

**Usability of nuclear single-copy genes compared
with plastid DNA on different phylogenetic levels of
and within the order Poales**

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SUMMARY

This thesis investigates the phylogenetic systematics at the ordinal level within the Poales, at the clade level within the restiids, at the familial level within the Bromeliaceae and Poaceae, and at the subfamilial level within one of the largest grass subfamilies, the Pooideae (c. 3,300 species), with the aim to answer questions that have as of yet been unresolved. It also compares different phylogenetic markers, tests the usefulness of selected nuclear single-copy gene regions for phylogenetic analyses on different systematic levels and establishes the methods for further investigations.

A plastid reference tree based on the *matK* gene (up to the *psbA* gene) was inferred for each set of investigated data. The nuclear single-copy genes topoisomerase 6 (*Topo6*; exon 8–11, exon 8–13 and exon 17–19), acetyl-CoA-carboxylase (*Acc1*, exon 9–13) and phytochrome B (*PhyB*, partial exon 1) were used to test whether these data track the same history as the chloroplast data on the different phylogenetic levels (see above). Each dataset was evaluated using maximum parsimony, maximum likelihood and Bayesian methods.

The tree inferred from *Topo6* (only exon regions) supported a congruent, but much less resolved phylogeny compared to *matK* and the common plastid based taxonomy of the order Poales. *PhyB*, however, indicated conflicting phylogenetic relationships, such as Flagellariaceae in a basal position.

Within the restiid clade, the *Anarthria* clade is diverging first in all analyses (*matK*, *Topo6* exon 8–11 and *PhyB*). *MatK* and *Topo6* are consistent with the generally recognized taxonomic order: Restionoideae, Sporadanthoideae, Centrolepidoideae and Leptocarpoideae, whereas *PhyB* shows Centrolepidoideae next, followed by paraphyletic Restionoideae with monophyletic Sporadanthoideae and Leptocarpoideae on the terminal branches. The inclusion of the former family Centrolepidaceae as a subfamily within Restionaceae, Centrolepidoideae, can be supported, but there is no phylogenetic evidence for or against the inclusion of Anarthriaceae in Restionaceae.

The basal position of *Brocchinia* within Bromeliaceae and the monophyly of Bromelioideae and Tillandsioideae were confirmed by the *Topo6* data. Detailed information about subfamilial and tribal constellation could not be gathered because of insufficient sampling.

SUMMARY

In contrast to the plastid data, the *Topo6* exon 8–11 data strongly support the monophyly of the basal subfamily Anomochlooideae and the major BOP clade (containing Bambusoideae, Oryzoideae and Pooideae) within the Poaceae phylogeny. The subfamily Pooideae is not supported by this nuclear data because *Brachyelytrum* is placed outside of the remaining Pooideae. Different amplification lengths of the *Topo6* region spanning exon 17–19 illustrate the tribe and subtribe classification of Pooideae. The combined nuclear analysis (*Acc1*, *PhyB* and two regions of *Topo6*) retrieves the following taxonomic order: *Brachyelytrum*, Nardeae (including subtribe Lygeinae), Duthieae, Meliceae, Stipeae, Diarrheneae, Brachypoideae and the ‘core’ Pooideae (Triticeae and the Aveneae/Poeae tribe complex). The tribes Diarrheneae and Duthieae are not supported in some individual marker analyses. The split of the Aveneae/Poeae tribe complex into two lineages (plastid results) is not supported by the nuclear data. The divergent position of some clone sequences of stipoid taxa *Trikeria pappiformis* and *Ampelodesmos mauritanicus* within the *Topo6* exon 8–13 and *Acc1* exon 9–13 data suggests a hybrid origin for these species. The results point to a potential ancestor within the tribe Duthieae.

STRUCTURE OF THE THESIS

This thesis is organized in four parts. It deals with the comparison of different phylogenetic markers, the test and establishment of nuclear single-copy gene markers, the molecular phylogeny of the order Poales, the restiid clade, the families Bromeliaceae and Poaceae and the subfamily Pooideae.

Part 1 comprises the current taxonomic composition and circumscription of the selected and analyzed systematic levels and points out several unanswered questions. It also gives a brief overview of the main characteristics of the phylogenetic markers used in this study (chloroplast vs. nuclear).

Part 2 represents the cumulative part of this dissertation. It contains three studies, which have been published in peer-reviewed journals. They are arranged in systematic, not in chronological order. The publications illuminate the main questions of this thesis: the first paper investigates the order Poales, the restiid clade and the Bromeliaceae, it is followed by the study of the grasses and the investigation of Poaceae subfamily Pooideae in the third article.

Part 3 includes the main results and overall discussion.

Part 4 contains the conclusion and a short outlook.

GENERAL INTRODUCTION

The monocotyledonous order Poales Small is probably the economically most important order of plants. It sits within the commelinid clade and contains c. 20,000 species, which is 7% of all angiosperms and approximately one third of monocots (Givnish & al., 2010; Bouchenak-Khelladi & al., 2014). Poales species are extremely varied in their morphological and biological characteristics. They populate nearly all ecosystems, both terrestrial and aquatic. The number of species within a family varies from only a few taxa in the Joinvilleaceae Toml. & A.C.Sm., Flagellariaceae Dumort. and Thurniaceae Engl. (four species each) to the very large family Poaceae (R.Br.) Barnhart (c. 12,000 species).

The order currently contains 14 families (Angiosperm Phylogeny Group IV, 2016): Bromeliaceae Juss., Cyperaceae Juss., Ecdeiocoleaceae D.F.Cutler & Airy Shaw, Eriocaulaceae Martinov, Flagellariaceae, Joinvilleaceae, Juncaceae Juss., Mayacaceae Aubl., Poaceae, Rapataceae Dumort., Restionaceae R.Br., Thurniaceae, Typhaceae Juss. and Xyridaceae C.Agardh. Major lineages within Poales have been referred to as bromeliad (the earliest diverging families), xyrid, cyperid, restiid and graminid clades (Bouchenak-Khelladi & al., 2014).

The fifth-largest plant family (in terms of number of species) and by far the most successful and economically important family of Poales is the grass family Poaceae. It includes the starchy food staples rice (*Oryza* L.), wheat (*Triticum* L.), barley (*Hordeum* L.), maize (*Zea* L.), rye (*Secale* L.), oat (*Avena* L.), sorghum (*Panicum* L.) and millet (cf. *Panicum* L.), as well as bamboo, sugarcane (*Saccharum* L.) and lemongrass (*Cymbopogon citratus* (DC.) Stapf). Grasses dominate many vegetation forms (called grasslands) such as steppes, prairies, savannahs, pampas and bamboo forests. Thus, they form the main source of food for humans and grazing animals. Grasses are also used in the manufacture of goods such as paper, thatch, insulation, fuel and clothing, they provide timber for furniture, fencing, scaffolding and construction, and are used to make baskets, floor matting and sports turf.

Pooideae Benth. is the largest subfamily of grasses and encompasses one third of all grasses with approximately 4,000 species in 200 genera (Soreng & al., 2017). They include most of the major cereal crops and many lawn and pasture grasses. All species

of this subfamily use the C₃ photosynthetic pathway and are distributed predominantly in the temperate climates of both hemispheres.

