Preslia 95: 185–213, 2023
doi: 10.23855/preslia.2023.185

Preslia

Evolution of genome size and GC content in the tribe *Carduinae* (*Asteraceae*): rare descending dysploidy and polyploidy, limited environmental control and strong phylogenetic signal

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Abstract: Genome size and GC content are basic species-specific attributes often delimiting genera or higher taxa, which enable the identification of polyploidy, hybridization and other modes of genome or karyotype evolution. The evolution of these genomic traits can often occur as a result of the selective pressure of the environment. Here, we reconstruct the evolution of these genomic traits in subtribe *Carduinae* (*Asteraceae*) in the context of changes in chromosome numbers. Using flow cytometry, genome size and GC content were estimated for 119 taxa and mapped onto a phylogenetic tree constructed using sequences from seven genetic markers. In addition, the genomic data were compared with the length of stomatal guard cells and achene size (length, weight) to evaluate the extent to which genomic characters could evolve adaptively in this subtribe. We found strong phylogenetic signals for the analysed genomic and phenotypic traits, which delimited most *Carduinae* genera or clades in agreement with the reconstructed phylogeny. Monoploid genome size was positively correlated with genomic GC content and stomatal guard cell length. In *Cirsium*, whose species were the focus of the majority of the analyses, the large-genomed subgen. *Lophiolepis* had smaller guard cells, which might be related to it occurring in more xeric habitats compared to subgen. *Cirsium*. In contrast, the achenes of the large-genomed subgen. *Lophiolepis* were larger, possibly in response to the summer drought, whereas achene weight and length were independent of genome size across the subtribe. Thus, genome size in the subtribe *Carduinae* might evolve under weak environmental control, at least under that mediated by the size of guard cells or achenes. Achene size was related positively to GC content, which could have evolved adaptively in response to summer drought. In *Carduus* and the North American *Cirsium* taxa, there is an increase in average chromosome size with reduction in monoploid chromosome number, suggesting descending dysploidy associated with chromosomal fusion. Polyploidy is relatively rare in this subtribe and was confirmed only in five of the species studied, including *Cirsium vulgare*, an invasive species that likely originated via distant (intersubgeneric) hybridization, as suggested by its intermediate genomic and achene features combined with the conflict between its morphology and phylogenetic position. Phylogenetic reconstruction, differences in genomic parameters, as well as stomatal guard cell and achene sizes support the separation of a monophyletic *Lophiolepis* from the remainder of *Cirsium*. Genome

Received: 6 Dec 2021; Revised: 20 Sep 2022; Accepted: 3 Dec 2022; Published: 27 Feb 2023

and achene size results also indicate that the early diverging *Cirsium italicum* can be separated from the rest of the monophyletic *Lophiolepis+Picnomon+Notobasis* clade.

Keywords: achene size, flow cytometry, genomic DNA base composition, nuclear DNA content, phylogeny, guard cell length

Introduction

Somatic (not replicated) nuclear DNA content (2C-value or holoploid genome size) and monoploid genome size (Cx-value = 2C/ploidy level; Greilhuber et al. 2005) are fundamental species-specific attributes that can be measured using flow cytometry. Not only does this method reveal differences in ploidy level among congeneric species and ploidy variation within species, it often also clearly distinguishes closely-related species despite identical chromosome numbers (Šmarda & Bureš 2006, Suda et al. 2007, Loureiro et al. 2010, Sliwinska et al. 2022). Furthermore, phylogenetic conservatism of monoploid genome size sometimes makes it possible to use this attribute to delimit supraspecific taxa (sections, genera, tribes, subfamilies, etc.; Šmarda et al. 2008, Pellicer & Leitch 2019, Sliwinska et al. 2022). In addition to the determination of genome size, flow cytometry also allows the estimation of another phylogenetically conservative genomic parameter, base composition (overall AT/GC ratio of nuclear DNA, usually expressed as GC content), when the sample is coprocessed with both base-specific and base-unspecific fluorochromes, typically with DAPI and propidium iodide (Šmarda et al. 2012, Sliwinska et al. 2022).

Genome size is a feature that can be used as an aid in species classifications and determinations, as well as a phenotypic property whose evolution can be causally linked to the environment in which a species occurs (Greilhuber & Leitch 2013, Faizullah et al. 2021, Elliott et al. 2022). Through the nuclear volume, genome size might be tightly linked to (minimum) cell size (Swanson et al. 1991, Herben et al. 2012, Šímová & Herben 2012, Doyle & Coate 2019). Therefore, genome size can be subject to natural selection, especially if selection is targeted at the size-related efficiency of organs or structures composed of one or few cells, such as plant stomata, spores, spermatozoids, or pollen grains (Beaulieu et al. 2008, Veselý et al. 2012, 2020, Faizullah et al. 2021). Environmentally-controlled evolution of genome size is thus often deduced from the correlation of holoploid (2C; Greilhuber et al. 2005) genome size with these traits, which can transform the effect of selection from the ecological level to the genome-size level and vice versa (Veselý et al. 2020). However, when genome size is small relative to the minimum cell size tolerated by natural selection in a given group of species, cell size can evolve independently of genome size and the correlation between genome size and cell size might be absent. Similarly, when cells are smaller than the maximum size tolerated by selection and there is a correlation between cell size and relatively large genome sizes, phenotypically-controlled evolution is not automatically implied. Taken together, environmentally-controlled evolution of genome size can be deduced only when there is (i) a correlation between genome and cell size, and (ii) an indication that cell size is naturally selected. In multicellular plant organs, such as seed or fruit, mass could also mediate genome size evolution, especially at fine phylogenetic scales such as within genera or

families (Beaulieu et al. 2007). This association might occur because the size of these organs is essential for reproductive success, dispersal, germination, survival in the seed bank and early establishment (Moles & Westoby 2004, Beaulieu et al. 2007, Bai et al. 2013, Suda et al. 2015, Chen et al. 2020, Gioria et al. 2020, Carta et al. 2022). Although the variability of genomic base composition is relatively narrow in plants compared to genome size variability (Šmarda & Bureš 2012), this trait seems to have some ecological significance (Šmarda et al. 2014, Veleba et al. 2017, Trávníček et al. 2019).

The genus *Cirsium* Mill. (*Asteraceae*) is composed of 448 species (WCVP 2022) distributed across the entire Northern Hemisphere (Meusel & Jäger 1992). Together with nine other genera [*Carduus* (92 spp.), *Cynara* (10 spp.), *Galactites* (3 spp.), *Lamyropsis* (6 spp.), *Notobasis* (1 sp.), *Picnomon* (1 sp.), *Ptilostemon* (15 spp.), *Silybum* (2 spp.), and *Tyrimnus* (1 sp.); WCVP (2022)], it constitutes a monophyletic subtribe *Carduinae*, with species diversity concentrated in the Mediterranean and Irano-Turanian regions (Susanna & Garcia-Jacas 2009, Barres et al. 2013, Herrando-Moraira et al. 2019). In the current most comprehensive phylogeny of *Carduinae* by Ackerfield et al. (2020), all traditionally recognized genera but *Cirsium* and *Carduus* constitute monophyletic lineages. While the morphology-based classification of all smaller genera is congruent with the current phylogeny, serious problems remain with the two genera that are most species rich: *Carduus* and *Cirsium*. Specifically, *Cirsium* and *Carduus* are polyphyletic in their current taxonomic delimitation (Ackerfield et al. 2020). Although two major groups of *Cirsium* recognized in Ackerfield et al. (2020) phylogeny largely overlap with traditional morphologically-defined subgenera in this genus, some species, such as *Cirsium vulgare* and *C. cephalotes,* are in conflict with this classification scheme (see Ackerfield et al. 2020). This conflict makes morphological delimitation of these groups impossible; however, genomic and/or micromorphological/anatomical traits could also be helpful in the delimitation of these groups. In *Carduus*, the East-African lineage delimited morphologically by Kazmi (1963) as *Carduus* subgenus. *Afrocarduus* diverged prior to the clade comprising genus *Tyrimnus* and the Eurasian lineage of the genus (*Carduus* subgen. *Carduus*), see Ackerfield et al. (2020).

While knowledge of chromosome numbers of 348 *Carduinae* species (Watanabe 2008, Rice et al. 2015) covers nearly 60% of the species diversity of this subtribe, genome sizes are known for only 28 species (Bureš et al. 2004, Garnatje et al. 2010, Bai et al. 2012, Garcia et al. 2013, Loureiro et al. 2013, Khaldi et al. 2014, Bureš et al. 2018, Michálková et al. 2018, Leitch et al. 2019, Šmarda et al. 2019). Thus, there is only genome size data for about 5% of species diversity and no data are available for taxa from the Irano-Turanian evolutionary centre of the subtribe. Moreover, there are even less data available for the genomic GC content of species from the *Carduinae* (Bureš et al. 2004, Šmarda et al. 2019). The chromosome number of 2n = 34, strongly predominant among *Carduinae*, is considered to be the ancestral diploid number of this subtribe, which is thought to be derived (via descending dysploidy) from paleopolyploid 2n = 4x = 36 (Mota et al. 2016). Furthemore, descending dysploidy might be occurring, as indicated by chromosome numbers 2n = 32, 30, 28, 24, 22, 20 or 16 detected in some genera, such as *Galactites*, *Lamyropsis* and *Ptilostemon* or for some species of *Carduus* and North American *Cirsium* (Watanabe 2008, Rice et al. 2015, Ackerfield et al. 2020), but no comparison has been made with the respective genome sizes of individual species to confirm this scenario of karyotype evolution. Polyploid chromosome numbers are rarely reported for

some representatives of *Carduus*, but they are reported more frequently in *Cirsium*, especially among its East-Asian species (Watanabe 2008). In addition, more than one ploidy level is also reported in some species of Eurasian *Cirsium* (Rice et al. 2015). To date, there is no broad cytometric screening of ploidy variation across the genus *Cirsium* or subtribe *Carduinae*. Because chromosome number and genome size do not evolve independently, both of these parameters should be analysed together, not separately. Such an approach not only allows for a more reliable identification of the respective modes of karyotype evolution, such as polyploidy, dysploidy (descending or ascending) or hybridization, but also the identification of possible errors in published chromosome numbers.

Together with most other *Asteraceae*, *Carduinae* share the same structural type of fruit, an achene (cypsela). The evolution of the size of an achene could be driven by changes in life history (monocarpic, polycarpic), differences in breeding systems (hermaphroditism, gynodioecy and dioecy), variation in ecological strategies and selection pressure of herbivores and seed predators such as weevils or tephritid flies (Zwölfer 1988, Fenner et al. 2002, Bureš et al. 2010). Since *Carduinae*, and more specifically, the most specious genera *Carduus* and *Cirsium* in this subtribe, occur in a wide range of open habitats often differing in moisture (Davis & Parris 1975, Werner 1976, Wagenitz 1987, Bureš 2004, Keil 2006, Yıldız et al. 2016), one might expect the size of their stomatal guard cells to be environmentally controlled. Therefore, achene size as well as size of stomatal guard cells could be traits that effectively translate various environmental pressures to genome size evolution in *Carduinae*.

In this study, the focus is on the evolution of genome size and genomic GC content in the genus *Cirsium* and related *Carduinae* in relation to chromosome number, stomatal guard cell length and achene size (weight, length) in order to address the following questions: (i) Is there a phylogenetic pattern in the variation of monoploid genome size and GC content that would support the delimitation of lineages recognized in the current phylogeny? (ii) Are monoploid genome size or average chromosome size related to GC-content or to monoploid chromosome number, reflecting particular modes of genome size and karyotype evolution? (iii) Are holoploid genome size and genomic GC content reflected in anatomical or morphological features, which could indicate adaptive evolution?

