

**Phylogeny and molecular evolution of oat-like grasses
(traditional Aveneae)**

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SUMMARY

This thesis deals with the systematics of the traditional grass tribe Aveneae, one of the largest tribes of the subfamily Pooideae with approximately 57 genera and 1,050 species. Its classification has been revised several times in recent studies. The phylogenetic analyses were conducted using molecular data of chloroplast and nuclear DNA sequences, complemented by chromosomal features, morphological characters and biogeography. The present study uses, for the first time, single-copy gene sequences to clarify relationships of traditional Aveneae species. Special attention has been paid to unanswered questions concerning the grass genus *Helictotrichon*. This thesis represents, compared with former investigations of the genus, the most densely sampled molecular sequence analyses of *Helictotrichon*. Its taxonomic history is rather complicated and in the past the circumscription of *Helictotrichon* was altered several times. The present thesis tries to clarify the relationships within the genus and between *Helictotrichon* and the genus *Trisetopsis* described by Wölk & Röser in 2013. The latter genus includes species formerly treated under *Helictotrichon* from tropical and subtropical Africa.

For the molecular phylogenetic analyses the plastid *matK* gene–3'*trnK* exon–*psbA* gene, the nuclear ITS1–5.8S gene–ITS2 and the single-copy gene topoisomerase 6 (exon 17–exon 19) were chosen.

The phylogenetic results and morphological data corroborate the exclusion of the African species from *Helictotrichon* and their inclusion into the newly described genus *Trisetopsis*. Two copy types of the single-copy gene topoisomerase 6 (Topo6), called A and B, are present in *Trisetopsis* but not in *Helictotrichon*. This single-copy gene allowed comprehensive analyses of the polyploid evolution in *Trisetopsis*. The copy type A probably descended from the South African species *T. longa* or *T. rogerellisii*. Copy type B is surprisingly found in New World species of *Calamagrostis*, *Grapphephorum*, *Peyritschia* and *Sphenopholis*.

The present study combined the nuclear ribosomal ITS and the single-copy gene Topo6 with chromosomal features to analyze the relationship within the European and especially Mediterranean species of *Helictotrichon*, including 14 species with numerous subspecies. The European and Mediterranean species are characterized by forming numerous polyploid complexes. There are three different copy types of Topo6 termed SAR, SET and PAR present in *Helictotrichon*. These different copy types supported an allopolyploid origin of several *Helictotrichon* species. A comparison of this molecular sequence data with chromosome and karyotype structure identified potential genome donors of the species affected by hybridization or polyploidy.

The combination of the three sequence markers with morphological characters led to a revision of *Helictotrichon* concerning its Southeast Asian species. The results revealed that, except for *Helictotrichon abietetorum*, *H. hideoi* and *H. leianthum*, all other Southeast Asian species were misplaced in *Helictotrichon*. The placement of *H. polyneurum* and *H. sumatrense* is not yet entirely clear and requires further analyses as shown by molecular and morphological data. Additionally, the nuclear marker ITS and Topo6 suggested a polyploid origin of several Southeast Asian species.

Following the results of the described analyses one new genus *Tzveleviochloa*, gen. nov., and one nothogenus, \times *Trisetopsotrichon*, nothogen. nov. were introduced and the following new combinations were made: *Trisetopsis aspera*, comb. nov., *T. imberbis*, comb. nov., *T. junghuhnii*, comb. nov., *T. virescens*, comb. nov., \times *Trisetopsotrichon altius*, comb. nov., *Tzveleviochloa burmanica*, comb. nov., *T. parviflora*, comb. nov., *T. potaninii*, comb. nov.

1 GENERAL INTRODUCTION

1.1 INTRODUCTION

The grass family Poaceae Barnhart with its approximately 10,000 species in over 700 genera (Clayton & Renvoize, 1986; Tzvelev, 1989; Watson & Dallwitz, 1992–; Kellogg, 2015) is one of the largest and most important families of the angiosperms. Representatives of the Poaceae are distributed worldwide. Native grasslands like the North American prairie, South American pampas, African savannah and Eurasian steppes comprise about one fifth of the earth's vegetation (Shantz, 1954). Additionally to the native ecosystems of grasslands also anthropogenic grasslands exist, which include cultivated taxa of Poaceae. Various species of that family have a great relevance in human economy. Particularly the cereals like maize (*Zea* L.), rice (*Oryza* L.), wheat (*Triticum* L.), barley (*Hordeum* L.), sorghum (*Panicum* L.), oat (*Avena* L.) and rye (*Secale* L.) but also forage crops like ryegrass (*Lolium* L.), fescue (*Festuca* L.), timothy (*Phleum* L.), oatgrass (*Arrhenatherum* P.Beauv.) and orchard grass (*Dactylis* L.) play an important role as source of carbohydrates (Lieberei & Reisdorf, 2012; FAO, 2014). Different millets like common millet (*Panicum* L.), pearl millet (*Pennisetum* Rich.) and foxtail millet (*Setaria* P.Beauv.) are grown as cereal crops in the African and Asian semiarid tropics. Additionally, the grass family includes with sugar cane (*Saccharum* L.) an important representative for the sugar production.

It should be noted that the subfamily Pooideae Benth. comprises most of the Poaceae species and has the greatest economical and ecological relevance of all temperate grasses. Its circumscription varied strongly between different treatments in its taxonomic history. While several classifications describe it rather narrowly (e.g. Macfarlane & Watson, 1980, 1982; Macfarlane, 1986; Watson & Dallwitz, 1992–) other previous and current circumscriptions of Pooideae define it more broadly (e.g. Clayton & Renvoize, 1986; Kellogg & Campbell, 1987; Tzvelev, 1989; GPWG, 2001; Kellogg, 2015; Soreng & al., 2015). Subsequently, this subfamily has received more attention in previous molecular phylogenetic works concerning its internal classification than others (Davis & Soreng, 1993, 2007; Kellogg & Watson, 1993; Catalán & al., 1997; Soreng & Davis, 1998, 2000; Hilu & al., 1999; Hsiao & al., 1999; Mathews & al., 2000; GPWG, 2001; Döring & al., 2007; Duvall & al., 2007; Soreng & al., 2007; Bouchenak-Khelladi & al., 2008; Döring, 2009; Schneider & al. 2009, 2011; GPWG II, 2012; Romaschenko & al., 2012; Schneider, 2013; Blaner & al., 2014; Hochbach & al., 2015; Saarela & al., 2015).

Tribal classification of the Aveneae and Poeae. — Traditional classifications of Pooideae based on morphological and anatomical characters, contain the widely recognized tribes Aveneae Dumort. and Poeae R.Br. (Pilger, 1954; Tzvelev, 1976; Clayton & Renvoize, 1986; Watson & Dallwitz, 1992–). These tribes are, in addition to Triticeae Dumort. and Bromeae Dumort., part of the so-called ‘core’ Pooideae (Soreng & Davis, 2000; Schneider & al., 2009; Kellogg, 2015). Recent molecular phylogenetic studies of the previous years recognized the tribe Aveneae, as traditionally described, as polyphyletic (Soreng & Davis, 2000; Davis & Soreng, 2007; Döring & al., 2007; Quintanar & al., 2007; Soreng & al., 2007; Bouchenak-Khelladi & al., 2008; Schneider & al., 2009, 2011; Saarela & al., 2010). Accordingly, the most recent treatments of the grass family subsume the traditional tribe Aveneae and some further smaller tribes into a broad, but monophyletic tribe Poeae (i.e., Kellogg, 2015; Soreng & al., 2015). This current circumscription of Poeae s.l. is highly similar to that suggested by Tzvelev (1989). However, several present studies referred Poeae s.l. to an unranked and so-called ‘Aveneae/Poeae’ tribe complex (Döring & al., 2007; Schneider & al., 2009, 2011, 2012; Blaner & al., 2014; Hochbach & al., 2015).

