

**Molecular phylogenetic analyses  
and classification  
of the Pooideae (Poaceae)**

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## Summary

The grass family Poaceae comprises a large number of crops and forage plants and plays an important ecological and economic role for humans and animals. Therefore, many scientific studies focus on phylogeny and evolutionary patterns within this plant family. Poaceae have been generally subdivided in subfamilies, tribes and sometimes further in subtribes, encompassing a large number of genera.

This study deals with the systematics of Pooideae, one of the largest subfamilies of grasses with approximately 150 genera and 3,300 species. Molecular data of chloroplast- and nucleus-encoded DNA sequences, partly combined with morphological characteristics, cytogenetics and biogeography were used to examine the phylogeny of Pooideae and to resolve as yet unanswered questions. In the past, the classification of this subfamily and the delimitation of the tribes have been revised several times. To better understand the evolutionary splits within the subfamily Pooideae representative number of taxa of all acknowledged tribes were analysed and several genera with uncertain placements or previous classification outside of Pooideae were included. Molecular phylogenetic analyses were carried out using sequence data of the chloroplast *matK* gene–3'*trnK* exon and nuclear ITS1–5.8S gene–ITS2. Additionally obtained new morphological information made it necessary to revise previous circumscriptions of major lineages within the subfamily.

The present thesis provides a well-resolved molecular phylogeny for the subfamily Pooideae with some re-alignments. The following preliminary classification of Pooideae is proposed: Brachyelytreae, Nardeae with the subtribes Nardinae and Lygeinae, Meliceae with the subtribes Brylkiniiinae and Melicinae, Duthieae, Phaenospermateae, Stipeae with the subtribes Ampelodesminae and Stipinae, Hordeae (syn. Triticeae) with the subtribes Brominae, Hordeinae and Littledaleinae and the Aveneae/Poeae tribe complex. The molecular phylogenetic analyses showed no significant incongruence between chloroplast and nuclear sequence data. In case of incongruent placements, these were weakly supported or unsupported.

Within the Pooideae, the phylogeny and delineation of the early diverging lineages were investigated in more detail. An interesting result is that all 'primitive' members of the original pooid tribe Aveneae subtribe Duthieinae as treated in some grass classifications, namely *Danthoniastrum*, *Duthiea*, *Metcalfia*, *Pseudodanthonia*, *Sinochasea*, and *Stephanachne*, grouped together with *Anisopogon*. The latter was traditionally placed outside of Pooideae. These genera formed a morphologically well-defined clade among the early evolutionary lineages. In this study, the genus *Phaenosperma* is closely related with this group based on chloroplast DNA sequence information, but differs strongly in structural

characters without any support for this position. It is proposed to preserve the Phaenospermateae as monotypic tribe encompassing only *Phaenosperma* and to accommodate the other genera under the newly described tribe Duthieeae mainly based on synapomorphic spikelet structures, which are not shared by *Phaenosperma*.

In addition, the molecular phylogenetic hypothesis demonstrates a clear delimitation of Duthieeae and Phaenospermateae against Stipeae and a taxonomic identification key for these tribes based on morphological characters is provided. Biogeographical analyses pointed to a warm and humid climatic niche as common origin of Phaenospermateae and Duthieeae. A shift of climatic adaptation to dry and cold conditions in only some genera of Duthieeae (*Sinochasea*, *Stephanachne*) indicates that the tribe was obviously less successful within the early diverging lineages of Pooideae than the species-rich tribe Stipeae, which has strongly diversified in dry and arid areas. Cytogenetic investigations confirmed high chromosome base numbers among the early diverging lineages, but indicated that the chromosome sizes differ more strongly than previously assumed.

Within the 'core' Pooideae, the splitting of the tribe complex of Aveneae and Poeae into two highly supported clades inferred from the chloroplast sequence data could not be supported by the nuclear ITS data. At present, unequivocal nuclear DNA sequence information is missing to propose a taxonomic treatment of the Aveneae/Poeae tribe complex under a common tribe Poeae or to support the maintenance of two separate tribes. Furthermore, the study shows that the small grass tribe Hainardieae is highly polyphyletic. Its genera (*Agropyropsis*, *Hainardia*, *Narduroides*, *Scribneria*, *Parapholis*, *Pholiurus*) were identified as members of different groups among the Aveneae/Poeae tribe complex based on molecular and morphological traits. Only *Hainardia* and *Parapholis* were confirmed as sister taxa with clearly synapomorphic characters, which deviate from the others. The cytogenetic analysis showed that chromosome base numbers differ strongly in the Aveneae/Poeae tribe complex with variations even within groups of closely related genera.

In conclusion, the thesis gives new insights into the classification of the grass subfamily Pooideae with strong evidence to redefine some tribes. Several changes in classification are proposed, including the description of the new tribe Duthieeae resolved among the early diverging lineages and a revised treatment of the tribes Nardeae and Hordeae.

## Structure of the thesis

The present thesis is organised into four chapters. DNA sequence data of the Pooideae are phylogenetically analysed and discussed especially in conjunction with morphological characters. Furthermore, the study deals with aspects of chromosome evolution and biogeography of Pooideae.

**Chapter 1** gives a general introduction into current knowledge and previous research on phylogeny and systematics of the grass subfamily Pooideae, and points out unanswered questions of evolutionary interest. In the last part of this chapter main results of the thesis are outlined and discussed with reference to the following three chapters, which are already published in peer-reviewed journals.

**Chapter 2** identifies the major evolutionary lineages within this subfamily of grasses and discusses their phylogenetic relationships based on chloroplast and nuclear DNA sequence data. The study investigates all currently recognised tribes and includes many insufficiently or not yet analysed genera, some of which had an uncertain placement or were treated differently in previous classifications. Morphological key characters are discussed with respect to molecular results and used to provide a more stable classification for the entire subfamily.

**Chapters 3 and 4** deal with the relationships within the early evolutionary lineages and the so-called 'core' Pooideae.

**Chapter 3** investigates in more detail the relationships within early diverging lineages of the grass subfamily Pooideae, with focus on the assemblage of *Phaenosperma*, *Anisopogon* and genera belonging in some classifications to the tribe Aveneae subtribe Duthieinae. All genera of Duthieinae are considered in this study along with a selection of presumably related genera that are relevant to identify the early evolutionary splits. Morphological, cytogenetic and biogeographical analyses are added to the molecular phylogenetic work based on chloroplast and nuclear sequences. Additionally, the delineation of the tribe Stipeae is discussed.

**Chapter 4** critically re-examines previous taxonomic treatments of genera that were occasionally unified under a separate tribe Hainardieae sensu Clayton & Renvoize (1986), due to their conspicuous spicate inflorescences. The true relationships of these genera are assessed based on molecular phylogeny, cytogenetic information and morphological traits. Additionally, many genera of the traditional Aveneae and Poeae were included for comparison.

## Chapter 1 – General Introduction

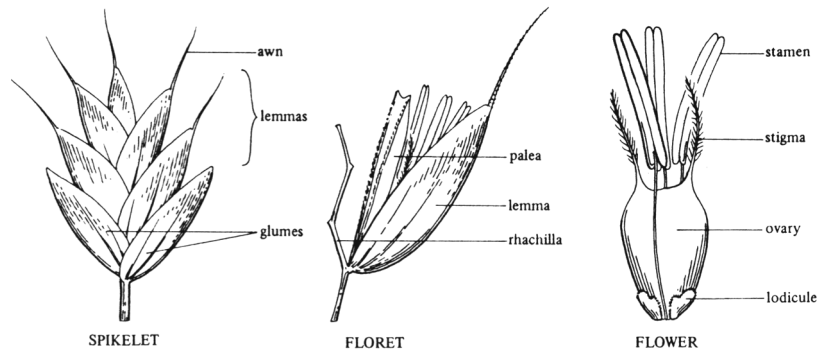
### Introduction

The grass family Poaceae Barnhart is one of the most important plant groups of fundamental interest to humans and animals. This family comprises 10,000 species and represents the fourth largest family ranking after Asteraceae, Fabaceae and Orchidaceae (Stevens 2001 onwards). The grass fruits (caryopsis) provide the dietary staple for humans. Essential cereal crops include barley (*Hordeum*), maize (*Zea*), oat (*Avena*), rice (*Oryza*), rye (*Secale*), sorghum (*Panicum*) and wheat (*Triticum*). Furthermore, grasses are of prime importance for grazing animals as forage plants such as bluegrasses (*Poa* species), false oat grass (*Arrhenatherum elatius*), fescues (*Festuca* species) and ryegrasses (*Lolium* species). Industrially, some parts of the plants are used as building and filling material or for the production of alcohol and fuel.

Species of the Poaceae are distributed worldwide and form large grassland ecosystems (prairies, savannas, steppes), which cover approximately 20% of the earth's land surface (Shantz 1954). Due to their enormous ecological and economical role, some scientists work on the evolutionary history of wild grasses as basis for breeding programs of cereals. In the past ten years, research groups have sequenced the complete genome of rice (International Rice Genome Sequencing Project 2005), maize (Schnable 2009), and recently even the wild grass *Brachypodium distachyon* (The International Brachypodium Initiative 2010).