Material and methods

Plant sampling and nomenclature

Genomic, morphometric and molecular data generated in this study were obtained from more than one thousand individuals representing all *Carduinae* genera. These samples were collected between 2005 and 2022 from natural populations (and occasionally from botanical garden collections) in the United States, Great Britain, Portugal (Madeira), Spain (incl. the Canary Islands), France, Switzerland, Germany, Austria, the Czech Republic, Slovakia, Italy (incl. Sicily), Slovenia, Croatia, Bosnia and Herzegovina, Serbia, Montenegro, Albania, North Macedonia, Greece, Romania, Bulgaria, Ukraine, Morocco, Tunisia, Turkey, Georgia, Iran, Russia and Japan (for full list of populations see Supplementary Table S1). The voucher specimens were deposited in the herbaria of Masaryk University (BRNU) and Artvin Coruh University (ARTH). Nomenclature of taxa analysed follows the accepted names listed in WCVP (2022).

Measurement of genome size, GC content and genomic data processing

Flow-cytometric samples were prepared from fully developed fresh leaves (Galbraith et al. 1983) and processed according to the two-step protocol of Otto et al. (1981), with the concentrations of buffers, dyes and other modifications described in detail by Šmarda et al. (2008). The stained nuclei suspensions were measured on two CyFlow flow cytometers (Partec GmbH) using the internal standards *Carex acutiformis* (2C = 799.93 Mbp, 36.46% GC), *Solanum lycopersicum* 'Stupické polní tyčkové rané' (2C = 1,696.81 Mbp, 38.72% GC), *Pisum sativum* 'Ctirad' (2C = 7,841.27 Mbp, 41.77% GC; Veselý et al. 2012), *Glycine max* 'Polanka' (2C = 2,030.89 Mbp, 37.89% GC) and *Bellis perennis* (2C = 3,089.89 Mbp, 39.54% GC; Šmarda et al. 2019) whose genome sizes and genomic GC contents were derived by comparison with the completely sequenced *Oryza sativa* subsp. *japonica* 'Nipponbare' (International Rice Genome Sequencing Project & Sasaki 2005); for details see also Temsch et al. (2022: Table 1). For the genome size and ploidy level estimations, propidium iodide was used as a fluorochrome, and for the genomic GC content estimation, the samples were coprocessed with DAPI (4',6-diamidino-2-phenylindole) fluorochrome (Šmarda et al. 2008, 2014, Sliwinska et al. 2022). The genomic GC content was calculated based on flow cytometric data using the ATGCFlow spreadsheet available at sci.muni.cz/botany/systemgr/download/Festuca/ATGCFlow.xls (Šmarda et al. 2008).

In order to achieve robust species' estimates, plants from several populations were measured, or several plants in cases where material from only a single population was available. The complete primary dataset (Supplementary Table S2) includes 1,809 flow cytometric analyses of 1,019 individuals (= 1,503 analyses of 786 individuals sampled for this study + extracted data). We repeated measurements until per-taxon CVs fell below 2% (thus all measured taxa have CV lower than 1.8% and 103 of 116 have CV lower than 1%). For the conversion of Mbp to pg the formula 1 pg = 978 Mbp (Doležel et al. 2003) was used. The primary data for genome size and genomic GC content were averaged for each of the 119 taxa analysed (Supplementary Table S3). The original extracted data were converted to sample/standard ratio and then the genome size and genomic GC content were recalculated using the genomic parameters of the standards used in this study when possible (see previous paragraph). For the estimation of average chromosome size (2C/2n), as reported in Supplementary Table S3, chromosome numbers were extracted from the Index to chromosome numbers in *Asteraceae* (Watanabe 2008) or from the Chromosome Counts Database – CCDB (Rice et al. 2015). When several aneuploid numbers were reported for a species, the median value was selected. In rare instances where chromosome numbers for several ploidy levels were reported for a species, the value congruent with the estimated DNA ploidy level was chosen, which was the most prevalent value in all cases. In the case of intraspecific variability, the chromosome number was selected based on material of the same geographic origin by referring to the original source of data when possible. All the chromosome numbers (including those in which consensual 2n was estimated) are clearly indicated (explained) in Supplementary Table S3, with the reference to the original source of data.

Chromosome counting

Because of the unusually small genome size of *Cirsium italicum* (see Results), the number of chromosomes in this species was recounted. For this purpose, achenes obtained

from cytometrically examined plants were germinated in the dark on wet filter paper at 21 °C with 1% (w/v) gibberellic acid in distilled water. Root tips were then collected and pre-treated for 24 h in ice-cold distilled water and then fixed for 25 min in ice-cold 4% (w/v) formaldehyde in 1× PBS (pH = 7.4). After washing in 1× PBS (three times for 15 min), root tips were digested in solution containing 0.085% (w/v) of cellulase R-10 (Duchefa Biochemie), 0.085% (w/v) of cellulysin cellulase (Merck Millipore), 0.12% (w/v) of pectolyase Y23 (Duchefa Biochemie) and 0.12% (w/v) of cytohelicase (Sigma-Aldrich) in 1 × PBS at 37 °C for 1 h. Thereafter, chromosome spreads were prepared by squashing and submerging in liquid nitrogen. After washing in 1× PBS (three times for 5 min) and dehydrating using a graded ethanol series (70, 85, 100% for 2 min each), chromosomes were counterstained with DAPI (1.5 µg/ml) in Vectashield and counted using an Olympus BX-51 microscope.

Measurement of stomatal guard cells and achenes and morphometric data processing

Lengths of stomatal guard cells on the abaxial (lower) surface of leaves of fresh plants or herbarium specimens were measured. In the case of fresh plants, surface impressions were made using a microrelief method: transparent nail polish was applied in a thin layer to the leaf surface and after it dried, the layer was removed with adhesive tape and placed onto a microscope slide. For herbarium specimens, a 0.5 × 0.5 cm section was taken from the specimen and rehydrated in 0.3% Triton X-100 (Sigma-Aldrich) in PBS solution for two hours and then transferred into chlorine bleach solution SAVO (NaClO 4.7 g / 100 g; Alfachem s.r.o.) for 2–4 hours, depending on leaf thickness. The bleach solution was then removed by washing with distilled water (2 × 10 min) and samples were mounted on microscope slides in glycerol solution (Sigma-Aldrich). Dense indumentum present on some of the leaves was always removed using a sharp razor prior to stomatal preparation. The slides were observed under an Olympus BX-51 microscope (objective 40; ~1,000× magnification on screen). Digitally documented slides were analysed manually in the Olympus 'Cell^F' program. Only the middle parts of the abaxial surface (underside) of fully developed, mature, median cauline leaves were used for the surface impressions. About 80–120 stomata were measured per species (20–40 stomata/slide; 1–2 slides/leaf; usually 3 leaves/species). The complete dataset (Supplementary Table S3) consists of mean stomatal guard cell lengths for 114 taxa (88 measured in this study and 26 obtained from Ozcan et al. 2015 and Bureš et al. 2018).

Achene weights for 48 taxa were obtained from the Seed Information Database (Royal Botanic Gardens Kew 2018) or from Hamzé & Jolls (2000). For 54 taxa, the achenes were weighed on a laboratory balance (Ohaus Adventurer Pro AV264C; accuracy 0.0001 g). At least 50 fully-developed achenes (cypselae) from three to five terminal capitula were analysed for each taxon. Before weighing, achenes were stored at room temperature for at least several months (see Supplementary Table S3 for the complete dataset of achene weights).

Achene length for 75 taxa were extracted from floras and other reliable sources (mostly from Ozcan 2017 or Köstekçi & Arabacı 2011; for a detailed list of all sources see Supplementary Table S3). For 43 taxa, the lengths of achenes were measured using an Olympus SZX-AS microscope equipped with a grid with a resolution of 0.01 mm (Supplementary Fig. S1). At least 30 achenes from three to five terminal capitula were measured for each taxon analysed. For the complete achene length dataset see Supplementary Table S3.

Phylogenetic tree preparation

Since the phylogeny of Ackerfield et al. (2020) includes only 58 of the 119 taxa analysed in this study, a new phylogeny based on the species analysed in this study, augmented with publicly available data for other *Carduinae* and selected *Cardueae*/*Asteraceae* outgroups (Supplementary Fig. S2) was produced. Genomic DNA was extracted from deep-frozen leaves using the commercial kit NucleoSpin Plant II (Marchery-Nagel) with extraction buffer PL2 according to the manufacturer's instructions. Sequences of nrDNA (ITS, ETS) and cpDNA (*trn*L-F, *rbc*L) were amplified with the primers listed in Supplementary Table S4. The 50-μL PCR mix consisted of 60–100 ng DNA, 1× PCR buffer (10 mM Tris–HCl, pH 8.8; 50 mM KCl; 0.1% Triton X-100; 1.5 mM $MgCl_2$), 2.5U Taq DNA polymerase, 150 μM dNTP, and 375 nM of each (forward and reverse) primer. The cycling conditions were 94 °C for 2 min and 30 cycles of 94 °C for 30 s, Ta °C (Supplementary Table S4) for 30 s and 72 °C for 1 min, followed by a final extension of 72 °C for 5 min. The specificity of the PCR and the lengths of the products were verified using a 1% agarose gel. Sequencing was done by Macrogen, Inc (Amsterdam, The Netherlands). Sequences for taxa from which we did not isolate DNA were obtained from GenBank (Benson et al. 2013; for accession numbers see Supplementary Table S5). For further analyses, we complemented our own sequences with ETS, ITS, *mat*K, *ndh*F, *psb*A-*trn*H, *rbc*L, *trn*L-F available in GenBank.

The sequences of each marker were aligned using the MAFFT algorithm (Katoh et al. 2002), as implemented in the GUIDANCE 2 web server (Sela et al. 2015). The external gaps were treated as missing data. A partition homogeneity test as implemented in PAUP 4.0a (Swofford 2003) with 500 iterations was used to test the congruence between the seven markers. The first run showed a significant incongruence (P = 0.008). By comparing phylogenetic trees based on nuclear (ETS, ITS) and chloroplast markers (*mat*K, *ndh*F, *psb*A-*trn*H, *rbc*L, *trn*L-F), constructed in the same way as described below, *Cirsium italicum* and two species of *Carduus* subgen. *Afrocarduus* (*Carduus keniensis* and *C. nyassanus*) were identified as potentially problematic taxa, because their placements in the nuclear and chloroplast trees differed. When these three species were excluded, the repeated partition homogeneity test no longer showed incongruence (P = 0.257). As there was no trait data for the two *Afrocarduus*-species, they were not included in the phylogenetic tree. In the case of *C. italicum*, it was split into two "taxa" (terminal nodes) before the construction of the phylogenetic tree: the first *C. italicum* "ITS ETS" with only nuclear markers and the second *C. italicum* "chloroplast markers" based only on chloroplast markers. After passing the partition homogeneity test, the seven aligned markers were concatenated into a supermatrix that was used for the construction of the phylogenetic tree in IQ-TREE v2.1.3 (Minh et al. 2020). A partition model analysis (Chernomor et al. 2016) was used, which allows each marker to have its own model of nucleotide substitution, using the PartitionFinder algorithm (option -m MFP + MERGE; Lanfear et al. 2012) that tries to merge partitions to reduce possible over-parameterization (Kalyaanamoorthy et al. 2017). The relaxed clustering algorithm was also used to reduce computations by only examining the top 10% partitioning schemes using (option -rcluster 10; Lanfear et al. 2014). The merge option resulted in a final partition of five character-sets and corresponding models: ETS (TVM+F+G4), ITS (GTR+F+I+G4), *mat*K + *trn*L-F (TVM+F+R2), *rbc*L (GTR+F+I+G4), *ndh*F + *psb*A-*trn*H (TPM3+F+R2).

Node support values were determined by calculating the fast-nonparametric version of the approximate likelihood ratio test (Shimodaira–Hasegawa [SH]-aLRT; Guindon et al. 2010) (option -alrt).

