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Analysis of karyological variability in *Taraxacum*

Ph.D. Thesis

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"I can do things you cannot,
you can do things I cannot;
together we can do great things."
Mother Teresa

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DECLARATION

I hereby declare that this thesis has been worked out by myself together with listed co-authors. All literary sources cited in this thesis are included in the References.

In Olomouc …………………

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Abstrakt:

Rod *Taraxacum* (pampelišky) představuje druhově velmi bohatou a celosvětově rozšířenou skupinu rostlin, která je řazena do čeledi Asteraceae, podčeledi Cichorioideae. Taxonomie tohoto rodu je známá svou složitostí, způsobenou retikulární evolucí, na níž se podílejí sexuální diploidní i apomiktické polyploidní druhy. Apomixie (nepohlavní rozmnožování za pomocí semen) je u pampelišek regulována za pomocí tří dominantních genů (pro meiotickou diplosporii, partenogenetický vývoj embrya a autonomní vývoj endospermu). Navíc o lokusu pro meiotickou diplosporii je známo, že se nachází alespoň na jednom z NOR-chromozomů. Nicméně, navzdory tomuto zjištění toho o struktuře a variabilitě karyotypů sexuálních a apomiktických pampelišek víme stále velice málo.

První část disertační práce je zaměřena na stanovení počtu chromozomů, ploidní úrovně a velikosti genomu pro vybrané zástupce apomiktických pampelišek ze sekce *Taraxacum* (*T. officinale* agg.). Cílem této studie bylo odhalit počet chromozomů v jádrech dvaceti osmi druhů pampelišek klasickou metodou roztlakových preparátů z jejich mitoticky aktivních kořenových špiček a zjistit míru variability velikosti genomu u dvaceti šesti druhů pomocí metody průtokové cytometrie. Výsledky studie potvrdily stejný počet chromozomů a ploidní úroveň (2n = 3x = 24) u všech dvaceti osmi druhů a u zhodnocených genomů odhalily pouze malou variabilitu v jejich obsahu DNA.

Druhá část této práce se primárně věnuje cytogenetickému mapování lokusů pro 45S a 5S rDNA na metafázních mitotických chromozomech za pomocí techniky FISH, a to v karyotypech třiceti osmi druhů sexuálních i apomiktických pampelišek, zastupujících sedmnáct sekcí. Cílem této studie bylo kromě popisu základních vlastností

jednotlivých karyotypů sexuálních a apomiktických pampelišek odhalit případné společné paterny v distribuci zmíněných lokusů rDNA na mitotických metafázních chromozomech, které by mohly být nápomocné při orientaci ve velmi složité taxonomii rodu. Naše výsledky odhalily ve většině studovaných karyotypů stejný poměr (1:2) v počtu lokusů pro 45S a 5S rDNA na haploidní sadu chromozomů. Na druhou stranu však byla zjištěna značná variabilita v pozicích obou lokusů rDNA na mitotických metafázních chromozomech, a to jak při porovnání karyotypů pampelišek zastupujících blízce příbuzné sekce, stejnou sekci, tak dokonce i vrámci jednoho karyotypu. Relativně vysoká variabilita získaných výsledků nejen z karyologické studie, ale i z výsledků doplňkových metod (určení velikosti genomu, obsahu GC bází a sekvenování ITS oblastí) je tedy pravděpodobně důsledkem složité retikulární evoluce tohoto rodu, zahrnující častou hybridizaci a polyploidizaci.

Klíčová slova:	5S rDNA, 45S rDNA, FISH, idiogram, karyologie, metafázní chromozomy, NOR, obsah GC bází, velikost genomu, *Taraxacum*
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Abstract:

Taraxacum F. H. Wiggers (dandelions) is a species-rich genus from family Asteraceae (subfamily Cichorioideae). It is well-known for its worldwide distribution and taxonomic complexity, which is caused by reticulate evolution including sexual diploid and apomictic polyploid species. Apomixis (asexual reproduction through seeds) in this genus is regulated by three dominant loci (for meiotic diplospory, parthenogenetic embryo development and autonomous development of endosperm), with the locus for meiotic diplospory located on at least one of the NOR-chromosomes. Despite this finding, there is still a lack of knowledge about the structure and variability of karyotypes of sexual and apomictic dandelions.

The first part of the thesis is focused on the determination of chromosome number, ploidy level and estimation of genome size for selected apomictic dandelions from section *Taraxacum* (*T. officinale* agg.). The aim of this study was to establish the number of chromosomes in the nuclei of twenty-eight dandelion species using the squash technique from their mitotically active root tips and to reveal the variability in genome size values for twenty-six dandelion species by flow cytometry. The results of the study confirmed the same chromosome number and ploidy level (2n = 3x = 24) for all twenty-eight *Taraxacum* species and revealed only minor differences in the DNA content of evaluated genomes.

The second part of this thesis deals mainly with the cytogenetic mapping of 45S and 5S rDNA loci on metaphase mitotic chromosomes using the FISH technique in karyotypes of thirty-eight sexual and apomictic *Taraxacum* species from seventeen

different sections. In addition to the description of the basic characteristics of individual karyotypes of sexual and apomictic dandelions, the main goal of this study was to detect any common patterns in the distribution of both types of rDNA loci on mitotic metaphase chromosomes, which could be helpful for the orientation in very complex taxonomy of the genus. Our results showed the same ratio (1:2) in the number of loci for 45S and 5S rDNA per haploid level in most of the studied karyotypes of sexual and apomictic dandelions. On the contrary, considerable variability in the positions of both rDNA loci was revealed, both when comparing two or more dandelion karyotypes representing different sections (or one section) and even within one evaluated karyotype. Relatively high variability in results was further found by using other cytogenetic and molecular techniques (estimation of genome size and GC content and sequence analysis of ITS), which all can be attributed to complex reticulate evolution in *Taraxacum* genus, involving frequent hybridization and polyploidization.

Keywords:	5S rDNA, 45S rDNA, FISH, GC content, genome size, idiogram, karyology, metaphase chromosomes, NOR, *Taraxacum*
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CHAPTER 1

GENERAL INTRODUCTION

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1.1 Characteristics of the genus *Taraxacum*

1.1.1 Taxonomic classification, distribution and modes of reproduction

The genus *Taraxacum* F. H. Wiggers (dandelions) represents a large genus of flowering plants. Based on the phylogenetic analysis, dandelions belong to the family Asteraceae (syn. Compositae), the subfamily Cichorioideae, the tribe Cichorieae and the subtribe Crepidinae (Stevens 2001; Kilian *et al.* 2009; Mandel *et al.* 2017). These common perennial rosulate herbs (hemicryptophytes) are able to grow in a wide range of habitats from subtropics to alpine and arctic biotopes around the world (with exception of Antarctica; Kirschner & Štěpánek 1996; Mártonfiová *et al.* 2007; Majeský *et al.* 2017). The evolutionary centre of this genus is likely to be found in the Himalayan Mountains of Central Asia, from where probably the genus gradually spread to the rest of the world (Richards 1973; Brock 2004; Majeský *et al.* 2012).

Taxonomic complexity of genus *Taraxacum* is a result of its three different strategies of reproduction - allogamy, autogamy and apomixis (Kirschner & Štěpánek 1994; Mártonfiová *et al.* 2010). In this genus, moreover, there is a known correlation between the mode of reproduction and ploidy level. Thus diploids and very rarely tetraploids are sexual (Kirschner *et al.* 1994; Kirschner & Štěpánek 1998), while other polyploids (ranging from the most common triploids to rare dodecaploids) are apomictic (Kirschner & Štěpánek 1996, Kirschner *et al.* 2003, Vašut 2003).

The co-occurrence of sexual and apomictic reproduction, along with hybridization and polyploidy have resulted in a large number of dandelion taxa. The infrageneric classification of the genus *Taraxacum* is based on groups of ecologically, morphologically, karyologically, and evolutionarily similar taxa forming individual sections. To date, the genus comprises about 2800 described (micro)species of dandelions grouped into approximately 60 sections (Záveská Drábková *et al.* 2009; Kirschner *et al*. 2015; Majeský *et al.* 2017). There are known some sections (such as *T.* sect. *Australasica*, *T.* sect. *Dioszegia, T.* sect. *Piesis* or *T.* sect. *Primigenia*) consisting exclusively sexual species and considered evolutionary ancestral (or precursor), whereas sections composed exclusively of apomicts (e.g. *T.* sect. *Hamata* or *T.* sect. *Naevosa*) are considered evolutionary derived (Kirschner *et al*. 2015). However, most of the dandelion sections

contain one or few sexual species and usually large number of polyploid apomictic taxa, traditionally referred to as microspecies (Majeský *et al.* 2017).

Generally, sexuals and apomicts in *Taraxacum* have most often relatively different geographical distribution, especially in Europe. This phenomenon is known as geographical parthenogenesis (Hörandl 2006). Diploid sexuals occupy only the southern and central regions of Europe, whereas polyploid apomicts inhabit larger areas. In the southern and central parts of Europe, polyploids usually co-occur with diploids, but they are exclusively distributed farther to northern regions of Europe (De Kovel & De Jong 2000; Verduijn *et al.* 2004; Kirschner *et al.* 2015; Mártonfiová 2015).

1.1.1.1 Apomixis in dandelions

The term apomixis in plants refers to natural form of asexual reproduction, that leads to production of genetically uniform offspring identical to their mother plant. Special type of apomixis is agamospermy, which is clonal reproduction through seeds and generally the term apomixis is meant in a meaning of agamospermy (Nogler 1984; Koltunow 1993). In apomictic plants, the embryo (Figure 1) can be formed either directly from somatic cells of the nucellus or the ovular integuments (sporophytic apomixis, syn. adventitious embryony) without interventing megagametophyte or from the egg cell of the megagametophyte (gametophytic apomixis). Gametophytic apomixis (Figure 1) can be further subdivided depending on whether the unreduced female gametophyte develops from mitotic divisions of the aposporous initial cell (apospory) or from mitotic (mitotic diplospory) or modified meiotic divisions (meiotic diplospory) of the megaspore mother cell (Van Dijk & Vijverberg 2005; Noyes 2007; Majeský *et al.* 2017).

Apomixis in dandelions is a type of meiotic diplospory, known as *Taraxacum* type (Figure 1), which is the most similar to sexual reproduction (Asker & Jerling 1992). In the case of *Taraxacum* type diplospory, during the megasporogenesis, the first division restitution is followed by a normal second meiotic division, which produces two unreduced megaspores. Similarly to sexual reproduction, only one megaspore is further mitotically divided giving to rise an unreduced megagametophyte (Vijverberg *et al.* 2004; Vašut *et al.* 2014; Janas *et al.* 2016). Subsequently, the unreduced egg cell of the embryo sac (megagametophyte) develops parthenogenetically into the embryo and the central cell of the unreduced embryo sac develops autonomously into the endosperm (Van Dijk 2003, Ozias-Akins & Van Dijk 2007). Some genetic studies indicate, that apomixis in the genus

Taraxacum is regulated by three dominant loci. The first locus named *DIPLOSPOROUS* (*DIP*) controls meiotic diplospory and the second one called *PARTHENOGENESIS* (*PAR*) encodes parthenogenetic embryo development (Van Dijk & Bakx-Schotman 2004; Vijverberg *et al*. 2004; Vijverberg *et al.* 2010). Both of them (*DIP, PAR*) are inherited as dominant traits, independently of each other. Moreover, it has been found, that the *DIP* locus in *Taraxacum* is located on at least one of the NOR chromosomes. Finally, the third locus for apomixis in dandelions, probably involved in endosperm development, remains unresolved so far (Ozias-Akins & Van Dijk 2007; Vijverberg & Van Dijk 2007; Vašut *et al.* 2014).

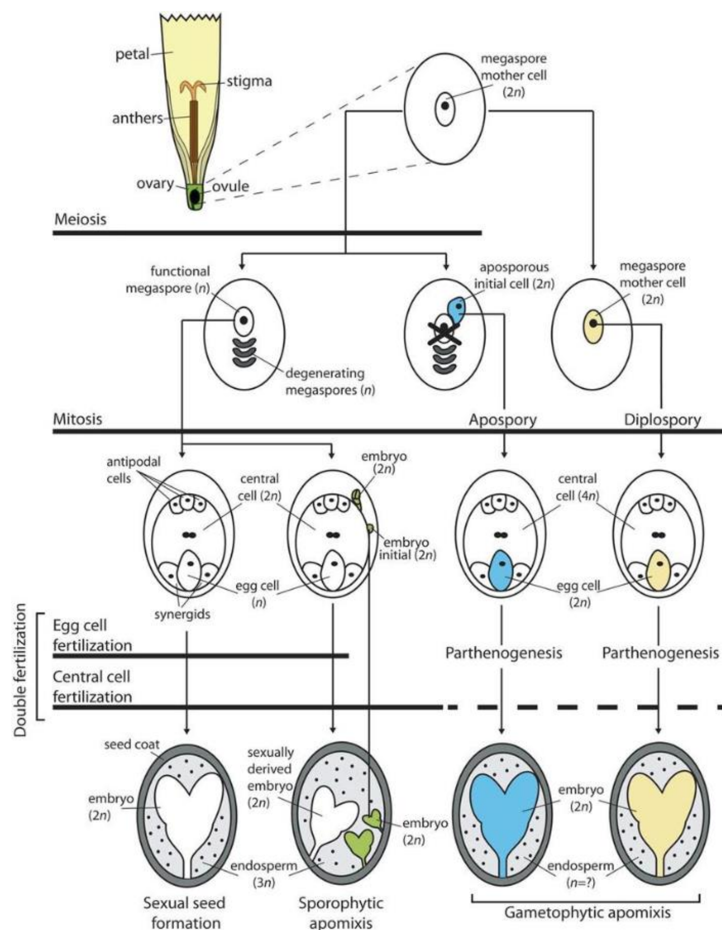


Figure 1. Schematic diagram of mechanisms of sexual and apomictic seed development (adapted from Hand & Koltunow 2014). This diagram compares the main differences in the seed development pathway for sexual seed formation and the apomictic mechanisms of sporophytic and gametophytic apomixis. Meiosis, mitosis, and double fertilization are the major components of the seed formation pathway. In gametophytic apomixis, embryo formation is initiated without the fertilization (parthenogenesis), however, endosperm formation can occur either with or without fertilization, which is represented by a dashed line. The ploidy level of endosperm formed through gametophytic apomixis is variable, depends on a number of factors, and is therefore represented by a question mark (?). In diagram are used different colours track the precursor cells that form the embryo for each pathway: sexual (white), sporophytic apomixis (green), diplospory (yellow), and apospory (blue).

1.2 Plant nuclear genome

A single copy of the complete genetic information of an organism (organelle) had already been termed genome in 1920 by Hans Winkler (Doležel & Greilhuber 2010; Peterson 2014). In plants, the hereditary information, stored in molecules of DNA, is usually contained in three different organelles. The cell nucleus contains the majority of the genetic material in the form of multiple linear molecules of DNA and represents the nuclear genome. The rest of the DNA in a plant genome is stored in semiautonomous organelles, mitochondria (mitochondrial genome) and chloroplasts (chloroplast genome), which contain their own DNA in forms of linear or circular molecules (Smith 2017; Kersey 2019).

1.2.1 Plant nuclear genome size and base content estimation

In literature, the term genome size is applied in two following meanings. Holoploid genome size is defined as the DNA content of the unreplicated haploid nuclear chromosome complement (with chromosome number n), regardless of the organism's ploidy and it is expressed by the C-value. On the other hand, the mass of nuclear DNA in a single chromosome set (with basic chromosome number x) of an organism is characterized as monoploid genome size, abbreviated with the C_x-value (Greilhuber *et al.* 2005; Doležel *et al.* 2007a). DNA content is quantified in absolute units, picograms of DNA (pg) or a number of base pairs (bp), where 1 pg ~ $9.78 \cdot 10^8$ bp (Doležel *et al.* 2003).

Nuclear genome size represents one of the general characteristics of species, important for many fields of research, including ecology, evolutionary biology, taxonomy and genomics (Bennett & Smith 1976; Doležel *et al.* 2007a, c; Kron *et al.* 2007). During the last 60 years, C-values have been estimated for more than 10 000 of eukaryotic species and obtained data is showing that holoploid genome sizes vary more than 66 000-fold across eukaryotes (Pellicer *et al.* 2010; Garcia *et al.* 2014). Similarly, C-values in angiosperms vary more than 2400-fold, with the smallest (so far) reported genome of 61 Mbp (1C = 0.062 pg) found in carnivorous taxa *Genlisea tuberosa* (Lentibulariaceae) and the largest genome of 150 Gbp (1C = 152.23 pg) in the octoploid rhizomatous geophyte *Paris japonica*, member of the family Melanthiaceae (Pellicer *et al.* 2010; Heslop-Harrison & Schwarzacher 2011; Fleischmann *et al.* 2014). Nevertheless, it has been found out very early, that the majority of eukaryotes contain in the nucleus vastly

more DNA than is necessary for coding and regulatory sequences and there is no linear relationship between DNA amount and the complexity of the organism (Mirsky & Ris 1951; Doležel *et al.* 1998). In 1971, C. A. Thomas (1971) named this phenomenon as the "C-value paradox". The discovery of non-coding, repetitive DNA provided a clue to the paradox, but some puzzles and unanswered questions remain enigmatic. For this reason, the phenomenon has been renamed the "C-value enigma" (Gregory 2001).

Another significant parameter of the plant nuclear genome is the DNA base composition, which in combination with genome size may provide a closer insight into the organization of the genome (Doležel *et al.* 2007a). For a particular region or the whole genome, base composition is usually expressed as the percentage of guanine (G) and cytosine (C) bases (GC content/genomic GC content). Within angiosperms, the highest GC contents were found so far in genomes of some grass taxa (Poaceae), usually more than 45% (Meister & Barow 2007; Veselý *et al.* 2012; Šmarda *et al*. 2019). It is also worth noting that in general, genomes of monocots have a statistically higher GC content compared to dicots (Šmarda & Bureš 2012). In addition, as reported by Veselý *et al.* (2012) and Šmarda *et al.* (2014, 2019) GC contents in plant genomes quadratically correlated with genome size, suggesting low genomic CG content that can be found in plants with both very small and large genomes. However, contrary to prokaryotic genomics, where the knowledge of GC content has a long tradition in their evolution and systematics, the details about genomic GC content of vast majority of higher plants are still lacking and its meaning in the ecology and evolution of particular taxa is still poorly known (Šmarda *et al*. 2012, 2014, 2019).

1.2.1.1 Methods for nuclear genome size and base content estimation

Several techniques have been employed to determine the DNA content of plant nuclei, but two pitoval methods in this field are Feulgen densitometry (hereafter FD) and flow cytometry (hereafter FCM). In history, the vast majority of genome size data in plants biology were acquired by FD, which was the prevailing method for genome size estimation until the late 1990s, when FCM has come to the fore of this field (Doležel *et al.* 2007a; Leitch & Bennet 2007; Greilhuber *et al.* 2010; Elliott & Gregory 2015). Nowadays, FD plays a rather marginal role of a supplementary technique in plant genome research, although in some respects has still several advantages over the FCM, such as the possibility to measure DNA amounts in single cells and very small DNA amounts

of single particles, there is a visual control, plant samples can be stored for a long time before their analysis, there is no debris in the histogram, because only nuclei are measured and the sample is not a liquid suspension of single particles so there is no risk of microbial contamination (Greilhuber 2008).

FCM was originally developed for the analysis of nuclear DNA content in human and animal blood cells, but due to easy and rapid sample preparation, fast sample processing and possibility, that material does not need to be actively dividing, FCM has become the method of choice in plant genome research (Doležel & Bartoš 2005; Doležel *et al.* 2007b). Generally, for FCM estimations of relative nuclear DNA content in plants (Figure 2), the suspension of intact nuclei is extracted together from a small amount of test sample tissue (T) and the tissue of the internal standard (S) by mechanical homogenization (e.g. using a sharp razor blade, method of Galbraith *et al.* (1983)) in an appropriate buffer solution. After filtration of the homogenate through a nylon mesh, the addition of intercalating DNA dye such as propidium iodide (PI) or ethidium bromide (EB) and elimination of RNA with RNAase (to provide meaningful DNA content measurements), the sample is ready for analysis. Then the amount of light emitted by the fluorochrome of stained nuclei is detected by flow cytometer and displayed in a form of a histogram of relative fluorescence intensity (Figure 2), which corresponds to relative nuclear DNA content (Doležel *et al.* 1992, 2007a; Kron *et al.* 2007; Greilhuber 2008).

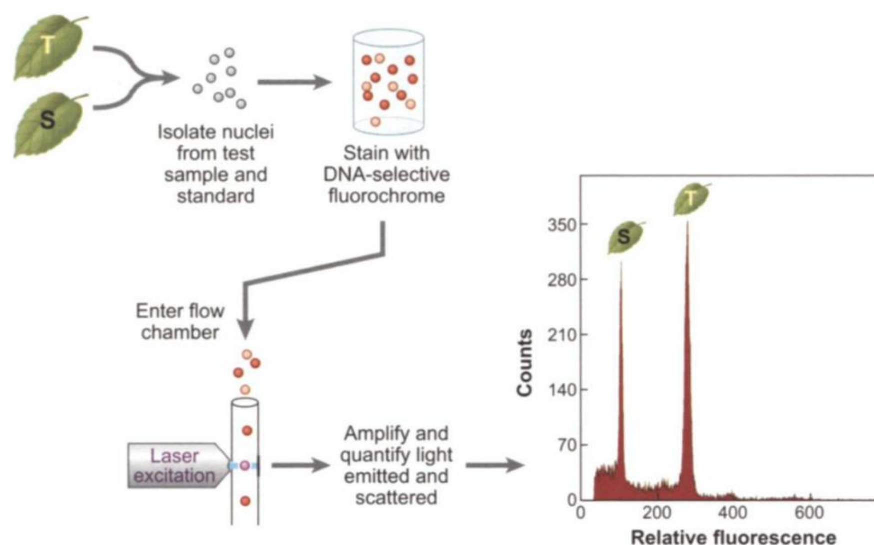


Figure 2. The procedure of estimation of genome size in the test sample by FCM (adapted from Kron *et al.* 2007). The absolute DNA content of the test sample (T) is estimated by a comparison of the mean values of fluorescence intensity of nuclei in the same phase of the cell cycle (typically in G1) between the target and an internal reference standard (S), whose genome size is known.

Subsequently, the absolute DNA content of the test sample (Figure 2) is calculated by comparing the relative positions of G1 peaks between the test sample and internal standard (whose genome size is known) following the formula (Doležel & Bartoš 2005):

$$sample\ 2C\ DNA\ content[pg\ DNA] = \frac{(sample\ G1\ peak\ mean) \cdot (standard\ 2C\ DNA\ content\ [pg\ DNA])}{standard\ G1\ peak\ mean}.$$

However, it is worth noting, that based on recommendations from Doležel *et al.* (2007a) several other conditions must be fulfilled to estimate the absolute DNA content of the test sample more reliably.

Generally, FCM has a broad range of applications in plant research. Except for the already mentioned nuclear genome size estimation, this method is also widely used for ploidy determination, identification of reproductive system, detection of mixoploidy, aneuploidy or endopolyploidy, chromosome sorting, study the position of a cell within the cell cycle, analysis of semiautonomous organelle (chloroplasts and mitochondria) or plant pathogens (e.g. viruses, bacteria and fungi) and last but not least for an approximation of base composition (GC content) in plant genomes (Doležel *et al.* 2007a, b).

Determination of genomic GC contents by FCS is founded on the comparison of parallel measurements of the sample and the internal standard stained with two different dyes: (1) intercalating dye (usually PI) for absolute DNA content estimation and (2) base-specific dye such as AT-specific 4′,6-diamidino-2phenylindole dihydrochloride (DAPI) or GC-specific mithramycin (MI) A for calculation of the GC content. Compared to the estimation of nuclear DNA content by FCM, the formula for determining of GC content is much more complex and its solving requires some mathematical approximation methods (e.g. *regula falsi* method; Barow & Meister 2002; Meister & Barow 2007). The reason for formula complexity is a non-linear relationship between the base-specific fluorescence and the base content of the genome, which depend on the property of individual base-specific dye requiring a specific number of consecutive base pairs of the same type (AT or GC) to bind to the DNA molecule. Unfortunately, the formula for determining the genomic GC content is valid only on the condition that the distribution of base pairs within the measured genomes is random. But in reality plant nuclear genomes are rich in AT- or GC-rich repetitive DNA sequences, which means that current determinations of GC content by FCM are only approximate (Barow & Meister 2002; Meister & Barow 2007; Šmarda *et al.* 2008, 2012; Šmarda & Bureš 2012). However,

notwithstanding some known limitations of this method, FCM is still irreplaceable in the field of DNA base composition research and as pointed out by Šmarda *et al.* (2012, 2019) and Veselý *et al.* (2012) flow cytometric estimates of GC content are usually highly correlated with estimates from other more demanding biochemical methods (e.g. thermomechanical analysis (TMA)) or sequence data.

1.2.1.2 Nuclear genome size and GC content estimation in dandelions

One of the first records of nuclear genome size in the genus *Taraxacum* were provided by Bennett & Smith (1976) and Bennett *et al.* (1982), who estimated the genome size of unknown *T. officinale* species as 2C = 2.60 pg and 2C = 2.55 pg, respectively, using Feulgen densitometric analysis. Only in 2005, Záveský *et al*. (2005) published the first more comprehensive study, in which genome size values of eighteen *Taraxacum* species from thirteen sections has been estimated using FCM. These values ranged from 2C = 1.74 pg (in diploid *T. linearisquameum*, sect. *Taraxacum*) to 2C = 6.91 pg (in tetraploid *T. albidum* sect. *Mongolica*). Although several other nuclear DNA content estimation studies in the genus *Taraxacum* were published between the years 2005 and 2018 (e.g. Vidic *et al.* 2009; Vašut *et al*. 2014; Iaffaldano *et al.* 2017; see Chapter 3.1), most of them contained only data from unspecified members of *T. officinale*. Only two recent studies, Macháčková *et al.* (2018) and Šmarda *et al.* (2019), have expanded the knowledge of nuclear C-values of dandelions. While the first-mentioned study reported 2C nuclear DNA content in 28 dandelion taxa from the section *Taraxacum* (for more details see Chapter 3.1), the second study maps genome size values of 28 dandelion species across different *Taraxacum* sections. In addition, Šmarda *et al.* (2019) is the only study so far, which has published the estimates of GC content for the genus *Taraxacum*, ranging from 38.8% GC content in diploid *T. linearisquameum* (sect. *Taraxacum*) to 40.9% GC content in triploid *T. hamatum* (sect. *Hamata*).

1.2.2 Structure and organization of plant nuclear genome

Although plants are known for large differences in genome size, they show a relatively high degree of similarity in respect to the nuclear genomic organization. The plant's nucleus is made up of the double-stranded genomic DNA, which together with associated histone and non-histone proteins form a functionally organized structure called chromatin (Alberts *et al.* 2002; Heslop-Harrison & Schwarzacher 2011).

1.2.2.1 Chromatin

Based on the level of compaction, chromatin appears in the nucleus in two different states, euchromatin or heterochromatin. Euchromatin occurs as a loosely packed, but gene-rich form of chromatin, which is usually transcriptionally active. On the contrary, highly condensed heterochromatin is relatively deficient in genes and mostly transcriptionally silent. Furthermore, heterochromatin can be subdivided into two types, constitutive and facultative heterochromatin. Constitutive heterochromatin is mainly formed at telomeres and pericentromeric regions of chromosomes, where it is permanently condensed and transcriptionally inert, whereas facultative heterochromatin is usually interspersed along chromosome arms and under certain circumstances, it may be either in the condensed or decondensed form (Grewal & Moazed 2003; Wegel & Shaw 2005; Huisinga *et al.* 2006; Saksouk *et al.* 2015).

Generally, at interphase of the cell cycle, plant nuclear genome exists in the state of decondensed chromatin fibres occupying specific territories within the nucleus and forming the loops active in gene expression and DNA replication, while at metaphase in the cell division (mitosis or meiosis) the chromatin is organized into the highly condensed structures called chromosomes (Heslop-Harrison & Schmidt 2007; Woodcock & Ghosh 2010).

1.2.2.2 Metaphase chromosomes

The term chromosome, which originates from the combination of two Greek words meaning coloured (*chrom*) body (*soma*), was firstly introduced in 1888 by German anatomist Henrich Wilhelm Gottfried von Waldeyer-Hartz (Schwarzacher 2003; Gardner *et al.* 2011). For most cytogenetic studies, condensed metaphase chromosomes obtained from either dividing somatic cells in mitosis or gametes division during meiosis have

been used, since at this stage the chromosomes are easily visualized under a light microscope. In general, metaphase chromosomes have two or three major structural features (Figure 3), including the primary constriction (i.e. centromere), telomeres and the secondary constriction (occurring only in some chromosomes).

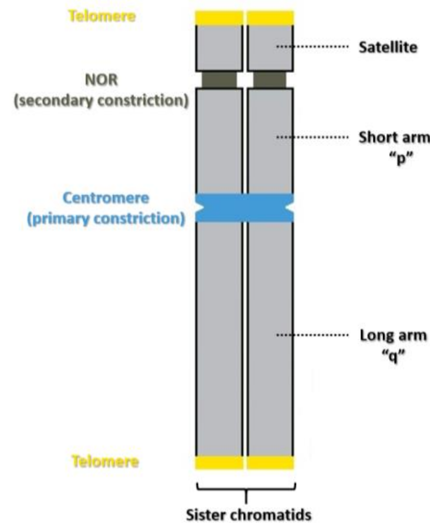


Figure 3. Schematic diagram of the main morphological features of a mitotic metaphase chromosome (adapted and modified from Heslop-Harrison & Schwarzacher 2011).

The centromere can be defined as a functional locus of the chromosome, which is responsible for sister chromatids cohesion due to the presence of a kinetochore complex and it is essential for their proper segregation during the cell division. Usually, the centromere can be distinguished from the remaining chromosomal regions by condensed heterochromatin constriction, that subdivides the chromosome into two arms (Figure 3) - the short arm (p, S) and the long arm (q, L). While in most plant species metaphase chromosomes are monocentric, there are also a few plant species, whose nuclear genomes contain chromosomes with more than one active centromere, such as dicentric chromosomes in maize or common wheat (Fu *et al.* 2012; Stimpson *et al.* 2012) or polycentric (holocentric) chromosomes in the genus *Carex* (Cuacos *et al.* 2015) or woodrush *Luzula elegans* (Heckmann *et al.* 2013), which exhibit centromere activity over nearly the entire chromatid length. It is also worth noting that, the DNA sequences located at centromere can remarkably differ and show considerable size variation, not only within closely related species, but furthermore, even between individual chromosomes within a single species (Schwarzacher 2003; Macas *et al.* 2010), whereas the function of the centromere is highly conserved. The centromere is usually composed of fibres arrays

of tandemly repeated DNA and retrotransposons (Małuszyńska & Heslop-Harrison 1991; Heslop-Harrison 2000; Ma *et al.* 2007). Nevertheless, it was also described some unusual configurations of the centromere containing single copy DNA, including even some transcribed genes (Mutti *et al*. 2010; Oliveira & Torres 2018).

On the other hand, telomeres, as terminal chromosomal domains, protect chromosome ends from degradation and fusion with neighbouring chromosomes and promote complete replication of DNA. The telomeric region in most plant species is highly conserved and composed of non-coding repetitive tandem repeats of seven-nucleotide sequences $(TTTAGGG)_n$. Nevertheless, as noted by Fajkus *et al.* (2016) and Procházková Schrumpfová *et al.* (2019), there are also plants such as *Allium* species, whose chromosomes contain atypical telomeric sequences $(CTCGGTTATGGG)_n$.

Apart from the primary constriction, some metaphase chromosomes may also contain secondary constrictions. The secondary constriction creates a button-like structure (satellite; Figure 3) at the end of the chromosome and usually also marks the site of the origin of nucleoli (nucleolar organizing region; NOR; Figure 3), a region containing multiple copies of the ribosomal genes (for more details see Chapter 1.2.3.1.1). Hence, these chromosomes are called satellite (SAT) chromosomes or NOR-bearing (NOR) chromosomes (Stace 2000; Stępiński 2014; Biscotti *et al.* 2015).

1.2.2.3 Karyotype

The karyotype generally represents the highest level of the structural and functional organization of the nuclear genome. The chromosome number, the size and morphology of individual metaphase chromosomes, along with the distribution of various types of chromosomal (primary and secondary constriction) and molecular markers (most often ribosomal RNA genes or various types of repetitive DNA) within a chromosome complement, are considered to be the features of the karyotype that are often used today for comparative evolutionary analyses in plants (Kato *et al*. 2004; Guerra 2008; Heslop-Harrison & Schwarzacher 2011). Finally, each karyotype and its features can be depicted by karyogram or idiogram.