In contrast to analyses of nuclear sequence data, phylogenetic analyses of plastid DNA always indicate two major strongly supported lineages within Poeae s.l., which largely correspond to traditional circumscriptions of the tribes Aveneae and Poeae (Soreng & Davis, 2000; Davis & Soreng, 2007; Döring & al., 2007; Quintanar & al., 2007; Soreng & al., 2007; Bouchenak-Khelladi & al., 2008; Schneider & al., 2009, 2011, 2012; Hochbach & al., 2015; Saarela & al., 2010, 2015). Previous analyses used different terms for these two plastid DNA lineages of Poeae s.l. (Saarela & al., 2015) and in some cases they are simply referred to as Poeae clade 1 and 2 (Schneider & al., 2009, 2011, 2012; Saarela & al., 2015). Here Poeae clade 1 contains the majority of traditional Aveneae taxa and Poeae clade 2 particular taxa traditionally belonging to the tribe Poeae. Actually in a previous work Saarela & al. (2010) used the designation ‘Aveneae-type plastid DNA’ and ‘Poeae-type plastid DNA’, based on the informal description of Soreng & Davis (2000), to describe the two plastid DNA lineages in Poeae s.l. The most complex description of Soreng & al. (2007) using the terms ‘plastid group 1 (Aveneae-type)’ and ‘plastid group 2 (Poeae-type)’ appears in a modified form in the recent classification of Soreng & al. (2015).

Subtribe Aveninae. — The tribe Poeae s.l. includes most of the species in the Pooideae and is defined in several treatments by a series of subtribes. The number of subtribes (15 to 21) depends on the respective classification (Soreng & al., 2007; Kellogg, 2015). The current phylogenetic classification of the Poaceae by Soreng & al. (2015) divided Poeae s.l.

into 19 subtribes, 12 of which are part of the informal Poeae chloroplast group 1 (Aveneae type) and 7 are ranked under Poeae chloroplast group 2 (Poeae type).

One of the larger subtribes of Poeae s.l. is the subtribe Aveninae Link, which has been placed in the tribe Aveneae by many traditional classifications (Pilger, 1954; Tzvelev, 1976; Clayton & Renvoize, 1986; Watson & Dallwitz, 1992–). Its circumscription was defined either broadly (e.g., Clayton & Renvoize, 1986; Watson & Dallwitz, 1992–; Soreng & al., 2007, 2015) as a morphologically heterogeneous group, or narrowly including only the genera *Arrhenatherum*, *Avena*, *Helictotrichon* and *Pseudarrhenatherum* Rouy (Quintanar & al., 2007, 2010). A broadly defined subtribe Aveninae comprised, among others, also the genera *Avellinia* Parl., *Gaudinia* P.Beauv., *Graphephorum* Desv., *Koeleria* Pers., *Lagurus* L., *Leptophyllochloa* C.E.Calderón, *Peyritschia* E.Fourn, *Rostraria* Trin., *Sphenopholis* Scribn., *Trisetaria* Forssk., *Tricholemma* (Röser) Röser and *Trisetum* Pers., whereas these genera were included in a separate subtribe Koeleriinae by Quintanar & al. (2007, 2010).

The recent classification of Kellogg (2015) introduced a broadly defined genus *Trisetaria* within subtribe Aveninae. The circumscription of that genus corresponds largely to subtribe Koeleriinae (Quintanar & al. 2010), except that the genera *Graphephorum* and *Sphenopholis* are kept separate (Kellogg & al., 2015). Therefore Aveninae sensu Kellogg includes, apart from the broadly described *Trisetaria* only six further genera (*Arrhenatherum*, *Avena*, *Graphephorum*, *Helictotrichon*, *Lagurus* and *Sphenopholis*).

***Helictotrichon* Besser.** — Traditional classifications of the subtribe Aveninae described *Helictotrichon* as one of its largest genera comprising up to 100 species (Tzvelev, 1976; Clayton & Renvoize, 1986; Watson & Dallwitz, 1992–). *Helictotrichon* species are mainly distributed in temperate Eurasia but extend along tropical mountains into several regions of the world (Fig.1). The circumscription of this genus varies greatly. Besser (1827) distinguished *Helictotrichon*, due to its perennial character and a more or less erect panicle, from *Avena*. However, subsequent classifications of the nineteenth and beginning of the twentieth centuries did not recognize *Helictotrichon* as a proper genus but included its species, among others, within *Avena*, *Avenastrum* Opiz or *Avenula* (Dumort.) Dumort. (e.g. Dumortier, 1868; Vierhapper, 1914; Saint-Yves, 1931). Since the genus *Helictotrichon* was reintroduced by Hubbard (1936) its taxonomic history has still been complicated. Different concepts defined *Helictotrichon* either narrowly segregating the genus into *Helictotrichon* s.str. and *Avenula* or *Avenochloa* Holub (e.g., Holub, 1962, 1976, 1980; Gervais, 1973; Romero Zarco 1984a,b; Tzvelev, 1989), based on the presence/absence of a ring of sclerenchyma that surrounds the endodermis, or more broadly including up to four different

subgenera, namely *Helictotrichon*, *Pubavenastrum* (Vierh.) Holub, *Pratavenastrum* (Vierh.) Holub and North African *Tricholemma* Röser (e.g., Holub, 1958; Tsvelev, 1976; Conert 1998; Röser, 1989; Lange, 1995). A chronological ordered overview of the most important former classification concepts of the genus *Helictotrichon*, is shown in detail by Röser (1989) and Lange (1995).

The gradually increasing number of molecular phylogenetic studies recognized the traditional genus *Helictotrichon* s.l. as a polyphyletic group. A satellite DNA sequence analysis of Grebenstein & al. (1996) proved a non-monophyletic origin of *Helictotrichon* s.l. by showing a different distribution of satellite DNA in the four subgenera (*Helictotrichon*, *Pubavenastrum*, *Pratavenastrum*, *Tricholemma*). A subsequent investigation (Grebenstein & al., 1998) used ITS1 and ITS2 sequences to support the assumption of *Helictotrichon* s.l. as paraphyletic or even polyphyletic by the identification of four different clades that correspond to the different subgenera. Further analyses, which led to the same conclusion like Grebenstein & al. (1996, 1998), included the physical mapping of 5S rDNA (Röser & al., 2001) and led as a first consequence to the introduction of the monotypical genus *Homalotrichon* Banfi (Conti & al., 2005).

These mentioned circumstances raised the question of the maintainance of four different subgenera under *Helictotrichon*. The recognition of separate genera was corroborated by different molecular systematic and cytogenetic approaches using satellite DNAs (Winterfeld, 2006, Winterfeld & Röser, 2007a,b), plastid sequence analyses based on the *matK* and *trnT-F* regions (Döring & al., 2007; Quintanar & al., 2007; Döring, 2009) and nuclear sequence analysis using ITS (Schneider & al., 2009; Winterfeld & al., 2009a). The comparison of molecular data together with anatomical and morphological characters support the relevance of smaller monophyletic genera like *Amphibromus* Nees, *Avenula* (syn. *Homalotrichon*, nom. illeg.), *Helictochloa* Romero Zarco, or *Tricholemma* (Röser & al., 2009; Romero Zarco, 2011).