The grass classification started about 200 years ago and systematists faced a great challenge because of the enormous number of species in this plant family. To date, evolutionary patterns in grasses are still only partially resolved and the taxonomic classification in several subfamilies, supertribes (used in few studies), tribes and partly further in subtribes changed many times.

Until molecular data were used in plant systematics, the classification of Poaceae was based mainly on morphology, especially on the structure of spikelets (Fig. 1). A lack of clear criteria or parallel evolution of inflorescence and spikelet architecture has made it difficult to identify true relationships (see Clayton & Renvoize 1986).



**Fig.1** General structure of the grass inflorescence with spikelet, floret and flower (Clayton & Renvoize 1986).

Anatomical, cytological and phytochemical traits were used later and compared with spikelet structure to find natural groups. Based on extensive sets of characters comprehensive classifications have been published, which partly differed from each other (e.g. Clayton & Renvoize 1986; Tzvelev 1989; Watson & Dallwitz 1992, 1992 onwards). The number of recognised subfamilies within the Poaceae varied between two (Tzvelev 1989) and 13 (Caro 1982).

Preliminary molecular studies have been initiated in the 1990s (Hamby & Zimmer 1988, Doebley et al. 1990, Soreng et al. 1990). Following molecular phylogenetic analyses used plastid DNA sequence data such as *rbcl* (Duvall & Morton 1996), *ndhF* (Clark et al. 1995), *rpoC2* (Cummings et al. 1994), *rps4* (Nadot et al. 1994), *matK* (Liang & Hilu 1996, Liang 1997; Hilu et al. 1999) or the nuclear DNA sequence data of ITS, GBSSI, and *phyB* (Hsiao et al. 1999; Mason-Gamer et al. 1998; Mathews et al. 2000) to better understand the phylogeny of Poaceae. The circumscriptions of the subfamilies and the contradictory taxonomic positions of many genera indicated substantial differences to the traditional classifications.

Recently, the Grass Phylogeny Working Group (GPWG II 2012), a network of scientists, has carried out the most comprehensive phylogenetic analysis to date and presented with highly support the division of grasses into the first branching, species-poor subfamilies Anomochlooideae, Pharoideae, Puelioideae and the two major later branching clades termed PACMAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae, und Danthonioideae) and BEP clade (Bambusoideae, Ehrhartoideae and Pooideae) with highly support. It confirmed largely the classification of grasses proposed by previous studies (cf. GPWG 2001; Duvall et al. 2007; Bouchenak-Khelladi et al. 2008).

### **Current classification of the subfamily Pooideae and unresolved issues**

The present thesis deals with the molecular phylogeny and systematics of the subfamily Pooideae, which was first described by Bentham (1861). It is the largest subfamily of the BEP clade. Members of the Pooideae are characterised by a  $C_3$  photosynthetic pathway,



parallel-sided subsidiary cells, the absence of microhairs (except *Nardus* and *Lygeum*) and nonvascularized lodicules (2–3). The pooid embryo structure is characterised by the presence of an epiblast and the lack of a scutellar cleft (Clayton & Renvoize 1986). The subfamily Pooideae encompasses one third of all grasses with approximately 3,300 species in 150 genera, which are distributed predominately in the cool temperate grasslands of both hemispheres and reach highest species richness in the northern hemisphere (Clayton & Renvoize 1986). The Pooideae were interpreted as an early radiation of grasses outside the tropics (Stebbins 1956, 1972). In the tropics, pooid grasses are distributed only on the tops of high mountains (Clayton & Renvoize 1986). The subfamily Pooideae includes many species-rich genera (e.g. *Calamagrostis*, *Festuca*, *Poa*, *Stipa*) and some of the most important cereals (*Avena*, *Hordeum*, *Secale*, *Triticum*). Therefore, this group plays an important role in systematics and evolutionary research besides mainly tropical distributed species of bamboo- and rice-relatives of the BEP clade. Studies on the cytogenetic evolution within Pooideae showed that the chromosome base numbers vary between  $x = 2$  and  $x = 13$  with reduction in the terminal lineages. Chromosome sizes range from ‘small’ to ‘quite large’ (e.g. Hsiao 1994; Kellogg 1998; Hilu 2004; Shchapova 2012).

The traditional circumscription of Pooideae differed considerably in terms of the number and delimitation of tribes based on anatomical and morphological characters (e.g. Tzvelev 1976, 1989; Macfarlane & Watson 1980, 1982; Clayton & Renvoize 1986; Watson & Dallwitz 1992, 1992 onwards). A number of genera were consistently difficult to classify.

Table 1 gives a historical overview of the classification within the subfamily including tribes, which have been traditionally placed outside of Pooideae in previous studies. In the past, the tribes Brachyelytreae, Diarrheneae and Phaenospermateae were placed as isolated groups within the Bambusoideae characterised by both bambusoid (embryo structure) and pooid (leaf anatomy or spikelets) structures (Clayton & Renvoize 1986). Alternatively, several tribes (Brachyelytreae, Lygeae, Nardeae, Stipeae, Ampelodesmeae, Anisopogoneae) were treated under a separate subfamily Stipoideae (Watson & Dallwitz 1992 onwards) or were partly classified under Arundinoideae (Watson & Dallwitz 1992). In the latter case, the subfamily Pooideae included two supertribes, i.e. Triticodae and Poodae. Phylogenetic studies based on structural characters and/or molecular data defined the subfamily Pooideae more broadly and included explicitly or implicitly Ampelodesmeae, Anisopogoneae, Brachyelytreae, Diarrheneae, Lygeae, Nardeae, Phaenospermateae, Stipeae (e.g. Kellogg & Campbell 1987; Davis & Soreng 1993; Kellogg & Watson 1993; Clark et al. 1995; Hsiao et al. 1995a, 1999; Kellogg & Linder 1995; Catalán et al. 1997; Soreng & Davis 1998, 2000; Hilu et al. 1999; Mathews et al. 2000; GPWG 2001).

**Table 1** Comparison of the different treatments of the tribes, which were assigned to the subfamily Pooideae by previous or current studies (subfamily, tribe, eventually supertribe or informal rank are indicated).

Tribes	Clayton & Renvoize (1986): morphology	Watson & Dallwitz (1992 onwards): morphology	GPWG (2001): molecular data, morphology	Davis & Soreng (2007): cpDNA sequences, morphology	Döring et al. (2007): cpDNA sequences	Bouchenak-Khelladi et al. (2008): cpDNA sequences
Ampelodesmeae (Conert) Tutin	<b>Pooideae:</b> in Poeae	<b>Stipoideae:</b> Ampelodesmeae	<b>Pooideae:</b> Ampelodesmeae	<b>Pooideae:</b> in Stipeae	<b>Pooideae:</b> in Stipeae	<b>Pooideae:</b> in Stipeae
Aveneae Dumort.	<b>Pooideae:</b> Aveneae	<b>Pooideae:</b> Poodae: Aveneae	<b>Pooideae:</b> in Poeae	<b>Pooideae:</b> in Poeae s.l.	<b>Pooideae:</b> in Aveneae/Poeae complex	<b>Pooideae:</b> in Aveneae + Poeae
Brachyelytreae Ohwi	<b>Bambusoideae:</b> Brachyelytreae	<b>Stipoideae:</b> Brachyelytreae	<b>Pooideae:</b> Brachyelytreae	<b>Pooideae:</b> Brachyelytreae	<b>Pooideae:</b> Brachyelytreae	<b>Pooideae:</b> Brachyelytreae
Brachypodieae Harz	<b>Pooideae:</b> in Triticeae	<b>Pooideae:</b> Triticodae: Brachypodieae	<b>Pooideae:</b> Brachypodieae	<b>Pooideae:</b> Brachypodieae	<b>Pooideae:</b> Brachypodieae	<b>Pooideae:</b> Brachypodieae
Bromeae Dumort.	<b>Pooideae:</b> Bromeae	<b>Pooideae:</b> Triticodae: Bromeae	<b>Pooideae:</b> Bromeae	<b>Pooideae:</b> Bromeae/Triticeae	<b>Pooideae:</b> in Bromeae/Triticeae complex	<b>Pooideae:</b> in Bromeae + Triticeae
Brylkinieae Tateoka	<b>Pooideae:</b> Brylkinieae	<b>Pooideae:</b> Poodae: in Meliceae	<b>Pooideae:</b> Brylkinieae	—	—	—
Diarrheneae (Ohwi) C. S. Campb.	<b>Bambusoideae:</b> Diarrheneae	<b>Bambusoideae:</b> Oryzodae: Diarrheneae	<b>Pooideae:</b> Diarrheneae	<b>Pooideae:</b> Diarrheneae	—	<b>Pooideae:</b> Diarrheneae
Hainardieae Greuter	<b>Pooideae:</b> Hainardieae	<b>Pooideae:</b> Poodae: in Aveneae or in Poeae	<b>Pooideae:</b> in Poeae	<b>Pooideae:</b> in Poeae s.l.	<b>Pooideae:</b> in Aveneae/Poeae complex	<b>Pooideae:</b> in Aveneae + Poeae
Hordeae <sup>1</sup> Martinov (syn. Triticeae Dumort.)	<b>Pooideae:</b> Triticeae	<b>Pooideae:</b> Triticodae: Triticeae	<b>Pooideae:</b> Triticeae	<b>Pooideae:</b> Bromeae/Triticeae	<b>Pooideae:</b> Bromeae/Triticeae complex	<b>Pooideae:</b> in Bromeae + Triticeae
Lygeae J. Presl	<b>Pooideae:</b> Lygeae	<b>Stipoideae:</b> Lygeae	<b>Pooideae:</b> Lygeae	<b>Pooideae:</b> Lygeae	<b>Pooideae:</b> in Nardeae	<b>Pooideae:</b> in Lygeae + Nardeae
Meliceae Rchb.	<b>Pooideae:</b> Meliceae	<b>Pooideae:</b> Poodae: Meliceae	<b>Pooideae:</b> Meliceae	<b>Pooideae:</b> Meliceae	<b>Pooideae:</b> Meliceae	<b>Pooideae:</b> Meliceae
Nardeae Koch	<b>Pooideae:</b> Nardeae	<b>Stipoideae:</b> Nardeae	<b>Pooideae:</b> Nardeae	<b>Pooideae:</b> Nardeae	<b>Pooideae:</b> Nardeae	<b>Pooideae:</b> in Lygeae + Nardeae
Phaenospemateae Renvoize & Clayton	<b>Bambusoideae:</b> Phaenospemateae	<b>Bambusoideae:</b> Oryzodae: Phaenospemateae	<b>Pooideae:</b> Phaenospemateae	<b>Pooideae:</b> Phaenospemateae	<b>Pooideae:</b> Phaenospemateae	<b>Pooideae:</b> Phaenospemateae
Poeae R. Br.	<b>Pooideae:</b> Poeae	<b>Pooideae:</b> Poodae: Poeae	<b>Pooideae:</b> Poeae	<b>Pooideae:</b> in Poeae s.l.	<b>Pooideae:</b> in Aveneae/Poeae complex	<b>Pooideae:</b> in Aveneae + Poeae
Seslerieae W. D. J. Koch	<b>Pooideae:</b> in Poeae	<b>Pooideae:</b> Poodae: Seslerieae	<b>Pooideae:</b> in Poeae	<b>Pooideae:</b> in Poeae s.l.	<b>Pooideae:</b> in Poeae	<b>Pooideae:</b> in Aveneae + Poeae
Stipeae Dumort.	<b>Pooideae:</b> Stipeae	<b>Stipoideae:</b> Stipeae	<b>Pooideae:</b> Stipeae	<b>Pooideae:</b> Stipeae	<b>Pooideae:</b> Stipeae	<b>Pooideae:</b> Stipeae