1.2.2.3.1 Chromosome number

The number of chromosomes in the karyotype varies considerably among plants and do not generally relate to their overall genome size (Kellogg & Bennetzen 2004).

Within the angiosperms, the lowest chromosome number (2n = 4) has been found so far in four monocots (*Colpodium versicolor, Ornithogalum tenuifolium, Rhynchospora tenuis, Zingeria biebersteiniana*) and two eudicots (*Brachyscome lineariloba, Haplopappus gracilis*; Vanzela *et al.* 1996; Cremonini 2005). Conversely, the highest chromosome number in monocotyledonous and dicotyledonous plants has been recorded so far for the palm, *Voanioala gerardii* (2n = c. 600; Johnson *et al.* 1989, Röser *et al.* 1997) and the stonecrop *Sedum suaveolens* (2n = c. 640; Uhl 1978), respectively. Nevertheless, the world record holder with the highest number of chromosomes in plant genome to date is a fern *Ophioglossum reticulatum* with 2n = c. 1440 (Khandelwal 1990; Patel & Reddy 2018).

This large variability in the chromosome number within angiosperms is likely to be the result of mechanisms such as (i) polyploidization (duplication of the whole genome; WGD) and/or (ii) descending or ascending dysploidization (or aneuploidization) responsible for losses or gains of single chromosomes in given karyotypes (Lysak & Schubert 2013; Weiss-Schneeweiss & Schneeweiss 2013; Escudero *et al.* 2014). In general, two main groups within the polyploid plants can be distinguished: (i) autopolyploids, which are produced by multiplication of the genome in one plant and (ii) allopolyploids, which are derived from the hybridization of two species doubling of the chromosomes of one or both species involved (Heslop-Harrison & Schwarzacher 2011). In addition, several studies (e.g. Dodsworth *et al.* 2016, Soltis & Soltis 2016) suggest that probably all angiosperm have undergone at least one WGD event followed by more or less extensive karyotype rearrangements towards diploid-like genomes (diploidization).

1.2.2.3.2 Chromosome size, shape and karyotype asymmetry

Relatively small (c. 1-3 µm) to medium-sized chromosomes are characteristic for most angiosperm species (Stace 2000, Medeiros-Neto *et al.* 2017). However, there are known also some plant genera with larger chromosomes (more than 20 µm). These are present especially among monocotyledons, e.g. *Lilium* (Peruzzi *et al.* 2009; Hwang *et al.* 2015) and *Paris* (Pellicer *et al.* 2010).

The shape of monocentric metaphase chromosomes is generally determined by the centromere position, usually expressed by centromeric index (CI) or by arm ratio (R, the ratio of the long arm to the short arm). The centromeric index is traditionally

defined as a percentage of the short arm length to the total length of the chromosome. According to a value of CI or R and the nomenclature of Levan *et al.* (1964), the chromosomes are classified as metacentric, submetacentric, subtelocentric (or acrocentric) and telocentric chromosomes (Figure 4).

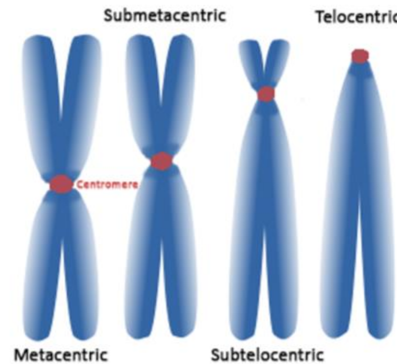


Figure 4. Schematic diagram of the classification of metaphase chromosomes based on the position of the centromere (adapted from https://mmegias.webs.uvigo.es/02-english/5-celulas/ampliaciones/8-cromosomas.php).

In general, most angiosperms possess symmetric karyotypes, consisted of similarly sized mostly metacentric or submetacentric chromosomes (Weiss-Schneeweiss & Schneeweiss 2013). Nevertheless, for some monocots such as Amaryllidaceae (*Hippeastrum, Rodophiala*), Asparagaceae (*Agave, Yucca*), Iridaceae (*Eleutherine*) and Xanthorrhoeacae (*Aloe, Haworthia, Gasteria*), a special case of asymmetric karyotype, the bimodal karyotype, is well known. It is characterized by the presence of two sets of chromosomes of contrasting size, without a gradual transition (Vosa 2005; Poggio *et al.* 2007; Báez *et al.* 2019).

The determination of karyotype symmetry or asymmetry is a widely discussed topic for many cytogeneticists and cytotaxononomists to date (Peruzzi & Eroğlu 2013; Eroğlu 2015; Medeiros-Neto *et al.* 2017). The search for an index that would reflect karyotype asymmetry in plants the best, began in 1931 by Levitsky (Levitsky 1931), followed by many others such as Huziwara (1962), Arano (1963), Stebbins (1971), Greilhuber & Speta (1976), Arano & Saito (1980), Zarco (1986), Lavania & Srivastava (1992), Šiljak-Yakovlev (1996), Watanabe *et al.* (1999) and Paszko (2006). The existing indices are divided into two groups depending on whether they describe intrachromosomal or interchromosomal karyotype asymmetry. While interchromosomal indices of asymmetry quantify dissimilarities in chromosome sizes within a given karyotype, intrachromosomal indices of asymmetry quantify relative differences

in the position of primary constriction among individual chromosomes of a complement (Peruzzi & Eroğlu 2013, Astuti *et al.* 2017). In several plant karyological studies (e.g. Hejazi *et al.* 2010, Techio *et al.* 2010, De Assis *et al.* 2013, Chiavegatto *et al.* 2016, Saensouk & Saensouk 2018), the most used interchromosomal asymmetry indices are the A_2 index (Zarco 1986) or its derived version expressed in percentage, the CV_{CL} index (Paszko 2006), due to their solid statistic basis. On the other hand, in the case of the estimation of intrachromosomal asymmetry, no consensus has been found yet among scientists in determining the most universal index for its expression (Zarco 1986, Paszko 2006, Medeiros-Neto *et al.* 2017). However, as stated by Peruzzi & Eroğlu (2013) and Astuti *et al.* (2017), among all known indices for estimating intrachromosomal asymmetry, the A index (Watanabe *et al.* 1999), or its derived version expressed in percentage, the M_{CA} index (Peruzzi & Eroğlu 2013) are statistically most accurate in its expression. For these reasons, the A2 and the A indices were also used in my study to express karyotype asymmetry in selected species of dandelions (for more details see Chapter 3.2)

1.2.2.3.3 Karyotype analysis in dandelions

The nuclear genome of dandelions is divided into 8 monocentric chromosomes in the haploid set and this basic chromosome number is common across all *Taraxacum* sections (Mogie & Richards 1983). As it has already been mentioned, the genus shows a considerable variation in the ploidy level, from commonly occurring diploids (2n = 2x = 16), triploids (2n = 3x = 24) and tetraploids (2n = 4x = 32) to uncommon pentaploids (2n = 5x = 40), hexaploids (2n = 6x = 48), heptaploids (2n = 7x = 56) and dodecaploids (2n = 12x = 96; Kirschner & Štěpánek 1996; Kirschner *et al*. 2003; Marciniuk *et al.* 2010). To date, plenty of karyological studies have been published (e.g. recently published Kula *et al.* 2013, Gedik *et al.* 2014, Sato *et al.* 2014, 2015, 2019; Wolanin & Musiał 2017, Gürdal *et al.* 2018, Gürdal & Özhatay 2018) to determine the total chromosome number in selected *Taraxacum* species of various sections (for more details see Chapter 3.1). However, due to a large number of known taxa in this genus, the amount of karyological data is still insufficient.

Conversely, knowledge of other karyotypic features, such as length and morphology of individual chromosomes, is scarce. The study by Sörensen & Gudjónsson (1946) is considered to be the first karyotype analysis devote to *Taraxacum*. Karyotypes

of one diploid *T. obtusifrons* and two triploids *T. polydon* and *T. laciniosifrons* from section *Taraxacum* have been reported in this study. To construct the karyotype formula for these three evaluated dandelion species, Sörensen and Gustavson described eight types of chromosomes (designated by letters A-H, Figure 5) based on their total length, presence and positions of primary and secondary constrictions.

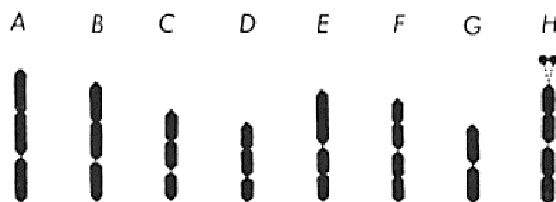


Figure 5. Idiograms of the eight chromosome types in dandelion karyotypes described by Sörensen & Gudjónsson (1946): A – the largest chromosome (c. 3.3 µm) with the primary constriction (PC) in subterminal position and secondary constriction (SC) situated at the middle of the long arm; B-D – similar chromosomes as type A but smaller in size (c. 3 µm (B), 2.3 µm (C) and 2 µm (D)); E – chromosome similar in total length (c. 2.8 µm) as type B with a medially placed PC and a SC on one arm; F – chromosome similar in total length (c. 2.6 µm) and position of PC as type E with a SC on both chromosome arms; G – chromosome similar in total length as type D but with a medially placed PC; H – chromosome the same size as types B and E but with a medially placed PC and a SC on each arm.

This classification of metaphase chromosomes in dandelion karyotypes was subsequently followed by three other karyological studies, Małecka (1962), Richards (1972) and Singh *et al.* (1974), in which the number of defined types of metaphase chromosomes for evaluating of individual dandelion karyotypes was changed from the previous eight types to six, ten and sixteen types, respectively. However, the karyotype formulas of individual *Taraxacum* species in recent karyological studies (e.g. Sato *et al.* 2007, 2011, 2012, 2014, 2015, 2019; Baeza *et al.* 2013; Gedik *et al.* 2014; Gürdal & Özhatay 2018) are expressed only by numbers of individual chromosome types, either based only on the position of their centromere (nomenclature by Levan *et al.* 1964) or additionally according to the presence of their secondary constriction.

Generally, the individual metaphase chromosomes in dandelion karyotypes are usually relatively small (c. 1-4 µm; e.g. Singh *et al.* 1974; Kirschner *et al.* 1994; Baeza *et al.* 2013; Gedik *et al.* 2014; Sato *et al.* 2007, 2011, 2012, 2014, 2015, 2019; Gürdal & Özhatay 2018). Dandelion karyotypes are most often highly symmetrical, with a predominance of metacentric chromosomes (e.g. Richards 1972; Kirschner *et al.* 1994; Baeza *et al.* 2013; Sato *et al.* 2007, 2011, 2012, 2014, 2015, 2019; Gürdal & Özhatay 2018) and total number of SAT-chromosomes in a complement is very diverse across

various dandelion sections (e.g. Den Nijs *et al.* 1978; Mogie & Richards 1983; Krahulcová 1993; Kirschner *et al.* 1994; Gürdal & Özhatay 2018).

1.2.3 Composition of plant nuclear DNA

Generally, plant nuclear DNA is composed of single or low-copy coding (exons) or non-coding sequences (such as introns, promoters and regulatory DNA sequences) and various types of repetitive DNA motifs (Figure 6; Kubis *et al.* 1998, Heslop-Harrison & Schmidt 2007). With the rapid development of sequencing methods in plant genomic research, it has been confirmed that protein-coding DNA makes up a minor part of the genome in a majority of angiosperms, while repetitive DNA may account in some species for over 80-90% of their genome (Narayan 1991; Barakat *et al.* 1998; Kelly *et al.* 2015). In addition, in most angiosperms with large genomes (e.g. maize, rice, barley), sequencing techniques have revealed that most of the genes are clustered in gene islands, that are isolated from each other by large regions of repetitive DNA (Barakat *et al.* 1998, Kellogg & Bennetzen 2004).

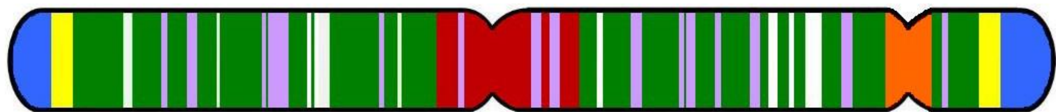


Figure 6. The general distribution of different types of repetitive sequences represented diagrammatically on plant chromosome with different colours (adapted and modified from Heslop-Harrison 2000; Mehrotra & Goyal 2014). Red, centromeric tandem repeats; blue (yellow), (sub-) telomeric repeats; orange, rDNA; green, intercalary tandem repeats; purple, dispersed repeats; white, genes and low-copy sequences.

1.2.3.1 Repetitive DNA sequences

Repetitive DNA forms the largest and most dynamic parts of the plant nuclear genomes. The length of repetitive units can vary from two nucleotide motifs to motifs longer than 10 kilobases, which are repeated hundreds, thousands or millions of times in the genome (Schwarzacher 2003; Heslop-Harrison & Schmidt 2007; Biscotti *et al.* 2015). Some repetitive sequences may be either species-specific or genus-specific or they can be universal in many species within a taxonomic family or various families (Mehrotra & Goyal 2014).

While a few repetitive elements have a known and important function, such as centromeric DNA, telomeric DNA (more details in chapter 1.2.2.2) or ribosomal DNA,

the role and function of a large number of repetitive sequences in plant genomes is still not completely known (Garrido-Ramos 2015; Robledillo *et al.* 2018). However, during the last few years it has been shown, that repetitive DNA, previously referred to as "junk DNA", is involved in many important functions such as the regulation of heterochromatin formation, influence of gene expression or contributing to epigenetic regulatory processes (Zakrzewski *et al.* 2010; Mehrotra & Goyal 2014). In addition, it is known that repetitive sequences play an important role in understanding the nature and consequences of genome size variation between different plant species and they are also useful tools for the investigation of evolutionary and phylogenetic relationships between individual plant species (Schwarzacher 2003; Garrido-Ramos 2015).

On the basis of the genomic organization, repetitive DNA sequences can be divided into two main groups, dispersed DNA and tandemly repeated DNA sequences (Figure 6). The former group, dispersed repetitive DNA elements, is mainly composed of transposable elements (TEs), in particular RNA transposons (retroelements or class I transposable elements) and DNA transposons (class II transposable elements), which are interspersed throughout the genome and distributed across all or most of the chromosomes in complement (Heslop-Harrison & Schmidt 2007). In angiosperms, the most abundant type of TEs are retroelements, which transpose and amplify through the "copy and paste" mechanism (Figure 7A) using the reverse transcription of an RNA intermediate of a source element (Kumar & Bennetzen 1999). On the other hand, DNA transposons move in the plant genomes usually by the "cut and paste" mechanism (Figure 7B) via a double-stranded (or single-stranded) DNA break (Feschotte & Pritham 2007; Munoz-Lopez & Garcia-Perez 2010).

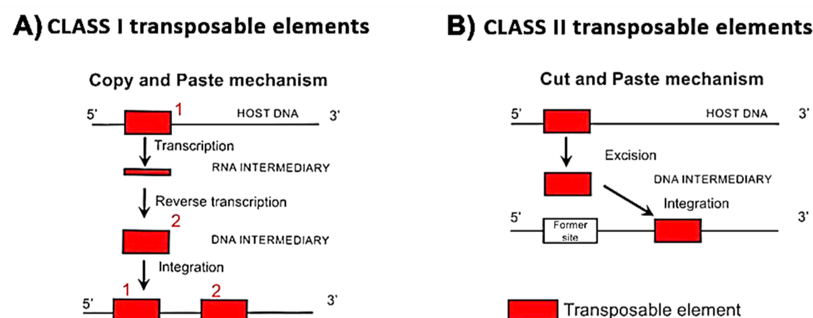


Figure 7. Two main types of transposable elements (TEs) depending on their mode of transposition (adapted from Serrato-Capuchina & Matute 2018). Class I TEs **(A)** transpose via an ssRNA (single-stranded RNA) intermediate, which is reverse transcribed to dsDNA (double-stranded DNA) prior to insertion of this copy in a new site in the genome. Class II TEs **(B)** do not involve an RNA intermediate and most of them are cut from their original location as dsDNA, prior to being inserted into a new site in the genome.

In addition to the above-mentioned modes of transposition, TEs can be further described as those having the ability to self-mobilize (autonomous) or those relying on co-mobilization by the enzymatic machinery of other TEs (non-autonomous) (Lee & Kim 2014; Naville *et al.* 2019). Generally, TEs are believed to be the major fraction of repetitive DNA in plant genomes and represent one of the main drivers of genome and chromosome size differentiation (Bennetzen *et al.* 2005; Hawkins *et al.* 2006).

In comparison to dispersed repetitive DNA elements, tandemly organized sequences include individual repetitive units (monomers) arranged adjacent to each other in monotonous arrays, which may be found at specific positions (pericentromeric, (sub)telomeric or intercalary regions) of the chromosomes (Schmidt & Heslop-Harrison 1998; Kubis *et al.* 1998). Pursuant to the monomers length and array size can be distinguished three groups of tandem repeated DNA sequences: microsatellites (2-5 bp long monomer, array size of 10-100 bp), minisatellites (6-100 bp long monomer, array size of 500 bp-30 kb) and satellite DNA (array size up to 100 Mb) (Schmidt & Heslop-Harrison 1996; Mehrotra & Goyal 2014). Microsatellites, also called simple sequence repeats (SSRs), are highly abundant within plant genomes, in which they are present in both coding and noncoding regions (Yu *et al.* 2017). Moreover, due to their typically high polymorphism, SSRs are widely used as molecular markers, for instance in population genetic studies, genomic mapping or identification of the parentage of polyploids (Hodel *et al.* 2016; Hosseinzadeh-Colagar *et al.* 2016). Minisatellites are often GC-rich, fast-evolving and compared to satellite DNA, they are still relatively little explored in plant genomes (Vergnaud & Denoeud 2000; Zakrzewski *et al.* 2010). Satellite DNA sequences generally represent the main component of genetically silent heterochromatic regions of plant chromosomes. The monomer length of this one of the most dynamic components of plant genomes ranges from 150 bp to 400 bp and most satellite repeat families in plants are species- or genus-specific. Thus, satellite DNA sequences are very often used as cytogenetic markers for studying chromosome and genome evolution (Mehrotra & Goyal 2014; Garrido-Ramos 2015).

1.2.3.1.1 Organization of nuclear ribosomal DNA

The ribosomal RNA (rRNA) genes are considered to be the essential housekeeping genes of plant genomes (Małuszyńska *et al*. 1998; Havlová et al. 2016). In contrast to the single-copy and low-copy of rRNA genes present in the plastidial and

mitochondrial genomes, the plant nuclear genomes contain hundreds to several thousand copies of each ribosomal gene family, 5S rDNA and 45S rDNA (Rosato *et al.* 2016). The 45S rDNA (Figure 8) is made up of tandem repeat units of the 18S, 5.8S and 26S rRNA genes, detached from each other by internal transcribed spacers (ITS1 and ITS2) and entire transcription unit is separated by an intergenic spacer (IGS), consisting of a non-transcribed spacer (NTS) and two external transcribed spacers (5´-ETS and 3´-ETS) on both sides of NTS. In most angiosperm genomes, the 5S rDNA is usually arranged in separate tandem arrays (hereafter S-type arrangement, Figure 8A) at chromosomal loci, that are independent of the 45S rDNA (Wicke *et al.* 2011; Weiss-Schneeweiss & Schneeweiss 2013) and its one repeat unit comprises highly conserved 120-bp-long coding regions (5S rRNA) with NTS of variable length (Kellogg & Appels 1995; Zhang *et al.* 2016). However, the results of several molecular and cytogenetic studies by Garcia *et al.* (2009a, 2009b, 2010, 2012) indicate that for nearly 25% of Asteraceae members have physically linked 45S and 5S rDNA loci in their genomes (hereafter L-type arrangements, Figure 8B), similar to the representatives of streptophyte algae and early-diverging green plants (liverworts, mosses, hornworts, lycophytes and monilophytes; Garcia & Kovařík 2013).

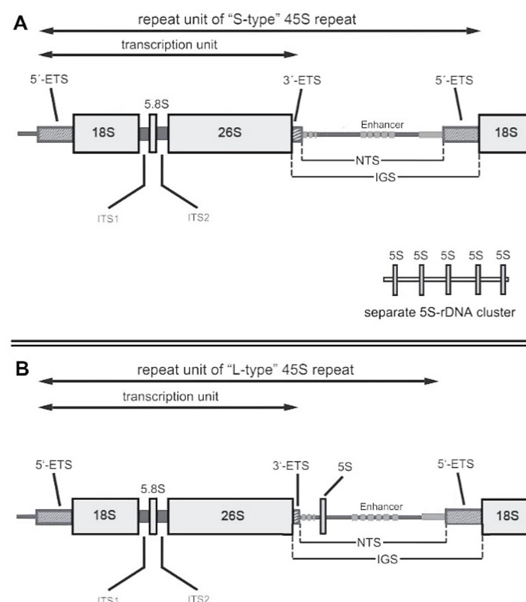


Figure 8. "S" and "L" type organization of the nuclear ribosomal DNA (adapted from Wicke *et al.* 2011). Generally, in both types, genes coding for 18S, 5.8S and 26S rRNA are separated by the internal transcribed spacers (ITS1 and ITS2) and each transcription unit is separated by intergenic spacer (IGS), which consists of a non-transcribed region (NTS) and two external transcribed spacers (5´-ETS and 3´-ETS) on both sides of NTS. However, while in S-type (**A**) lineages the 5S rDNA is localized in a separate cluster, in L- type (**B**) lineages, the 5S rDNA is localized within the NTS of the 45S repeat unit.

While the polycistronic 18S-5.8S-26S rRNA genes are transcribed in the NORs as one unit by RNA polymerase I and after further processing, they form mature 18S, 5.8S and 26S rRNA molecules, the 5S rRNA genes are the only rRNA genes, that are transcribed separately by RNA polymerase III in nucleoplasm (Garcia & Kovařík 2013; Weiss-Schneeweiss & Schneeweiss 2013). These four types of rRNA together with ribosomal proteins are essential structural components of ribosomes, which consist of two subunits. In plants, the large 60S ribosomal subunit consists of three rRNA molecules (26S, 5.8S and 5S) and approximately 46 proteins, whereas the small 40S subunit includes only 18S rRNA molecule and around 33 proteins (Layat *et al.* 2012; Mahelka *et al.* 2013).

Ribosomal genes, as one of the best-known members of a multigene family, have the fascinating ability to spread the same set of mutations in all their units and maintain their sequence homogeneity within the genome, using a highly synchronized manner, called concerted evolution (Álvarez & Wendel 2003; Rooney & Ward 2005). The mechanisms of concerted evolution are not entirely clear, although, it is assumed, that unequal crossing-over and gene conversion are its prominent drivers (Eickbush & Eickbush 2007; Volkov *et al.* 2017). In contrast to the highly conserved sequences of the 5S, 18S, 5.8S, and 26S rRNA genes, that are subjected to high selection pressure, the non-coding regions of rDNA (the ITS of 45S and the NTS of 5S) can be more polymorphic, due to frequently occurring nucleotide polymorphisms and common insertions and/or deletions in its sequence (Álvarez & Wendel 2003). Especially, the ITS region of 45S rDNA, owing to its several unique features (biparental inheritance, easy PCR amplification, multicopy structure and moderate size varying between 500 and 700 bp in angiosperm allowing easy sequencing) has become one of the most popular genomic loci, that can be useful for resolving phylogenetic relationship in many plants at the specific or generic levels (Baldwin *et al.* 1995; Coleman 2003; Stępiński 2014).

On the other hand, it is worth noting, that concerted evolution may not keep step with variation generating processes such as hybridization, polyploidization and gene and chromosome segment duplication. In these cases, pseudogenes and/or divergent copies of rDNA can be present in individual plant genomes, which in turn greatly limits the usefulness of their ITS regions for phylogenetic analysis in such taxa (Buckler *et al.* 1997; Bailey *et al.* 2003). Interestingly, several recent studies have shown, that pseudogenes can be an effective tool in a phylogenetic analysis of related species, where the functional ITS regions provide a limited source of variation (Ochieng *et al.* 2007; Prade *et al*. 2018).

1.2.4 Cytogenetic analysis of plant nuclear genome

The first attempts to identify metaphase chromosomes in the nucleus under the light microscope were based on the monochromatic staining using classical chromatin-specific dyes, e.g. acetocarmine, lacto-propionic orcein (or aceto-orcein), gentian violet and hematoxylin. These staining methods are still ideal to determine the total number of chromosomes in the nucleus and to highlight their gross morphological features, but they do not allow unambiguous identification of individual chromosomes in the karyotype, mainly because of the small size and/or similar morphology of the chromosomes in karyotype in most angiosperm species (Stace 2000; Fedak & Kim 2008). However, at the turn of the 1960s and 1970s, two milestone techniques for chromosome identification and karyotype analysis were discovered, namely chromosome banding and *in situ* hybridization (Ansari *et al.* 1999).

1.2.4.1 Chromosome banding

The concept of chromosome banding was born in 1968 when Torbjorn Oskar Caspersson and his colleagues observed characteristic patterns of dark and light banding regions due to differences in DNA base composition along the length of plant mitotic chromosomes after using fluorescent DNA-binding dye applied to chromosome spreads (Caspersson *et al.* 1968). For this chromosome banding technique (Q-banding) was used fluorochrome called quinacrine, which specifically interacts in the form of light bands (Q-bands) with AT-rich regions of the nuclear genome (Weisblum & De Haseth 1972). Nevertheless, also other fluorescent stains produce similar Q-bands to that of quinacrine, such as Hoechst, DAPI or diimidazolinophenylindole (DIPI). Q-banding as one of the first and the simplest banding techniques has allowed the identification of individual chromosomes and their homologues in most plant species. However, due to its several disadvantages, Q-banding was soon replaced by other banding methods such as G-, R-, C- or N-banding (Moore & Best 2001).

Between the years 1970 and 1990, Giemsa (G)-banding became another commonly used chromosome banding technique. In comparison to Q-banding, G-banding produces long-lived permanent slides, that do not require UV light for their studying and dark G-bands similar to light Q-bands are located in late replicated regions with AT-rich base content (Stace 2000). A pattern that is approximately the opposite of Q- and

G-banding is produced by Reverse (R)-banding, which predominantly stains the GC-rich regions of a genome and it is useful for the detection of structural rearrangements involving telomeric ends of chromosomes (Bickmore 2001, Fedak & Kim 2008). On the other hand, the C-banding method stains noncoding constitutive heterochromatin surrounding mainly the centromeres as dark C-bands and all other chromatin remains pale (Jellen 2016). Finally, the Nucleolar organizing region (N)-banding, as well as the silver staining technique, was initially developed to study NORs in the plant and animal metaphase chromosomes (Zoshchuk *et al.* 2003). Nevertheless, Gerlach (1977) and subsequently others, noted that the N-bands do not necessarily correspond to NORs in several plant species, but they can occupy similar positions as C-bands.

In general, although chromosome banding techniques have been widely used for karyotyping, identifying abnormalities in chromosome numbers, revealing chromosome rearrangements in karyotypes of many plant species, their distinction has been often limited and they have not always yielded reliable results (Bickmore 2001, Fedak & Kim 2008).

1.2.4.2 In situ hybridization

The development of *in situ* hybridization (ISH) techniques marked the beginning of a new era of cytogenetics that filled the gap between classical cytogenetic and molecular-genetic methods (Raff & Schwanitz 2001). The ISH techniques allow, inter alia, detection and localization of nucleotide sequences on chromosomes, interphase nuclei and extended chromatin fibres, based on the principle of complementary binding of a labelled DNA (RNA) probe to a specific target sequence in cytological preparations (Kato *et al.* 2005). These techniques provide wide practical applications in plant nuclear genome research, they are very useful for chromosome mapping, genome analysis, clarification of phylogenetic relationships, detection of chromosomal aberrations and alien chromatin in plant genomes and study of chromosome organization at interphase nuclei (Devi *et al*. 2005).

The initial ISH techniques used radiolabelled probes, which were visualized by autoradiography (Gall & Pardue 1969, John *et al.* 1969). However, due to the disadvantages of radiolabelled probes as for example time-consuming exposition, instability and negative impact on human health, the sensitive, rapid, and nonradioactive

variations of ISH with enzymatic, metal or subsequently fluorochrome-labelled probes have been developed (Langer-Safer *et al.* 1982).

The ISH technique using fluorescence to detect DNA probes is referred to as Fluorescence *in situ* hybridization (FISH). The first application of FISH was introduced in the early 1980s, but only a single probe was used (Bauman *et al.* 1980; Ratan *et al.* 2017). Approximately a decade later, Leitch *et al.* (1991) used for the first time a multi-coloured FISH on plant chromosomes, which making FISH an essential technique in karyotyping and chromosome analysis for many plant species.

Probes for a FISH experiment (Figure 9) are usually labelled either directly, using fluorescent-labelled (e.g. Cy3, Cy5, Fluorescein, Rhodamine, Texas red, etc.) nucleotides or indirectly by incorporation of reporter molecules (most commonly used biotin and digoxigenin), that are subsequently detected by fluorochrome-conjugated avidin, streptavidin or other antibodies (Lavania 1998; Devi *et al*. 2005). While direct labelling methods of FISH probes are the best choice for detecting tandemly arranged gene sequences (rDNA), tandemly arranged and dispersed repetitive sequences, clones of large-insert DNA libraries and the whole genomic DNA, by reason of the speed and ease of detection, the indirect labelling methods are able to detect single- or low-copy sequences and genes, even in cases when the direct methods are most often not enough sensitive, due to possibility of signal amplification (Wiegant *et al.* 1991; Kato *et al.* 2006; Ehtisham *et al.* 2016).

The development of the sensitivity, specificity and resolution of the basic FISH technique, which to date comprises a large number of its various modifications, has gone side by side the advances in the fields of fluorescence microscopy and digital imaging (Volpi & Bridger 2008). Some of today's modern FISH techniques allow the detection of probes as small as 1–3 kb, which have been reported for instance in petunia, rice and tobacco (Moscone *et al.* 1996; Jiang & Gill 2006; Ohmido *et al*. 2010). Furthermore, there are some reports (Desel *et al.* 2001; Stephens *et al.* 2004; Khrustaleva *et al.* 2016), where unique DNA probes less than 1 kb have been detected by FISH. Nevertheless, as reported by Jiang (2019), these very small probes are usually detected at low frequencies, thus they are not enough robust markers for routine chromosome identification or cytogenetic studies in plants. On the other hand, the modified FISH technique, which uses labelled total genomic DNA(s) of plant species as a probe(s), is known as Genomic *in situ* hybridization (GISH; Schwarzacher *et al.* 1989). Compared to the basic FISH technique, GISH can offer new insight into the phylogenetic origin and taxonomic relationships

of many plant species by identifying the parental origin of chromatin in natural or artificial hybrids (interspecific or intergeneric) and recombination locations in chromosomes of allopolyploids and interspecific introgression lines (Jiang & Gill 1994; Markova & Vyskot 2010).