For the "complete tree" (Supplementary Data S1, Supplementary Fig. S2), one sequence of each of seven markers was used for each species/intraspecific taxon; accession numbers are listed in Supplementary Table S5. The complete tree, including more than one third of *Carduinae* species diversity and all their genera, comprises 226 terminal nodes (species/intraspecific taxa). To obtain an ultrametric tree for the subsequent phylogenetic analyses, the complete tree was dated in MEGA X (Kumar et al. 2018) using the RelTime-Branch-lengths option (Tamura et al. 2012, Mello 2018) and pruned to the final tree composed of 106 tips corresponding to the taxa analysed (Supplementary Data S2, Supplementary Fig. S3). All divergence times in the inferred ultrametric tree are relative times as they were not calibrated.

Statistical analyses

For the testing of associations between genomic parameters and phenotypic traits, we used linear models that account for phylogenetic relatedness (PGLS). Specifically, the *phylolm* function was used as implemented in the *phylolm* package (Ho & Ane 2014) in R4.1.0 (R Core Team 2020) and is indicated by "PGLS" in lower index when P-values are mentioned. Results/parameters of all PGLS analyses are reported in Supplementary Table S6. Since there was no substantial difference in the PGLS analyses between the phylogenies differing in the position of *Cirsium italicum* (based on chloroplast or nuclear markers; Supplementary Table S6), only the result based on the phylogeny with the "nuclear" position of this species (P_{PGLS}) is reported. In each analysis, response variables and predictors were chosen according to their biological relevance (see Results). For statistical analyses, genome size and achene weight were log-transformed (base 10) to achieve a normal distribution of regression residuals. Differences between the clades/genera were tested using the Mann-Whitney test as indicated (by "M-W" in lower index) when P-values are mentioned.

Results

Carduinae phylogeny

The phylogeny presented is based on seven sequence markers composed of 226 terminal nodes: 197 representing taxa of the subtribe *Carduinae* (~1/3 of its species diversity) and 29 of other *Cardueae*/*Asteraceae* outgroups (for Newick format see Supplementary Data S1, for tree Supplementary Fig. S2). Information on the partitions including best-fit substitution models and number of parsimony informative sites are reported in Supplementary Table S7. The subtree of *Carduinae* includes 106 taxa/terminal nodes with known genome size (Fig. 1, Supplementary Data S2, Supplementary Fig. S3). All of the traditionally recognized genera, except *Cirsium,* are monophyletic lineages (Fig. 1, Supplementary Fig. S2, S3). *Cirsium* is polyphyletic in its current taxonomic delimitation as *Cirsium* subgen. *Lophiolepis* (incl. *C. cephalotes*) is a monophyletic clade with *Picnomon*, *Notobasis* and *Cirsium italicum* diverging earlier. In addition, *Cirsium* subgen. *Cirsium*

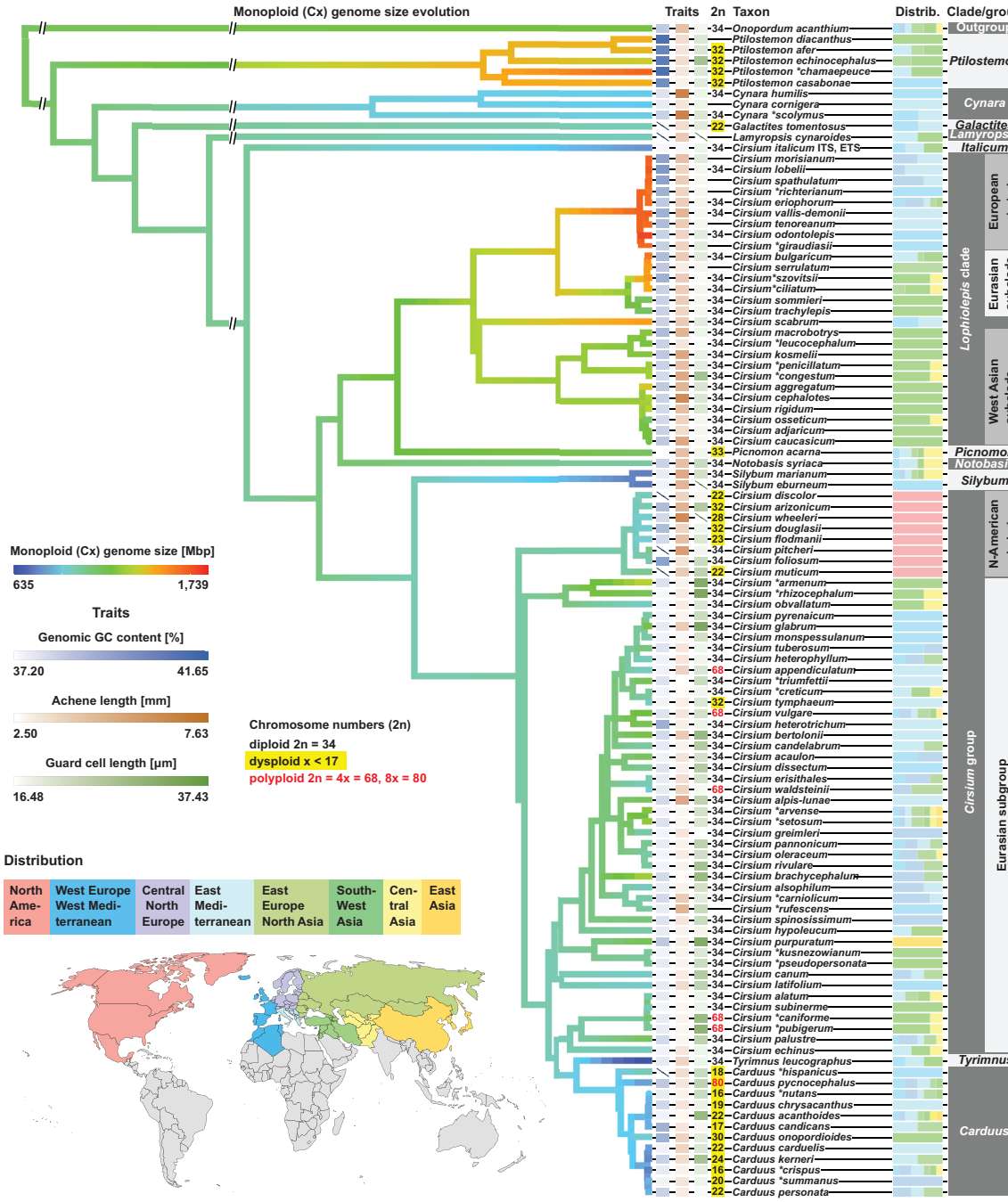

Fig. 1. Evolution of monoploid (Cx) genome size in subtribe *Carduinae* inferred using ancestral state reconstruction and mapped on a dated phylogeny and compared to the estimated genomic GC content, achene length, guard cell length and geographic distribution of extant species (terminal nodes).

(incl. *C. vulgare*) form a paraphyletic group with the earlier diverging clade of *Silybum* and the monophyletic *Tyrimnus-Carduus* subgen. *Carduus* clade is in the terminal crown position (Fig. 1, Supplementary Fig. S2, S3).

Genome size and polyploidy

Genome size and genomic GC content were: (i) newly estimated for 91 taxa, and (ii) re-estimated, based on larger sample sets, for 12 taxa (Table 1). Together with the extracted genome sizes (16 taxa), this represents 119 taxa and 105 species (Table 1).

Holoploid (2C) genome size varied 4.95-fold in subtribe *Carduinae*, ranging from 1.30 pg in *Tyrimnus leucographus* to 6.43 pg in *Carduus pycnocephalus*. Within the Eurasian *Cirsium* subgroup, the 2C-value clearly differed between diploids (2n = 2x = 34; 1.89–2.74 pg) and tetraploids (2n = 4x = 68; 3.77–4.89 pg). These data verify the tetraploid status of *Cirsium vulgare* (2C = 4.89 pg), *C. appendiculatum* (2C = 3.85 pg), *C. pubigerum* (2C = 4.62 and 2C = 4.17 pg for *C. pubigerum* var. *caniforme* and *C. pubigerum* var. *pubigerum*, respectively) and *C. waldsteinii* (2C = 3.77 pg). No polyploids were recorded in the *Lophiolepis* and *Italicum* clades, which recently were classified in the genus *Cirsium*. Furthermore, there were no polyploids recorded in all other clades of *Carduinae*, with the exception of *Carduus pycnocephalus* (*Carduus* subgen. *Carduus*), whose holoploid genome (2C = 6.43 pg) is about four times bigger than that of its close relatives, which indicates this species (measured sample) is octoploid (Table 1). No intraspecific ploidy variation was detected in the species studied.

The genome of *Cirsium odontolepis* (1Cx = 1,739 Mbp) is the largest and that of *Tyrimnus leucographus* (1Cx = 635 Mbp) the smallest; thus, the range in variation of Cx in the subtribe is 2.74-fold. Despite the relatively narrow variation in 1Cx, the phylogenetic signal of this parameter was strong (Pagel's $\lambda = 0.971$; Supplementary Table S8) and effectively delimits most of clades/genera of *Carduinae* (Fig. 1). While monoploid genomes are relatively large in the genus *Ptilostemon* and the *Lophiolepis* clade (specifically the European subclade; Fig. 1), smaller monoploid genome sizes were recorded for the genera *Cynar*a, *Silybum*, *Carduus*, *Tyrimnus* and the *Italicum* (= *Cirsium italicum*; Fig. 1) clade. Within the recently recognized polyphyletic genus *Cirsium*, the Eurasian *Cirsium* subgroup differ in monoploid genome size from the *Lophiolepis* clade ($P_{M\text{-}W} < 0.001$).

Genomic GC content

Genomic GC content varied from 37.20% in *Picnomon acarna* to 41.65% in *Ptilostemon diacanthus* (Table 1). Genomic base composition (GC/AT) thus ranged 1.21-fold [= (41.65/58.35)/(37.20/62.80)] across the subtribe. Genomic GC content also had strong phylogenetic signal (Pagel's $\lambda = 0.907$; Supplementary Table S8) and effectively delimited several lineages of *Carduinae* (Fig. 1). Genomic GC content was smaller in the Eurasian *Cirsium* subgroup than the *Lophiolepis* clade ($P_{M\text{-}W} < 0.001$; Fig. 1). A higher GC content was detected in *Ptilostemon* and lower content in the *Picnomon*, *Silybum* and *Italicum* clades (Fig. 1). Across the whole subtribe, genomic GC content increased with monoploid genome size ($P_{PGLS} < 0.001$; Supplementary Table S6). Similarly, genomic GC content was positively correlated with average chromosome size (2C/2n; $P_{PGLS} = 0.006$; Supplementary Table S6) in the *Carduinae* subtribe.

Table 1. Genome size and genomic GC content of *Carduinae* (including one outgroup): newly estimated values for 91 taxa (Source = A). Sources of genome size: A = this study; B = Bureš et al. (2004); C = Michálková et al. (2018); D = Bai et al. (2012); E = Bureš et al. (2018); F = Šmarda et al. (2019); G = Loureiro et al. (2013); H = Garcia et al. (2013); I = Khaldi et al. (2014). Distribution: Afr = Africa; As = Asia; Eu = Europe; N Am = North America; ^{E} = East; ^{N} = North; ^{S} = South; ^{W} = West; $_{Penins.}$ = Peninsula. For populations sampled see Supplementary Table S1; for standards and their ratios of samples with particular measurements see Supplementary Table S2.