<sup>1</sup> The tribe name Hordeae Martinov (1820) has nomenclatural priority against Triticeae Dumort. (1824), see also Reveal (2012).

The chloroplast gene *matK* and the intron region between *matK* and 3'*trnK* has proven particularly successful to analyse relationships within Pooideae (Hilu et al. 1999; Davis & Soreng 2007; Döring et al. 2007; Döring 2009). These and other investigations, which accumulated a large set of chloroplast sequence data for combined analyses, provided a similar phylogenetic structure of Pooideae, but the taxonomic treatment of some tribes remained unclear (cf. Table 1; GPWG 2001; Davis & Soreng 2007; Döring et al. 2007; Bouchenak-Khelladi et al. 2008; Döring 2009). Molecular phylogenetic analyses of the entire subfamily Pooideae using nuclear sequence data, for example, from the internal transcribed spacer (ITS) region or the gene phytochrome B (*phyB*) sampled only a limited number of taxa (Hsiao et al. 1994, 1995a, 1999; Mathews et al. 2000). However, DNA sequence data of the nuclear genome were extensively used in a number of studies that analysed the relationships within the tribes Triticeae (syn. Hordeae) and Aveneae/Poeae (e.g. Baum & Appels 1992; Hsiao et al. 1995b; Kellogg & Appels 1995; Grebenstein et al. 1998; Mason-Gamer & Kellogg 2000; Mason-Gamer 2001, 2005; Röser et al. 2001; Torrecilla & Catalán 2002; Blattner 2004; Quintanar et al. 2007; Chiapella 2007; Gillespie et al. 2008, 2010; Hand et al. 2010; Jakob & Blattner 2010; Saarela et al. 2010; Brassac 2012).

Based on molecular phylogenetic reconstructions the tribe Brachyelytreae was consistently identified as earliest diverging lineage within the Pooideae (Clark et al. 1995; Kellogg & Linder 1995; Catalán et al. 1997; Hilu et al. 1999; Hsiao et al. 1999; Davis & Soreng 2007; Döring et al. 2007; Bouchenak-Khelladi et al. 2008, Döring 2009).

Several studies showed the tribes Nardeae and Lygeae branching in a clade after Brachyelytreae among the early diverging lineages and supported a close relationship of these tribes (cf. Cummings et al. 1994; Catalán et al. 1997; Soreng & Davis 1998, 2000; Barker et al. 1999; Hsiao et al. 1999; Mathews et al. 2000; Döring et al. 2007; Bouchenak-Khelladi et al. 2008). Nardeae and Lygeae differ clearly in morphological structures of their inflorescences. The occurrence of synapomorphic spikelet characters, however, provided an evidence on a common origin and it was suggested to accommodate these under the tribe Nardeae (Döring et al. 2007).

Based on morphology the genera *Phaenosperma* and *Anisopogon* have been mostly placed outside of the Pooideae, but appeared in molecular phylogenetic studies in a common lineage with some genera (*Danthoniastrum*, *Duthiea* and *Sinochasea*) of the tribe Aveneae subtribe Duthieinae sensu Clayton & Renvoize (1986) among the early diverging lineages of Pooideae (cf. Davis & Soreng 2007; Döring et al. 2007; Döring 2009). However, some of these genera (*Anisopogon*, *Danthoniastrum*, *Duthiea*, *Sinochasea*) share spikelet features (e.g. the awn arising from an apical incision of the lemma and frequent occurrence of three lodicules) with Stipeae and thus were placed in Stipoideae, Pooideae or Arundinoideae (cf.

Watson & Dallwitz 1992, 1992 onwards). In the 'Flora of China' treatment *Duthiea*, *Sinochasea* and *Stephanachne* were classified within Stipeae (Wu & Phillips 2006).

The molecular phylogenetic structure of the species-rich Stipeae was considered in particular detail by several studies, which revealed Stipeae as a monophyletic tribe and revised the generic boundaries (e.g. Jacobs et al. 2007; Barkworth et al. 2008; Romaschenko et al. 2008, 2010, 2012; Cialdella et al. 2010; Hamasha et al. 2012). The morphologically odd genus *Ampelodesmos* was always difficult to classify and has been placed in close relation to Stipeae by chloroplast sequence data (e.g. Soreng & Davis 1998, 2000; GWPG 2001; Döring et al. 2007; Bouchenak-Khelladi et al. 2008; Döring 2009). Because of different assessments of structural traits (plesiomorphic versus autapomorphic characters), *Ampelodesmos* was either treated under the tribe Poeae (Clayton & Renvoize 1986), accommodated under a monotypic tribe Ampelodesmeae (e.g. Watson & Dallwitz 1992; GPWG 2001; CNWG 2000 onwards) or included in Stipeae (Decker 1964b; Barkworth 2007).

Among the early evolutionary splits Meliceae and the monotypic tribe Brylkinieae were classified based on morphological characters either under a common tribe or maintained as independent lineages (cf. Clayton & Renvoize 1986; Tzvelev 1989; Watson & Dallwitz 1992; Mejia-Saulés & Bisby 2000; GPWG 2001). There was a lack of suitable molecular information to address taxonomic treatment of these tribes.

The traditionally circumscribed tribes Bromeae, Hordeae (syn. Triticeae), Aveneae and Poeae were informally defined as 'core' Pooideae (Davis & Soreng 1993). Aveneae and Poeae comprise together about 70% of all genera within Pooideae. Early molecular analyses have established a monophyletic origin of a clade containing Aveneae and Poeae (e.g. Soreng et al. 1990; Davis & Soreng 1993; Nadot et al. 1994; Hsiao et al. 1995a, 1999; Catalán et al. 1997; Soreng & Davis 1998, 2000; Hilu et al. 1999; GPWG 2001; Quintanar et al. 2007; Döring et al. 2007; Soreng et al. 2007; Bouchenak-Khelladi et al. 2008; Döring 2009; Saarela et al. 2010). In the last years, extensive works were carried out to study the relationships among these tribes. Chloroplast DNA data suggested a clear split of Aveneae/Poeae, which did not correspond to morphologically based tribal circumscriptions (e.g. Soreng & Davis 2000; Soreng et al. 2007; Döring et al. 2007; Quintanar et al. 2007; Bouchenak-Khelladi et al. 2008; Döring 2009; Saarela et al. 2010). Additionally, it seemed that some members of the occasionally acknowledged tribes Seslerieae and Hainardieae were close to Aveneae and Poeae and thus should be included there (cf. Tzvelev 1989; GPWG 2001). In contrast to phylogenetic reconstructions of chloroplast DNA data, analyses of nuclear sequence data could not confirm the bifurcation of the Aveneae/Poeae clade and showed a partly deviated topology, but with weak to no support (e.g. Quintanar et al. 2007; Saarela et al. 2010). Some studies discussed intertribal hybridisation between taxa of

Aveneae and Poeae as possible cause for these conflicts and suggested to merge them under a common tribe Poeae s.l. subdivided into up to 20 subtribes depending on the authors (cf. Soreng & Davis 2000; CNWG 2000 onwards; Davis & Soreng 2007; Soreng et al. 2007; Quintanar et al. 2007). Furthermore, the loss of the nuclear GBSSI intron 10 was indicated as synapomorphic trait for Poeae s.l. (Davis & Soreng 2007). Other chloroplast-based analyses recommended to maintain Aveneae and Poeae for the two chloroplast DNA lineages within the Aveneae/Poeae tribe complex until more comprehensive nuclear sequence data would be obtained (Döring et al. 2007; Döring 2009).