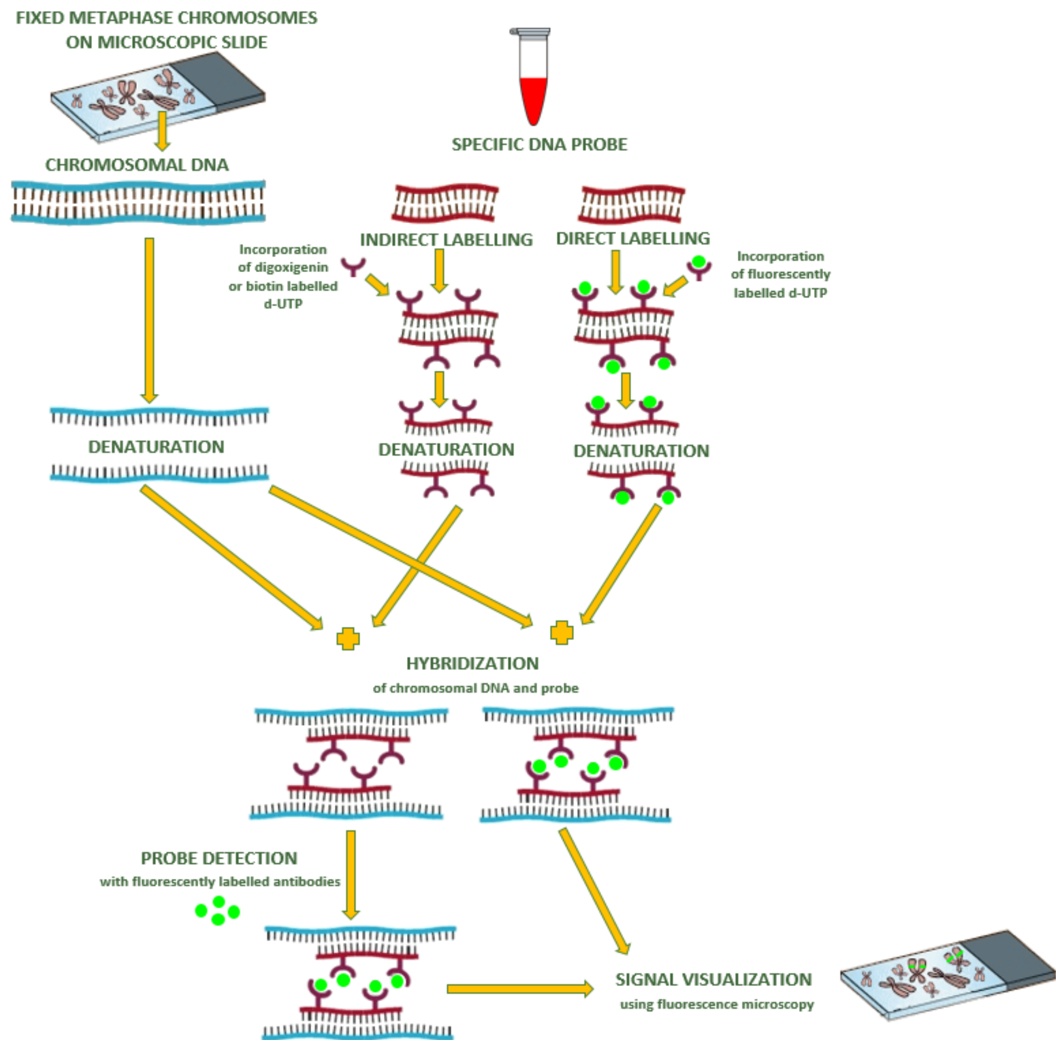
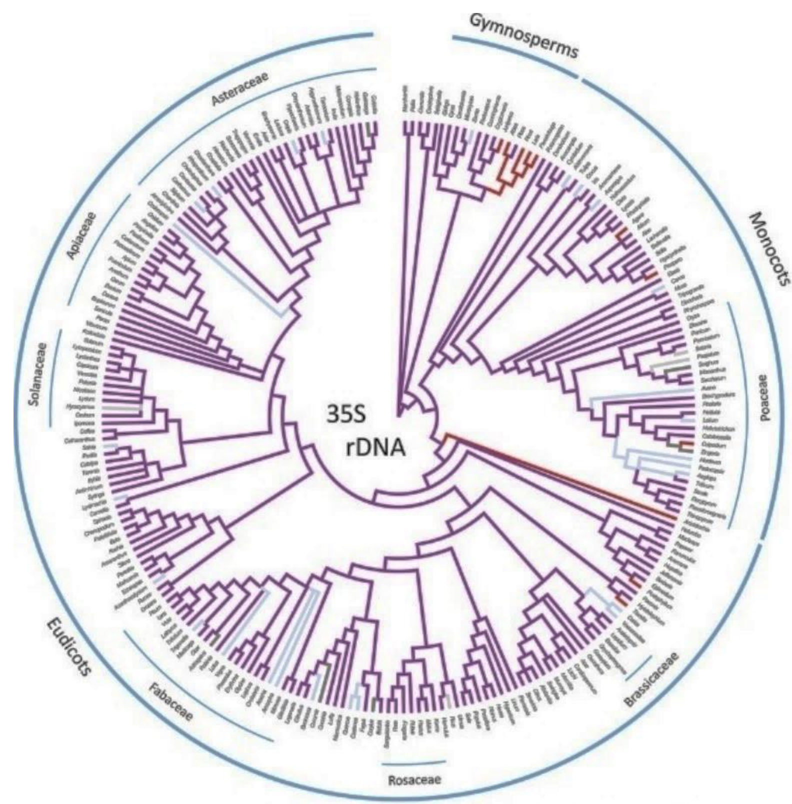


Figure 9. The principle of FISH technique on metaphase chromosomes with a direct or/and indirect DNA labelled probe. The basic elements of FISH are a DNA probe and a target sequence of chromosomes fixed on a slide. Various molecular methods (e.g. nick translation, random priming, PCR, etc.) can be used to label different input DNA probes. Two labelling strategies are commonly used, indirect labelling (left panel) and direct labelling (right panel). For indirect labelling, probes are labelled with modified nucleotides that contain a hapten, whereas direct labelling uses nucleotides that have been directly modified to contain a fluorophore. Subsequently, the labelled probe and the target DNA are denatured to produce single-stranded DNA. Denaturation is followed with the hybridization of the probe with the complementary target DNA sequence. Finally, the discrete fluorescent signal is detected at the site(s) of the hybridization probe and target sequences using fluorescence microscopy. In case of the indirectly labelled probe, an extra step is required for visualization of the nonfluorescent haptens (Speicher & Carter 2005; Ratan *et al.* 2017).

1.2.4.3 Cytogenetic mapping of 5S and 45S rDNA in Asteraceae

In general, cytogenetic mapping of the 5S and 45S rDNA by FISH technique provides universally applicable markers for identification of individual chromosomes in a complement, investigation of karyotype evolution and genome characterization in many plant species (e.g. Ali *et al*. 2005; Berjano *et al.* 2009; Kolano *et al.* 2012; Roa & Guerra 2012; Garcia *et al*. 2017). However, it is quite surprising, that relatively little has been published about the distribution of 5S and 45S rDNA loci in the karyotypes of the individual genera of Asteraceae, the largest angiosperm family. Known data about the distribution of rDNA loci within the Asteraceae family represents only 11.81% from the entire plant rDNA database, in which the genus *Artemisia* is the most abundant in terms of the number of records (Release 3, March 2017; Vitales *et al.* 2017). While karyotypes of several *Artemisia* species are characterized by the prevailing L-type organisation of the 45S and 5S rDNA loci (Garcia *et al.* 2007, 2009 a, b; Matoba & Uchiyama 2009), the S-type arrangement of these loci predominate in the genomes of most of the other evaluated genera of Asteraceae (e.g. *Tragopogon* (Pires *et al.* 2004), *Centaurea* (Dydak *et al*. 2009), *Helianthus* (Garcia *et al.* 2010), *Hypochaeris* (Weiss-Schneeweiss *et al*. 2003; Ruas *et al.* 2005), *Brachyscome* (Adachi *et al.* 1997), etc.; Garcia *et al.* 2010). As shown in Figure 10, the most common position of 45S rDNA on the metaphase chromosomes in evaluated karyotypes within Asteraceae family is terminal, whereas the 5S rDNA sites are more variable in their distribution with predominant localization at interstitial regions of metaphase chromosomes (Garcia *et al.* 2010, 2017).

In case of the genus *Taraxacum*, Vašut *et al*. (2014) is the only study so far, which has reported data on the distribution of 45S rDNA. In this study of twelve dandelion species from seven different sections, the number of loci for 45S rDNA corresponds to the ploidy level of each evaluated dandelion species and their positions are without exception in the NOR of SAT-chromosomes. However, a detailed study on the distribution of both the 5S and 45S rDNA on metaphase chromosomes across the genus *Taraxacum* has not yet been published.



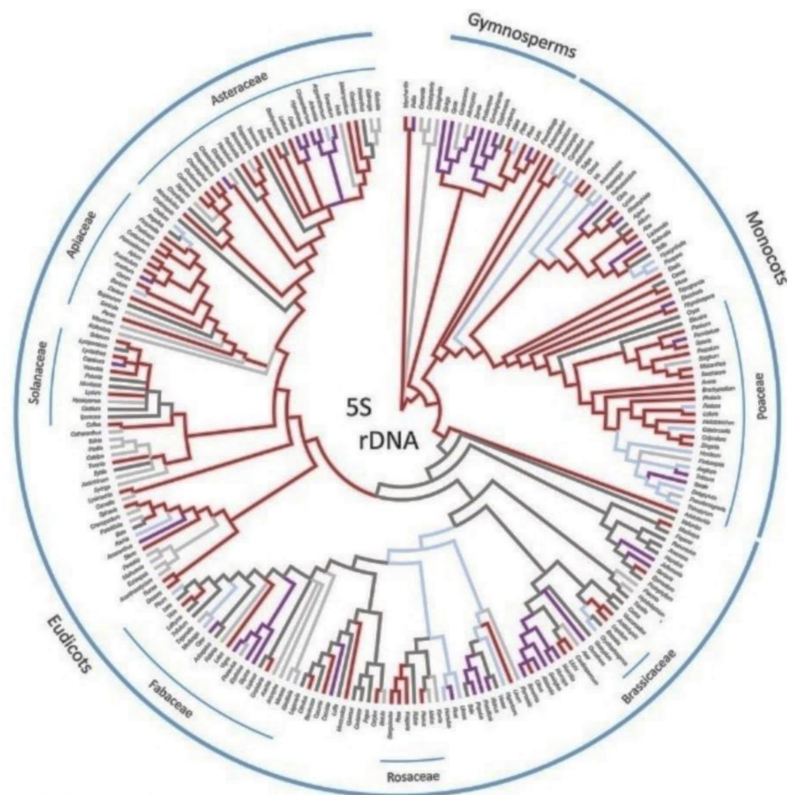


Figure 10. Distribution of the terminal (including subterminal), interstitial, centromeric (including pericentromeric) and mixed positions (when at least two of foregoing categories were found in the same karyotype) of 5S and 35S rDNA loci on metaphase chromosomes in evaluated karyotypes across eudicots, monocots and gymnosperms from the Plant rDNA database (adapted from Garcia *et al.* 2017).

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CHAPTER 2

AIMS OF THE THESIS

Aims of the Thesis

The main aim of the presented Ph.D. thesis was to provide new perspectives on the structure and variability of sexual and apomictic dandelion genomes across various *Taraxacum* sections using standard karyological method or modern molecular cytogenetic technique and to put these data to broader context of cytogenetic and molecular analyses.

<u>At the centre of interest were the following specific aims:</u>

- To establish the chromosome number, ploidy level and to estimate the genome size for 28 selected species of *Taraxacum* sect. *Taraxacum.*
- To compare the karyotypes with fluorescently labelled 45S and 5S rDNA loci between sexual and apomictic dandelions within one or more evaluated *Taraxacum* sections.
    - ➢ To determine the chromosome number, the karyotype formula, the number and localization of 45S and 5S rDNA loci on mitotic metaphase chromosomes and to create haploid idiogram for each evaluated *Taraxacum* taxa.
    - ➢ To detect whether the number of satellite chromosomes corresponds to the ploidy level and the loci for 45S rDNA on metaphase chromosomes are distributed only in the NOR in each evaluated dandelion karyotype.
    - ➢ To reveal whether a pattern of the distribution of 45S and 5S rDNA loci on metaphase chromosomes is associated with the reproduction strategy of studied *Taraxacum* taxa (i.e. allogamy, autogamy and apomixis).
    - ➢ To test whether there is a correlation between the number of 45S and 5S rDNA loci and the estimations of genome size or GC base content in evaluated *Taraxacum* taxa.
    - ➢ To bring new insights into the complex evolution in *Taraxacum* genomes by studying their dynamic changes in rDNA.

The chromosome preparation technique, FISH, flow cytometry and various standard molecular methods have been used to meet the above-mentioned aims and their detailed descriptions are given in the individual parts of Chapter 3.

CHAPTER 3

INDIVIDUAL PAPERS

3.1 New chromosome counts and genome size estimates for 28 species of *Taraxacum* sect. *Taraxacum*

Macháčková P, Majeský Ľ, Hroneš M, Hřibová E, Trávníček B, Vašut RJ

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[Ľuboš Majeský (ĽM) conceived the idea of the study. Radim Jan Vašut (RJV) and Petra Macháčková (PM) designed the study. Plant material was collected by Bohumil Trávníček (BT) and RJV. Seedlings were cultivated by PM and ĽM. PM and Eva Hřibová (EH) designed the karyological analyses. PM performed all karyological experiments and compilation of all image collages. Michal Hroneš (MH) contributed to flow-cytometric analyses. PM wrote the first draft, and MH and RJV improved subsequent version of the manuscript. All authors read and approved the final manuscript.]

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New chromosome counts and genome size estimates for 28 species of *Taraxacum* sect. *Taraxacum*

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Abstract

The species-rich and widespread genus *Taraxacum* F. H. Wiggers, 1780 (Asteraceae subfamily Cichorioideae) is one of the most taxonomically complex plant genera in the world, mainly due to its combination of different sexual and asexual reproduction strategies. Polyploidy is usually confined to apomictic microspecies, varying from 3x to 6x (rarely 10x). In this study, we focused on *Taraxacum* sect. *Taraxacum* (= *T.* sect. *Ruderalia*; *T. officinale* group), i.e., the largest group within the genus. We counted chromosome numbers and measured the DNA content for species sampled in Central Europe, mainly in Czechia. The chromosome number of the 28 species (*T. aberrans* Hagendijk, Soest & Zevenbergen, 1974, *T. atroviride* Štěpánek & Trávníček, 2008, *T. atrox* Kirschner & Štěpánek, 1997, *T. baeckiiforme* Sahlin, 1971, *T. chrysophaenum* Railonsala, 1957, *T. coartatum* G.E. Haglund, 1942, *T. corynodes* G.E. Haglund, 1943, *T. crassum* H. Øllgaard & Trávníček, 2003, *T. deltoidifrons* H. Øllgaard, 2003, *T. diastematicum* Marklund, 1940, *T. gesticulans* H. Øllgaard, 1978, *T. glossodon* Sonck & H. Øllgaard, 1999, *T. guttigestans* H. Øllgaard in Kirschner & Štěpánek, 1992, *T. huelphersianum* G.E. Haglund, 1935, *T. ingens* Palmgren, 1910, *T. jugiferum* H. Øllgaard, 2003, *T. laticordatum* Marklund, 1938, *T. lojoense* H. Lindberg, 1944 (= *T. debrayi* Hagendijk, Soest & Zevenbergen, 1972, *T. lippertianum* Sahlin, 1979), *T. lucidifrons* Trávníček, ineditus, *T. obtusifrons* Marklund, 1938, *T. ochrochlorum* G.E. Haglund, 1942, *T. ohlsenii* G.E. Haglund, 1936, *T. perdubium* Trávníček, ineditus, *T. praestabile* Railonsala, 1962, *T. sepulcrilobum* Trávníček, inedi-

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tus, *T. sertatum* Kirschner, H. Øllgaard & Štěpánek, 1997, *T. subhuelphersianum* M.P. Christiansen, 1971, *T. valens* Marklund, 1938) is 2n = 3x = 24. The DNA content ranged from 2C = 2.60 pg (*T. atrox*) to 2C = 2.86 pg (*T. perdubium*), with an average value of 2C = 2.72 pg. Chromosome numbers are reported for the first time for 26 species (all but *T. diastematicum* and *T. obtusifrons*), and genome size estimates for 26 species are now published for the first time.

Keywords

Asteraceae, chromosome number, flow cytometry, karyology, *Taraxacum officinale*

Introduction

Taraxacum F. H. Wiggers, 1780 (Asteraceae subfamily Cichorioideae) is a species-rich genus of common and widespread perennial grassland herbs growing from the subtropics to subarctic (arctic/alpine) regions across the world. Rough estimates suggest the genus contains approximately 2,800 species in approximately 60 sections (Kirschner et al. 2015), with the higher diversity in the mountains of Eurasia (Ge et al. 2011); a total of 1,900 species in 35 sections are listed for Europe (Kirschner et al. 2007). The complexity of *Taraxacum* taxonomy is caused by its combination of different reproduction strategies, including sexual reproduction (mainly outcrossing, less frequently selfing) and apomixis (meiotic diplospory; Richards 1973, Asker and Jerling 1992, Kirschner and Štěpánek 1994, Kirschner et al. 1994, Majeský et al. 2017). The vast majority of *Taraxacum* taxa are apomictic polyploid microspecies, only a few species are sexual diploids. The phenomenon of apomixis itself (i.e. clonal reproduction by seeds) attracts the attention of plant systematists as well as plant breeders for its possible application in crop breeding.

The basic chromosome number in *Taraxacum* is x = 8, and it is constant across all the sections. The diploid number (2n = 2x = 16) is confined to only sexually reproducing species, and sexual species are nearly all diploids, with only a few exceptions of sexual tetraploids known in section *Piesis* (Kirschner and Štěpánek 1994, 1998a, Trávníček et al. 2013). In contrast, apomictic species are never diploids but always polyploids (Majeský et al. 2017), having one of the genes involved in regulation of apomixis (*DIPLOSPOROUS*) located on the NOR chromosome (Vašut et al. 2014). Most of the known chromosome numbers for apomictic *Taraxacum* species are at a triploid level (2n = 3x = 24), especially those of the widespread European sections *Taraxacum* sect. *Taraxacum* (Mártonfiová 2006, Kula et al. 2013), *T.* sect. *Erythrosperma* (Małecka 1967, 1969, Vašut 2003, Schmid et al. 2004, Vašut et al. 2005, Uhlemann 2007, 2010, Vašut and Majeský 2015, Wolanin and Musiał 2017), *T.* sect. *Palustria* (Małecka 1972, 1973, 1978, Kirschner and Štěpánek 1998b, Marciniuk et al. 2010) and *T.* sect. *Hamata* (Mogie and Richards 1983, Øllgaard 1983). However, tetraploids (2n = 4x = 32) also occur quite frequently in some sections, such as the European dandelions in sections *T.* sect. *Palustria* (e.g., *T. vindobonense* Soest, 1965, *T. brandenburgicum* Hudziok, 1969 and *T. portentosum* Kirschner & Štěpánek, 1998), *T.* sect. *Erythrosperma* (e.g., *T. tortilobum* Florström, 1914, *T. fulvum* Raunkiaer, 1906

and *T. bifurcatum* Hagendijk et al., ineditus), *T.* sect. *Naevosa* (e.g., *T. euryphyllum* (Dahlstedt, 1911) M. P. Christiansen, 1940 and *T. naevosum* Dahlstedt, 1900), *T.* sect. *Scariosa* and *T.* sect. *Celtica* (*T. unguilobum* Dahlstedt, 1912 and *T. fulvicarpum* Dahlstedt, 1927). Higher ploidy levels are uncommon in *Taraxacum*, while natural pentaploids (2n = 5x = 40; e.g., in the European species *T. skalinskanum* Małecka & Soest, 1972 and *T. zajacii* J. Marciniuk et P. Marciniuk, 2012 and 6 other species of section *Palustria*, *T. faeroense* Dahlstedt in H. H. Johnston, 1926 of *T.* sect. *Spectabilia*, *T. caledonicum* A. J. Richards, 1972 of section *Celtica* and *T. albidum* Dahlstedt, 1907 of section *Mongolica* from Japan), hexaploids (2n = 6x = 48 for *T. ranunculus* Kirschner & Štěpánek, 1998 of section *Palustria* and *T. nordstedtii* Dahlstedt, 1911 of section *Celtica*), and aberrant heptaploid (2n = 7x = 56) or decaploid (2n = 10x = 80) mutants of natural species have been documented (Richards 1969, Małecka 1973, Mogie and Richards 1983, Kirschner and Štěpánek 1984, 1998b, Sato et al. 2011, Marciniuk et al. 2012). The geographic distribution of diploids and polyploids in Europe is more or less separated, with polyploids mainly distributed in the colder regions of mountains in the north and with diploid sexuals distributed in warmer regions of the south, which results in the phenomenon of geographic parthenogenesis (den Nijs et al. 1990, den Nijs and van der Hulst 1988, Uhlemann 2001, Verduijn et al. 2004a).

Genome size estimation (plant genome C-value) (Greilhuber et al. 2005) is a rapid cytogenetic method that helps provide a better understanding of the evolutionary relationships among studied taxa. The method itself has methodological limitations (multiple factors can affect the measurements; the method does not provide any information on repetitive sequences involved; etc.); however, patterns of genome size estimates in species groups provide additional information on possible pathways of evolution (Soltis et al. 2003, Leitch et al. 2005, Šmarda et al. 2012). Although flow cytometry was widely used in *Taraxacum* research for rapidly identifying the ploidy level in mixed apomictic-sexual populations (e.g., Meirmans et al. 1999, Verduijn et al. 2004a, 2004b, Mártonfiová 2006, 2015, Mártonfiová et al. 2007, 2010) or in taxonomic revisions (e.g., Vašut 2003), genome size estimates are very limited. Genome size (C-value) in *Taraxacum* varies (in known species) between 2C = 1.74 pg in diploid *T. linearisquameum* Soest, 1966 and 2C = 6.91 pg in tetraploid *T. albidum* (Záveský et al. 2005, Siljak-Yakovlev et al. 2010); European triploid apomicts have a value of 2C ≈ 2.4–2.76 pg (Bennett et al. 1982, Záveský et al. 2005, Bainard et al. 2011, Iaffaldano et al. 2017). Considerable variation (~1.2-fold difference) in DNA content, measured as the C-value, was observed in *T. stenocephalum* Boissier et Kotschy ex Boissier, 1875 (Trávníček et al. 2013) and in a sample of an unidentified species of the *Taraxacum officinale* group in North America (Iaffaldano et al. 2017).

Taraxacum sect. *Taraxacum* (formerly known as *T.* sect. *Ruderalia*; generally known as *Taraxacum officinale* group; see Kirschner and Štěpánek 2011) has a strongly prevailing triploid ploidy level of 2n = 3x = 24, by which it differs from other closely related sections (*Erythrosperma*, *Palustria*, and *Celtica*) with known ploidies of 3x and 4x or even higher. In this study, we aimed to count the chromosome number of 28 species for which knowledge was lacking and to detect the ploidy level for these species. Fur-

thermore, we searched for variability in genome size among these species to determine whether we can detect variation in DNA content among species similar to that found in a sample of unidentified taxa of *T. officinale* group.

Material and methods

Plant Material

We studied a total of 28 *Taraxacum* species (25 formally described and three still undescribed, referred to by their working names) belonging to *Taraxacum* sect. *Taraxacum* (Table 1). Plants and achenes of the investigated species were collected in natural habitats of several localities of Central Europe in the period 2014–2016. A detailed description of the localities, date, and collectors of samples is provided in Table 1. The studied plant material was documented by herbarium specimens and is deposited in the herbarium of the Department of Botany, Palacký University in Olomouc, Czech Republic (OL). All studied species are apomictic (agamospermous); thus, maternal plants and offspring plants (grown from seeds) are taxonomically (genetically) identical.

For karyological analyses, achenes were sown in Petri dishes containing 1% agar solution and germinated at room temperature. Fresh young leaves for flow cytometric analyses were collected from juvenile plants cultivated in a greenhouse at the Department of Botany, Faculty of Science, Palacký University in Olomouc.

Karyology

For chromosome counts, we used mitotically active root tip meristems of dandelion seedlings. Seedlings of the investigated species with 1–2 cm long roots were collected in the morning. To obtain the desired metaphase index, the roots were pre-treated in a 2 mM solution of 8-hydroxyquinoline for two hours at room temperature and an additional two hours at 4 °C in the dark. Then, the material was fixed in Carnoy's fixative (a mixture (3:1, v/v) of absolute ethanol and acetic acid) and stored in a refrigerator (4 °C) until further processing (Hasterok and Maluszynska 2000). For slide preparation, a combination of protocols in Hasterok and Maluszynska (2000) and van Baarlen et al. (2000) was used with the following changes for the investigated species of dandelions. Fixed root tips were washed in citrate buffer (0.01 M, pH 4.8) for 5 min and then enzymatically digested in a mixture of 0.1% cellulose Onozuka RS (*Trichoderma* Persoon, 1794; Sigma), 0.1% pectolyase (*Aspergillus japonicus* Saito, 1906; Sigma) and 0.1% cytohelicase (*Helix pomatia* Linnaeus, 1758; Sigma) in the citrate buffer for 90 min at 37–40 °C. To remove trace amounts of the enzymatic mixture, the root tips were then gently washed in citrate buffer for 5 min. Only the mitotically active meristematic tissue of a root tip was cut off under a stereoscopic microscope, transferred into a drop of 50% acetic acid on a slide and covered by a cov-

Table 1. List of species used in this study, with sampling details. Country codes according to ISO 3166-1 alpha-2 (AT = Austria; CZ = Czechia, DE = Germany, HU = Hungary, IT = Italy, SK = Slovakia); Collectors: BT = Bohumil Trávníček; RJV = Radim Jan Vašut.

Taxon	Country	Locality; GPS; Date; Collector
T. aberrans Hagendijk, Soest & Zevenbergen, 1974	AT	Upper Austria, Obernberg am Inn town, lawn in the street of Therese-Riggle-Strasse; 48°19'14"N; 13°19'52"E; 10.05.2015; BT
T. atroviride Štěpánek & Trávníček, 2008	AT	Altaussee village (near Bad Aussee town), lawns and roadsides in the ski resort NNW from the village (valley of Augstbach brook); 47°39'42"N; 13°44'38"E; 08.05.2014; BT
T. atrox Kirschner & Štěpánek, 1997	IT	Cave del Predil settlement (S from Tarvisio town), lawns at the road no SP76 (at lake of Lago di Predil); 46°25'11"N; 13°33'42"E; 16.05.2015; BT
T. baeckiiforme Sahlin, 1971	HU	Felsöcsatár village (W from the Szombathely town), grassy roadsides at the road towards Vaskeresztes village; 250 m a.s.l.; 47°12'20"N; 16°26'51"E; 26.04.2015; BT
T. chrysophaenum Railonsala, 1957	CZ	Bartošovice village (near Nový Jičín town), lawns in park in central part of the village; 49°40'15"N, 18°02'59"E; 23.04.2014; BT
T. coartatum G. E. Haglund, 1942	CZ	Lubná village (near Polička town), grassy places at brook in E part of the village; 480 m a.s.l.; 49°46'26"N, 16°13'57"E; 17.05.2016; BT & RJV
T. corynodes G. E. Haglund, 1943	CZ	Hanušovice town, lawns at the railway station; 50°04'18"N, 16°55'52"E; 19.05.2015; BT
T. crassum H. Øllgaard & Trávníček, 2003	CZ	Nové Město na Moravě town, grassy places at brook in the town, ca 0.6 km ESE from railway station of "Nové Město na Moravě-zastávka"; 600 m a.s.l.; 49°33'45"N, 16°04'04"E; 17.05.2016; BT & RJV
T. deltoidifrons H. Øllgaard, 2003	CZ	Jimramov town, grassy places in the park of Bludník in N part of the town; 500 m a.s.l.; 49°38'19"N, 16°13'25"E; 17.05.2016; BT & RJV
T. diastematicum Marklund, 1940	CZ	Svratka village, meadows and grassy places at NW margin of the settlement of Česká Cikánka; 630 m a.s.l.; 49°42'35"N, 16°03'01"E; 17.05.2016; BT & RJV
T. gesticulans H. Øllgaard, 1978	CZ	Hanušovice town, lawns at the railway station; 50°04'18"N, 16°55'52"E; 19.05.2015; BT
T. glossodon Sonck & H. Øllgaard, 1999	CZ	Studnice village (N from Nové Město na Moravě town), meadow at road near the Paseky settlement ca 1 km NNW from the village; 780 m a.s.l.; 49°36'51"N, 16°05'17"E; 17.05.2016; BT & RJV
T. guttigestans H. Øllgaard in Kirschner & Štěpánek, 1992	CZ	Nové Město na Moravě town, grassy places at brook in the town, ca 0.6 km ESE from railway station of "Nové Město na Moravě-zastávka"; 600 m a.s.l.; 49°33'45"N, 16°04'04"E; 17.05.2016; BT & RJV
T. huelphersianum G. E. Haglund, 1935	CZ	Pekařov settlement (near Hanušovice town), lawns and meadows in the settlement; 50°04'41"N, 17°01'31"E; 19.05.2015; BT
T. ingens Palmgren, 1910	CZ	Svratka village, meadows and grassy places at NW margin of the settlement of Česká Cikánka; 630 m a.s.l.; 49°42'35"N, 16°03'01"E; 17.05.2016; BT & RJV
T. jugiferum H. Øllgaard, 2003	CZ	Jedlí village (NW from Zábřeh town), lawns and roadsides in central part of the village; 49°55'54"N, 16°47'45"E; 19.05.2015; BT
T. laticordatum Marklund, 1938	CZ	C Moravia, Hlinsko pod Hostýnem village, roadside at road towards Prusinovice village; 49°22'34"N, 17°36'47.8"E; 20.05.2016; BT
T. lojoense H. Lindberg, 1944 †	CZ	Úterý village (near Konstantinovy Lázně town), lawns at the brook on the eastern village margin; 510 m a.s.l.; 49°56'24"N, 13°00'21"E; 25.04.2014; BT
T. lucidifrons Trávníček, ineditus	CZ	Kunín village (near Nový Jičín town), lawns in chateau park; 49°38'39"N, 17°59'18"E, 23.04.2014; BT
T. obtusifrons Marklund, 1938	CZ	Lubná village (near Polička town), grassy places at brook in E part of the village; 480 m a.s.l.; 49°46'26"N, 16°13'57"E; 17.05.2016; BT & RJV

Taxon	Country	Locality; GPS; Date; Collector
T. ochrochlorum G. E. Haglund, 1942	CZ	Svratka village, meadows and grassy places at NW margin of the settlement of Česká Cikánka; 630 m a.s.l.; 49°42'35"N, 16°03'01"E; 17.05.2016; BT & RJV
T. ohlsenii G. E. Haglund, 1936	DE	Schönwald village (near Hof town), wet meadow and adjacent roadsides at the road (no. 15) towards Rehau village; 550 m a.s.l.; 50°13'37"N, 12°04'57"E; 27.04.2014; BT
T. perdubium Trávníček, ineditus	CZ	Záhlinice village (near Hulín town), wet meadow 1.3 km SSW from the railway station; 190 m a.s.l.; 49°16'52"N, 17°28'58"E; 20.04.2016; BT
T. praestabile Railonsala, 1962	IT	Sella Nevea settlement (SW from Tarvisio town), lawns near hotel of Canin, road no. SP76; 46°23'19"N, 13°28'25"E; 16.05.2015; BT
T. sepulcrilobum Trávníček, ineditus	CZ	Záhlinice village (near Hulín town), wet meadow 1.3 km SSW from the railway station; 190 m a.s.l.; 49°16'52"N, 17°28'58"E; 20.04.2016; BT
T. sertatum Kirschner, H. Øllgaard & Štěpánek, 1997	CZ	Svratka village, meadows and grassy places at NW margin of the settlement of Česká Cikánka; 630 m a.s.l.; 49°42'35"N, 16°03'01"E; 17.05.2016; BT & RJV
T. subhuelphersianum M. P. Christiansen, 1971	SK	Spišské Podhradie village (near Levoča town), lawn at road not far from Sivá brada travertine spring; 49°00'28"N, 20°43'26"E; 01.05.2014; BT
T. valens Marklund, 1938	HU	Szombathely town, lawns in the Szent István park (at the street of Jókai Mór); 225 m a.s.l.; 47°13'45"N, 16°36'15"E; 26.04.2015; BT

† The taxon traditionally identified as *T. lippertianum* Sahlin, 1979 in Central Europe and recently considered a synonym of *T. debrayi* Hagendijk, Soest & Zevenbergen, 1972. According to BT, both taxa are synonyms of *T. lojoense* (B. Trávníček unpubl., H. Øllgaard pers. comm.).

erslip. After heating the preparation to 42 °C for 1–2 min, cells were spread between a glass slide and coverslip in a drop of 50% acetic acid. The coverslip was mechanically removed by a razor blade after deep freezing in liquid nitrogen, and the slide was air dried. To increase the contrast of metaphase chromosomes for counting, the preparations were stained with DAPI (4',6-Diamidine-2'-phenylindole dihydrochloride; Vectashield Mounting Medium with DAPI, Vector Laboratories). For each species, at least ten metaphases were analysed to determine the chromosome number. Well-spread metaphase images were captured using Olympus BX 60 and Axio Imager Z.2 Zeiss fluorescence microscopes, both equipped with a CCD camera and ISIS software (Metasystems, Altlussheim, Germany).

Genome size estimation

The absolute genome size (2C-value; Doležel et al. 2007) of the fresh plant samples was quantified using a BD Accuri C6 flow cytometer (BD Biosciences, San Jose) equipped with a blue laser (488 nm, 20 mW, BD Accuri; BD Biosciences, San Jose). Sample preparation followed the standard protocol using LB01 isolation buffer supplemented with PVP (polyvinylpyrrolidone, 10 g/500 ml of buffer) to suppress interference of phenolic compounds with DNA staining (Doležel and Bartoš 2005, Doležel et al. 2007). Approximately 0.2 cm^2 of the plant tissue between secondary veins was chopped in 500 μl of LB01 buffer together with a similar amount of tissue

of an internal standard. Due to peak overlap in some accessions, *Solanum lycopersicum* Linnaeus, 1753 'Stupické polní rané' (2C = 1.96 pg; Doležel et al. 2007) served as the primary reference standard, and *Glycine max* (Linnaeus, 1753) Merrill, 1917 'Polanka' (2C = 2.33 pg, re-calculated against a primary standard) served as the secondary standard. The suspension was filtered through a 42 µm nylon mesh, supplemented with 20 µl of RNase A type II-A (with a final concentration of 50 µg/ml) and incubated at room temperature for approximately 10 min. The sample was then stained with 20 µl of propidium iodide (PI; final concentration of 50 µg/ml) and incubated with occasional shaking for approximately 5 min at room temperature. A flow-through fraction was then run on the flow cytometer, and the relative fluorescence intensity of at least 5,000 particles was recorded. Each sample was analysed at least three times. If the range of variation in the three measurements exceeded the 2% threshold, then the outlying value was discarded, and the sample was re-analysed. Only G0/G1 peaks with coefficients of variation < 4% were accepted. The 2C-value was calculated by multiplying the 2C-value of the standard with the sample/standard fluorescence ratio. Monoploid genome size (1Cx-value) was calculated by dividing the 2C-value by the inferred chromosome number.