Taxon	2C±S.D. (pg)	DNA ploidy level	1Cx (Mbp)	GC content ± s.d. (%)	Source	Clade/Group	Distribution
Carduus acanthoides	1.65±0.03	2	805	38.25	F	*Carduus*	**Eu-As**
Carduus candicans	1.66±0.05	2	811	39.92±0.41	A	*Carduus*	^{SE}**Eu**$_{(E\ Alps–Balkanids)}$
Carduus carduelis	1.65±0.03	2	808	38.40±0.23	A	*Carduus*	$^{S–SE}$**Eu**$_{(Italy–Bulgaria)}$
Carduus carlinoides subsp. *hispanicus*	2.04±0.05	2	996	n.a.	G	*Carduus*	^{SW}**Eu**$_{(Spain)}$
Carduus collinus	1.64±0.04	2	803	39.26±0.72	A	*Carduus*	$^{S–E}$**Eu**$_{(Italy–Ukraine)}$
Carduus crispus subsp. *crispus*	1.51±0.01	2	738	38.54	F	*Carduus*	**Eu-As**
Carduus crispus subsp. *multiflorus*	1.65±0.03	2	807	n.a.	H	*Carduus*	$^{W–N}$**Eu**$_{(Spain–Sweden)}$
Carduus chrysacanthus	1.84±0.06	2	902	39.17±1.09	A	*Carduus*	^{S}**Eu**$_{(Italy–Bosnia)}$
Carduus defloratus subsp. *glaucus*	1.77±0.06	2	863	39.07±0.32	A	*Carduus*	$^{C–E}$**Eu**$_{(Austria–Ukraine)}$
Carduus defloratus subsp. *summanus*	1.69±0.03	2	828	38.26±0.11	A	*Carduus*	$^{C–S}$**Eu**$_{(France–Austria)}$
Carduus kerneri	1.55±0.05	2	758	39.27±0.30	A	*Carduus*	$^{S–E}$**Eu**$_{(Montenegro–Ukraine)}$
Carduus litigiosus	1.60±0.02	2	783	38.15±0.21	A	*Carduus*	^{SW}**Eu**$_{(France–Italy)}$
Carduus nutans subsp. *granatensis*	1.86±0.08	2	910	n.a.	H	*Carduus*	^{SW}**Eu**$_{(Spain)}$
Carduus nutans subsp. *nutans*	1.57±0.01	2	768	38.58	F	*Carduus*	**Eu-As**
Carduus nutans subsp. *platylepis*	1.59±0.02	2	776	38.75±0.17	A	*Carduus*	^{S}**Eu**$_{(France–Croatia)}$
Carduus onopordioides	1.99±0.04	2	971	39.95±0.23	A	*Carduus*	$^{SW–C}$**As**$_{(Georgia–Iran)}$
Carduus personata	1.69±0.02	2	824	39.38	F	*Carduus*	**Eu**$_{(France-Ukraine)}$
Carduus poliochrus	1.60±0.03	2	783	38.82±0.32	A	*Carduus*	^{SW}**As**$_{(Caucasus)}$
Carduus pycnocephalus	6.43±0.14	8?	786?	39.52±0.36	A	*Carduus*	^{S}**Eu**–^{N}**Afr**–^{SW}**As**
Cirsium acaulon	2.28±0.04	2	1113	38.29±0.40	A, B	EuAs-*Cirsium*	^{W}**Eu**
Cirsium adjaricum	2.29±0.05	2	1120	39.36±0.28	A	*Lophiolepis*	^{SW}**As**
Cirsium aggregatum	3.01±0.05	2	1474	39.54±0.19	A	*Lophiolepis*	^{SW}**As**
Cirsium alatum	2.00±0.06	2	976	38.65±0.49	A	EuAs-*Cirsium*	^{SE}**Eu**–$^{SW–C}$**As**
Cirsium alpis-lunae	2.26±0.04	2	1107	39.00±0.32	A	EuAs-*Cirsium*	^{S}**Eu**$_{(N\ Apennines)}$
Cirsium alsophilum	1.95±0.07	2	954	38.65±0.42	A	EuAs-*Cirsium*	**Eu**$_{(E\ Alps–Dinarids)}$
Cirsium appendiculatum	3.85±0.09	4	941	38.45±0.29	A	EuAs-*Cirsium*	^{SE}**Eu**$_{(Balkan)}$
Cirsium arizonicum	2.04±0.03	2	997	39.73±0.12	A	EuAs-*Cirsium*	**N Am**$_{(SW\ USA–Mex.)}$

Taxon	2C±S.D. (pg)	DNA ploidy level	1Cx (Mbp)	GC content ± s.d. (%)	Source	Clade/Group	Distribution
Cirsium arvense var. *arvense*	2.52±0.05	2	1234	38.46±0.41	A, B	EuAs-*Cirsium*	Cosmopolitan
Cirsium arvense var. *setosum*	2.59±0.03	2	1269	38.90±0.21	A	EuAs-*Cirsium*	Cosmopolitan
Cirsium bertolonii	2.32±0.05	2	1133	39.08±0.44	A	EuAs-*Cirsium*	^{S}**Eu**$_{(N\ Apennines)}$
Cirsium brachycephalum	2.67±0.05	2	1307	39.20±0.47	A, B	EuAs-*Cirsium*	^{SE}**Eu**
Cirsium bulgaricum	3.36±0.06	2	1643	39.82±0.29	A	*Lophiolepis*	^{SE}**Eu**–^{SW}**As**$_{(Black\ Sea\ shores)}$
Cirsium candelabrum	2.05±0.05	2	1001	38.56±0.31	A	EuAs-*Cirsium*	^{SE}**Eu**–^{SW}**As**$_{(Thrace)}$
Cirsium canum	1.94±0.03	2	950	38.38±0.27	A, B	EuAs-*Cirsium*	^{SE}**Eu**–$^{SW–C}$**As**$_{(–W\ Siberia)}$
Cirsium carniolicum subsp. *carniolicum*	2.03±0.04	2	992	39.02±0.29	C	EuAs-*Cirsium*	**Eu**$_{(E\ Alps)}$
Cirsium carniolicum subsp. *rufescens*	2.08±0.06	2	1016	38.65±0.44	A	EuAs-*Cirsium*	**Eu**$_{(Pyrenees)}$
Cirsium caucasicum	2.34±0.07	2	1143	39.57±0.47	A	*Lophiolepis*	^{SW}**As**$_{(NE\ Turkey–Caucasus)}$
Cirsium cephalotes	2.65±0.10	2	1297	39.38±0.71	A	*Lophiolepis*	^{SW}**As**$_{(NE\ Turkey–Caucasus)}$
Cirsium ciliatum subsp. *ciliatum*	2.91±0.03	2	1425	39.38±0.21	A	*Lophiolepis*	^{E}**Eu**–^{C}**As**
Cirsium ciliatum subsp. *szovitsii*	3.08±0.04	2	1504	39.99±0.21	A	*Lophiolepis*	$^{SW–C}$**As**$_{(E\ Turkey–Iran)}$
Cirsium creticum subsp. *creticum*	2.15±0.03	2	1051	38.34±0.28	A	EuAs-*Cirsium*	^{S}**Eu**–^{SW}**As**
Cirsium creticum subsp. *triumfettii*	2.32±0.05	2	1134	38.97±0.32	A	EuAs-*Cirsium*	^{S}**Eu**$_{(Apennine\ Penins.)}$
Cirsium discolor	2.00	2	976	n.a.	D	NAm-*Cirsium*	**N Am**
Cirsium dissectum	2.09±0.04	2	1023	38.78±0.24	A	EuAs-*Cirsium*	^{W}**Eu**
Cirsium douglasii	1.90±0.04	2	931	39.77±0.32	A	NAm-*Cirsium*	**N Am**
Cirsium echinus	1.99±0.05	2	972	38.46±0.32	A	EuAs-*Cirsium*	$^{SW–C}$**As**$_{(NE\ Turkey–Afghanistan)}$
Cirsium eriophorum	3.26±0.05	2	1596	39.61±0.31	A, B	*Lophiolepis*	**Eu**
Cirsium erisithales	2.06±0.04	2	1009	38.66±0.35	A, B	EuAs-*Cirsium*	**Eu**$_{(mountains)}$
Cirsium flodmanii	1.87±0.02	2	915	39.01±0.38	A	NAm-*Cirsium*	**N Am**$_{(Can–N\ USA)}$
Cirsium foliosum	2.00±0.04	2	978	40.10±0.39	A	NAm-*Cirsium*	**N Am**$_{(Can–NW\ USA)}$
Cirsium glabrum	2.41±0.05	2	1181	38.62±0.32	A	EuAs-*Cirsium*	^{SW}**Eu**$_{(Pyrenees)}$
Cirsium greimleri	1.97±0.06	2	965	38.51±0.27	E	EuAs-*Cirsium*	**Eu**$_{(E\ Alps–Dinarids)}$
Cirsium heterophyllum	1.91±0.03	2	932	38.19±0.26	A, B	EuAs-*Cirsium*	**Eu–As**
Cirsium heterotrichum	2.25±0.05	2	1100	39.89±0.43	A	EuAs-*Cirsium*	^{SE}**Eu**$_{(Balkan)}$
Cirsium hypoleucum	2.04±0.05	2	997	38.66±0.47	A	EuAs-*Cirsium*	^{SW}**As**$_{(N\ Turkey–Caucasus)}$
Cirsium italicum	1.61±0.02	2	787	37.65±0.16	A	*Italicum*	^{S}**Eu**–^{SW}**As**$_{(Turkey)}$
Cirsium kosmelii	2.53±0.03	2	1237	39.48±0.36	A	*Lophiolepis*	^{SW}**As**$_{(N\ Turkey–Caucasus)}$
Cirsium latifolium	1.93±0.04	2	944	38.58±0.26	A	EuAs-*Cirsium*	**Eu**$_{(Madeira)}$
Cirsium leucocephalum subsp. *leucocephalum*	2.46±0.05	2	1203	39.25±0.33	A	*Lophiolepis*	^{SW}**As**$_{(Turkey–NW\ Iran)}$