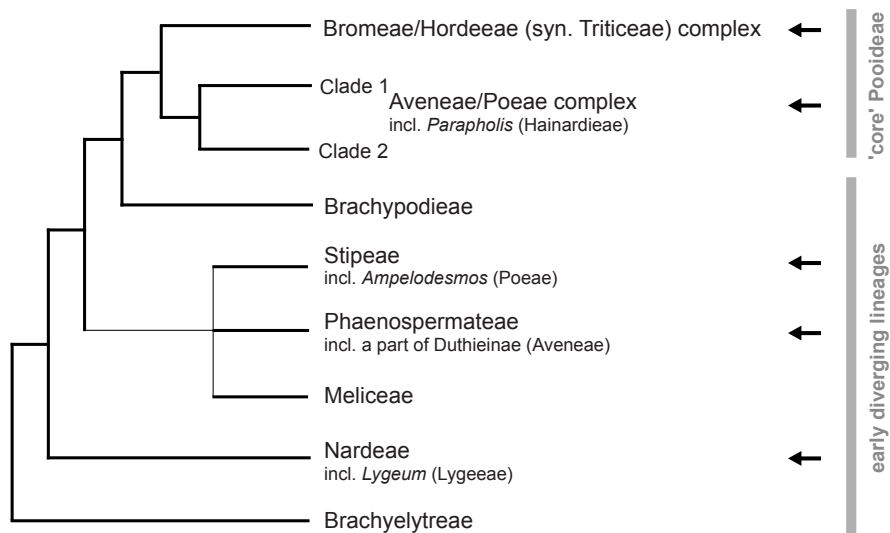
Taxonomic conflicts were also observed concerning the delimitation of the two other tribes of the 'core' Pooideae Bromeae and Hordeae (syn. Triticeae) based on differences between molecular and morphological data. Molecular phylogenetic investigations identified the tribes Bromeae and Hordeae clearly as a monophyletic group as sister to the Aveneae/Poeae clade (e.g. Davis & Soreng 1993; Nadot et al. 1994; Hsiao et al. 1995a, 1999; Catalán et al. 1997; Soreng & Davis 1998, 2000; Hilu et al. 1999; Mathews et al. 2000; GPWG 2001; Döring et al. 2007; Soreng et al. 2007; Bouchenak-Khelladi et al. 2008). Recently, the genus *Littledalea*, traditionally affiliated to Bromeae, was consistently revealed as sister to the remainder of the Bromeae/Hordeae (Davis & Soreng 2007; Döring et al. 2007; Soreng et al. 2007; Döring 2009). On the other hand two genera (*Hordelymus*, *Psathyrostachys*) have been placed only with uncertainty within Hordeae depending on the molecular marker used (chloroplast versus nuclear data; cf. Hsiao et al. 1995b; Catalán et al. 1997; Döring 2009). For these reasons, the taxonomic treatment of this phylogenetic group remained unresolved and was provisionally termed as Bromeae/Triticeae complex (Döring et al. 2007; cf. Davis & Soreng 2007; Bouchenak-Khelladi et al. 2008).

Among Pooideae the small tribe Hainardieae was composed of only six genera (*Agropyropsis*, *Hainardia*, *Narduroides*, *Parapholis*, *Pholiurus*, *Scribneria*) and approximately 10 species according to Clayton & Renvoize (1986). The species are mostly annuals adapted to moist saline soils. Morphologically, Hainardieae seemed well-defined by morphological characters such as spicate inflorescence and spikelets arranged into two rows (Clayton and Renvoize 1986). Other taxonomic treatments placed all of these genera in Poeae (Tzvelev 1989). Alternatively, *Scribneria* was ranked under Aveneae and the other genera were placed in Poeae (cf. Macfarlane 1987; Watson and Dallwitz 1992, 1992 onwards). Molecular phylogenetic studies corroborated a close relationship of Hainardieae to Aveneae and Poeae, but only some genera of Hainardieae had been sampled. *Hainardia* and *Parapholis* were identified as closely related taxa, which belonged to Poeae subtribe Parapholiinae (Soreng & Davis 2000; Catalán et al. 2004, 2007; Soreng et al. 2007). *Narduroides* was accommodated under the subtribe Loliinae (Torrecilla et al. 2004; Catalán et al. 2004, 2007; Soreng et al. 2007). A further previous study based on chloroplast DNA

restriction site analysis suggested to classify *Scribneria* under a new monotypic subtribe Scribneriinae (Soreng et al. 2003, 2007).

## Thesis questions

The thesis aims to gain new insights into the phylogeny and systematics of the grass subfamily Pooideae. Previous studies were contradictory in the delineation of Pooideae and the taxonomic treatments of some lineages remained uncertain (see Table 1). Our previous study (Döring et al. 2007) is used as starting point for the present thesis to analyse unresolved relationships in this grass subfamily. Different phylogenetic and systematic aspects, which have not yet been completely understood, are illustrated in Fig. 2.



**Fig. 2** Modified cladogram of phylogenetic relationships within Pooideae showing the main lineages (the tribe Diarrheneae was not analysed) based on sequence data of the *matK* gene (Döring et al. 2007). Arrows indicate clades with uncertain circumscription and unresolved phylogenetic placement. Their tribal placements according to Clayton & Renvoize (1986) are shown in brackets. Supported branches are in bold print, branches without support in light print.

In an attempt to resolve these systematic and taxonomic questions new sequence data of the chloroplast gene *matK* now including the intron between *matK* and 3'*trnK* and the 3'*trnK* exon were analysed.

Furthermore, it was asked whether the incongruence between morphological and chloroplast data noticed among the major lineages of Pooideae, especially within Aveneae and Poeae, could be caused by past hybridisation or reticulate evolution (Soreng et al. 2000, Quintanar et al. 2007, Soreng et al. 2007). Differences between chloroplast and nuclear phylogenetic reconstructions based on different inheritance patterns (maternal versus biparental) may unveil evolutionary processes that were involved in the evolutionary history of the Pooideae. The nuclear region ITS1–5.8S gene–ITS2, most commonly used in plant systematics since its establishment more than 20 years ago (Baldwin et al. 1992), was

selected as nuclear marker. This DNA region can be amplified relatively easy and is suitable even for molecular analyses of severely degraded DNA from poorly preserved plant tissues such as herbarium material (Blattner 1999). In the present thesis mainly plant material from herbarium specimens was used.

In addition, cytogenetic, morphological and biogeographical data were analysed with focus on taxa, which had not yet been sufficiently considered.

The following questions of interest are addressed:

- Can phylogenetic analyses based on sequences of the chloroplast *matK* gene–3'*trnK* exon and the nuclear ITS1–5.8S gene–ITS2 contribute to a reliable classification of the subfamily Pooideae with well-defined tribes? Does the creation of a taxonomically completely overlapping set of chloroplast and nuclear DNA sequence data reveal clear indications of past hybridisation or reticulation within the Pooideae? Which evolutionary trends in cytogenetic characters (e.g. chromosome number or genome size), morphological structure and biogeographical patterns can be found within the subfamily?
- Which evolutionary patterns can be identified within the early diverging lineages of Pooideae with focus on the circumscription of the tribes Stipeae, Meliceae and Phaenospermateae? Are all genera of the traditionally circumscribed Duthieinae (Aveneae) sensu Clayton and Renvoize (1986) closely related to Phaenospermateae?
- What is the most appropriate taxonomic treatment suggested by morphological and molecular data for the genera *Lygeum* and *Nardus*?
- What insight can be obtained from the nuclear and combined nuclear and chloroplast DNA data on the delineation and systematics of the 'core' Pooideae?
- Are Bromeae and Hordeae (syn. Triticeae) supported as separated tribes or should they be better accommodated under a common tribe?
- Does the new sequence information confirm the delineation of the Aveneae/Poeae tribe complex with a clear split into two strongly supported clades?
- A further topic is to re-examine all genera of the small pooid tribe Hainardieae sensu Clayton & Renvoize (1986) and to test their relationships among the Aveneae/Poeae tribe complex.