Current classification of the order Poales. — One of the earliest molecular phylogenetic analyses of Poales is based on plastid *rbcL* sequence information (Duvall & al., 1993) and these results were combined with a morphological analysis by Kellogg & Linder (1995). The currently acknowledged family constellation of Poales was described by the Angiosperm Phylogeny Group (APG, 1998). Several studies mainly based on chloroplast DNA sequence information identified five different clades among Poales (Chase & al., 2000; Bremer, 2002; Linder & Rudall, 2005): Bromeliaceae, Rapateaceae and Typhaceae at the base, followed by the ‘core’ Poales. The monophyletic cyperid clade includes Cyperaceae, Juncaceae and Thurniaceae, and is sister to the not clearly defined xyrid clade (Eriocaulaceae, Mayacaceae and Xyridaceae). The robust restiid clade (Anarthriaceae D.F.Cutler & Airy Shaw, Centrolepidaceae Endl. and Restionaceae) is sister to the graminid clade, which contains Ectepiocoleaceae, Flagellariaceae, Joinvilleaceae and Poaceae. A large monocot phylogeny based on 81 plastid DNA regions has confirmed the previous systematics with basal families Bromeliaceae, Typhaceae, Rapateaceae and a paraphyletic xyrid clade (Givnish & al., 2010). In the largest phylogenetic analysis of the order Poales to date (chloroplast data from *rbcL* and *ndhF* marker for 545 species), Mayacaceae and Rapateaceae were added to the cyperid clade, but without strong support values (Bouchenak-Khelladi & al., 2014). A matrix of 75 protein coding genes of the plastome was analyzed by Barrett & al. (2016) and showed Bromeliaceae, Typhaceae, Rapateaceae, Mayacaceae as the basal group. They could retrieve the graminid, restiid and cyperid clade, but not the xyrid clade.

The analysis of Chase & al. (2006) contained 26S rDNA sequence information for the first time, but only as small proportion of the combined dataset. In 2015, Hertweck & al. analyzed the first monocot dataset based on the nuclear low-copy gene *PhyC* and a combined analysis. They could not show the relationships among the poalean families, but received a support increase along the backbone of the tree. The phylogenetic succession Typhaceae, Bromeliaceae, Rapateaceae, cyperids, paraphyletic xyrids, restiids and graminids was found by a concatenated and coalescence-based analysis of 234 single-copy orthogroups (McKain & al., 2016).

All of these studies established a robust and common phylogenetic hypothesis for the order Poales. The support of the xyrid clade and particularly the placement of

Mayacaceae remain subject to debate, and the phylogenetic sequence of the basal families is not yet fully resolved.

Current classification of the restiid clade. — Morphological and molecular phylogenetic studies based on chloroplast genome regions generally recognize three families in the restiid clade of the order Poales: Anarthriaceae, Centrolepidaceae and Restionaceae (Kellogg & Linder, 1995, Briggs & al., 2000, 2010; Bremer, 2002; Chase, 2004, APG III, 2009, Givnish & al., 2010, Bouchenak-Khelladi & al., 2014). They consist of tufted or rhizomatous, herbaceous plants, rush-like or bamboo-like in overall appearance, with small wind-pollinated flowers and mostly with reduced leaves (Briggs & al., 2014).

The small Western Australian family Anarthriaceae comprises three genera, *Anarthria* R.Br., *Lyginia* R.Br. and *Hopkinsia* W.Fitzg. Previous studies have supported the monophyly of this family and the sister group relationship to Restionaceae and Centrolepidaceae (Briggs & al., 2000; Briggs & Johnson, 2000; Bremer, 2002; Michelangeli & al., 2003; Linder & Rudall, 2005; Chase & al., 2006; Bouchenak-Khelladi & al., 2014). APG IV (2016) proposed the inclusion of Anarthriaceae in the Restionaceae as *Anarthria* clade.

The phylogenetic placement of Australasian centrolepids (three genera, c. 35 species) is difficult to determine. Most broad-scale molecular studies based on chloroplast DNA sequence data have placed Centrolepidaceae as sister group to Restionaceae (e.g. Michelangeli & al., 2003; Marchant & Briggs, 2007). Others have suggested a position within Restionaceae, usually as sister group to the subfamily Sporadanthoideae B.G.Briggs & H.P.Linder (e.g. Bremer, 2002; Briggs & al., 2010, 2014), whereas Briggs & Linder (2009) reported an unresolved position. Briggs & al. (2014) proposed the inclusion of centrolepids as subfamily Centrolepidoideae Burnett in Restionaceae, which was confirmed by APG IV (2016).

Within Restionaceae three subfamilies are supported by anatomical, morphological and molecular phylogenetic investigations (Briggs & al., 2000, 2010; Linder & al., 2000, 2003; Briggs & Linder, 2009). The largest subfamily Restionoideae Arnott (c. 350 species) is mainly distributed in Africa, with one species in Madagascar, and comprises two strongly supported tribes: Restioneae Bartl. and Willdenowieae Mast. (Linder & al., 2000, 2003; Hardy & al., 2008). The smallest subfamily Sporadanthoideae (three genera) is limited to Australia and New Zealand and is monophyletic in all molecular phylogenetic studies (e.g. Briggs & al., 2000, 2010,

2014; Briggs & Linder, 2009). Leptocarpoideae B.G.Briggs & H.P.Linder is the most widespread subfamily, occurring mainly in Australia and New Zealand, New Guinea, Southeast Asia and South America. It comprises five clades (*Winifredia* L.A.S.Johnson & B.G.Briggs, *Leptocarpus* R.Br., *Loxocarya* R.Br., *Baloskion* Raf. and *Desmocladus* Nees; Briggs & al., 2014) and is also monophyletic (Briggs & al., 2000, 2010, 2014; Briggs & Linder, 2009).

Current classification of the Poaceae. — The systematics and classification of the large, diverse and economically important grass family have been studied for a long time. Early studies were based on anatomical, biochemical, cytological and morphological data (Clayton & Renvoize, 1986; Tzvelev, 1989; Watson & Dallwitz, 1992). Since then, molecular phylogenetic methods have become available and have deepened our understanding of the phylogenetic relationships among the grasses. First studies used plastid marker regions to reconstruct phylogenetic trees, such as *rbcL* (Hamby & Zimmer, 1988; Doebley & al., 1990), *ndhF* (Clark & al., 1995, Catalán & al., 1997), *rpoC2* (Barker & al., 1999) and *matK* (Hilu & al., 1999). In addition to these chloroplast markers, nuclear genome regions such as *waxy* (Mason-Gamer & al., 1998; GPWG, 2001), *PhyB* (Mathews & al., 2000) and ITS (Hsiao & al., 1999) have been used.

A comprehensive investigation based on morphological and molecular data has resulted in a new subfamilial classification being published in 2001 by the Grass Phylogeny Working Group (GPWG). Anomochloideae Pilg. ex Potztl, Pharoideae L.G.Clark & Judz. and Puelioideae L.G.Clark, M.Kobay, S.Mathews, Spangler & E.A.Kellogg formed the basal lineages, which were segregated from the traditional bamboo grasses. These subfamilies are sister to a group of the major clades BOP (syn. BEP; Soreng & al., 2015) containing Bambusoideae Luerss., Oryzoideae Kunth ex Beilschm. (syn. Ehrhartoideae Caro) and Pooideae, and PACCAD (Panicoideae Link, Arundinoideae Burmeist., Chloridoideae Kunth ex Beilschm., Centrothecoideae Soderst., Aristidoideae Caro and Danthonioideae H.P.Linder & N.P.Barker).

Further studies have suggested the inclusion of Centrothecoideae in Panicoideae, and the introduction of an additional subfamily Micrairoideae Pilg. (PACMAD; Duvall & al., 2007, Sánchez-Ken & al., 2007). The latest comprehensive grass phylogenies based on chloroplast DNA regions (GPWG II, 2012; Soreng & al., 2015, 2017) and a 250 plastome phylogeny (Saarela & al., 2018) have confirmed this subfamilial structure. However, a number of studies suggested a paraphyletic subfamily Anomochloideae

(Hilu & al., 1999; Zhang & al., 2000, Davis & Soreng, 2007). The BOP clade was also paraphyletic according to several studies (e.g. Davis & Soreng, 1993; Soreng & Davis, 1998; Hilu & al., 1999 Hsiao & al., 1999) or insufficiently resolved (Clark & al., 2000; Mathews & al., 2000; Zhang, 2000). A potential relationship of the Pooideae and the PACMAD clade, for example, was found by Soreng & Davis (1998), Hsiao & al. (1999) and Duvall & al. (2007). The PACMAD clade was generally monophyletic and strongly supported. The phylogenetic sequence of the subfamilies within the clades is partially still difficult to determine, as is the structure of the genera and species within each subfamily.