Currently the genus *Helictotrichon* is delineated rather narrowly after the exclusion of the subgenera *Pratavenastrum*, *Pubavenastrum* and *Tricholemma* from *Helictotrichon* s.l. and their following transfer to separate genera. The present circumscription of *Helictotrichon* includes taxa of the former subgenus *Helictotrichon* expanded by the species of the previous genus *Pseudarrhenatherum* (*H. pallens* syn. *P. pallens* and *H. thorei* syn. *P. longifolium*), which were nested within the species of *Helictotrichon* based on plastid and nuclear DNA analyses (Röser & al., 2001; Quintanar & al., 2007; Schneider & al., 2009; Winterfeld & al., 2009a). As a consequence of the results from the nuclear DNA sequence data (Röser & al.,

2001; Quintanar & al., 2007; Schneider & al., 2009; Winterfeld & al., 2009a) a generic realignment of the genus *Helictotrichon* was made (Röser & al., 2009) and has been adopted by the recent phylogenetic classification in Soreng & al. (2015). An inclusion of *Pseudarrhenatherum* within *Arrhenatherum* as suggested by Clayton & Renvoize (1986) and also by the current classification of Kellogg (2015) could not be supported.



Fig. 1. Distribution of *Helictotrichon* excluding *Avenula*, *Helictochloa* and *Tricholemma* but not *Trisetopsis* (Röser & al., 2009) based on own data and information taken from Hooker (1896), Hubbard (1936), Hutchinson & Dalziel (1936), Schweickert (1937), Bor (1951, 1960, 1970), Holub (1962), Ohwi (1965), Tzvelev (1968, 1976), Clayton (1970, 1972), Launert (1971), Wickens (1976), Cope (1982), Sevenster & Veldkamp (1983), Röser (1989, 1996), Gibbs Russell (1991), Davidse (1994), Lange (1995), Conert (1998), Broome & Noltie (2000), Cope (2006, 2007), Winterfeld (2006), Wu & Phillips (2006), Tucker (2007), Mashau & al. (2010).

Former investigations of the genus *Helictotrichon* and its taxonomical classification systems mainly focused on Palearctic, especially Mediterranean species (Saint-Yves, 1931; Holub, 1959a,b, 1962, 1976, 1977, 1980; Gervais, 1973; Tzvelev, 1976; Romero Zarco 1984a,b, 2011; Conert, 1998; Röser, 1989, 1996, 1997; Lange, 1995; Grebenstein & al., 1996, 1998; Röser & al., 2001, 2009, 2014; Winterfeld, 2006; Döring & al., 2007; Winterfeld & Röser, 2007a,b; Döring, 2009; Winterfeld & al., 2009a, 2016). Several of these studies pointed out differences between Palearctic *Helictotrichon* species in comparison with Paletropical and South African species of that genus (e.g. Romero Zarco, 2011). In regard to

the numerous studies on Mediterranean *Helictotrichon* species, comparatively few studies have focused on South African, East African and Southeast Asian species of *Helictotrichon*. The few existing studies on tropical and subtropical *Helictotrichon* species mainly refer to floristic literature and identification keys (e.g., Hooker 1896; Hubbard, 1936, 1937; Hutchinson & Dalziel, 1936; Schweickerdt, 1937; Bor, 1951, 1960, 1970; Ohwi, 1965; Monod de Froideville, 1968; Tzvelev, 1968; Clayton, 1970; 1972; Launert, 1971; Cope, 1982; Gibbs Russell, 1991; Davidse, 1994; Moulik, 1997; Broome & Noltie, 2000; Cope, 2006, 2007; Wu & Phillips, 2006; Mashau & al., 2010). Studies based on morphological or chromosomal data are comparatively rare (e.g., Henrard, 1940; Tateoka, 1954, 1965; Mehra & Sharma, 1977; Parkash, 1979; Sharma & Sharma, 1979; Sevenster & Veldkamp, 1983; Hoshino & Davidse, 1988; Gibbs Russell, 1991; Morton, 1993; Spies & al., 1996; Mashau & al., 2010, 2013, 2015) not to mention molecular phylogenetic sequence analyses.

Hybridization and Polyploidy. — Polyploidy, or genome doubling, is a very common phenomenon in plants, especially in the family of Poaceae (Stebbins 1956; Levy & Feldman, 2002). Previous and recent investigations estimated that approximately 70–80% of the angiosperms (Goldblatt, 1980; Lewis, 1980; Masterson, 1994) and more than 80% of grasses (De Wet, 1987) are polyploid.

Indeed, the increase of the number of chromosome sets caused by polyploidization is an important factor in the origin of new plant species (Grant, 1981; Soltis & Soltis, 1993; Masterson, 1994; Leitch & Bennett, 1997). There are two different general types of polyploids recognized, the autopolyploids and the allopolyploids. Autopolyploids are characterized by fully homologous but multiple chromosome sets which originated of a simple doubling of a single genome. In contrast, allopolyploids have sets from two or more different species and ended in homeologous but different chromosome sets. Thereby, two important evolutionary events are involved in allopolyploid evolution. First, interspecific hybridization which cause two divergent (homeologous) nuclear genomes and second polyploidization where whole genomes are duplicated (Ainouche & al., 2009).

The genus *Helictotrichon* in its traditional (e.g. Röser, 1989; Lange, 1995) but also in its current circumscription (e.g. Röser & al., 2009; Romero Zarco, 2011) includes numerous ‘polyploid complexes’ according to the definition of Stebbins (1956). The chromosome base number is $x = 7$ (Röser, 1989; Winterfeld, 2006) but varied in the polyploids of *Helictotrichon* in its narrow sense (Röser & al., 2009) from $4x = 28$ up to $13x = 91$ (Winterfeld, 2006). Polyploid evolution of European especially Mediterranean *Helictotrichon* species has well been documented over the last twenty years (Röser, 1989; Winterfeld, 2006; Winterfeld &

Röser, 2007a,b; Winterfeld & al., 2009a,b, 2012). In contrast, similar studies for Tropical African, South African and Southeast Asian *Helictotrichon* species concerning the origin of polyploids are completely lacking. Only a few analyses have dealt with cytogenetic aspects comprising especially chromosome counts of a limited number of *Helictotrichon* species (Tateoka, 1954, 1965; Mehra & Sharma, 1977; Parkash, 1979; Sharma & Sharma, 1979; Christopher & Samraj, 1985; Hoshino & Davidse, 1988; Morton, 1993; Spies & al., 1996).

1.2 THESIS QUESTIONS

As mentioned above the taxonomic history of the genus *Helictotrichon* is rather complicated and the genus circumscription remains unclear to date. The aim of this thesis is to elucidate the phylogeny and systematics of traditional Aveneae, especially the genus *Helictotrichon* in its narrow circumscription (Röser & al., 2009; Romero Zarco, 2011) and selected closely related representatives of the subtribe Aveninae (see Soreng & al., 2015).