## Main results and overall discussion

### Phylogeny and classification of the Pooideae

To address unresolved questions concerning phylogeny and systematics of the subfamily Pooideae chloroplast (*matK* gene–3'*trnK* exon) and nuclear (ITS1–5.8S gene–ITS2) DNA sequence datasets were generated for representative genera of all tribes recognised by GWPG (2001). Phylogenetic reconstructions provided evidences to update the classification of Pooideae. The combined analysis showed a largely well-resolved series of clades representing major lineages of the subfamily (chapter 2). Furthermore, new morphological informations obtained by own observations and compared with data from the literature made it necessary to change the previous classifications in several aspects (cf. Table 1; Clayton & Renvoize 1986; Watson & Dallwitz 1992 onwards; GPWG 2001; Davis & Soreng 2007; Bouchenak-Khelladi et al. 2008). Especially the taxonomic treatment of the tribes Nardeae, Lygeae, Brylkinieae, Meliceae, Ampelodesmeae, Stipeae, Bromeae, Hordeae (syn. Triticeae), Hainardieae, and the subtribe Duthieinae (Aveneae) was revised relative to their original circumscriptions (Fig. 3).

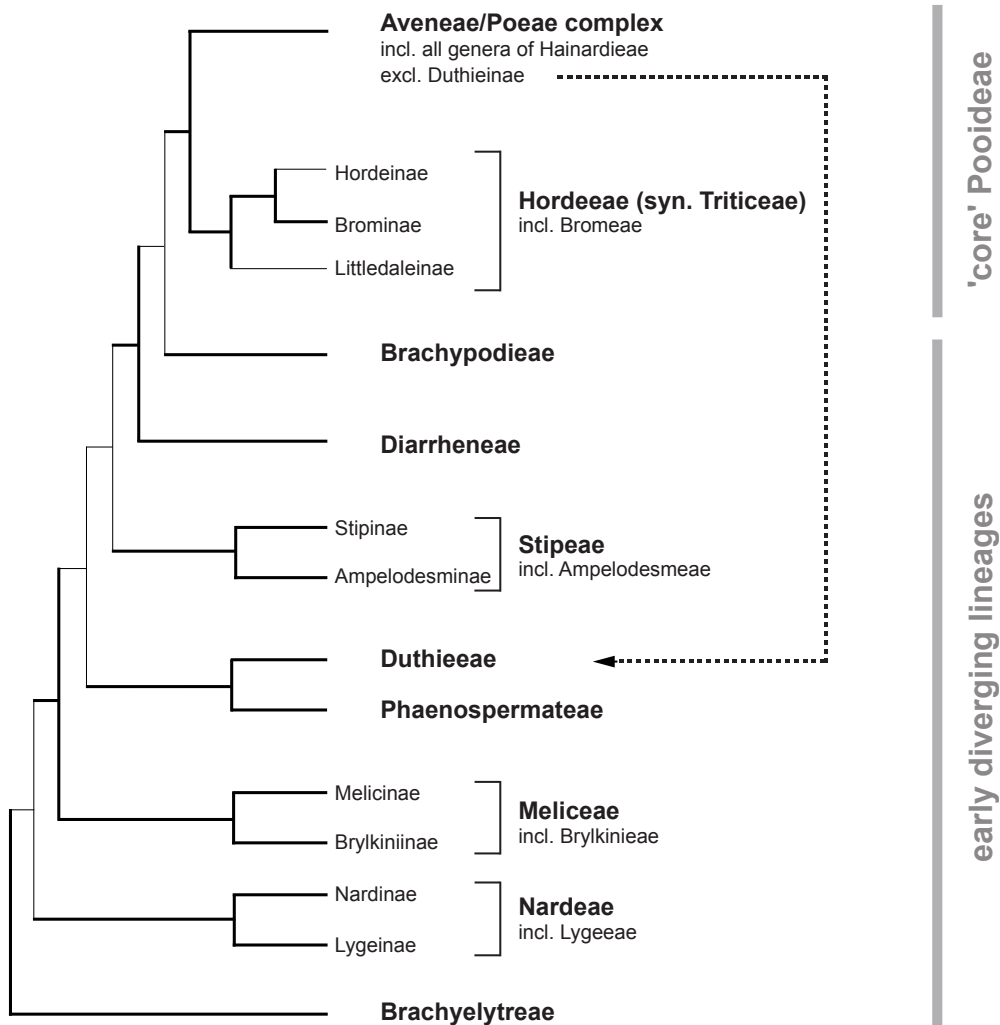
**Early diverging lineages.** — Within the early diverging lineages the molecular phylogenetic analysis based on the combined chloroplast and nuclear DNA dataset revealed the traditionally described lineages Nardeae/Lygeae, Meliceae/Brylkinieae and Stipeae/Ampelodesmeae as highly supported sister taxa (chapter 2).

The monotypic tribes Nardeae and Lygeae were confirmed as early diverging lineage grouping after the Brachyelytreae (e.g. Barker et al. 1999; Hilu et al. 1999; Hsiao et al. 1999; Soreng & Davis 2000; Davis & Soreng 2007, 2010; Döring et al. 2007; Bouchenak-Khelladi et al. 2008; Döring 2009; Hamasha et al. 2012; Romaschenko et al. 2012). The clearly synapomorphic characters (bicellular, though differently shaped microhairs, absence of lodicules, and solitary stigmas) give good morphological evidence to classify the genera *Nardus* and *Lygeum* under a common tribe Nardeae (Döring et al. 2007, Döring 2009). The maintenance of these genera under the separate subtribes Nardinae and Lygeinae as suggested in this study (chapter 2) is based on several inflorescence traits (i.e. sheathed or not, number of spikelets, different shape of glumes and lemmas).

In the molecular phylogenetic analyses presented here (chapters 2, 3), the originally monotypic tribe Brylkinieae was resolved as sister clade to the tribe Meliceae. Both tribes share several morphological features (connate margins of the leaf sheaths and partial fusion and fleshy structure of the lodicules), but differ clearly in spikelet and gynoeceum characters. The sister position of *Brylkinia* to Meliceae contradicts a cladistic analysis of morphological characters (Mejia-Saulés & Bisby 2000) that showed *Brylkinia* in close relationship to



*Diarrhena* (Diarrheneae) and *Brachypodium* (Brachypodieae), but was corroborated by subsequent molecular phylogenetic studies (Döring 2009; Davis & Soreng 2010; Romaschenko et al. 2012). In consideration of molecular information and critical evaluation of morphological characters, this study suggests that the tribe Meliceae should be broadly defined to encompass the subtribes Brylkiniinae and Melicinae (chapter 2).



**Fig. 3** Schematic overview of the phylogenetic hypothesis and classification within the Pooideae, including the changes of tribe and subtribe delineations proposed in this thesis (chapter 2–4). The branches indicated by bold lines are highly supported by the combined analyses of nuclear ITS1–5.8S gene–ITS2 and chloroplast *matK* gene–3'*trnK* exon DNA sequences or by using chloroplast sequence data alone.

Furthermore, the genus *Ampelodesmos* appeared in close relationship to the tribe Stipeae as already shown in previous studies (e.g. Davis & Soreng 2007, 2010; Döring et al. 2007; Barkworth et al. 2008; Bouchenak-Khelladi et al. 2008; Romaschenko et al. 2008; Döring 2009; Hamasha et al. 2012; Romaschenko et al. 2012). The monophyly of Ampelodesmeae/Stipeae was mainly suggested by the combined analysis of chloroplast and nuclear DNA sequences or chloroplast sequence data alone (chapters 2). Morphological information (mostly three lodicules, apex of the lemma entire and with a terminal awn or

incised, awn arising from the sinus) provided further arguments to merge *Ampelodesmeae* and *Stipeae* under a common tribe (chapter 3). The cytogenetic investigation of *Ampelodesmos mauritanicus* corroborated a high chromosome base number ( $x = 12$ ) and small chromosome size, which represent characteristics shared with *Stipeae* (cf. Barkworth et al. 2008; Romaschenko et al. 2008, 2012). The provisional classification under the two subtribes *Ampelodesminae* and *Stipinae* proposed here is based on the fact that spikelets with several florets and hairy ovary only occur in the genus *Ampelodesmos* as opposed to the remainder of *Stipeae*, which are characterised by spikelets with single florets, without rachilla extension and glabrous ovary (chapters 2, 3). In addition, a morphological key to the major groups within *Stipeae* is presented (see also chapter 3).