Current classification of the grass subfamily Pooideae. — Early studies again used anatomical and morphological features to classify this large subfamily. However, the delimitation and number of tribes or subtribes and the systematic position of several genera could not be conclusively determined (e.g. Macfarlane & Watson, 1982; Clayton & Renvoize, 1986; Tzvelev, 1989; Watson & Dallwitz, 1992). Davis & Soreng (1993) described the so-called ‘core’ Pooideae, comprising Bromeae Dumort., Triticeae Dumort., Aveneae Dumort. and Poeae R.Br.. The species and genera belonging to this group have been placed within the Pooideae in all relevant studies. The tribes Ampelodesmeae Tutin, Anisopogoneae, Brachyleytreae Ohwi, Diarrheneae C.S.Campb., Lygeae J.Presl, Nardeae W.D.J.Koch and Stipeae Dumort have been united under a separate subfamily Stipoideae Burmeister. by some studies (Watson & Dallwitz, 1992 onwards), partially classified as members of the subfamily Arundinoideae by others (Watson & Dallwitz, 1992), or placed within the bamboo grasses (Clayton & Renvoize, 1986). In particular, the position of the tribes Diarrheneae, Phaenospermateae Renvoize & Clayton and Brachyleytreae has been difficult to determine because they exhibit both poid and bambusoid structures (Clayton & Renvoize, 1986).

All of these tribes are included in a more broadly defined subfamily Pooideae according to studies based on structural and molecular data (Catalán & al., 1997; Soreng & Davis 1998, 2000; Hilu & al., 1999; Hsiao & al., 1999; Davis & Soreng, 2007; Döring & al., 2007; Duvall & al., 2007; Bouchenak-Khelladi & al., 2008; Schneider & al., 2009, 2011). This classification was subsequently extended by the tribe Duthieae M.Röser & J.Schneider, described by Schneider & al. (2011).

Previous investigations generally used chloroplast DNA sequence data and/or nuclear ribosomal internal transcribed spacer (nrITS; cf. Duarte & al., 2010), with the

exception of Mathews & al. (2000) and GPWG (2001), which analyzed nuclear single-copy gene regions (*PhyB* and GBSSI) for the whole grass family, as mentioned above. Numerous studies have also been conducted using nuclear marker regions at the tribe or genus level within grasses and Pooideae (Huang & al., 2002; Mason-Gamer, 2005, 2013; Fan & al., 2007, 2009; Gillespie & al., 2010; Hand & al., 2010; Jakob & Blattner, 2010; Saarela & al., 2010; Sha & al., 2010; Brassac & al., 2012; Wölk & Röser, 2014, 2017; Brassac & Blattner, 2015; Wölk & al., 2015).

Table 1 gives a comparison of the different treatments of the tribes, which are assigned to the subfamily Pooideae based on selected molecular phylogenetic analyses. The taxonomic sequence of these tribes and the associated species or genera is not yet entirely clear. Previous studies based on molecular sequence information have generally recognized *Brachyleytrum* P.Beauv. as diverging first, followed by *Nardus* L. and/or *Lygeum* Loefl. ex L., and Phaenospermateae respectively Duthieae in the basal lineages of Pooideae (Catalán & al., 1997; Soreng & Davis, 1998, 2000; Hisao & al., 1999; Mathews & al., 2000; Davis & Soreng, 2007; Döring & al., 2007; Schneider & al., 2009, 2011). Detailed molecular studies investigated the monophyly and phylogeny of the large tribe Stipeae (Barkworth & al., 2008; Romaschenko & al., 2008, 2010, 2012; Hamasha & al., 2012). The genus *Ampelodesmos* Link has been classified in a close relationship with the tribe Stipeae (Soreng & Davis, 1998, 2000; GPWG, 2001; Döring & al., 2007; Bouchenak-Khelladi & al., 2008), placed within the Poeae (Clayton & Renvoize, 1986), as monotypic tribe Ampelodesmeae (Watson & Dallwitz, 1992; GPWG, 2001; Soreng & al., 2015, 2017), or within the tribe Stipeae (Barkworth, 2008; Schneider & al., 2009, 2011). The taxonomy of the tribes Brylkinieae Tateoka. and Meliceae Rchb. is as of yet undetermined because of a lack of comprehensive molecular studies; they are generally treated separately or in a common tribe (Clayton & Renvoize, 1986; GPWG, 2001; Schneider & al., 2009, 2011; Kellogg, 2015; Soreng & al., 2017). The small tribe Diarrheneae is recognized by several molecular phylogenetic investigations, but its taxonomic placement varies between studies (Catalán & al., 1997; Hsiao & al., 1999; Mathews & al., 2000; GPWG, 2001, Schneider & al., 2009, 2011; Soreng & al., 2015). The monotypic tribe Brachypodieae is mostly closely related to the ‘core’ Pooideae (Hilu & al., 1999; Hsiao & al., 1999; Mathews & al., 2000; Döring & al., 2007; Duvall & al., 2007; Bouchenak-Khelladi & al., 2008; Schneider & al., 2009, 2011; Soreng & al., 2015). A combined lineage consisting of traditional Bromeae and Triticeae was strongly supported in previous studies based on molecular data (Catalán

& al., 1997, Davis & Soreng, 1993; Döring & al., 2007; Duvall & al., 2007; Bouchenak-Khelladi & al., 2008; Soreng & al., 2007; Schneider & al., 2009, 2011; Soreng & al., 2017). The genus *Littledalea* Hemsl. is included in Bromeae (Kellogg, 2015), recognized as sister tribe (Littledaleae Soreng & J.I.Davis) of Bromeae and Triticeae (Soreng & al., 2015, 2017) or treated as one of three subtribes in Triticeae (Schneider & al., 2009). The species-rich Aveneae/Poeae tribe complex (Schneider & al., 2009) is split into two strongly supported lineages (Clade 1 and 2) in several molecular phylogenetic studies based on chloroplast sequence data (Catalán & al., 1997; Hilu & al., 1999; Soreng & Davis, 2000; Davis & Soreng, 2007; Döring & al., 2007; Soreng & al., 2007; Bouchenak-Khelladi & al., 2008; Schneider & al., 2009, 2011; Soreng & al., 2015, 2017). This bifurcation was not supported by phylogenetic trees based on nuclear sequence data (Quintanar & al., 2007; Schneider & al., 2009, 2011).

Table 1. An overview of the classification within the subfamily Pooideae by previous and current molecular phylogenetic studies.

Tribes	GPWG (2001)	Bouchenak-Khelladi & al. (2008)	Schneider & al. (2011)	Soreng & al. (2017)
Ampelodesmeae (Conert) Tutin	Ampelodesmeae	Ampelodesmeae	Stipeae	Ampelodesmeae
Aveneae Dumort.	Poeae	Aveneae + Poeae	Aveneae/Poeae complex	Poeae
Brachyleytreae Ohwi	Brachyleytreae	Brachyleytreae	Brachyleytreae	Brachyleytreae
Brachypodieae Harz	Brachypodieae	Brachypodieae	Brachypodieae	Brachypodieae
Bromeae Dumort.	Bromeae	Bromeae + Triticeae	Triticeae	Bromeae
Brylkinieae Tateoka	Brylkinieae	-	Meliceae	Brylkinieae
Diarrheneae (Ohwi) C.S.Campb.	Diarrheneae	Diarrheneae	Diarrheneae	Diarrheneae
Duthieae Röser & J. Schneider	Phaenospermateae	-	Duthieae	Duthieae
Triticeae Dumort.	Triticeae	Bromeae + Triticeae	Triticeae	Triticeae
Littledaleae Soreng & J.I. Davis	-	-	Triticeae	Littledaleae
Lygeae J.Presl.	Lygeae	Lygeae + Nardeae	Nardeae	Lygeae
Meliceae Rchb.	Meliceae	Meliceae	Meliceae	Meliceae
Nardeae Koch	Nardeae	Lygeae + Nardeae	Nardeae	Nardeae
Phaenospermateae Renvoize & Clayton	Phaenospermateae	Phaenospermateae	Phaenospermateae	Phaenospermateae
Poeae R.Br.	Poeae	Aveneae + Poeae	Aveneae/Poeae complex	Poeae
Stipeae Dumort.	Stipeae	Stipeae	Stipeae	Stipeae

