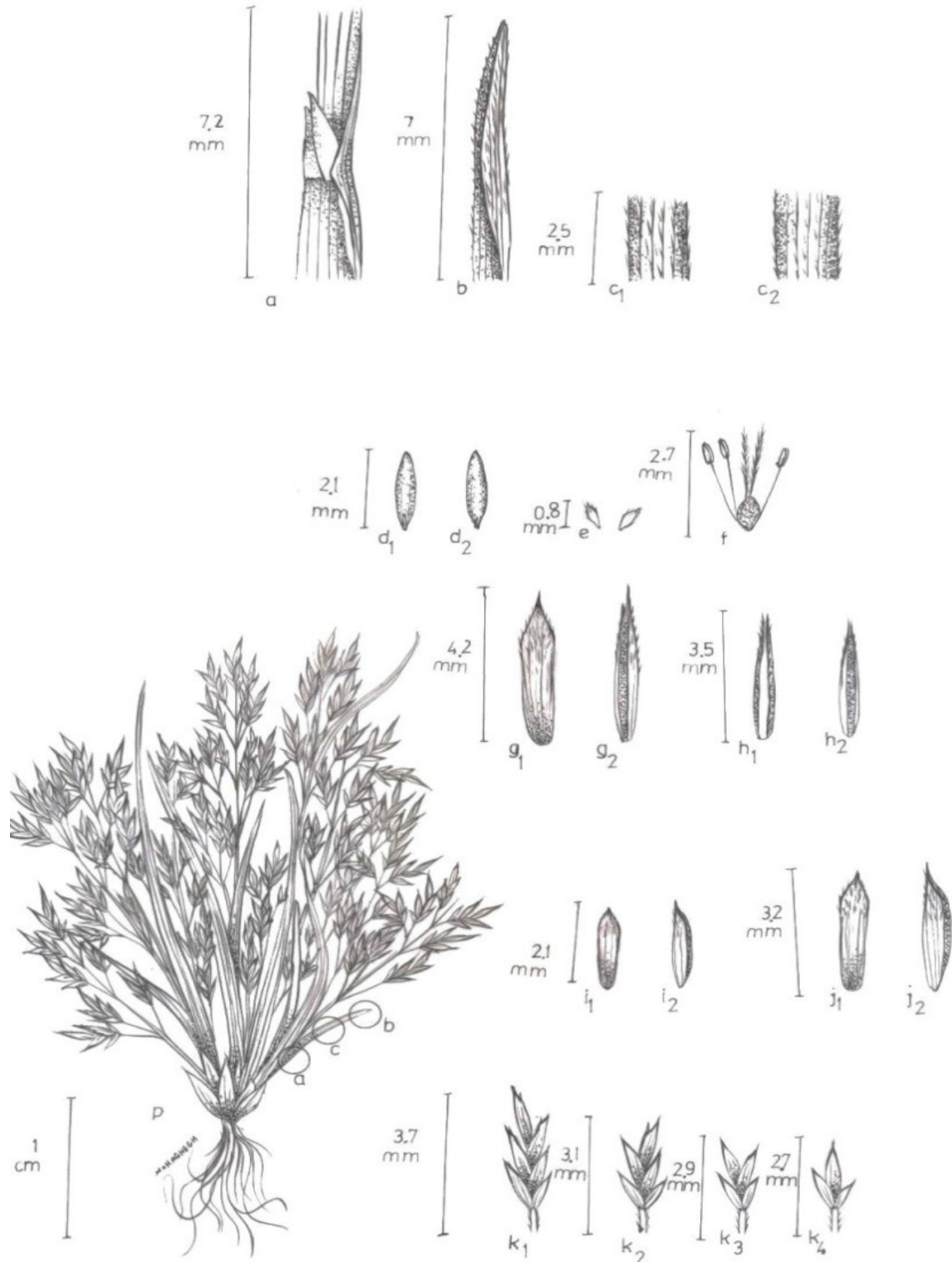


Figure 5. Schematic drawings of *Eremopoa medica* in Iran and the illustration shows morphological features as: a culm surface; b rachilla surface; c1 upper surface of leaves; c2 lower surface of leaves; d lemma; e spikelet; f glum; g palea; p habit. (Adapted from Rahmanian S. et al. 2014).

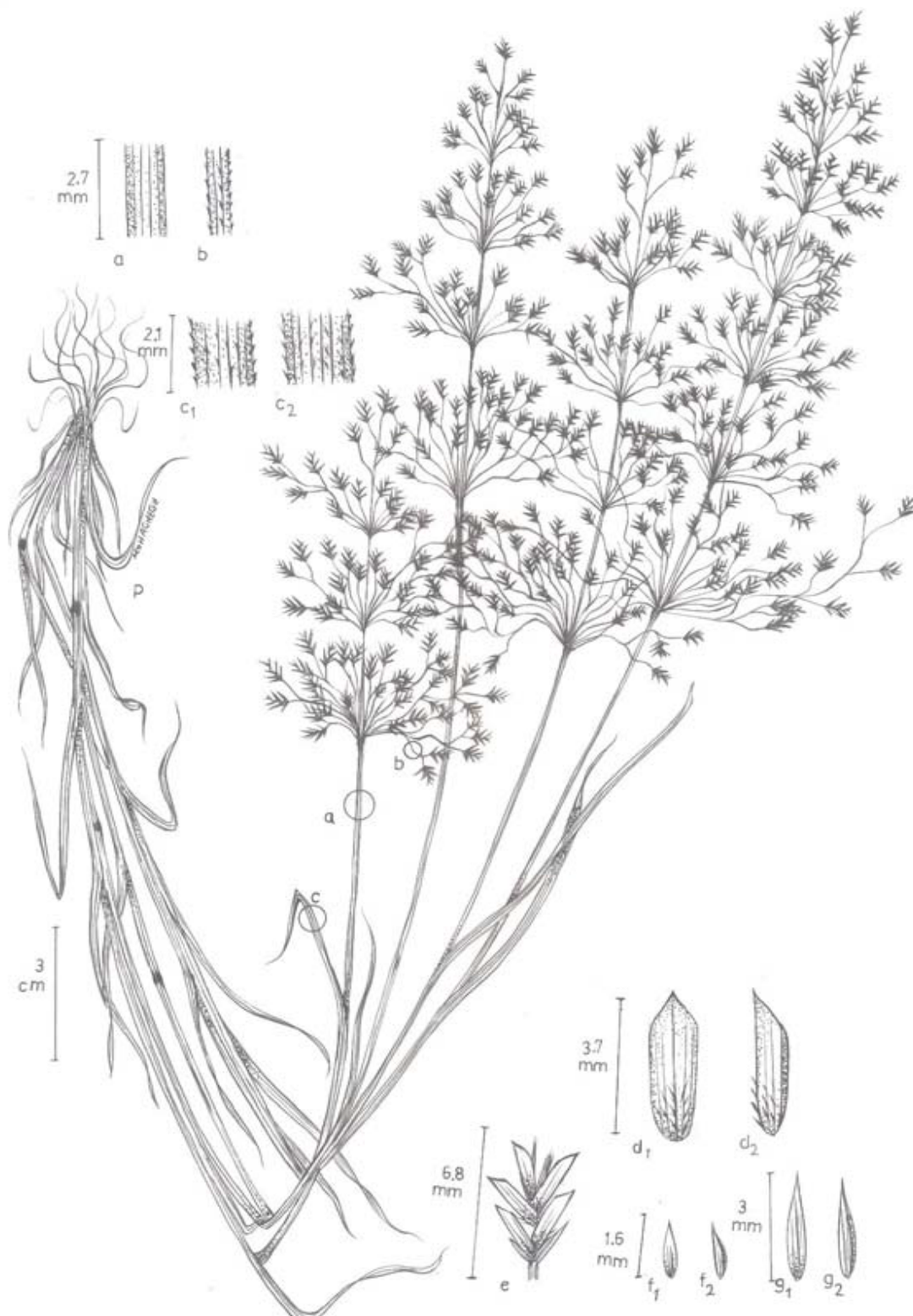


Figure 6. Schematic drawings of *Eremopoa nephelochloides* in Iran and the illustration shows morphological features as: a node of culm; b leaf apex; c1 upper surface of leaf; c2 lower surface of leaf; d ligule; e flower; f lodicule; g grain; h upper glume; i lower glume; j palea; k lemma; l rachilla surface; n spikelet; p habit. (Adapted from Rahmanian S. et al. 2014).