A further early diverging lineage as shown in this thesis comprises genera with very different previous taxonomic treatments. For the first time, all genera considered by Clayton & Renvoize (1986) as 'primitive' representatives of the tribe *Aveneae*, in which they were summarised as subtribe *Duthieinae* (*Danthoniastrum*, *Duthiea*, *Metcalfia*, *Pseudodanthonia*, *Sinochasea*, *Stephanachne*), were identified as a well-defined group together with the monotypic genus *Anisopogon* (chapter 3). The latter had sometimes been classified as a member of the subfamily *Arundinoideae* (Clayton & Renvoize 1986; Watson & Dallwitz 1992) or *Stipoideae* (Watson & Dallwitz 1992 onwards). *Phaenosperma*, the only genus of the tribe *Phaenospermateae* and previously treated under the subfamily *Bambusoideae*, appeared in close relationship to this assemblage based on chloroplast data as similarly found in other molecular phylogenetic studies (Davis & Soreng 2007; Döring et al. 2007; Döring 2009; Romaschenko et al. 2012; Blaner 2012; chapter 2). The phylogenetic analyses based on ITS sequences presented in chapter 3 could not sufficiently resolve this heterogeneous group (cf. Romaschenko et al. 2012). The results of the extensive investigation of morphological characters indicated, however, that *Phaenosperma* (characterised by spikelets disarticulating below the glumes and falling entire; lemma without awn; caryopsis globose; leaves pseudopetiolate with inverted lamina) differs strongly from the other genera, which are characterised by clearly synapomorphic spikelet traits (spikelets disarticulating below the florets; lemma awned; caryopsis linear, elliptical or fusiform, not globose; leaves 'grass-like', apex of the lemma deeply incised and awn arising from the sinus). A possible taxonomic treatment of all mentioned genera under a common broad tribe *Phaenospermateae* (CNWG 2000 onwards; Davis & Soreng 2007; Romaschenko et al. 2012) is not supported due to the lack of any morphological synapomorphy. The thesis gives strong arguments to keep *Phaenosperma* under the monotypic tribe *Phaenospermateae* sensu Renvoize & Clayton (1985) and unifies the other genera under a newly described tribe *Duthieae*. Interestingly, recent information from different regions of the nuclear single-copy gene topoisomerase 6 (*Topo6*) also places *Phaenosperma* clearly outside of the *Duthieae* (Blaner 2012). In

addition, the tribes Duthieae and Phaenospermateae differ clearly from the tribe Stipeae. In this study, the biogeographical data indicated that Phaenospermateae and Duthieae most likely originated under warm and humid climatic conditions. Some genera of Duthieae (*Sinochasea*, *Stephanachne*) became adapted to drought and cold conditions like many taxa of the species-rich tribe Stipeae. However, Duthieae were obviously less successful than the latter judging from their seemingly relict distribution (only small areas in SE Asia, Mexico, Australia, Balkan/Caucasus). In contrast to the previous classification of Soreng et al. (2003), the genera *Podophorus* and *Megalachne* are not related to members of the Duthieae. Based on molecular phylogenetics and morphological characteristics they turned out clearly associated with the Aveneae/Poeae tribe complex as suggested by Clayton and Renvoize (1986).

In chapter 3 the species-poor tribe Diarrheneae is almost completely sampled in our (four of five species sampled) and there is some evidence that it is more distantly related to the 'core' Pooideae than the tribe Brachypodieae, but closer than Stipeae (Fig. 3). The molecular phylogenetic analyses indicated further that North American *Diarrhena americana* is clearly segregated from the well-supported East Asian cluster consisting of three species, which should be treated under the genus *Neomolinia* as proposed by Tzvelev (1976, 1989).

The tribe Brachypodieae appeared mostly as sister to the 'core' Pooideae as similarly found by several other studies (e.g. Catalán et al. 1997; Döring et al. 2007; Davis & Soreng 2007, 2010). The branching order with regard to the position of *Diarrhena*, however, requires further study (chapters 2, 3; cf. Döring 2009; Hamasha et al. 2012).

**'Core' Pooideae.** — All molecular phylogenetic analyses, presented here, identified the tribe complexes of Aveneae/Poeae and Bromeae/Hordeae as sister clades forming a highly supported monophyletic group termed 'core' Pooideae (Davis & Soreng 1993). The relationships and classification of this very large group was investigated in some detail using molecular, morphological and cytogenetic characters (chapters 2, 4).

The tribes Bromeae and Hordeae are not corroborated as monophyletic lineages in this thesis, in agreement with other studies (e.g. Davis & Soreng 2007; Döring et al. 2007; Soreng et al. 2007; Döring 2009; Davis & Soreng 2010). The genus *Littledalea* was not a member of the traditional Bromeae (chapter 2), but appeared as sister to Bromeae and Hordeae (chapter 3). The traditional delineation of Bromeae against Hordeae was mainly based on the presence of an appendage at the ovary apex and the subterminal position of the stigmas in Bromeae (e.g. Clayton & Renvoize 1986; Tzvelev 1989). The results of the morphological re-examination indicated, however, that *Littledalea* has no such appendage and the styles are inserted directly at the ovary apex and not subterminal as in typical Bromeae (*Boissiera* and *Bromus*). In conclusion, the molecular phylogeny and differences in morphological key characters provided sufficient evidence to exclude *Littledalea* from

Bromeae and define a broader tribe Hordeae (syn. Triticeae) with subtribes Brominae, Hordeinae and the newly described Littledaleinae.

In chapter 2 the tribes Aveneae plus Poeae were verified as a second monophyletic clade of the 'core' Pooideae, termed Aveneae/Poeae tribe complex. Based on chloroplast data they formed two highly supported major lineages, which differed from the traditional circumscription of Aveneae and Poeae in compliance with many other molecular phylogenetic studies (e.g. Davis & Soreng 2007; Döring et al. 2007; Soreng et al. 2007; Bouchenak-Khelladi et al. 2008; Döring 2009; Saarela et al. 2010). The same deep split between these two clades, however, was not retrieved from the analysis of sequence data from the nuclear rDNA ITS region (chapters 2, 4), due to insufficient resolution or weak support. Only the more distal branches gained support and they were the same as in chloroplast DNA sequence analyses. The current molecular phylogenetic data give no unequivocal evidence of incongruity between chloroplast and nuclear phylogenetic trees. Whether past hybridisation or reticulation might have played a role in the evolution of major lineages within Aveneae/Poeae, as discussed in several studies (Soreng et al. 2000; Quintanar 2007; Soreng et al. 2007; Gillespie et al. 2010), still is an unanswered question.

The results of the present thesis do not allow to draw firm conclusions with regards to the taxonomic treatment of the Aveneae/Poeae tribe complex, i.e. as two distinct tribes or as a broad tribe Poeae, which has been suggested by some studies (e.g. Davis & Soreng 2007; Soreng et al. 2007; Saarela et al. 2010).

In chapter 4, the tribe Hainardieae, as originally defined, was resolved as a highly polyphyletic group based on molecular phylogenetics and morphology. Its genera (*Agropyropsis*, *Hainardia*, *Narduroides*, *Scribneria*, *Parapholis*, *Pholiurus*) were placed in different clades within the Aveneae/Poeae tribe complex. The present study proposes a provisional classification under four different subtribes, whose precise circumscriptions depend on further studies with a more complete sampling of genera belonging to the Aveneae/Poeae tribe complex. Only *Hainardia* and *Parapholis* were identified as sister taxa within the subtribe Parapholiinae and are characterised by synapomorphic traits (disarticulation of the inflorescence axis at maturity of the fruits into segments, palea with glabrous keels). *Pholiurus* is characterised by disarticulation below the glumes (entirely falling spikelets) and its placement in the subtribe Poinae was strongly supported by the molecular phylogenetic analyses. Furthermore, this study showed a close relationship between *Scribneria* and *Deschampsia*. These genera share some morphological characters (awned lemma, a conspicuous hairy callus) and it was suggested to accommodate them provisionally under Aristaveninae or Holcinae. The classification of *Narduroides* in Loliinae was corroborated. Likewise, *Agropyropsis* resembles taxa of Loliinae in morphology (spike-like inflorescences and prolonged upper rachilla internodes) and should be treated as

member of this subtribe. The seemingly well-defined inflorescence characters (the spicate form and spikelets arranged in two rows) and the adaptation to saline soils have obviously evolved several times in parallel within the Aveneae/Poeae tribe complex.

In conclusion, the phylogenetic history of the Aveneae/Poeae tribe complex remains insufficiently resolved and requires further investigations. It can be assumed that a more detailed analysis using a more complete taxon sampling and sequence information of other nuclear regions (single- or low-copy genes; see below) compared with analyses of morphological data will be the key to decipher the evolutionary pattern of this species-rich group of grasses.

### **Comparison of chloroplast and nuclear DNA sequence data**

The phylogenetic analyses based on sequence data of chloroplast *matK* gene–3'*trnK* exon data provided highly supported lineages within the subfamily Pooideae, even though the branching order of the early diverging lineages could not be sufficiently resolved (chapters 2, 3). The phylogenetic analysis based on nuclear ITS revealed in some cases only weakly supported branches, especially with regard to the early evolutionary branching. The limited significance of ITS may be explained by the higher degree of homoplasy compared with other markers, which makes it difficult to obtain a sufficient phylogenetic signal as noticed in the past (Álvarez & Wendel 2003, Feliner & Rosselló 2007). However, the analyses based on *matK* confirmed nearly all strongly supported lineages inferred by nuclear ITS data (chapters 2–4). Indeed, the phylogeny of Pooideae benefits from the combined analysis of *matK* and ITS data because it provided a robust topology for most lineages of the entire subfamily (chapter 2).

In future studies sequence information from nuclear single- or low-copy genes should be gathered to obtain a well-supported molecular phylogeny of Pooideae, especially in the Aveneae/Poeae tribe complex. Different exon and intron regions of the nuclear genes topoisomerase 6 (*Topo6*) and acetyl-CoA-carboxylase (*Acc1*) have recently proved to be suitable for phylogenetic analyses at the tribe or genus level of the Pooideae (*Topo6*: Jakob & Blattner 2010; Brassac et al. 2012; Blanner 2012; *Acc1*: Fan et al. 2009; Hand et al. 2010; Sha et al. 2010; own unpublished data).