Taxon	2C±S.D. (pg)	DNA ploidy level	1Cx (Mbp)	GC content ± s.d. (%)	Source	Clade/Group	Distribution
Cirsium leucocephalum subsp. *penicillatum*	2.61±0.03	2	1277	39.40±0.28	A	*Lophiolepis*	$^{SW–C}$**As**(N Turkey–Caucasus)
Cirsium ligulare	3.36±0.07	2	1644	40.00±0.11	A	*Lophiolepis*	^{SE}**Eu**
Cirsium lobelii	3.42±0.11	2	1671	40.33±0.46	A	*Lophiolepis*	^{S}**Eu**(Apennines)
Cirsium macrobotrys	2.56±0.03	2	1252	39.77±0.32	A	*Lophiolepis*	^{SW}**As**(E Turkey–N Iran)
Cirsium monspessulanum	1.99±0.06	2	972	38.43±0.44	A	EuAs-*Cirsium*	^{SW}**Eu**(Portugal–N Italy)
Cirsium morisianum	3.35±0.05	2	1641	40.30±0.28	A	*Lophiolepis*	^{SW}**Eu**(France–Italy)
Cirsium muticum	2.14	2	1046	n.a.	D	NAm-*Cirsium*	**N Am**
Cirsium obvallatum	1.94±0.07	2	947	38.44±0.40	A	EuAs-*Cirsium*	$^{SW–C}$**As**(NE Turkey–NW Iran)
Cirsium odontolepis	3.56±0.04	2	1739	39.31±0.21	A	*Lophiolepis*	^{SW}**Eu**(S France–Spain)
Cirsium oleraceum	2.03±0.03	2	995	38.50±0.28	A, B	EuAs-*Cirsium*	**Eu–As**
Cirsium osseticum	2.12±0.05	2	1037	39.18±0.17	A	*Lophiolepis*	$^{SW–C}$**As**(NE Turkey–NW Iran)
Cirsium palustre	2.26±0.06	2	1103	39.00±0.35	A, B	EuAs-*Cirsium*	**Eu–As**
Cirsium pannonicum	2.14±0.03	2	1045	38.59±0.27	A, B	EuAs-*Cirsium*	**Eu**
Cirsium pitcheri	2.19	2	1072	n.a.	D	NAm-*Cirsium*	**N Am**(Great Lakes region)
Cirsium pseudopersonata subsp. *kusnezowianum*	2.07±0.03	2	1014	38.46±0.36	A	EuAs-*Cirsium*	^{SW}**As**(NE Turkey–Caucasus)
Cirsium pseudopersonata subsp. *pseudopersonata*	2.09±0.03	2	1021	38.73±0.26	A	EuAs-*Cirsium*	^{SW}**As**(NE Turkey)
Cirsium pubigerum var. *caniforme*	4.62±0.07	4	1131	38.57±0.22	A	EuAs-*Cirsium*	$^{SW–C}$**As**(NE Turkey–N Iraq)
Cirsium pubigerum var. *pubigerum*	4.17±0.11	4	1020	38.63±0.13	A	EuAs-*Cirsium*	$^{SW–C}$**As**
Cirsium pugnax	2.61±0.07	2	1278	39.17±0.34	A	*Lophiolepis*	^{SW}**As**(NE Turkey)
Cirsium purpuratum	2.28±0.01	2	1116	39.50±0.27	A	EuAs-*Cirsium*	^{E}**As**(Japan)
Cirsium pyrenaicum	1.99±0.06	2	971	38.37±0.66	A	EuAs-*Cirsium*	^{SW}**Eu**(Iberian Penins.)
Cirsium rhizocephalum subsp. *rhizocephalum*	2.26±0.09	2	1103	37.76±0.20	A	EuAs-*Cirsium*	$^{SW–C}$**As**(NE Turkey–Afghanistan)
Cirsium rigidum	2.72±0.02	2	1332	39.11±0.15	A	*Lophiolepis*	^{SW}**As**(NE Turkey–Caucasus)
Cirsium richterianum subsp. *giraudiasii*	3.24±0.07	2	1587	39.28±0.27	A	*Lophiolepis*	^{SW}**Eu**(Iberian Penins.)
Cirsium richterianum subsp. *richterianum*	3.19±0.08	2	1561	39.91±0.52	A	*Lophiolepis*	^{SW}**Eu**(Iberian Penins.)
Cirsium rivulare	2.08±0.05	2	1018	38.37±0.32	A, B	EuAs-*Cirsium*	**Eu**
Cirsium scabrum	3.04±0.05	2	1488	39.25±0.27	A	*Lophiolepis*	^{S}**Eu**–^{N}**Afr**
Cirsium serrulatum	2.95±0.07	2	1442	39.64±0.34	A	*Lophiolepis*	^{E}**Eu**–^{C}**As**(–W Siberia)
Cirsium simplex subsp. *armenum*	2.74±0.06	2	1339	38.93±0.21	A	EuAs-*Cirsium*	^{SW}**As**(E Turkey–Caucasus)
Cirsium sommieri	2.19±0.04	2	1072	39.30±0.21	A	*Lophiolepis*	^{SW}**As**(E Turkey)
Cirsium sorocephalum subsp. *congestum*	2.65±0.05	2	1295	39.16±0.31	A	*Lophiolepis*	$^{SW–C}$**As**(SE Turkey–W Iran)
Cirsium spathulatum	3.32±0.06	2	1622	40.10±0.17	A	*Lophiolepis*	**Eu**(Switz.–NW Italy)

Taxon	2C±S.D. (pg)	DNA ploidy level	1Cx (Mbp)	GC content ± s.d. (%)	Source	Clade/Group	Distribution
Cirsium spinosissimum	2.18±0.09	2	1064	38.84±0.38	A	EuAs-*Cirsium*	**Eu**$_{(Alps)}$
Cirsium subinerme	1.92±0.05	2	938	38.31±0.32	A	EuAs-*Cirsium*	^{E}**Eu**–$^{SW–C}$**As**$_{(–W\ Iran)}$
Cirsium tenoreanum	3.26±0.08	2	1597	39.77±0.44	A	*Lophiolepis*	^{S}**Eu**$_{(Apennine\ Penins.)}$
Cirsium trachylepis	2.16±0.04	2	1058	39.23±0.28	A	*Lophiolepis*	^{SW}**As**$_{(NE\ Turkey)}$
Cirsium tuberosum	2.23±0.07	2	1091	38.87±0.49	A	EuAs-*Cirsium*	^{W}**Eu**
Cirsium tymphaeum	1.89±0.04	2	924	38.20±0.22	A	EuAs-*Cirsium*	^{SE}**Eu**$_{(Balkan)}$
Cirsium vallis-demonii	3.34±0.05	2	1636	39.97±0.26	A	*Lophiolepis*	^{S}**Eu**$_{(Sicily)}$
Cirsium vulgare	4.89±0.16	4	1196	39.02±0.29	A, B	EuAs-*Cirsium*	Cosmopolitan
Cirsium waldsteinii	3.77±0.07	4	922	38.61±0.29	E	EuAs-*Cirsium*	^{E}**Eu**$_{(Carpathians)}$
Cirsium wheeleri	1.88±0.02	2	920	39.14±0.19	A	NAm-*Cirsium*	**N Am**$_{(SW\ USA–Mexico)}$
Cynara cardunculus subsp. *cardunculus*	2.06±0.06	2	1006	n.a.	I	*Cynara*	^{S}**Eu**–^{N}**Afr**
Cynara cardunculus subsp. *scolymus*	1.83±0.01	2	897	39.30±0.05	A	*Cynara*	^{S}**Eu**–^{N}**Afr**–^{SW}**As**
Cynara cornigera	1.77±0.02	2	864	38.68±0.07	A	*Cynara*	^{SE}**Eu**–^{NE}**Afr**
Cynara humilis	1.78±0.02	2	871	38.91±0.34	A	*Cynara*	^{SW}**Eu**–^{NW}**Afr**
Galactites tomentosus	2.00±0.09	2	978	n.a.	H	*Galactites*	^{SW}**Eu**–^{NW}**Afr**
Lamyropsis cynaroides	1,98±0.09	2	968	n.a.	H	*Lamyropsis*	^{SE}**Eu**–^{SW}**As**
Notobasis syriaca	2.07±0.04	2	1011	39.33±0.47	A	*Notobasis*	^{S}**Eu**–^{N}**Afr**$_{(Mediterranean)}$
Picnomon acarna	2.25±0.03	2	1100	37.20±0.16	A	*Picnomon*	^{SW}**Eu**–^{NW}**Afr**–$^{SW–C}$**As**
Ptilostemon afer	2.90±0.07	2	1418	40.89±0.29	A	*Ptilostemon*	^{SE}**Eu**–^{SW}**As**
Ptilostemon casabonae	2.94±0.05	2	1438	40.55±0.50	A	*Ptilostemon*	^{SW}**Eu**$_{(France–Italy)}$
Ptilostemon chamaepeuce var. *chamaepeuce*	3.32±0.03	2	1625	41.57±0.33	A	*Ptilostemon*	^{SE}**Eu**–^{SW}**As**
Ptilostemon chamaepeuce var. *cyprius*	2.99±0.09	2	1461	40.69±0,52	A	*Ptilostemon*	^{SE}**Eu**$_{(Cyprus)}$
Ptilostemon diacanthus	2.95±0.09	2	1442	41.65±0.54	A	*Ptilostemon*	^{SW}**As**
Ptilostemon echinocephalus	2.76±0.09	2	1349	41.13±0.33	A	*Ptilostemon*	^{E}**Eu**–^{SW}**As**
Silybum eburneum	1.54±0.05	2	754	38.44±0.59	A	*Silybum*	^{SW}**Eu**–^{NW}**Afr**
Silybum marianum	1.55±0.02	2	760	38.64±0.26	A	*Silybum*	^{S}**Eu**–^{N}**Afr**–$^{SW–C}$**As**
Tyrimnus leucographus	1.30±0.04	2	635	38.55±0.58	A	*Tyrimnus*	^{SW}**Eu**–^{N}**Afr** –^{SW}**As**$_{(Mediterranean)}$
Outgroup (subtribe *Onopordinae*)							
Onopordum acanthium	2.32±0.01	2	1134	39.52±0.23	A	Onopordinae	^{W}**Eu**–^{NW}**Afr**–^{C}**As**

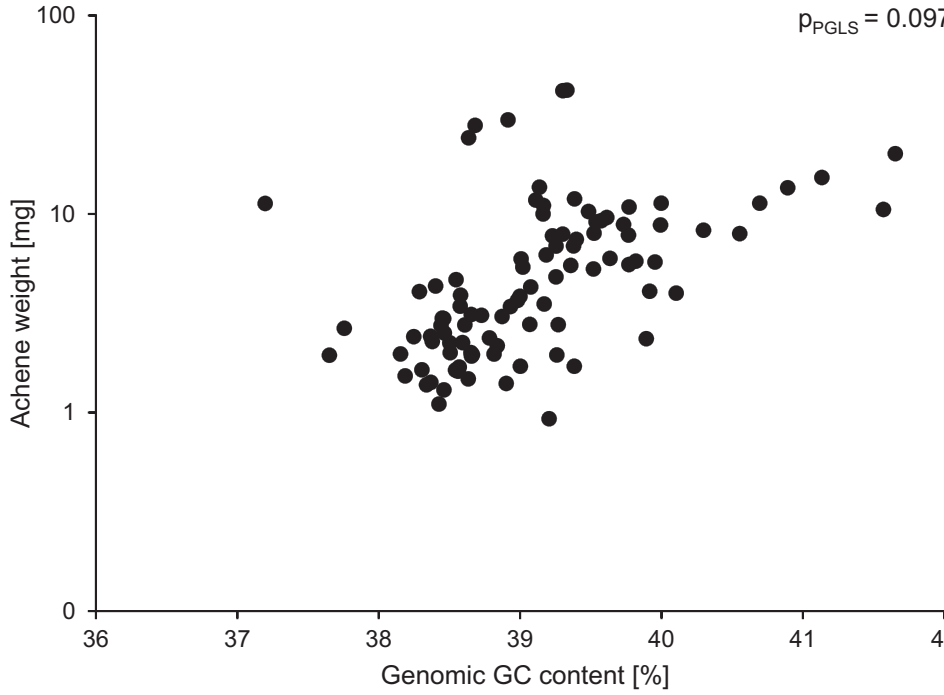

Fig. 2. Achene size is positively related to GC content, which could be an adaptive response to summer drought in subtribe *Carduinae*.

Achene and stomatal guard cell size in relation to genomic parameters

The average achene weight per species varied 45.2-fold in the *Carduinae* subtribe, ranging from 0.93 mg in *Cirsium brachycephalum* to 42.04 mg in *Notobasis syriaca,* whereas the average length of achenes (Supplementary Fig. S1A) varied 3.05-fold from 2.50 mm in *Cirsium creticum* subsp. *triumfettii* to 7.63 mm in *Cynara humilis* (Supplementary Table S3). Strong phylogenetic signal was detected for both achene weight and length (Pagel's λ = 0.944 and 0.820, respectively; Supplementary Table S8). Although the achenes were lighter and shorter in the Eurasian *Cirsium* subgroup with small genomes than in the *Lophiolepis* clade with large genomes (Fig. 1; $P_{M\text{-}W} < 0.001$ for both achene weight and length), there was no relationship between the holoploid (2C) genome size and size (weight or length) of achenes in the *Carduinae* subtribe (P_{PGLS} = 0.247 for weight; P_{PGLS} = 0.833 for length; Supplementary Table S6). However, some marginal support was detected when genomic GC content was tested as a predictor of achene weight (Fig. 2; P_{PGLS} = 0.097).

The average stomatal guard cell length per species varied 2.27-fold in the *Carduinae* subtribe, ranging from 16.48 μm in *Cirsium greimleri* to 37.43 μm in *Cirsium rhizocephalum*. The phylogenetic signal of the size of stomatal guard cells was weaker than that of the genomic or achene parameters (Pagel's λ = 0.323; Supplementary Table S8). Although stomatal guard cells were shorter in the *Lophiolepis* clade, with a large genome,

than in the Eurasian *Cirsium* subgroup ($P_{M\text{-}W} < 0.001$), with a small genome, which indicates a possible negative correlation between stomata and genome sizes, there was some support for holoploid genome size (2C) increasing with the length of stomatal guard cell in the *Carduinae* subtribe ($P_{PGLS} = 0.024$; Fig. 1; Supplementary Table S6). No relationship was detected when GC content was tested as a predictor for stomatal guard cell length ($P_{PGLS} = 0.702$; Supplementary Table S6).