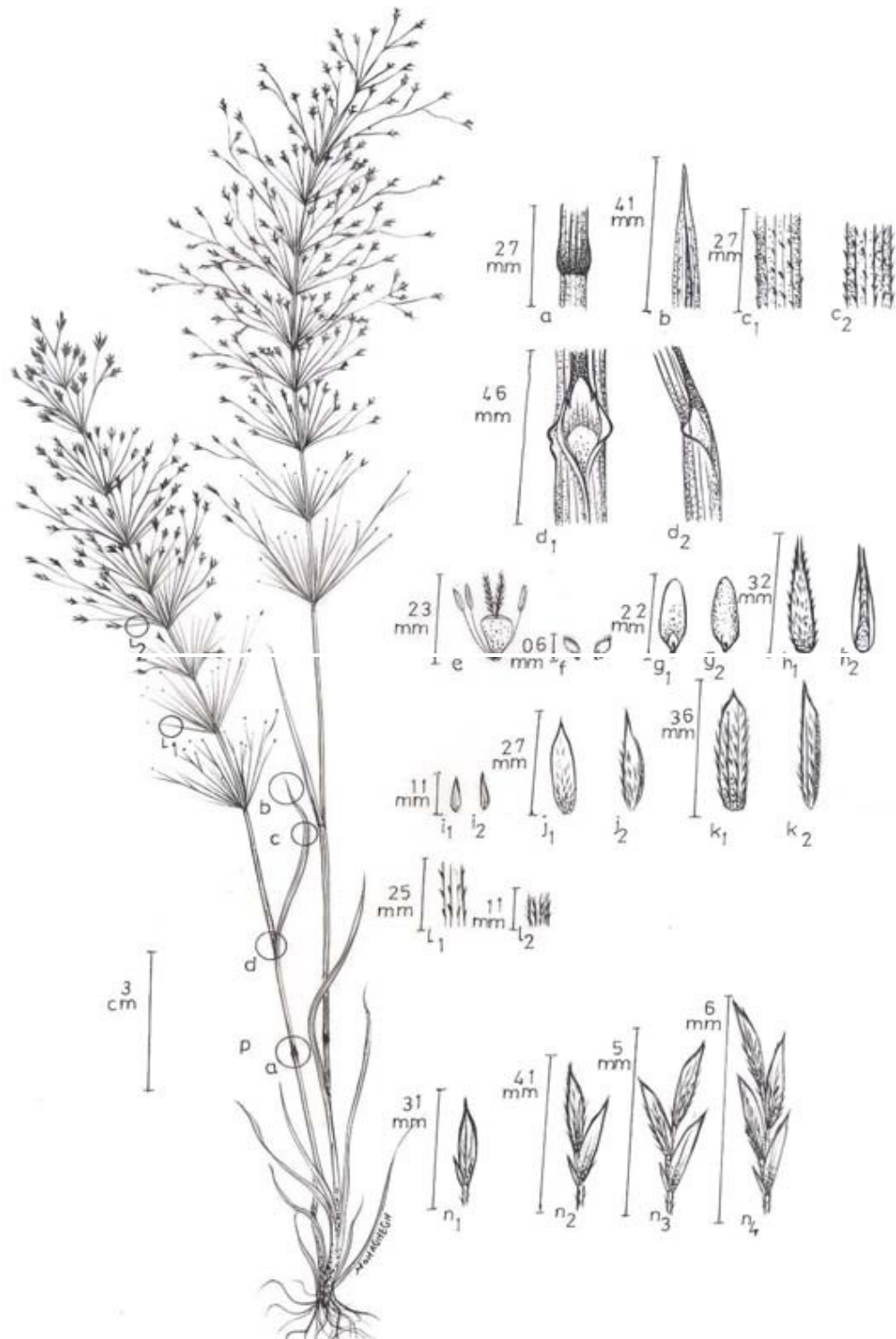


Figure 7. Strict consensus of 100,000 trees from maximum parsimony analysis on *trnT-trnL-trnF* (plastid DNA region) with bootstrap values > 50 displayed above each branch. The color coding for *Poa* subgenera & supersections, and for *Oreopoa* is: aqua for *Sylvestres*; blue for *Ochlopoa*; magenta for *Stenopoa*; green and amber for supersections of subg. *Poa*, *Poa* and *Homalopoa*, Purple for subg. *Pseudopoa* (Eremopoa) and orange for *Oreopoa*.

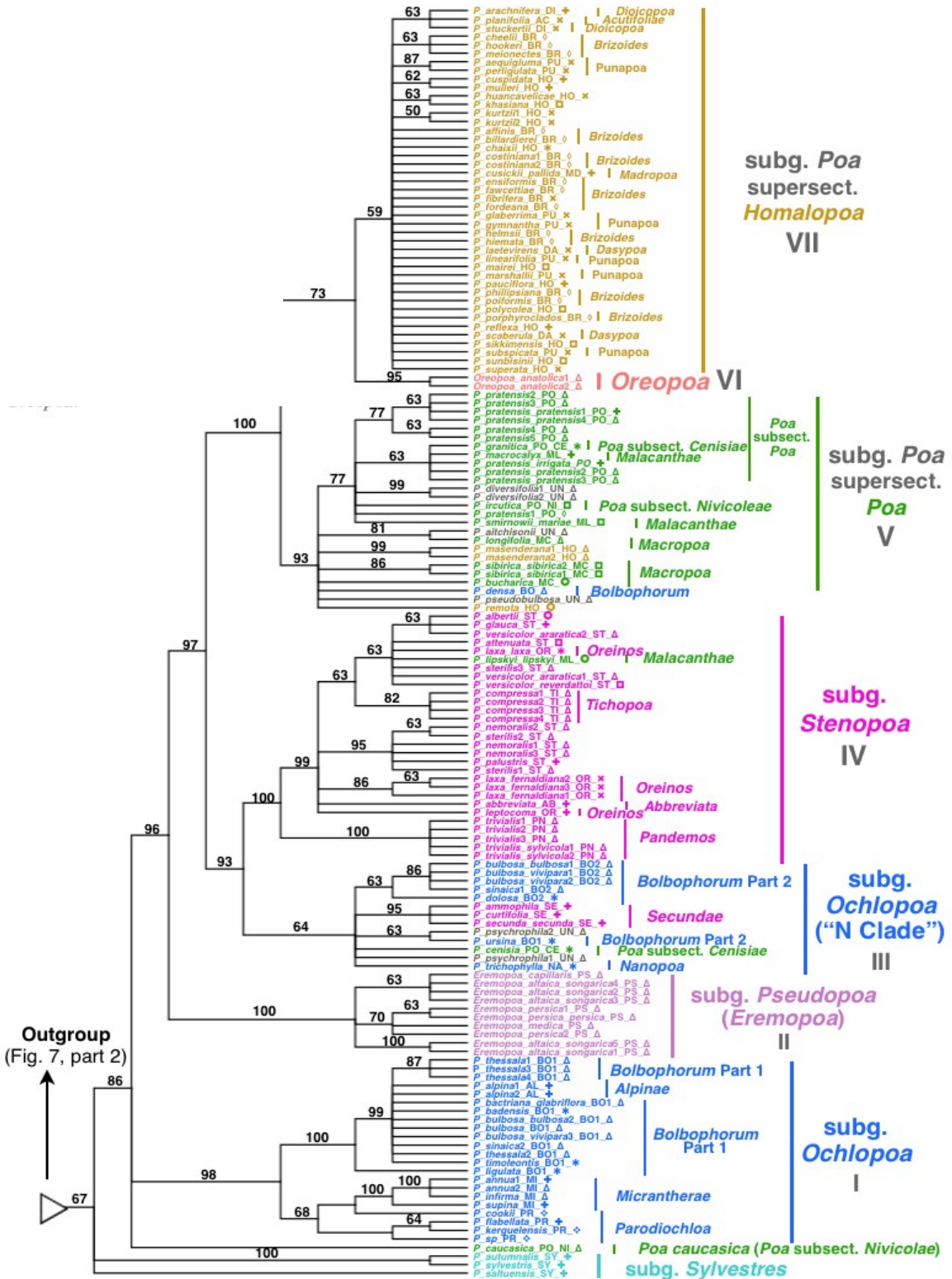


Figure 7. Continued. Outgroup relationships.

