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RESEARCH

Reductional dysploidy and genome size diversity in Pooideae, the largest subfamily of grasses (Poaceae)

Grit Winterfeld[1] · Natalia Tkach[1] · Martin Röser[1]

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Abstract

The nuclear genome sizes of 59 species from 33 genera of the Poaceae subfamily Pooideae were investigated by flow cytometry. This subfamily is characterized by a wide range of holoploid (2C values) and monoploid (1Cx values) genome sizes and mean chromosome sizes, including both the highest and some of the lowest values of the entire grass family. For example, the tribe Brachypodieae has the smallest monoploid genomes and chromosomes, followed by the majority of Stipeae and individual representatives of the tribes Ampelodesmeae, Duthieeae and Meliceae, which belong to the phylogenetically 'early-diverging' lineages. Comparatively large genome and chromosome sizes were found in the Lygeeae and some Meliceae. The 'core Pooideae' had the largest values in the subfamily, with the greatest variation in Aveneae, Festuceae and Poeae. The tribes Bromeae and especially Triticeae, which includes wheat and related crops, had larger minimum monoploid genome and chromosome sizes compared to the other 'core Pooideae' tribes. It appears that the occurrence of exclusively rather large monoploid genomes (>3.4 pg/1Cx) and chromosomes (MC ≥ 0.5 pg) is restricted to Triticeae. The origin of $x=7$ of the 'core Pooideae' from $x=12$ of the 'early-diverging' Pooideae lineages was apparently not related to an increase in genome size, whereas chromosome fusion caused an increase in chromosome size. The evolutionary aspects of chromosome base number variation in Pooideae are discussed, and new chromosome numbers are presented, including the first polyploid ($2n=4x=20$) of the model plant *Brachypodium distachyon* s.s.

Keywords C-value · Chromosome base number · Dysploidy · Genome · Genome size · Polyploidy

Introduction

With approximately 11,800 species and 790 genera (Soreng et al. 2022), the grasses (Poaceae) are the fifth largest family of seed plants. Grasses are almost globally distributed, occurring on all continents including Antarctica. The Poaceae comprise 12 subfamilies (GPWG II 2012; Kellogg 2015a; Hodkinson 2018; Soreng et al. 2022), which are not only morphologically distinct, but also have different centers of distribution, climatic adaptations, and ecological preferences.

The largest subfamily, Pooideae, which includes about 4,130 species in 220 genera, has the C_3 photosynthetic pathway throughout and is preferentially distributed in the temperate climates of the northern and, to a lesser extent, the southern hemisphere. Pooideae species are represented in the tropics and subtropics in the high mountains, but are scarce in the tropical or subtropical lowland areas, whereas they are abundant in various types of temperate zone vegetation, including the vast Eurasian steppes (grasslands). The plants are usually hemicryptophytic perennials, but the Pooideae have also evolved numerous annual species as an adaptation to highly seasonal climates, especially in regions with an annual dry season. For example, in the winter rain region of the Fertile Crescent in the Near East, which has dry and hot summers, the Pooideae have evolved the ancestors of some of the world's most important crops, notably wheat with several cultivated relatives (*Triticum aestivum* with many varieties and other *Triticum* species), rye (*Secale cereale*), barley (*Hordeum vulgare*), oats (*Avena sativa* and related species). Since the 1980s, ×*Triticale* (×*Triticosecale*),

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✉ Martin Röser
martin.roeser@botanik.uni-halle.de

[1] Institute of Biology, Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

an intergenic hybrid between *Triticum* and *Secale*, has also been increasingly cultivated in agriculture.

Accordingly, these cultivated grasses are the subject of intensive breeding, physiological, genetic, and molecular genetic research, and the wild grasses related to them are also extensively studied. The genome sizes of many Pooideae genera, especially the tribe Triticeae, which includes wheat, barley, and rye, among many others, have also been studied in this context (Bennett and Smith 1976; Doležel et al. 1998; Jakob et al. 2004; Martis et al. 2013; Yan et al. 2016; Bauer et al. 2017; Avni et al. 2022; Chen et al. 2022; Li et al. 2022; Feldman and Levy 2023a, b), and the largest genome size of 55.15 pg/2C ever found in grasses belongs to an artificially generated octoploid ×*Triticale* (Bennett 1972), which also belongs to this tribe.

The continued interest in studying plant genome size is not only related to obtaining baseline data for the large number of species that have not been studied in this regard, but also has applied and practical aspects, such as the selection of additional wild species for genome sequencing projects in grasses, preferably species with small genome sizes and diploidy to compare genome organization in different grass lineages. For example, *Brachypodium distachyon* was selected for the first wild grass genome sequencing project, in part because its genome was known to be small based on DNA content measurements (The International Brachypodium Initiative 2010; Catalán et al. 2012; Li and Harkess 2018; Diakostefani et al. 2023).

It is also particularly important to estimate genome sizes of wild grass species of breeding interest that are related to different cultivated grasses. In addition, genome sizes are in many cases associated with ecomorphological, physiological, phenological and phenotypic characteristics (cell size, stomatal size, cell cycle duration, flowering time, embryo size, seed size, etc.), so that genome sizes and their evolutionary changes also may have an adaptive and functional significance and ultimately influence the ecological behavior of the species as well as their macroecological and biogeographical patterns (Knight et al. 2005; Knight and Beaulieu 2008; Leitch and Leitch 2012; Herben et al. 2012; Šímová and Herben 2012; Greilhuber and Leitch 2013; Suda et al. 2015; Simonin and Roddy 2018; Hoang et al. 2019; Šmarda et al. 2019; Veselý et al. 2020; Faizullah et al. 2021; Shao et al. 2021; Carta et al. 2022; Chen et al. 2022; Bhadra et al. 2023; Bitomský et al. 2023; Bureš et al. 2023, 2024; Jiang et al. 2023; Pyšek et al. 2023; Zhang and Qiu 2023; Cang et al. 2024; Las Peñas et al. 2024). For ‘polyploid complexes’, i.e. groups of closely related individual species or microtaxa/microspecies differing by their ploidy levels, such differences in distribution or ecological behavior have long been known and are among the classic examples of ‘cytogeography’. DNA quantification, especially by flow cytometry, which is well established as a reliable and accurate method, provides a time-saving and efficient way to study such differentiation processes at different scales, from variation within populations to large areas of distribution (Paule et al. 2018; Savaş Tuna et al. 2019; Bednářová et al. 2021; Becher et al. 2022; Kobrlová et al. 2022; Greimler et al. 2022; Garnatje et al. 2023; Horák et al. 2023; Hutang et al. 2023; Pustahija et al. 2024; Bureš et al. 2024). In addition to the saltational changes caused by polyploidization, gradual, more or less continuous changes in genome size can also be detected, sometimes even between populations or closely related taxa, which typically result primarily from the amplification of mobile elements in the genome and their gradual loss over longer periods of time, leading to genome enlargement or reduction (Zedek et al. 2010; Michael 2014; Winterfeld et al. 2020; Wang et al. 2021; Wang et al. 2021; Dai et al. 2022; Mhiri et al. 2022; Moreno-Aguilar et al. 2022; Yu et al. 2023).

Many genera of Pooideae show a strong proliferation of annual species, a well-known process of angiosperm life form evolution (Hjertaas et al. 2023) that is often associated with changes in genome size (Bennett and Leitch 2005; Knight et al. 2005; Leitch and Bennett 2007). However, the Pooideae are not only characterized by many annuals in Mediterranean-type climates, but also by particularly cold-adapted species in the boreal to Arctic, and sub-Antarctic to Antarctic regions, including one of only two native flowering plant species of Antarctica, *Deschampsia antarctica* (Greene and Holtom 1971). In addition, Pooideae also dominate in the alpine zone in the high mountains of the Northern Hemisphere, where they constitute extensive natural grasslands. A combination of morphological and physiological adaptive traits allows these grasses to withstand such harsh environmental conditions, for example, sufficient frost tolerance and tolerance to water stress, ability to able to grow and maintain substantial photosynthetic rates at low temperatures, adequate management of excess photosynthetically active radiation, etc. (Cavieres et al. 2016; Saéz et al. 2018; Ramírez et al. 2024). A multidisciplinary approach using the interplay of physiology, cytogenetics, genomics, and phylogenetic research is needed to fill critical knowledge gaps in the physiological, biochemical and molecular basis of adaptation to extreme low temperature environments (Herben et al. 2012; Lütz 2012; Schubert et al. 2019, 2020; Hüner et al. 2022; Sklenář et al. 2022). Species of Pooideae are iconic examples of one of the four major growth forms of flowering plants beyond the tree line, namely the tussock-forming graminoids (Körner 2023). All this makes Pooideae an ideal taxonomic group to study the genomic, cellular, physiological and developmental basis of adaptation to seasonally changing climatic conditions (periodic cold, drought) and plant survival strategies.

Taxonomically, the subfamily Pooideae has mostly been divided into 10–15 tribes (Clayton and Renvoize 1986;

Table 1 Subfamily Pooideae and its subordinate taxa as treated in classifications of Soreng et al. (2022) (left) and previous studies of the authors (right). The linear sequence of taxa largely follows the patterns of molecular phylogenetic studies (Schneider et al. 2009, 2011; Blaner et al. 2014; Hochbach et al. 2015; Saarela et al. 2015, 2018; Tkach et al. 2020; Orton 2021; Gallaher et al. 2019, 2022; Huang et al. 2022; Zhang et al. 2022)

Early-diverging Pooideae lineages	**Early-diverging Pooideae lineages**
Tribe Brachyelytreae Ohwi	Tribe Brachyelytreae Ohwi
Supertribe Nardodae Soreng	Tribe Nardeae W.D.J.Koch
Tribe Nardeae W.D.J.Koch	Subtribe Nardinae Kromb.
Tribe Lygeeae J.Presl	Subtribe Lygeinae Röser
Tribe Duthieeae Röser & Jul.Schneider	Tribe Duthieeae Röser & Jul.Schneider
Tribe Phaenospermateae Renvoize & Clayton	Tribe Phaenospermateae Renvoize & Clayton
Supertribe Melicodae Soreng	Tribe Meliceae Link ex Endl.
Tribe Brylkinieae Tateoka	Subtribe Brylkiniinae Ohwi
Tribe Meliceae Link ex Endl.	Subtribe Melicinae Fr.
Supertribe Stipodae L.Liu	Tribe Stipeae Dumort.
Tribe Ampelodesmeae Tutin	Subtribe Ampelodesminae Conert
Tribe Stipeae Dumort.	Subtribe Stipinae Griseb.
Tribe Diarrheneae C.S.Campb.	Tribe Diarrheneae C.S.Campb.
Tribe Brachypodieae Harz	Tribe Brachypodieae Harz
Core Pooideae	**Core Pooideae**
Supertribe Poodae L.Liu	Supertribe Poodae L.Liu
Tribe Poeae R.Br. (s.l.)	Tribe Aveneae Dumort.
Supertribe Triticodae T.D.Macfarl. & L.Watson	Tribe Festuceae Dumort.
Tribe Littledaleeae Hemsl.	Tribe Poeae R.Br. (s.s.)
Tribe Bromeae Dumort.	Intertribe hybrids of Aveneae/Festuceae/Poeae
Tribe Triticeae Dumort.	Supertribe Triticodae T.D.Macfarl. & L.Watson
	Tribe Triticeae
	Subtribe Littledaleinae Röser
	Subtribe Brominae Dumort.

Kellogg 2015a; Soreng et al. 2022), except for the very different taxonomic concept of Tzvelev (1989), who recognized only two subfamilies of grasses, the bambusoid grasses as subf. Bambusoideae and the 'true grasses' as subf. Pooideae, which included not only 14 tribes commonly recognized as members of Pooideae, but also 13 other tribes that most authors assigned to other subfamilies (Oryzoideae, Panicoideae, etc.). Molecular phylogenetic studies place Pooideae, in its usual delimitation, together with the subfamilies Bambusoideae (bamboos) and Oryzoideae (rice subfamily) firmly in the 'BOP clade', one of the major lineages of grasses (GPWG 2001; Bouchenak-Khelladi et al. 2008; GPWG II 2012; Blaner et al. 2014; Saarela et al. 2018; Gallaher et al. 2019, 2022; Baker et al. 2022; Huang et al. 2022). The BOP clade began to diversify at about at least 50 Ma ago (Hodkinson 2018), which is a very conservative estimate; other estimates ranged from 67–95 Ma (Upper Cretaceous), depending on whether or not microfossils were included and which calibration method was used (Gallaher et al. 2019, 2022; Schubert et al. 2019; Orton et al. 2021; Huang et al. 2022; Zhang et al. 2022).