The previous diploma thesis of Wölk (2011) and the description of the genus *Trisetopsis* in 2013 (Wölk & Röser) are the starting points of the present thesis. The former thesis (Wölk, 2011) demonstrated that even the remaining species of *Helictotrichon* were polyphyletic concerning South African and two Asian species of *Helictotrichon*. Differences of the Palearctic and the Paleotropical/South African species were already indicated by several former morphological studies (Schweickerdt, 1937; Sevenster & Veldkamp, 1983; Romero Zarco, 2011). Thus as a first consequence, based on molecular data (Wölk, 2011) and morphological observations of South African *Helictotrichon* species by Schweickerdt (1937) and Wölk & Röser (2013), the genus *Trisetopsis* was introduced (Wölk & Röser, 2013), segregating the tropical and subtropical African species from *Helictotrichon*. Nevertheless, these previous data leave more unanswered questions concerning the tropical African species of *Trisetopsis* and Southeast Asian species of *Helictotrichon* that had to be resolved. Therefore special attention was paid to the European species of *Helictotrichon*, tropical and South African species of *Trisetopsis* and Southeast Asian species treated under *Helictotrichon*. Species of the whole distribution area of *Helictotrichon* inclusive *Trisetopsis* were molecularly analyzed for the first time.

The present thesis compared the results from DNA sequencing, morphology and cytogenetics to (1) examine phylogenetic relationships among the different genera of the subtribe Aveninae; (2) clarify the phylogenetic relationships between, firstly, *Helictotrichon* and the African *Trisetopsis*, secondly, the European and Southeast Asian species of *Helictotrichon*; (3) determine the relationships among European species of *Helictotrichon*; (4) clarify the phylogenetic relationships among species of *Trisetopsis* and with Southeast Asian species; (5) identify parental genome donors that contributed to the origin of allopolyploids in *Helictotrichon*, *Trisetopsis* and the Southeast Asian species; (6) clarify the allopolyploid origins of hybrids and polyploids; (7) consider speciation events on the polyploid level; (8) test the utility of single-copy topoisomerase 6 to identify phylogenetic relationships for the first time in genera of traditional Aveneae and Poeae.

2 CUMULATIVE PART

2.1 Publication 1 - Polyploid evolution, intercontinental biogeographical relationships and morphology of the recently described African oat genus *Trisetopsis* (Poaceae)

Alexandra Wölk & Martin Röser

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Polyploid evolution, intercontinental biogeographical relationships and morphology of the recently described African oat genus *Trisetopsis* (Poaceae)**Wölk, Alexandra & Röser, Martin**

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Abstract

We examined the morphology and molecular phylogeny of several genera of the traditional grass tribe Aveneae, with focus on *Helictotrichon* and the recently described genus *Trisetopsis* from tropical and subtropical Africa. Altogether we surveyed 34 taxa for structural characters of the spikelets including lemmas, lodicules and ovaries. Sequences of the nuclear single-copy gene topoisomerase 6 (Topo6) spanning two introns, nuclear ribosomal ITS and the plastid *matK-psbA* region were generated for 81 taxa and analyzed by maximum parsimony and Bayesian methods. The phylogenetic results show African *Trisetopsis* in all molecular markers investigated clearly distinct from *Helictotrichon*, which in its new circumscription encompasses only Northern Hemisphere species. Morphological data support exclusion of the African species from *Helictotrichon* as a separate genus. The Topo6 sequences reveal a strong geographical pattern of variation which allows for comprehensive phylogeographic analyses. Two copy types of Topo6 are present in *Trisetopsis* (A and B). They are strongly different from that found in the Eurasian species of *Helictotrichon*. Copy type A probably descended from South African *T. longa* or *T. rogerellisii*, which appear to be the only African species with only type A. All other species of *Trisetopsis* contain both types A and B. Unexpectedly, type B is widespread in the New World (*Calamagrostis*, *Grappophorum*, *Peyritschia*, *Sphenopholis*) but, apart from the *Trisetopsis* allopolyploids, is absent from Africa. *Trisetopsis imberbis* is introduced as a new combination.

Keywords Aveneae, *Helictotrichon*, ITS, *matK-psbA*, topoisomerase 6, *Trisetopsis*

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Supplementary Material <https://www.ingentaconnect.com/content/iapt/tax>

2.2 Publication 2 - Genome evolution in a Mediterranean species complex: phylogeny and cytogenetics of *Helictotrichon* (Poaceae) allopolyploids based on nuclearDNA sequences (rDNA, topoisomerase gene) and FISH

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Genome evolution in a Mediterranean species complex: phylogeny and cytogenetics of *Helictotrichon* (Poaceae) allopolyploids based on nuclear DNA sequences (rDNA, topoisomerase gene) and FISH

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Abstract

We examined the molecular phylogeny and chromosomal features of European *Helictotrichon* species to explore the relationships within the genus and to investigate the origin of several polyploids. Using both approaches, molecular and cytogenetic, reveal the strong impact of allopolyploidisation on genome organisation from chromosome structure to sequence level. Our research focused on Mediterranean and endemic species of the Alps. Altogether, the molecular phylogenetic analyses include a sample of 17 *Helictotrichon* species and subspecies, used DNA sequences from the nuclear ribosomal (nr) internal transcribed spacer region (ITS) and the single copy gene topoisomerase 6 (Topo6), and were analyzed by maximum parsimony and Bayesian methods. Karyotype structures were investigated by fluorescence *in situ* hybridisation (FISH) and fluorochrome banding. Cytogenetic characters were mapped on the combined phylogenetic tree. The absence or comparatively rare occurrence of different ITS sequence types in some (allo-) polyploid species of *Helictotrichon* suggests frequent intergenomic homogenisation of ribosomal DNA (rDNA) loci due to the phenomenon of concerted evolution. This result implies that the ITS region is not an ideal marker to study polyploid evolution of these grasses. The phylogenetic analysis of the Topo6 region revealed three major clades that concur with three different copy types (termed SAR, SET, PAR), representing the major genome groups in *Helictotrichon*. A comparison of the molecular phylogenetic trees with the chromosome and karyotype structure supports allopolyploidy of several *Helictotrichon* species and identifies potential genome donors. A correlation between molecular phylogenetic/cytogenetic results and geographical distribution is expressed by a west-east disjunction, in the narrower or wider sense, of the analyzed species. While SAR represents a geographically narrowly distributed southwest

Mediterranean genome group, PAR and SET are very widespread (Mediterranean to Asia) and encompass several instances of west-east disjunctions.

Keywords chromosomes, cytogenetics, FISH, *Helictotrichon*, Poaceae, polyploidy, ribosomal DNA, satellite DNA, topoisomerase 6

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2.3 Publication 3 - Hybridization and long-distance colonization in oat-like grasses of south and east Asia, including an amended circumscription of *Helictotrichon* and the description of the new genus *Tzveleviochloa* (Poaceae)

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Hybridization and long-distance colonization in oat-like grasses of south and east Asia, including an amended circumscription of *Helictotrichon* and the description of the new genus *Tzveleviochloa* (Poaceae)

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Abstract

The phylogenetic relationships of 31 genera of the traditional grass tribe Aveneae, with focus on Southeast Asian species of *Helictotrichon*, were examined using DNA sequences of the plastid *matK-psbA* region, nuclear ITS, the nuclear single-copy gene topoisomerase 6 (Topo6) spanning two introns and morphology. A molecular phylogenetic analysis was performed for 122 species applying maximum parsimony and Bayesian methods. Additionally we surveyed 11 Southeast Asian taxa for structural spikelet characters including lemmas, lodicules and ovaries. The phylogenetic results of the three molecular markers revealed that several Southeast Asian species traditionally accommodated in *Helictotrichon* are misplaced in this genus except for *H. abietetorum*, *H. hideoi* and *H. leianthum*. The placement of *H. polyneurum* and *H. sumatrense* in the genus *Helictotrichon* requires further investigation as suggested by morphological and preliminary molecular data. Due to the occurrence of different sequence types of ITS and Topo6, both nuclear DNA markers suggested an allopolyploid origin for several Southeast Asian taxa. (1) In *Helictotrichon parviflorum* and *H. potaninii* two different sequence types of Topo6 occur, closely similar to those of *Trisetum* and *Helictotrichon*, respectively. Nuclear ITS data of both species point to affinities with *Trisetum* and related genera but not to *Helictotrichon*. Both species belong to a hitherto unknown genus that is morphologically distinct from *Trisetum* and *Helictotrichon* and is named *Tzveleviochloa*. (2) Two copy types (A and B) of Topo6 previously identified in