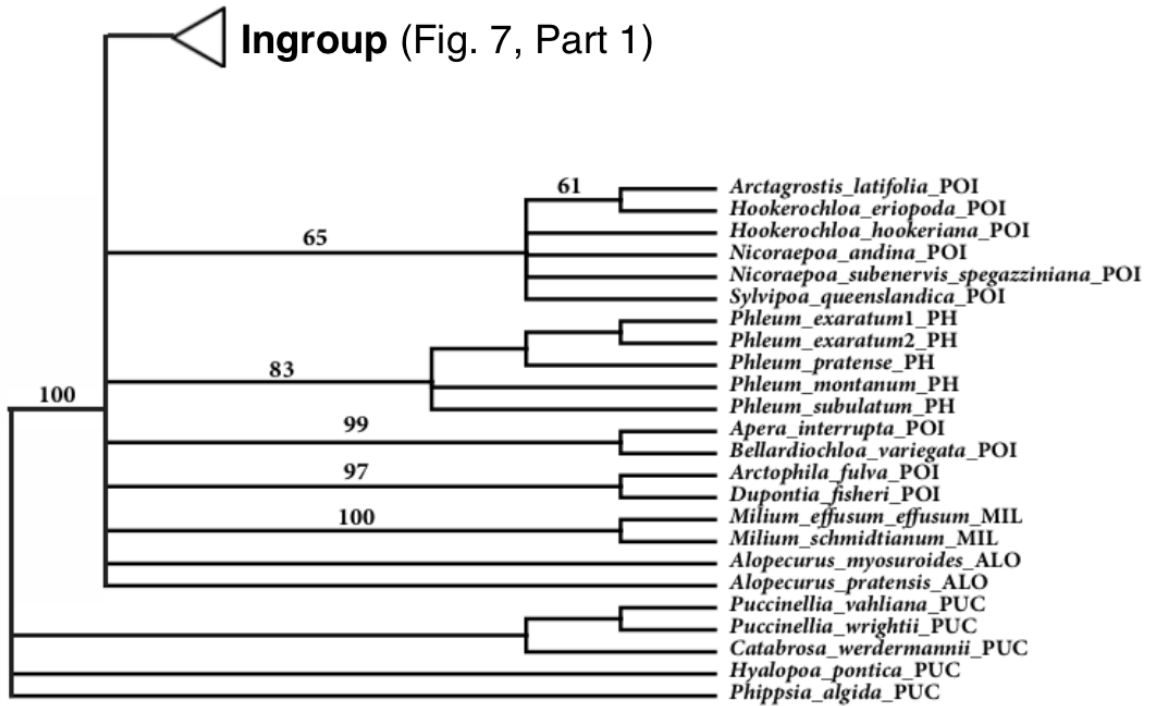


Figure 9. Strict consensus of 100,000 trees from maximum parsimony analysis on *matK* (plastid DNA region) excluding *P. fibrifera* and *P. ursina* with bootstrap values > 50 displayed above each branch. The color coding for *Poa* subgenera & supersections, and for *Oreopoa* is: aqua for *Sylvestres*; blue for *Ochlopoa*; magenta for *Stenopoa*; green and amber for supersections of subg. *Poa*, *Poa* and *Homalopoa*, Purple for subg. *Pseudopoa* (*Eremopoa*) and orange for *Oreopoa*.

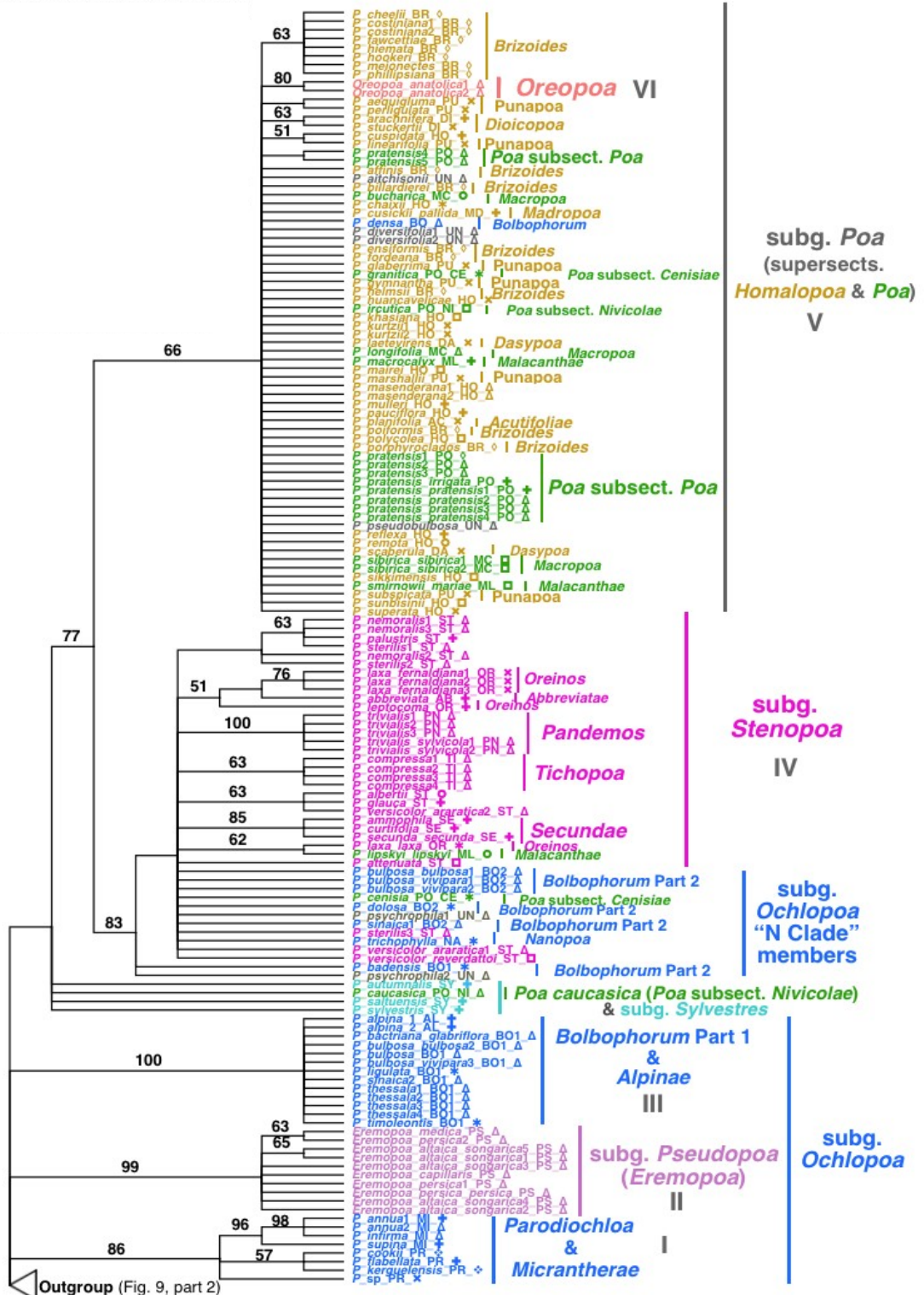


Figure 9. Continued. Outgroup relationships.

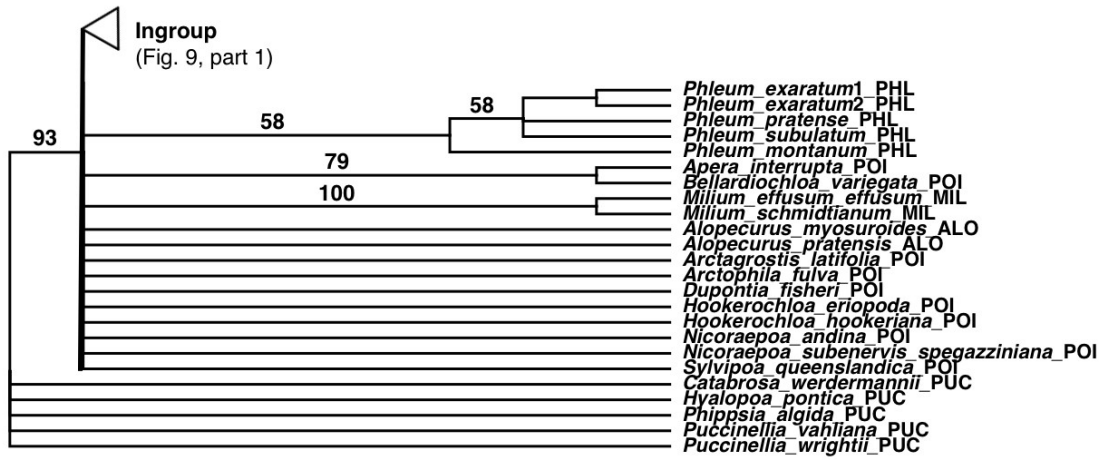


Figure 10. Continued. Outgroup relationships.