The phylogenetic structure of the Pooideae (cf. Table 1) is now largely resolved and well supported by molecular DNA studies of Sanger sequences and phylogenomic work on plastid and nuclear DNA (Table 1) (Davis and Soreng 2007; Bouchenak-Khelladi et al. 2008; Schneider et al. 2009, 2011, 2012; GPWG II 2012; Blaner et al. 2014; Hochbach et al. 2015; Saarela et al. 2015; Pimentel et al. 2017; Sancho et al. 2018; Gallaher et al. 2019, 2022; Tkach et al. 2020; Orton et al. 2021; Huang et al. 2022; Zhang et al. 2022). The phylograms typically show a series of phylogenetically 'early-diverging' lineages, some with higher chromosome base numbers of $x = 9$–13, followed by a larger monophyletic group known as the 'core Pooideae' (Davis and Soreng 1993; Soreng and Davis 1998; Kellogg 2015a, b) which is characterized by an almost uniform $x = 7$ if the sister lineage Brachypodieae is not included, although this has sometimes been done (Soreng et al. 2007, 2015; Saarela et al. 2015).

The tribe Brachyelytreae is sister to the rest of the subfamily Pooideae, as consistently found in nuclear and plastid DNA phylogenetic analyses (Soreng and Davis 1998; Schneider et al. 2009; Blaner et al. 2014; Hochbach et al. 2015; Pimentel et al. 2017; Saarela et al. 2018; Gallaher et al. 2019, 2022; Schubert et al. 2019; Orton et al. 2021; Huang et al. 2022; Zhang et al. 2022). The tribes Nardeae and Lygeeae, if they are to be considered as separate and not as one tribe as previously proposed (Schneider et al. 2009), diverge next in the phylogenies, followed by the tribe Duthieeae, as suggested by recent nuclear transcriptome- and genome-based phylogenetic studies (Huang et al.

2022; Zhang et al. 2022). This has already been established by Sanger sequence analyses of single or low copy number nuclear genes (Hochbach et al. 2015: Fig. 2, tree labeled as 'nuclear DNA combined'). However, plastid DNA sequence data place the Duthieeae closer to the tribe Phaenospermateae (Hochbach et al. 2015; Schubert et al. 2019). Unfortunately, the Duthieeae have not yet been sampled in plastome-based phylogenomic studies of the subfamily Pooideae (e.g., Saarela et al. 2018; Orton et al. 2021). The tribe Phaenospermateae is close to the tribe Meliceae, which is the next concordantly diverged tribe in both the plastid and nuclear phylogenomic trees of the Pooideae (Saarela et al. 2018; Orton et al. 2021; Huang et al. 2022; Zhang et al. 2022). Molecular phylogenetic analyses suggest the tribe Ampelodesmeae with only *Ampelodesmos* is most likely an ancient hybrid taxon, as plastid DNA Sanger sequence and plastome sequencing data show *A. mauritanicus* embedded in the Stipeae (e.g., Schneider et al. 2009, 2011; Blaner et al. 2014; Hochbach et al. 2015; Saarela et al. 2018; Orton et al. 2021), while nuclear single or low-copy gene data show commonalities with both the Stipeae and the Duthieeae (Blaner et al. 2014; Hochbach et al. 2015). Recent nuclear phylogenomic data placed *Ampelodesmos* as a sister taxon to the tribe Stipeae (Huang et al. 2022; Zhang et al. 2022). This suggests an extended tribe Stipeae with two subtribes: Ampelodesminae and the Stipinae, as previously proposed (Schneider et al. 2009, 2011). Previous analyses have not consistently resolved the tribe Diarrheneae as monophyletic. Plastome data placed *Diarrhena obovata* either separately or in a common lineage together with *Brachypodium* from the monogeneric tribe Brachypodieae, depending on the analysis (with or without gapped sites in the non-coding alignment partition) (Saarela et al. 2018; Orton et al. 2021). Analyses of plastid Sanger sequence data showed *N. japonica* in this position and *D. americana*/*D. obovata* distinct (Hochbach et al. 2015) or suggested both genera to be monophyletic, albeit with moderate support (Schneider et al. 2011). However, according to nuclear phylogenomic data, *D. obovata* is either sister to the Stipeae s.s. (Huang et al. 2022) or sister to the Stipeae s.s. plus Ampelodesmeae (Zhang et al. 2022). In addition, *Neomolinia* (*N. japonica*, *N. fauriei*) was found to be sister to the Brachypodieae plus 'core Pooideae' (Zhang et al. 2022), a position that was also revealed by some nuclear Sanger datasets (Hochbach et al. 2015). The monophyly of the Diarrheneae was supported by topoisomerase, phytochrome B, ITS of ribosomal DNA and all nuclear Sanger datasets combined (Schneider et al. 2011; Hochbach et al. 2015). The small tribe Brachypodieae is sister to the large 'core Pooideae' lineage, whose monophyly has been confirmed in all molecular phylogenetic studies to date. The major lineages proposed as supertribes are Poodae and Triticodae. The former has been considered as a single tribe, Poeae s.l. (GPWG 2001; GPWG II 2012; Kellogg 2015a; Soreng et al. 2022). Alternatively, a previous study proposed three tribes (Aveneae, Festuceae, and Poeae) and several groups of reticulate origin between these tribes, mostly between Aveneae and Festuceae (Tkach et al. 2020). Further research is needed to classify the reticulate groups. In particular, it is important to obtain more genomic data from nuclear DNA. The Triticodae comprise three lineages, which are treated as either tribes or subtribes. The tribe Littledaleeae or subtribe Littledaleinae if a broad tribe Triticeae is accepted, as in one of our previous publications (Schneider et al. 2009), is sister to the rest of Triticodae, namely the tribes Triticeae and Bromeae, alternatively subtribes Hordeinae (sic) and Brominae, in molecular phylogenetic DNA analyses (Davis and Soreng 2007; Döring et al. 2007; Schneider et al. 2009; Orton et al. 2021; Huang et al. 2022; Zhang et al. 2022).The age of diversification of the Pooideae is 59–80 Ma (Paleocene/Upper Cretaceous) in most analyses, that of the 'core Pooideae' about 35–48 Ma (Eocene) (Pimentel et al. 2017; Gallaher et al. 2019, 2022; Schubert et al. 2019; Orton et al. 2021; Huang et al. 2022; Zhang et al. 2022). Both diversifications may have been facilitated and benefited from paleoclimatic and vegetation changes, particularly the emergence of colder and more open habitats at higher latitudes while atmospheric CO_2 concentrations were still high until they began to decline in the late Eocene, favoring C photosynthesis (Edwards et al. 2010; Strömberg 2011; Spriggs et al. 2014; Schubert et al. 2019; Gallaher et al. 2022; Strömberg and Staver 2022; CenCO2PIP 2023; Sage et al. 2023).

Chromosome base numbers in Pooideae have been proposed to follow a general trend from $x = 10$, 11, and 13, characteristic of the 'basal' genera and tribes of Pooideae, to smaller numbers of $x = 2$–7, which appeared "later in the subfamily, probably in concert with accelerated speciation and radiation into new habitats" (Hilu 2004: p. 17). It has been argued that the predominance of $x = 7$ and the comparatively large chromosomes in the 'core Pooideae' could be derived from the higher monoploid numbers represented in what are, according to current understanding, phylogenetically early-diverging lineages (Avdulov 1931; Stebbins 1956, 1982; Clayton and Renvoize 1986; Soreng et al. 1990; Kellogg 1998; Hilu 2004; Kellogg and Bennetzen 2004). Interestingly, the large chromosome and, implicitly, genome sizes of the 'core Pooideae' with $x = 7$ were already interpreted ecologically by Avdulov (1931), i.e. in connection with the adaptation of these grasses to cool or cold climates. However, the underlying mechanisms or physiological reasons are not known, as argued by Stebbins (1956). In addition, the common chromosomal features of the 'core Pooideae' ($x = 7$, large chromosomes) may predate the split of the 'core Pooideae' into their major lineages (see Table 1), providing evidence for their monophyly (Kellogg and Campbell 1987; Soreng et al. 1990; Kellogg 2015a). In terms of genome size, a large increase has already been observed in the Pooideae, with a particularly significant increase in the Triticeae (Bennetzen and Kellogg 1997; Kellogg and Bennetzen 2004; Kellogg 2015a). The tribe Triticeae includes the major cereals wheat, barley, rye, etc., and is undoubtedly by far the best-studied Pooideae lineage regarding genome and chromosome organization (Miedaner and Korzun 2018; Pont et al. 2019; Tadesse et al. 2019; Bernhardt et al. 2020; Walkowiak et al. 2020; Levy and Feldman 2022; Feldman and Levy 2023c), while the other lineages of Pooideae have been studied much less extensively, and some of them even lack baseline data.

Our study on the genome sizes therefore has the following objectives: (1) obtain genome size (C-value) estimates for most subordinate taxonomic groups of Pooideae, (2) use this taxonomic sampling as complete as possible to address variation within Pooideae and its potential evolutionary significance, (3) provide chromosome numbers and ploidy levels of the taxa studied, since polyploidy as an important factor of holoploid genome size variation is common in this subfamily, (4) calculate monoploid genome sizes (1Cx) to compare taxa with different chromosome base numbers *x*, (5) find out whether the different chromosome sets in Pooideae, such as $x=7$ or $x=12$, are associated with a proportional difference in 1Cx genome size, since in principle it should not matter to an organism whether its genetic information is distributed over a few or many chromosomes in its monoploid chromosome set, and (6) contribute to a better understanding of the genomic and chromosomal evolution of the BOP clade and in particular of the Pooideae in comparison to their related subfamilies Oryzoideae and Bambusoideae.

Material and methods

Plant material

Our sample included 63 specimens from 59 species and 33 genera. One to three accessions per species were examined, which are listed individually in Online Resource 1. Fresh leaves for the genome size analyses were collected in the field, from living potted plants of our greenhouse research collection or from the plant collections of the Botanical Garden of the University of Halle-Wittenberg. Leaf samples were either processed immediately or stored in plastic bags with moist tissue in a refrigerator at 4 °C for up to five days until processing. In other cases, silica gel-dried leaves, preferably stored at -20 °C or -80 °C, were successfully used. Voucher specimens of most accessions are deposited in the herbarium of the University of Halle-Wittenberg (HAL). Details on the collections of the analyzed taxa can be found in the Online Resource 1.

Measurement of genome sizes

Relative genome sizes were estimated by FCM following the protocols of Doležel et al. (2007), Sliwinska et al. (2022), Koutecký et al. (2023) and Loureiro et al. (2023) with minor modifications. Tissue preparation, propidium iodide (PI) staining, measurements and equipment used followed our previous studies (Tkach et al. 2025; Winterfeld et al. 2025). The determined genome sizes and the standard species provided by the Institute of Experimental Botany, Academy of Sciences of the Czech Republic, grown in our greenhouses and used for the measurements, are listed in Online Resource 1. Genome sizes are given in picograms [pg] and can be converted to DNA content in base pairs [bp] by multiplication with the conversion factor 0.978×10^9 (Doležel et al. 2003). Previously published DNA C-values were obtained from the ‘Plant DNA C-values Database’ (Leitch et al. 2019; Henniges et al. 2023; https://cvalues.science.kew.org/) and from the original publications if these have been published after 2019 (see Results and discussion, References). Preference was given to genome sizes estimated by FCM using an internal standard as reference genome together with the sample and PI as fluorescent dye (Doležel and Bartoš 2005), hereafter abbreviated as FCM + PI. The additional literature data are listed in Online Resource 2.

Chromosome numbers, monoploid genome sizes (1Cx values), and mean chromosome sizes (MC)

Chromosome counts were taken from the Chromosome Counts Database (CCDB 2023) (see Rice et al. 2015; Rice and Mayrose 2023) or from original publications. For several accessions, chromosomes were counted in this study or in previous studies from our laboratory (Online Resource 1). Root tips were harvested from cultured potted plants. Enzymatically digested root tips were squashed and stained in 45% propionic acid with 2% carmine (Winterfeld et al. 2018). Monoploid genome sizes (1Cx values) were calculated for species with known chromosome number or ploidy by dividing the 2C values by the respective ploidy level (Greilhuber et al. 2005). The mean DNA content per chromosome (MC) in picograms was calculated by dividing the 2C values by the diplophasic (sporophytic) chromosome number (2*n*) or by dividing the 1C values by the haplophasic (gametophytic) chromosome number (*n*), i.e. 2C/2*n* or 1C/*n*, respectively.

Systematics and taxonomy of the study group

The subfamily Pooideae includes several subgroups, some of which have different taxonomic circumscriptions and ranks depending on the author, such as supertribe, tribe, and subtribe. An overview of the classifications used for the analyzed taxa is given in Table 1.