African *Trisetopsis*, a genus encompassing predominantly subsaharan species that traditionally had been ascribed to *Helictotrichon*, also occur in two Southeast Asian species, i.e. *H. junghuhnii* and *H. virescens*. In agreement with morphological data both species need to be transferred to *Trisetopsis*. This expands the hitherto known eastern edge of the range of *Trisetopsis* from the Arabian Peninsula to China. (3) Additionally, the Chinese *H. altius* has a copy type sequence of Topo6 similar to that of *Trisetopsis* but not of *Helictotrichon* in its genome, whereas its plastid DNA fits *Helictotrichon* and relatives. Along with morphological characters this supports intermediacy between *Trisetopsis* and *Helictotrichon*. Therefore *H. altius* is probably an intergeneric hybrid, here named \times *Trisetopsotrichon altius*. The taxonomically difficult genus *Helictotrichon* s.l. in tropical to temperate southeastern Asia becomes disassembled into *Helictotrichon* s.str., the new genus *Tzveleviochloa*, gen. nov., and the nothogenus \times *Trisetopsotrichon*, nothogen. nov. The following combinations are introduced: *Trisetopsis aspera*, comb. nov., *T. junghuhnii*, comb. nov., *T. virescens*, comb. nov., \times *Trisetopsotrichon altius*, comb. nov., *Tzveleviochloa burmanica*, comb. nov., *T. parviflora*, comb. nov., *T. potaninii*, comb. nov.

Keywords ITS, *matK-psbA* region, taxonomy, topoisomerase 6, *Trisetopsis*, \times *Trisetopsotrichon*

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3 MAIN RESULTS AND OVERALL DISCUSSION

To clarify the phylogeny and systematics of selected genera of the traditional tribe Aveneae (inter alia species of the subtribe Aveninae sensu Soreng & al. (2007) like *Arrhenatherum*, *Avena*, *Avellinia*, *Gaudinia*, *Grapphorum*, *Helictotrichon* incl. *Pseudarrhenatherum*, *Koeleria*, *Lagurus*, *Leptophyllochloa*, *Peyritschia*, *Rostraria*, *Sphenopholis*, *Trisetaria*, *Tricholemma* and *Trisetum*) and especially unresolved questions of the circumscription of the genus *Helictotrichon*, the plastid *matK-psbA* region, nuclear ITS and the nuclear single-copy gene topoisomerase 6 (Topo6) spanning two introns were used. Phylogenetic analyses based on sequence data combined with morphological investigations of structural characters of the inflorescences and spikelets (lemma, lodicule, ovary) provided evidence to exclude several Southeast Asian species of *Helictotrichon* and transfer them into the new genus *Tzveleviochloa* and the recently described *Trisetopsis*.

Subtribe Aveninae. — The here suggested circumscription of subtribe Aveninae, supports as far as possible the classification of Soreng & al. (2015). The genera *Arrhenatherum*, *Avellinia*, *Avena*, *Gaudinia*, *Grapphorum*, *Helictotrichon*, *Koeleria*, *Lagurus*, *Peyritschia*, *Rostraria*, *Sphenopholis*, *Trisetaria*, *Tricholemma* and *Trisetum* are part of the Aveninae in all marker analyses of the present study (publication 1 and 3). Also the newly described genera *Trisetopsis*, *Tzveleviochloa* and *×Trisetopsotrichon*, including species formerly treated under *Helictotrichon*, appeared as part of the large Aveninae lineage (publication 3). Interesting results are found for the large genus *Calamagrostis* (subtribe Agrostidinae Fr.; Kellogg, 2015; Soreng & al., 2015). Similar to findings of Saarela & al. (2010) the present study examined *Calamagrostis* from Mexico, Central and South America as closely related to *Peyritschia* and *Sphenopholis* and therefore as member of subtribe Aveninae (publication 1 and 3). The Aveninae are part of the tribe Poeae chloroplast group 1 (Aveneae type) as defined by Soreng & al. (2015). The latter classification placed the genera *Avenula* and *Helictochloa*, including former *Helictotrichon* s.l. species, in subtribes of the Poeae chloroplast group 2 (Poeae type) as corroborated by the *matK-psbA*, ITS and Topo6 analyses of the present thesis (publications 1 and 3). This study dealt, for the first time, with molecular data of the genus *Leptophyllochloa*. Marker analyses of *matK-psbA* and ITS (publication 3) showed that *Leptophyllochloa* is not part of the Aveninae as supposed by recent classifications (Kellogg, 2015; Soreng & al., 2015). On the contrary, it is part of the Poeae chloroplast group 2 (Poeae type) like *Avenula* and *Helictochloa*. Further investigations (Wölk, unpublished data) showed that *Leptophyllochloa* is part of the subtribes Holcinae Dumort. (Soreng & al., 2015) or Airinae Fr. (Kellogg, 2015). In the classification of Kellogg (2015) *Leptophyllochloa* is synonym, based on morphological data, of a broadly defined

genus *Trisetaria*. This genus *Trisetaria* corresponds in the broadest sense to the so-called *Trisetum* group of the present investigation in publication 3.

***Helictotrichon*.** — The phylogenetic reconstructions in the present study showed *Helictotrichon* even in the narrow sense (Röser & al., 2009; Romero Zarco, 2011) as polyphyletic. In all phylogenies (publication 1, 2 and 3) the type species *H. sempervirens* (Schweickert, 1937), grouped together with Eurasian, North African, North American and a few Southeast Asian species of the genus. The removal of tropical and subtropical African, Arabian and Malagasy species from *Helictotrichon* into a new genus *Trisetopsis* (Wölk & Röser, 2013) is supported in the present thesis due to molecular and morphological results (publication 1 and 3). All three marker used support *Trisetopsis* as separate lineage distinct from *Helictotrichon*. Except for *H. abietetorum*, *H. hideoi* and *H. leianthum* the other examined Southeast Asian species appear in several different lineages distinct from *Helictotrichon* in the *matK-psbA*, ITS and Topo6 trees (publication 3). The placement of *H. polyneurum* and *H. sumatrense* in the genus *Helictotrichon* appears uncertain and requires further investigation as suggested by morphological and preliminary molecular data. Morphological investigations of the spikelets corroborate a distinction of tropical Asian from Eurasian *Helictotrichon* species. Therefore the majority of Southeast Asian species had to be removed from the genus *Helictotrichon* into smaller genera (*Trisetopsis*, *Tzveleviochloa*, \times *Trisetopsotrichon*). The inclusion of the former genus *Pseudarrhenatherum* (*H. thorei* syn. *P. longifolium*, *H. pallens* syn. *P. pallens*; Röser & al., 2009) into *Helictotrichon* is supported in all phylogenies of the present thesis (publication 1, 2 and 3).