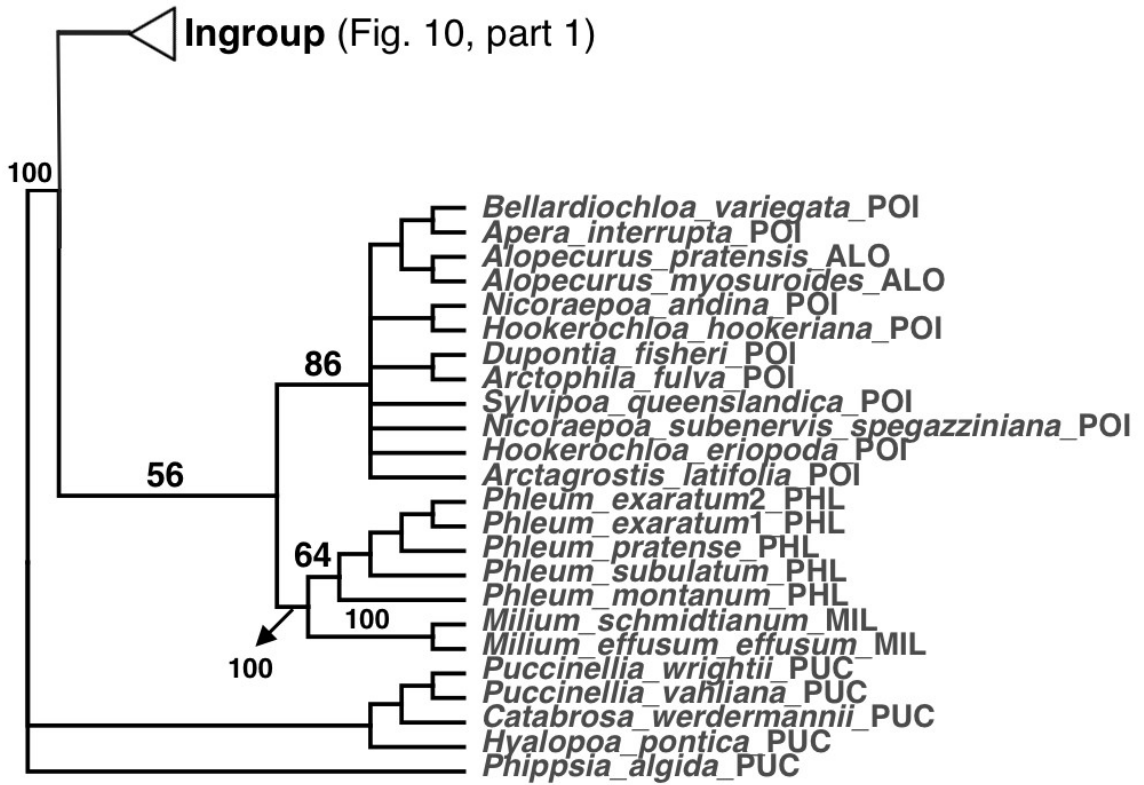


Figure 12. Continued. Outgroup relationships.

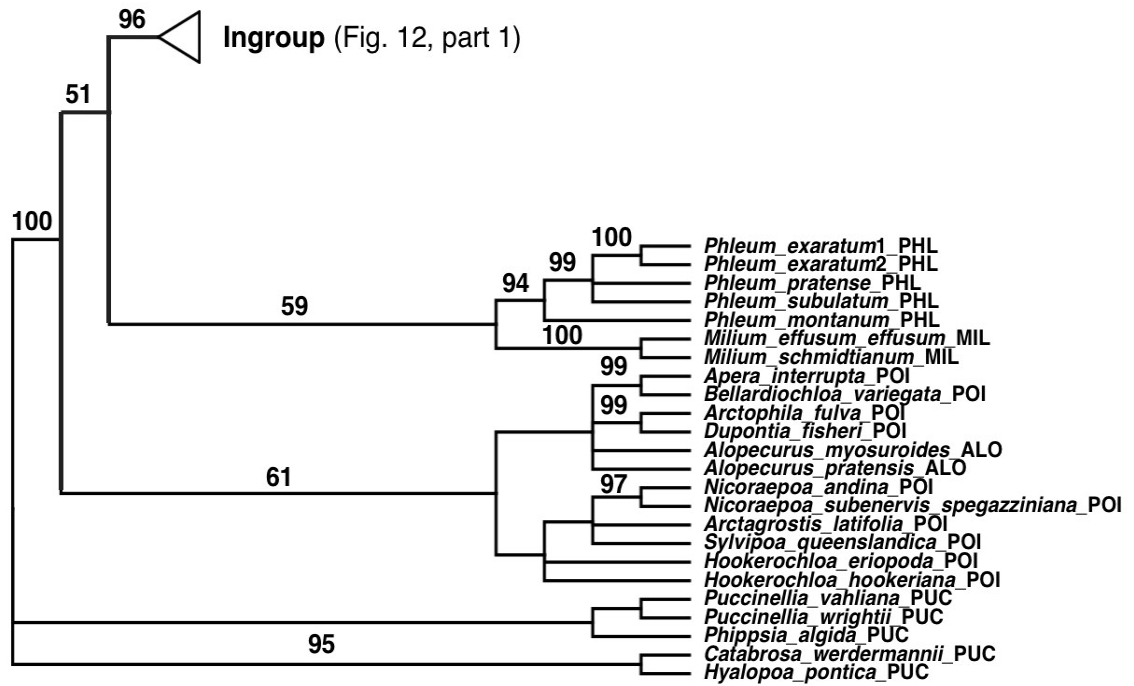


Figure 13. Strict consensus of 100,000 trees from maximum parsimony analysis on ITS (nuclear DNA region) with bootstrap values > 50 displayed above each branch. The color coding for *Poa* subgenera & supersections, and for *Oreopoa* is: aqua for *Sylvestres*; blue for *Ochlopoa*; magenta for *Stenopoa*; green and amber for supersections of subg. *Poa*, *Poa* and *Homalopoa*, Purple for subg. *Pseudopoa* (*Eremopoa*) and orange for *Oreopoa*.



Figure 13. Continued. Outgroup relationships.

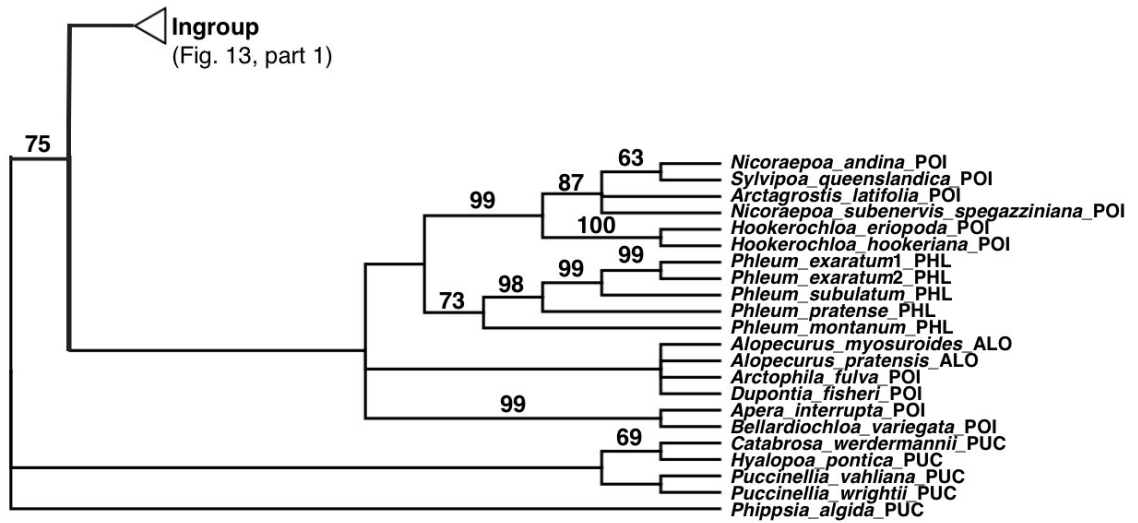


Figure 14. Continued. Outgroup relationships.