Results

The chromosome number of all 28 studied species of *Taraxacum* sect. *Taraxacum* (*T. aberrans*, *T. atroviride*, *T. atrox*, *T. baeckiiforme*, *T. chrysophaenum*, *T. coartatum*, *T. corynodes*, *T. crassum*, *T. deltoidifrons*, *T. diastematicum*, *T. gesticulans*, *T. glossodon*, *T. guttigestans*, *T. huelphersianum*, *T. ingens*, *T. jugiferum*, *T. laticordatum*, *T. lojoense*, *T. lucidifrons*, *T. obtusifrons*, *T. ochrochlorum*, *T. ohlsenii*, *T. perdubium*, *T. praestabile*, *T. sepulcrilobum*, *T. sertatum*, *T. subhuelphersianum*, *T. valens*) was counted invariably as 2n = 3x = 24 (Figs 1, 2). With respect to the position of the centromere, the chromosomes of all studied species were predominantly submetacentric or metacentric. The chromosome sizes were relatively small (Figs 1, 2). The smallest chromosome size in this study was 1.02 µm (*T. ochrochlorum*), and the largest one was 4.94 µm (*T. baeckiiforme*).

The DNA content of the twenty-six studied *Taraxacum* species (two species, i.e., *T. chrysophaenum* and *T. subhuelphersianum*, were not analysed due to low-quality fresh material) ranged 1.08-fold from 2C = 2.60 pg in *T. atrox* to 2C = 2.86 pg in *T. perdubium* (Table 2). The average and median 2C-values for *Taraxacum* sect. *Taraxacum* (based on these 26 species) are 2.72 pg and 2.71 pg, respectively.

Discussion

Chromosome number variation differs among sections of the genus *Taraxacum* and more frequently occurs in sections such as *Palustria* or *Celtica*, whereas in section

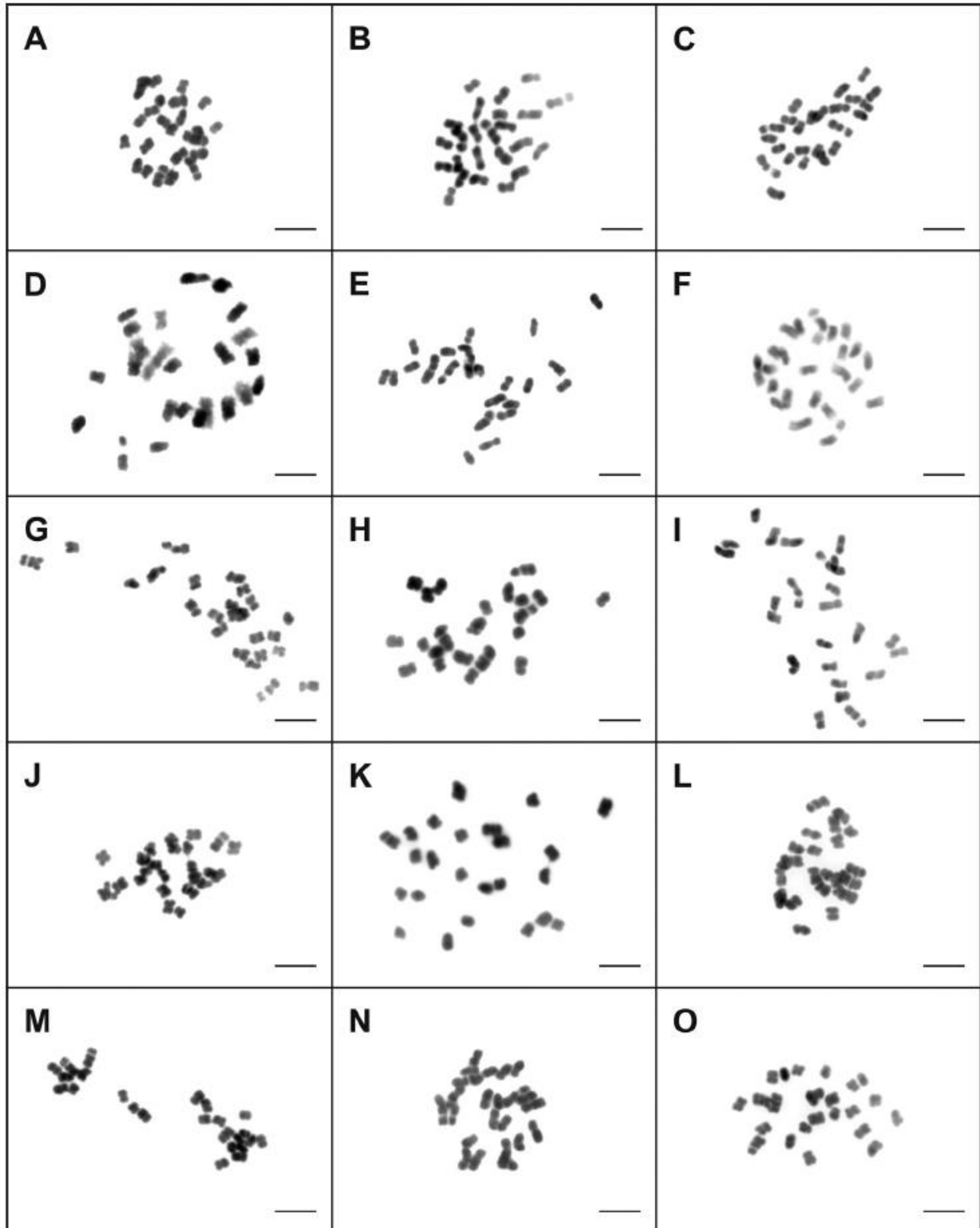

Figure 1. Mitotic metaphase chromosomes of studied triploid species (2n=3x=24) of *Taraxacum* sect. *Taraxacum*. **A** *T. aberrans* **B** *T. atroviride* **C** *T. atrox* **D** *T. baeckiiforme* **E** *T. chrysophaenum* **F** *T. coartatum* **G** *T. corynodes* **H** *T. crassum* **I** *T. deltoidifrons* **J** *T. diastematicum* **K** *T. gesticulans* **L** *T. glossodon* **M** *T. guttigestans* **N** *T. huelphersianum* **O** *T. ingens*. Scale Bar: 5µm.

Taraxacum (and also section *Hamata*), it is nearly unknown. In our study, we aimed to either find variation in ploidy or confirm the prevailing triploid level. Our findings confirmed previously published records of 2n = 3x = 24 for *T. diastematicum* and *T.*

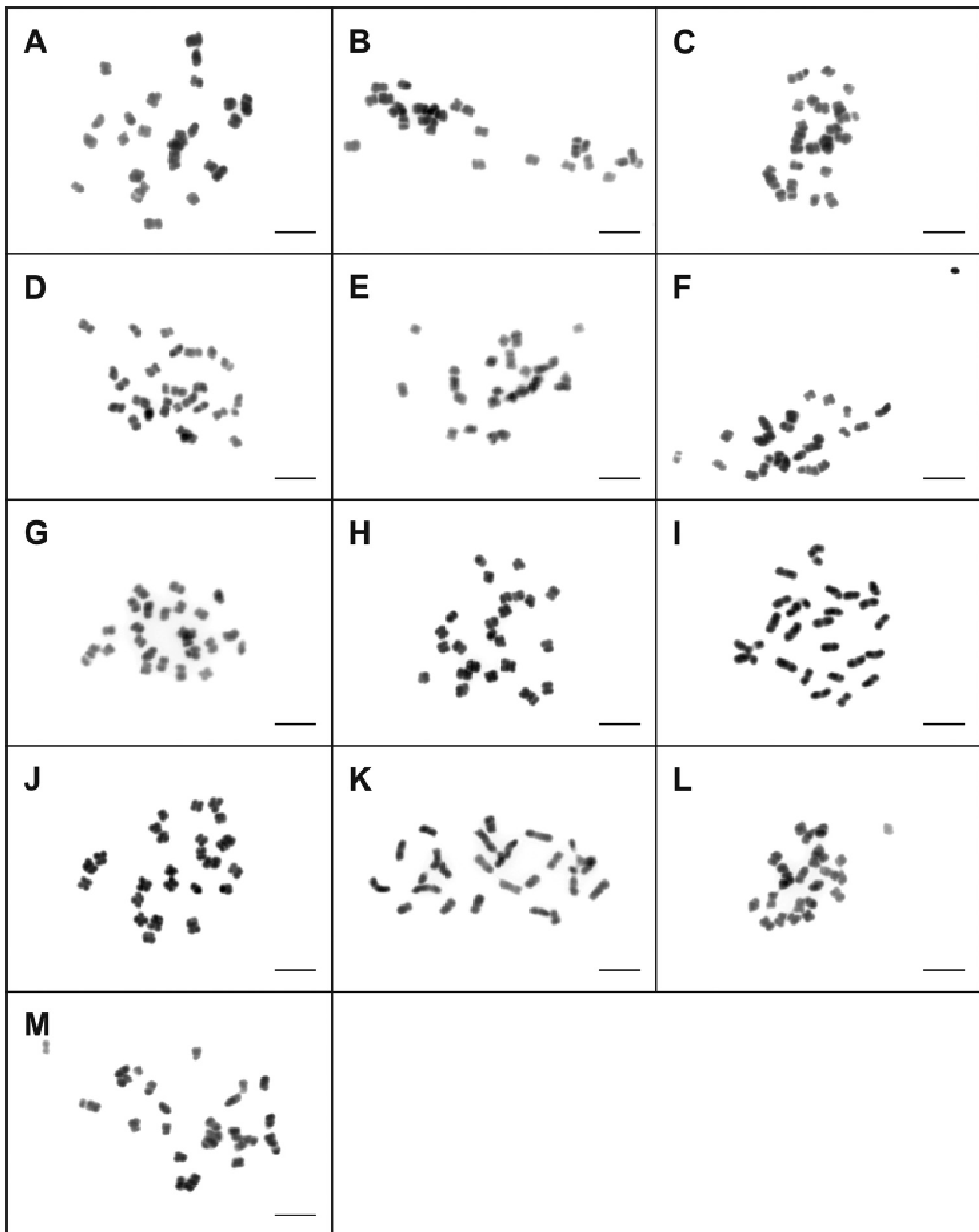

Figure 2. Mitotic metaphase chromosomes of studied triploid species (2n=3x=24) of *Taraxacum* sect. *Taraxacum*. **A** *T. jugiferum* **B** *T. laticordatum* **C** *T. lojoense* **D** *T. lucidifrons* **E** *T. obtusifrons* **F** *T. ochrochlorum* **G** *T. ohlsenii* **H** *T. perdubium* **I** *T. praestabile* **J** *T. sepulcrilobum* **K** *T. sertatum* **L** *T. subhuelphersianum* **M** *T. valens*. Scale Bar: 5µm.

obtusifrons (Uhlemann 2001, Salih et al. 2017); the chromosome numbers for all other 26 species are new findings. The ploidy level measured by flow cytometry was previously documented for 11 species (*T. atrox*, *T. baeckiiforme*, *T. corynodes*, *T. crassum*, *T.*

glossodon, *T. guttigestans*, *T. ingens*, *T. laticordatum*, *T. ohlsenii*, *T. sertatum* and *T. valens*; Trávníček et al. 2010); we now provide exact information on chromosome numbers and genome size estimations.

A tetraploid chromosome number (2n = 4x = 32) was counted for only a few species of the 165 species of *T.* sect. *Taraxacum* with known chromosome numbers in the Chromosome Counts Database (CDDB, version 1.45; Rice et al. 2015). None of the records can be considered fully reliable due to frequent misidentifications of the *Taraxacum* microspecies (lack of identification by specialists). Den Nijs and Sterk (1984) published two chromosome counts, i.e., triploid (2n = 3x = 24) and tetraploid (2n = 4x = 32), for species named as *T. lacistrum* Sahlin, 1982, and collected in France; however, the tetraploid number is listed as a question mark, and this chromosome number must therefore be considered dubious (due to the apomictic behaviour of microspecies, it is implausible to have 2 different ploidy levels for the same species). The chromosome number for a species from the High Atlas, *T. atlantis-majoris* H. Lindberg, 1932 was counted as tetraploid, but the species identification is mentioned as "*T.* cf. *atlantis-majoris*", and misidentification as other species (even from other sections, such as *Piesis*) cannot be excluded (Oberprieler and Vogt 1993). The tetraploid record for *T. albertshoferi* Sahlin, 1984 (Sahlin 1984) cannot be accepted without doubt either, because in the same paper, *T. franconicum* Sahlin, 1984 (which is now considered a synonym of *T. plumbeum* Dahlstedt, 1911) is also described with a tetraploid chromosome number, which was confirmed to be erroneous; the correct one is triploid (e.g., Vašut 2003). The tetraploid record for *T. mediterraneum* Soest, 1954 (Cardona and Contandriopoulos 1983; identified as *T. balearicum* Soest, 1961) does not refer how the taxon was determined. Rousi et al. (1985) published a tetraploid record for *T. penicilliforme* H. Lindberg, 1907 as a member of *T.* sect. *Vulgaria* (= *T.* sect. *Taraxacum*), but this species belongs to *T.* sect. *Borea*. Thus, the only somewhat reliable record of a tetraploid in *Taraxacum* sect. *Taraxacum* is for the alpine species *T. venticola* A. J. Richards, 1972 (Richards 1972).

Our list of species of *T.* sect. *Taraxacum* mainly includes typical members of the section, which differ slightly in their eco-geographic preferences. Some species have (in Central Europe) a preference for wet and sub-oceanic regions (such as *T. corynodes*, *T. chrysophaenum*, *T. lucidifrons* and *T. ochrochlorum*); on the other hand, some occupy more xerothermic regions (e.g., *T. atrox*, *T. baeckiiforme,* and *T. lojoense*). Some species resemble members of *T.* sect. *Celtica* (*T. lucidifrons*) or *T.* sect. *Palustria* (*T. perdubium* and *T. sepulcrilobum*). However, although the species in our study differ somewhat in ecology and geography, there is no variation in their ploidy levels. This is in agreement with previous studies in which only a triploid level was undoubtedly recorded for Nordic ("Atlantic") and Pannonian or Mediterranean species.

Genome size estimates in *Taraxacum* sect. *Taraxacum* are very limited. Only a few papers dealt with its genome size (Bennett et al. 1982, Záveský et al. 2005, Bainard et al. 2011, Iaffaldano et al. 2017), but none of these papers studied known apomictic microspecies; only unknown species of the *T. officinale* group were measured. Gener-

Table 2. Nuclear DNA content of studied *Taraxacum* sect. *Taraxacum* species (Lyc = *Solanum lycopersicon* 'Stupické polní rané'; Gly = *Glycine max* 'Polanka'; n.a. = not analysed, N = number of plants analysed; 1Cx = monoploid genome size, 2C = DNA amount/ploidy level).

Species	2C DNA amount [pg] (mean ± s.d.)	N	Ploidy	1Cx [pg]	Standard
T. aberrans	2.71 ± 0.010	3	3x	0.90	Lyc
T. atroviride	2.70 ± 0.020	2	3x	0.90	Lyc
T. atrox	2.60 ± 0.002	2	3x	0.87	Lyc
T. baeckiiforme	2.62 ± 0	1	3x	0.87	Lyc
T. chrysophaenum	n.a.	n.a.	3x	n.a.	n.a.
T. coartatum	2.72 ± 0.070	2	3x	0.91	Lyc
T. corynodes	2.67 ± 0.001	2	3x	0.89	Lyc
T. crassum	2.62 ± 0.020	2	3x	0.87	Lyc
T. deltoidifrons	2.69 ± 0.007	3	3x	0.90	Lyc
T. diastematicum	2.67 ± 0	1	3x	0.89	Lyc
T. gesticulans	2.83 ± 0.040	2	3x	0.94	Lyc
T. glossodon	2.77 ± 0.010	2	3x	0.92	Lyc
T. guttigestans	2.74 ± 0.004	2	3x	0.91	Lyc
T. huelphersianum	2.79 ± 0.006	2	3x	0.93	Lyc
T. ingens	2.68 ± 0.013	3	3x	0.89	Gly + Lyc
T. jugiferum	2.71 ± 0.001	2	3x	0.90	Lyc
T. laticordatum	2.84 ± 0.008	2	3x	0.95	Lyc
T. lojoense	2.62 ± 0.020	4	3x	0.87	Lyc
T. lucidifrons	2.81 ± 0	1	3x	0.94	Lyc
T. obtusifrons	2.75 ± 0.03	2	3x	0.92	Lyc
T. ochrochlorum	2.67 ± 0	1	3x	0.95	Gly
T. ohlsenii	2.63 ± 0	1	3x	0.88	Lyc
T. perdubium	2.86 ± 0	1	3x	0.95	Lyc
T. praestabile	2.73 ± 0.050	3	3x	0.91	Lyc
T. sepulcrilobum	2.72 ± 0	1	3x	0.91	Lyc
T. sertatum	2.69 ± 0.010	2	3x	0.90	Lyc
T. subhuelphersianum	n.a.	n.a.	3x	n.a.	n.a.
T. valens	2.70 ± 0	1	3x	0.90	Lyc

Table 3. Genome size estimates of *T. officinale* group in literature record. Values with asterisk (*) indicate re-calculated values according to conversion rate of 1 pg ~ 9.78×10^{8} bp (Doležel et al. 2003).

Literature	2C [pg]	2C [Gbp]
Bennett et al. 1982	2.55	2.49*
Záveský et al. 2005	1.74–2.70	1.70–2.64*
Vidic et al. 2009	2.56*	2.50
Temsch et al. 2010	2.51	2.45*
Bainard et al. 2011	2.67	2.61*
Iaffaldano et al. 2017	1.65–3.09* (2.45–2.76*)	1.61–3.02 (2.40–2.70)
this study	2.60–2.86	2.54–2.80*

ally, the genome size of the *T. officinale* group varies between 2C = 1.65 pg and 2C = 3.09 pg (Bennett et al. 1982, Záveský et al. 2005, Vidic et al. 2009, Temsch et al. 2010, Bainard et al. 2011, Iaffaldano et al. 2017; summarized in Table 3); values between 2C = 1.65–1.74 pg (Záveský et al. 2005, Iaffaldano et al. 2017) are equal to a diploid ploidy level (i.e., the species *T. linearisquameum*). The genome size of triploid apomicts apparently ranges from 2C = 2.45 pg to 2.76 (3.09) pg (see literature above). Our results are among the highest recorded values. The overall variation in recorded values is approximately 16 % (excluding the highest value of 2C = 3.09 pg, which may represent an aneuploid or tetraploid plant). Such variation can reflect real genome size variation among different species (individuals). Within a single species, *Taraxacum stenocephalum* (*T.* sect. *Piesis*), an ~1.2-fold difference in DNA content is documented (1.194-fold difference for DAPI and 1.219-fold difference for PI; Trávníček et al. 2013). Greater variation in DNA content can be attributed to the sexual reproduction of the species (in contrast to the apomictic reproduction of the species in our study). Even greater variation in DNA content was documented in *Picris hieracioides* Linnaeus, 1753 (Asteraceae, Cichorioideae, Cichorieae); in diploid sexual species, it ranged from 2C = 2.26 to 3.11 pg (1.37-fold difference; Slovák et al. 2009). In other genera of Asteraceae with the occurrence of apomictic taxa, such as *Hieracium* Linnaeus, 1753 and *Pilosella* Hill, 1756 DNA content variation is considerably larger than the known variation in *Taraxacum* sect. *Taraxacum*, i.e., 2.37-fold and 4.3-fold, respectively (Suda et al. 2007, Chrtek et al. 2009).

Genome size estimates vary in all taxa. Multiple factors can affect the measurement of genome size, e.g., differences in instrument settings among the instruments used (Doležel et al. 1998), using inadequate dye (DAPI vs. PI; Doležel et al. 1992), interactions between the dye and other molecules that lead to cytosolic effects (Noirot et al. 2000), and discrepancies in standardization (Doležel and Greilhuber 2010). Applying different laboratory procedures to the same species can lead to up to <10% variation; in the *T. officinale* group, different treatments led to a difference of up to 8.7% (Bainard et al. 2011). Therefore, at least part of the difference among published records can be attributed to a bias due to differences in laboratory procedures. We used a standardized procedure (buffers, tissue treatments, etc.) in our lab; therefore, the observed variation among the species used in this study likely reflects the real variation in DNA content.

Our study provided new data for 26 species of *T.* sect. *Taraxacum*, which confirmed no variation in chromosome number and ploidy level (2n = 3x = 24) and revealed only minor variation in DNA content that roughly equalled a possible methodological bias. The species sampled cover variation within the section: a sample of typical *T.* sect. *Taraxacum* species (most of the studied species) but also species that by morphology or ecology are intermediates of other sections, i.e., *T. perdubium* and *T. sepulcrilobum*, which are morphological and ecological intermediates between the studied section and *T.* sect. *Palustria*; or *T. lucidifrons*, which is morphologically similar to *T.* sect. *Celtica* or species resembling members of *T.* sect. *Borea* (*T. ohlsenii*, *T. lojoense* and *T. atrox*). Two species in our list are apolliniferous (*T. atrox* and *T. subhuelphersianum*). Such unusual homogeneity among species in *T.* sect. *Taraxacum*

rather than great morphological (and ecological) variability might reflect a young evolutionary origin, which is likely in contrast to sections *Palustria*, *Erythrosperma* and others that may partly consist of evolutionarily older species (Wittzell 1999, Majeský et al. 2012, Kirschner et al. 2015). Although there is no evidence for the potential evolutionary scenario in European *Taraxacum* sections, we can speculate that the origin of apomictic species of *T.* sect. *Taraxacum* (*T. officinale* group) may be a result of "recent" hybridization between triploid apomicts and diploid sexuals in the sexual-asexual cycle in a mixed dandelion population, a phenomenon experimentally described in this group (Tas and van Dijk 1999, van Dijk 2003, van Dijk and Vijverberg 2005). In a mixed population (2x and 3x cytotypes; sexual and apomictic types), triploids are results of hybridization between triploid apomicts (diploid pollen) and diploid sexuals (haploid egg cell); however, a rare occurrence of tetraploidy (probably of temporary occurrence) can accelerate the formation of novel triploids (Verduijn et al. 2004b). These tetraploids probably occur in nature as a (rare) product of hybridization in mixed populations (probably discovered in the papers of Sato et al. 2014 or Iaffaldano et al. 2017; Ľ. Majeský, unpublished results) and function as a bridge in the formation of novel stable apomictic microspecies, but probably no such temporary tetraploid hybrids evolved in stable microspecies.

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3.2 New insights into rDNA variation in apomictic and sexual *Taraxacum* (Asteraceae)

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New insights into rDNA variation in apomictic and sexual *Taraxacum* (Asteraceae)

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Abstract

Background and Aims Apomictic genera have a complex evolutionary history, mostly including reticulate hybridization, and variation in reproduction modes. In sexually reproducing taxa, evolutionary events leading to their origin may be quickly masked through the homogenization of tandemly organized arrays of nrDNA. In contrast, restriction from the functional meiosis considerably hampers the gene conversion in polyploid apomictic taxa, leaving footprints of past hybridization events in their genomes. Rapid chromosomal rearrangements and transposable elements (TEs) play an important role in shaping the genome diversity. *Taraxacum*, a genus with apomictic taxa, has still hazy and considerably complex reticulate evolutionary history; earlier investigation of rDNA sequences proved the complex pattern of the evolution, but without direct evidence for particular processes.

Methods The distribution and number of rDNA loci were investigated in 38 *Taraxacum* taxa (covering different reproduction modes, geographic regions, and putative phylogenetic groups) using FISH. Additionally, genome size and GC content were measured and tested for correlation with karyotype patterns. The ITS1-5.8S-ITS2 region of four sexual and five apomictic taxa was sequenced to investigate inter- and intra-individual variation.

Key Results Most of the studied species considerably differ in chromosome position of loci and karyotype patterns, but conserved in the number of 45S and 5S rDNA loci with 1:2 ratio per haploid genome. Six species differ in the number of rDNA loci, the most deviating *T. eriopodum* has 4:2 ratio of rDNA loci per haploid genome. Genome size (2C) varies 6-fold and together with GC-content, partly distinguishes the evolutionary basal taxa from the derived ones. Sexual taxa showed limited variation in the sequenced region (with 2-8 variants), but apomictic taxa varied significantly (with 20-36 variants).

Conclusions Extensive reticulate evolution in *Taraxacum* and consequent phenomena like genome repatterning, non-effective concerted evolution, are likely causing dynamic nature of *Taraxacum* karyotypes, as well as the large variation in genome size and rDNA sequences.

Keywords: apomixis, *Asteraceae*, concerted evolution, FISH, GC content, genome size, hybridization, karyotype, metaphase chromosomes, rDNA, *Taraxacum.*

Introduction

Polyploidy and hybridization are among the major evolutionary forces in plants (Ramsey and Schemske, 1998). Both phenomena have a substantial impact on genome organization, and changes at the chromosomal level have a strong impact on evolution (Wendel, 2000; Soltis and Soltis, 2009; Wendel *et al.*, 2016). Allopolyploidy (merging of two different genomes) generates genomic changes, e.g., via chromosome re-pattering, epigenomic modifications, gene neo- and sub-functionalization, etc. (Doyle *et al.*, 2008; Leitch and Leitch, 2008; Vicient and Casacuberta, 2017; Wendel *et al.*, 2018). We can learn the dynamics of karyotype evolution on an example of the mustard family (*Brassicaceae*): extensive genome changes such as multiple independent whole-genome duplications/triplications, re-diploidizations, and genomic blocks shuffling shaped the evolution of the family (Lysak *et al.*, 2016; Mandáková *et al.*, 2017). Similarly, genomic changes shaped the evolution of allopolyploid wheat (e.g., Feldman and Levy, 2012) or play an important role in a recently formed polyploid hybrid of *Tragopogon* (Lim *et al.*, 2008). Recurrent sexual process stabilizes hybridogeneous karyotypes, and alien chromosomes can disappear in a few generations (Perničková *et al.*, 2019). However, various aberrant karyotypes can be further fixed by clonal reproduction–apomixis (e.g. genus *Boechera*; Mandáková *et al.*, 2015).

Apomixis, i.e. asexual reproduction through seeds occurs in less than 1% across all major clades of flowering plants (Whitton *et al.*, 2008; Hojsgaard *et al.*, 2014). Due to loss of sexual recombination and consequent higher mutation load (Muller, 1963; 1964), apomictic organisms are expected to be blind evolutionary ends (Darlington, 1939). On the contrary, due potential for rapid expansion of their distribution areas, (at least rare) ability to hybridize with sexuals (in *Taraxacum*, see e.g. Verduijn *et al.*, 2004) and other factors, apomicts can act as facilitators for genetic and phenotypic diversification in sexual-apomictic complexes and thus survive on long-time evolutionary scale (van Dijk, 2003; Hojsgaard and Hörandl, 2015). Apomictic plants represent mostly polyploids (both auto- and allopolyploids) of supposed young evolutionary origin and putatively having their closest relatives in diploid sexuals (e.g., Majeský *et al.*, 2017). However, solid age estimates for apomictic groups are hardly missing; analysis of loci under divergent selection from flower-specific transcriptomes revealed Pleistocene hybrid origin of two apomictic taxa of the *Ranunculus auricomus* complex (Pellino *et al.*, 2013). The lack of recombination causes the allelic divergence (Meselson effect),

due to unequal mutation load, which was also confirmed in *Ranunculus* (Pellino *et al.*, 2013). Apomixis can further enhance allelic divergence by fixing unusual cytotypes. Unlike in obligate sexual plants, the high number of triploid (or higher odd-ploidy level) taxa are known in apomictic genera like *Taraxacum*, *Rubus, Hieracium* and others (e.g. Chrtek *et al.*, 2007; Krahulcová *et al.*, 2013; Macháčková *et al.*, 2018 and literature cited therein). Stable karyotypes with supernumerous chromosomes in diploid *Boechera* (Mandáková *et al.*, 2015) or hemizygous regions in grasses (Ozias-Akins *et al.*, 1998; Akiyama *et al.*, 2005) are associated with apomixis as well.

One of the prominent apomictic genera is *Taraxacum* (dandelions; Asteraceae, Cichorioideae) a genus with worldwide distribution, reaching the number of taxa up to 2800 classified into 60 sections—putative phylogenetic groups (Kirschner *et al.*, 2015). Due to the low level of morphological variation, reticulate distribution of this variation, contrasting reproduction systems and hybridization, the evolution, and systematics of the genus is still not well understood (Majeský *et al.*, 2017). Major outcome from all four attempts to reconstruct the *Taraxacum* phylogeny (Wittzell, 1999; Kirschner *et al.*, 2003; Záveská-Drábková *et al.*, 2009; Kirschner *et al.*, 2015) is that cladistic phylogeny is nearly impossible for this genus and the only solid division is based on the evolutionary age: ancestral and derived species. Wittzell (1999) found 31 bp insertion in *trn*L–*trn*F intergenic spacer clearly distinguishing group of ancestral species from the derived ones; the most recent approach based on nrDNA polymorphism (Kirschner et al., 2015) distinguishes 3 major groups of species: ancestral (A), precursor (P) and derived (D), which all are clustered in seven clades—we follow these categories in our study (see also Richards, 1973).

Nearly 90% of the genus species diversity represents polyploid taxa, which reproduce clonally via autonomous diplospory. Sexual reproduction is strictly confined to diploids (with few tetraploid exceptions only) (e.g., Majeský *et al.*, 2017). High level of known complexity regarding the phylogeny (Kirschner *et al.*, 2015), ploidy and genome size variation (e.g. Záveský *et al.*, 2005) or epigenetic variation (e.g. de Verhoeven *et al.*, 2010; de Carvalho *et al.*, 2016) are attributed to three major forces: reticulate evolution due to extensive hybridization and polyploidization (Witzell, 1999; Kirschner *et al.*, 2015), and (nearly) obligate apomixis (Richards, 1973). The proposed genetic control of apomixis in *Taraxacum* assumes the presence of three genetic loci: *DIPLOSPOROUS* (*DIP*), *PARTHENOGENESIS* (*PAR*), *AUTONOMOUS* (*AUT*) (Tas and van Dijk, 1999). Only the *DIP* locus was genetically identified

and physically positioned on the distal arm of one of the NOR chromosomes (van Dijk and Bakx-Schotman, 2004; Vijverberg *et al.*, 2004; Vašut *et al.,* 2014).

Nucleolus organizer region (NOR) are chromosomal segments harbouring genes for the formation of the ribosomes that are crucial components of living organisms for protein synthesis. They are identified under the microscope as chromosomes possessing a secondary constriction and are essential for the nucleolus formation (Heslop-Harrison and Schwarzacher, 2011). Each eukaryotic genome contains multiple copies of ribosomal DNA (rDNA) arranged in tandem arrays. Ribosomal (rRNA) genes represent one of the most abundant gene families within the genome of different organisms. There are two types of rDNA loci: the 45S rDNA – containing genes for 18S-5.8S-25S rRNA separated by internal transcribed spacers (ITS1 and ITS2), while these clusters are separated by Intergenic Spacer (IGS); and 5S rDNA – containing genes for 5S rRNA separated by Non-transcribed Spacer (NTS) (Rogers and Bendich, 1987). Both types are organized in tandemly arranged repetitive units on one or several chromosomes. Loci for 5S and 45S rDNA may have a different constitution: may be physically separated (S-type) or linked (L-type) (Garcia *et al.*, 2017). However, most seed plants possess an S-type arrangement of rDNA loci (Wicke *et al.*, 2011; Garcia *et al.*, 2017); in *Asteraceae* only around 25 % of species possess the L-type (Garcia et al., 2010). Both 5S and 45S loci greatly vary in copy number among different species as well as in the number of loci within the genome, and there is no correlation between the number of loci and number of chromosomes (Rogers and Bendich, 1987; Garcia *et al.*, 2017). A low but significant correlation was observed between the genome size and the number of 5S and 45S rDNA loci, as well as a significant positive correlation of the number of these loci and ploidy level (Garcia *et al.*, 2017). One of the prominent and from biosystematics points of view interesting feature of 45S rDNA locus is their high homogeny even though it represents the multicopy gene family. This is referred to as "concerted evolution" (Elder and Turner, 1995)—the model for evolution of multigene families, which leads to homogenization of numerous repeats within the genome (Liao, 1999). The process of sequence homogenization is very effective; however, maintaining divergent copies of rDNA and pseudogenes is not rare among plants and occurs mainly between evolutionary young hybrid and polyploid taxa (Kovařík *et al.*, 2005; Xu *et al.*, 2016; Morales-Briones and Tank, 2019). Effectiveness of concerted evolution can be further suppressed by apomixis and numerous variants of rDNA thus leave the footprint of past hybridization events in the genome (Campbell *et al.*, 1997;

Fehrer *et al.*, 2009; Zarrei *et al.*, 2014; Sochor *et al.*, 2015). Faster mutation rate of non-genic parts as ITS and NTS in comparison to high conservation of coding rRNA genes make the locus ideal for preparation of universal primers for PCR amplification. The rDNA loci thus represent one of the most utilized cytogenetic and molecular marker in a wide array of studies: phylogenetic studies (Baldwin *et al.*, 1999; Álvarez and Wendel, 2003), for tracking the hybrid origin (e.g., Fehrer *et al.*, 2009; Hřibová *et al.*, 2011; Sochor *et al.*, 2015) or for the divergence time estimation (e.g. Tremetsberger *et al.*, 2013; Nasrollahi *et al.*, 2019; Zhang *et al.*, 2019).