Overview of molecular phylogenetic marker regions. — The molecular marker regions primarily used in plant phylogenies at present are still the ones obtained from chloroplast DNA. Plastid DNA is predominantly inherited uniparentally and maternally in angiosperms (Knoop & Müller, 2009), and most advantageous for molecular investigations of single-copy nature. Additionally, many studies have used the internal transcribed spacer nuclear ribosomal DNA (ITS nrDNA) of the 18S-5.8S-26S nrDNA repeat unit because this well-homogenized region infected by concerted evolution makes it suitable for phylogenetic studies. These markers are generally easy to amplify and sequence, and can be aligned across lower taxonomic levels (Barfuss, 2012). The number of available published sequences also facilitates the design of new primers. However, the relatively slow evolutionary rate of these regions leads to difficulties in resolving the phylogenetic relationships at all taxonomic levels, especially the lower taxonomy, even in multi-locus and combined studies (Small & al., 2004). Nuclear genes evolve more rapidly, they are biparentally inherited and the introns are relatively stable (Small & al., 2004; Knoop & Müller, 2009). Because of this, nuclear single- and low-copy genes promise a better phylogenetic solution, particularly in plant groups with low variation in the plastid DNA sequences. Ludeña & al. (2011) show that heterozygosity, PCR constraints and paralogy make it difficult to optimize nuclear gene markers. The interpretation of results based on nuclear single- or low-copy sequence data is also complicated by processes such as incomplete lineage sorting, hybridization and introgression (Piñeiro & al., 2009), particularly when low-copy regions are used to investigate multi-copy nuclear genes and polyploid species (e.g. Sang & al., 2004). Despite these limitations, single-copy nuclear markers are useful for tracing the evolution of allopolyploids and for identifying parental taxa of polyploids (Brassac & al., 2012; Triplett & al., 2012; Zimmer & Wen, 2012).

THESIS QUESTIONS

As is evident from the Introduction, there are several contentious issues within the different phylogenetic levels of the Poales. This thesis aims to provide new information regarding the phylogeny and systematics of the large order Poales, the restiid clade, the Poaceae family and the grass subfamily Pooideae. It also compares plastid and nuclear markers, and tests and establishes nuclear single-copy gene markers on different phylogenetic levels.

The diploma thesis Blaner (2012) formed the basis of the present thesis. For the first time two different *Topo6* regions have been used to investigate phylogenetic questions on the family level within the Poaceae, with a more detailed look at the subfamily Pooideae and the extension to poalean species of Joinvilleaceae and Restionaceae.

The present thesis compares the results of chloroplast and nuclear single-copy sequencing to:

- ◆ examine phylogenetic relationships within the order Poales and try to clarify the structure of the basal families, as well as the position of Mayacaceae and the xyrid clade,
- ◆ resolve relationships within the restiid clade such as the number of families included and the topological position of Centrolepidaceae,
- ◆ clarify phylogenetic relationships within the Poaceae, in particular the monophyly of the basal subfamilies and the BOP clade,
- ◆ investigate conflicting tribal classifications within the grass subfamily Pooideae, such as Duthieae, Triticeae, Meliceae, Nardeae and Phaenospermateae, as well as the strongly supported split of the Aveae/Poeae clade into two lineages,
- ◆ test the utility of selected nuclear single-copy gene markers on different phylogenetic levels (order, clade, family, subfamily) for the first time,
- ◆ establish these markers for phylogenetic investigations,
- ◆ compare the results based on nuclear sequences with plastid DNA sequence data (*matK*).

CUMULATIVE PART

PUBLICATION 1

Nuclear genes, *matK* and the phylogeny of the Poales

Anne Hochbach, H. Peter Linder & Martin Röser

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Nuclear genes, *matK* and the phylogeny of the Poales

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Abstract

Phylogenetic relationships within the monocot order Poales have been well studied, but several unrelated questions remain. These include the relationships among the basal families in the order, family delimitations within the restiid clade, and the search for nuclear single-copy gene loci to test the relationships based on chloroplast loci. To this end two nuclear loci (*PhyB*, *Topo6*) were explored both at the ordinal level, and within the Bromeliaceae and the restiid clade. First, a plastid reference tree was inferred based on *matK*, using 140 taxa covering all APG IV families of Poales, and analyzed using parsimony, maximum likelihood and Bayesian methods. The trees inferred from *matK* closely approach the published phylogeny based on whole-plastome sequencing. Of the two nuclear loci, *Topo6* supported a congruent, but much less resolved phylogeny. By contrast, *PhyB* indicated different phylogenetic relationships, with, inter alia, Mayacaceae and Typhaceae sister to Poaceae, and Flagellariaceae in a basally branching position within the Poales. Within the restiid clade the differences between the three markers appear less serious. The *Anarthria* clade is first diverging in all analyses, followed by Restionoideae, Sporadanthoideae, Centrolepidoideae and Leptocarpoideae in the *matK* and *Topo6* data, but in the *PhyB* data Centrolepidoideae diverges next, followed by a paraphyletic Restionoideae with a clade consisting of the monophyletic Sporadanthoideae and Leptocarpoideae nested within them. The Bromeliaceae phylogeny obtained from *Topo6* is insufficiently sampled to make reliable statements, but indicates a good starting point for further investigations. We

find that *matK* is remarkably good at retrieving the chloroplast phylogeny, that *Topo6*, despite low resolution, is suitable to test the generality of the plastid phylogeny as a taxic phylogeny, that *PhyB* might be too complex to be really useful at the level of families within an order, that the inclusion of the centrolepids in Restionaceae might be valid, but that there is no phylogenetic support for or against including the Anarthria clade in Restionaceae. The basal arrangement of families in the Poales (Bromeliaceae, Typhaceae, Rapateaceae) remains unresolved.

Keywords Bromeliaceae; *matK*; molecular phylogeny; nuclear loci; Poales; Restionaceae

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PUBLICATION 2

Phylogenetic relationships in the grass family (Poaceae) based on single copy locus topoisomerase 6 compared with chloroplast DNA

Anne Blaner, Julia Schneider & Martin Röser

Systematics and Biodiversity (2014) 12: 111--124

Phylogenetic relationships in the grass family (Poaceae) based on the nuclear single copy locus topoisomerase 6 compared with chloroplast DNA

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Abstract

Phylogenetic relationships within the grass family were studied using a newly obtained locus of the nuclear single copy gene topoisomerase 6 (*Topo6*) spanning the four exons 8–11 and the chloroplast *matK* gene. Data were evaluated using maximum parsimony, maximum likelihood and Bayesian methods. All analyses showed genera *Streptochaeta* and *Anomochloa* as early diverging, followed by *Pharus* as sister to the rest of the Poaceae, and monophyly of the subfamily Anomochlooideae was supported by the nuclear dataset. The remaining grasses formed a strongly supported and monophyletic group, which split into the major clades BEP and PACMAD in the *Topo6* analyses. Monophyly of the BEP clade was strongly supported by the *Topo6* data. The results showed clearly incongruity between the two sets of data, such as the different subfamilial relationships of Bambusoideae, Ehrhartoideae and Pooideae. Most of the analysed species are representatives of subfamily Pooideae, which was analysed in more detail by PCR fragment length differences of another *Topo6* region spanning the exons 17–19. Monophyly of Pooideae was strongly supported by the *matK* data, whereas the

nuclear data placed *Brachyelytrum* outside of the remaining Pooideae. Relationships within the early evolutionary lineages remained largely unresolved in the phylogenetic trees, but the ‘core’ Pooideae (Aveneae/Poeae tribe complex and Hordeae) were highly supported in all analyses. The differences in amplification lengths illustrate the tribe and subtribe classification of Pooideae. The comparatively conserved structure of the newly studied *Topo6* region makes it a promising marker from the nuclear genome that could

be successfully PCR-amplified to study higher-level phylogenetic relationships within grasses and perhaps between families within the order Poales.