Discussion

The monoploid genome sizes (Cx) and genomic GC contents included in this study represent more than 18% of the known *Cirsium* or *Carduinae* species in Europe and Western Asia (i.e. the Mediterranean and Irano-Turanian evolutionary centres of the subtribe and all its recently recognized genera).

Genomic and morphologic data support the division of the genus Cirsium

The topology of the phylogeny constructed for the cytometrically analysed *Carduinae* resembles that published by Ackerfield et al. (2020). The only topological differences between the two phylogenies is the position of the earlier diverging *Cirsium italicum* in the presented phylogeny (earlier than *Notobasis* and *Picnomon*) and slightly modified relationships within the *Cirsium* group. The relationship of all main clades/groups mostly representing current *Carduinae* genera (or subgenera) is the same in both phylogenies (Supplementary Fig. S4), such as: *Carduus* subgen. *Carduus*, *Tyrimnus*, *Cirsium* subgen. *Cirsium* (= *Cirsium* group), *Silybum*, *Cirsium* subgen. *Lophiolepis* (= *Lophiolepis* clade), *Picnomon*, *Notobasis*, *Galactites*, *Lamyropsis*, *Cynara* and *Ptilostemon*; *Onopordum* is included as an outgroup. In contrast to the phylogeny of Ackerfield et al. (2020), *Cirsium cephalotes* is now embedded within the *Lophiolepis* clade, which is congruent with its morphology and previous classification (e.g. Davis & Parris 1975). The phylogenetic reconstruction presented here places *Cirsium vulgare* within the *Cirsium* group, despite its *Lophiolepis* morphology, which is consistent with Ackerfield et al. (2020) phylogeny.

The strong phylogenetic signal of the genomic (monoploid genome size, average chromosome size, genomic GC content) and morphological (achene weight and length) traits indicates that closely related species are similar in these parameters, which are, therefore, useful for taxonomic delimitation of higher taxa in this subtribe. The substantial differences in monoploid genome size and genomic GC content between the *Lophiolepis* clade and *Cirsium* group (incl. *C. vulgare*) detected in this study (Fig. 1, 2A, B) provide additional support for their taxonomic separation at the generic level, as already indicated by the phylogenetic relationships (Ackerfield et al. 2020) and resolved in a parallel study (Del Guacchio et al. 2022). In the reconstructed phylogeny presented, *Lophiolepis* is also a monophyletic clade, with two monotypic genera (*Picnomon* and *Notobasis*) outside of the clade (Fig. 1, Supplementary Fig. S2). Subsequently, the remainder of the genus *Cirsium* is paraphyletic with *Silybum* as a sister/basal lineage and with the monophyletic *Tyrimnus* + *Carduus* subgen. *Carduus* clade nested inside as a terminal clade (Fig. 1, Supplementary Fig. S2). The possible new generic delimitation of the *Lophiolepis*-clade and *Cirsium*-group is further supported by differences in the size (weight, length) and shape of achenes, as well as the length of their stomatal guard cells (Fig. 3C–E, 4G–Q).

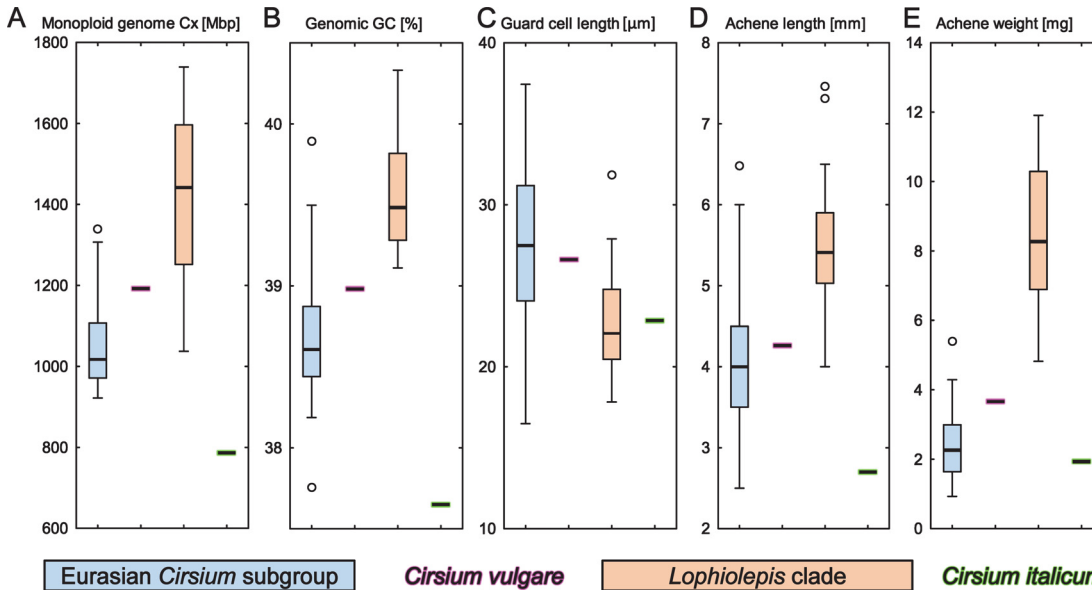

Fig. 3. The intermediate position of *Cirsium vulgare* (highlighted in magenta) for five traits compared to the Eurasian *Cirsium* subgroup (light blue) and *Lophiolepis* clade (light red), as well as *Cirsium italicum* (highlighted in bright green). Boxplots show median (thick line), interquartile range (boxes), non-outlier range (whiskers) and outliers (circles) of monoploid (1C) genome. (A) monoploid genome, (B) genomic GC content, (C) guard cell length, (D) achene length, (E) achene weight.

Interestingly, *Cirsium italicum* (the *Italicum* clade, Fig. 1, 3A) was estimated to have a small 2C genome. This species, traditionally classified in *Cirsium* subgen. *Lophiolepis*, has a holoploid genome half the size of other consistently diploid taxa in the *Lophiolepis* clade (Table 1). Both diploid (2n = 34; Kuzmanov 1985) and tetraploid (2n = 68; Romano et al. 1994) chromosome numbers are reported for *Cirsium italicum*. Only 2n = 34 was recorded in the root tips of germinated ripe achenes obtained from the plants analysed cytometrically (Fig. 5). The separated phylogenetic position of this species outside the *Notobasis+Picnomon+Lophiolepis* clade (Fig 1), combined with its unusually small genome and lower genomic GC content (Table 1, Fig. 3A, B), support its taxonomic separation at the generic level from the rest of *Cirsium*. This separation is also supported by its tiny achenes (Fig. 3D, E), the shape (but not size) of which resembles the achenes of *Picnomon* (Fig. 4T, U) and not those of the *Lophiolepis* clade (Fig. 4M–Q). This indicates that it is likely that the report of a tetraploid chromosome number for this species is erroneous and could have arisen from confusions in the identification of plants with a small capitula in the morphologically similar *Cirsium vulgare*, which was noted during a previous herbarium revision (P. Bureš, unpublished).

Cirsium vulgare could be an "intersubgeneric" allopolyploid

The placement of *Cirsium vulgare* among lineages of the paraphyletic *Cirsium* group (this study – Fig. 1; Ackerfield et al. 2020) is contrary to most of its morphological features,

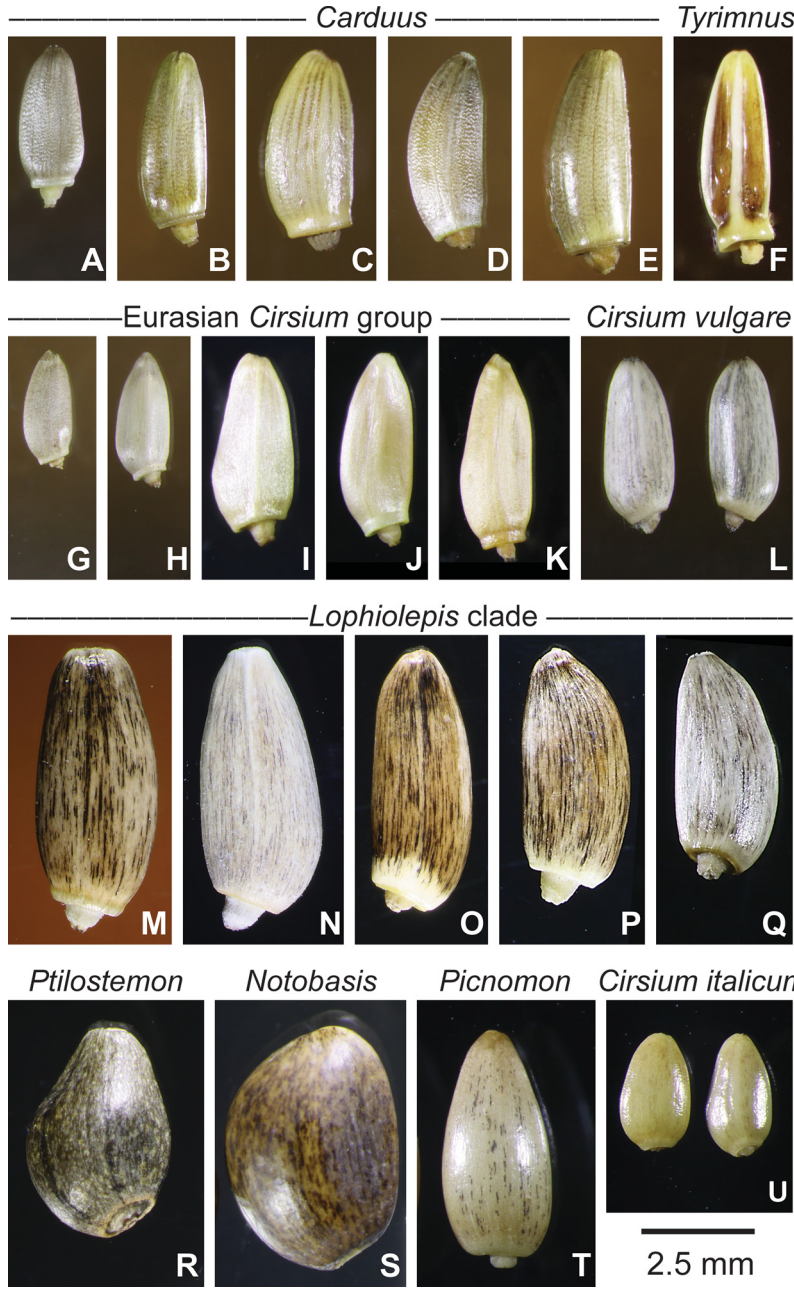

Fig. 4. Achene size and shape for: (A) *Carduus acanthoides*, (B) *Carduus poliochrus*, (C) *Carduus candicans*, (D) *Carduus kerneri*, (E) *Carduus onopordioides*, (F) *Tyrimnus leucographus*, (G) *Cirsium brachycephalum*, (H) *Cirsium palustre*, (I) *Cirsium spinosissimum*, (J) *Cirsium echinus*, (K) *Cirsium obvallatum*, (L) *Cirsium vulgare*, (M) *Cirsium eriophorum*, (N) *Cirsium morisianum*, (O) *Cirsium ligulare*, (P) *Cirsium caucasicum*, (Q) *Cirsium tenoreanum*, (R) *Ptilostemon echinocephalus*, (S) *Notobasis syriaca*, (T) *Picnomon acarna*, (U) *Cirsium italicum*. (A–F) *Tyrimnus-Carduus* subgen. *Carduus* clade, (G–L) Eurasian *Cirsium* subgroup, (M–Q) *Lophiolepis* clade, (R) *Ptilostemon* clade, (S) *Notobasis* clade, (T) *Picnomon* clade, (U) *Italicum* clade.