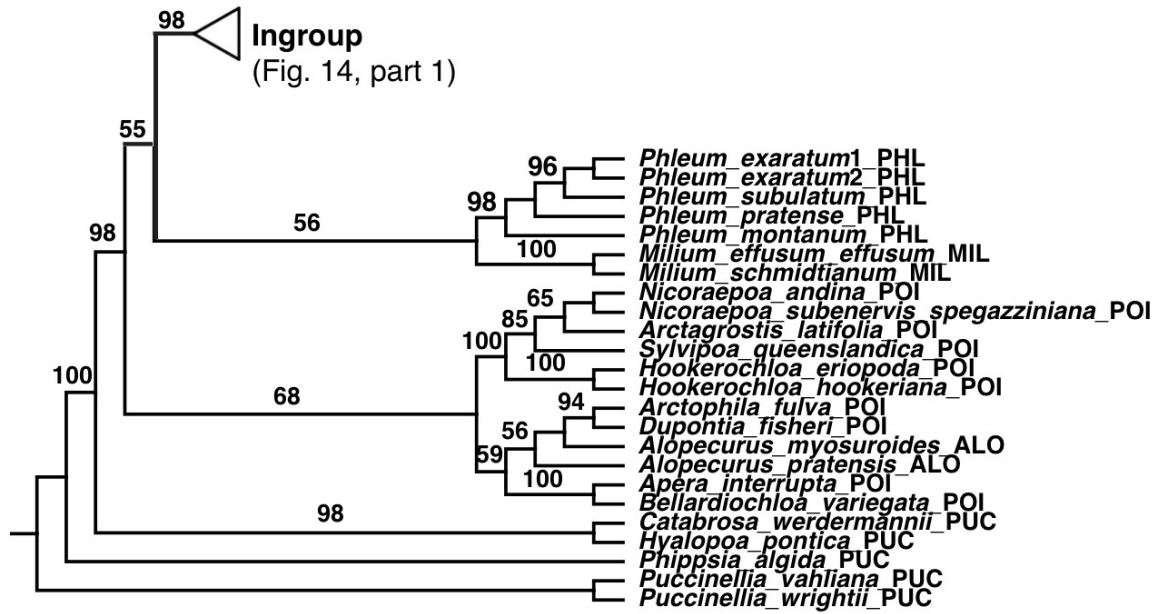
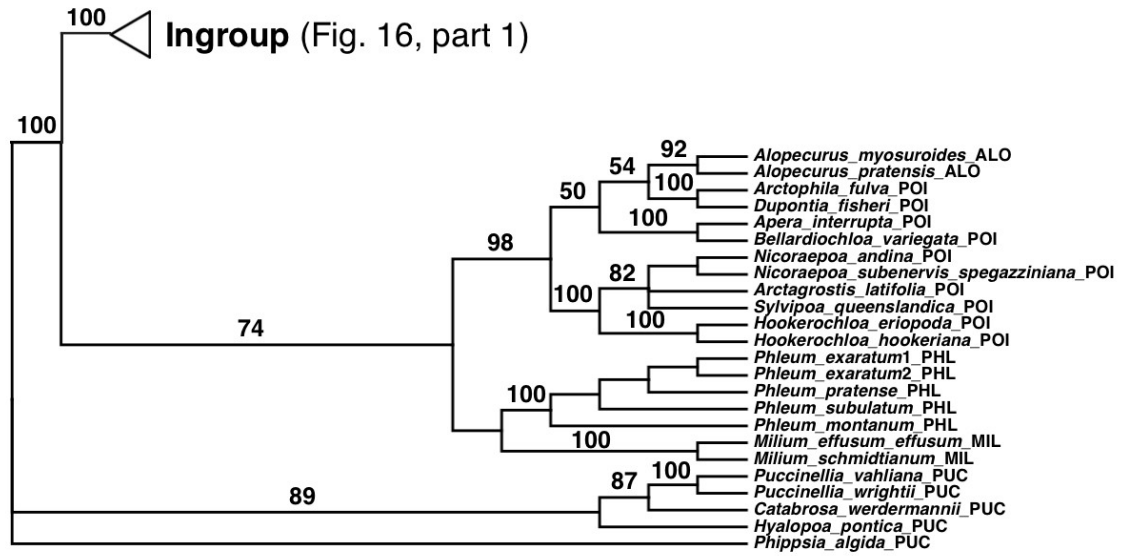


Figure 16. Continued. Outgroup relationships.



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Appendix

Taxon; DNA ID; Voucher information (herbarium); Country of origin; GenBank numbers respectively for ETS, ITS and *trn*TLF are provided for all examined specimens in this study. The sequences with GenBank number are from Gillespie *et al.* (2007, 2008, 2009, 2010), while the other obtained DNA sequences that are marked with — will be submitted to GenBank. Also the sequences for two plastid regions, *matK* and *rpoB-trnC*, will be submitted to GenBank. The classification of Gillespie *et al.* (2007, 2008 & 2009) has been followed here.

1. Subgenus *Ochlopoa* (Asch. & Graebn.) Hyl.

Section *Alpinae* (Hegetschw. ex Nyman) Stapf

Poa alpina 1 L., GIL_0173, Gillespie 6299 CAN, USA: Colorado, GQ324287, GQ324483, DQ353985; *Poa alpina* 2 L., GIL_0298, Gillespie *et al.* 6749-1 CAN, Canada: Nunavut, —, EU792390, DQ353986

Section *Bolbophorum* Asch. & Graebn.

Poa bactriana Roshev. subsp. *glabriflora* (Roshev.) Tzvelev, GIL_2171, Gauba 21237 IRAN, Iran, —, —, —; *Poa badensis* Haenke ex Willd., GIL_1279, Hajkova 2004-12 US, Bulgaria, GQ324295, GQ324490, GQ324402; *Poa bulbosa* L., GIL_2104, Gillespie & Levin 10314 CAN, Turkey, —, —, —; *Poa bulbosa* L. subsp. *bulbosa* 1, GIL_2123, Gillespie *et al.* 10492 CAN, Turkey, —, —, —; *Poa bulbosa* L. subsp. *bulbosa* 2, GIL_2141, Gillespie *et al.* 10591 CAN, Turkey, —, —, —; *Poa bulbosa* L. subsp. *vivipara* (Koeler) Arcang. 1, GIL_2105, Gillespie & Cabi 10326 CAN, Turkey, —, —, —; *Poa bulbosa* L. subsp. *vivipara* (Koeler) Arcang. 2, GIL_2125; Gillespie *et al.* 10495 CAN, Turkey, —, —, —; *Poa bulbosa* L. subsp. *vivipara* (Koeler) Arcang. 3, GIL_2149, Gillespie *et al.* 10631 CAN, Turkey, —, —, —; *Poa densa* Troitsky, GIL_2218, Mohsenian 25247 TARI, Iran, —, —, —; *Poa dolosa* Boiss. & Heldr., GIL_1244, Soreng *et al.* 7495-1 US, Greece, GQ324312, GQ324502, GQ324414; *Poa ligulata* Boiss., GIL_1274, Segarra s.n., Spain; *Poa sinaica* Steud., GIL_2124, Gillespie *et al.* 10493 CAN, Turkey, —, —, —; *Poa sinaica* Steud., GIL_2191, Assadi &