Keywords BEP, grasses, *matK*, phylogenetics, Poaceae, Pooideae, *Topo6*, topoisomerase

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PUBLICATION 3

A multi-locus analysis of phylogenetic relationships within grass subfamily Pooideae (Poaceae) inferred from sequences of nuclear single copy gene regions compared with plastid DNA

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Molecular Phylogenetics and Evolution (2015) 87: 14--27

A multi-locus analysis of phylogenetic relationships within grass subfamily Pooideae (Poaceae) inferred from sequences of nuclear single copy gene regions compared with plastid DNA

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Abstract

To investigate phylogenetic relationships within the grass subfamily Pooideae we studied about 50 taxa covering all recognized tribes, using one plastid DNA (cpDNA) marker (*matK* gene–*trnK* exon) and for the first time four nuclear single copy gene loci. DNA sequence information from two parts of the nuclear genes topoisomerase 6 (*Topo6*) spanning the exons 8–13 and 17–19, the exons 9–13 encoding plastid acetyl-CoA-carboxylase (*Acc1*) and the partial exon 1 of phytochrome B (*PhyB*) were generated.

Individual and nuclear combined data were evaluated using maximum parsimony, maximum likelihood and Bayesian methods. All of the phylogenetic results show *Brachyelytrum* and the tribe Nardeae as earliest diverging lineages within the subfamily. The ‘core’ Pooideae (Hordeae and the Aveneae/Poeae tribe complex) are also strongly supported, as well as the monophyly of the tribes Brachypodieae, Meliceae and Stipeae (except *PhyB*). The beak grass tribe Diarrheneae and the tribe Duthieae are not monophyletic in some of the analyses. However, the combined nuclear DNA (nDNA) tree yields the highest resolution and the best delimitation of the tribes, and provides the following evolutionary hypothesis for the tribes: *Brachyelytrum*, Nardeae, Duthieae, Meliceae, Stipeae, Diarrheneae, Brachypodieae and the ‘core’ Pooideae. Within the individual datasets, the phylogenetic trees obtained from *Topo6* exon 8–13 shows the most interesting results. The divergent positions of some clone sequences of *Ampelodesmos mauritanicus* and *Trikeria pappiformis*, for instance, may indicate a hybrid origin of these stipoid taxa.

Keywords *matK*, Nuclear single copy, Phylogeny, Poaceae, Pooideae, *Topob*

DOI <http://dx.doi.org/10.1016/j.ympev.2015.03.010>

Supplementary Material <http://dx.doi.org/10.1016/j.ympev.2015.03.010>.

MAIN RESULTS AND OVERALL DISCUSSION

Selected nuclear single-copy genes in comparison with plastid marker region *matK* were used to address open questions regarding the phylogeny and systematics of the order Poales, the restiid clade, the family Poaceae and the grass subfamily Pooideae, and to test the utility for phylogenetic analyses within these different systematic levels. The chloroplast *matK* region (up to the *psbA* gene) has already been proven to be meaningful in the investigated phylogenetic groups (Hilu & al., 1999; Tamura & al., 2004; Döring & al., 2007; Marchant & Briggs, 2007; Hardy & al., 2008; Schneider & al., 2009, 2011; Givnish & al., 2010; Evans & al., 2015; Hertweck & al., 2015). The topoisomerase 6 gene (*Topo6*) is an important house-keeping gene and involved in the repair of double-strand breaks (Hartung & al., 2002). It was previously utilized in studies of the genus *Hordeum* L. (Jakob & Blattner, 2010; Brassac & al., 2012; Brassac & Blattner, 2015) and the oat-like grasses (traditional Aveneae; Wölk & Röser, 2014, 2017; Wölk & al. 2015). The phytochrome B gene (*PhyB*) is a photoreceptive signaling protein and involved in light-sensitive processes (Ludeña & al., 2011). It has been used successfully in studies on angiosperms (Mathews & al., 1995), palms (Ludeña & al., 2011), Restionaceae (Litsios & al., 2014) and Poaceae (Mathews & al., 2000). The protein-coding gene *Acc1* (encoding plastid acetyl-CoA carboxylase) has proved to be useful in phylogenetic studies of the Aveae/Poeae tribe complex (Hand & al., 2010), the tribe Triticeae (Huang & al., 2002; Fan & al., 2007, 2009; Sha & al., 2010) and the genus *Panicum* L. (Triplett & al., 2012). Table 2 gives an overview of the nuclear single-copy genes and their application.

Table 2. Overview of systematic groups and the nuclear single-copy gene regions used within this thesis.

Systematic group	<i>Topo6</i> exon 8–11	<i>Topo6</i> exon 8–13	<i>Topo6</i> exon 17–19	<i>PhyB</i> partial exon 1	<i>Acc1</i> exon 9–13
Poales	only exons			X	
Restiid clade	X			X	
Bromeliaceae	X				
Poaceae	X				
Pooideae		X	X	X	X

Poales basal group. — Most plastid DNA analyses place Typhaceae and Bromeliaceae as sister groups (Bremer, 2002; Davis & al., 2004; Givnish & al., 2006, 2011; Bouchenak-Khelladi & al., 2014; Hertweck & al., 2015; Magallón & al., 2015), but some suggest that Bromeliaceae diverge first, followed by Typhaceae (Chase & al., 2000, 2006; Soltis & al., 2000; Givnish & al., 2010). The *matK* tree shows Bromeliaceae and Typhaceae in a polytomy as diverging first, followed by Rapateaceae and the remaining poalean taxa (publication 1), which corroborates Hertweck & al. (2015). Because of insufficient resolution neither the plastid marker *matK* nor the nuclear markers *PhyB* and *Topo6* could provide the clarification of the basal structure within Poales (publication 1).

Bromeliaceae. — Bromeliaceae is monophyletic in all present datasets (publication 1). *Brocchinia* Schult.f. is the sister group to all remaining taxa (publication 1; Terry & al., 1997; Horres & al., 2000; Crayn & al., 2004, 2015; Givnish & al., 2011, 2014; Escobedo-Sarti & al., 2013). The subfamily Tillandsioideae Burnett is monophyletic in the *matK* and the *Topo6* tree (publication 1; Terry & al., 1997; Horres & al., 2000; Crayn & al., 2004; Escobedo-Sarti & al., 2013). Following Terry & al. (1997) and Givnish & al. (2011) the subfamily Pitcairnioideae Harms ex Engler & Prantl is supported by the *matK* data, but polyphyletic in the *Topo6* analysis (publication 1; Horres & al., 2000; Crayn & al., 2004; Escobedo-Sarti & al., 2013). The most terminal subfamily Bromelioideae Burnett is not monophyletic in the chloroplast data, however, it is strongly supported in the *Topo6* tree, where *Dyckia* Schult.f. is the sister group to Bromelioideae (publication 1). The most commonly reported sister group relationship of Bromelioideae and Puyoideae Givnish (Terry & al., 1997; Crayn & al., 2004; Givnish & al., 2011, 2014; Escobedo-Sarti & al., 2013) is not supported by the present study. Because of the lack of backbone support values, the *Topo6* marker could not fully resolve the subfamilial relationships among the Bromeliaceae (publication 1), but it generally performed well in this family.

Mayacaceae and the xyrid clade. — The family Mayacaceae consists only of the marsh/aquatic genus *Mayaca* Aubl. and still has a controversial phylogenetic position within the order Poales. Previous studies have placed this family within or in close relationship with the xyrid clade (Michelangeli & al., 2003; Linder & Rudall, 2005; Givnish & al., 2010), within the cyperid clade (Chase & al., 2000, 2006; Janssen & Bremer, 2004; Bouchenak-Khelladi & al., 2014) or between these two clades (Davis & al., 2004; Hertweck & al., 2015; McKain & al., 2016). The plastid *matK* data weakly

supports the structure between xyrid (Ericaulaceae and Xyridaceae) and cyperid clade, whereas *Topo6* could not resolve the position of the Mayacaceae (publication 1). The *PhyB* tree shows *Mayaca* grouping with Poaceae, Typhaceae and Eriocaulaceae (publication 1). This taxonomic placement has not been proposed before and needs to be treated with caution because of the lack of bootstrap support. The xyrid clade is the sister group to the strongly supported relationship of restiid and graminid clades in the *matK* analyses (cf. Bremer, 2002; Janssen & Bremer, 2004; Bouchenak-Khelladi & al., 2014; Hertweck & al., 2015; McKain & al., 2016), but it is paraphyletic and unsupported in all of the present analyses (publication 1).