Results and discussion

Genome and chromosome sizes of the Pooideae

2C values. The holoploid genome sizes (diplophasic, i.e., sporophytic genome sizes of the non-replicated nuclear DNA) of the Pooideae (Tables 2, 3; Figs. 1, 2; Online Resource 1) ranged from 0.67 pg/2C in *Brachypodium stacei* ($2n = 2x = 20$) to 45.26 pg/2C recorded for the Triticeae species *Thinopyrum ponticum* ($2n = 10x = 70$) (Vogel et al. 1999), implying approximately 68-fold variation. The values thus spanned the genome size categories "very small" (≤ 2.8 pg/2C) to "large" (≥ 28 and < 70.0 pg/2C) (Leitch et al. 1998). Consistently low 2C values ("very small") occurred in the tribe Brachypodieae, followed by tribes Ampelodesmeae, Brachyelytreae, Duthieeae, Nardeae, Phaenospermateae with "small" (≥ 2.8 and ≤ 7.0 pg/2C), and both Stipeae with very small and Diarrheneae with small to just "medium-sized" values (> 7.0 and < 28.0 pg/2C), respectively. Medium-sized genomes were also found in Littledaleeae (*Littledalea racemosa*), and several other tribes, whose genome sizes, however, varied widely, ranging from "very small" to "large" such as in Aveneae, Bromeae, Festuceae, Meliceae, Poeae, Triticeae and presumed ancient hybrids between some of these tribes (Tables 2, 3). Lygeeae (only *Lygeum spartum* with two cytotypes) had a consistent "medium" to "large" genome size. No data on genome sizes were available for the Brylkinieae.

1Cx values. The sizes of the monoploid non-replicated chromosome sets with the base number x (1Cx value) ranged in subf. Pooideae from 0.33 pg/1Cx in *Austrostipa scabra* subsp. *scabra* (Stipeae) to 9.19 pg/1Cx in *Anthoxanthum gracile* (Aveneae), representing a 27.8-fold variation (Tables 2, 3; Figs. 1, 2; Online Resource 1) (Chumová et al. 2015; Tkach et al. 2024). The tribe Brachypodieae (genus *Brachypodium* only) was characterized by consistently low 1Cx values of 0.34–0.52 pg/1Cx, followed by Stipeae, Ampelodesmeae, Duthieeae, Diarrheneae, Phaenospermateae and Nardeae with up to 2.01 pg/1Cx. Aveneae, Bromeae, Festuceae, Meliceae, Poeae and Triticeae were more variable. The Triticeae lacked genomes of < 3.43 pg/1Cx and were characterized by larger genomes of up to 9.45 pg/1Cx. Aveneae, Festuceae, Poeae and their presumed hybrid taxa had predominantly medium-sized monoploid genomes of mostly about 1.2–4.0 pg/1Cx, but included, besides *Anthoxanthum gracile*, two other taxa with large genomes of > 8.0 pg/1Cx, *Echinaria capitata* (Seslerieae) and *Tricholemma jahandiezii* (Aveneae) (Table 3; Figs. 1, 2) (Tkach et al. 2024). The monogeneric Lygeeae (*Lygeum spartum* only) had a medium-sized monoploid genome of about 5.45 pg/1Cx.

Mean chromosome DNA content (MC). Chromosomes sizes of the Pooideae varied altogether between 0.02 pg (*Austrostipa scabra* subsp. *scabra*) and 1.84 pg (*Tricholemma jahandiezii*), representing a 92.0-fold variation (Tables 2, 3; Figs. 1, 2; Online Resource 1). The smallest chromosomes with MC < 0.1 pg occurred in Ampelodesmeae, Brachypodieae, Duthieeae and all sampled Stipeae except for *Macrochloa tenacissima*, which had an MC of 0.16 pg. Chromosomes of 0.1–0.2 pg also occurred in the sampled Brachyelytreae, Diarrheneae, Nardeae and Phaenospermateae. Meliceae and even more so Aveneae, Bromeae, Festuceae, Poeae, the presumed hybrid taxa and Triticeae showed a comparatively wide range of MCs of altogether 0.11–1.84 pg. Poeae had predominantly small chromosomes (MC 0.11–0.90 pg), whereas Triticeae were characterized by predominantly larger chromosomes (MC 0.49–1.35 pg). Lygeeae (*Lygeum spartum*) had medium-sized chromosomes of about 0.54 pg. As no chromosome numbers were available for Littledaleeae, the MC data for this tribe are missing.

Characteristics of the Pooideae tribes

'Early-diverging' lineages

The **tribe Brachyelytreae** comprises only one genus, *Brachyelytrum*, which occurs in a disjunction between the temperate regions of eastern North America and East Asia (Saarela et al. 2003). Its two American species have been studied, *B. aristosum* and *B. erectum*, which had identical of 3.13 pg/2C and therefore most likely have the same ploidy level and chromosome number. Considering $x = 11$ obtained from $2n = 22$ of *B. erectum* counted in this study, the 1Cx value would be 1.57 pg and the MC would be 0.14 pg in both species (Tables 2, 3; Figs. 1, 2; Online Resource 1). For *B. erectum* we unfortunately gave the incorrect number $2n = 130–133$ in a previous publication (Schneider et al. 2011). Another grass species had grown unnoticed in the pot with *Brachyelytrum*, whose root tips were used for chromosome counting in our previous study.

The 2C values of 2.9 pg and 3.0 pg in two *B. erectum* accessions, also determined by FCM + PI (Bai et al. 2012), are in good agreement with our results.

The **tribes Nardeae and Lygeeae** contain only the two monospecific genera *Nardus* and *Lygeum*.

Table 2 Summary of the subfamily Pooideae taxa examined with 2C values, chromosome numbers, ploidy level, 1Cx values and mean chromosome DNA content (MC). In the case of several accessions of the same taxon and cytotype, the mean values are given. Chromosome numbers were counted in our laboratory (asterisk) or were taken from the CCDB (2023) and original literature. Square brackets indicate inferred chromosome numbers and ploidy levels based on 2C values and available congeneric species data. The dash indicates values that were not calculated due to allopolyploidy. Online Resource 1 provides complete details of the analyzed samples and measurements. *N/A* not available

Taxon	2C value [pg]	2*n* chromosome number	Ploidy level	1Cx value [pg]	MC [pg]
Ampelodesmeae					
Ampelodesmos mauritanicus (Poir.) T.Durand & Schinz	4.46	48*	4*x*	1.12	0.09
Brachyelytreae					
Brachyelytrum aristosum (Michx.) P.Beauv. ex Trel.	3.13	N/A	N/A	N/A	N/A
Brachyelytrum erectum (Schreb.) P.Beauv.	3.13	22*	2*x*	1.57	0.14
Brachypodieae					
Brachypodium distachyon (L.) P.Beauv.	0.71	10*	2*x*	0.36	0.07
Brachypodium distachyon	1.46	20*	4*x*	0.37	0.07
Brachypodium hybridum Catalán, Joch.Müll., Hasterok & G.Jenkins	1.39	30*	4*x*	–	–
Brachypodium pinnatum (L.) P.Beauv.	1.61	28	4*x*	–	–
Brachypodium stacei Catalán, Joch.Müll., L.A.J.Mur & T.Langdon	0.67	20*	2*x*	0.34	0.03
Brachypodium sylvaticum (Huds.) P.Beauv.	1.04	18*	2*x*	0.52	0.06
Bromeae					
Boissiera squarrosa (Sol.) Nevski	3.81	14*	2*x*	1.91	0.27
Bromus epilis Keng f.	8.21	14	2*x*	4.11	0.59
Diarrheneae					
Diarrhena americana P.Beauv.	7.82	60*	6*x*	1.30	0.13
Neomolinia fauriei (Hack.) Honda	4.77	38*	4*x*	1.19	0.13
Neomolinia mandshurica (Maxim.) Honda	4.81	38*	4*x*	1.20	0.13
Duthieeae					
Danthoniastrum compactum (Boiss. & Heldr.) Holub	1.27	24*	2*x*	0.64	0.05
Duthiea bromoides Hack.	2.60	24*	2*x*	1.30	0.11
Metcalfia mexicana (Scribn.) Conert	3.38	N/A	N/A	N/A	N/A
Littledaleeae					
Littledalea racemosa Keng	17.19	N/A	N/A	N/A	N/A
Lygeeae					
Lygeum spartum Loefl. ex L.	21.80	40*	4*x*	5.45	0.55
Lygeum spartum	32.78	62*	6*x*	5.46	N/A
Meliceae					
Glyceria maxima (Hartm.) Holmb.	13.44	60	6*x*	2.24	0.22
Melica altissima L.	6.97	18*	2*x*	3.49	0.39
Melica ciliata L.	4.83	18*	2*x*	2.42	0.27
Melica hyalina Döll	10.82	36*	4*x*	2.71	0.30
Melica nutans L.	6.21	18*	2*x*	3.11	0.35
Melica picta K.Koch	7.06	18*	2*x*	3.53	0.39
Melica transsilvanica Schur	4.89	18*	2*x*	2.45	0.27
Schizachne purpurascens subsp. *callosa* (Turcz. ex Griseb.) T.Koyama & Kawano	1.61	20*	2*x*	0.81	0.08
Nardeae					
Nardus stricta L.	4.01	24*	2*x*	2.01	0.17
Phaenospermateae					
Phaenosperma globosum Munro ex Benth.	3.61	24*	2*x*	1.81	0.15
Stipeae					
Achnatherum calamagrostis (L.) P.Beauv.	1.66	24	2*x*	0.83	0.07

Table 2 (continued)

Taxon	2C value [pg]	$2n$ chromosome number	Ploidy level	1Cx value [pg]	MC [pg]
Achnatherum henryi var. *acutum* (L.Liu ex Z.L.Wu) S.M.Phillips & Z.L.Wu	1.95	[24]	[$2x$]	0.98	0.08
Achnatherum inebrians (Hance) Keng	4.01	[48]	[$4x$]	1.00	0.08
Achnatherum paradoxum (L.) Banfi, Galasso & Bartolucci	1.26	24	$2x$	0.63	0.05
Achnatherum pekinense (Hance) Ohwi	1.69	24	$2x$	0.85	0.07
Anemanthele lessoniana (Steud.) Veldkamp	1.58	44*	$4x$	0.40	0.04
Austrostipa acrociliata (Reader) S.W.L.Jacobs & J.Everett	1.58	44*	$4x$	0.40	0.04
Austrostipa blackii (C.E.Hubb.) S.W.L.Jacobs & J.Everett	2.40	44*	$4x$	0.60	0.05
Austrostipa breviglumis (J.M.Black) S.W.L.Jacobs & J.Everett	1.83	44*	$4x$	0.46	0.04
Austrostipa exilis (Vickery) S.W.L.Jacobs & J.Everett	1.50	[44]	[$4x$]	0.38	0.03
Austrostipa ramosissima (Trin.) S.W.L.Jacobs & J.Everett	1.94	44*	$4x$	0.49	0.04
Austrostipa scabra (Lindl.) S.W.L.Jacobs & J.Everett subsp. *scabra*	1.30	62*	$4x$	0.33	0.02
Austrostipa scabra subsp. *falcata* (Hughes) S.W.L.Jacobs & J.Everett	1.37	44*	$4x$	0.34	0.03
Austrostipa stipoides (Hook.f.) S.W.L. Jacobs & Everett	2.89	44*	$4x$	0.72	0.07
Austrostipa stuposa (Hughes) S.W.L.Jacobs & J.Everett	2.57	66*	$6x$	0.43	0.04
Austrostipa verticillata (Nees ex Spreng.) S.W.L.Jacobs & J.Everett	1.89	44*	$4x$	0.47	0.04
Celtica gigantea (Link) F.M.Vázquez & Barkworth	8.29	[96]	$8x$	1.04	0.09
Macrochloa tenacissima (L.) Kunth	3.82	24*	$2x$	1.91	0.16
Neotrinia splendens (Trin.) M.Nobis, P.D.Gudkova & A.Nowak	2.69	44*	$4x$	0.67	0.06
Oloptum miliaceum (L.) Röser & Hamasha	0.85	24	$2x$	0.43	0.04
Oloptum miliaceum	1.12	[36]	[$3x$]	0.37	0.03
Piptatherum holciforme (M.Bieb.) Roem. & Schult.	0.95	24	$2x$	0.48	0.04
Ptilagrostis dichotoma Keng ex Tzvelev	4.07	N/A	N/A	N/A	N/A
Stipa capillata L.	2.83	44	$4x$	0.71	0.06
Stipa tirsa Steven	2.69	44*	$4x$	0.67	0.06
Triticeae					
Agropyron cristatum (L.) Gaertn.	31.24	28	$4x$	7.81	1.12
Elymus hystrix L.	21.07	28*	$4x$	5.27	0.75
Elymus sibiricus L.	26.56	28	$4x$	6.64	0.95
Hordelymus europaeus (L.) Jess. ex Harz	20.11	28	$4x$	5.03	0.72
Kengyilia hirsuta (Keng) J.L.Yang, C.Yen & B.R.Baum	33.13	42	$6x$	5.52	0.79
Kengyilia thoroldiana (Oliv.) J.L.Yang, C.Yen & B.R.Baum	32.75	42	$6x$	5.46	0.78
Leymus angustus (Trin.) Pilg.	26.11	28	$4x$	6.53	0.93
Psathyrostachys huashanica Keng f. ex P.C.Kuo	17.89	14	$2x$	8.95	1.28

Nardus stricta, a species widespread in the Holarctic, had a mean value of 4.02 pg/2C based on three studied accessions (Table 2; Online Resource 1). This is consistent with previous studies that also used FCM + PI, which recorded values of 3.71–4.16 pg/2C (Šmarda et al. 2013, 2019; Zonneveld 2019). Although the chromosome number of this species appears to be slightly variable, $2n = 24$ was the most frequently observed count (G. Winterfeld unpublished data). Previous studies have reported $2n = 26$ as most common (CCDB 2023), although Rychlewski's extensive study (1967) found different numbers ranging from $2n = 22–28$, with $2n = 26$ still being the most frequent. The base number can only be speculated upon, but it is possible that it is $x = 12$ and *Nardus stricta* is diploid. Assuming this, the 1Cx value (Table 2) would be 2.01 pg, with an MC of 0.17 pg.