Sardabi 41769 TARI, Iran, —, —, —; *Poa thessala* Boiss. & Orph. 1, GIL_2111, Gillespie *et al.* 10400 CAN, Turkey, —, —, —; *Poa thessala* Boiss. & Orph. 2, GIL_2113, Gillespie *et al.* 10407 CAN, Turkey, —, —, —; *Poa thessala* Boiss. & Orph. 3, GIL_2139, Gillespie *et al.* 10589 CAN, Turkey, —, —, —; *Poa thessala* Boiss. & Orph. 4, GIL_2144, Gillespie *et al.* 10596 CAN, Turkey —, —, —; *Poa timoleontis* Heldr. *ex* Boiss., GIL_1409, Soreng *et al.* 7509-1 US, Greece, —, —, —; *Poa ursina* Velen., GIL_1334, Stoneberg SH17 US, Bulgaria, GQ324352, GQ324527, GQ324437

Section *Micrantherae* Stapf

Poa annua L. 1, GIL_0164, Gillespie 6284 CAN, Canada: Ontario, —, EU792386, DQ353983; *Poa annua* L. 2, GIL_2195, 66 IRAN, Iran, —, —, —; *Poa infirma* Kunth, GIL_2110, Gillespie *et al.* 10370 CAN, Turkey, —, —, —; *Poa supina* Schrad., GIL_1088, Soreng & Cayouette 5950-2 US, USA: Cult. (from Europe), GQ324383, EU792387, DQ353984

Section *Nanopoa* J.R. Edm

Poa trichophylla Heldr. & Sart. *ex* Boiss., GIL_1249, Soreng *et al.* 7508-2 US, Greece, GQ324386, GQ324554, GQ324461

Section *Parodiochloa* (C.E. Hubb.) Soreng

Poa cooki (Hook. f.) Hook.f., GIL_0888, Hennion Gen8, Subantarctic Islands: Kerguelen Islands, GQ324307, EU792382, EU792455; *Poa flabellata* (Lam.) Raspail, GIL_1357, Wright 9NSG, South Georgia Islands, GQ324321, EU792381, EU792453; *Poa kerguelensis* (Hook. f.) Steud, GIL_0886, Hennion Gen5, Subantarctic Islands: Kerguelen Islands, GQ324336, EU792385, EU792457; *Poa sp.*, GIL_1742, Peterson *et al.* 19606 US, Argentina, —, —, —

2. Subgenus *Poa*

❖ Supersection *Homalopoa* (Dumort.) Soreng & L.J. Gillespie)

Section *Acutifoliae* Pilg. *ex* Potztal

Poa planifolia Kuntze, GIL_1011, Peterson 19233 US, Argentina, —, —, —

Section *Brizoides* Pilg. ex Potztl subsect. *Australopoa* Soreng, L.J. Gillespie & S.W.L. Jacob

Poa cheelii Vickery, GIL_0604, Gillespie & Jacobs 7332-1 CAN, Australia, GQ324301, GQ324494, GQ324406; *Poa costiniana* Vickery 1, GIL_0615, Gillespie *et al.* 7356-1 CAN, Australia, GQ324308, GQ324499, GQ324411; *Poa costiniana* Vickery 2, GIL_0617, Gillespie *et al.* 7367 CAN, Australia, GQ324309, GQ324500, GQ324412; *Poa ensiformis* Vickery, GIL_0622, Gillespie *et al.* 7379 CAN, Australia, GQ324315, GQ324504, GQ324415; *Poa fawcettiae* Vickery, GIL_0607, Gillespie *et al.* 7344 CAN, Australia, —, —, —; *Poa fordeana* F. Muell., GIL_0603, Gillespie & Jacobs 7326-2 CAN, Australia, GQ324323, GQ324508, GQ324420; *Poa helmsii* Vickery, GIL_0609, Gillespie *et al.* 7339 CAN, Australia, —, —, —; *Poa hiemata* Vickery, GIL_0614, Gillespie *et al.* 7354 CAN, Australia, GQ324329, GQ324511, GQ324424; *Poa hookeri* Vickery, GIL_0619, Gillespie *et al.* 7370 CAN, Australia, GQ324332, GQ324514, GQ324426; *Poa meionectes* J. Vickery, GIL_0599, Gillespie & Jacobs 7316-1 CAN, Australia, GQ324353, GQ324528, GQ324438; *Poa phillipsiana* J. Vickery, GIL_0618, Gillespie *et al.*, 7369 CAN, Australia, GQ324360, GQ324533, GQ324444; *Poa poiformis* (Labill.) Druce, GIL_0623, Gillespie *et al.* 7381 CAN, Australia, GQ324361, GQ324534, GQ324445; *Poa porphyroclados* Nees, GIL_0988, Peterson *et al.* 14476 US, Australia, GQ324364, GQ324537, GQ324448

Section *Brizoides* Pilg. ex Potztl subsect. *Austrofestuca* (Tzvelev) Soreng, J. Gillespie & S.W.L. Jacobs

Poa billardierei St.-Yves, GIL_1446, Gillespie *et al.* 7382 CAN, Australia, GQ324296, GQ324491, GQ324403

Section *Dasypoa* (Pilg.) Soreng

Poa laetevirens E. Fr., GIL_0997, Peterson & Soreng 15641 US, Chile, —, —, DQ354019; *Poa scaberula* Hook. f., GIL_0994, Peterson & Soreng 15575 US, Chile, GQ324375, EU792412, EU792461 (GQ324454)

Section *Dioicopoa* E. Desv.

Poa arachnifera Torrey, GIL_1066, Soreng 5801 US, USA: Oklahoma, GQ324290, GQ324486, DQ354021; *Poa stuckertii* (Hack.) Parodi, GIL_1208, Soreng & Soreng 7132 US, Chile, GQ324381, EU792414, DQ354022

Group "Punapoa J. Edm."

Poa aequigluma Tovar, GIL_1752, Peterson *et al.* 21684 US, Peru, —, —, —; *Poa glaberrima* Tovar, GIL_1014, Peterson *et al.* 19577 US, Argentina, —, —, —; *Poa gymnantha* Pilg., GIL_1002, Peterson & Soreng 15730 US, Chile, —, —, —; *Poa linearifolia* N.F. Reulio- Rodriguez, GIL_1757, Peterson *et al.* 21904 US, Peru, —, —, —; *Poa marshallii* Tovar, GIL_1749, Peterson *et al.* 21546 US, Peru, —, —, —; *Poa perligulata* Pilg., GIL_1744, Peterson *et al.* 20414 US, Peru, —, —, —; *Poa subspicata* (J. Presl) Kunth, GIL_1747, Peterson *et al.* 21486 US, Peru, —, —, —

Section *Homalopoa* Dumort

Poa affinis Br., GIL_1442, Gillespie & Jacobs 7311-2 CAN, Australia, GQ324286, GQ324482, GQ324399; *Poa chaixii* Vill., GIL_1049, Soreng 4677 US, Russia, GQ324299, EU792404, —; *Poa cuspidata* Nutt., GIL_1051, Soreng 4679-3 US, USA: Maryland, —, —, —; *Poa fibrifera* Pilg., GIL_1758, Peterson *et al.* 21907 US, Peru, —, —, —; *Poa huacavelicae* Tovar, GIL_1748, Peterson *et al.* 21540 US, Peru, —, —, —; *Poa khasiana* Stapf, GIL_1371, Wen *et al.* (TME) 1753 US, China: Sichuan, —, —, —; *Poa kurtzii* E. Fr. 1, GIL_0998, Peterson & Soreng 15654 US, Chile, GQ324340, EU792413, DQ354018; *Poa kurtzii* E. Fr. 2, GIL_1000, Peterson & Soreng 15676 US, Chile, —, JF904854, DQ354017; *Poa mairei* Hack., GIL_1368, Wen *et al.* (TME) 1657 US, China: Yunnan, —, —, —; *Poa masenderana* Freyn & Sint. 1, GIL_2184, Assadi 73254 TARI, Iran, —, —, —; *Poa masenderana* Freyn & Sint. 2, GIL_2186, Assadi & Shahmohammadi 60053 TARI, Iran, —, —, —; *Poa mulleri* Swallen, GIL_2026, Peterson 21459 US, Mexico, —, —, —; *Poa pauciflora* Roem. & Schult., GIL_1755, Peterson *et al.* 21777 US, Peru, —, —, —; *Poa polycolea* Stapf, GIL_1364, Wen *et al.* (TME)1453 US, China: Yunnan, —, —, —; *Poa reflexa* Vasey & Scribn., GIL_1229, Soreng 7422 US, USA: Colorado, —, GQ324543, GQ324450; *Poa remota* Forselles, GIL_1254, Soreng

et al. 7540 US, Kyrgyzstan Republic, GQ324372, GQ324545, GQ324452; *Poa sikkimensis* (Stapf) Bor, GIL_1064, Soreng & Peterson 5676 US, China: Tibet, —, —, —; *Poa sunbisinii* Soreng & G.H. Zhu, GIL_2023, Wen 1179, China, —, —, —; *Poa superata* Hack., GIL_0996, Peterson & Soreng 15615 US, Chile, —, —, —