The genus *Helictotrichon* also in its narrow sense is characterized by frequent polyploidy and a certain degree of hybridization (Winterfeld, 2006; Winterfeld & al., 2009a, Winterfeld & al., 2016). Hybridization and polyploidization can radically impact genome organization from sequence level to chromosome structure (Winterfeld & al., 2016). In the present study Topo6 appears to be the most powerful sequence marker to resolve the relationships among the remaining species of *Helictotrichon* s.str. and its polyploid complexes. The Topo6 datasets contained three different copy types termed SAR, SET and PAR (publication 1, 2 and 3) in *Helictotrichon* s.str., which represents the major genome groups. The SAR copy type of Topo6 was found in *Helictotrichon sarracenorum*, and both subspecies of *H. filifolium*. The SET copy type occurred in both subspecies of *H. setaceum* and *H. desertorum* and also in *H. cantabricum*, *H. convolutum*, *H. \times krischae*, *H. pallens*, *H. thorei*, *H. sempervirens* and *H. filifolium* subsp. *arundanum*. The PAR copy type was found in

H. cantabricum, *H. decorum*, *H. filifolium* subsp. *filifolium*, *H. ×krischae*, *H. parlatorei*, *H. sempervirens* and both subspecies of *H. sedenense*.

Investigations on the Mediterranean species of *Helictotrichon* (publication 2) connected the three different copy types with different marker chromosomes (termed set, par I, par II, tho I and tho II) that were detected by fluorescence in situ hybridization (FISH). These three different identified clades (SAR, SET, PAR) are defined neither by morphological nor by consistent biogeographic characters. Molecular phylogenetics was compared with chromosome and karyotype structure to investigate the origin of several *Helictotrichon* allopolyploids (publication 2). Diploid lineages were identified that were involved in the formation of polyploid complexes as potential genome donors in Mediterranean and Alpine species.

A lack of common morphological characters or geographic distribution among the species of each of the three copy types was most obvious in the Topo6 dataset including additionally to the Mediterranean species of *Helictotrichon*, the Southeast Asian and temperate Asiatic species (publication 3). The copy type PAR is very widespread and is found not only in Mediterranean species but also in the three Southeast Asian species *H. abietetorum*, *H. hideoi*, *H. leianthum* and the temperate Asiatic *H. krylovii*. On the other hand, the remaining temperate Asiatic species have the widespread SET copy type of Topo6 in their genomes. This copy type is shared also by both subspecies of temperate Eurasian *H. desertorum* and the North American *H. mortonianum*. This result supports the assumption of *H. mortonianum* as closest relative to Central Asian *H. mongolicum* (Tzvelev, 1976).

***Trisetopsis*.** — Both, the chloroplast *matK-psbA* and the nuclear ITS and Topo6 analyses placed the species of the genus *Trisetopsis* in groups distinct from the *Helictotrichon* lineage (publication 1 and 3). The investigation of numerous Southeast Asian species formerly included into *Helictotrichon* shows that *Trisetopsis* is not only limited to African species (publication 3). The sequences of former *Helictotrichon virescens* and *H. junghuhnii* from south Asia form a lineage together with the African species of *Trisetopsis* based on plastid *matK-psbA* and nuclear Topo6. In the phylogeny of ITS only three clone sequences of *H. junghuhnii* cluster together with *Trisetopsis*. The others form a separate clade together with the south Asian species *Trisetopsis virescens* and *×Trisetopsotrichon altius*. However, the preponderant molecular results and also common morphological traits (the apically deeply bifid lemma, the sparsely ciliate ovary and the bifid lodicules that are typical for species of *Trisetopsis*) led to the transfer of *H. virescens* and *H. junghuhnii* into *Trisetopsis*. Furthermore, *H. asperum* was transferred to *Trisetopsis*. Although sequence data of *H.*

asperum are lacking in the present study own observations and current marker analyses (Wölk unpublished) support the transfer of *H. asperum* to *Trisetopsis*. The species *H. asperum* and *H. virescens* were even synonymized in some previous studies (Hooker, 1896; Sevenster & Veldkamp, 1983; Davidse, 1994; Broome & Noltie, 2000). Finally, the new combinations *T. aspera*, *T. junghuhnii* and *T. virescens* were made (publication 3).

Unexpectedly, the species of the genus *Trisetopsis* formed a monophyletic group with New World species of *Calamagrostis*, *Grappophorum*, *Peyritschia* and *Sphenopholis* in the plastid DNA trees (publication 1 and 3). There are two different copy types of Topo6 in *Trisetopsis*, called A and B (publication 1 and 3). The copy type B is also found in the mentioned New World species. To find possible migration routes and a consistent link between the New World species and *Trisetopsis* biogeographical approaches and analyses are necessary.

Southeast Asian species former included in *Helictotrichon*. — The last analysis within the present thesis shows that not only the excluded species of *T. aspera*, *T. junghuhnii* and *T. virescens* were previously misplaced in *Helictotrichon*. Numerous further species from India, China, Bhutan, Nepal, Farther India and the Malay Archipelago appeared to be neither a part of *Helictotrichon*, nor of *Trisetopsis* based on molecular and morphological data. However, there are certain similarities of morphological characters of several of these species with *Helictotrichon*. In the past these similarities led to the inclusion of those species into *Helictotrichon*. The present investigation points out that processes like hybridization and polyploidization are responsible for those similarities. The species *Helictotrichon altius*, as an example, showed an intermediate position between *Trisetopsis* and *Helictotrichon*. In the *matK-psbA* phylogeny *H. altius* is part of the *Helictotrichon* lineage. But in ITS and Topo6 it is closely related to species of *Trisetopsis*, especially *T. junghuhnii* and *T. virescens*. In contrast to the species of *Trisetopsis* it has only copy type A in its genome. The ovary pubescence of *H. altius* appeared also intermediate between *Helictotrichon* and *Trisetopsis*. It has, on the one hand, an entirely pubescent ovary that is typical for *Helictotrichon* but, on the other hand, the hairs are fine and short like that one typically found on the apex of the sparsely ciliate *Trisetopsis* ovaries. Due to these molecular and morphological findings a hybrid origin with parental genomes of *Helictotrichon* and *Trisetopsis* are assumed for *H. altius*. As a consequence the nothogenus \times *Trisetopsotrichon* and the new combination \times *Trisetopsotrichon altius* were introduced (publication 3).

In addition the former *Helictotrichon parviflorum* and *H. potaninii* are also not part of the *Helictotrichon* lineage based on results from the investigated *matK-psbA* region and ITS.

Interestingly, these species show a close relationship to *Trisetum* and allied species (*Trisetum* group in publication 3). Chromosome counts were not available for *H. parviflorum* and *H. potaninii*, but the Topo6 data point to a hybrid origin with possible subsequent allopolyploidization of these two species. Two different copy types, on the one hand the PAR copy type of *Helictotrichon* and on the other hand a *Trisetum*-like copy type, were found in *H. parviflorum* and *H. potaninii*. In contrast to \times *Trisetopsotrichon altius*, with lemmas and lodicules like the parental *Trisetopsis* species, the intermediate morphology of *H. parviflorum* and *H. potaninii* is clearly shown in the lemma shape and ovary pubescence. As a consequence of the molecular results and the intermediate character of *H. parviflorum* and *H. potaninii*, these two species belong to a hitherto overlooked genus, which was named *Tzveleviochloa*. In the present study the names *Tzveleviochloa parviflora* and *T. potaninii* were introduced.