However, it cannot be ruled out that *Nardus* is triploid or hypotriploid, based on $x = 9$ or 10. This could explain the apomictic, or more precisely agamospermous, reproduction that has been demonstrated in this species (Rychlewski 1961; Kissling et al. 2006). It is not yet known whether *Nardus* is

Table 3 Genome sizes (holoploid 2C and monoploid 1Cx values) and mean chromosome DNA content (MC) of the examined representatives of the tribes of Poaceae subfamily Pooideae. The most frequent chromosome base numbers, if there are several in a tribe, are printed in bold. For details on our data see Table 2 and Online Resource 1. For further data as specified in Material and Methods see the individual tribes in Results and Discussion. Data for the tribes Aveneae, Festuceae, Poeae and their hybrids are from Tkach et al. (2024)

Tribes and chromosome base numbers	2C value [pg]	1Cx value [pg]	MC [pg]
Ampelodesmeae ($x=12$)	4.46	1.12	0.09
Aveneae ($x=4$, 5, 6, **7**)	2.44–44.75	1.22–9.19	0.18–1.84
Brachyelytreae ($x=11$)	3.13	1.57	0.14
Brachypodieae ($x=5$, 7, 8, 9, 10)	0.67–1.61	0.34–0.52	0.03–0.07
Bromeae ($x=7$)	3.80–40.50	1.90–5.82	0.27–0.83
Brylkinieae ($x=10$)	N/A	N/A	N/A
Diarrheneae ($x=9$, 10)	4.77–7.82	1.19–1.30	0.13
Duthieeae ($x=12$)	1.27–3.38	0.64–1.30	0.05–0.11
Festuceae ($x=7$)	3.01–30.48	1.51–5.44	0.22–0.78
Littledaleeae ($x=?$)	17.19	N/A	N/A
Lygeeae ($x=8?$, 10)	21.80–32.78	5.45–5.46	0.54–0.55
Meliceae ($x=8$, 9, **10**)	1.61–13.44	0.81–3.53	0.08–0.39
Nardeae ($x=12?$)	4.02	2.01	0.17
Phaenospermateae ($x=12$)	3.61	1.81	0.15
Poeae ($x=2$, 4, 5, **7**)	1.49–36.73	0.75–6.07	0.11–0.90
Stipeae ($x=7$, 8, 9, 10, **11**, **12**, 14)	0.85–8.29	0.33–1.91	0.02–0.16
Triticeae ($x=7$)	6.85–45.91	3.43–9.45	0.49–1.35
Intertribe hybrids of Aveneae/Festuceae/Poeae ($x=4$, **7**, 9)	2.86–38.89	1.13–8.30	0.16–0.92

N/A not available

an obligate apomict, producing caryopses asexually in all cases, or a facultative apomictic, producing agamospermous fruits only in some cases. Since sexual reproduction is infrequent in diplosporic apomicts like *Nardus*, Kissling et al. (2006) suggested that *Nardus* primarily reproduces through agamospermy, a common way for triploid plants to avoid meiosis failure due to incorrect chromosome segregation.

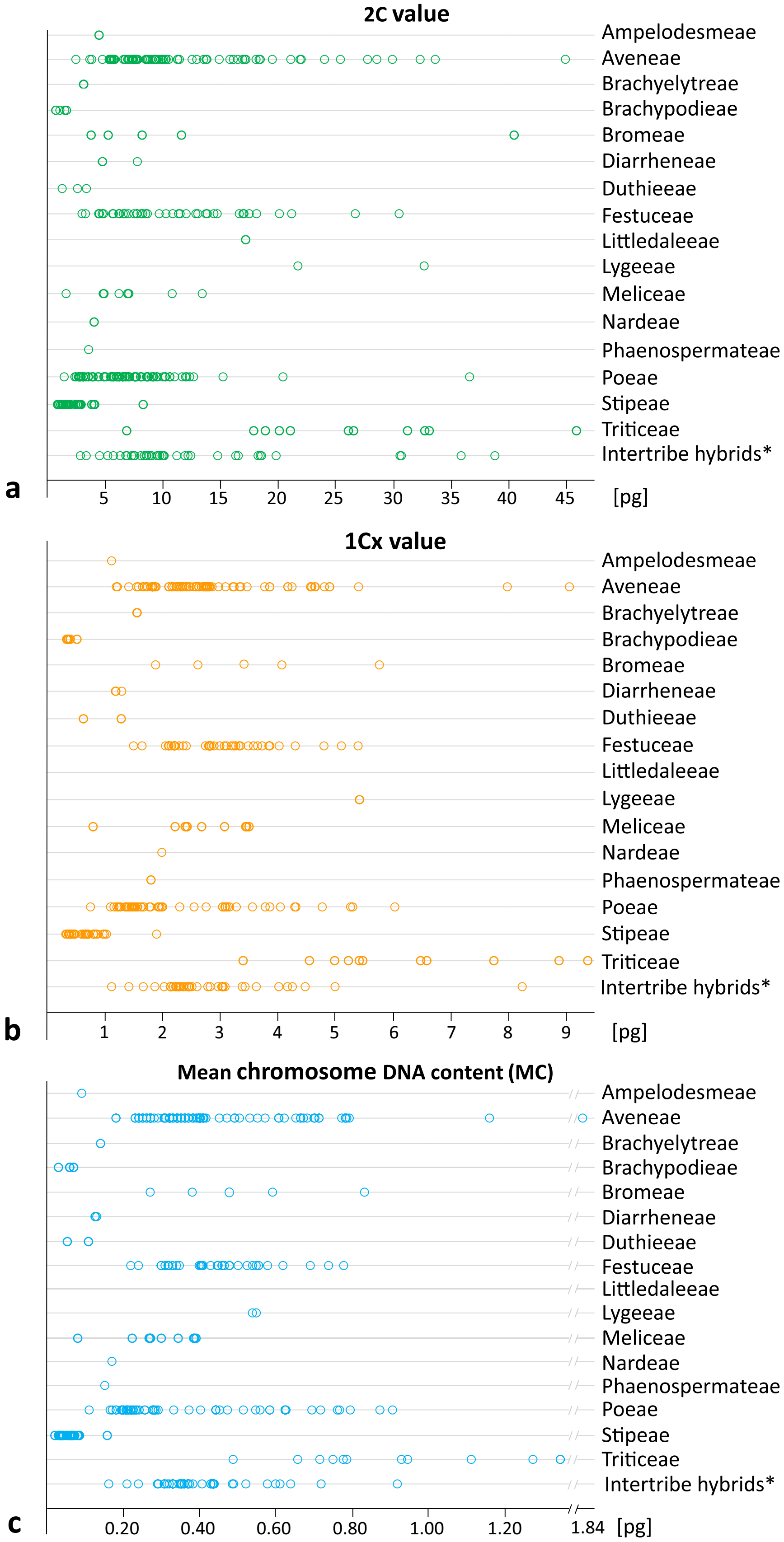
In the Mediterranean *Lygeum spartum* we sampled two accessions with $2n=40$ and one with $2n=$ ca. 62, which can best be interpreted as $2n=60$ plus two accessory or B-chromosomes. The plants with $2n=40$ had 2C values of 21.80 pg (mean value), broadly consistent with the previously estimated value of 19.75–21.53 pg in seven accessions of this cytotype, also by using FCM + PI (Abdeddaim-Boughanmi et al. 2019). The plants with $2n=62$ had 32.78 pg/2C. The base number is probably $x=10$, so that the 1Cx values for the presumably tetraploid accessions are about 5.45 pg and for the hexaploid accession 5.46 pg. The 1Cx values of the two cytotypes are therefore relatively uniform, as is the MC, which is almost consistently 0.55 pg. Interestingly, *Lygeum* was previously found to have $2n=16$ in two accessions from Algeria, in addition to the $2n=40$ plants that are more common in the Mediterranean region (Abdeddaim-Boughanmi et al. 2019). Therefore, it has been suggested that *Lygeum spartum* has two different base numbers, namely $x=8$ and $x=10$. The present study cannot contribute to this question, but it seems that the numbers and genome sizes found in this study are consistently based on $x=10$, although numerically they could also be based on $x=8$, assuming pentaploidy ($2n=5x=40$) and hypo-octoploidy ($2n=8x=64$) for our two cytotypes. In this case, however, one would expect more deviating 1Cx values than are found, namely 4.36 pg and 4.10 pg. Due to the almost consistent 1Cx values of about 5.45 pg, the assumption of tetra- and hexaploidy it is more plausible, suggesting that both cytotypes uniformly have a monoploid chromosome set of $x=10$.

The **tribe Duthieeae** consists of seven genera and has a unique disjunctive distribution across Central Asia (*Sinochasea*, *Stephanachne*), Southeast Australia (*Anisopogon*), the Himalayas (*Duthiea*, *Pseudodanthonia*), the Balkan Peninsula, the Caucasus (*Danthoniastrum*) and Mexico (*Metcalfia*). *Pappagrostis* Roshev. is sometimes considered as the eighth genus (e.g. Soreng et al. 2022), but it is actually a superfluous name for *Stephanache* Keng, as Roshevits himself acknowledged (Schneider et al. 2011: p. 41).

The sampled Duthieeae taxa, including *Danthoniastrum compactum*, *Duthiea bromoides* and *Metcalfia mexicana*, had 2C values of 1.27 pg, 2.60 pg and 3.38 pg, respectively (Tables 2, 3; Figs. 1, 2; Online Resource 1). The former two taxa have $2n=2x=24$, while the chromosome number of *Metcalfia* is unknown. Therefore, the 1Cx values were 0.64 pg and 1.30 pg, respectively. The MC of *Danthoniastrum compactum* (0.05 pg) was about half that of *Duthiea bromoides* (0.11 pg).

The **tribe Phaenospermateae** comprises only *Phaenosperma globosum*, a species found in the forests of subtropical to temperate eastern Asia. *Phaenosperma globosum* is diploid ($2n=24$) with a 2C value of 3.61 pg, a 1Cx value

Fig. 1 Variation of genome size and chromosome DNA content in the studied tribes of Poaceae subfamily Pooideae. **a** Holoploid 2C genome sizes. **b** Monoploid 1Cx genome sizes. **c** Mean chromosome DNA contents (MC). For our data see Table 2 and Online Resource 1, for further data as specified in Material and methods see the individual tribes in Results and discussion. Data for the Bromeae include genome size estimates of Joachimiak et al. (2001), for the Meliceae of Bai et al. (2012), for the Triticeae of Vogel et al. (1999), Jakob et al. (2004) and Eilam et al. (2007). These additional data are listed in Online Resource 2. Data for the tribes Aveneae, Festuceae, Poeae and their probable inter-tribe hybrids (asterisk) are from Tkach et al. (2024)



of 1.81 pg and an MC of 0.15 pg (Tables 2, 3; Figs. 1, 2; Online Resource 1).

The **tribe Meliceae** is mainly distributed in temperate regions of the world and comprises about 8 genera and 160 species (Soreng et al. 2017).

The 2C values in the Meliceae ranged from 1.61 pg in diploid *Schizachne purpurascens* subsp. *callosa* ($2n=20$) to 13.44 pg in hexaploid *Glyceria maxima* ($2n=60$) (Tables 2, 3; Figs. 1, 2; Online Resource 1). Both genera have $x=10$, but their 1Cx values and MC differ greatly, with 0.81 pg versus 2.24 pg and 0.08 pg versus 0.22 pg, respectively. *Schizachne* belonged to the taxa with the smallest 2C values and chromosome sizes (MC) among the taxa studied, along with the tribe Brachypodieae, most Stipeae and *Danthoniastrum compactum* of the Duthieeae (Table 2; Online Resource 1). Bai et al. (2012) recorded 2C values of 1.8–2.8 pg for *S. purpurascens* subsp. *purpurascens* from North America using FCM + PI, which is higher than in our accession from the Altai Mts.

Several diploid species of *Melica*, a genus with $x=9$, had 2C values of 4.83–6.98 pg, while tetraploid *M. hyalina* had 10.82 pg. The 1Cx values in *Melica* ranged from 2.42 pg to 3.49 pg. Comparatively small monoploid genomes (1Cx of 2.42–2.45 pg) occurred in *M. ciliata* and *M. transsilvanica*, which are morphologically similar Eurasian species of dry, open habitats, and were taxonomically classified under *M.* sect. *Dalycum* Dumort. subsect. *Ciliatae* (Lavrenko) Bor (Hempel 2012) or belonged to the ‘Ciliata clade’ according to molecular phylogenetic analyses (Khodaverdi et al. 2023). The largest monoploid genomes (1Cx of 3.11–3.53 pg) were found in *M. altissima*, *M. nutans* and *M. picta*, which are all Eurasian forest understory species and were assigned to different sections: the former to sect. *Altimelica* W.Hempel, while both *M. nutans* and *M. picta* belong to sect. *Melica* subsect. *Melica* (Hempel 2012). All three species were placed in the same ‘Nutans clade’ (Khodaverdi et al. 2023).