The primary goal of the present study was to investigate and describe the position of the rDNA loci (45S and 5S) as well as the variability of metaphase chromosomes in our model study system, the genus *Taraxacum*. For this purpose, we finely selected taxonomically well defined sexual and apomictic taxa with respect to supposed evolutionary age and covering the whole genus distribution range. We aimed to find the pattern of rDNA loci distribution within the genus; chromosome/genomic signs reflecting complex reticulate and polyploidization history of the genus; relationship between sexuals and apomicts within same sections (putative evolutionary group), and finally to describe the pattern of evolution of the loci within different genetic background (sexual vs. apomictic). Revealed unprecedented variability in the positioning of rDNA loci led us to broaden our research question to whether the variation in number of rDNA sites is linked to genome size variation. And further, we investigated if the multiple rDNA clusters represent homeologous (thus stemming from hybridization event) or paralogous (arising through duplication or chromosome/genomic rearrangements) sequences.

Material and methods

Plant Material

We studied 38 *Taraxacum* taxa belonging to 17 different sections (putative evolutionary groups). The taxon selection was made in order to completely cover natural distribution range of the genus (Europe, Asia, Africa, Australia, and America), and putative contrasting evolutionary age (ancestral, precursor and derived; see Kirschner *et al.*, 2015). Investigated sample-set includes 13 diploid sexual taxa (native to five continents) and 25 polyploids (triploid and tetraploid) apomictic taxa (native to Europe and Asia). Besides the investigation of rDNA loci among different evolutionary lineages

(represented by different sections), the sample-set was “fine-tuned” to allow also the investigation of variation within the sections. This was achieved by including 8+1 and 5+1 taxa from the two of the most common and widespread dandelion groups in Europe: i.e., *T.* sect. *Erythrosperma* (lesser dandelions, clade II according to Kirschner *et al.*, 2015) and *T.* sect. *Taraxacum* (*T. officinale* group, clade VI according to Kirschner *et al.*, 2015). Information on taxonomy and origin of the studied plant material is summarized in Table 1.

Genome size estimation

Genome size (2C-value; Doležel *et al.,* 2007) of all analyzed taxa was established using a BD Accuri C6 flow cytometer (BD Biosciences, San Jose) equipped with a blue laser (488 nm, 20 mW, BD Accuri; BD Biosciences, San Jose). Sample preparation followed the standard protocol using LB01 isolation buffer supplemented with PVP (polyvinylpyrrolidone, 10 g/500 ml of buffer) to suppress interference of phenolic compounds with DNA staining (Doležel and Bartoš, 2005; Doležel *et al.,* 2007). Samples were supplemented with 50 µl of RNAse and stained by 50 µl of propidium iodide (PI). For details of sample preparation, see Macháčková *et al.* (2018). Several internal standards were used due to peak overlap in some investigated taxa. *Solanum lycopersicum* ‘Stupické polní rané’ (2C = 1.96 pg; Doležel *et al.,* 2007) served as the primary reference standard, and *Glycine max* ‘Polanka’ (2C = 2.33 pg, re-calculated against a primary standard), *Raphanus sativus* ‘Saxa’ (2C = 1.12 pg, re-calculated against a primary standard), and *Solanum pseudocapsicum* (2C = 2.58 pg, re-calculated against a primary standard) served as secondary standards. The fluorescence intensity of at least 5,000 particles was recorded. Each sample was analyzed at least three times. If the range of variation in the three measurements exceeded the 2% threshold, then the outlying value was discarded, and the sample was re-analyzed. Only G0/G1 peaks with the coefficient of variation < 4.5% were accepted.

Table 1. List of studied *Taraxacum* taxa with sampling details. Evolutionary age: A = ancestral, P = precursor, D = derived; type of sexual reproduction: o = outcrossing (allogamous), s = selfing (autogamous). Country names abbreviated according to ISO 3166-1 alpha-2 codes. Collector's initials: BT = B. Trávníček, JK = J. Kirschner, JŠ = J. Štěpánek, LK = L. Klimeš, LKo and MH = L. Kobrlová and M. Hroneš, ĽM = Ľ. Majeský, MD = M. Dančák, PŠ = P. Šarhanová, RJV = R. J. Vašut and ZŠ = Z. Špíšek.

Section (*Taraxacum* sect. …)		Species	Origin of studied plants
Sexual taxa			
Alpina	P, o	*T. bulgaricum* Soest	BG, Rila Mountains, Sedemte rilski lake (LKo and MH)
Antarctica	P, s	*T. gilliesii* Hook. and Arn.	AR, Tierra del Fuego, Estancia Haberton (PŠ)
Australasica	P, s	*T. cygnorum* Hand.-Mazz.	AU, SW Victoria (donated by RBG Melbourne)
Dioszegia	A, o	*T. haussknechtii* Hausskn.	MK, Skopje, Skopje (ĽM and RJV)
Dioszegia	A, o	*T. serotinum* (W. and K.) Fisch.	BG, Pernik, Dolna Dikanya village (ĽM)
Erythrosperma	D, o	*T. erythrospermum* Andrz.	SK, Rožňava, Krásnohorské podhradie village (ĽM)
Glacialia	A, o	*T. glaciale* Hand.-Mazz.	IT, Central Apennini Mts., Caramanico (JŠ)
Obliqua	P, o	*T. pyrenaicum* Reut.	AD, Encamp, El Pas de la Casa (MD)
Piesis	A, a	*T. atlanticum* Pomel	MA, Atlas Mts. (JK)
Piesis	A, s	*T. bessarabicum* (Hornem.) HM	HU, Bács-Kiskun, Orgovány village (RJV)
Piesis s.l.	A, o	*T. cylleneum* Fürnkranz	GR (plants from cultivation) (JŠ)
Taraxacum	D, o	*T. linearisquameum* Soest	CZ, Olomouc, Olomouc (ĽM)
Tibetana	P, s	*T. eriopodum* (D. Don) Candolle	IN, Ladakh (LK)
Apomictic taxa			
Alpestria	D	*T.* sp. 1 [not determined]	RO, Bucegi Mountains, Buşteni (ĽM)
Alpina	P	*T.* sp. 2 [not determined]	AT, Oberösterreich, Dachstein (MD)
Crocea	D	*T.* sp. 3 [not determined]	NO, NP Stabursdalen (ZŠ)
Erythrosperma	D	*T. aspectabile* Štěpánek *et al.*	CZ, Southern Moravia, Velké Bílovice (RJV)
Erythrosperma	D	*T. bellicum* Sonck[1]	SK, Malacky, Plavecký Štvrtok village (ĽM)
Erythrosperma	D	*T. cristatum* Kirschner *et al.*	SK, Rimavská Sobota, Drienčany village (ĽM)
Erythrosperma	D	*T. lacistophylloides* Dahlst.	AT, Lower Austria, Sulz im Wienerwald village (BT)
Erythrosperma	D	*T. maricum* Vašut *et al.*	CZ, Břeclav, Pouzdřany village (ĽM)
Erythrosperma	D	*T. pudicum* Vašut and Majeský	CZ, S Moravia, Bojanovice near Znojmo (RJV)
Erythrosperma	D	*T. scanicum* Dahlst.	NL, Gelderland, Wageningen (RJV)
Erythrosperma	D	*T. zeisticum*, ined.	NL, Gelderland, Wageningen (RJV)
Hamata	D	*T. boekmanii* Borgv.	CZ, Žďár na Sázavou, Jimramov (BT and RJV)
Hamata	D	*T. lamprophyllum* M.P.Christ.	CZ, N Bohemia, Liberec, Fojtka (BT)
Naevosa s.l.	D	*T. gelertii* Raunk.	DE, Sachsen, Jägersgrün village (BT)
Naevosa s.str.	D	*T.* sp. 4 [not determined]	NO, Kirkenes (ZŠ)
Naevosa s.str.	D	*T.* sp. 5 [not determined]	NO, Nordland, road (BT)
Taraxacum	D	*T. alatum* H. Lindb.	CZ, Přerov, Kojetín (BT)
Taraxacum	D	*T. amplum* Markl.	CZ, Přerov, Jezernice (BT)
Taraxacum	D	*T. copidophyllum*	CZ, E Bohemia, Strašov pond near Chlumec n/C (BT)
Taraxacum	D	*T. obtusifrons* Markl.	CZ, Písek, Vepice (BT)
Taraxacum	D	*T. fascinans* Kirschner *et al.*	CZ, Jičín, (BT)
Leucantha	P	*T. candidatum* Kirschner *et al.*	IN, Ladakh: Yaye Tso; 4700m a.s.l. (LK)
cf. *Biennia*	P	*T.* sp. 6 [not determined]	IN, Ladakh: Achinathang to Beema; 2750-2900 m (LK)
Suavia	P	*T. tricolor* Soest	IN, Ladakh: Zara Vy, SE of Spangchen; 5200 m (LK)
Suavia	P	*T.* sp. 7 [not determined]	IN, Ladakh: Chumatang Phu valley, 5000-5500m (LK)

[1] Taxon widely known as *T. prunicolor* M. Schmid *et al.* (see Schmid *et al.*, 2004), but recently synonymized with *T. bellicum*

GC content estimation

For estimation of genomic guanine+cytosine (GC) content, samples were processed with two fluorochromes, the intercalating PI and the AT-selective DAPI (4′,6-diamidino-2phenylindole dihydrochloride). Samples were prepared equally to genome size estimation described above, then split into two, stained by either 50 µl of PI or 50 µl of DAPI and analyzed using two different flow cytometers. PI-stained samples were analyzed using the Accuri C6 flow cytometer, and DAPI-stained samples were analyzed using the Partec CyFlow ML flow cytometer (Partec GmbH, Münster, Germany) equipped with a Partec UV LED kit (365 nm, 10 mW). *Solanum lycopersicum* 'Stupické polní rané', *Glycine max* 'Polanka', *Raphanus sativus* 'Saxa' and *Solanum pseudocapsicum* served as reference standards. Calculation of % GC content followed the procedure described in Šmarda *et al.* (2008, 2012). The GC content was plotted against the monoploid genome size in R using ggplot2 and ggrepel libraries.

Preparation and labelling of probes for FISH

Genomic DNA of *Taraxacum linearisquameum* van Soest (i.e., diploid sexual taxon of *T.* sect *Taraxacum*, or *T. officinale* agg.) was isolated from lyophilized leaves using NucleoSpin Plant II kit (Macherey–Nagel GmbH and Co. KG, Düren, Germany) following the manufacturer's recommendations and further used as template DNA for preparation of FISH probes. The 5S and 45S rDNA probes were amplified using specific primers (5S-F: 5′-AAACGGCTACCACATCCAAG-3′; 5S-R: 5′-CGAAGGCCAACGTAATAGGA-3′; and 18S-F: 5′-GATCCCATCAGAACTCCGAAG-3′; 18S-R: 5′-CGGTGCTTTAGTGCTGGTATG-3′). The PCR mix contained 25 ng of genomic DNA, 0.2 mM digoxigenin-11-dUTP or biotin-16-dUTP (Sigma-Aldrich, Saint Louis, Missouri, USA), 1 µM of specific primers, 1X PCR buffer with 1.5 mM $MgCl_2$ and 2U of *Taq* DNA polymerase (New England Biolabs, Ipswick, Massachusetts, USA) in a final volume of 25 µl. The amplification was performed with the following conditions: 94 °C for 5 min (1 cycle), 94 °C for 50 s, 55 °C for 50 s and 72 °C for 1 min (35 cycles) and 72 °C for 5 min (1 cycle). Finally, the quality of both probes was resolved in 1.2% agarose gels.

Chromosome spreads preparations and FISH

Actively growing root tips of dandelion seedlings were used to prepare the slides with chromosomal spreads following the same procedure presented in a previous study

by Macháčková *et al.* (2018). The air-dried slides of chromosome preparations were counterstained with DAPI in Vectashield media (Vector Laboratories, Peterborough, UK) and checked using fluorescent microscope Olympus BX 60 prior to FISH analysis. The preparations containing the best chromosomal figures were selected for FISH, destained from DAPI and post-fixed by following conditions at RT (room temperature): washed in 2 x SSC (2 x 5min), 4% (v/v) formaldehyde solution (1 x 10 min), 2 x SSC (3 x 4 min), dehydrated in an increasing ethanol series (70, 90 and 96% ethanol, 2 min each) and air-dried.

Hybridization mixture consisting of 50% (v/v) formamide, 10% (v/v) dextran sulfate, 2 × SSC and 200-400 ng of each labelled probe was applied onto slides, covered with a glass coverslip and denatured at 80 °C for 3 min. The hybridization was carried out at 37 °C overnight in a humidified chamber. The digoxigenin-labelled (5S rDNA) and biotin-labelled (45S rDNA) probes were detected using anti-digoxigenin-FITC (Roche Applied Science) or anti-streptavidin-Cy3 (Vector Laboratories, Burlingame, USA), respectively. The antibodies were applied in concentrations recommended by manufacturers. Finally, chromosomes were counterstained with DAPI plus Vectashield antifade mounting medium (15 µl/slide) and covered with a coverslip.

Metaphase spreads were acquired with Olympus BX 60 and Axio Imager Z.2 Zeiss fluorescence microscopes, both equipped with Cooled Cube 1 camera (Metasystems, Altlussheim, Germany), appropriate optical filters and ISIS software (Metasystems). Recalculating the scale bar for each FISH image and pseudo colouring the DAPI channel in grey was done using the Fiji software (Schindelin *et al.,* 2012). The final images adjustment was made in Adobe Photoshop CC software (Adobe Systems Incorporated, San Jose, USA).

Chromosome measurements and karyotype analysis

At least five mitotic metaphase spreads per each investigated taxon (obtained at 100x magnification) were analyzed to obtain dataset of the following chromosome features: the length of short (S) and long (L) chromosome arm, the total chromosome length (TL), centromeric index (CI), arm ratio (r) and the distance of FISH signals from centromere. The individual chromosomes were classified according to centromeric index as metacentric (m: $50\% \geq CI > 37.5\%$), submetacentric (sm: $37.5\% \geq CI > 25\%$) and subtelocentric (st: $25\% \geq CI > 12.5\%$) chromosomes, followed the nomenclature of Levan *et al.* (1964).

The degree of karyotype asymmetry for each dandelion taxon was estimated using the formulas for the interchromosomal asymmetry index A2 (Zarco, 1986), describing heterogeneity among chromosome sizes in a complement and coefficient of intrachromosomal asymmetry A (Watanabe *et al.*, 1999), quantifying the relative differences in the centromere position among chromosomes of a complement.

For the preparation of haploid idiogram per each investigated taxon, morphological features of individual chromosomes, and positions of 5S and 45S rDNA sites were measured using ISIS software (Metasystems). Chromosomes of individual metaphase were first divided into a total of 8 groups based on the total length of chromosomes, their similar arm ratio and FISH signals distribution. Subsequently, these eight chromosome groups were arranged in descending order based on the mean values of total chromosome lengths and identified either only by numbers or moreover by small letters. Numbers ranging from 1 to 8 indicated the order of individual chromosome groups, while letters represented individual variants in the distribution of the 5S and 45S rDNA loci on morphologically similar chromosomes throughout the karyotype. The resulting haploid idiogram for each taxon was prepared in DRAWID software version 0.26 (Kirov *et al.*, 2017) and Adobe Photoshop CC software based on average values determined from five measurements on different metaphase spreads.

DNA extraction PCR amplification, cloning, and sequencing of ITS1-5.8S-ITS2 region

To investigate the level of genetic variation within the investigated 45S rDNA locus, possibility of pseudogenization, and origin of multiple loci (homeologs vs. paralogs), we investigated ITS (ITS1-5.8S-ITS2) region in selected representatives of sexual (*T. bessarabicum* – BES; *T. eriopodum* – TIB; *T. cylleneum* – CYL; *T. erythrospermum* – ERY) and apomictic taxa (*T. boekmanii* – BOE; *T.* sect. *Crocea* [*T.* sp. 3] – CRO; *T. maricum* – MAR; *T.* sect. *Naevosa* [*T.* sp. 5] – NAE; *T. zeisticum* – ZEI). Total genomic DNA was extracted from fresh leaves using a modified CTAB protocol of Doyle and Doyle (1987). ITS region was amplified using universal primers ITS1 and ITS4 (White *et al.* 1990). The PCR reactions were performed in a total of 25 µl with 1mM dNTPs, 1X PCR reaction buffer (containing 3 mM of Mg^{2+} in final volume), and 1U of MyTaq Red DNA Polymerase (Bioline Reagents Ltd, UK) and 20-50 ng of genomic DNA. The reaction conditions were: 94°C for 3 min; 36 cycles with 94°C for 30 s, 52°C for the 30s, 72°C for 30s; followed by 5 min at 72°C.

Firstly, the ITS region was amplified in selected accessions; products were directly sequenced from both (forward and reverse) direction, and sequences were investigated for polymorphism. Next, PCR products were purified using the High Pure PCR Product Purification Kit (Roche, Mannheim, Germany) and purified products were cloned using TOPO™ TA Cloning Kit™ (Invitrogen, Carlsbad, USA). For each of the nine selected taxa, 37 to 61 clones were selected and sequenced on the ABI 3730xl DNA analyzer (Applied Biosystems) at the Institute of Experimental Botany AS CR (Olomouc, Czech Republic).

Sequence treatment, alignment, and definition of unique ribotypes

Geneious 7.1.8. (Biomatters Ltd., Auckland, New Zealand) was used for sequence analysis (assembly of partial reads, base editing, alignment, and concatenation). Firstly, assembled sequences were aligned for each accession separately and inspected for polymorphism. Random single nucleotide polymorphisms (SNPs) occurring in less than three sequences were considered as polymerase mismatch and were corrected to the dominant nucleotide. Then, all corrected ribotypes were pooled, and the number of unique ribotypes was defined using the online web tool FABOX (Villesen, 2007).

ITS sequence analysis and secondary structure reconstruction

Boundaries of 18S, ITS1, and 5.8S, were defined based on homology assessment with Genbank accession KY671127 (*Taraxacum* sp. RHS-2016 – Salih *et al.*, 2017). For the definition of 5.8S and ITS2 as well as ITS2 and 26S rDNA boundaries (Keller *et al.*, 2009), the ITS2 database was used (http://its2.bioapps.biozentrum.uni-wuerzburg.de/; Ankenbrand *et al.*, 2015). GC content (%) was calculated in Geneious 7.1.8.; the number of conserved and variable (parsimony-informative/singleton) sites were identified using MEGA version 6 (Tamura *et al.*, 2013) and nucleotide diversity (π), number of mutations and ribotype diversity (Hd) were calculated in DnaSP (Rozas *et al.*, 2003). To estimate the functionality of the 5.8S rDNA locus and presence of pseudogenes, the 5.8S region was checked for the presence of the four conserved motifs: M1 – M3 (Harpke and Peterson, 2008), M4 (Liston *et al.*, 1996). The secondary structure of the 5.8S region was assessed using mFOLD Web Server (Zucker, 2003; Mathews *et al.*, 2007). ITS2 region was checked for the presence of the UGGU motif and U-U mismatch (Schultz *et al.,* 2005) using the ITS2 database. The database was also used for prediction of the presence of a conserved four-helices structure based on homology

modelling (Wolf *et al.*, 2005). Presence of mutations within the M1 – M4 and UGGU conserved motifs, U-U mismatch, and/or inability of 5.8S rRNA and/or ITS2 region to fold into the conserved secondary structure would be considered as a sign of pseudogene presence. All sequences were deposited in Genbank (https://www.ncbi.nlm.nih.gov/genbank/), corresponding Genbank accessions numbers can be found in Supplementary data Table S1.

Identification of homeologs and paralogs

To visualize genealogical relationships and to identify possibly homeologous (stemming from hybridization) and paralogous (derived from duplication) ribotypes, the distance-based split network was constructed (Neighbor-net; p-distances) using SplitsTree4 4.10 (Huson and Bryant, 2006). Additional graphics were performed in R (ggplot2 library).

Results

Karyotype analysis

Mitotic metaphase chromosomes of all studied taxa were relatively small, ranging from 1.19 µm (*T. cristatum*) to 5.36 µm (*T. amplum*), chromosome sets characteristics of all taxa are summarized in Supplementary data Table S2. The smallest difference in total length between the largest and the smallest chromosome in karyotype was found in diploid *T. serotinum* and triploid *T. copidophyllum*, where the value of interchromosomal asymmetry was 0.11. On the contrary, the highest level of interchromosomal asymmetry (A2 = 0.27) was found in triploid *T. boekmanii*.

Generally, karyotypes of all examined *Taraxacum* taxa were relatively symmetrical due to the presence of predominantly metacentric or submetacentric chromosomes (Supplementary data Table S2). The only exception was the tetraploid *T.* sect. *Alpina* (*T.* sp. 2)—its karyotype contains an additional group of subtelocentric chromosomes. Because of the presence of subtelocentric chromosomes, the tetraploid *T.* sect. *Alpina* [*T.* sp. 2] also had the highest level of intrachromosomal asymmetry (A = 0.24). On the contrary, karyotypes with the lowest level of intrachromosomal asymmetry (A = 0.09) were found in two taxa, sexual diploid *T. glaciale,* and apomictic triploid *T. bellicum*.

Number and distribution of 5S rDNA and 45S rDNA loci

Simultaneous FISH localization of the 45S and 5S rDNA loci on mitotic metaphase chromosomes was assessed for 38 studied *Taraxacum* taxa (Figs 1-3, Supplementary data Figs S1-S3). In general, the number of 5S and 45S rDNA loci was conserved among all examined taxa, unlike its positions that varied considerably.

The median karyotype of diploid sexual dandelions (based on nine taxa) consists of two signals of 45S and four signals of 5S rDNA (thus 1× 45S + 2× 5S rDNA loci per haploid genome; Fig. 1, Supplementary data Fig. S1 and Table S2). Anomalous number of loci are unique and represent the following patterns: 4× 45S + 4× 5S rDNA loci (*T. cylleneum* – Fig. 1F, Supplementary data Fig. S1F), 4× 45S + 6× 5S rDNA loci (*T. bessarabicum* – Fig. 1E, Supplementary data Fig. S1E), 3× 45S + 4× 5S rDNA loci (*T. linearisquameum* – Fig. 1I, Supplementary data Fig. S1I), and 8× 45S + 4× 5S rDNA loci (*T. eriopodum* – Figs. 1K, Supplementary data Fig. S1K). Median karyotype of polyploid apomicts corresponds to the median karyotype of diploid sexuals, thus possessing 1× 45S + 2× 5S rDNA loci per haploid genome (Figs 2, 3, Supplementary data Figs S2, S3 and Table S2). All the investigated triploids did not deviate from this median karyotype, while two tetraploid apomictic taxa showed rather anomalous karyotype composition: 5× 45S + 8× 5S rDNA (*T.* sect. *Alpina* [sp. 2] – Fig. 3G, Supplementary data Fig. S3); 3× 45S + 8× 5S rDNA (*T.* sect. *Crocea* [sp.3] – Figs. 3F, Supplementary data Fig. S3).

Compared to the relatively stable number of the investigated rDNA loci, their chromosomal distribution was much more variable across the studied dandelions. In the majority of studied taxa, the 5S rDNA locus was predominantly localized in the (peri)centromeric region (Figs 1-3, Supplementary data Figs S1-S3). While, some taxa (Figs 1E,K-M, 2A and 3A, Supplementary data Figs S1E,K-M, S2A and S3A) show signals for 5S rDNA in the interstitial parts of short or/and long arms of non-satellite chromosomes. The only exception was the tetraploid *T.* sect. *Naevosa* [sp. 5], which had one of the total eight loci of 5S rDNA localized at a terminal position on the long arm of one metacentric chromosome (Fig. 3L, Supplementary data Fig. S3). As expected, 45S rDNA loci were predominantly detected in the nucleolar organizing region (NOR). In *T.* sect. *Crocea* [sp. 3] (Fig. 3F, Supplementary data Fig. S3F) 45S rRNA genes were additionally located in (peri)centromeric region of long arm of one satellite chromosome, while in three other investigated taxa (*T. cristatum, T. bellicum,* and *T.* sect. *Naevosa*

[sp. 4]) 45S rDNA sites were exclusively or additionally localized in (sub)telomeric regions (Figs 2BC, Supplementary data Figs 2BC) or interstitial parts (Fig. 3K, Supplementary data Fig. S3K) of short arms of one to three satellite chromosomes. Interestingly, samples *T. boekmanii* (Fig. 3H, Supplementary data Fig. S3H) and *T. tricolor* (Fig. 3C, Supplementary data Fig. S3C) contained two and three satellite chromosomes respectively without the signals of 45S rDNA.

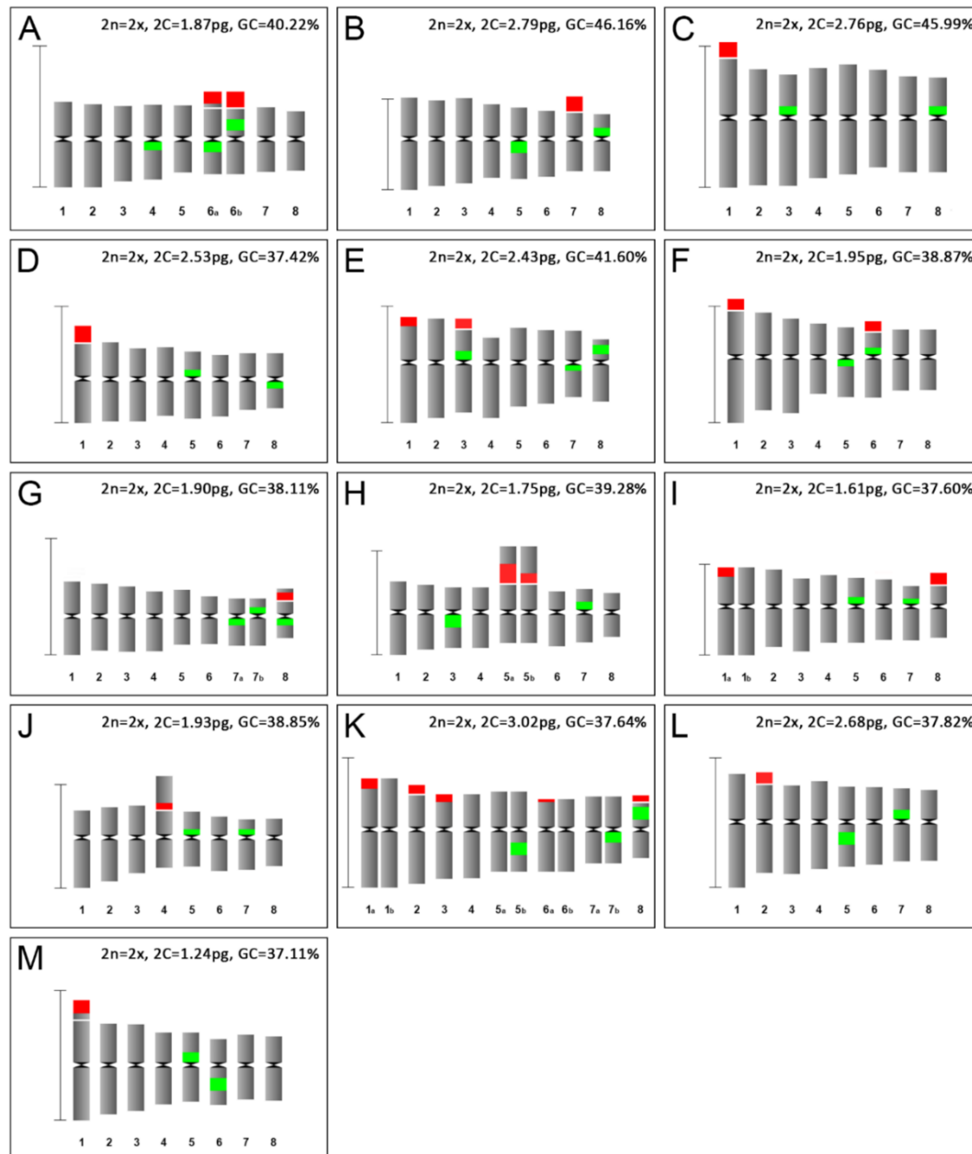

Fig. 1 Idiograms of haploid chromosome set at metaphase and the chromosomal locations of the 45S (red signal) and 5S (green signal) rDNA loci of sexual diploid (2n = 16) *Taraxacum* taxa. Bar = 5 µm. (A) *T. glaciale* (sect. *Glacialia*). (B) *T. haussknechtii* (sect. *Dioszegia*). (C) *T. serotinum* (sect. *Dioszegia*). (D) *T. atlanticum* (sect. *Piesis*). (E) *T. bessarabicum* (sect. *Piesis*). (F) *T. cylleneum* (sect. *Piesis* s.l.). (G) *T. pyrenaicum* (sect. *Obliqua*). (H) *T. erythrospermum* (sect. *Erythrosperma*). (I) *T. linearisquameum* (sect. *Taraxacum*). (J) *T. bulgaricum* (sect. *Alpina*). (K) *T. eriopodum* (sect. *Tibetana*). (L) *T. gilliesii* (sect. *Antarctica*). (M) *T. cygnorum* (sect. *Australasica*).