Restiid clade. — The APG IV (2016) enlarged the Restionaceae to re-include Anarthriaceae and Centrolepidaceae to stabilize the taxonomy of the poalean order. As a result, the restiid clade consists only of the family Restionaceae. The *Anarthria* clade is monophyletic and the sister group to the remaining Restionaceae in all current analyses (publication 1). As mentioned above, this is the most commonly recognized relationship (Briggs & al., 2000; Briggs & Johnson, 2000; Bremer, 2002; Michelangeli & al., 2003; Linder & Rudall, 2005; Chase & al., 2006; Bouchenak-Khelladi & al., 2014; Briggs & al., 2014), therefore, there is no phylogenetic reason to include Anarthriaceae in the Restionaceae.

The phylogenetic placement of the centrolepids (cf. Michelangeli & al., 2003; Marchant & Briggs, 2007; Briggs & al., 2014) presents a similar problem. In publication 1 the morphologically highly divergent previous family Centrolepidaceae is represented by *Gaimardia* Gaudich. It is the sister group to Leptocarpoideae in the *matK* tree, to Sporadanthoideae + Leptocarpoideae in the *Topo6* tree and to Restionaceae s.s. in the *PhyB* tree (publication 1). The present results do not contribute to a clarification on this issue.

The *matK* and *Topo6* data retrieved the monophyly of all three recognized subfamilies within the Restionaceae s.s., whereas the *PhyB* data suggest that Restionoideae could be paraphyletic (publication 1). Overall, the results of this study confirm the commonly accepted subfamily and tribe structure of the Restionaceae (Briggs & al., 2000, 2010, 2014; Briggs & Linder, 2009).

Poaceae. — Within the monophyletic grass family, *Streptochaeta* Schrad. and *Anomochlora* Brongn. are the genera to branch off first in the plastid and the nuclear *Topo6* trees, followed by *Pharus* P.Browne (publication 2; Clark & al., 1995; Soreng & Davis, 1998; Hilu & al., 1999; Hsiao & al., 1999; Mathews & al., 2000; Zhang, 2000;

GPWG, 2001; Michelangeli & al., 2003; Davis & Soreng, 2007; Duvall & al., 2007; Bouchenak-Khelladi & al., 2008; Givnish & al., 2010; Saarela & Graham, 2010; Soreng & al., 2015, 2017; Saarela & al., 2018). The subfamily Anomochlooideae is only supported by the nuclear *Topo6* data (Clark & Judziewicz, 1996; GPWG, 2001; Givnish & al., 2010; Saarela & Graham, 2010), but is paraphyletic according to the *matK* data (publication 2; Hilu & al., 1999; Zhang, 2000; Davis & Soreng, 2007, Soreng & al., 2017).

The major clades BOP and PACMAD form a strongly supported relationship in the current and most of the previous studies (publication 2; Hilu & al., 1999, 2003; GPWG, 2001; Michelangeli & al., 2003; Davis & Soreng, 2007; Marchant & Briggs, 2007; Bouchenak-Khelladi & al., 2008; Givnish & al., 2010; Saarela & Graham, 2010; GPWG II, 2012; Soreng & al., 2017; Saarela & al., 2018). Monophyly of the BOP clade is also confirmed by the present plastid and nuclear data, but the support values vary from very weak in the *matK* results to very strong in the *Topo6* analysis (publication 2). The taxonomic order of the subfamilies within these large clades could not be clarified because of the small sampling size and lack of backbone support (publication 2; Hillis, 1996; Wiens, 1998, 2003a, 2003b, 2005; Hillis & al., 2003; Salamin & al., 2005; Knoop & Müller, 2009; Crawley & Hilu, 2012). The PACMAD clade is paraphyletic, but the subfamilies Micrairoideae and Panicoideae are strongly supported by *matK* and *Topo6* (publication 2). All three subfamilies within the BOP clade are strongly supported in the plastid tree, whereas the *Topo6* data show Pooideae paraphyletic and Oryzoideae weakly supported (publication 2).

Pooideae. — The morphologically ambiguous genus *Brachyeleytrum* combines poid and bambusoid characters (e.g. Kellogg & Campbell, 1987; Clayton & Renvoize, 1996; Watson & Dallwitz, 1992). The nuclear tree in the Poaceae analysis show this genus to be separated from the Pooideae within the BOP clade (publication 2). The present *matK*, *PhyB*, *Topo6* exon 8–13 and the nuclear combined analyses, however, confirm previous studies and place *Brachyeleytrum* as diverging first within the Pooideae (publications 2 and 3; cf. Catalán & al., 1997; Soreng & Davis, 1998; GPWG, 2001; Duvall & al., 2007; Bouchenak-Khelladi & al., 2008; GPWG II, 2012; Soreng & al., 2017; Saarela & al., 2018).

The monotypic genera *Lygeum* and *Nardus* are in a relationship with maximum support and are part of the basal poid lineages in all phylogenetic trees of this study (publications 2 and 3), which agrees with the generally acknowledged taxonomy

(Catalán & al., 1997; Hsiao & al., 1999; Mathews & al., 2000; GPWG, 2001; Davis & Soreng, 2007; Döring & al., 2007; Bouchenak-Khelladi & al., 2008; Schneider & al., 2009, 2011; Romaschenko & al., 2012; Soreng & al., 2017; Saarela & al., 2018). Schneider & al. (2009) suggested the inclusion of *Lygeum* in the tribe Nardeae, which was confirmed by Kellogg (2015) and this study (publication 3).

A common broad tribe Phaenospermateae (see plastid data in publication 3; Davis & Soreng, 2007; Döring & al., 2007; Schneider & al., 2009; Romaschenko & al., 2012, 2014) was not supported by Schneider & al. (2011) because there is no morphological synapomorphic character, leading them to separate *Phaenosperma* in a monotypic tribe from the remaining genera unified under the tribe Duthieae. The present nuclear dataset resulted in a relationship of *Phaenosperma* and the tribe Meliceae or left this genus in an unresolved polytomy, which confirms the recommendation for a monotypic Phaenospermateae (publication 3; Soreng & al. 2017). The present combined nuclear analysis strongly supported the tribe Duthieae, whereas all other trees showed this tribe to be polyphyletic (publication 3). It is worth noting that some *Ampelodesmos* sequences cluster together with the all or part of the tribe Duthieae and share some characters with *Danthoniastrum* (Holub) Holub in the *Acc1* and *Topo6* exon 8–13 dataset (publication 3; cf. Romaschenko & al., 2014). A similar pattern emerged for *Trikeria pappiformis* (Keng) P.C.Kuo & S.L.Lu, and because of this placement, the present results propose a putative hybrid origin of these stipoid taxa between the tribe Stipeae and an ancestor of the Duthieae (publication 3). The inclusion of *Ampelodesmos* in the Stipeae (subtribe Ampelodesminae Conert) rather than in their own monotypic tribe is also supported by all phylogenetic analyses (publication 3; Schneider & al., 2009; Kellogg, 2015).

All trees in this study show a highly supported relationship of *Brylkinia* F.Schmidt and the remaining Meliceae (publications 2 and 3; cf. Schneider & al., 2009, 2011; Romaschenko & al., 2012; Soreng & al., 2017). Following Schneider & al. (2009), the present results confirm an admission of *Brylkinia* under a separate subtribe Brylkiniinae Ohwi within the Meliceae (publication 3; Kellogg, 2015).