In previous studies using FCM + PI, the genome sizes of the sampled Meliceae taxa were 10.56 pg/2C and 13.3 pg/2C for *G. maxima*, 6.08 pg/2C for *M. altissima*, 4.06 pg/2C and 4.66 pg/2C for *M. ciliata*, 5.39 pg/2C and 5.7 pg/2C for *M. nutans* and 4.25 pg/2C for *M. transsilvanica* (Pustahija et al. 2013; Šmarda et al. 2019; Zonneveld 2019), which is in good to very good agreement with our results.

Ampelodesmos mauritanicus, the only member of the **tribe Ampelodesmeae** (Soreng et al. 2022), is a tall tussock grass found in the Western and Central Mediterranean. The accession examined was tetraploid with $2n=4x=48$ and had a genome size of 4.46 pg/2C, implying a 1Cx value of 1.12 pg and an MC of 0.09 pg (Tables 2, 3; Figs. 1, 2; Online Resource 1).

We examined 26 accessions from ten genera of the worldwide distributed **tribe Stipeae** s.s., which comprises about 28 genera and 530 species (Kellogg 2015a; Soreng et al. 2017). The 2C values, including diploids and polyploids, spanned 0.85–8.29 pg. Most of the sampled taxa had smaller monoploid genomes (1Cx values of 0.33–1.00 pg) and MCs (0.02–0.08 pg) than *Ampelodesmos*. However, the Ibero-Mauritanian, monogeneric genus *Celtica* (1.04 pg/1Cx and MC of 0.09 pg) had values that were similar to those of *Ampelodesmos* (Tables 2, 3; Figs. 1, 2; Online Resource 1). The examined accession of *C. gigantea* had a 1Cx value of 1.04 pg and an MC of 0.09 pg, and probably had $2n=8x=96$ (see CCDB 2023 and the review of chromosome numbers in the Stipeae by Tkach et al. 2021: Supplementary Appendix 2).

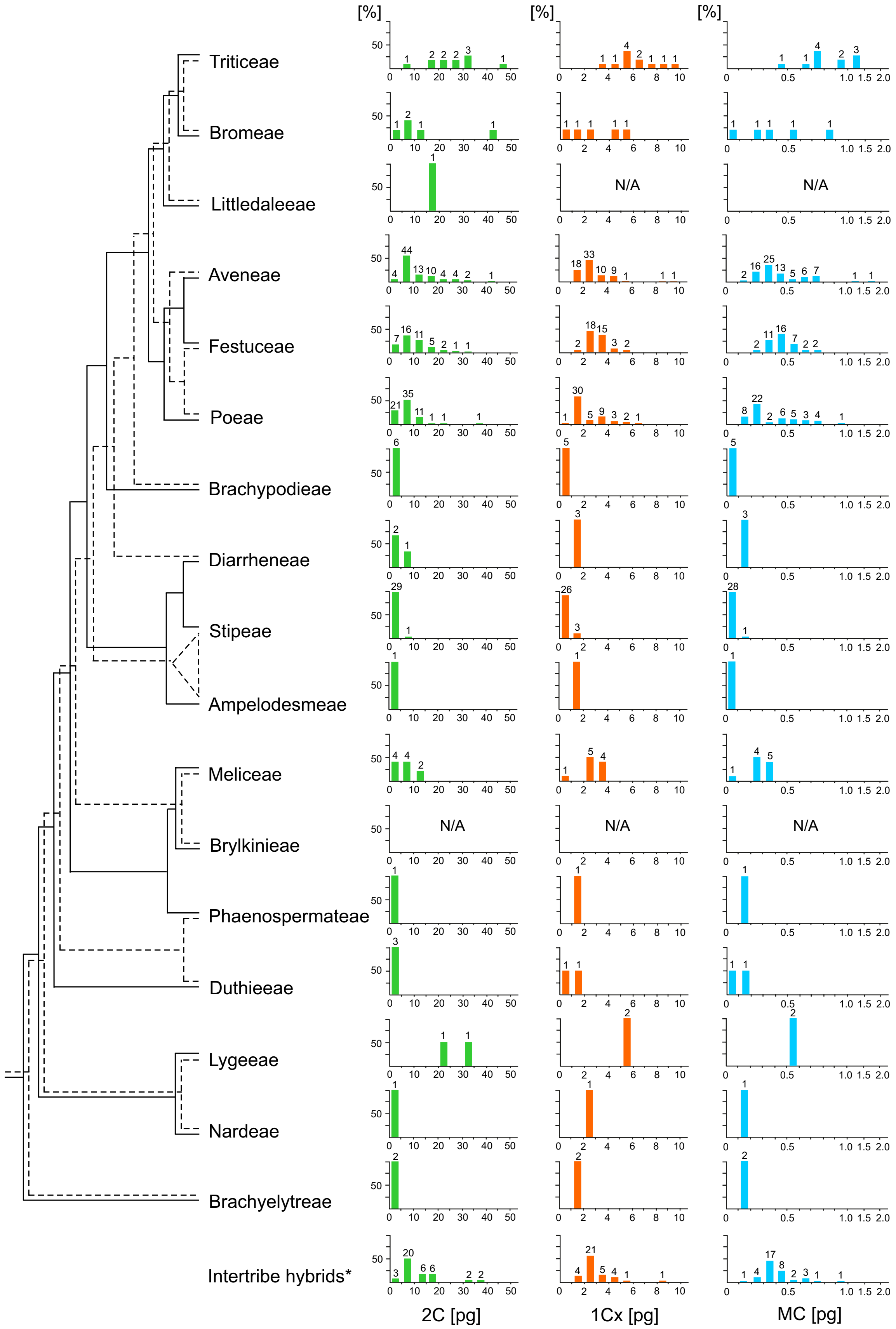
Macrochloa tenacissima is the only taxon of the Stipeae whose monoploid genome size (1Cx of 1.91 pg) and MC (0.16 pg) significantly exceeds the values of *Ampelodesmos*, *Celtica* and the rest of Stipeae (Table 2; Online Resource 1). *Macrochloa* is a genus from the southwest Mediterranean that harbors only 1–2 species. The more widespread of the two, *M. tenacissima*, has $2n=2x=24$, which was verified in the same accession that we examined for genome size.

The relatively large monoploid genome size of *M. tenacissima* and its monoploid number $x=12$ are significant from a phylogenetic perspective. This is because *Macrochloa* is sister to the rest of Stipeae with strong support according to the well-resolved molecular phylogenetic tree based on plastid DNA data (Romaschenko et al. 2012). The newly counted $2n=24$ in *Macrochloa* suggests that $x=12$ is likely the original base number of the tribe Stipeae supporting our previous reconstruction of chromosome number evolution in this tribe (Tkach et al. 2021: Fig. 7). Furthermore, Stipeae may have had a comparatively large genome size as an ancestral trait, despite their generally small genome sizes.

A reduction in genome size seems to have occurred in the Australasian genera *Austrostipa* and *Anemanthele*. These genera had mostly low 1Cx values of 0.33–0.60 pg and small MCs of 0.02–0.05 pg (Tables 2, 3; Figs. 1, 2; Online Resource 1). These Stipeae outliers are conspicuous in their chorology and are also characterized by a monoploid chromosome number $x=11$, which is exceptional within their major phylogenetic lineage. This lineage predominantly has $x=12$ (Tkach et al. 2021). Among *Austrostipa* species, only *A. stipoides* has a comparatively high 1Cx value of 0.66 pg and an MC of 0.07 pg, confirming previously noted unusually large chromosome sizes of this species (Winterfeld et al. 2015: Fig. 2a).

Small monoploids genomes of 0.37–0.48 pg/1Cx and MCs of 0.03–0.04 pg were found also in *Oloptum* and *Piptatherum*. It is worth noting that the two studied accessions of *O. miliaceum* differed in their 2C values, which may reflect different cytotypes (2*x*, 3*x*) previously found in this species (CCDB 2023).

Our data on genome sizes in the Stipeae (Tables 2, 3; Figs. 1, 2; Online Resource 1) largely confirm previous


[%]
[%]
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50
Triticeae
1 2 2 2 3 1
1 1 4 2 1 1 1
1 1 4 2 3
Bromeae
1 2 1 1
1 1 1 1 1
1 1 1 1 1
Littledaleeae
1
N/A
N/A
Aveneae
4 44 13 10 4 4 2 1
18 33 10 9 1 1 1
2 16 25 13 5 6 7 1 1
Festuceae
7 16 11 5 2 1 1
2 18 15 3 2
2 11 16 7 2 2
Poeae
21 35 11 1 1 1
1 30 5 9 3 2 1
8 22 2 6 5 3 4 1
Brachypodieae
6
5
5
Diarrheneae
2 1
3
3
Stipeae
29 1
26 3
28 1
Ampelodesmeae
1
1
1
Meliceae
4 4 2
1 5 4
1 4 5
Brylkinieae
N/A
N/A
N/A
Phaenospermateae
1
1
1
Duthieeae
3
1 1
1 1
Lygeeae
1 1
2
2
Nardeae
1
1
1
Brachyelytreae
2
2
2
Intertribe hybrids*
3 20 6 6 2 2
4 21 5 4 1 1
1 4 17 8 2 3 1 1
0 10 20 30 40 50
0 2 4 6 8 10
0 0.5 1.0 1.5 2.0
2C [pg]
1Cx [pg]
MC [pg]

◀**Fig. 2** Holoploid (2C) and monoploid (1Cx) genome sizes and mean chromosome DNA content (MC) arranged according to a phylogenetic tree of Poaceae subfamily Pooideae. DNA content intervals are shown on the x-axis of the bar graphs, while the y-axis represents the corresponding percentage estimates, which sum to 100% for each tribe. The number of estimates falling within each interval is displayed above the corresponding bar. The simplified phylogenetic tree is adapted from plastome-based phylogenetic analyses (Gallaher et al. 2019, 2022; Saarela et al. 2018; Schubert et al. 2019; Orton et al. 2021). Dashed lines indicate relationships based on nuclear Sanger sequence and phylogenomic analyses (Tkach et al. 2020; Baker et al. 2022; Huang et al. 2022; Zhang et al. 2022). For our data see Table 2 and Online Resource 1, for further data as specified in Material and methods see the individual tribes in Results and discussion. Data for the Bromeae include genome size estimates of Joachimiak et al. (2001), for the Meliceae of Bai et al. (2012), for the Triticeae of Vogel et al. (1999), Jakob et al. (2004) and Eilam et al. (2007). These additional data are listed in Online Resource 2. Data for the tribes Aveneae, Festuceae, Poeae and their probable intertribe hybrids (asterisk) from Tkach et al. (2024)

estimates of 2C values obtained by using FCM + PI, specifically *Achnatherum calamagrostis* (as *Lasiagrostis calamagrostis* or *Stipa calamagrostis* with 1.36 pg/2C and 1.74 pg/2C), *Anemanthele lessoniana* (1.89 pg/2C), *Austrostipa stipoides* (3.15 pg/2C) and several species of *Stipa* (2.24–2.74 pg/2C) (Murray et al. 2005; Pustahija et al. 2013; Šmarda et al. 2014, 2019).

The genome size of *S. capillata*, the first sequenced species from tribe Stipeae was 1,004 Mbp in length (≈ 2.05 pg/2C) (Baiakhmetov et al. 2021). This is lower than the genome sizes of 2.41 pg/2C (Šmarda et al. 2019) and 2.83 pg/2C (Tables 2; Online Resource 1) estimated for this species by FCM + PI. For *S. brevifolia*, a species not included in this study, similar genome sizes of approximately 1,060–1,090 Mbp sequence length (≈ 2.16–2.22 pg/2C) were recorded (Yun et al. 2023).

The **tribe Diarrheneae** comprises five species that exhibit a disjunctive distribution between eastern Asia and southeastern North America. This distribution is typical of many elements of broad-leaved deciduous forests of the northern temperate climate zone. The species are taxonomically grouped either in a single genus, *Diarrhena* s.l. (Clayton and Renvoize 1986; Kellogg 2015a) or in two genera, American *Diarrhena* and Asian *Neomolinia* (Tzvelev 1976, 1989; Schneider et al. 2009; Tzvelev and Probatova 2019; Soreng et al. 2022).

The representatives of the Diarrheneae all had relatively large 2C values (Tables 2, 3; Figs. 1, 2; Online Resource 1). *Diarrhena americana* had a 2C value of 7.82 pg with $2n = 6x = 60$ found in the same accession (Schneider et al. 2011). *Neomolinia fauriei* and *N. mandshurica* had 4.77 pg and 4.81 pg, respectively, both with $2n = 4x = 38$ verified in this study (Tables 2; Online Resource 1). The chromosome number of *Neomolinia* species likely resulted from allopolyploidy, involving crosses between ancestors with $x = 9$ and $x = 10$, or from reductive dysploidy, starting from a tetraploid ancestor with $2n = 40$. The 1Cx values of the Diarrheneae taxa showed minimal variation (1.19–1.30 pg), and the MC was consistently 0.13 pg (Table 1). The seemingly only available previous FCM estimate of genome size in the Diarrheneae was 7.8 pg/2C in *Diarrhena obovata* (Bai et al. 2012), which is consistent with our results.