A controversial result in the present study was delivered by the former *Helictotrichon polyneurum* and *H. sumatrense* (publication 3). The analysis of ITS identified two different clone sequences in *Helictotrichon sumatrense*. One that is related to *Helictotrichon* and other clone sequences, which are nested between taxa of *Trisetum* and relatives (*Trisetum* group in publication 3). The occurrence of this different ITS copies lead to the assumption of an allopolyploid origin and a complicated polyploid evolution, with parentage from two different lineages, for *H. sumatrense* (publication 3). On the other hand the present study identifies a *Helictotrichon*-like ITS sequence of *Helictotrichon polyneurum*. The known gametophytic chromosome number of $n = 2x = 14$ for *H. polyneurum* also supports a polyploid origin of that species. Both, an autopolyploid and an allopolyploid origin, caused by sequence homogenization, were discussed for the formation of *H. polyneurum* (publication 3). Molecular data for the plastid and single-copy marker are lacking probably because of degraded DNA in the old plant material. In consequence, no general statement for the other investigated DNA marker (*matK-psbA* region and Topo6) could be made with regard to *H. polyneurum* and *H. sumatrense*. Also the investigated morphological characters show a distinct look of *H. polyneurum* and *H. sumatrense* in contrast to the remaining *Helictotrichon* species. This supports the assumption that both species are doubtful members of *Helictotrichon* as discussed in publication 3.

Marker discussion. — The phylogenetic analyses of the present thesis are based on molecular datasets of the plastid *matK-psbA* region, the ribosomal ITS and the nuclear single-copy gene topoisomerase 6 (Topo6). Compared with nuclear DNA the structurally stable, haploid and non-recombinant chloroplast genome is numerically overrepresented in

plant cells and therefore easily available for DNA isolation (Knoop & Müller, 2009; Hochbach & al., 2015). In angiosperms it is characterized by primarily uniparental, usually maternal inheritance (Small & al., 2004). The plastid region *matK-psbA* has been used to detect hybridization events in comparison with the two used nuclear regions. The plastid region *matK-psbA* was already used repeatedly in studies of Poaceae (Liang & Hilu, 1996; Hilu & al., 1999; Döring & al., 2007; Soreng & al., 2007; Döring 2009; Schneider & al., 2009, 2011, 2012; Blaner & al., 2014; Hochbach & al., 2015). In contrast to these numerous former studies the present analyses only comprised a small part (about 280 nucleotides) of the *matK* gene focusing on the two non-coding regions 3'*trnK* intron and *trnK-psbA* spacer. These non-coding DNA regions evolved more rapidly than the coding regions of *matK* (*matK* gene, *trnK* exon) and therefore are more powerful for investigations at lower taxonomic levels like the genus rank (Small & al., 2004). But despite of that the plastid *matK-psbA* datasets in this thesis had, compared to the used nuclear markers, a low percentage of potentially parsimony-informative characters. Similar observations were made in a multi-locus analysis of Hochbach (2015) on the grass subfamily Pooideae. In comparison with the used nuclear DNA marker the plastid analyses of the *matK-psbA* region showed well and strongly supported lineages in the early diverging branches of the phylogenies. In contrast, neither ITS nor Topo6 showed a clear and well supported hierarchical structure of the early diverging genera of Poaceae s.l., but have a well supported resolution of the external branches.

Nuclear genes are characterized by biparental inheritance and a faster rate of sequence evolution than it is usually observed in chloroplast genomes (Knoop & Müller, 2009). The ribosomal ITS consists of intergenic spacers as part of the 18S–5.8S–26S rDNA cistron arranged in tandem arrays at one or more chromosomal loci (Alvarez & Wendel, 2003). Therefore it is also comparatively easily available for DNA isolation because ribosomal genes appear as hundreds to thousands of copies per array (Small & al., 2004). Polyploid evolution within *Helictotrichon* and related genera was one of the main issues in the present thesis and the results showed that ITS was not the most ideal marker to solve these problems. Obviously it is affected by homogenization caused by a process called ‘concerted evolution’ (Wendel, 2000; Alvarez & Wendel, 2003; Kovarik & al., 2004, 2005, 2008; Dadejova & al., 2007; Lim & al., 2007; Mason-Gamer, 2008; Jakob & Blattner, 2010; Mason-Gamer & al., 2010) that in some cases result in a single dominant sequence across all copies and arrays. The present thesis shows evidence for such homogenization processes in several species of *Helictotrichon* concerning nearly every western African species of *Trisetopsis* and some species of *Koeleria* and relatives, which finally allowed the PCR products to be directly sequenced. However,

concerted evolution is not a fixed phenomenon across repeated units or in any taxa. Therefore different sequence variants of ITS are possibly detectable in the absence of concerted evolution (Alvarez & Wendel, 2003) as the different copy types for South African *Trisetopsis*, Southeast Asian *Helictotrichon sumatrense* and some other species showed. Still, the ability of ITS to identify genome donors is given to a certain degree (Solti & Soltis, 1991; Sang & al., 1995; Widmer & Baltisberger, 1999; Hughes & al., 2002; Rauscher & al., 2002; Tate & Simpson, 2003; Guggisberg & al., 2006; Mason-Gamer, 2008; Winterfeld & al., 2009b). An advantage of ITS at lower taxonomic levels is that the sequences evolved at a fast rate leading to more phylogenetically informative characters for the data analyses (Small & al., 2004).

Low- or single-copy nuclear genes are less affected by gene conversion and therefore able to identify parental genome donors of hybrids or polyploids more reliably (Alvarez & Wendel, 2003; Mason-Gamer & al., 2010; Zimmer & Wen, 2012). A gene that mostly occurs in a single-copy state is the nuclear *Topo6* (Blattner, 2015). It was the third marker for the performed phylogenetic analyses and was used to identify the origins of hybrids or allopolyploid species in the present study. It was previously utilized in investigations of *Hordeum* (Jakob & Blattner, 2010; Brassac & al., 2012; Brassac & Blattner, 2015), the grass subfamily Pooideae (Hochbach & al., 2015) and relationships of the whole family Poaceae (Blaner & al., 2014). In the present thesis *Topo6* (exon 17 – exon 19) was used for the first time in the tribe Poeae s.l. It was suitable for investigations at lower taxonomic level due to the large intron regions between exon 17 and 18 and exon 18 and 19. Therefore the most parsimony-informative characters of the used datasets were found in the nuclear single-copy intron regions of *Topo6*. However these variable intron regions ended up in a low backbone resolution for the *topo6* tree, as identified also in investigations of Pooidee (Hochbach & al., 2015). In contrast, the external tribes and branches were well to strongly supported.