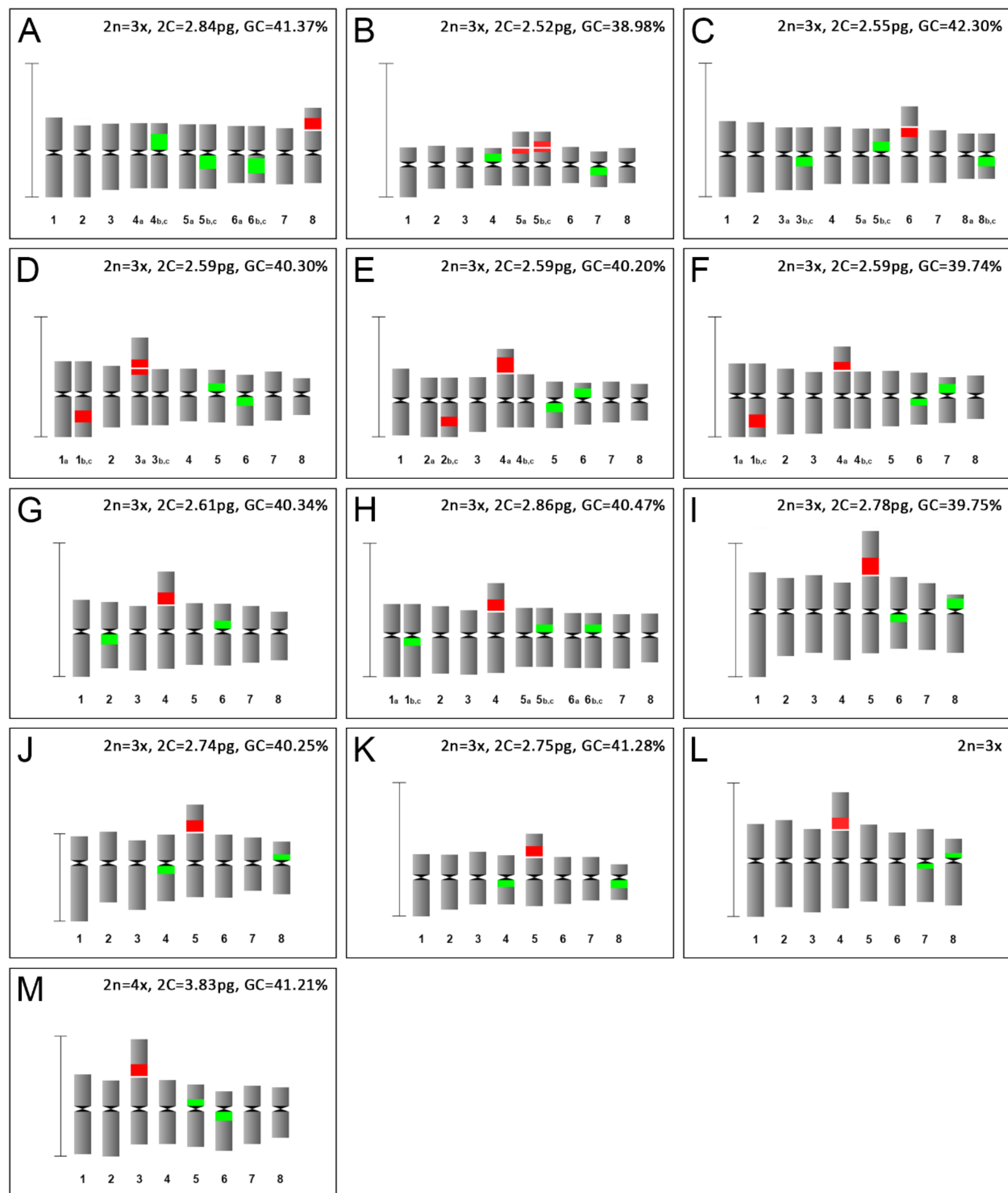

Fig. 2 Idiograms of haploid chromosome set at metaphase and the chromosomal locations of the 45S (red signal) and 5S (green signal) rDNA loci of apomictic triploid (2n = 24) *Taraxacum* taxa of European widespread sections *Erythrosperma* (A-H) and *Taraxacum* (I-L). Bar = 5 µm. (A) *T. pudicum.* (B) *T. cristatum.* (C) *T. bellicum.* (D) *T. scanicum*. (E) *T. maricum*. (F) *T. zeisticum.* (G) *T. aspectabile.* (H) *T. lacistophylloides*. (I) *T. alatum.* (J) *T. amplum* (K) *T. obtusifrons.* (L) *T. copidophyllum.* (M) *T. fascinans.*

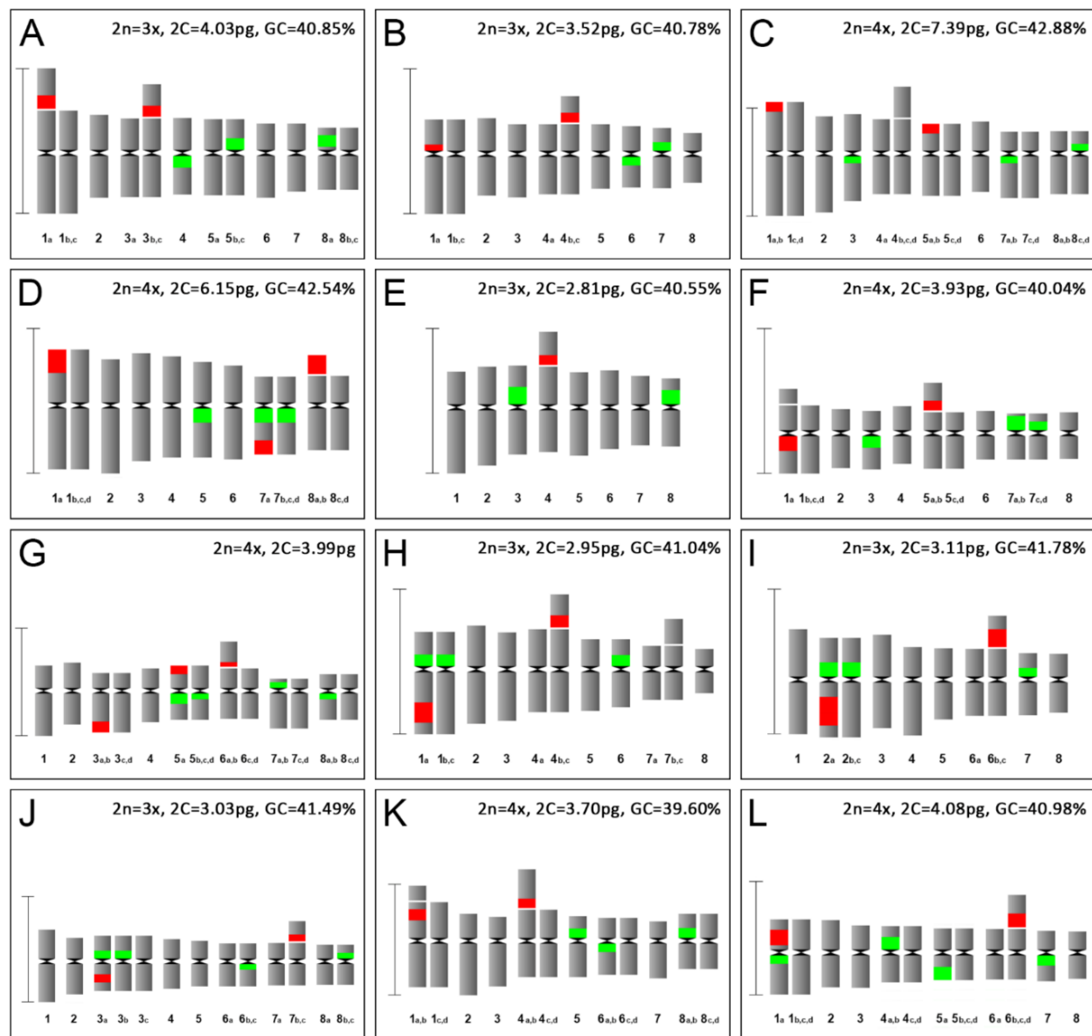

Fig. 3 Idiograms of haploid chromosome set at metaphase and the chromosomal locations of the 45S (red signal) and 5S (green signal) rDNA loci of other apomictic polyploid *Taraxacum* taxa. Bar = 5 µm. (A) *T. candidatum* (sect. *Leucantha*). (B) *T.* sp. 6 (sect. *cf. Biennia*). (C) *T. tricolor* (sect. *Suavia*). (D) *T.* sp. 7 (sect. *Suavia*). (E) *T.* sp. 1 (sect. *Alpestria*). (F) *T.* sp. 3 (sect. *Crocea*). (G) *T. alpinum* (sect. *Alpina*). (H) *T. boekmanii* (sect. *Hamata*). (I) *T. lamprophyllum* (sect. *Hamata*). (J) *T. gelertii* (sect. *Naevosa* s.l.). (K) *T.* sp. 4 (sect. *Naevosa*). (L) *T.* sp. 5 (sect. *Naevosa*).

Apart from the large cluster of 45S rDNA localized on satellite chromosomes, seven investigated taxa contained additional 45S rDNA sites which were intercalated mainly in (sub)telomeric (*T. bessarabicum, T. linearisquameum*, *T. eriopodum, T. tricolor, T.* sect. *Suavia* [sp. 7] and *T.* sect. *Alpina* [sp. 2]*;* Figs 1EIK, 3CDG, Supplementary data Figs S1EIK, S3CDG) or (peri)centromeric (*T.* sect. *Biennia* [sp. 6]*;* Fig. 3B, Supplementary data S3B) regions of non-satellite chromosomes. Similarly, three representatives of the section *Erythrosperma* (*T. scanicum, T. maricum and T. zeisticum*) showed additional 45S rDNA signals but in the interstitial chromosome regions of long arms of two non-satellite chromosomes (Figs. 2DEF, Supplementary data Figs S2DEF).

Except for a total of eleven *Taraxacum* taxa, 45S and 5S rDNA loci were located on different chromosomes. Tetraploid, *T.* sect. *Suavia* [sp. 7] (Fig. 3D, Supplementary data Fig. S3D) and three diploids (*T. bessarabicum, T. cylleneum* and *T. eriopodum* – Figs 1EFK, Supplementary data Figs. S1EFK) showed signals of both investigated rDNA loci on the same chromosome arm, while in six other taxa, including one diploid (*T. pyrenaicum* – Fig. 1G, Supplementary data Fig. S1G), three triploids (*T. boekmanii, T. lamprophyllum and T. gelertii*– Figs. 3HIJ, Supplementary data Figs. S3HIJ) and two tetraploids (*T.* sect. *Alpina* [sp. 2] and *T.* sect *Naevosa* [sp. 5] – Figs. 3GL, Supplementary data Figs. S3GL) the rDNA loci were located on the opposite chromosomal arm of one non-satellite chromosome or one NOR chromosome pair. Interestingly, *T. glaciale* (Fig. 1A, Supplementary data S1A) was the only taxon, which showed a signal for both 5S + 45S on the same arm as well as on the opposite arm within one NOR chromosome pair.

There were no similar karyotypes among all the studied diploid sexuals, even between (putatively) closely related taxa; the only similarity was found between karyotypes of Patagonian *T. gilliesii* and south-Australian *T. cygnorum* (both taxa belong to a different evolutionary group, Figs. 1LM, Supplementary data S1LM). Variability occurs even between homologous chromosomes in five diploid taxa: *T. glaciale*, *T. pyrenaicum, T. erythrospermum*, *T. linearisquameum,* and *T. eriopodum*, suggesting a highly dynamic nature of rDNA pattern in *Taraxacum* (Figs. 1AGHIK, Supplementary data S1 AGHIK). Similarly to diploids, the karyotype pattern of investigated polyploids also differed considerably among taxa, even within closely related taxa (i.e., members of the same group). Some members of section *Erythrosperma* form two groups of taxa, which have superficially similar karyotypes (see Figs 2AC and Figs 2DEF), however, the variability between homologous chromosomes bearing 5S or 45S rDNA (or both) loci is without any logic pattern reflecting their alleged relationship. Similarly, five taxa of section *Taraxacum* (i.e. all species except *T. obtusifrons*) and two taxa of section *Erythrosperma* (*T. aspectabile* and *T. lacistophylloides*) represent the third group of similar karyotypes (Figs 2GHIJLM, Supplementary data Figs 2GHIIJLM). The studied members of the sect. *Hamata* (*T. boekmanii* and *T. lamprophyllum*; Figs 3HI, Supplementary data Figs S3HI) possessed similar karyotypes, except the presence of one extra satellite chromosome in *T. boekmanii* without signal for 45S locus. This chromosome was lacking in the *T. lamprophyllum*. Two tetraploid taxa from the sect. *Naevosa* (*T. gelertii* and *T.* sp. 5; Figs 3JL, Supplementary data Figs S3JL)

showed similarity with the *T.* sect. *Hamata* karyotype, while karyotype of the third investigated taxon (*T.* sp. 4; Fig. 3K, Supplementary data Fig. S3K) from this group, was remarkably different. The rest of the investigated species showed no similarity in the karyotype. There is no apparent similarity between sexuals and apomicts of appropriate sections; however remarkable is the diploid *T. erythrospermum*, which signal for 45S rDNA is different between the two homologous chromosomes (Fig. 1H, Supplementary data Fig. S1H). The different strength of the fluorescent signal seems like a change in the copy number of rDNA (CNV – copy number variation), which is similar to the triploid apomictic taxon *T. cristatum* (Fig. 2B, Supplementary data Fig. S2B) from the same section. There is an overall high similarity of karyotypes and distribution of rDNA between the diploid sexual and polyploid apomictic taxa from this group (*T.* sect. *Erythrosperma*).

Genome size and GC content estimations

The genome size of 37 analyzed taxa varied 6-fold with the average ± s.d. of 3.01 ± 1.15 pg. The smallest genome size (2C = 1.24 pg) has diploid *T. cygnorum* (sect. *Australasica*) and the largest one (2C = 7.39 pg) tetraploid *T. tricolor* (sect. *Suavia*). The monoploid genome size varied 3-fold with the average ± s.d. of 1.05 ± 0.25 pg, and with extreme values found in same taxa, i.e. the lowest monoploid value in *T. cygnorum* ($1C_x$ = 0.62 pg), and the highest one in *T. tricolor* ($1C_x$ = 1.85 pg). The GC content varied 1.2-fold with the average ± s.d. 40.44 ± 2.00 %, the lowest values (37.11 %) were found again in *T. cygnorum* and additionally in *T. bellicum* (sect. *Erythrosperma*), whereas the highest values (45.99 % and 46.16 %) belong to two taxa of section *Dioszegia* (*T. serotinum* and *T. haussknechtii*, respectively). GC content significantly correlates with genome size (r_s = 0.43, P = 0.009) but not with ploidy level (r_s = 0.27, P = 0.106) and number of 45S loci (r_s = 0.077, P = 0.651); non-significant correlation is between GC content and number of 5S loci (r_s = 0.301, P = 0.071). Genome size correlates significantly with number of 5S rDNA (r_s = 0.741, $P < 0.001$) and 45S rDNA loci (r_s = 0.386, P = 0.018) but not with the number of satellites (r_s = 0.101, P = 0.557). Genome size estimates, monoploid values, and GC content are summarized for 37 taxa in Table 2. Monoploid genome size plotted against GC content (Fig. 4) revealed relaxed clusters of taxa related to ploidy level (sexual diploid taxa with prevailing low GC content), geography (all four apomictic taxa from Himalaya—i.e., *T. candidatum*, *T. tricolor*, *T.* sp. 6 and *T.* sp. 7—have large monoploid genome size

but the GC content around or slightly above the average value) and taxonomy (rather relaxed clusters of taxa belonging to the same section; see, e.g., *T.* sect. *Erythrosperma*).

Table 2. Nuclear DNA and CG content of 37 *Taraxacum* taxa. Internal standards: G = *Glycine max*, L = *Solanum lycopersicon*, R = *Raphanus sativus*, S = *Solanum pseudocapsicum*; n/a = not analysed, N = number of plants analysed by flow cytometry, 1 C_x = monoploid genome size, 2C = total DNA amount.

Section	Species	Chromosome number and ploidy	2C DNA amount (mean ± SD) [pg]	$1C_x$ [pg]	GC cont. [%]	Std	N
Diploid sexuals							
Alpina	*T. bulgaricum*	2n = 2x = 16	1.93 ± 0.01	0.97	38.85	G	3
Antarctica	*T. gilliesii*	2n = 2x = 16	2.68 ± 0.09	1.34	37.82	L	2
Australasica	*T. cygnorum*	2n = 2x = 16	1.24 ± 0.02	0.62	37.11	G	2
Dioszegia	*T. haussknechtii*	2n = 2x = 16	2.79 ± 0.00	1.40	46.16	G	1
Dioszegia	*T. serotinum*	2n = 2x = 16	2.76 ± 0.00	1.38	45.99	G	1
Erythrosperma	*T. erythrospermum*	2n = 2x = 16	1.75 ± 0.00	0.88	39.28	G	1
Glacialia	*T. glaciale*	2n = 2x = 16	1.87 ± 0.00	0.94	40.22	G	1
Obliqua	*T. pyrenaicum*	2n = 2x = 16	1.90 ± 0.02	0.95	38.11	G	2
Piesis	*T. atlanticum*	2n = 2x = 16	2.53 ± 0.00	1.27	37.42	L	1
Piesis	*T. bessarabicum*	2n = 2x = 16	2.43 ± 0.09	1.22	41.60	R	2
Piesis s.l.	*T. cylleneum*	2n = 2x = 16	1.95 ± 0.001	0.98	38.87	G	2
Taraxacum	*T. linearisquameum*	2n = 2x = 16	1.61 ± 0.008	0.81	37.60	G	2
Tibetana	*T. eriopodum*	2n = 2x = 16	3.02 ± 0.02	1.51	37.64	G	2
Polyploid apomicts							
Alpestria	*T.* sp. 1	2n = 3x = 24	2.81 ± 0.02	0.94	40.55	L	2
cf. *Biennia*	*T.* sp. 6	2n = 3x = 24	3.52 ± 0.001	1.17	40.78	L	2
Erythrosperma	*T. zeisticum*	2n = 3x = 24	2.59 ± 0.03	0.86	39.74	L	2
Erythrosperma	*T. aspectabile*	2n = 3x = 24	2.61 ± 0.01	0.87	40.34	L	2
Erythrosperma	*T. bellicum*	2n = 3x = 24	2.55 ± 0.00	0.85	42.30	L	1
Erythrosperma	*T. cristatum*	2n = 3x = 24	2.52 ± 0.01	0.84	38.98	L	2
Erythrosperma	*T. lacistophylloides*	2n = 3x = 24	2.86 ± 0.04	0.95	40.47	L	2
Erythrosperma	*T. maricum*	2n = 3x = 24	2.59 ± 0.00	0.86	40.20	L	1
Erythrosperma	*T. pudicum*	2n = 3x = 24	2.84 ± 0.02	0.95	41.37	L	2
Erythrosperma	*T. scanicum*	2n = 3x = 24	2.59 ± 0.03	0.86	40.30	L	2
Hamata	*T. boekmanii*	2n = 3x = 24	2.95 ± 0.00	0.98	41.04	G	1
Hamata	*T. lamprophyllum*	2n = 3x = 24	3.11 ± 0.005	1.04	41.78	G	2
Leucantha	*T. candidatum*	2n = 3x = 24	4.03 ± 0.01	1.34	40.85	G	2
Naevosa s.l.	*T. gelertii*	2n = 3x = 24	3.03 ± 0.04	1.01	41.49	L	2
Taraxacum	*T. alatum*	2n = 3x = 24	2.78 ± 0.03	0.93	39.75	L	2
Taraxacum	*T. amplum*	2n = 3x = 24	2.74 ± 0.00	0.91	40.25	L	1
Taraxacum	*T. copidophyllum*	2n = 3x = 24	n/a	n/a	n/a	n/a	0
Taraxacum	*T. obtusifrons*	2n = 3x = 24	2.75 ± 0.03	0.92	41.28	L	2
Alpina	*T.* sp. 2	2n = 4x = 32	3.99 ± 0.02	1.00	n/a	S	2
Crocea	*T.* sp. 3	2n = 4x = 32	3.93 ± 0.005	0.98	40.04	G	2
Naevosa s.str.	*T.* sp. 4	2n = 4x = 32	3.70 ± 0.004	0.93	39.60	G	2
Naevosa s.str.	*T.* sp. 5	2n = 4x = 32	4.08 ± 0.04	1.02	40.98	G	2
Suavia	*T. tricolor*	2n = 4x = 32	7.39 ± 0.06	1.85	42.88	G	2
Suavia	*T.* sp. 7	2n = 4x = 32	6.15 ± 0.20	1.54	42.54	G	2
Taraxacum	*T. fascinans*	2n = 4x = 32	3.83 ± 0.03	0.96	41.21	L	2

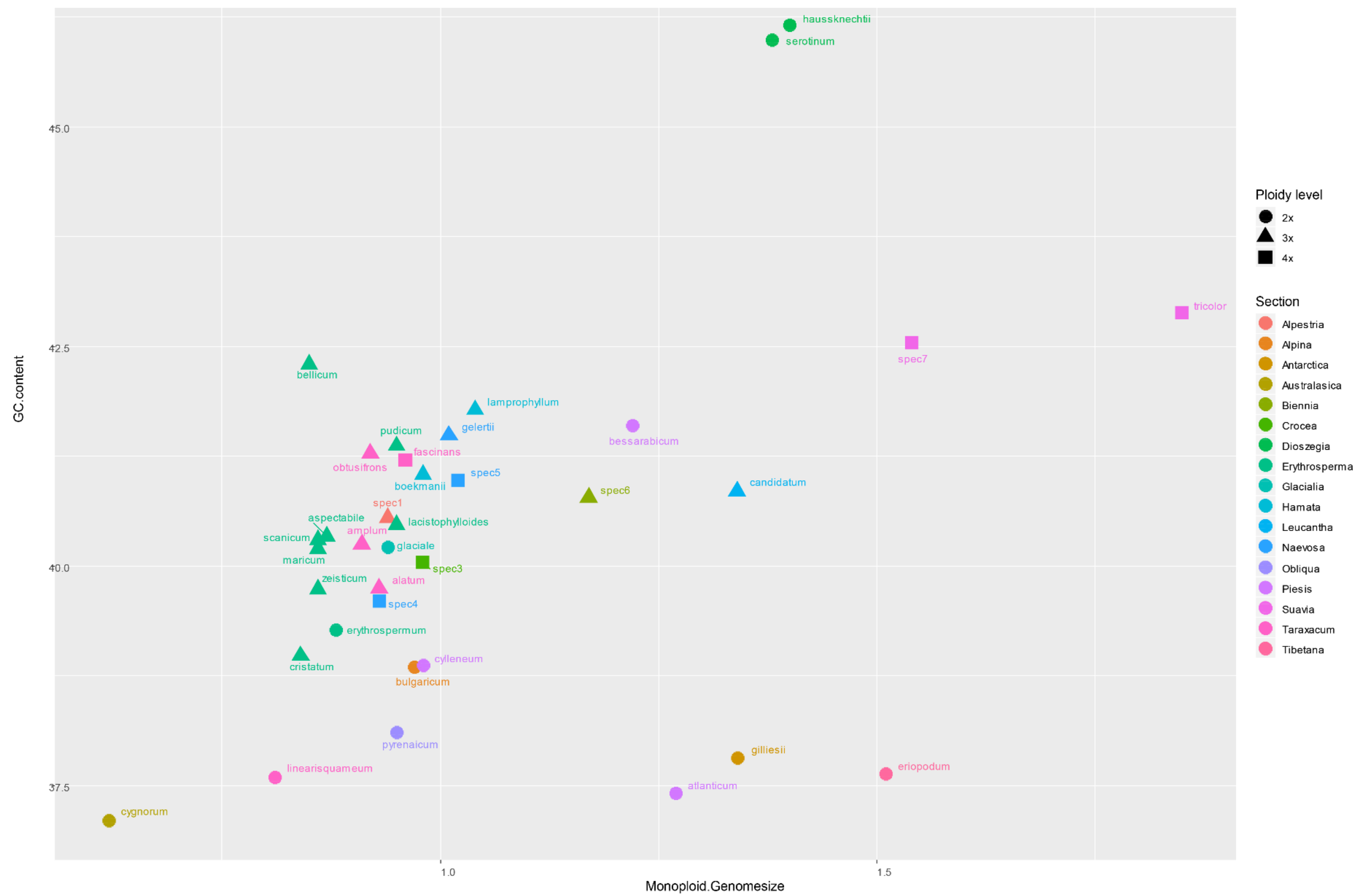

Fig. 4 Relationship between monoploid genome size and GC content. Monoploid genome-size [pg] plotted against guanosine+cytosine (GC) content [%].

Sequence characteristics, nucleotide diversity and GC content of the ITS locus

Characterization of the nucleotide diversity for the entire ITS1-5.8S-ITS2 region as well as for its three parts separately for the sequenced samples is summarized in Supplementary data Table S3. In total, 477 ITS clones were obtained from the nine investigated taxa, from which 146 represented distinct clones. The overall length of the final alignment of ITS1-5.8S-ITS2 was 646 bp (partial sequences of 18S and 26S were disregarded), with 83 variable sites (12.9%) from which 78 were parsimony informative and five were singletons. The overall nucleotide diversity (π) was 0.0196, ribotype diversity (Hd) 0.999, and a total of 87 mutations were identified (Supplementary data Table S3). When the three parts (ITS1, 5.8S, ITS2) were evaluated separately, the level of nucleotide (π) and ribotype diversity (Hd) was similar for ITS1 and ITS2, while 5.8S region was the least variable.

Characterization of the entire locus, as well as its three parts separately for each investigated accession, is present in Table 3. No length variation was observed for 5.8S (156 bp), and only minor variation was observed for the two spacers; ITS1: 259 – 257 bp; ITS2: 230 – 229 bp (Table 3). GC content varied from 49.6 % (*T. bessarabicum*) to 47.3 % (*T. erythrospermum*) for ITS1, from 53.8 % (*T. bessarabicum*, *T. cylleneum*) to 54.5 % (*T. eriopodum*, *T. erythrospermum*, *T. maricum*) for 5.8S and from 52.4 % (*T. maricum*) to 55 % (*T. cylleneum*). The four sequenced sexual taxa were less variable (in terms of the number of distinct clones and number of variable sites), comparing to the apomicts, suggesting more efficient concerted evolution. Sexual taxa were characterized by two (*T. bessarabicum*, *T. erythrospermum*), six (*T. cylleneum*) and eight (*T. eriopodum*) distinct ribotypes, which differed only by a few SNPs in ITS1 or ITS2 region but not in 5.8S region. The apomictic taxa were much more variable, possessing from 20 (*T. boekmanii*) to 36 (*T.* sect. *Crocea* [sp. 3]) distinct ribotypes. The variability of the two spacers (ITS1, ITS2) was very similar, while the 5.8S region was more conserved with maximally 2 SNPs (Table 3). Eleven ribotypes, from the total number of 146 distinct ribotypes, were shared among the five apomictic accessions (Supplementary data Table S4). However, no sharing was observed for sexual accessions.

Table 3. Characterization of the diversity of the investigated ITS locus within the investigated sexual and apomictic taxa. S – sexual taxa; A – apomictic taxa. GC content was calculated as an average value over all different ribotypes; BES – *T. bessarabicum*; TIB – *T. eriopodum* (sect. *Tibetana*); CYL – *T. cylleneum*; ERY – *T. erythrospermum*; BOE – *T. boekmanii*; CRO – *T.* sect. *Crocea* [*T.* sp. 3]; MAR – *T. maricum*; NAE – *T.* sect. *Naevosa* [*T.* sp. 5]; ZEI – *T. zeisticum*

	accession	STIB	SBES	SCYL	SERY	ABOE	ANAE	ACRO	AMAR	AZEI
	total N° of ribotypes	37	43	51	55	54	55	54	55	61
	N° of different ribotypes	8	2	6	2	20	35	36	22	28
ITS1	length (bp)	258-259	258	257	258	258	258	258	258	258
	N° of conserved	257	258	253	258	255	252	253	248	246
	variable	2	0	4	0	3	6	5	10	12
	parsimony informative	2	0	2	0	3	5	5	10	12
	singletons	0	0	2	0	0	1	0	0	0
5.8S	length (bp)	156	156	156	156	156	156	156	156	156
	N° of conserved	156	156	156	156	155	155	154	155	154
	variable	0	0	0	0	1	1	2	1	2
	parsimony informative	0	0	0	0	1	1	2	1	2
	singletons	0	0	0	0	0	0	0	0	0
ITS2	length (bp)	230-229	230	230	230	230-229	230-229	230	230	230
	N° of conserved	230	248	249	229	224	225	223	219	220
	variable	0	2	1	1	6	5	7	11	10
	parsimony informative	0	0	1	0	5	4	7	11	10
	singletons	0	0	0	0	1	1	0	0	0
whole region	length (bp)	645-643	644	643	644	644-643	644-643	644	644	644
	N° of conserved	643	642	638	643	634	632	630	622	620
	variable	2	2	5	1	10	12	14	22	24
	parsimony informative	2	0	3	0	9	10	14	22	24
	singletons	0	0	2	0	1	2	0	0	0
GC content [%]	ITS1	48,8	49,6	48,6	47,3	48,2	48,4	48,3	47,9	47,9
	5.8S	54,5	53,8	53,8	54,5	53,9	54,1	54	54,5	54,4
	ITS2	53,8	53,9	55	52	52,9	53	52,9	52,4	52,7

Presence of conserved motifs, secondary structures and identification of pseudogenes

Typical secondary structure of 5.8S rRNA, as referred in Goertzen, *et al.* (2003), was reconstructed for all the investigated distinct ribotypes. No mutation in the four inspected conserved motifs (M1-M4) was observed (Supplementary data Table S5). In the case of ITS2, all distinct sequences formed a specific four-helices structure, with the presence of conserved motifs (Supplementary data Table S5). Because all the investigated sequences folded into typical conserved structures, and no mutations were observed within the conserved motifs, no pseudogenes were confirmed within the investigated 146 distinct ITS clones.

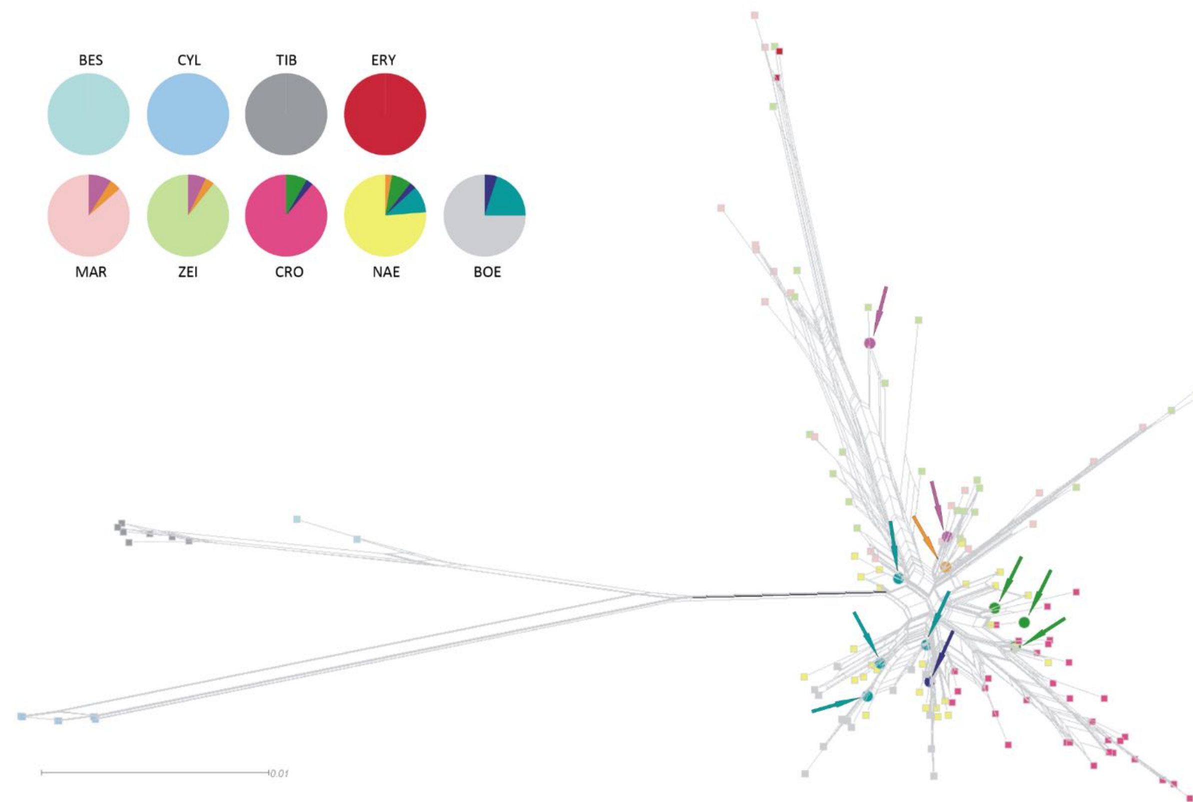

Fig. 5 Neighbor-net (based on p-distances) showing suggested relationships among the 146 distinct ITS ribotypes. The 11 ribotypes shared among the five investigated apomictic taxa are depicted by a circle (and highlighted by arrow), while squares represent unshared ribotypes. Pie-charts shows the proportion of shared and unshared ribotypes. Colour coding: light turquoise – *T. bessarabicum* (BES); light blue – *T. cylleneum* (CYL); grey – *T. eriopodum* (TIB); red – *T. erythrospermum* (ERY); body pink – *T. maricum* (MAR); light green – *T. zeisticum* (ZEI); red-purple – *T.* sect. *Crocea* [*T.* sp. 3] (CRO); yellow – *T.* sect. *Naevosa* [*T.* sp. 5] (NAE); light grey – *T. boekmanii* (BOE); dark turquoise – ribotypes shared between *T. boekmanii* and *T.* sect. *Naevosa* [*T.* sp. 5]; blue – ribotype shared among *T. boekmanii*, *T.* sect. *Naevosa* [*T.* sp. 5] and *T.* sect. *Crocea* [*T.* sp. 3]; green – ribotypes shared between *T.* sect. *Naevosa* [*T.* sp. 5] and *T.* sect. *Crocea* [*T.* sp. 3]; purple – ribotype shared between *T. maricum* and *T. zeisticum*; orange – ribotype shared among *T. maricum*, *T. zeisticum* and *T.* sect. *Naevosa* [*T.* sp. 5]

Neighbor-net analysis and identification of orthologous sequences

The three sexual accessions (*T. eriopodum*, *T. bessarabicum*, *T. cylleneum*) appeared in well defined separated clusters (Fig. 5). The fourth sexual accession *T. erythrospermum* was nested within a cluster together with two apomictic accessions, *T. maricum*, and *T. zeisticum*, i.e., apomictic taxa belonging to same section *Erythrosperma*, and it is presumed they shared a common ancestry. However, ITS clones of none of the five apomictic taxa formed one distinct cluster but instead appeared in several intermixed clusters separated by many splits. Each of the nine ribotypes shared among apomicts (Table S4) appeared instead at basal splits (Fig. 5).