Based on current classifications, the **tribe Brachypodieae** comprises solely the genus *Brachypodium* (Catalán et al. 2016a; Kellogg 2015a; Soreng et al. 2022), which consists of 16–22 species primarily located in Eurasia, with some outliers in Africa and America south of Mexico. *Brachypodium* exhibits significant variation in chromosome numbers, ranging from $2n = 10$ to $2n = 48$, including many intermediate numbers that demonstrate dysploid and polyploid variation. Several base numbers $x = 5$, 8, 9, and 10 have been identified, while the occurrence of $x = 7$ has not been confirmed (Robertson 1981). The only taxa with $2n = 28$ are *B. phoenicoides* and one of the cytotypes of *B. pinnatum* and *B. rupestre*. These taxa are not polyploids with $x = 7$, but rather dibasic allotetraploids based on $x = 9$ and $x = 5$ (Wolny and Hasterok 2009; Catalán et al. 2016a; Hasterok et al. 2022). It is possible that they are also based on $x = 9$ and $x = 8$, which implies a chromosome number reduction after polyploidization (Díaz-Pérez et al. 2018). Phylogenomic and comparative chromosome analyses suggest that the $x = 10$ such as found in *B. mexicanum* was ancestral in *Brachypodium* and the lower numbers were derived from it (Catalán et al. 2012, 2016a; Betekhtin et al. 2014; Lusinska et al. 2019; Hasterok et al. 2022; Sancho et al. 2022).

The **annual** *Brachypodium distachyon* s.l., formerly considered a single species with three cytotypes (Robertson 1981), has meanwhile been divided into *B. distachyon* s.s. ($2n = 2x = 10$), *B. stacei* ($2n = 2x = 20$) and *B. hybridum* ($2n = 4x = 30$) (Catalán et al. 2012, 2016b). *Brachypodium hybridum* is an allotetraploid hybrid that combines the $x = 5$ and $x = 10$ genomes of *B. distachyon* and *B. stacei* (Catalán et al. 2012; Hasterok et al. 2015; Lusinska et al. 2018).

The annual *Brachypodium* species in this study had 2C values of 0.71 pg in 2*x B. distachyon* s.s. (excluding the first found tetraploid; see below), 0.67 pg (mean value) in 2*x B. stacei*, and 1.39 pg (mean value) in 4*x B. hybridum*. In these accessions, kindly provided by P. Catalán, $2n = 10$, 20 and 30, respectively, were confirmed. A further accession of *B. stacei* from the Aegean Islands (Kos, Greece) had 0.70 pg/2C (Tables 2, 3; Figs. 1, 2; Online Resource 1).

Earlier genome size estimates using FCM + PI were 0.63 pg/2C in *B. distachyon* (Wolny and Hasterok 2009), 0.56 pg/2C in *B. stacei* and 1.27 pg/2C in *B. hybridum* (Catalán et al. 2012). Our data also shows the extensive additivity of the genome sizes of diploid *B. distachyon* and *B. stacei* in the tetraploid *B. hybridum*. Although these absolute values in this study differ by approximately 10–12% from the ones previously reported, the ratio of the genome size estimates (1:0.89:2.24) is largely consistent with ours (1:0.94:2.10). Further estimates of 2C genome sizes for *B. distachyon* and *B. hybridum* were 0.74 pg (mean value) and 1.43 pg (mean value), respectively (Savaş Tuna et al. 2019), which closely aligns with the values obtained in this study.

Genome sequencing studies have recorded the following sizes: 544 Mbp (≈ 0.56 pg/2C) for *B. distachyon* (IBI 2010), 461 Mbp (≈ 0.47 pg/2C) for *B. stacei* and 1,006 Mbp (≈ 1.03 pg/2C) for *B. hybridum* (Gordon et al. 2020). The absolute values are lower than the FCM estimates, as is frequently the case, but their ratio (1:0.84:2.19) is similar. Recently, comparable sequence lengths of 527 Mbp (≈ 0.54 pg/2C) for *B. stacei* and of 1,056–1,057 Mbp (≈ 1.08 pg/2C) for B. *hybridum*, respectively, were recorded (Mu et al. 2023a, b; Scarlett et al. 2023).

Interestingly, an accession of *B. distachyon* s.s. from Evvoia, Greece, consisted of tetraploid specimens with $2n = 4x = 20$, which is new for this species (Tables 2, 3; Online Resource 1). Two of three examined specimens had 2C values of 1.39 pg, the third one had 1.59 pg. The shape and length of the chromosomes under the microscope were typical for *B. distachyon* s.s. and not as small as in *B. stacei* (G. Winterfeld unpublished data). Therefore, autopolyploidy seems likely in this case, which is supported by the largely concordant 1Cx values of 0.35–0.40 pg and the MC of 0.07–0.08 pg of the widespread diploid and the new tetraploid cytotype of *B. distachyon* s.s.

Two **perennial** species of *Brachypodium* were additionally sampled, namely two accessions of *B. pinnatum*, which, according to the genome size probably represent the allotetraploid cytotype with $2n = 28$ based on $x = 5 + 9$ (Wolny and Hasterok 2009), and *B. sylvaticum* ($2n = 2x = 18$ verified in this accession), which had 1.61 pg/2C (mean value), and 1.04 pg/2C, respectively, which are reasonably consistent with the previously recorded values of 1.33–1.57 pg/2C and 0.78–0.95 pg/2C, respectively, also estimated using FCM + PI (Wolny and Hasterok 2009; Šmarda et al. 2019; Zonneveld 2019; Decena et al. 2024). For *B. sylvaticum*, the sequenced genome was found to be 717 Mbp (≈ 0.73 pg/2C) (Lei et al. 2024), which is therefore compatible with the FCM estimates. The 1Cx value in *B. sylvaticum* was 0.52 pg and the MC was 0.06 pg, while these values cannot be calculated for the allopolyploid *B. pinnatum*. The genome size parameters of both perennial species were comparable in magnitude to the values found in the annuals *B. distachyon*, *B. stacei* and *B. hybridum* but were consistently lower, suggesting that the change from perennial to annual life form in *Brachypodium* may have been associated with genome shrinkage, which would support the frequently observed correlation between annual life form and possession of small genomes (Carta et al. 2022).

'Core Pooideae'

The **'core Pooideae'** are the largest group within the subfamily Pooideae, consisting of approximately 170 genera and 3,250 species (Soreng et al. 2022). This group has a cosmopolitan distribution, with a focus on temperate regions that have a strongly seasonal climate, rather than the Tropics and subtropics. About 370 genome size estimates have been made for the species of the **supertribe Poodae** alone. They are only summarized in Table 3 and Figs. 1 and 2 and are discussed in more detail in a separate publication (Tkach et al. 2024).

The previously circumscribed **tribe Aveneae** (Tkach et al. 2020) comprises approximately 44 genera and 873 species, recalculated using data from Soreng et al. (2022), and has a cosmopolitan distribution. The 2C values ranged from 2.48 pg (mean value) in *Torreyochloa pallida* to 33.69 pg in *Helictotrichon filifolium* when both diploids and polyploids are included. The 1Cx values of diploids, based only on $x = 7$, ranged from 1.24 pg (mean value) in *T. pallida* to 4.98 pg in *Avena hispanica*. Their MCs were 0.18 pg (mean value) and 0.71 pg, respectively. In the diploids of *Anthoxanthum* with $x = 5$, *A. gracile* was reported to have an even higher 2C value of 18.38 pg (Table 3; Figs. 1, 2). Polyploids ($16x$–$18x$) had 2C values reaching up to 44.75 pg (Chumová et al. 2015).

The **tribe Festuceae** has approximately 23 genera and 702 species with a worldwide distribution. The 2C values range from 3.01 pg (mean value) in diploid *Lamarckia aurea* to 20.11 pg in polyploid *Lolium giganteum* ($6x$). The highest 2C value of 30.48 pg was found in $14x$ *Festuca yvesii* (Martínez-Sagarra et al. 2021). The diploids had consistently $x = 7$ in this tribe, and their 1Cx values ranged from 1.51 pg (mean value) in *Lamarckia aurea* to 5.44 pg (mean value) in *Festuca lachenalii* (syn. *Micropyrum tenellum*). The MCs were 0.22 pg and 0.78 pg, respectively (Table 3; Figs. 1, 2).

In the cosmopolitan **tribe Poeae**, which contains about 42 genera and 833 species, the 2C values ranged from 2.41 pg in *Poa persica* to 36.73 pg in the $12x$ cytotype of *Arctagrostis latifolia*, when both diploids and polyploids were included (Tables 2, 3; Figs. 1, 2; Online Resource 1). The 1Cx values of the diploids, considering only those with $2n = 14$, ranged from 1.21 pg in *Poa persica* to 6.07 pg in *Ventenata macra*. Their MC were 0.17 pg and 0.87 pg, respectively. The smallest genome size recorded for the Poeae was 1.49 pg/2C for *P. supina*, implying a 1Cx value of 0.75 pg and an MC of 0.11 pg (Mao and Huff 2012). The largest chromosomes in this tribe occurred in the dysploid *Colpodium biebersteinianum* ($x = 2$) with an MC (mean value) of 0.90 pg (Houben et al. 2003 and Kotseruba et al. 2003, 2010 as *Zingeria biebersteiniana*).

The taxonomically unplaced **groups of presumed hybrid origin** among the three tribes of the supertribe

Poodae mentioned above comprise 19 genera and 173 species. They had 2C values ranging from 2.86 (mean value) pg in *Corynephorus canescens* to 38.89 pg in *Helictochloa pratensis* when both diploids and polyploids are included (Table 3; Figs. 1, 2). The 1Cx values of diploids with $x = 7$ ranged from 1.43 pg (mean value) in *Corynephorus canescens* to 5.03 pg (mean value) in *Mibora minima*. The MC was 0.20 pg and 0.72 pg, respectively. Interestingly, *M. minima* is a diminutive, short-lived annual from the winter-mild regions of Western Europe that germinates in the fall and survives the winter as a plantlet. Its large genome size may be related to such life history and ecological traits as discussed for the 'neotenic' centrolepids of the grass-like family Restionaceae (Winterfeld et al. 2025) and other examples mentioned there. Even higher 1Cx values of 8.30 pg (mean value) were found in the $x = 9$ species *Echinaria capitata* with an MC of 0.92 pg (mean value), also a small winter annual of the Mediterranean and Near East.

The studied representatives of the **supertribe Triticodae** came from all three recognized phylogenetic lineages of this group. The only genus of the **tribe Littledaleeae**, *Littledalea*, distributed with four species in Central Asia, had 17.19 pg/2C found in two examined accessions of *L. racemosa*, but its chromosome number is not known (Tables 2, 3; Figs. 1, 2; Online Resource 1).

The **tribe Bromeae** is distributed with 1–2 genera and about 165 species in temperate regions of both hemispheres. Two *Boissiera* accessions studied had 3.80 pg/2C, a 1Cx value of 1.91 pg and an MC of 0.27 pg (mean values) (Tables 2, 3; Figs. 1, 2; Online Resource 1). Previous studies using FCM + PI for diploid species of *Bromus*, a genus that consistently has $x = 7$, yielded 2C values ranging from 5.27 pg (*B. tectorum*) to 11.63 pg (*B. arvensis*) (Joachimiak et al. 2001; Pustahija et al. 2013; Šmarda et al. 2019; Zonneveld 2019 partly sub *Anisantha* K.Koch), which resulted in larger 1Cx values of 2.64–5.82 pg and larger MCs of 0.38–0.83 than in *Boissiera*. The 2C value of 8.21 pg found for *B. epilis* (Table 2),whose chromosome number was not verified, corresponds to that of diploid *Bromus* species. Its 1Cx value would therefore be 4.11 pg and the MC 0.59 pg. Polyploid species had up to 40.50 pg/2C, as recorded for a dodecaploid South American *Bromus* sp. ($2n = 84$) (Joachimiak et al. 2001; Tuna et al. 2001, 2006; Kłos et al. 2009; Pustahija et al. 2013), whose 1Cx would be 3.38 pg and the MC 0.48 pg.