Within the ‘core’ Pooideae (Davis & Soreng, 1993), the tribe Triticeae and the Aveneae/Poeae tribe complex are clearly monophyletic (publications 2 and 3; e.g. Hilu & al., 1999; Duvall & al., 2007; Bouchenak-Khelladi & al., 2008; Schneider & al., 2009, 2011; Soreng & al., 2017; Saarela & al., 2018). In all results of the present study the tribe Triticeae is divided into Brominae Dumort., Hordeinae Dumort. and

Littledalea (publications 2 and 3). Schneider & al. (2009) proposed a broader tribe Triticeae with the subtribes Brominae, Hordeinae and Littledaleinae Röser, and this taxonomic treatment was followed by this study (publications 2 and 3). In contrast, Soreng & al. (2015, 2017) used the rank of the supertribe Triticodae T.D.Macfarl. & L.Watson to unite the tribes Bromeae, Littledaleae and Triticeae.

The Aveneae/Poeae tribe complex also referred to as supertribe Poodae is strongly supported by the present phylogenetic trees (publication 3) and the plastid data confirmed the conclusions of former studies, supporting a clear split of this group into two lineages (publication 3; cf. Catalán & al., 1997; Hilu & al., 1999; Soreng & Davis, 2000; Davis & Soreng 2007; Döring & al., 2007; Soreng & al., 2007, 2017; Quintanar & al., 2007; Schneider & al., 2009, 2011; Saarela & al., 2018). Up to now no nuclear marker analysis could retrieve this split (publication 3; Quintanar & al., 2007; Schneider & al., 2009).

Utility of plastid *matK* and nuclear *Acc1*, *Topo6* and *PhyB*. — Within the order Poales the generated *matK* phylogeny (publication 1) broadly agrees with previous plastid trees (e.g. Bouchenak-Khelladi & al., 2014) and nuclear trees (e.g. McKain & al., 2016). It resolves a graminid clade, a restiid clade, a cyperid clade and a xyrid clade without *Mayaca*. It also supports the monophyly of the families recognized by APG IV (2016). These results support the usability of *matK* for phylogenetic investigation of the order Poales and confirm previous works at the ordinal level and above (Givnish & al., 2010; Evans & al., 2015; Hertweck & al., 2015). By contrast, there are only minor discrepancies to the trees obtained from nuclear or multi-locus analyses, such as placing the Micrairoideae in a sister relationship with the Danthonioideae (publication 1; Micrairoideae and Arundinoideae in GWPG II, 2012). The *Topo6* tree of the order Poales is characterized by a poorly resolved and supported backbone, caused by the short length of this locus (only alignable exon regions) and the resulting low number of informative sites (publication 1). Overall it is largely congruent with the plastid and transcriptome trees (Givnish & al., 2010; Bouchenak-Khelladi & al., 2014; Hertweck & al., 2015; Barrett & al., 2016; McKain & al., 2016) and supports the relationship of Ecdeiocoleaceae + Joinvilleaceae, the cyperids (Cyperaceae + Juncaceae) and the restiids (Anarthria clade + Restionaceae). In contrast, the *PhyB* data result in an unusual topology (publication 1), with several groupings (e.g. Mayacaceae, Typhaceae and Eriocaulaceae) supported by posterior probability = 1.0, but no bootstrap support (likelihood or maximum parsimony). It is assumed that posterior probability and

bootstrap are fundamentally different values for phylogenetic accuracy, whereby the posterior probability is over-parameterized when the priors are incorrect (Erixon & al., 2003; Huelsenbeck & Rannala, 2004; Yang & Rannala, 2005). The *PhyB* nodes supported by bootstrap are congruent with the *Topo6* data and can be treated as reliable support (publication 1).

Within the restiid clade, the differences between the three markers used in this study are much smaller than within the order Poales (publication 1). *MatK* and *Topo6* show *Gaimardia* as a sister group to the Leptocarpoideae, whereas *PhyB* places it as a sister group to the Restionaceae s.s. (publication 1). These positions and a sister relationship to the subfamily Sporadanthoideae have been reported previously (Bremer, 2002; Michelangeli & al., 2003; Marchant & Briggs, 2007, Briggs & al., 2014). *PhyB* data suggest that the subfamily Restionoideae could be paraphyletic. Generally, the plastid *matK* region and both nuclear markers performed well within the restiid clade and resulted in usable phylogenies.

The chloroplast *matK* region leads to meaningful topologies at the family level, as mentioned above (publications 1 and 2). The *Topo6* marker region exon 8–11 compares c. 700 base pairs, and the sampling size of the large families Bromeliaceae (publication 1) and Poaceae (publication 2) was less than 1% of all recognized taxa. The impact of missing data on the accuracy and robustness of phylogenetic trees was already mentioned (Hillis 1996; Wiens 1998, 2003a, 2003b, 2005; Hillis & al., 2003, Salamin & al., 2005; Knoop & Müller, 2009; Crawley & Hilu, 2012). These studies suggested that phylogenetic accuracy benefits from adding species and/or characters or gene regions with different tempo and modes of evolution. Nevertheless, *Topo6* generated good results at the family level, given that it supported the monophyly of the grass subfamily Anomochlooideae, the major BOP clade and some subfamilial nodes within the bromeliads (publications 1 and 2).

The plastid region *matK* gene–3'*trnK* exon combines coding regions and non-coding regions and generated a well resolved phylogenetic tree of the grass subfamily Pooideae, with strongly supported tribes (except for Duthieae and Diarrheneae), but did not contribute significant information at a lower taxonomic level (publication 3). The backbone resolution of the *Topo6* exon 8–13 tree is relatively good and even the nodes within the tribes are strongly supported (publication 3). The divergent phylogenetic positions of *Ampelodesmos* and *Trikeria* are also shown by this data (publication 3). The tribes and subtribes are also largely strongly supported within the

Topo6 exon 17–19 dataset, but the backbone support is very weak because of the high proportion of intron characters (publication 3). The tree obtained from *Acc1* shows a level of resolution similar to the one obtained from *Topo6* exon 17–19, but it retrieves the same interesting position of *Ampelodesmos*, as does the *Topo6* exon 8–13 tree (publication 3). The *PhyB* region contains only a part of exon 1 and resulted in the least resolved phylogenetic tree, whereas the combined nuclear tree benefits from the additionally included characters and yields the highest resolution without any polytomies (cf. Wiens 1998, 2003a, 2003b, 2005; Duarte & al., 2010; Crawley & Hilu, 2012).

CONCLUSION AND OUTLOOK

The current study demonstrates that the analyses of the plastid *matK* marker and the nuclear single-copy DNA markers provide satisfactory support in some parts of the phylogenetic trees at different taxonomic levels within the large order Poales.

The *matK* locus retrieves the currently accepted plastid phylogeny of the order Poales very well and *Topo6* shows an almost match with these results, however, with less support. By contrast, the *PhyB* data show numerous mismatches from these results but lack bootstrap support. Neither plastid nor nuclear data could answer the outstanding questions regarding the phylogenetic relationships of taxa within Poales. To clarify the structural sequence of the basal families, the placement of Mayacaceae and the position and extent of the xyrid clade, more nuclear-encoded data are required. A similar picture emerged within the restiid clade, where the delimitation of the families could not be clarified in detail. All present results confirm the inclusion of the Centrolepidaceae as a subfamily Centrolepidoideae within the Restionaceae, but there is no support for or against including the Anarthriaceae in the Restionaceae. Apart from this, many nodes of the Poales phylogeny are resolved and supported by the present plastid and nuclear data.

At the family level, *matK* and *Topo6* exon 8–11 were used to investigate the Bromeliaceae and Poaceae. The nuclear marker region in particular provided some meaningful results, such as the supported monophyly of the major BOP clade and the basal subfamily Anomochlooideae, and some supported nodes within Bromeliaceae. Because of the small sampling size and the fact that only one nuclear marker was investigated, more conclusive statements are not possible. Nevertheless, the results provide a starting point for further investigations, because the analyses of additional marker regions and the inclusion of more taxa will significantly improve the tree topologies.