Discussion

The pattern of the chromosomal distribution and number of the rDNA loci in plants is considerably variable across different evolutionary lineages (e.g., Roa and Guerra, 2012; Garcia *et al.*, 2017). Some genera exhibit high conservation, whereas others show even considerable intraspecific variation. For example, in grasses, genera like *Oryza*, *Paspalum*, *Aegilops* have a conserved pattern of rDNA loci distribution (Vaio *et al.*, 2005, Chung *et al.*, 2008, Abdolmalaki *et al.*, 2019, etc.), whereas, e.g., *Elymus* s.l. the number and distribution of rDNA loci vary even among accessions of the same species (Mahelka *et al.*, 2013), probably as a result of hybrid origin.

Generally, the majority of flowering plants have two or four 45S rDNA loci per diploid karyotype and only one pair of 5S rDNA loci (Roa and Guerra, 2012). Two pairs of 5S rDNA loci are known only in a small fraction of higher plants, which indicates a strong trend to the reduction of 5S rDNA loci in plants karyotypes (Roa and Guerra, 2015). Based on analysis of 2949 karyotypes of 1791 plant species, Garcia *et al.* (2017) proposed general rDNA patterns in karyotypes of land plants: (*i*) typical plant karyotype has more 35S rDNA loci than 5S rDNA loci; (ii) 35S rDNA loci have mostly terminal position on chromosomes; (iii) 5S rDNA loci are predominantly interstitial or centromeric (especially apparent in species with a single locus). Hypothetical ancestral karyotype of land plants (2n = 16) has one terminal 35S sites, and one interstitial 5S loci, the median karyotype of land plants (2n = 24), has two terminal 35S sites and one to two interstitial 5S sites (Garcia *et al.*, 2017).

Our results from the analysis of 5S and 45S rDNA patterns in *Taraxacum* partly fit general expectations. However, we detected variation in the number and chromosome

position of rDNA loci, genome size, and sequence diversity of ITS loci. At first glance, the results do not provide a clear pattern related to any of the considered traits (such as evolutionary origin, putative phylogenetic relationships, geography, genome size variation, reproduction strategy). Below we discuss the observed pattern of rDNA, genome size, and sequence variation from different points of view.

Chromosome distribution pattern of rDNA loci in Taraxacum taxa

All the investigated taxa of *Taraxacum* across different phylogenetic groups show relatively constant karyotype patterns regarding the number of 5S and 45S rDNA loci. Unlike most of the land plants (*Garcia et al.*, 2017), dandelions have predominantly more 5S than 45S rDNA loci: i.e. most of the studied taxa have two 5S loci and one 45S rDNA locus per haploid genome. Only sexual diploid *T. eriopodum* is exceptional in having more 45S loci than 5S rDNA loci (i.e. 2 × 5S + 4 × 45S rDNA loci per haploid genome, Supplementary data Table S1). All studied taxa have the S-type of 5S rDNA locus.

However, the karyotype pattern of rDNA loci (chromosome position of rDNA loci) sharply differs among studied taxa. Even closely related taxa do not share the same karyotype pattern (*T.* sect *Dioszegia* represented: by *T. hausknechtii* and *T. serotinum*; or *T.* sect. *Piesis* represented by *T. atlanticum*, *T. bessarabicum* and *T. cylleneum*; Figs 1BCDEF, Supplementary data Figs S1BCDEF) and only low similarity between sexuals and apomicts of the same section was observed (see Supplementary data Table S1 and groups *T.* sect. *Taraxacum* and sect. *Erythrosperma* and Figs. 1, 2, and Supplementary data Figs. S1, S2). The highest number of rDNA loci (per haploid genome) was found in two autogamic diploid taxa, *T. bessarabicum*, and *T. eriopodum*. The autogamy itself is, evidently, not the key factor for interpreting the variability, as autogamy increases the level of homozygosity – thus would not support unbalanced karyotype as present in e.g., *T. eriopodum*. Another autogamic taxa (*T. cygnorum*, *T. gilliesii*, and putatively *T. atlanticum*) possessed a fully balanced karyotype, with loci number typical for median *Taraxacum* karyotype: 2 × 5S + 1 × 45S rDNA.

Unexpected is the odd number of 45S rDNA loci in three advanced (precursor) taxa, i.e. diploid sexual *T. linearisquameum* (Fig. 1I, Supplementary data Fig. S1I), and tetraploid apomictic *T.* sect. *Alpina* [sp. 2] (Fig. 3G, Supplementary data Fig. S3G), and *T.* sect. *Crocea* [sp. 3]) (Fig. 1I, Supplementary data Fig. S1I) and differences in positions of rDNA loci between pairs of homologous chromosomes (Figs. 1-3, Supplementary data Figs. S1-S3). Further, two taxa contain satellite chromosomes

lacking the 45S rDNA signal (*T. tricolor and T. boekmanii*; Figs. 3CH, Supplementary data Figs S3CH). The variability of karyotypes was observed across different ploidy levels (diploids as both polyploids tri- and tetra-) as well as both reproductive modes (apomictic and sexual) in the present study. We think that hybridization following by genome repatterning and chromosomal rearrangements, strengthened by non-functional meiosis and clonal reproduction, can be accounted for the observed variability. A strong reticulate hybridization history of dandelions is frequently blamed for obstructing reconstruction of the genus phylogeny (e.g., Witzell, 1999; Kirschner *et al.*, 2015). Cytoembryological observations of both sexual and apomictic dandelions showed that the meiosis of sexual dandelions is fully balanced, while polyploids showed numerous disturbances during the first meiotic division with the frequent formation of univalents, bivalents, and multivalent with chiasmata, while the second meiotic division completely fails (Małecka, 1973; 1982; van Baarlen *et al.*, 2000; 2002). These meiotic aberrations were observed for restitution female gametogenesis as well as for reductional male meiosis. The result of such aberrations may be mostly unreduced female gametophyte (but also partially reduced), and unbalanced from reduced to the unreduced male gametophyte (Małecka, 1973; Koul and Singh, 1982) with chromosomal rearrangements. The newly formed hybrid (and does not matter on the direction of hybridization, if it is sex♀ × apo♂ or apo♀ × sex♂) will possess differently refolded karyotype concerning its parents, and some homeologous chromosome pairs may have not equal parental contribution (from monosomy to polysomy), as seen, e.g., in *Tragopogon* hybrids (Lim *et al.* 2008). Following genome rearrangement (which is expected to occur mainly in newly arising polyploids) will further reorganize the newly arising genome (e.g., Levy and Feldman, 2002; Lim *et al.*, 2008; Raskina *et al.*, 2008; Wendel *et al.*, 2016). The change in the copy number (CNV – copy number variation), which may act rapidly within a few generations, can be seen as another process generating variability between rDNA loci (e.g., Rogers and Bendich, 1987; Raskina *et al.*, 2008; Rabanal *et al.*, 2017). This may also reflect in the genome size (e.g., Prokopowich *et al.*, 2003). It is expected that not all rDNA loci will be active, and some will be silenced through the process of nucleolar dominance (Reeder, 1985) when transcriptionally inactive NORs become fully condensed at metaphase, what will result in the loss of secondary constriction in silenced 45S rDNA loci (McStay and Grummt, 2008). Besides that, 5S rDNA loci may undergo substantial

repatterning in hybrid species such as preservation of parental loci (e.g., Mahelka *et al.*, 2013), or nearly complete loss of loci of one parent (e.g., Volkov *et al.*, 2017).

However, there are more processes playing synergistically, especially in polyploids, which may be responsible for the observed variability of rDNA loci. The 45S rDNA clusters were suggested to represent fragile genomic sites, which are frequently subjected to mutations and rearrangements associated with epigenetic alterations (e.g., Huang *et al.*, 2008; Huang *et al.* 2012). Moreover, Transposable elements (TEs) are frequently found close to rDNA clusters or are evenly dispersed within the arrays of rDNA loci, and their presence increases the possibility of chromosomal rearrangements (e.g., Raskina *et al.*, 2008 and references therein). The repetitive manner in which the clusters of rDNA loci are present in the genome may be commonplace for heterologous and homologous recombinations, which may change locus size, as well as the distribution and number of loci (Raskina *et al.*, 2008). Thus if the rDNA clusters represent fragile sites, they represent also hot spots for chromosome rearrangements (Eickbush and Eickbush, 2007; Raskina *et al.*, 2008; Cazaux *et al.*, 2011). Investigation of heritable gene expression in apomictic *T. macranthoides* (*T.* sect. *Taraxacum*) showed, large non-random differences among the studied individuals, from which one third was accounted to the heritable TE and TE-related gene activity (de Carvalho *et al.*, 2016).

Genome size and genomic GC content

The dynamic nature of the *Taraxacum* genome also indicates the genome size variation, which is in monoploid C-value nearly 3-fold among studied taxa of dandelions. The present data increases the known variability of previously observed 2-fold variation in the genus *Taraxacum* (Záveský *et al.*, 2005; Macháčková *et al.*, 2018). Genome size estimations for dandelions are not numerous (Záveský *et al.*, 2005; Trávníček *et al.*, 2013; Macháčková *et al.*, 2018; Šmarda *et al.*, 2019), but this is the first study which considers the genome size variability together with the distribution of rDNA sequences in nuclear genome of dandelions. Correlation between the genome size and the number of rDNA loci was documented (Prokopowich *et al.*, 2003; Garcia *et al.*, 2017), what has support in the present study as well. Comparing the genome sizes among investigated taxa, especially interesting are those diploid species, which are considered to represent evolutionary basal taxa; i.e., *Taraxacum* sect. *Piesis*, *T.* sect. *Dioszegia* and sect. *Tibetana* (Witzell, 1999, Kirschner *et al.*, 2005; Záveská-Drábková *et al.* 2009). When comparing the genome size of these groups with groups of supposedly phylogenetically younger

diploid but also polyploid taxa (e.g., *T.* sect. *Taraxacum*, and *T.* sect. *Erythrosperma*) the genome downsizing is evident (Fig. 4) as well as the difference in the number of rDNA loci compared to evolutionary ancestral ones (Table 2). Although there was a positive correlation between the genome size and the number of rDNA loci within the present study, the number of the rDNA clusters itself cannot be responsible for the high variations. More plausible sources of the observed variation can be differences in extensions of various repeats (variation in copy number) among investigated taxa, including also rDNA stretches (e.g., Roger and Bendich, 1987; Wang *et al.*, 2019) and transposable elements (TEs), as both these structures accounted for the large part of the plant genome (Wendel *et al.*, 2016, Vicient and Casacuberta, 2017) as well as large part of the transcriptome of dandelions (de Carvalho *et al.*, 2016).

Contrasting to the genome size, genomic GC-content estimates have quite conservative value in studied taxa; and observed 1.2-fold variation is attributed to rare extremes. Despite relatively low variability, the GC content reveals several patterns in studied taxa. The most significant is the difference between sexual and apomictic taxa (median 38.6 for sexuals while 40.7 for apomicts). This difference may be attributed to genomic and chromosomal rearrangements after supposed polyploidization event and/or increase/decrease of highly repetitive genomic regions during these processes (Šmarda and Bureš, 2012). Most of the diploid taxa possessed genomes with slightly lower GC content (Table 2; Fig. 4). Among sexual taxa, only *T. bessarabicum* (sect. *Piesis*) and a two taxa *T. serotinum* and *T. haussknechtii* (both from section *Dioszegia*) remarkably deviate from the pattern (see Fig. 4). We cannot provide a direct explanation for the observed genome size variation, but from our flow-cytometric together with karyologic data it is evident that genome of dandelions underwent genomic rearrangements not only after polyploidization events but also at the diploid level (see also ancestral vs. derived diploids), and chromosome rearrangements, as well as variation in clusters of repetitive DNA (rDNA), are actively shaping the genome of dandelions. Relatively high GC content in the two above mentioned taxa from the section *Dioszegia* may be accounted for their putatively evolutionary basal status within the genus (Kirschner *et al.*, 2015), thus their genomes may contain more GC-rich regions.

Similarly, also the section *Piesis*, of which *T. bessarabicum* is in Fig. 4 positioned in "the halfway" between *Dioszegia* sexuals and other diploid sexuals, are representing a rather basal evolutionary group of dandelions. The isolated position of three apomictic

taxa (*T. candidatum* – *T.* sect. *Leucantha*; *T.* sp. 7 + *T. tricolor* – *T.* sect. *Suavia*; Fig. 4) are due to their large genome size; however, GC content falls within other apomicts. Taxa of these two sections are important because they combine ancestral and derived characters (so-called precursor species, see Richards, 1973; Kirschner and Štěpánek, 2005; Kirschner *et al.*, 2006) and represent intermediate steps in the genus evolution (Záveská-Drábková *et al.*, 2009). Some similar situation with clear separation of basal versus derived phylogenetic groups coupled with the change in genome size and GC content was described e.g., in *Festuca* (Šmarda *et al.*, 2008) and *Cirsium* (Bureš *et al.*, 2004).

The ITS sequence variation.

The rDNA locus is usually highly uniform within an individual due to the process of sequence homogenization (Dover, 1982), a phenomenon defined as concerted evolution (Zimmer *et al.*, 1980). The process of sequence homogenization is usually highly effective; however, maintaining of divergent (but homogenized) copies of rDNA is not rare among plants and is frequently found among different taxa (e.g., *Mammillaria* – Harpke and Peterson, 2006; *Pyrus* – Zheng *et al.*, 2008; or *Musa* – Hřibová *et al.* 2011) and especially in apomictic genera like *Crataegus* (Zarrei *et al.*, 2014), *Hieracium* (Fehrer *et al.*, 2009) or *Rubus* (Sochor *et al.*, 2015). Homogenization of divergent arrays of rDNA depends on many factors (summarized e.g., in Kovařík *et al.*, 2004): *i*) the evolutionary age of taxa – evolutionary older taxa may have had more time to undergo the complete concerted evolution; *ii*) chromosomal location – sequences located close to telomeres have a higher likelihood to interact with non-homologous chromosomes; *iii*) evolutionary distance between hybridizing parental species – hybridization of evolutionary distant taxa may suppress the sequence homogenization; or *iv*) the occurrence of homeologous pairing—when it is suppressed the gene conversion is less likely to occur. However, all the processes of sequence homogenization highly rely on pairing of homologous chromosomes, considering the non-functional meiosis and highly disturbed homologous pairing (especially in allopolyploids); the concerted evolution in apomicts cannot operate. In the present study, we detected some minor variations in the sequenced locus among sexual taxa, varying between two and eight variants, differing by a few SNPs in ITS1 or ITS2 region only (Table 3). Highest variation (eight ITS1-5.8S-ITS2 variants) was found in *T. eriopodum*, which is unique (among investigated sexuals) by a high number of rDNA loci and large genome size. The homogenizing effect of concerted evolution is more effective within locus than among loci (e.g., Mahelka *et al.*, 2013), thus

preservation of distinct ribotypes variants within the sequenced sexuals may represent different loci of 45S rDNA, as the number of recognized ribotypes roughly corresponds to the number of identified 45S rDNA loci (Tabs. 2+5). However, it was showed that apomicts possessed higher mutation rate than sexuals (Lovell *et al.*, 2017), the operation of concerted evolution may be too slow to cope with generated variability, or the gene conversion is disrupted by non-functional meiosis.

The recent investigation of European apomictic brambles showed extensive hybridization, that nearly all the apomictic polyploid taxa of *Rubus* subg. *Rubus* native to Europe share ribotypes of only six ancestral taxa (Sochor *et al.*, 2015). These parental ribotypes are variably mixed in descending hybrid polyploid taxa. A similar pattern was also documented for *Crataegus* taxa, in which variation in ITS2 sequences occurs not only in apomicts but also in sexual taxa of hybrid origin (Zarrei *et al.*, 2014; 2015). Extensive variation within the ETS of 45S rDNA clusters was also observed in *Hieracium* (Fehrer *et al.*, 2009). In dandelions, the situation is more complicated because exact parental combinations for particular polyploid apomictic taxa are unknown and are just guessed by taxonomists. Thus, direct molecular or cytogenetic comparison of particular progenitor taxa with their polyploid hybrid descendant is not possible in this case and could be inferred only from "managed crosses". Previous studies of apomictic dandelions detected shared sequences among different polyploid taxa of Asian sections *Suavia, Leucantha and Stenoloba* (Záveská-Drábková *et al.*, 2009), and this observation was further confirmed in the present study (Supplementary data, Table S4). Any of the investigated apomictic taxa formed one well-defined cluster in neighbor-net analysis, and ribotypes of apomictic taxa were spread in several clusters, while the ribotypes shared among the four apomictic taxa (Supplementary data, Table S4) occupy basal splits (Fig. 5). From the Neighbor-net analysis, it is evident that the ITS1-5.8S-ITS2 locus in apomictic dandelions represents a mixture of homeologous (originated from hybridization) and paralogous (originated from duplication) variants, both of which possess several mutant varieties. Shared sequences, even between morphologically and ecologically unrelated taxa, actively support the suggested extensive reticulate evolution of *Taraxacum* (e.g., Richards, 1973; Wittzell, 1999; Záveská-Drábková *et al.*, 2009). From the general point of view, such dynamic sequence variation of polyploid apomictic dandelions may be accounted to the presumably (only) recent reticulate evolution in the genus (Richards, 1973). Unlike in sexual species,

generally genomic variation in apomicts is accompanied by non-concerted evolution, chromosomal rearrangements and increased mutation load.

Pseudogenes are being commonly detected within complex arrays of rDNA sequences in polyploid hybrid taxa (e.g., Hřibová *et al.*, 2011; Mahelka *et al.*, 2013; Sochor *et al.*, 2015); thus, the lack of pseudogenes within the sequenced dandelion taxa may be surprising. On the other side from the present study but also from other studies of rDNA polymorphism in dandelions (King, 1993; Záveská-Drábková *et al.*, 2009; Kirschner *et al.*, 2015) it is evident that the number of detected different ribotypes increases with the number of sequenced clones. A possible explanation is that although we sequenced a large number of clones, we did not sequence enough to discover pseudogenes. On the other side, only a small fraction of rDNA loci are active, and rapid silencing of redundant arrays of 45S rDNA [which may cause the divergent arrays will escape the concerted evolution (Dadejová *et al.*, 2007; Kovařík *et al.*, 2004)], may prevent the pseudogenization.

Conclusion

Our investigation of rDNA loci within the genus *Taraxacum* revealed high plasticity in all investigated traits: chromosome length, number and chromosome location of rDNA loci, genome size, genomic GC-content, ITS1-5.8S-ITS2 sequence diversity across the different reproduction modes (sexual and apomictic) and different ploidy levels (from diploid to tetraploid). The observed variability in the number of rDNA loci and especially in the chromosomal position of these loci may result from dynamic changes in a karyotype. Theoretically, it can be caused by TEs and extensive chromosomal rearrangements, however, we don't have such evidence and it would require further study. Especially polyploid dandelions may exhibit a high dynamic evolution of karyotype after their formation, which may erase the footprints of progenitor taxa. Generally, the strong reticulate hybridization history—with subsequent striking chromosome rearrangements-hampers the reconstruction of the phylogeny, which apparently happened also in *Taraxacum*. Polyploid dandelions are thought to be of auto- as well as allopolyploid origin (e.g., Richards, 1973), and our data support these formation pathways. Investigated polyploid dandelions with balanced karyotype could result from autopolyploidization of a particular sexual taxon (which may be extinct or entered into a relic area) or may represent backcross of primary autopolyploid with sexual taxon.

While those taxa with an unbalanced karyotype (with different homeologous chromosomes) could have originated in allopolyploidization events. Despite the fact that concerted evolution might be still an ongoing process in dandelions, it seems the concerted evolution of divergent arrays of 45S rDNA locus is disrupted in polyploid apomictic dandelions, most probably due to disrupted meiosis. Within sexuals, the gene conversion is much more effective, although operating mainly within loci, thus still leaving different arrays of sequences.

Supplementary data

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: Genbank accession numbers for ITS1-5.8S-ITS2 locus of investigated *Taraxacum* taxa. Table S2: Karyotype morphometric analysis of 38 *Taraxacum* species. Table S3: Characterization of the diversity of the investigated ITS locus. Table S4: Pairwise table showing the pattern of ribotype sharing among the investigated sexual and apomictic taxa. Table S5: Characterization of the 5.8S and ITS2 region for the presence of proposed conserved motifs. Fig. S1: Distribution of 5S and 45S rDNA sites detected by FISH on mitotic metaphase chromosomes of sexual diploid *Taraxacum* taxa. Fig. S2: Distribution of 5S and 45S rDNA sites detected by FISH on mitotic metaphase chromosomes of widespread European apomictic triploid *Taraxacum* taxa. Fig S3: Distribution of 5S and 45S rDNA sites detected by FISH on mitotic metaphase chromosomes of other apomictic polyploid *Taraxacum* taxa

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3.2.1 Supplementary data

Figure S1 Distribution of 5S rDNA (green signals) and 45S rDNA (red signals) sites detected by FISH on mitotic metaphase chromosomes of sexual diploid (2n = 16) *Taraxacum* taxa. The positions of 5S rDNA and 45S rDNA signals are shown by white or yellow arrowheads, respectively. Purple arrowheads indicate satellite without a signal for 45S rDNA. Bar = 5µm. (A) *T. glaciale* (sect. *Glacialia*). (B) *T. haussknechtii* (sect. *Dioszegia*). (C) *T. serotinum* (sect. *Dioszegia*). (D) *T. atlanticum* (sect. *Piesis*). (E) *T. bessarabicum* (sect. *Piesis*). (F) *T. cylleneum* (sect. *Piesis* s.l.). (G) *T. pyrenaicum* (sect. *Obliqua*). (H) *T. erythrospermum* (sect. *Erythrosperma*). (I) *T. linearisquameum* (sect. *Taraxacum*). (J) *T. bulgaricum* (sect. *Alpina*). (K) *T. eriopodum* (sect. *Tibetana*). (L) *T. gilliesii* (sect. *Antarctica*). (M) *T. cygnorum* (sect. *Australasica*).

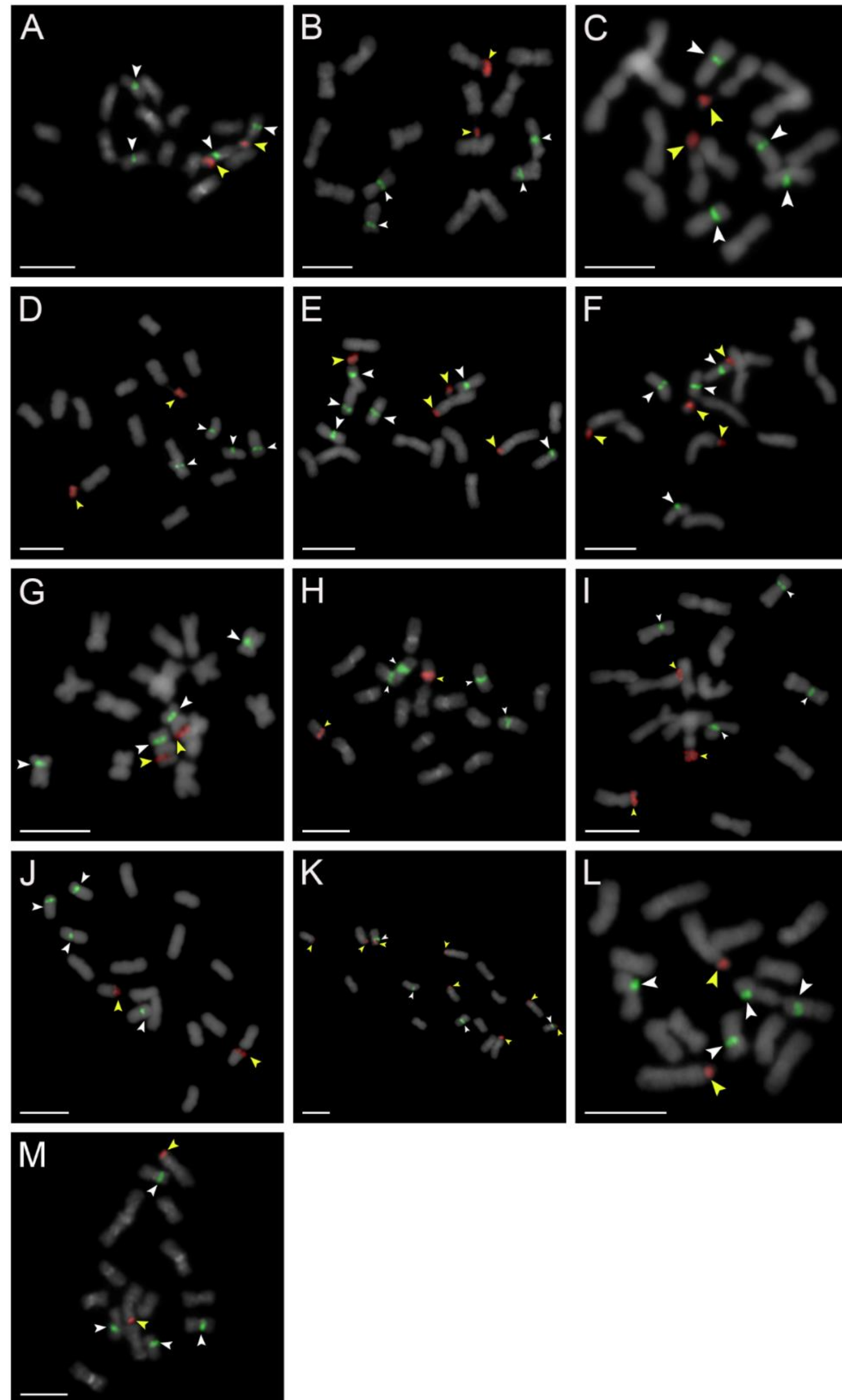

Figure S2 Distribution of 5S rDNA (green signals) and 45S rDNA (red signals) sites detected by FISH on mitotic metaphase chromosomes of apomictic triploid (2n = 24) *Taraxacum* taxa of European widespread sections *Erythrosperma* (A-H) and *Taraxacum* (I-L). The positions of 5S rDNA and 45S rDNA signals are shown by white or yellow arrowheads, respectively. Purple arrowheads indicate satellite without a signal for 45S rDNA. Bar = 5μm. (A) *T. pudicum.* (B) *T. cristatum.* (C) *T. bellicum.* (D) *T. scanicum*. (E) *T. maricum*. (F) *T. zeisticum.* (G) *T. aspectabile.* (H) *T. lacistophylloides*. (I) *T. alatum.* (J) *T. amplum* (K) *T. obtusifrons.* (L) *T. copidophyllum.* (M) *T. fascinans.*

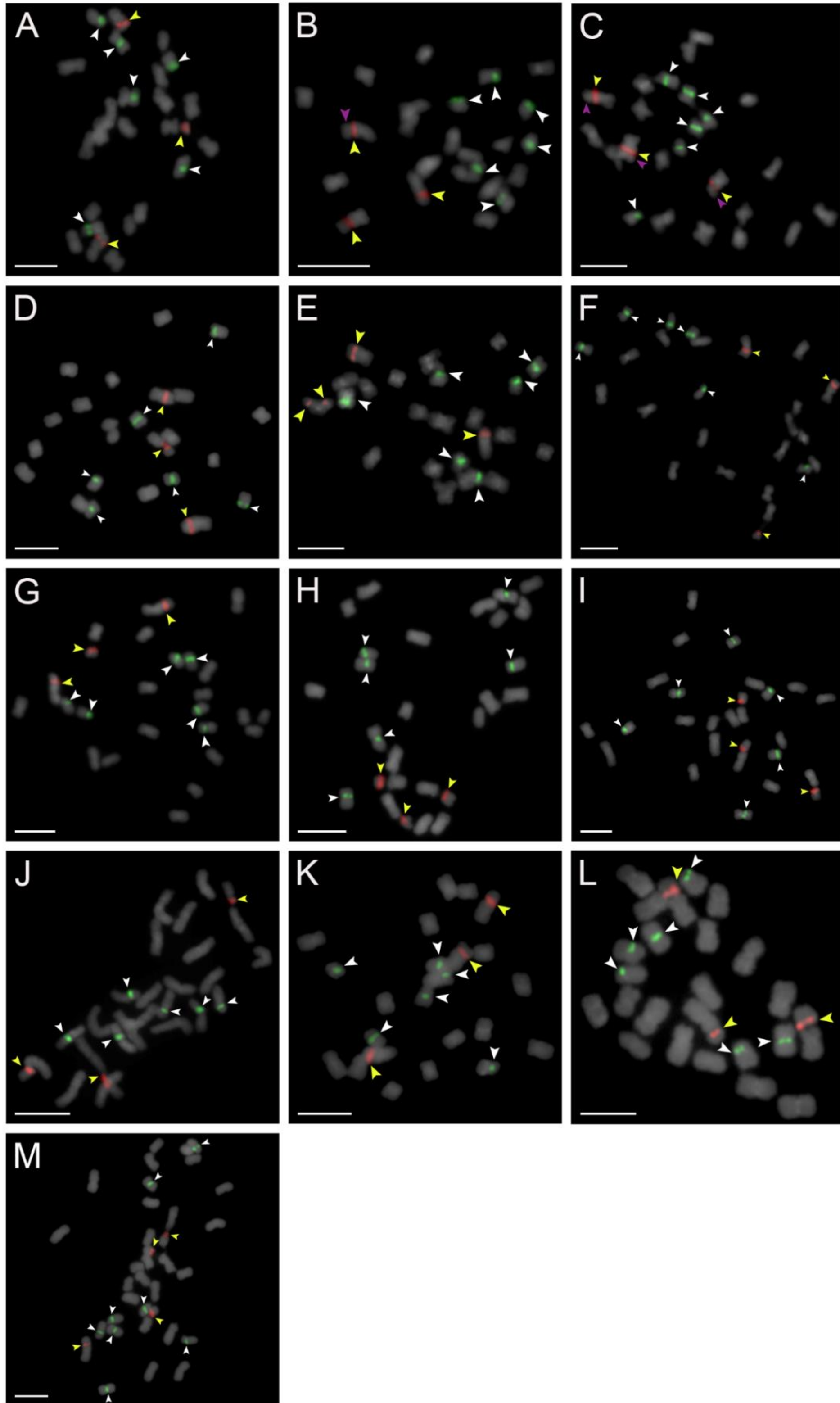

Figure S3 Distribution of 5S rDNA (green signals) and 45S rDNA (red signals) sites detected by FISH on mitotic metaphase chromosomes of other apomictic polyploid *Taraxacum* taxa. The positions of 5S rDNA and 45S rDNA signals are shown by white or yellow arrowheads, respectively. Purple arrowheads indicate satellite without a signal for 45S rDNA. Bar = 5µm. (A) *T. candidatum* (2n = 3x = 24; sect. *Leucantha*). (B) *T.* sp. 6 (2n = 3x = 24; sect. *cf. Biennia*). (C) *T. tricolor* (2n = 4x = 32; sect. *Suavia*). (D) *T.* sp. 7 (2n = 4x = 32; sect. *Suavia*). (E) *T.* sp. 1 (2n = 3x = 24; sect. *Alpestria*). (F) *T.* sp. 3 (2n = 4x = 32; sect. *Crocea*). (G) *T. alpinum* (2n = 4x = 32; sect. *Alpina*). (H) *T. boekmanii* (2n = 3x = 24; sect. *Hamata*). (I) *T. lamprophyllum* (2n = 3x = 24; sect. *Hamata*). (J) *T. gelertii* (2n = 3x = 24; sect. *Naevosa* s.l.). (K) *T.* sp. 4 (2n = 4x = 32; sect. *Naevosa*). (L) *T.* sp. 5 (2n = 4x = 32; sect. *Naevosa*).