Section *Madropoa* Soreng

Poa cusickii Vasey subsp. *cusickii*, GIL_1077, Soreng 5829 US, USA: Nevada, GQ324310, GQ324501, DQ354029

❖ Supersection *Poa*

Section *Macropoa* F. Herm. ex Tzvelev

Poa bucharica Roshev., GIL_1261, Soreng *et al.* 7662 US, Kyrgyzstan Republic, —, —, —; *Poa longifolia* Trin., GIL_1892, Soreng 7945 US, Russia: Cabardino-Balkaria, —, —, —; *Poa sibirica* Roshev. subsp. *sibirica* 1, GIL_0957, Olonova 2002-1 CAN, Russia: Khakasia, GQ324376, EU792401, DQ354044 (DQ354045); *Poa sibirica* Roshev. subsp. *sibirica* 2, GIL_0977, Olonova 2003-45 CAN, Russia: Khakasia, —, GQ324547, GQ324455

Section *Malacanthae* (Roshev.) Olon.

Poa lipskyi Roshev. subsp. *lipskyi*, GIL_1262, Soreng *et al.* 7686 US, Kyrgyzstan Republic, —, —, —; *Poa macrocalyx* Trautv. & C.A. Mey., GIL_1123, Soreng & Soreng 6059-1 US, USA: Alaska, —, —, DQ354008; *Poa smirnowii* subsp. *mariae* (Reverd.) Tzvelev, GIL_0959, Olonova 2002-07 CAN, Russia: Khakasia, GQ324380, GQ324551, DQ354005

Section *Poa* subsect. *Cenisiae* (Asch. & Graebn.) V. Jirásek

Poa cenisia All., GIL_1242, Soreng *et al.* 7493-2 US, Greece, —, —, —; *Poa granitica* Braun-Blanq., GIL_1324, Stoneberg MC7, Slovakia, —, —, —; *Poa psychrophila* Boiss. & Heldr. 1, GIL_2114, Gillespie *et al.* 10409 CAN, Turkey, —, —, —; *Poa psychrophila* Boiss. & Heldr. 2, GIL_2115, Gillespie *et al.* 10410 CAN, Turkey, —, —, —

Section *Poa* subsect. *Nivicolae* (Roshev.) Tzvelev

Poa caucasica Trin., GIL_1909, Soreng 7992a US, Russia, —, —, —; *Poa ircutica* Roshev., GIL_0910, Kasanovskiy 2002-7 CAN, Russia: Irkutsk, GQ324335, EU792402, DQ354007

Section *Poa* subsect. *Poa*

Poa pratensis L. 1, GIL_0621, Gillespie *et al.* 7375 CAN, Australia, —, —, —; *Poa pratensis* L. 2, GIL_2122, Gillespie *et al.* 10491 CAN, Turkey, —, —, —; *Poa pratensis* L. 3, GIL_2126, Gillespie *et al.* 10502 CAN, Turkey, —, —, —; *Poa pratensis* L. 4, GIL_2131, Gillespie *et al.* 10568 CAN, Turkey, —, —, —; *Poa pratensis* L. 5, GIL_2142, Gillespie *et al.* 10592 CAN, Turkey, —, —, —; *Poa pratensis* L. subsp. *irrigata* (Lindm.) H. Lindb, GIL_0444, Gillespie 7005 CAN, Canada: Newfoundland, —, —, —; *Poa pratensis* L. subsp. *pratensis* 1, GIL_0178, Gillespie 6310 CAN, USA: Colorado, —, —, DQ354011; *Poa pratensis* L. subsp. *pratensis* 2, GIL_2119, Gillespie *et al.* 10469 CAN, Turkey, —, —, —; *Poa pratensis* L. subsp. *pratensis* 3, GIL_2200, Runemark & Assadi 21870 TARI, Iran, —, —, —; *Poa pratensis* L. subsp. *pratensis* 4, GIL_2213, Runemark & Assadi 20797 TARI, Iran, —, —, —

3. Subgenus *Pseudopoa* (K. & Koch) Stapf

Eremopoa altaica (Trin.) Roshev. subsp. *songarica* Tzvelev 1, GIL_1044, Soreng & Güney 4165 US, Turkey, GQ324311, EU792400, DQ353987 & DQ353988; *Eremopoa altaica* (Trin.) Roshev. subsp. *songarica* Tzvelev 2, GIL_2103, Gillespie & Levin 10313 CAN, Turkey, —, —, —; *Eremopoa altaica* (Trin.) Roshev. subsp. *songarica* Tzvelev 3, GIL_2138, Gillespie *et al.* 10584 CAN, Turkey, —, —, —; *Eremopoa altaica* (Trin.) Roshev. subsp. *songarica* Tzvelev 4, GIL_2169, Iranshahr 20357 IRAN, Iran, —, —, —; *Eremopoa altaica* (Trin.) Roshev. subsp. *songarica* Tzvelev 5, GIL_2196, Assadi & Mozaffarian 36867 TARI, Iran, —, —, —; *Eremopoa capillaris* R. Mill, GIL_2136, Gillespie *et al.* 10578 CAN, Turkey, —, —, —; *Eremopoa medica* H. Scholz, GIL_2190, Mozaffarian & Nowrozi 35082 TARI, Iran, —, —, —; *Eremopoa persica*

(Trin.) Roshev. 1, GIL_2177, Mozaffarian 53671 TARI, Iran, —, —, —; *Eremopoa persica* (Trin.) Roshev. 2, GIL_2183, Assadi & Vosoughi 24939 TARI, Iran, —, —, —; *Eremopoa persica* (Trin.) Roshev. subsp. *persica*, GIL_2175, Yazdanfard 51968 IRAN, Iran, —, —, —

4. Subgenus *Stenopoa* (Dumort.) Soreng & L.J. Gillespie

Section *Abbreviatae* Nannf. ex Tzvelev

Poa abbreviata L., GIL_0111, Gillespie & Chatenoud 5957 CAN, Canada: Nunavut, —, GQ324481, DQ353996

Section *Oreinos* Asch. & Graebn.

Poa laxa Haenke subsp. *fernaldiana* (Nannf.) Hyl. 1, GIL_0455, Gillespie 7013 CAN, USA: New Hampshire, —, —, —; *Poa laxa* Haenke subsp. *fernaldiana* (Nannf.) Hyl. 2, GIL_0457, Gillespie 7015-1 CAN, USA: New Hampshire, —, —, —; *Poa laxa* Haenke subsp. *fernaldiana* (Nannf.) Hyl. 3, GIL_0458, Gillespie 7015-3 CAN, USA: New Hampshire, —, EU792399, DQ353995, *Poa laxa* Haenke subsp. *laxa*, GIL_1339, Stoneberg SH7, Czech Republic, —, —, —; *Poa leptocoma* Trin., GIL_1145, Soreng & Soreng 6100 US, USA: Alaska, —, —, DQ353999

Section *Pandemos* Asch. & Graebn.

Poa trivialis L. 1, GIL_2121, Gillespie *et al.* 10490 CAN, Turkey, —, —, —; *Poa trivialis* L. 2, GIL_2132, Gillespie *et al.* 10571 CAN, Turkey, —, —, —; *Poa trivialis* L. 3, GIL_2135, Gillespie *et al.* 10577 CAN, Turkey, —, —, —; *Poa trivialis* L. subsp. *syvicola* (Guss.) H. Lindb. 1, GIL_2109, Gillespie *et al.* 10368 CAN, Turkey, —, —, —; *Poa trivialis* L. subsp. *syvicola* (Guss.) H. Lindb. 2, GIL_2127, Gillespie *et al.* 10503 CAN, Turkey, —, —, —

Section *Secundae* V.L. Marsh ex Soreng

Poa ammophila A.E. Porsild, GIL_0068, Gillespie 5851 CAN, Canada: Northwest Territories, —, EU792392, DQ353992; *Poa curtifolia* Scribn., GIL_1204, Soreng & Soreng 6347c-1 US, USA: Washington, —, EU792394, DQ353994; *Poa secunda* J.