Within the grass subfamily Pooideae more nuclear marker regions were studied and the most robust results were found. The combined nuclear tree shows the most strongly supported tribe sequence: Brachyelytreae, Nardeae (with subtribes Lygeinae and Nardinae), Duthieae, Meliceae (subtribes Brylkiniinae and Melicinae) + Phaenospermatatae, Stipeae, Diarrheneae, Brachypodieae and the ‘core’ Pooideae. The divergent position of some clone sequences of *Ampelodesmos mauritanicus* and

Trikeria pappiformis within the *Topo6* exon 8–13 and/or *Acc1* dataset suggested a putative hybrid origin of these stipoid taxa with a potential ancestor in the tribe Duthieae. The strongly supported and well defined split of the Aveneae/Poeae tribe complex into two lineages based on plastid data could not be confirmed by any of the present nuclear investigations.

However, progress has been made in exploring phylogenetic relationships of the Poales, restiids, Bromeliaceae, Poaceae and Pooideae by investigating nuclear single-copy genes. Some taxonomic problems still persist in a number of groups, mainly as a result of poor sampling, limited resolution and because it is impossible to combine different marker regions. To get a fully resolved picture of relationships within the order Poales, more DNA data (taxa and characters) and a careful revision of morphological, cytological and biogeographical aspects are needed. A major challenge that remains within the Poales is the large number of species, for which it is sometimes impossible to get plant material of sufficient quality for genetic analyses. Continuously improving next generation sequencing technologies, enabling the analysis of herbarium specimens with highly degraded DNA, will play an important role in future phylogenetic studies of the order Poales and the lower taxonomic levels (Staats & al., 2011; Beck & Semple, 2015). The investigation of polyploids using nuclear single-copy genes will provide crucial information about the evolution and origin of poalean species and could help to uncover hybridization events.

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<https://doi.org/10.1016/j.ympev.2012.07.015>

APPENDIX

Publication list

Publications

Hochbach, A., Linder, H.P. & Röser, M. (2018). Nuclear genes, *matK*, and the phylogeny of the Poales. *Taxon* 67: 521--536.

Hochbach, A., Schneider, J. & Röser, M. (2015). A multi-locus analysis of phylogenetic relationships within grass subfamily Pooideae (Poaceae) inferred from sequences of nuclear single copy gene regions compared with plastid DNA. *Molecular Phylogenetics and Evolution* 87: 14--27.

Blaner, A., Schneider, J. & Röser, M. (2014). Phylogenetic relationships in the grass family (Poaceae) based on the nuclear single copy locus topoisomerase 6 compared with chloroplast DNA. *Systematics and Biodiversity* 12: 111--124.

Contributions to conferences

Poster Presentations

Blaner, A., Schneider, J. & Röser, M. (2014). Molecular phylogenetic structure of grass subfamily Pooideae based on a nuclear single copy gene. 15th Annual Meeting of the Society of Biological Systematics (GfBS) and the 22nd International Symposium “Biodiversity and Evolutionary Biology” of the German Botanical Society (DBG). Dresden.

Blaner, A., Schneider, J. & Röser, M. (2013). Phylogenetic relationships in the grass family (Poaceae) based on the nuclear single-copy locus topoisomerase 6. MONOCOTS V: 5th International Conference on Comparative Biology of Monocotyledons. New York.

Blaner, A., Schneider, J. & Röser, M. (2012). Molecular phylogenetic relationships within grasses (family Poaceae) and the order Poales based on a nuclear single copy gene. 21st International Symposium “Biodiversity and Evolutionary Biology” of the German Botanical Society (DBG). Mainz

Talks

Schneider, J., Hoffmann, M.H., **Blaner, A.**, Winterfeld, G. & Röser, M. (2014). Molecular phylogenetics meets morphology: New insights into classification of the grass subfamily Pooideae (Poaceae). 15th Annual Meeting of the Society of Biological Systematics (GfBS) and the 22nd International Symposium “Biodiversity and Evolutionary Biology” of the German Botanical Society (DBG). Dresden.

Schneider, J., Winterfeld, G., Hoffmann, M., **Blaner, A.** & Röser, M. (2013). Duthieae, a new tribe of subfamily Pooideae, and its relationships. MONOCOTS V: 5th International Conference on Comparative Biology of Monocotyledons. New York.

Curriculum vitae

Name	Anne Hochbach, née Blaner
Academic degree	Dipl. biol.
Date and place of birth	13.03.1987 in Halle (Saale), Germany
Marital status	married, one child
Nationality	German

School education

1997 – 2005	Secondary School, Neuhaus am Rennweg
1993 – 1997	Primary School, Neuhaus am Rennweg

University education

06/2012 – present	PhD student, Martin-Luther-University Halle-Wittenberg, Institute of Biology, Geobotany and Botanical Garden, supervisor: Prof. Dr. Martin Röser, Title: “Usability of nuclear single copy genes compared with plastid DNA on different phylogenetic levels of and within the order Poales”
08/2015 – 09/2016	Maternity leave
07/2011 – 05/2012	Diploma thesis, Martin-Luther-University Halle-Wittenberg, Institute of Biology, Geobotany and Botanical Garden, supervisor: Prof. Dr. Martin Röser, Title: “Molekular-phylogenetische Untersuchungen an den Unterfamilien der Gräser (Poaceae)”
10/2006 – 05/2012	Study of Biology, Martin-Luther-University Halle-Wittenberg Subjects: Geobotany and Molecular Biology, Plant Physiology, Plant Breeding, Pedology

APPENDIX

Scientific work experience

10/2013 – 02/2016	Research assistant, Martin-Luther-University Halle-Wittenberg, Institute of Biology, Geobotany and Botanical Garden
05/2012 – 09/2013	Graduate assistant, Martin-Luther-University Halle-Wittenberg, Institute of Biology, Geobotany and Botanical Garden, Department Systematics and Biodiversity, supervised by Prof. Dr. Martin Röser
03/2011 – 07/2011	Student assistant, Martin-Luther-University Halle-Wittenberg, Institute of Biology, Geobotany and Botanical Garden, Project: “Latin American Plant Initiative (LAPI) herbarium, supervised by Prof. Dr. Uwe Braun

Teaching experience

SS 2014 and 2015	Practical course on plant identification (Bachelor students) Botanical excursions (Bachelor students) Practical course on molecular phylogenetic methods (Bachelor students)
WS 2013/2014	Practical course on cytogenetic methods (Master students)
WS 2012/2013	Seminar General Botany for Geosciences (Bachelor students)
WS 2012/2013 – SS 2015	Support of bachelor and diploma thesis

Erklärung über den persönlichen Anteil an den Publikationen

Publication I	<p>Hochbach, A., Linder, H.P. & Röser, M. (2018). Nuclear genes, <i>matK</i>, and the phylogeny of the Poales. <i>Taxon</i> 67: 521--536.</p> <p>Data collection: Anne Hochbach (95%), Martin Röser (5%)</p> <p>Analysis: Anne Hochbach (100%)</p> <p>Writing: Anne Hochbach (70%), Peter Linder (30%), corrections by Martin Röser</p>
Publication II	<p>Blaner, A., Schneider, J. & Röser, M. (2014). Phylogenetic relationships in the grass family (Poaceae) based on the nuclear single copy locus topoisomerase 6 compared with chloroplast DNA. <i>Systematics and Biodiversity</i> 12: 111--124.</p> <p>Data collection: Anne Hochbach (85%), Julia Schneider (10%), Martin Röser (5%)</p> <p>Analysis: Anne Hochbach (90%), Julia Schneider (10%)</p> <p>Writing: Anne Hochbach (90%), Martin Röser (10%)</p>
Publication III	<p>Hochbach, A., Schneider, J. & Röser, M. (2015). A multi-locus analysis of phylogenetic relationships within grass subfamily Pooideae (Poaceae) inferred from sequences of nuclear single copy gene regions compared with plastid DNA. <i>Molecular Phylogenetics and Evolution</i> 87: 14--27.</p> <p>Data collection: Anne Hochbach (85%), Julia Schneider (10%), Martin Röser (5%)</p> <p>Analysis: Anne Hochbach (90%), Julia Schneider (10%)</p> <p>Writing: Anne Hochbach (90%), Martin Röser (10%)</p>

Haldensleben, den 24.07.2018

Anne Hochbach

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 habe. Ich versichere, dass ich keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Haldensleben, den 24.07.2018

Anne Hochbach