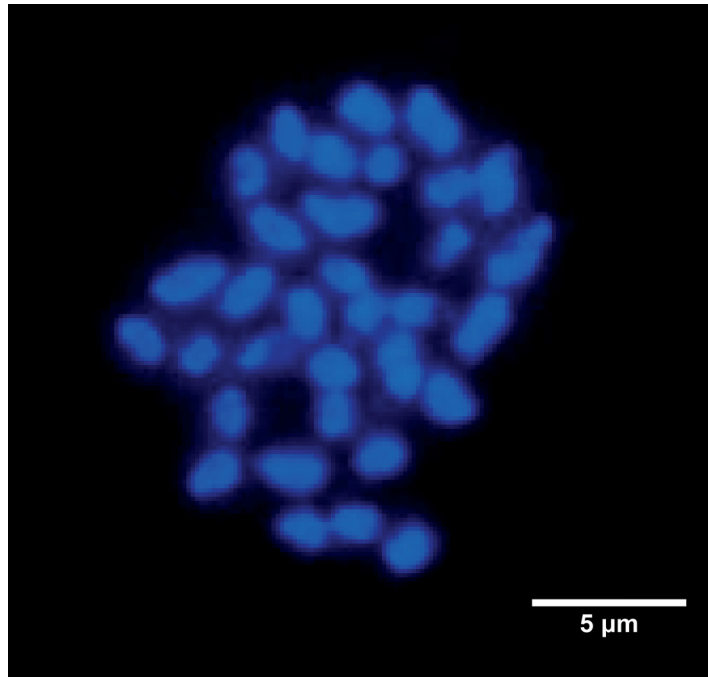

Fig. 5. Image showing the chromosomes in the small-genomed *Cirsium italicum* (2n = 34).

specifically, rigid setae on its adaxial leaf surface and involucres with protruding terminal spines, which are typical for the *Lophiolepis* clade where *C. vulgare* is traditionally classified. On the other hand, the decurrent leaves of *C. vulgare* are quite unusual for the *Lophiolepis* clade, but can be found in a number of representatives of the Eurasian *Cirsium* subgroup (*C. palustre*, *C. brachycephalum*, *C. canum*, *C. creticum*, *C. monspessulanum*) and in most species in the *Carduus* clade. The monoploid genome size, genomic GC content and size of chromosomes of *C. vulgare* are intermediate between the values typical for the Eurasian *Cirsium* subgroup and the *Lophiolepis* clade (Fig. 3A, B; Supplementary Table S3); its achene weight and stomatal guard cell length are also clearly intermediate (between Eurasian *Cirsium* and *Lophiolepis*; Fig. 3C, E). The intermediate morphological, anatomical and genomic values indicate that *C. vulgare* could be a hybrid (allotetraploid), with the parental species belonging to the Eurasian *Cirsium* subgroup and *Lophiolepis* clade. Moreover, *C. vulgare* is reported to have hybridized with many taxa of both the Eurasian *Cirsium* subgroup and *Lophiolepis* clade (Wagenitz 1987, Bureš 2004). However, the confirmation of a putative distant hybrid origin for this species, one of the most invasive species in the World (CABI 2021), needs to be verified by phylogenomic analysis. Alternatively, *C. vulgare*'s morphological similarity to species in the *Lophiolepis* clade could be the result of convergent evolution. One prominent example illustrating how *Lophiolepis*-like morphology can evolve in the subgenus *Cirsium* is the North American species *Cirsium andrewsii*, which is clearly embedded in the North American *Cirsium* subclade (Ackerfield et al. 2020), even though its morphology (leaves, involucres) strongly resembles representatives of the *Lophiolepis* clade (see Keil 2006).

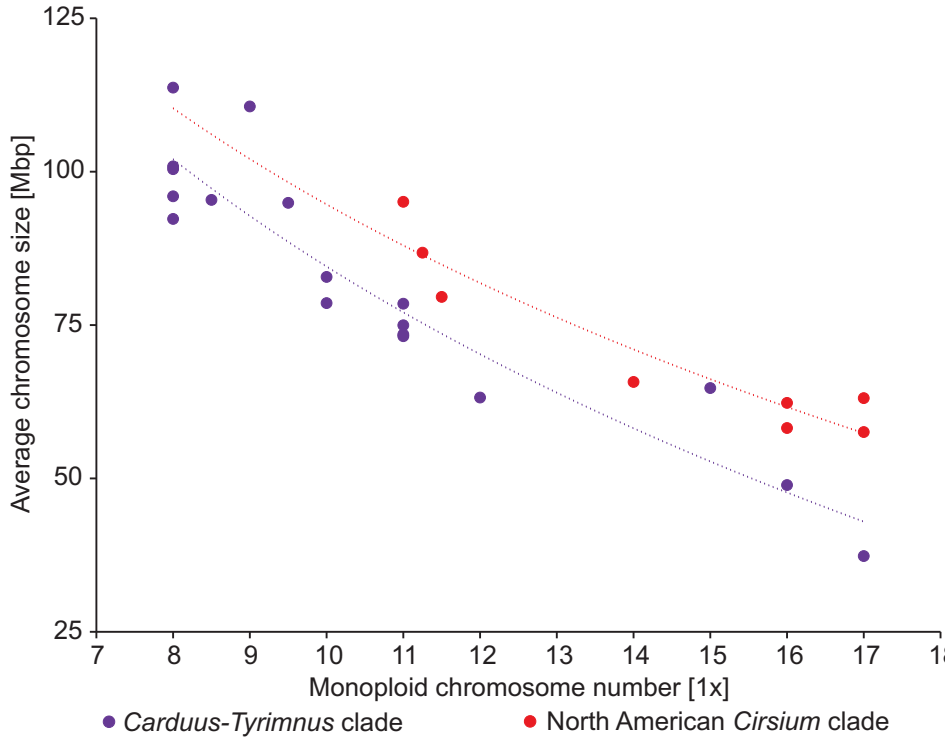

Fig. 6. Average chromosome size increases with decreasing monoploid chromosome number in the *Tyrimnus-Carduus* subgen. *Carduus* clade and the North American *Cirsium* clade.

Descending dysploidy in Carduus and Cirsium

The reconstructed ancestral monoploid genome size of *Carduinae* (1Cx~1100 Mbp; based on fastML method in Phytools R package, Revell 2012) does not change substantially along the backbone of the subtribe (Fig. 1) based on the ancestral monoploid chromosome number (n = 17; Mota et al. 2016, Ackerfield et al. 2020). The smaller genome size reported for North American *Cirsium* taxa (compared to their Eurasian relatives, which all have n = 17), might be associated with a decrease in their chromosome numbers (n = x = 17→10). Similarly, as in the *Carduus* clade, which have a smaller number of chromosomes (n = x = 16→8), the genome size is also small (Fig. 1). However, in these clades this reduction is proportionally smaller than the decrease in monoploid chromosome numbers, which indicates there is an increase in the size of the chromosomes associated with the decrease in the monoploid chromosome number (Fig. 6). This trend indicates descending dysploidy, which might have resulted from the combination of chromosomal fusions and small deletions during/due to chromosome translocations (Mayrose & Lysak 2020) in North American *Cirsium* and *Carduus*. Also, the karyotypes of *Lamyropsis cynaroides* (n = x = 13) and *Galactites tomentosa* (n = x = 11), whose corresponding genomes are slightly smaller (1Cx = 978 or 968 Mbp, respectively) than the *Carduinae* ancestral state, possibly evolved by a similar type of descending dysploidy.

These results support the widely accepted view that descending dysploidy is the predominant type of karyotype evolution in *Cardueae* (Sánchez-Jiménez et al. 2009, Vallès et al. 2012, Mota et al. 2016). In contrast, substantial genome downsizing in the *Cynara*, *Silybum*, *Tyrimnus* and *Italicum* clades is not associated with any change in chromosome number or genome enlargement in the *Lophiolepis* clade. In *Ptilostemon*, a reduction in chromosome number (n = 17→16) is even associated with an increase in genome size.

Correlation of genome size and GC content

Genomic GC content is often found to be positively correlated with monoploid genome size, especially in groups of plants composed of species with relatively small genomes (Lipnerová et al. 2013, Veleba et al. 2014, 2017, Šmarda et al. 2019). A positive relation between these two variables across the entire subtribe *Carduinae* was recorded in this study. One explanation for such a pattern is that genome enlargement or shrinkage is driven by amplification or removal of GC-rich repetitive DNA, as previously documented, for example, in genomes of the grass species *Brachypodium distachyon* (Stritt et al. 2019) and several panicoid species (SanMiguel &Vitte 2009, Estep et al. 2013), as well as in *Tanacetum* in the *Asteraceae* (Olanj et al. 2015). Although most of the major evolutionary transitions in *Asteraceae* were most likely associated with shifts in transposable element abundance (Staton & Burke 2015), a more detailed study evaluating the role of GC-rich repetitive DNA proliferation/removal in the evolution of genome size in *Carduinae* is needed.

Neopolyploidy is rare in spinose species

Tetraploid status has been cytometrically confirmed in four species within the Eurasian *Cirsium* subgroup (~10% of the species studied in this subgroup). Although this study included few *Cirsium* species from East Asia and North America, a comparison with published chromosomal counts suggests that the low prevalence of polyploid species in Europe and western and central Asia contrasts with the numerous polyploids (4x, 5x, 6x) reported in *Cirsium* in East Asia, where it was detected in 27 mostly soft-leaved poorly- or non-prickly species (Watanabe 2008, Rice et al. 2015). Among the pricklier species in the *Lophiolepis* clade, only diploids were detected (Table 1), even though tetraploid chromosome numbers (2n = 68) are occasionally reported in this clade for four of the species analysed: *Cirsium bulgaricum* (Tonian 1982), *C. ciliatum* (Kuzmanov et al. 1991), *C. leucocephalum* (Nouroozi et al. 2010) and *C. ligulare* (Kuzmanov et al. 1986, 1991). Although it is possible that variation in intraspecific ploidy exists in these taxa and by chance we only analysed diploid individuals in this study, it is more likely that tetraploid counts for these species are erroneous due to the confusion over the widely distributed and morphologically polymorphic *C. vulgare*. The low frequency or absence of polyploidy among pricklier compared to the high frequency among less prickly species of *Cirsium* s. l. is more likely to reflect a similar pattern across the whole tribe *Cardueae,* of an absence or very low incidence of polyploidy in prickly *Cousinia*, *Carlina*, *Echinops*, *Ptilostemon*, *Cynara* versus its presence in soft-leaved *Centaurea*, *Saussurea*, *Xeranthemum*, *Psephellus*, *Klasea* and *Carthamus* (cf. Watanabe 2008, Vallès et al. 2012, Rice et al. 2015).

Since prickliness primarily reflects herbivore pressure and is associated with a high proportion of sclerenchyma, it is likely that similar factors responsible for the low incidence

of polyploidy among woody species (Knight & Beaulieu 2008, Leitch & Leitch 2012, Rice et al. 2019) could also constrain the incidence of polyploidy among prickly species. However, this hypothesis needs to be tested at a broader phylogenetic scale across families and genera comprising both prickly and herbaceous species. Interestingly, there is also a geographical and altitudinal trend in plant prickliness: with prickly species predominant in the Mediterranean and Irano-Turanian regions and soft-leaved herbaceous species more common in temperate areas or at high altitudes. Thus, the differences in the incidence of polyploidy between prickly and non-prickly *Carduinae* (or *Cardueae*) lineages only indirectly reflect the global trend in the geographical distribution of polyploids in which frequency increases with latitude and altitude (Rice et al. 2019).

Could genomic parameters evolve adaptively?