## Character evolution within the Pooideae

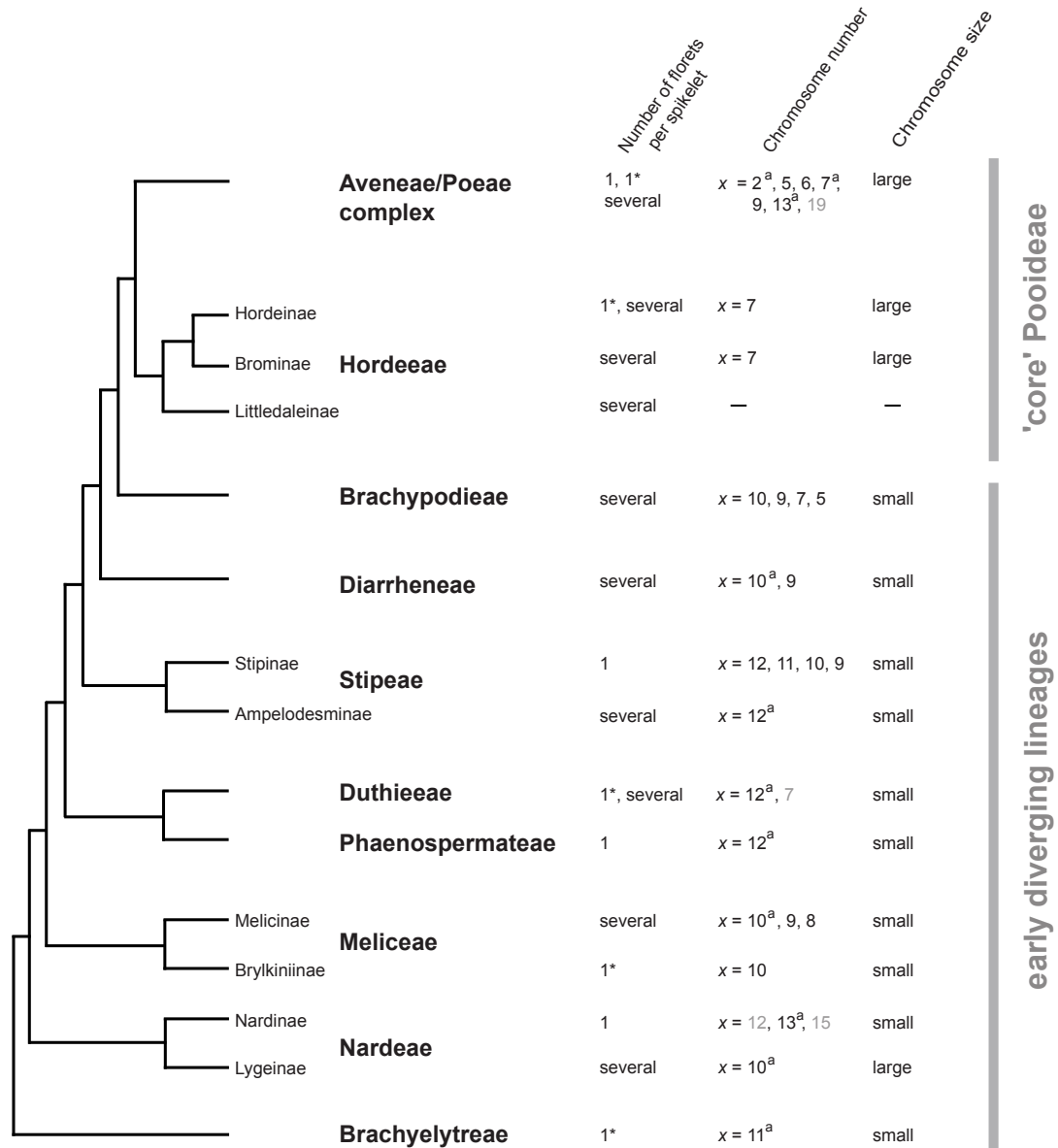
**Numbers of florets per spikelet.** — In addition to the molecular phylogeny and revised classification of the Pooideae this thesis deals also with the evolutionary significant question whether multiple florets or a single floret per spikelet represent the plesiomorphic character state of the entire Pooideae. Previous studies supposed that a single floret per spikelet could be original and multiple florets thus would have originated several times in parallel (cf. Catalán et al. 1997; GPWG 2001). The molecular phylogenetic results reported here (chapters 2, 3), however, imply that single florets per spikelet may rather be derived from spikelets with originally several florets (cf. Fig. 4). A considerable number of genera nested in the early diverging lineages are characterised by several florets (Nardeae subtribe Lygeinae, Meliceae subtribe Melicinae, Stipeae subtribe Ampelodesminae, Duthieae (genera *Danthoniastrum*, *Metcalfia*, *Pseudodanthonia*, and most species of *Duthiea*). Genera characterised by a single floret per spikelet are partly grouping in the same lineage (Nardeae subtribe Nardinae, Meliceae subtribe Brylkiniinae, some taxa of Duthieae (genera *Anisopogon*, *Sinochasea*, *Sinochasea*, *Stephanachne*, and *Duthiea brachypodium*), Phaenospermateae and Stipeae subtribe Stipinae). The presence of a rachilla extension in taxa having single spikelets (Brachyelytreae, Meliceae subtribe Brylkiniinae, Nardeae subtribe Nardinae, Duthieae) additionally indicates that spikelets with a single floret are derived from spikelets with several florets (chapter 2).

**Chromosome size and chromosome base numbers.** — The cytogenetic studies represented in this thesis give a good overview of the evolutionary patterns of chromosome base numbers and sizes within the Pooideae (Fig. 4). The analysis of exemplary genera from different lineages of Pooideae revealed a tendency from high chromosome base numbers in the early evolutionary lineages ( $x = 12$  to  $x = 5$ ) towards lower numbers (most frequent numbers of  $x = 7$  to  $x = 2$ ) in the 'core' Pooideae (chapter 3). Chromosome sizes (from 'small' to 'large') are more varied in the subfamily than supposed before (see also chapter 3).

The cytogenetic investigation of exemplary genera from the Aveneae/Poeae tribe complex confirmed the most frequent monoploid chromosome number of  $x = 7$  and large chromosome sizes (chapter 4). The result including data from the literature showed that the strongest variation of chromosome numbers is encountered within the Aveneae/Poeae tribe complex. Numbers vary between  $x = 2$  and  $x = 13$  (cf. Rodionov 2007; Shchapova 2012).

Chromosome numbers are extremely variable even among smaller groups as documented in the *Parapholis* lineage ( $x = 7$  to  $x = 19$ ) and the *Puccinellia* lineage ( $x = 2$  to  $x = 7$ ). The highest chromosome base number of  $x = 19$  was reported for the genus *Parapholis* (Watson & Dallwitz 1992 onwards), but this is supposedly not a strictly monoploid chromosome set. Actually, the unusual high gametophytic chromosome numbers

( $n = 21, 19, 18$ ) found in different specimens of *Parapholis incurva* were interpreted as polyploid ( $3x = 21$ ) or polyploid and aneuploid ( $n = 18, 19$ ) and derived from monoploid chromosome sets of  $x = 7$ , as typical for Aveneae/Poeae species (Spies et al. 1997).



**Fig. 4** Simplified phylogenetic tree of Pooideae based on this thesis with some spikelet and cytogenetic traits. Presence of a rachilla extension in spikelets with single florets is indicated by \*. Chromosome base numbers and sizes are summarised according to chapters 3, 4). <sup>a</sup>Monoploid chromosome number obtained in this thesis (chapters 3, 4). Chromosome numbers represented in grey require further verification. — information not available until now.

In conclusion, there is a tendency of reduction of the chromosome base number within Pooideae starting with comparatively high numbers, but no consistent trend within the early diverging evolutionary lineages and within the 'core' Pooideae. The considerable variation of chromosome base numbers within the clades, especially in the highly diverse Aveneae/Poeae tribe complex, should be further verified. Further studies at lower taxonomic

level with detailed information on the actual type of chromosomal changes in individual genera would be essential to make more accurate statements on trends in chromosome evolution regarding the entire subfamily.

## Conclusions and outlook

This thesis provides insight into the evolutionary patterns within Pooideae and many questions could be answered. The most significant outcome is a comparatively robust phylogenetic hypothesis of the major lineages of this subfamily. Morphological characters were extensively investigated, mainly on herbarium material. The molecular phylogenetic results are largely in agreement with data on morphological structures, especially inflorescence characters, which have formerly been partly not or only insufficiently analysed. Some have apparently not been considered to be meaningful as taxonomic character or simple been overlooked in the past.

As a consequence of the new findings, the merging of some widely accepted traditional tribes within the Pooideae is suggested, such as Nardeae and Lygeae, Meliceae and Brylkinieae, Stipeae and Ampelodesmeae, Hordeae (syn. Triticeae) and Bromeae, the Aveneae/Poeae tribe complex and Hainardieae. Duthieae is described as a new tribe, and kept separate from the tribe Phaenospermateae.