In the **tribe Triticeae**, which comprises about 27 genera and 500 species principally mainly in temperate to warm regions of the northern hemisphere, the 2C value of the diploid *Psathyrostachys huashanica* ($2n = 14$ according to CCDB 2023) examined was 17.89 pg (Tables 2, 3; Figs. 1, 2; Online Resource 1). Its 1Cx value was thus 8.95 pg, and the MC was 1.28 pg. This agrees well with the 14.98–17.91 pg/2C recorded for other diploid *Psathyrostachys* species and also determined by FCM + PI (Vogel et al. 1999; Bernhardt 2016). Genome size estimates (FCM + PI) for many other wild diploid species of the tribe Triticeae that consistently have $x = 7$ yielded 2C values ranging from 6.85 pg (*Hordeum euclaston*) to 18.90 pg (*Secale montanum*) (Vogel et al. 1999; Jakob et al. 2004; Eilam et al. 2007; Bernhardt 2016). The 1Cx values of the diploid Triticeae therefore ranged from 3.43 pg to 9.45 pg and their MCs from 0.49 pg to 1.35 pg.

The polyploids examined in this study were probably tetraploid (*Agropyron cristatum*, *Elymus hystrix*, *E. sibiricus*, *Hordelymus europaeus*) or hexaploid (*Kengyilia hirsuta*, *K. thoroldiana*, *Leymus angustus*) according to CCDB (2023), with 20.11–33.13 pg/2C, 1Cx values of 5.03–7.81, and MCs of 0.72–1.12 pg (Table 2). The apparently highest 2C-genome size of the Triticeae recorded so far was in the decaploid *Thinopyrum ponticum*, averaging 45.26 pg, estimated by FCM + PI (Vogel et al. 1999).

Evolutionary patterns of genome size in the Pooideae

The variation of the holoploid genome sizes in subf. Pooideae is responsible for almost all known variation in the whole grass family (Tkach et al. 2025). In the Poaceae, no higher 2C values than in the Pooideae have been recorded so far, whereas lower values of 0.42 pg/2C and 0.48 pg/2C were found in two annual species of *Panicum* (subf. Panicoideae), viz., *P. gilvum* (chromosome number unknown) and *P. hillmanii* ($2n = 2x = 18$), and of 0.50 pg/2C (≈ sequenced 245 Mbp) in *Oropetium thomaeum* ($2n = 2x = 18$) (subf. Chloridoideae), which is also annual, respectively (VanBuren et al. 2015; Chen et al. 2021). In addition, a value of 0.5 pg/2C was obtained for the perennial to annual *Paspalum fimbriatum* ($2n = 2x = 20$) of subf. Panicoideae, but DAPI instead of PI was used as the fluorescent dye for FCM in this study (Galdeano et al. 2016), questioning the comparability of the measured value as DAPI preferentially binds to AT-rich DNA (Schweizer and Nagl 1976; Kapuściński and Szer 1979; Sumner 1990).

Most of the **'early-diverging' Pooideae lineages** had 2C values < 4 pg, 1Cx values < 2 pg and MCs < 0.2 pg, as exemplified by the tribes Ampelodesmeae, Brachyelytreae, Duthieeae, Nardeae, Phaenospermateae and Stipeae (Figs. 2, 3). The values were therefore relatively small, but not extremely small. The values in the tribe Meliceae were more variable and, for the most part, much larger. Especially the Lygeeae, with the only genus *Lygeum*, stood out among the 'early-diverging' lineages by their exceptionally large genomes (both 2C and 1Cx values) and chromosome sizes (MC).

Among the 'early-diverging' Pooideae lineages, the Brachypodieae with the single genus *Brachypodium* had

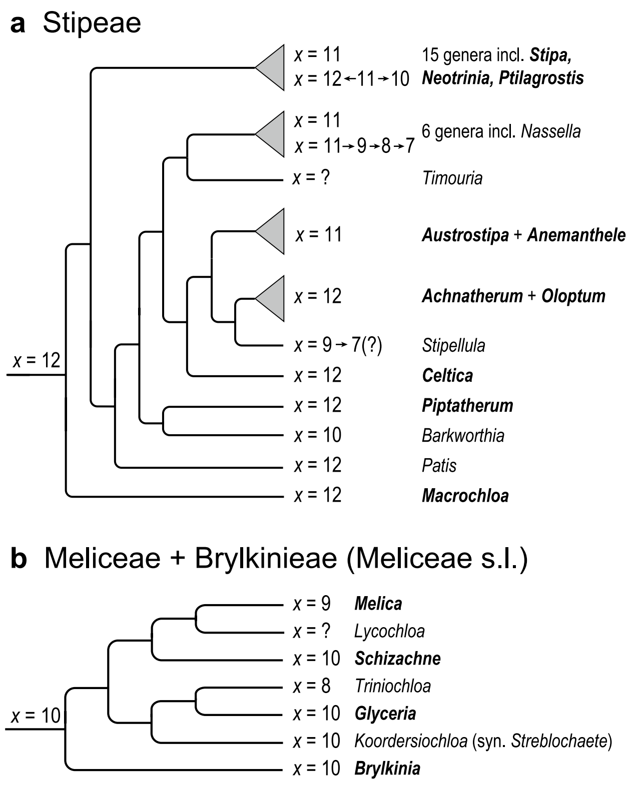

Fig. 3 Chromosome base numbers *x* in the tribes **a** Stipeae and **b** Meliceae and Brylkinieae (Meliceae s.l.) mapped on simplified phylograms showing the sister group relations of the genera and major clades. The parsimony-based reconstructions suggest $x=12$ in Stipeae and $x=10$ Meliceae and Brylkinieae as the most likely ancestral chromosome base numbers, from which the lower numbers were derived by reductional dysploidy. Genera analyzed in this study for genome sizes printed in bold. Phylogenetic trees modified from Schneider et al. (2009, 2011) and Tkach et al. (2021). For sources of chromosome numbers see Table 2 and Online Resource 1; otherwise see CCDB (2023), Pohl and Davidse (1971), Schneider et al. (2011), Tkach et al. (2021: Supplementary Appendix 2)

the smallest monoploid genomes (1Cx values < 0.52 pg) and chromosomes (MC < 0.07 pg), and possibly also holoploid genomes (2C < 1.61 pg); however, only taxa with up to 4*x* ploidy have been sampled in this study. The small monoploid genome sizes and small chromosomes in Brachypodieae are particularly interesting from an evolutionary point of view because, according to the available molecular phylogenetic data, this lineage is undoubtedly sister to the ‘core Pooideae’ with significantly larger 1Cx and MC sizes. Brachypodieae and ‘core Pooideae’ therefore differ markedly in genome and chromosome size, which in principle gives no indication of what these characters were like in their common ancestor. However, it is likely that the genome size of their ancestor was similar to that of the majority of ‘early-diverging’ lineages, and that the very small genomes of *Brachypodium* are due to an ‘autapomorphic’ reduction of the genomes. The same is probably true for a part of the Stipeae, where the Australasian outlier of this otherwise Eurasian-American tribe is characterized by particularly small genomes, which probably also evolved secondarily from larger ones (see below).

The **‘core Pooideae’** and the Brachypodieae (*Brachypodium*) have in common that both clades deviate from the otherwise almost universal $x=12$, which prevails in the ‘early-diverging’ lineages of the subfamily Pooideae. The ‘core Pooideae’ have mainly $x=7$ and smaller numbers $x=5$, 4, 2 in their phylogenetically derived groups. The Brachypodieae (*Brachypodium*) have among others, $x=10$ in the phylogenetically rather original species *B. mexicanum*, while $x=9$, 8, 5 are derived from $x=10$ (see above).

The question would be whether the chromosomes of Brachypodieae and ‘core Pooideae’ share any structural chromosome features that facilitated their fusion, implicitly reducing the base number from $x=12$ to $x=10$ (Brachypodieae) and $x=7$ (‘core Pooideae’), respectively. Both may have followed the model of karyotype evolution demonstrated for the origin of low chromosome number genomes within the genus *Brachypodium*, namely the predominance of nested chromosome fusions, i.e., the insertion of one chromosome into another, while a simultaneous relatively low incidence of other types of chromosome rearrangements was observed (Lusinska et al. 2018, 2019; Gordon et al. 2020; Hasterok et al. 2020; Sancho et al. 2022). Phylogenomic and comparative chromosome barcoding studies could help elucidate the evolutionary origin of the ‘core Pooideae’ genome. A comparison of the genomes of rice ($x=12$), *Brachypodium distachyon* ($x=5$) and species of *Avena* ($x=7$) revealed ancestral synteny defined by blocks of shared gene sequences in the chromosomes that were conserved among these taxa (Liu et al. 2023). Some rearrangements were shared between *Brachypodium* and the *Avena* species but not with rice, adding to the previously shown similarity of the gene order in *Brachypodium* and species from the tribe Triticeae (The International Brachypodium Initiative 2010; Kellogg 2015b). Further insights into the genome organization and synteny in the chromosomes of Littledaleeae/Bromeae/Titiceae and Aveneae/Festuceae/Poeae would be of great value for understanding the evolutionary origin and development of these important grass groups.

The genome size of Brachypodieae of 0.3–0.5 pg/1Cx (Table 2), which is similar to, or in some cases even smaller than that of rice (*Oryza sativa*; subf. Oryzoideae) of 0.5 pg/1Cx (Tkach et al. 2025), could be based on an autapomorphic genome miniaturization and does not represent the ancestral state of both Brachypodieae and Poeae. Our data on the monoploid genome sizes (1Cx) of ‘core Pooideae’ are therefore ambiguous with respect to a possible whole genome duplication at the origin of this lineage. Although 1Cx values < 1 pg are virtually absent and those < 2 pg are comparatively rare in the 'core Pooideae', in contrast to most of the tribes of the 'early-diverging' lineages with the exception of Lygeeae and Meliceae, the overall predominantly rather small monoploid genome sizes (1Cx), especially of the tribe Poeae (Fig. 2, middle column) do not support the hypothesis of a whole-genome duplication (WGD) for the ancestor of the 'core Pooideae', whereas such a WGD could not be excluded if only the mostly higher 1Cx values of Aveneae, Festuceae and especially Triticeae were considered. The absence of a 'core Pooideae'-specific WGD is further supported by the analysis of genomic synteny in the Pooideae (Zhang et al. 2022), in which nearly all gene duplications found were due to tandem duplication or transposon-mediated duplication and did not originate from WGD, with the exception of allopolyploidization events following hybridization, such as found in the 'core Pooideae' genera *Agrostis*, *Deschampsia*, *Poa* and *Sesleria* (Zhang et al. 2022).

The origin of the 'core Pooideae' chromosome set of $x = 7$ was therefore likely based on structural changes of the chromosome set leading to reduction of chromosomes, e.g. by different types of chromosome fusions (Schubert and Lysak 2011), without a major increase in DNA content. As expected from such fusions, this reduction in chromosome number was apparently accompanied by an increase in chromosome size, evidenced by increased MCs, for example in Aveneae, Festuceae and also Poeae (Fig. 2: right column), compared to the 'early-diverging' lineages of the Pooideae.

The dramatic increase of monoploid genome sizes (1Cx) and chromosome sizes (MC), which occurred in the Triticeae, while seemingly not in its sister tribe Bromeae (monoploid genome size of Littledaleeae unknown), was therefore not linked with, or caused by, the transition from $x = 12$ to $x = 7$ of the 'core Pooideae' and the involved structural chromosome re-organization, but only occurred only later.

Chromosome base numbers, paleogenomics and the origin of the Pooideae

Variation in the chromosome base number *x*. Although $x = 7$ is widespread, occurring in an estimated 75% of the total 4,130 species of the Pooideae, this is not the phylogenetically ancestral number. The more likely candidate is $\boldsymbol{x = 12}$, as suggested previously (e.g. Hilu 2004), which occurs in many of the 'early-diverging' lineages such as the monogeneric Ampelodesmeae (*Ampelodesmos mauritanicus*), Phaenospermateae (*Phaenosperma globosum*) (Avdulov 1931: p. 92; Myers 1947; Tateoka 1954, 1955, 1956; Decker 1964; Nilsson and Lassen 1971; Schneider et al. 2011; Winterfeld et al. 2015; Zhang et al. 2018) and also in Nardeae (*Nardus stricta*), where it is probably also the actual monoploid chromosome number (see above) instead of $x = 13$ as sometimes suggested. $2n = 2x = 24$ was also recorded for all taxa of the Duthieeae studied so far (*Danthoniastrum compactum*, *Duthiea brachypodium*, *Sinochasea trigyna*, *Stephanachne monandra*, *S. pappophorea* (Fedorov 1969: p. 565 citing an unpublished count of L.A. Alexandrova; Winterfeld 2006; Schneider et al. 2011; Zhang et al. 2018), whereas $2n = 14$ or $n = 14$ is incorrect (for discussion see Winterfeld 2006; Tkach et al. 2021). $2n = 24$ also occurs frequently in the tribe Stipeae, which appears to have more diverse monoploid chromosome numbers, as $x = 11$ and even $x = 8$, 9, 10 were also recorded (Winterfeld et al. 2015; Tkach et al. 2021; Supplementary Appendix 2). The base numbers mapped on a molecular phylogeny of the genera of Stipeae (Fig. 3a) support $x = 12$ as primary in this tribe, which is strongly supported by $2n = 2x = 24$ newly counted in *Macrochloa tenacissima* (Table 2; Online Resource 1). This species is the widespread one of the possibly two species of the western to southwestern Mediterranean genus *Macrochloa*, in molecular phylogenetic analysis the strongly supported sister to the rest of the tribe Stipeae (Romaschenko et al. 2012).