4 CONCLUSION AND OUTLOOK

The present thesis focused on the interrelations between (1) the African species removed from *Helictotrichon* to the recently described genus *Trisetopsis* (Wölk & Röser, 2013), (2) the Palearctic and Nearctic species of *Helictotrichon* excluding *Avenula* Besser, *Helictochloa* Romero Zarco and *Tricholemma* (Röser) Röser and (3) Southeast Asian species still included in *Helictotrichon*. This thesis additionally dealt with (4) the phylogeny of several related species in the subtribe Aveninae and (5) considered speciation events in hybrids and polyploids of the analyzed species. It used molecular sequence data from three different DNA regions (plastid *matK-psbA*, nuclear ITS and Topo6) partly combined with morphological, cytogenetic and karyotype data. It was shown that *Helictotrichon* even in its narrow sense (Röser & al., 2009; Romero Zarco, 2011) and after the removal of *Trisetopsis* is polyphyletic. The molecular results show clearly that the (sub)tropical African and Southeast Asian species traditionally treated under *Helictotrichon* form separate lineages distinct from *Helictotrichon* s.str. Morphological characters, especially the characteristic shape of lemmas, lodicules and the ovary pubescence, corroborate the recognition of the genus *Trisetopsis* and the analyses of nuclear DNA combined with morphological data provided evidence for a new Southeast Asian genus *Tzveleviochloa* and the Asian nothogenus \times *Trisetopsotrichon*. ITS and Topo6 suggested an allopolyploid origin of nearly all species of *Trisetopsis* and the species of *Tzveleviochloa*. The present study showed that ITS in some cases is affected by sequence homogenization concerning in particular the polyploid tropical East African species of *Trisetopsis*, the Southeast Asian *Tzveleviochloa* and nearly all *Helictotrichon* polyploids. This indicates that ribosomal ITS is not in any case an ideal marker to study polyploid evolution, nevertheless one *Helictotrichon* species had two different sequence types of ITS in its genome with affinities to different unrelated *Trisetum* species. In contrast, the Topo6 sequences reveal a strong pattern of genetic variation and had the ability to identify genome donors of polyploids more reliably. It recognized different copy types in *Trisetopsis*, *Helictotrichon* and *Tzveleviochloa*, but also in other genera of Aveninae like *Graphephorum* and *Koeleria*. Indeed, based on the single copy gene it was possible to identify different parental genomes.

The present study gives evidence of a complicated evolutionary history of *Trisetum* and allied genera that could still not be fully resolved in this thesis caused the rare species sampling of the large genus *Trisetum*. This unresolved history of *Trisetum* and allies could be an interesting subject for further studies. Additionally, the analyses of further Southeast Asian species associated with *Helictotrichon* has not been fully completed in the present study. For example, single-copy data for numerous Southeast Asian species are completely lacking. Also biogeographical analyses could be very interesting to resolve the migration history in

Helictotrichon and *Trisetopsis*. However, the investigations of allopolyploids using nuclear protein coding single- or low-copy genes delivered interesting insights into the polyploid evolution of grasses. Further studies should be made using multiple single- or low-copy genes because of the possible different evolutionary histories of single markers. Using only Topo6 may provides only little insight into species relationships. Here next-generation sequencing (NGS) approaches would be appropriate to analyze multiple single- or low-copy genes even when only highly degraded DNA of herbarium material is available (Beck & Semple, 2015).

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6 APPENDIX

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Publication list

Publications

Wölk, A. & Röser, M. (accepted 2016): Hybridization and long-distance colonization in oat-like grasses of south and east Asia, including an amended circumscription of *Helictotrichon* and the description of the new genus *Tzveleviochloa* (Poaceae).

Winterfeld, G., **Wölk, A.** & Röser, M. (2016): Genome evolution in alpine oat-like grasses through homoploid hybridization and polyploidy. AoB PLANTS doi:10.1093/aobpla/plw039.

Wölk, A., Winterfeld, G. & Röser, M. (2015): Genome evolution in a Mediterranean species complex: phylogeny and cytogenetics of *Helictotrichon* (Poaceae) allopolyploids based on nuclear DNA sequences (rDNA, topoisomerase gene) and FISH. *Systematics and Biodiversity* 13: 326–345.

Wölk, A. & Röser, M. (2014): Polyploid evolution, intercontinental biogeographical relationships and morphology of the recently described African oat genus *Trisetopsis* (Poaceae). *Taxon* 63: 773–788.

Braun, U., **Wölk, A.** & Heuchert, B. (2014): Second collection of *Tilletia avenastri* discovered in type material of *Trisetum imberbe*. *Schlechtendalia* 27: 15–16.

Wölk, A. & Röser, M. (2013): The new genus *Trisetopsis* and new combinations in oat-like grasses (Poaceae). *Schlechtendalia* 25: 57–61.

Contributions to conferences

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Wölk, A., Winterfeld, G. & Röser, M. (2015): Phylogenetics and genome evolution of Mediterranean *Helictotrichon* (Poaceae, grasses) based on two nuclear gene regions (ITS, Topo6) and FISH. Black Forest Summer School on NGS data for phylogenetics, September 14th-17th 2015; Poster Abstracts, p. 28. Marburg.
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Wölk, A. & Röser, M. (2013): *Trisetopsis*, a new genus of oat-like grasses (Poaceae). 55th Phylogenetic Symposium 2013 ‘‘The time for phylogenetics: inferring and applying timetrees in evolutionary biology’’, November 22th-24th 2013; Poster presentations, p. 21. Oldenburg.

Wölk, A. & Röser, M. (2013): *Trisetopsis*, a new genus of oat-like grasses (Poaceae). 5th International Conference on Comparative Biology of Monocotyledons, July 7th-13th 2013; Abstracts, p. 181. New York.

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Talks

Wölk, A. & Röser, M. (2014): Polyploid evolution, intercontinental biogeographical relations and morphology of the newly described African oat genus *Trisetopsis* (Poaceae). 15th Annual Meeting of the Society of Biological Systematics (GfBS) and the 22nd International Symposium of the German Botanical Society (DBG), “Biodiversity and Evolutionary Biology”, March 24th-27th 2014; Abstracts, p. 49. Dresden.

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WS/2015	Practical on Next Generation Sequencing (Master students)

Erklärung über den persönlichen Anteil an den Publikationen

Publication I	<p>Wölk, A. & Röser, M. (2014): Polyploid evolution, intercontinental biogeographical relationships and morphology of the recently described African oat genus <i>Trisetopsis</i> (Poaceae). <i>Taxon</i> 63: 773–788.</p> <p>Data collection: Alexandra Wölk (90%), Martin Röser (10%) Analysis: Alexandra Wölk (95%), Martin Röser (5%) Writing: Alexandra Wölk (90%), Martin Röser (10%)</p>
Publication II	<p>Wölk, A., Winterfeld, G. & Röser, M. (2015): Genome evolution in a Mediterranean species complex: phylogeny and cytogenetics of <i>Helictotrichon</i> (Poaceae) allopolyploids based on nuclear DNA sequences (rDNA, topoisomerase gene) and FISH. <i>Systematics and Biodiversity</i> 13: 326–345.</p> <p>Data collection: Alexandra Wölk (70%), Grit Winterfeld (30%) Analysis: Alexandra Wölk (80%), Grit Winterfeld (20%) Writing: Alexandra Wölk (90%), Grit Winterfeld (10%), corrections by Martin Röser</p>
Publication III	<p>Wölk, A. & Röser, M. (accepted for publication in <i>Taxon</i> on 01.11.16): Hybridization and long-distance colonization in oat-like grasses of south and east Asia, including an amended circumscription of <i>Helictotrichon</i> and the description of the new genus <i>Tzveleviochloa</i> (Poaceae).</p> <p>Data collection: Alexandra Wölk (95%), Martin Röser (5%) Analysis: Alexandra Wölk (100%) Writing: Alexandra Wölk (90%), Martin Röser (10%)</p>

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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 habe. Ich versichere, dass ich die Arbeit nur unter Verwendung der von mir angegebenen Quellen und Hilfsmittel angefertigt habe und ich die wörtlich oder inhaltlich entnommen Stellen benutzter Werke, als solche kenntlich gemacht habe.

Halle (Saale), 18.11.16

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