The following preliminary classification of tribes and subtribes is suggested: 1) Brachyelytreae; 2) Nardeae with subtribes Nardinae and Lygeinae; 3) Meliceae with subtribes Brylkiniinae and Melicinae; 4) Phaenospermateae; 5) Duthieae; 6) Stipeae with subtribes Ampelodesminae and Stipinae; 7) Diarrheneae; 8) Brachypodieae; 9) Hordeae with subtribes Brominae, Hordeinae and Littledaleinae; 10) the Aveneae/Poeae tribe complex. As part of the re-alignments Littledaleinae and Lygeinae were described as new subtribes. Furthermore, the results revealed the tribe Hainardieae as highly polyphyletic. Its genera belong to four different lineages within the Aveneae/Poeae tribe complex that can be treated as subtribes (Parapholiinae, Loliinae, Poinae, Aristaveninae or Holcinae). Hainardieae should be abandoned as separate tribe.

Even though the phylogenetic relationships among major lineages within the Pooideae could be much better resolved than in all previous studies, some evolutionary questions could not yet be fully answered and should be addressed in future studies. Firstly, it is recommended to examine further the still uncertain branching order of the early diverging lineages of Pooideae. Secondly, further studies will be necessary to evaluate in more detail the molecular phylogenetic and taxonomic treatment of the Aveneae/Poeae tribe complex using a more complete taxon sampling. To analyse these highly important systematic issues nuclear single- or low-copy genes should be used. Phylogenetic approaches can benefit from novel sequencing technologies, generally termed next-generation sequencing (NGS), now



and in the future. NGS provides enormous opportunities for plant systematics such as timesaving in screening of further suitable single- or low-copy nuclear genes. Furthermore, NSG offers an excellent perspective to analyse highly fragmented DNA that solve current problems in working with degraded DNA of herbarium material (Staats et al. 2011). Much more comprehensive nuclear and chloroplast, eventually also mitochondrial DNA data are needed to achieve significant progress in phylogenetic reconstructions and to test, e.g. the role of hybridisation as driving force in the evolution of Pooideae.

The present work also demonstrates that the interpretation of molecular phylogenetic hypotheses particularly benefits from in-depth analyses of morphological, cytogenetic and biogeographical aspects. Currently, the work is being continued as a part of a more extended project funded by the DFG (Deutsche Forschungsgemeinschaft).

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## Chapter 2 – Phylogenetic structure of the grass subfamily Pooideae based on comparison of plastid *matK* gene–3'*trnK* exon and nuclear ITS sequences

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### Abstract

The grass subfamily Pooideae was studied using DNA sequence information from the chloroplast (cp) *matK* gene–3'*trnK* exon and the nuclear ribosomal (nr) ITS1–5.8S gene–ITS2 in a sample of 67 taxa covering all of its tribes. Branches with strong bootstrap support are consistently resolved in both datasets, whereas discrepancy is confined to low-support or unsupported nodes in one of the datasets. The results do not reveal a significant role of past hybridisation, plastid lineage sorting or reticulation in the evolutionary diversification of the major lineages of the subfamily. The combined analysis of the plastid and nuclear datasets results in a largely well-supported pattern of divergence for the major lineages of the subfamily. Some re-alignments of tribes and subtribes are proposed and discussed with reference to relevant morphological and structural characters. We propose the recognition of broader tribes Nardeae with subtribes Nardinae and Lygeinae, Meliceae with subtribes Brylkiniinae and Melicinae, Stipeae with subtribes Ampelodesminae and Stipinae, and Triticeae with subtribes Littledaleinae, Brominae and Hordeinae. For the tribe complex of Aveneae and Poeae, the clear-cut split into two major clades and further resolution into some high-support lineages depicted by cpDNA is not contradicted by nuclear ITS and their taxonomic treatment as separate tribes or a single tribe remains an unanswered question.

Keywords: Aveneae, grasses, Poaceae, Poeae, Pooideae, ITS, *matK* gene–3'*trnK* exon, systematics

## Chapter 3 – Duthieae, a new tribe of grasses (Poaceae) identified among the early diverging lineages of subfamily Pooideae: molecular phylogenetics, morphological delineation, cytogenetics, and biogeography

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### Abstract

The phylogeny of Pooideae, one of the largest subfamilies of grasses, has been intensively studied during the past years. To investigate the early evolutionary splits in Pooideae we used a broad sample of genera with uncertain placement, some of which have not been studied in molecular phylogenetics before, complemented by representatives from other lineages of this subfamily. Morphological, cytogenetic and biogeographical analyses were added to the molecular sequence work on chloroplast *matK*–*3'trnK* and nuclear ITS. According to chloroplast DNA data, a new and well-supported lineage was identified among the early branches. It consisted of *Phaenosperma* and a larger group of genera encompassing *Anisopogon*, *Danthoniastrum*, *Duthiea*, *Metcalfia*, *Pseudodanthonia* (inclusion resting on ITS and morphology), *Sinochasea* and *Stephanachne*. Based on structural characters we suggest to keep *Phaenosperma* under the monotypic tribe Phaenospermateae and to accommodate the other genera under a new tribe Duthieae, which is morphologically well-defined by synapomorphic spikelet features. *Megalachne* and *Podophorus* were not part of the early diverging Pooideae lineages but belong to the Aveneae/Poeae complex. Morphological characteristics of Duthieae are discussed with respect especially to Stipeae and reveal consistent differences between both tribes. The genera of Duthieae and the major lineages of Stipeae are keyed. A cytogenetic survey of exemplary taxa corroborates high chromosome base numbers as prevailing within the early diverging lineages of Pooideae, but chromosome sizes are more highly varied than previously reported. Ecogeographical analyses point to warm and humid conditions as the ancestral bioclimatic niche of *Phaenosperma* and Duthieae, whereas adaptation to cold and drought occurred only in a part of Duthieae but was obviously less successful than in the widespread and much more species-rich tribe Stipeae.

The distribution of Duthieae with species-poor or monotypic genera in mountains of the northern hemisphere and *Anisopogon* as an outlier in Australia suggests relict character.

Keywords: biogeography, cytogenetics, Duthieae, grasses, ITS, *matK*, morphology, *Phaenosperma*, phylogenetics, Poaceae

## Chapter 4 – Polyphyly of the grass tribe Hainardieae (Poaceae: Pooideae): identification of its different lineages based on molecular phylogenetics, including morphological and cytogenetic characteristics

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### Abstract

The small pooid grass tribe Hainardieae comprises six genera with approximately ten species; however, this tribe was not accepted by all previous taxonomic treatments. To study the relationships among these genera and to infer the phylogeny and evolutionary patterns, we used sequence variation of the internal transcribed spacers (ITS) of nuclear ribosomal and chloroplast (cp) *matK* DNA and morphology. Many genera of the Aveneae/Poeae tribe complex additionally were included. Both molecular datasets showed Hainardieae to be highly polyphyletic, and its genera to branch with different groups of the Aveneae/Poeae. *Parapholis* and *Hainardia* are corroborated as being closely related, and belonging to a firmly supported Eurasian clade together with *Catapodium* incl. *Scleropoa*, *Cutandia*, *Desmazeria*, *Sphenopus*, *Vulpiella* (subtribe Parapholiinae) and with *Cynosurus* as sister to this assemblage. The other genera of traditionally recognised Hainardieae are positioned phylogenetically distant: Mediterranean *Narduroides* is verified as more or less related to *Festuca* and relatives (subtribe Loliinae), whereas the west Eurasian *Pholiurus* is close to the lineage of *Poa* and relatives (subtribe Poinae). North American *Scribneria* is sister to *Deschampsia* and both genera should be unified under a common subtribe (Aristaveninae or Holcinae). The phylogenetic position of the Algerian genus *Agropyropsis* (close to *Narduroides* and within the Loliinae) is suggested on morphology only, because no molecular data was obtained for it. Considering classification, we support the abandonment of tribe Hainardieae and argue to abandon Poeae subtribe Scribneriinae. Poeae subtribe Parapholiinae is redefined with a novel genus content, due to the exclusion of *Agropyropsis* and *Pholiurus* and the inclusion of *Vulpiella*.

Keywords: cytogenetics, grasses, Hainardieae, ITS, *matK*, Poaceae

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## Publication list

### Publications

#### 2013

Hoffmann, M.H., **Schneider J.**, Hase, P. & Röser, M. (2013). Rapid and recent world-wide diversification of bluegrasses (*Poa*, Poaceae) and related genera. *PLoS ONE* 8 (3): e60061. doi:10.1371/journal.pone.0060061.

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#### 2011

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#### 2007

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## Contributions to conferences

### 2013

**Schneider, J.**, Winterfeld, G., Hoffmann, M.H., Blaner, A. & Röser, M. (in press). Duthieae, a new tribe of subfamily Pooideae, and its relationships. Monocots V: 5th International Conference on Comparative Biology of Monocotyledons, to be held July 7–13, 2013, New York, United States. (Oral presentation)

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### 2012

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**2008**

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**2007**

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**2006**

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## **2005**

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## **Eigenständigkeitserklärung**

Hiermit erkläre ich an Eides statt, dass diese Arbeit bisher weder der Naturwissenschaftlichen Fakultät I der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde. Ich erkläre, dass ich mich bisher noch nie um den Doktorgrad beworben habe, und dass ich meine Arbeit selbstständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Halle (Saale), 29.04.2013

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