The monoploid number $\boldsymbol{x = 11}$ occurs not only in Stipeae but interestingly also in the monogeneric Brachyelytreae, which is sister to the rest of the Pooideae. This number therefore cannot be excluded out a priori as primary in the Pooideae, but it is unlikely because of the relationship of the Pooideae to the other subfamilies of the grasses, especially to the BOP clade (see below).

The number $\boldsymbol{x = 10}$ occurs in the tribe Meliceae, which also has $x = 8$ and $x = 9$. However, the primary number of this tribe is most likely $x = 10$, since this higher number is also found in the firmly established phylogenetic sister of Meliceae s.s., the monogeneric tribe Brylkinieae (Schneider et al. 2009, 2011) (Table 2). The Meliceae s.s. contains two sister lineages, one with *Koordersiochloa* (syn. *Streblochaete*) with $x = 10$ as sister to *Glyceria* ($x = 10$) and *Triniochloa* ($x = 8$), the other with *Schizachne* ($x = 10$) as sister to *Melica* ($x = 9$) and *Lycochloa* (chromosome number unknown), this all supporting $x = 10$ as ancestral and the lower numbers $x = 9$ and 8 as derived in Meliceae s.l. (Fig. 3b).

The monogeneric tribe Lygeeae, which includes only *Lygeum spartum*, has probably also $x = 10$, according to $2n = 40$ and the newly found $2n = 60$ (+ 2B?) (Online Resource 1). However, due to the occurrence of populations with $2n = 16$ at the southern edge of the range of this species in the western Mediterranean (see above), a further monoploid number $x = 8$ is also possible.

The Diarrheneae with $2n = 60$ in *Diarrhena* and $2n = 38$ in *Neomolinia* (Table 2; Online Resource (1). probably also go back to $x = 10$, whereby $2n = 38$ would then have to be regarded as hypotetraploid or attributed to an allopolyploid

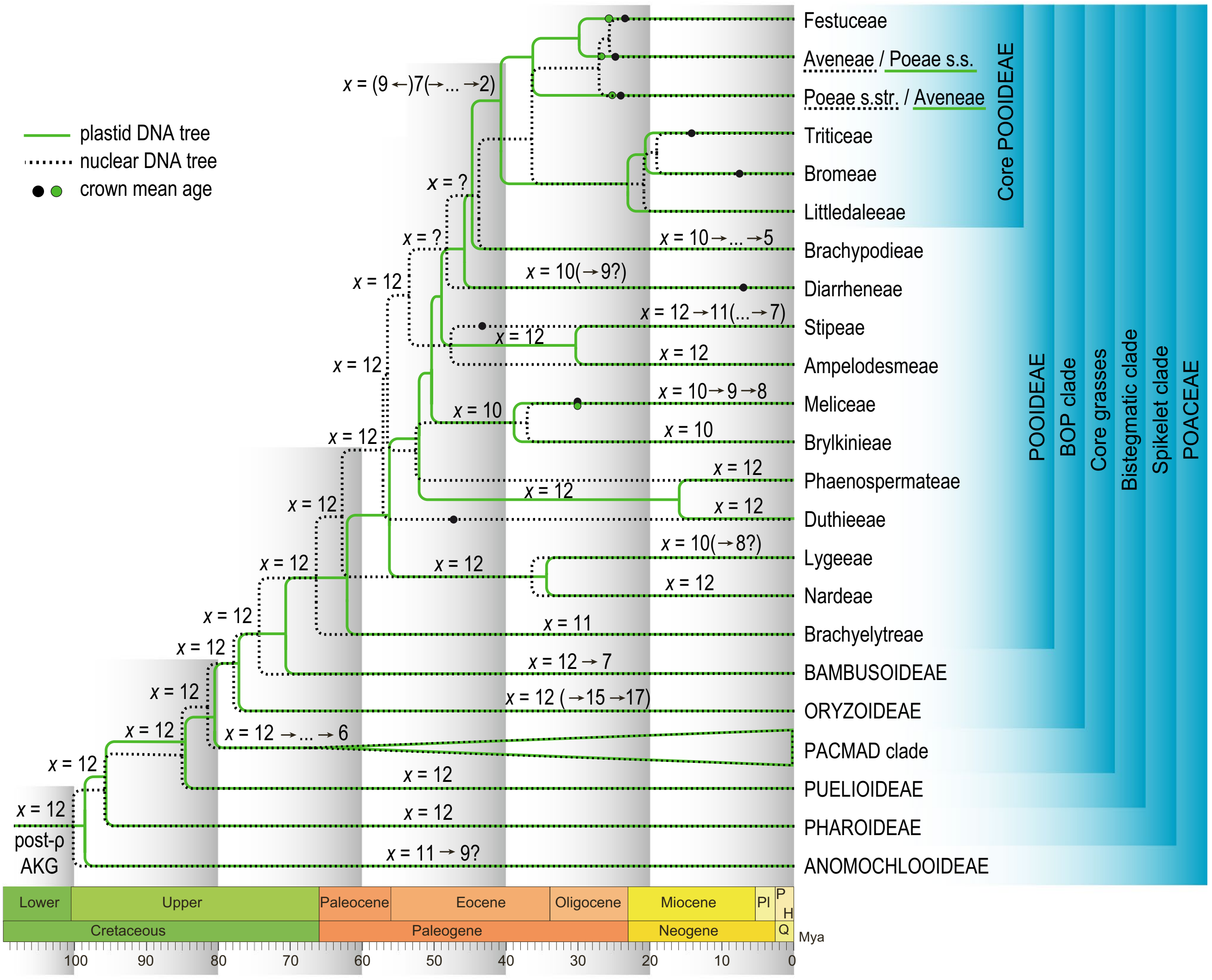

Fig. 4 Chromosome base numbers *x* and events of descending dysploidy in Pooideae plotted on a simplified phylogenetic tree showing its major lineages. The monoploid numbers (x) are given for each lineage, and the directions of dysploid change are indicated by arrows. The ancestral number of the subfamily Pooideae was most likely $x=12$, which is supported by the fact that it is shared by the subfamilies Bambusoideae and Oryzoideae. These are the sister subfamilies of Pooideae, and all three together form the BOP clade. The base number $x=12$ also occurs in the PACMAD clade, which is sister to the BOP clade, but it is less common. Outside of these 'core grasses', $x=12$ is also present in the subfamilies Puelioideae and Pharoideae, which belong to the phylogenetically 'early-diverging' grass lineages, while the Anomochlooideae, the most 'primitive' grasses lacking typical grass spikelets, have $x=11$ and possibly $x=9$, which may represent a 'derived', apomorphic character (Tkach et al. 2025). Dated phylogenetic trees based on plastid DNA sequence and nuclear phylogenomic analyses, adapted from Gallaher et al. (2022) and Huang et al. (2022). H = Holocene, P = Pleistocene, Pl = Pliocene, Q = Quaternary, post-ρ AKG = 'ancestral grass karyotype' with 12 chromosomes formed after the whole-genome duplication characteristic of all grasses. See Results and discussion for further explanation and Tkach et al. (2025) for details on the AKG

origin from chromosome sets with base numbers $x=10$ and $x=9$.

The base number $x=10$ is also primary in the Brachypodieae (*Brachypodium*), while $x=9$, 8, 5, which are found in several diploid and partly polyploid species of *Brachypodium*, are derived from it (see above).

Monoploid genome sizes (1Cx values). Assuming 2C values < 4 pg, 1Cx values < 2 pg, and MCs < 0.2 pg as ancestral in the subfamily Pooideae (see above), a comparison of these parameters, especially of the tribe Brachyelytreae, sister to the rest of the subfamily Pooideae, reveals some similarities between the 'early-diverging' Pooideae and the closely related subfamilies Bambusoideae (bamboos) and Oryzoideae (rice subfamily), with which Pooideae is united in the BOP clade. While bamboos show a much wider range of 2C values (about 3.2–7.0 pg/2C) due to their frequent polyploidy, their monoploid genome sizes of about 0.5–1.8 pg/1Cx and MCs < 0.15 pg are similar to those of Brachyelytreae and most other 'the early-diverging' Pooideae lineages. The Oryzoideae, however, are more divergent due to their overall smaller genome size parameters of < 1.8 pg/2C, about 0.5–1.75 pg/1Cx and MCs of 0.04 pg (Tkach et al. 2025: Table 2), which makes them similar to the tribe Brachypodieae with also 'miniaturized genomes' with rather few repetitive DNA in their genomes. This is shown by the average of only 36 Mbp of repetitive DNA present in the entire 375 Mbp rice genome (3,010 accessions of *Oryza sativa* analyzed) and 42 Mbp in the 234 Mbp genome of *Brachypodium stacei*, respectively (Wang et al. 2018; Lei et al. 2024). These very small amounts of repetitive DNA compared to other grasses may represent an evolutionarily 'derived' feature of their genomes rather than an ancestral trait. Instead, the putative ancestral genome parameters of the subfamily Pooideae are remarkably similar also to those of the most 'ancient' lineages of extant grasses, i.e. the subfamilies Anomochlooideae and Pharoideae, which have monoploid genome sizes of about 1.2–1.8 pg and MCs of about 0.1–0.2 pg (Tkach et al. 2025), while no genome size data are available for the subfamily Puelioideae, the next-diverging lineage in the phylogeny. Some of these 'early-diverging lineages' are also characterized by $x = 12$ (*Pharus*, *Puelia*), while the Anomochlooideae genus *Streptochaeta* ($2n = 22$) has $x = 11$ and *Anomochloa* ($2n = 36$) may have $x = 9$ (Tkach et al. 2025).

The base number $x = 12$ was probably also present in the sister lineage of the Pharoideae, i.e. the 'core grasses', which split into the BOP and the PACMAD clades, which are the two major lineages of present-day grasses (Fig. 4). While this number is present only in some extant lineages of the PACMAD clade, such as the subfamily Aristidoideae, which interestingly is sister to the rest of the PACMAD clade according to nuclear DNA phylogenetic analysis (Huang et al. 2022), whereas relationships among the subfamilies have been partly inconclusive and sensitive to the phylogenetic methods when using plastid DNA data (Teisher et al. 2017; Saarela et al. 2018; Duvall et al. 2020). However, most other lineages of the PACMAD clade have lower numbers $x = 9$ or $x = 10$ (Tkach et al. 2025: Fig. 3).

In the BOP clade, however, $x = 12$ predominates and is characteristic not only of the 'basal' Pooideae but also of the subfamily Oryzoideae, with the exception of *Zizania* with $x = 15$, 17, whose derivation from $x = 12$ is well understood (Kennard et al. 2000; Haas et al. 2021); and most lineages of the Bambusoideae, i.e. the tribes Bambuseae and Arundinarieae, which are woody and with few exceptions have $x = 12$, whereas the New World herbaceous tribe Olyreae mostly has $x = 20$, 22 (Tkach et al. 2025).

Chromosomal synteny analyses revealed that the genomes of *Oryza sativa* (Oryzoideae) and *Pharus latifolius* (Pharoideae), each with $x = 12$, differ by only a few rearrangements from the reconstructed ancestral grass karyotype (AGK) (Murat et al. 2017; Ma et al. 2021), respectively, and also found an extensive whole-genome gene collinearity between the bamboo and rice (Guo et al. 2019). The evolutionary stasis of the AGK until 42 Ma after the split of *Pharus latifolius* and the lineage leading to Pooideae and the other 'core grass' lineages noted previously (Ma et al. 2021) correlates with $x = 12$ as a nearly unchanged chromosome base number (Fig. 4).

Conclusions

The subfamily Pooideae shows the largest variation of holoploid genome sizes (2C values) of all grass subfamilies. The monoploid genome sizes (1Cx values of single chromosome sets) and the average DNA content of the chromosomes (MC values) are also highly variable. The maximum values of grasses with respect to these size parameters are found in the subfamily Pooideae, while the minimum values found in some panicoid grasses are still slightly lower than the smallest values in the Pooideae.

Within the Pooideae, the phylogenetically 'early-diverging' lineages are characterized by relatively small genome sizes and base numbers of predominantly $x = 12$. In this respect, they are similar to their most closely related grass subfamilies, the Bambusoideae and Oryzoideae. Moreover, these features of the BOP clade resemble those of the most primitive subfamilies of grasses, as far as they have been studied in this respect (*Streptochaeta* of the Anomochlooideae and *Pharus* of the Pharoideae). The features appear to be a retention of the 'original' characteristics of the ancestral grass karyotype (AGK), accordingly appears to have been fairly static in evolutionary terms.

In contrast, the Brachypodieae represent a tribe of the Pooideae, in which a striking reduction in genome size has occurred, which also distinguishes the Brachypodieae from their phylogenetic sister group, the 'core Pooideae'. The latter have predominantly larger monoploid genomes than the 'early-diverging' lineages and have a base number of mostly $x = 7$ with larger chromosomes. They probably originated by fusions of chromosomes from the $x = 12$ sets. Using the genome sizes data, there is no evidence for a WGD at the origin of the $x = 7$ clade.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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