The *Lophiolepis* clade with large genomes has larger achenes than the Eurasian *Cirsium* subgroup and *Carduus* clade with small genomes (Fig. 3D, E; $P_{M\text{-}W} < 0.001$ both for length and weight). Although, this pattern seemingly indicates a positive association between achene size and genome size, such a relationship is absent across the subtribe. Heavier and larger achenes of the *Lophiolepis* clade and North American *Cirsium* subclade probably reflect the need for larger nutrient reserves, which are necessary for rapid germination and seedling survival during critically short moist periods in xeric and more disturbed habitats (Moles & Westoby 2004) typical for most representatives of these two clades (Davis & Parris 1975, Werner 1976, Keil 2006). Similarly, across the whole subtribe *Carduinae*, Mediterranean and Irano-Turanian species also appear to have heavier (often more rounded) achenes than other temperate or mountain species, independent of their genome sizes. This pattern is possibly a consequence of the better survival of large achenes during the summer drought period typical of the Mediterranean climate, as has been demonstrated in field observations and glasshouse experiments focusing on the seed mass of various Western Australian *Fabaceae*, *Myrtaceae* and *Proteaceae* by Hallett et al. (2011). Achene size thus could have evolved adaptively in *Carduinae,* but independently of genome size, as has been suggested for multicellular plant structures such as leaves, flowers, fruits and seeds (see Knight & Beaulieu 2008, Krahulcová et al. 2017). However, achene weight was weakly correlated with genomic GC content in our study. Interestingly, GC content has been previously reported to be positively associated with dryer climates in the families *Droseraceae* (Veleba et al. 2017), *Orchidaceae* (Trávníček et al. 2019) and the whole monocot clade of angiosperms (Šmarda et al. 2014). Therefore, it is possible that GC content evolved in response to aridity also in *Carduinae*. The correlation of achene weight with GC content in this subtribe might thus be mediated by the independent evolution of both these traits driven by drought conditions, a hypothesis that requires further testing.

The small genomes of *Cirsium italicum*, *Galactites*, *Notobasis*, *Silybum* and *Tyrimnus* could be conditioned by their short life cycles (cf. Supplementary Table S3) in which the long-lasting replication of large genomes might present a significant selective disadvantage (Bennett 1972). However, this relationship was not tested because for many *Carduinae* it is difficult to distinguish between true annuals and occasionally annual/biennial/shortly perennial monocarpic species. Furthermore, it is difficult to distinguish between true monocarpic and monocarpic species occasionally forming rhizomes with daughter leaf

rosettes that are actually facultatively polycarpic species and whose life history is determined by the conditions in which the plant grows (Supplementary Table S3).

In plants with small genomes, the size of the genome does not necessarily determine minimum guard cell size when compared to plants with large genomes (Veselý et al. 2012, Faizullah et al. 2021). This could also apply to *Carduinae*, as their genome size is relatively small compared with other flowering plants (Leitch et al. 2005, 2019, Faizullah et al. 2021). Indeed, the results presented only reveal a weak correlation between genome and stomatal size, which could indicate that stomatal size could also be significantly driven by environmental conditions. Similar to the achene example mentioned above, this could be a response to habitat xericity. Dry conditions are assumed to favour small stomata, due to their higher responsiveness and efficiency of CO_2 uptake (Franks & Farquhar 2007, Lawson & Blatt 2014). In addition, small stomata are typically found in the xerophilous *Lophiolepis* clade (Davis & Parris 1975, Werner 1976) and not in the Eurasian *Cirsium* subgroup, which frequently occur in mesophilous and alpine meadows or open park-like forests in temperate climates (Werner 1976, Bureš 2004, Yıldız et al. 2016), despite the present finding that genome sizes in *Lophiolepis* are generally larger than in European *Cirsium* (Fig. 1).

Supplementary materials

Data S1. – Complete phylogenetic tree of *Carduinae* with 226 terminal nodes (species/intraspecific taxa) in Newick format.
Data S2. – Final (pruned) phylogenetic tree of *Carduinae* with 106 terminal nodes (analysed taxa) in Newick format.
Fig. S1. – Measurement of achene length.
Fig. S2. – Complete phylogenetic tree of *Carduinae* with 226 terminal nodes (species/intraspecific taxa).
Fig. S3. – Final (pruned) phylogenetic tree of *Carduinae* with 106 terminal nodes (analysed taxa).
Fig. S4. – Comparison of the phylogeny constructed for *Carduinae* using samples analysed cytometrically with the phylogeny for *Carduinae* by Ackerfield et al. (2020).
Table S1. – Populations and taxa studied.
Table S2. – Primary flow cytometric measurements of individuals, populations and taxa.
Table S3. – Data for analysed taxa; names, 2n, ploidy level, holoploid 2C genome size, monoploid 1Cx genome size, genomic GC content, average chromosome size, achene weight, achene length, stomatal guard cell length.
Table S4. – PCR primers' sequences.
Table S5. – GenBank accessions of analysed sequences.
Table S6. – Flow-cytometric standards.
Table S7. – Phylogenetic signals.
Table S8. – Regression models.

Supplementary materials are available at www.preslia.cz

Acknowledgements

We would like to thank Daniela Bunde and Albert-Dieter Stevens (Berlin-Dahlem), Vladimir Khlobystov (Southern Federal Univ. Rostov-on-Don), Kerstin Rapp (Georg-August Univ., Göttingen), Vlastik Rybka (Troja, Prague) and Andreas Titze (Philipps-Univ., Marburg) for providing seed or leaf material form their Botanical Garden collections. We also thank to Andrea Gálová, Hana Galušková, Helena Chytrá, Kryštof Chytrý, Milan Chytrý, Jiří Danihelka, Corrado Marceno and Pavel Novák (Dept. Bot. & Zool., Masaryk Univ., Brno) for collecting field samples. For providing the locations of natural populations, we are indebted to Gianmaria Bonari (Univ. Sienna), Melania Marchi (Tourist Company Escursioni Apuane) and Sierra Dawn Stoneberg Holt (Montana, U.S.). Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA CZ LM2018140, supported by the Ministry of Education, Youth and Sports of the Czech Republic)

and by the ELIXIR-CZ project (LM2018131), part of the international ELIXIR infrastructure. This work was financially supported by the Czech Science Foundation (project no. GA20-15989S).

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Evoluce velikosti genomu a obsahu GC bází v tribu *Carduinae*: vzácná sestupná dysploidie a polyploidie, slabá kontrola prostředím a silný fylogenetický signál

Velikost genomu a genomický obsah bází jsou základní druhově specifické atributy, které často vymezují také rody nebo vyšší taxony. V kombinaci s chromosomovými počty tyto znaky umožňují také identifikovat polyploidii, dysploidii, hybridizaci a další mechanismy evoluce genomu či karyotypu. Evoluce může u těchto genomových znaků často probíhat adaptivně, pod selekčním tlakem prostředí. V naší studii jsme rekonstruovali evoluci těchto genomových znaků u podtribu *Carduinae* (*Asteraceae*) v kontextu změn počtů jejich chromosomů. Velikost genomu a genomický obsah GC bází u 119 taxonů, jsme vymapovali na fylogenetický strom, konstruovaný pomocí sedmi sekvenčních markerů. Tato genomická data jsme dále porovnali s naměřenou délkou svěracích buněk průduchů a délkou a hmotností nažek, abychom identifikovali, zda se studované genomové znaky mohly vyvíjet adaptivně. Detekovali jsme silný fylogenetický signál jak u genomických znaků, tak u znaků na nažkách a svěracích průduchových buňkách. Tyto znaky proto poměrně dobře vymezují většinu rodů a dalších linií v podtribu *Carduinae*. Zjistili jsme, že velikost monoploidního genomu a průměrná velikost chromosomů pozitivně korelují s genomickým obsahem GC bází a s délkou svěracích buněk. Velikost genomu se tedy mohla v podtribu *Carduinae* vyvíjet adaptivně pod kontrolou efektivity/velikosti svěracích buněk průduchů, ale jen do určité míry. V rodě *Cirsium* mají totiž druhy z podrodu *Lophiolepis* (= monofyletická linie) menší svěrací buňky (navzdory svým větším genomům), což může souviset právě s jejich větší afinitou k sušším stanovištím ve srovnání s většinou druhů typového podrodu (= parafyletická skupina *Cirsium*), které mají svěrací buňky větší a genomy menší. Velikost nažek se vyvíjela nezávisle na velikosti genomu pravděpodobně v závislosti na rozsahu suché letní periody typické pro mediterránní klima, v němž se většina *Carduinae* s velkými nažkami vyskytuje. Z tohoto důvodu pravděpodobně zprostředkovaně koreluje velikost nažek s genomickým obsahem GC bází, který by rovněž měl pozitivně odrážet afinitu k suchému prostředí, jak se již dříve ukázalo u čeledí *Droseraceae*, *Orchidaceae* resp. napříč celou linií jednoděložných rostlin, nicméně tato hypotéza by

měla být ještě podrobněji testována. U druhů rodu *Carduus* nebo u severoamerických druhů rodu *Cirsium* se jejich chromosomy mají tendenci zvětšovat se při poklesu základního chromosomového čísla (x = 16→8, resp. 2n = 17→10), což naznačuje přítomnost descendentní dysploidie spojené s delecemi částí fúzovaných chromosomů. Podobně je snížení počtu chromosomů provázeno proporčně menší redukcí genomu také v rodech *Galactites* (2n = 22) a *Lamyropsis* (2n = 26). Naproti tomu v rodě *Ptilostemon* narostla velikost genomu přesto, že počet chromosomů zde poklesl (2n = 34→32). Konečně u rodů *Silybum*, *Tyrimnus*, *Cynara* a u druhu *Cirsium italicum* se velikost genomu zmenšila navzdory jejich stabilnímu počtu chromosomů (2n = 34). To naznačuje, že karyotypová evoluce neprobíhá v rámci podtribu pouze descendentní dysploidií spojenou s delecemi částí fúzovaných chromosomů. Korelace genomického obsahu GC bází s průměrnou velikostí chromosomů, resp. velikostí genomů, naznačuje, že jedním z mechanizmů evoluce velikosti genomu u *Carduinae* mohla být také proliferace a eliminace GC-bohatých repetitivních úseků DNA. Polyploidie je v podtribu *Carduinae* poměrně vzácná a byla potvrzena pouze u pěti studovaných druhů, včetně invazního *Cirsium vulgare*, u kterého by mohla být důsledkem vzdálené (mezipodrodové) hybridizace, jak naznačují jeho intermediární genomické a anatomické znaky, konfliktní morfologie a fylogenetická pozice, ale také jeho ochota hybridizovat s druhy obou podrodů – *Lophiolepis* i *Cirsium*. V podtribu *Carduinae*, ale zejména napříč celým tribem *Cardueae*, se polyploidie jeví méně častou u více ostnitých linií. Takový rozdíl ve výskytu polyploidie však může nepřímo odrážet i dlouho známý globální trend v geografickém rozšíření polyploidů, neboť ostnitější linie dominují v oblastech se sušším mediteránním klimatem, zatímco neostnité linie upřednostňují spíše temperátní nebo horské oblasti. Proto by tento trend měl by být podrobněji analyzován napříč těmi liniemi krytosemenných, které zahrnují ostnité i neostnité druhy. Rekonstruovaná fylogeneze, rozdíly v genomových parametrech, velikosti svěracích buněk průduchů i nažek podporují oddělení monofyletického kladu/podrodu *Lophiolepis* od zbytku rodu *Cirsium*. Také *Cirsium italicum* lze pravděpodobně oddělit od zbytku monofyletického kladu *Lophiolepis*+*Picnomon*+*Notobasis* na základě velikosti genomu, genomického obsahu bází a délky, hmotnosti a tvaru nažek.

How to cite: Bureš P., Ozcan M., Šmerda J., Michálková E., Horová L., Plačková K., Šmarda P., Elliott T. L., Veselý P., Ćato S., Norouzi M., Sheidai M. & Zedek F. (2023) Evolution of genome size and GC content in the tribe *Carduinae* (*Asteraceae*): rare descending dysploidy and polyploidy, limited environmental control and strong phylogenetic signal. – Preslia 95: 185–213.

Preslia, a journal of the Czech Botanical Society

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