Presl. subsp. *secunda*, GIL_1073, Soreng 5812 US, USA: Nevada, —, EU792393, DQ353991

Section *Stenopoa* Dumort

Poa albertii Regel, GIL_1263, Soreng *et al.* 7696b US, Kyrgyzstan Republic, —, —, —; *Poa attenuata* Trin., GIL_0963, Olonova 2003-16 CAN, Russia: Khakasia, —, —, —; *Poa glauca* Vahl, GIL_0039, Gillespie 5804 CAN, Canada: Nunavut, GQ324324, AY237839, GQ324421; *Poa nemoralis* L. 1, GIL_2116, Gillespie *et al.* 10411 CAN, Turkey, —, —, —; *Poa nemoralis* L. 2, GIL_2118, Gillespie *et al.* 10468 CAN, Turkey, —, —, —; *Poa nemoralis* L. 3, GIL_2179, Assadi 73922 TARI, Iran, —, —, —; *Poa palustris* L., GIL_0211, Gillespie 6461 CAN, Canada: Ontario, —, EU792396, DQ354000; *Poa sterilis* M. Bieb. 1, GIL_2106, Gillespie & Cabi 10327 CAN, Turkey, —, —, —; *Poa sterilis* M. Bieb. 2, GIL_2120, Gillespie *et al.* 10472 CAN, Turkey, —, —, —; *Poa sterilis* M. Bieb. 3, GIL_2128, Gillespie *et al.* 10527 CAN, Turkey, —, —, —; *Poa versicolor* Besser subsp. *araratica* 1, GIL_2203, Aminirad & Moussavi 11199 IRAN, Iran, —, —, —; *Poa versicolor* Besser subsp. *araratica* 2, GIL_2211, Mozaffarian 48533 TARI, Iran, —, —, —; *Poa versicolor* Besser subsp. *reverdattoi* (Roshev.) Olonova & G. Zhu, GIL_0972, Olonova 2003-29 CAN, Russia: Khakasia, —, —, —

Section *Tichopoa* Dumort

Poa compressa L. 1, GIL_2107, Gillespie *et al.* 10338 CAN, Turkey, —, —, —; *Poa compressa* L. 2, GIL_2108, Gillespie *et al.* 10364 CAN, Turkey, —, —, —; *Poa compressa* L. 3, GIL_2133, Gillespie *et al.* 10574 CAN, Turkey, —, —, —; *Poa compressa* L. 4, GIL_2148, Gillespie *et al.* 10625 CAN, Turkey, —, —, —

5. Subgenus *Sylvestres* (V.L. Marsh *ex* Soreng) Soreng & L.J. Gillespie

Section *Sylvestres* V.L. Marsh *ex* Soreng

Poa autumnalis Elliott, GIL_1052, Soreng 4680-1 US, USA: Maryland, GQ324294, EU792379, DQ353979; *Poa saltuensis* Fernald & Wiegand, GIL_0497, Gillespie 7043

CAN, Canada: Ontario, GQ324374, EU792378, EU792451; *Poa sylvestris* A. Gray, GIL_1050, Soreng 4678-3 US, USA: Maryland, GQ324384, EU792375, DQ353980

Unclassified *Poa* Species

Poa aitchisonii Boiss., GIL_2170, 21174 IRAN, Iran, —, —, —; *Poa diversifolia* (Boiss. & Balansa) Hack. ex Boiss. 1, GIL_2129, Gillespie *et al.* 10529 CAN, Turkey, —, —, —; *Poa diversifolia* (Boiss. & Balansa) Hack. ex Boiss. 2, GIL_2145, Gillespie *et al.* 10611 CAN, Turkey, —, —, —; *Poa pseudobulbosa* Bor, GIL_2137, Gillespie *et al.* 10581 CAN, Turkey, —, —, —

Genus *Oreopoa*

Oreopoa anatolica H. Scholz & Parolly 1, GIL_2140, Gillespie *et al.* 10590 CAN, Turkey, —, —, —; *Oreopoa anatolica* H. Scholz & Parolly 2, GIL_2143, Gillespie *et al.* 10593 CAN, Turkey, —, —, —

❖ Outgroups

Subtribe Alopecurinae

Alopecurus pratensis L., GIL_1395, Soreng *et al.* 7651b US, Kyrgyzstan Republic, —, —, —; *Alopecurus myosuroides* Huds., GIL_2084, Gillespie *et al.* 10561 CAN, Turkey, —, —, —

Subtribe Phleinae

Phleum exaratum Hochst. ex Griseb. 1, GIL_2074, Gillespie *et al.* 10498 CAN, Turkey, —, —, —; *Phleum exaratum* Hochst. ex Griseb. 2, GIL_2083, Gillespie *et al.* 10554 CAN, Turkey, —, —, —; *Phleum montanum* K. Koch, GIL_1913, Soreng 8006 US, Russia: Karachay-Cherkessia, —, —, —; *Phleum pratense* L., GIL_1891, Soreng 7943 US, Russia, —, —, —; *Phleum subulatum* (Savi) Asch. & Graebn., GIL_2093, Gillespie *et al.* 10628 CAN, Turkey, —, —, —

Subtribe Miliinae

Milium effusum L. subsp. *effusum*, GIL_1904, Soreng 7978 US, Russia: Cabardino-Balkaria, —, —, —; *Milium schmidtianum* K. Koch, GIL_1920, Soreng 8019 US, Russia, —, —, —

Subtribe Poinae

Apera interrupta (L.) P. Beauv., GIL_1008, Peterson *et al.* 19173 US, Argentina, GQ324242, EU792364, EU792439; *Arctagrostis latifolia* (R. Br.) Griseb., GIL_0226, Gillespie *et al.* 6586-1 CAN, Canada: Nunavut, GQ324245, EU792351, DQ353969; *Arctophila fulva* (Trin.) Rupr., GIL_065,1Aiken 99-230 CAN, Canada: Northwest Territories, GQ324246, EU792347, DQ354058; *Bellardiochloa variegata* (Lam.) Kerguelen, GIL_1251, Soreng *et al.* 7519-1 US, Greece, GQ324257, EU792361, EU792438; *Dupontia fisheri* R. Br., GIL_0229, Gillespie *et al.* 6589 CAN, Canada: Nunavut, GQ324266, EU792346, DQ353967; *Hookerchloa eriopoda* (Vickery) Jacobs, GIL_0898, Jacobs 9128 NSW, Australia, GQ324270, EU792349, EU792433; *Hookerchloa hookeriana* (F. Muell. ex Hook.f.) E.B. Alexeev, GIL_1854, Gillespie *et al.* 7352 CAN, Australia, —, —, —; *Nicoraepoa andina* (Trin.) Soreng & Gillespie, GIL_1217, Soreng & Soreng 7182 US, Chile, GQ324275, EU792354, DQ353971; *Nicoraepoa subenervis* subsp. *Spegazziniana* (Nicora) Soreng & L.J. Gillespie, GIL_1009, Peterson *et al.* 19186 US, Argentina, GQ324281, EU792358, EU792443; *Sylvipoa queenslandicae* (C.E. Hubbard) Soreng, Gillespie & Jacob, GIL_0601, Gillespie *et al.* 7320 CAN, Australia, GQ324393, GQ324559, GQ324466

Subtribe Puccinelliinae

Catabrosa werdermannii (Pilg.) Nicora & Rùgolo, GIL_1209, Soreng & Soreng 7150 US, Chile, GQ324258, EU792334, DQ353958; *Hyalopoa pontica* (Bal.) Tzvelev, GIL_1917, Soreng 8014 US, Russia: Adygea, —, —, —; *Phippisia algida* (Sol.) Br., GIL_0161, Gillespie 6253 CAN, Canada: Nunavut, —, EU792331, DQ353949 (DQ353950); *Puccinellia vahliana* (Leibm.) Scribn., GIL_0042, Gillespie 5808 CAN, Canada: Nunavut, GQ324285, EU792336, EU854591; *Puccinellia wrightii* (Scribn. & Merr.) Tzvelev, GIL_1451, Elven SUP03-9-1, ?, —, —, —