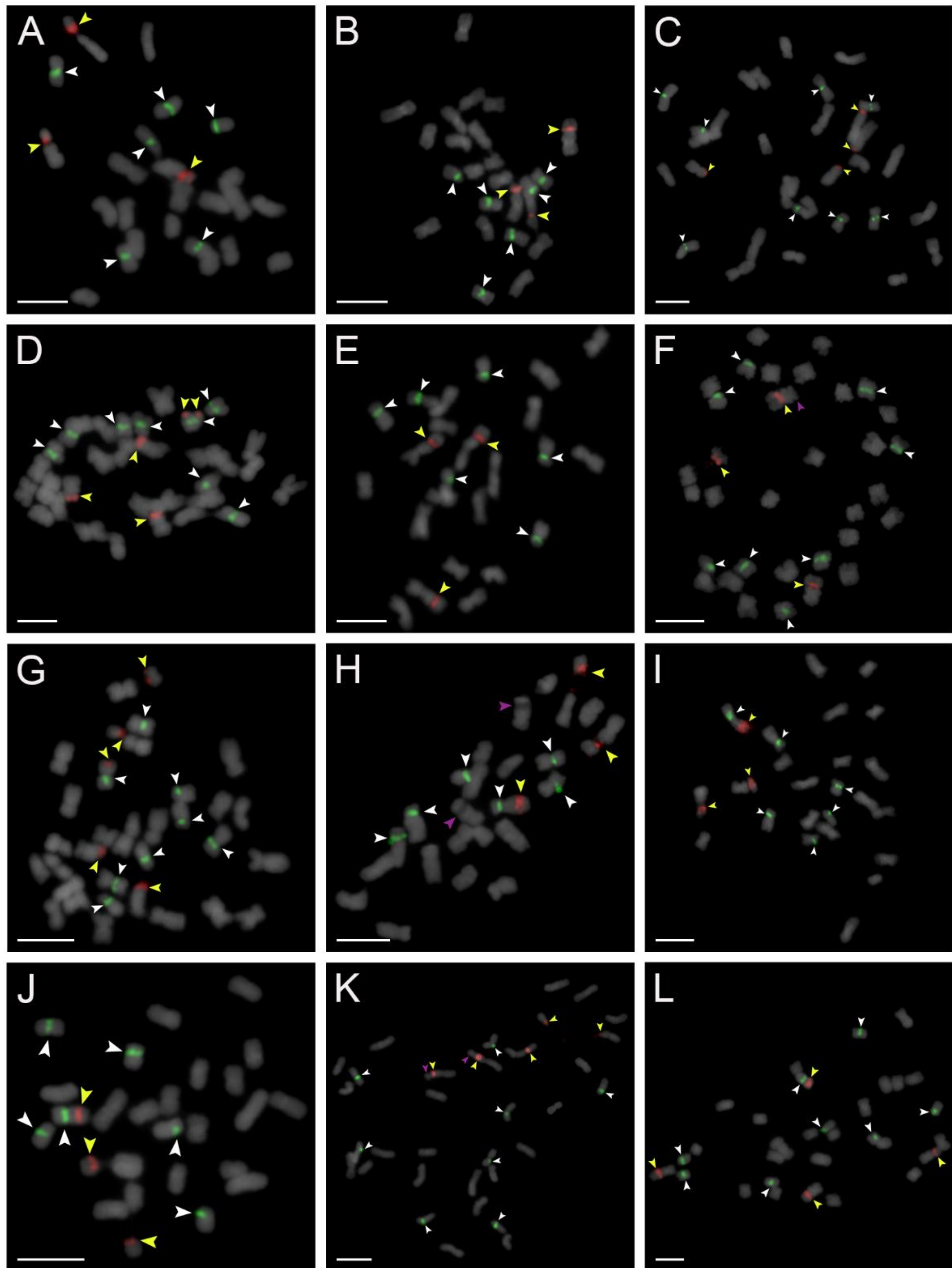

Table S1. Genbank accession numbers for ITS1-5.8S-ITS2 locus of investigated *Taraxacum* taxa

Sequenced taxon	Genbank accession number
T. bessarabicum	MN480849, MN480850, MN480851, MN480852, MN480853, MN480854, MN480855, MN480856, MN480857, MN480858, MN480859, MN480860, MN480861, MN480862, MN480863, MN480864, MN480865, MN480866, MN480867, MN480868, MN480869, MN480870, MN480871, MN480872, MN480873, MN480874, MN480875, MN480876, MN480877, MN480878, MN480879, MN480880, MN480881, MN480882, MN480883, MN480884, MN480885, MN480886, MN480887, MN480888, MN480889, MN480890, MN480891
T. cylleneum	MN480968, MN480954, MN480989, MN480974, MN480964, MN480973, MN480978, MN480967, MN480993, MN480987, MN480965, MN480957, MN480969, MN480971, MN480948, MN480981, MN480970, MN480959, MN480979, MN480982, MN480951, MN480955, MN480960, MN480961, MN480947, MN480976, MN480958, MN480995, MN480952, MN480988, MN480962, MN480994, MN480996, MN480950, MN480949, MN480985, MN480986, MN480977, MN480997, MN480966, MN480975, MN480984, MN480953, MN480956, MN480963, MN480992, MN480990, MN480980, MN480991, MN480972, MN480983
T. eriopodum	MN493462, MN493475, MN493458, MN493480, MN493488, MN493470, MN493477, MN493468, MN493478, MN493487, MN493465, MN493476, MN493459, MN493456, MN493482, MN493460, MN493457, MN493464, MN493461, MN493479, MN493471, MN493469, MN493485, MN493455, MN493484, MN493463, MN493473, MN493490, MN493454, MN493481, MN493466, MN493467, MN493486, MN493489, MN493474, MN493472, MN493483
T. erythrospermum	MN480946, MN480930, MN480901, MN480932, MN480894, MN480903, MN480937, MN480904, MN480919, MN480934, MN480895, MN480911, MN480907, MN480942, MN480892, MN480902, MN480936, MN480920, MN480943, MN480915, MN480921, MN480944, MN480899, MN480897, MN480939, MN480912, MN480916, MN480896, MN480914, MN480923, MN480935, MN480917, MN480928, MN480900, MN480908, MN480893, MN480906, MN480913, MN480940, MN480929, MN480945, MN480938, MN480898, MN480924, MN480910, MN480905, MN480918, MN480941, MN480927, MN480922, MN480925, MN480933, MN480931, MN480926, MN480909
T. boekmanii	MN493271, MN493260, MN493239, MN493268, MN493231, MN493242, MN493266, MN493247, MN493259, MN493254, MN493237, MN493244, MN493267, MN493261, MN493241, MN493240, MN493249, MN493265, MN493262, MN493274, MN493235, MN493234, MN493246, MN493273, MN493251, MN493272, MN493250, MN493256, MN493236, MN493279, MN493230, MN493255, MN493233, MN493258, MN493248, MN493263, MN493278, MN493276, MN493280, MN493232, MN493281, MN493264, MN493253, MN493252, MN493269, MN493275, MN493257, MN493270, MN493229, MN493243, MN493277, MN493282, MN493238, MN493245

Table S1. Continued

Sequenced taxon	Genbank accession number
T. sect. Crocea [*T*. sp. 3]	MN493211, MN493191, MN493215, MN493212, MN493176, MN493193, MN493227, MN493207, MN493196, MN493209, MN493203, MN493217, MN493205, MN493185, MN493225, MN493198, MN493218, MN493179, MN493228, MN493210, MN493177, MN493187, MN493219, MN493190, MN493184, MN493180, MN493226, MN493216, MN493178, MN493224, MN493175, MN493213, MN493194, MN493222, MN493199, MN493204, MN493181, MN493221, MN493183, MN493197, MN493200, MN493189, MN493188, MN493192, MN493220, MN493206, MN493208, MN493214, MN493182, MN493201, MN493223, MN493195, MN493202, MN493186
T. maricum	MN493414, MN493417, MN493419, MN493423, MN493430, MN493428, MN493449, MN493404, MN493426, MN493416, MN493409, MN493442, MN493422, MN493408, MN493453, MN493445, MN493407, MN493433, MN493405, MN493400, MN493412, MN493451, MN493446, MN493402, MN493429, MN493439, MN493401, MN493438, MN493440, MN493434, MN493447, MN493410, MN493425, MN493418, MN493436, MN493450, MN493411, MN493431, MN493424, MN493448, MN493421, MN493443, MN493413, MN493399, MN493437, MN493444, MN493432, MN493441, MN493452, MN493406, MN493420, MN493427, MN493435, MN493415, MN493403
T. sect. *Naevosa* [*T.* sp. 5]	MN493357, MN493346, MN493386, MN493391, MN493353, MN493369, MN493374, MN493388, MN493370, MN493344, MN493389, MN493396, MN493375, MN493350, MN493363, MN493362, MN493373, MN493376, MN493365, MN493378, MN493392, MN493351, MN493358, MN493364, MN493385, MN493359, MN493372, MN493366, MN493390, MN493360, MN493371, MN493397, MN493367, MN493394, MN493379, MN493395, MN493345, MN493354, MN493384, MN493347, MN493361, MN493368, MN493382, MN493398, MN493355, MN493377, MN493383, MN493356, MN493387, MN493393, MN493381, MN493348, MN493380, MN493349, MN493352
T. zeisticum	MN493325, MN493317, MN493295, MN493299, MN493288, MN493335, MN493328, MN493311, MN493319, MN493341, MN493285, MN493290, MN493289, MN493310, MN493283, MN493304, MN493287, MN493296, MN493329, MN493312, MN493297, MN493337, MN493291, MN493307, MN493326, MN493286, MN493336, MN493303, MN493292, MN493324, MN493318, MN493284, MN493321, MN493339, MN493293, MN493322, MN493327, MN493332, MN493333, MN493316, MN493338, MN493309, MN493331, MN493298, MN493300, MN493308, MN493301, MN493320, MN493342, MN493306, MN493330, MN493323, MN493315, MN493313, MN493314, MN493302, MN493294, MN493340, MN493334, MN493343, MN493305

Table S2. Karyotype morphometric analysis of 38 *Taraxacum* taxa.

Section	Species	Chrom. number and ploidy	Karyotype					Range of mean values of			Asymmetry index	
			5S[1]	45S[2]	both[3]	SAT[4]	formula[5]	TL[6] (± SD) [µm]	R[7]	CI[8] [%]	A[9]	A2[10]
Alpina	*T. bulgaricum*	2n = 2x =16	4	2	-	2	6m + 2sm	3.73 (± 0.10) - 2.28 (± 0.27)	1.12-1.87	34.85-47.24	0.18	0.18
Antarctica	*T. gilliesii*	2n = 2x =16	4	2	-	2	8m	4.39 (± 0.10) - 2.83 (± 0.21)	1.17-1.43	40.37-46.99	0.13	0.12
Australasica	*T. cygnorum*	2n = 2x =16	4	2	-	2	8m	3.83 (± 0.12) - 2.48 (± 0.10)	1.13-1.53	39.56-46.91	0.10	0.18
Dioszegia	*T. haussknechtii*	2n = 2x =16	4	2	-	2	8m	5.08 (± 0.33) - 3.15 (± 0.10)	1.09-1.35	42.55-47.74	0.11	0.18
Dioszegia	*T. serotinum*	2n = 2x =16	4	2	-	2	8m	4.57 (± 0.16) - 3.34 (± 0.29)	1.03-1.56	39.13-49.16	0.11	0.11
Erythrosperma	*T. erythrospermum*	2n = 2x =16	4	2	-	2	8m	3.53 (± 0.29) - 2.13 (± 0.10)	1.12-1.62	38.26-47.27	0.15	0.15
Glacialia	*T. glaciale*	2n = 2x =16	4	2	2	2	8m	3.03 (± 0.10) - 2.09 (± 0.10)	1.00-1.40	41.71-49.80	0.09	0.13
Obliqua	*T. pyrenaicum*	2n = 2x =16	4	2	2	2	8m	3.14 (± 0.11) - 1.57 (± 0.10)	1.07-1.65	37.80-48.27	0.13	0.22
Piesis	*T. atlanticum*	2n = 2x =16	4	2	-	2	8m	3.38 (± 0.25) - 2.37 (± 0.05)	1.12-1.56	39.02-47.25	0.12	0.14
Piesis	*T. bessarabicum*	2n = 2x =16	6	4	2	2	6m+2sm	4.59 (± 0.13) - 2.68 (± 0.39)	1.08-2.27	30.60-47.96	0.17	0.19
Piesis s.l.	*T. cylleneum*	2n = 2x =16	4	4	2	4	7m+1sm	4.80 (± 0.10) - 2.59 (± 0.10)	1.10-1.85	37.00-47.49	0.14	0.25
Taraxacum	*T. linearisquameum*	2n = 2x =16	4	3	-	2	7m+1sm	4.82 (± 0.23) - 2.82 (± 0.15)	1.11-1.68	37.35-47.41	0.15	0.18
Tibetana	*T. eriopodum*	2n = 2x =16	4	8	2	4	8m	4.39 (± 0.10) - 2.18 (± 0.10)	1.03-1.61	38.37-49.33	0.12	0.21
Alpestria	*T.* sp. 1	2n = 3x = 24	6	3	-	3	7m+1sm	3.51 (± 0.14) - 2.37 (± 0.10)	1.10-1.87	34.90-47.54	0.13	0.15
cf. *Biennia*	*T.* sp. 6	2n = 3x = 24	6	3	-	2	7m+1sm	3.26 (± 0.10) - 1.72 (± 0.26)	1.15-1.76	36.21-46.43	0.15	0.20
Erythrosperma	*T. zeisticum*	2n = 3x = 24	6	3	-	1	8m	3.07 (± 0.10) - 1.83 (± 0.18)	1.14-1.60	38.50-46.78	0.14	0.18
Erythrosperma	*T. aspectabile*	2n = 3x = 24	6	3	-	3	8m	2.86 (± 0.24) - 1.80 (± 0.11)	1.16-1.54	39.37-46.25	0.14	0.13
Erythrosperma	*T. bellicum*	2n = 3x = 24	6	3	-	3	8m	2.85 (± 0.17) - 1.68 (± 0.17)	1.10-1.35	42.51-47.58	0.09	0.17
Erythrosperma	*T. cristatum*	2n = 3x = 24	6	3	-	3	6m+2sm	1.85 (± 0.10) - 1.29 (± 0.10)	1.12-1.91	34.36-47.25	0.16	0.13
Erythrosperma	*T. lacistophylloides*	2n = 3x = 24	6	3	-	3	7m+1sm	2.73 (± 0.10) - 1.81 (± 0.15)	1.15-1.69	37.11-46.53	0.17	0.13
Erythrosperma	*T. maricum*	2n = 3x = 24	6	3	-	1	8m	2.77 (± 0.28) - 1.54 (± 0.11)	1.10-1.64	37.86-48.44	0.13	0.21
Erythrosperma	*T. pudicum*	2n = 3x = 24	6	3	-	3	8m	2.96 (± 0.10) - 1.94 (± 0.20)	1.14-1.64	37.89-46.75	0.13	0.14
Erythrosperma	*T. scanicum*	2n = 3x = 24	6	3	-	1	8m	3.16 (± 0.27) - 1.52 (± 0.13)	1.07-1.65	37.77-48.24	0.11	0.21

Table S2. Continued

Section	Species	Chrom. number and ploidy	5S[1]	45S[2]	both[3]	SAT[4]	Karyotype formula[5]	Range of mean values of TL[6] (± SD) [µm]	R[7]	CI[8] [%]	Asymmetry index A[9]	A2[10]
Hamata	*T. boekmanii*	2n = 3x = 24	6	3	1	4	7m+1sm	3.53 (± 0.10) - 1.54 (± 0.16)	1.08-1.75	36.40-48.05	0.14	0.27
Hamata	*T. lamprophyllum*	2n = 3x = 24	6	3	1	2	7m+1sm	3.64 (± 0.10) - 2.04 (± 0.17)	1.07-1.71	36.93-48.30	0.12	0.22
Leucantha	*T. candidatum*	2n = 3x = 24	6	3	-	3	8m	3.54 (± 0.13) - 2.15 (± 0.10)	1.18-1.54	39.34-45.80	0.14	0.15
Naevosa s.l.	*T. gelertii*	2n = 3x = 24	6	3	1	2	8m	3.43 (± 0.12) - 1.92 (± 0.12)	1.14-1.43	41.13-46.68	0.13	0.21
Taraxacum	*T. alatum*	2n = 3x = 24	6	3	-	3	5m+3sm	3.93 (± 0.38) - 2.17 (± 0.11)	1.09-2.50	47.98-28.58	0.18	0.18
Taraxacum	*T. amplum*	2n = 3x = 24	6	3	-	3	6m+2sm	4.86 (± 0.35) - 2.98 (± 0.25)	1.09-2.19	31.39-47.94	0.17	0.16
Taraxacum	*T. copidophyllum*	2n = 3x = 24	6	3	-	3	7m+1sm	3.48 (± 0.16) - 2.50 (± 0.10)	1.13-1.61	32.96-46.89	0.19	0.11
Taraxacum	*T. obtusifrons*	2n = 3x = 24	6	3	-	3	6m+2sm	2.35 (± 0.14) - 1.34 (± 0.16)	1.04-1.71	36.92-49.10	0.15	0.16
Alpina	*T.* sp. 2	2n = 4x = 32	8	5	1	2	4m+3sm+1st	2.87 (± 0.10) - 2.13 (± 0.29)	1.21-3.18	23.95-46.17	0.24	0.14
Crocea	*T.* sp. 3	2n = 4x = 32	8	3	-	3	7m+1sm	2.33 (± 0.14) - 1.59 (± 0.10)	1.14-1.72	36.81-46.75	0.16	0.13
Naevosa s.str.	*T.* sp. 4	2n = 4x = 32	8	4	-	4	5m+3sm	3.84 (± 0.30) - 2.47 (± 0.22)	1.06-2.03	33.04-48.56	0.19	0.17
Naevosa s.str.	*T.* sp. 5	2n = 4x = 32	8	4	1	3	8m	2.95 (± 0.10) - 2.08 (± 0.17)	1.07-1.35	42.60-48.23	0.10	0.17
Suavia	*T.* sp. 7	2n = 4x = 32	8	4	1	2	7m+1sm	4.12 (± 0.19) - 2.58 (± 0.32)	1.05-1.72	36.75-48.77	0.13	0.16
Suavia	*T. tricolor*	2n = 4x = 32	8	4	-	2	6m+2sm	5.26 (± 0.10) - 2.91 (± 0.16)	1.19-2.03	33.02-45.61	0.18	0.22
Taraxacum	*T. fascinans*	2n = 4x = 32	8	4	-	4	7m+1sm	3.30 (± 0.30) - 2.07 (± 0.19)	1.15-2.44	29.10-46.62	0.19	0.15

[1] Total number of 5S rDNA loci in a karyotype
[2] Total number of 45S rDNA loci in a karyotype
[3] Number of chromosomes in a complement with both loci, for 45S and 5S rDNA
[4] Number of satellite chromosomes in a complement
[5] Chromosomes were classified using the nomenclature of Levan (1964) as metacentric (m), submetacentric (sm) and subtelocentric (st) chromosomes
[6] The total chromosome length
[7] Arm ratio
[8] Centromeric index
[9] Intrachomosomal asymmetry index (Watanabe et al., 1999)
[10] Interchromosomal asymmetry index (Zarco 1986)

Table S3. Characterization of the diversity of the investigated ITS locus.

whole region				
Total N° of ribotypes/N° of diff. ribotypes	477/146	**ITS1**	**5.8S**	**ITS2**
N° of sites	646	259	156	230
N° of variable sites	83	36	5	42
N° of parsimony - informative/singleton sites	78/5	34/2	5/0	39/3
nucleotide diversity (π)	0,0196	0,026	0,0057	0,0274
N° of mutations	87	37	6	44
ribotype diversity (Hd)	0,999	0,948	0,714	0,947

Table S4. Pairwise table showing the pattern of ribotype sharing among the investigated sexual and apomictic taxa. ^{S} – sexual taxa; ^{A} – apomictic taxa; BES – *T. bessarabicum*; TIB – *T. eriopodum* (sect. *Tibetana*); CYL – *T. cylleneum*; ERY – *T. erythrospermum*; BOE – *T. boekmanii*; CRO – *T.* sect. *Crocea* [*T.* sp. 3]; MAR – *T. maricum*; NAE – *T.* sect. *Naevosa* [*T.* sp. 5]; ZEI – *T. zeisticum*.

	^{S}TIB	^{S}BES	^{S}CYL	^{S}ERY	^{A}BOE	^{A}NAE	^{A}CRO	^{A}MAR	^{A}ZEI
^{S}TIB	-	0	0	0	0	0	0	0	0
^{S}BES	0	-	0	0	0	0	0	0	0
^{S}CYL	0	0	-	0	0	0	0	0	0
^{S}ERY	0	0	0	-	0	0	0	0	0
^{A}BOE	0	0	0	0	-	5	1	0	0
^{A}NAE	0	0	0	0	5	-	4	1	1
^{A}CRO	0	0	0	0	1	4	-	0	0
^{A}MAR	0	0	0	0	0	1	0	-	3
^{A}ZEI	0	0	0	0	0	1	0	3	-

Table S5. Characterization of the 5.8S and ITS2 region for the presence of proposed conserved motifs. M1—CGATGAAGAACGTAGC; M2—GAATTGCAGAAT ; M3— TTTGAACGCA ; M4—GATATC ; [S] –designate sexual accessions; [A] – designate apomictic accessions; BES – *T. bessarabicum*; TIB – *T. eriopodum* (sect. *Tibetana*); CYL – *T. cylleneum*; ERY – *T. erythrospermum*; BOE – *T. boekmanii*; CRO – *T.* sect. *Crocea* [*T.* sp. 3]; MAR – *T. maricum*; NAE – *T.* sect. *Naevosa* [*T.* sp. 5]; ZEI – *T. zeisticum*. In the case of conserved motifs in the ITS2 region, entries within the parentheses refer to the specific presence of particular motif within II or III helices (for reference, see Material and methods).

accession / motif	presence of conserved motifs in 5.8S region				presence of motifs in ITS2 region		
	M1	**M2**	**M3**	**M4**	**U-U mismatch (II, left)**	**U-U (II, right) mismatch AAA (btw. II, III)**	**UGGU (III, 5´side)**
[S]TIB	conserved	conserved	conserved	conserved	present	present	present
[S]BES	conserved	conserved	conserved	conserved	present	present	present
[S]CYL	conserved	conserved	conserved	conserved	present	present	present
[S]ERY	conserved	conserved	conserved	conserved	present	present	present
[A]BOE	conserved	conserved	conserved	conserved	present	present	present
[A]NAE	conserved	conserved	conserved	conserved	present	present	present

CHAPTER 4

PUBLISHED ABSTRACT

4.1 Comparative cytogenetic analyses in 38 *Taraxacum* species revealed considerable variation among both, sexual and apomictic taxa

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[Poster presentation]

Comparative cytogenetic analyses in 38 *Taraxacum* species revealed considerable variation among both, sexual and apomictic taxa

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Genus *Taraxacum* (dandelion; *Asteraceae*) has several reproduction modes, the vast majority of taxa are either obligate sexuals or apomicts. Typical reproduction mode confined to polyploids is diplosporous apomixis. One of the genes (DIP; diplospory) involved in its regulation is positioned on distal arm of one of the NOR chromosomes in apomictic common dandelions (*Taraxacum officinale* agg.) and in lesser dandelions (*T. erythrospermum* agg.). In this study, we analysed the variability of metaphase chromosomes in karyotypes of sexual and apomictic taxa across the globe. In addition to 45S rDNA probes, we also used 5S rDNA probes and analysed 38 species from major evolutionary clades and geographic regions. We expected stability in a number of 45S and 5S rDNA loci, which was partly confirmed in evolutionary advanced groups: most of such taxa have one locus for 45S rDNA and two loci for 5S rDNA, which multiplies according to ploidy level. However, we have found variability in the position of studied loci even among closely related taxa within the same section. Especially evolutionary ancestral species (such as *T. bessarabicum*) showed a considerable increase in the number of both loci significantly deviating from the expected "simple" karyotype. We also detected variants among homologous chromosomes. The overall genome size (detected by flow-cytometry) revealed 6.1-fold variability in the studied set of species. However, genome size variability did not correlate with a number of 45S / 5S rDNA loci. Now we work on sequencing the ITS regions of selected species in order to better understand the variability of 45S rDNA loci. However, known data revealed considerable karyotype and genome size variation among sexual as well as apomictic dandelion species, indicating very extensive hybridization in the evolutionary past and also recent.

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Comparative cytogenetic analyses in 38 *Taraxacum* species revealed considerable variation among both, sexual and apomictic taxa

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Introduction

***Taraxacum* WIGG.**
(family *Asteraceae*, subfamily *Cichorioideae*)

- a species-rich genus (2000+ taxa in ~ 60 sections) with worldwide very complicated taxonomy
- common perennial grassland herbs with worldwide distribution spanning both hemispheres
- combination of three reproductive strategies: autogamy, allogamy and apomixis (meiotic diplospory)
- genus forms an agamic complex: apomicts mostly triploids (or tetraploids), sexuals predominantly diploids (rarely tetraploids)
- basic chromosome number x = 8
- one of the genes (DIP; diplospory) involved in apomixis regulation positioned on distal arm of one of the NOR chromosomes

Aims of the study

- How large is the genome size of evaluated *Taraxacum* taxa?
- What is the chromosome number and chromosome morphology in selected *Taraxacum* taxa?
- What is the number and position of 45S and 5S rDNA loci in the studied *Taraxacum* genomes?
- Does the number of 45S and 5S rDNA loci correlate with genome size and ploidy level in examined dandelion taxa?
- What can be inferred from our data about evolution of *Taraxacum* taxa?

Plant material

Subgeneric classification	Species	Chromosome number (2n)
T. sect. *Alpestria* Soest	T. sp.	24
T. sect. *Alpina* G. E. Haglund	T. bulgaricum Soest	16
	T. sp.	32
T. sect. *Antarctica* Hand. - Mazz.	T. gilliesii Hook. & Arn.	16
T. sect. *Australasica* Kirschner, Saarlem & Štěpánek	T. cygnorum Hand. - Mazz.	16
T. sect. *Crocea* M. P. Christ.	T. sp.	32
T. sect. *Dioszegia* (Hazsl.) Hazsl.	T. haussknechtii Hausskn.	16
	T. serotinum (Waldst. & Kit.) Poir.	16
T. sect. *Erythrosperma* (H. Lindb.) Dahlst.	T. aspectabile	24
	T. cristatum Kirschner & al.	24
	T. erythrospermum Andrz. ex Besser	16
	T. lacistophylloides Dahlst.	24
	T. marcum Vašut & al.	24
	T. piceicolor M. Schmid, Vašut & Oosterv.	24
	T. pudicum Vašut & Majeský	24
	T. scanicum Dahlst.	24
	T. zeiticum Uhlemann	24
T. sect. *Glacialia* Hand. - Mazz.	T. glaciale Hand. - Mazz.	16
T. sect. *Hamata* H. Øllg.	T. sp.	24
	T. lamprophyllum M. P. Christ.	24
T. sect. *Kashmirana* Soest ex R. Doll.	T. sp.	32
T. sect. *Leucantha* Soest	T. sp.	24
T. sect. *Naevosa* M. P. Christ.	T. gelertii Raunk.	24
	T. sp.	32
	T. sp.	32
T. sect. *Obliqua* Dahlst.	T. pyrropappum Boiss.	16
T. sect. *Oligantha* Soest / Dimmole	T. sp.	32
T. sect. *Parvula* Hand. - Mazz.	T. sp.	24
T. sect. *Piesis* (DC.) Kirschner & Štěpánek	T. atlanticum Pomel	16
	T. bessarabicum (Hornem.) Hand. - Mazz.	16
	T. cylleneum Pörtner	16
T. sect. *Taraxacum* F. H. Wigg.	T. alatum H. Lindb.	24
	T. amplum Markl.	24
	T. copidophyllum Dahlst.	24
	T. lacinians Kirschner, Mikoláš & Štěpánek	32
	T. linearisquameum Soest	16
	T. obtusifrons Markl.	24
T. sect. *Tibetana* Soest	T. eriopodum (D. Don) DC.	16

Methods

FISH (Fluorescence *In Situ* Hybridization)

Flow cytometry

- sample preparation followed the standard protocol using LB01 isolation buffer with polyvinylpyrroline (PVP)
- due to peak overlap several internal standards were used:
  - *Solanum lycopersicum* 'Stupnické polní rané' (2C=1.96pg) as primary standard
  - *Glycine max* 'Polanka' (2C=2.50pg) + *Raphanus sativus* 'Saxa' (2C=1.11pg) as secondary standards
- samples were stained with propidium iodide
- for more details see Macháčková et al. 2018[3]

BD Accuri ™ C6 Flow Cytometer[4]

Results

Sexual *Taraxacum* species

Distribution of 45S (yellow arrowheads) and 5S (white arrowheads) rDNA loci detected by FISH on mitotic metaphase chromosomes (bar=5 µm), ploidy level and nuclear DNA content of selected dandelion taxa:
(A) *T. bulgaricum* (sect. *Alpina*) (B) *T. gilliesii* (sect. *Antarctica*) (C) *T. cygnorum* (sect. *Australasica*) (D) *T. haussknechtii* (sect. *Dioszegia*) (E) *T. serotinum* (sect. *Dioszegia*) (F) *T. erythrospermum* (sect. *Erythrosperma*) (G) *T. glaciale* (sect. *Glacialia*) (H) *T. pyrropappum* (sect. *Obliqua*) (I) *T. atlanticum* (sect. *Piesis*) (J) *T. bessarabicum* (sect. *Piesis*) (K) *T. cylleneum* (sect. *Piesis*) (L) *T. linearisquameum* (sect. *Taraxacum*) (M) *T. eriopodum* (sect. *Tibetana*)

Apomictic *Taraxacum* species

Idiograms of haploid chromosome set at metaphase with locations of 45S and 5S rDNA loci, ploidy level and nuclear DNA content of selected *Taraxacum* apomicts from following sections: (A) *T.* sect. *Alpestria*, (B) *T.* sect. *Alpina*, (C) *T.* sect. *Crocea*, (D-K) *T.* sect. *Erythrosperma*, (L,M) *T.* sect. *Hamata*, (N) *T.* sect. *Kasmirana*, (O) *T.* sect. *Leucantha*, (P-R) *T.* sect. *Naevosa*, (S) *T.* sect. *Oligantha*, (T) *T.* sect. *Parvula*, (U-X) *T.* sect. *Taraxacum*. Bar is 5 µm.

Conclusions and future work

- The overall genome size revealed 6.1-fold variability in studied set of dandelions taxa.
- Genome size variability did not correlate either with a number of 45S or 5S rDNA loci.
- Chromosomes of all studied taxa were relatively small (ranging from 1.19 µm to 5.36 µm) and symmetrical (mostly metacentric or submetacentric).
- Majority of selected *Taraxacum* taxa have one locus for 45S rDNA and two loci for 5S rDNA (in haploid chromosome set), which multiplies according to ploidy level.
- The stability in number of both rDNA loci was partly confirmed in evolutionary advanced groups, whereas evolutionary ancestral taxa (e.g. *T. bessarabicum*) showed a considerable increase in number of both loci.
- FISH analysis also revealed significant variability in the position of both studied loci even among closely related taxa within the same section.
- Our data revealed considerable karyotype and genome size variation among sexual as well as apomictic dandelion species, indicating very extensive hybridization in the evolutionary past and also recent.
- Now we work on sequencing the ITS regions of selected species in order to better understand the variability of 45S rDNA loci.

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[4] Picture was retrieved from: https://bdbiosciences.com/documents/BD_Accuri_C6Flow_Cyto_Instrument_Manual.pdf

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CHAPTER 5

SUMMARY AND CONCLUSIONS

This Ph.D. thesis brought new insights into the structure and variability of sexual and apomictic dandelion genomes using standard and modern karyological methods.

The first part of the thesis was focused on section *Taraxacum* (syn. *T.* sect. *Ruderalia*, *T. officinale* agg.), which is considered to be one of the largest dandelion sections in terms of the number of its members. In this study, twenty-eight apomicts with slightly different eco-geographic preferences were selected to determine their chromosome number and ploidy level using a standard squash technique from their mitotically active root tips. The chromosome numbers of all studied dandelion species absolutely confirmed the previous assumption that apomicts of this section have a strongly prevalent triploid ploidy (2n = 3x = 24), by which they differ from the apomicts of other closely related sections (*Erythrosperma, Palustria* and *Celtica*) with known ploidies of 3x and 4x or even higher. Additionally, karyotypes of all evaluated dandelion species were described as relatively symmetrical with a predominance of small metacentric chromosomes. Similarly to the uniformity of chromosome counts, for twenty-six apomicts of this study were revealed only minor variation of DNA content in their genomes, ranged 1.08-fold from 2C = 2.60 pg in *T. atrox* to 2C = 2.86 pg in *T. perdubium*.

In the second part of the thesis, basic karyotype features along with cytogenetic mapping of 45S and 5S rDNA loci by FISH technique on mitotic metaphase chromosomes were used with the aim to reveal any common karyological features, which could be helpful for the orientation in very complex taxonomy of the genus. A database of haploid idiograms was created for straightforward comparison of a total of thirty-eight karyotypes of sexual and apomictic dandelions belonging to the seventeen different putative evolutionary groups (sections). Similarly to the previous study, most of the investigated karyotypes were relatively symmetrical, with the predominance of small to medium size metacentric chromosomes. While in twenty-four dandelion karyotypes the number of SAT-chromosomes corresponded to ploidy level of evaluated taxa, in the remaining karyotypes, probably due to nucleolar dominance, the number of SAT-chromosomes was increased or decreased. Our study revealed the stable ratio (1:2) in the number of 45S and 5S rDNA loci per haploid level in most of the studied karyotypes and furthermore, the chromosomal positions of both rDNA loci were found separately from each other (S-type arrangement) in all investigated karyotypes. However, the karyotype pattern in chromosomal positions of rDNA loci significantly varied among studied taxa and only low similarity was observed in a few (putatively) closely related

taxa (i.e., members of the same group). In general, the 5S rDNA loci were preferentially localized to the (peri)centromeric regions of the non-satellite chromosomes, whereas the 45S rDNA were predominantly detected in NOR of SAT-chromosomes. In this study, the genome size estimations for thirty-seven dandelion taxa varied 6-fold from 2C = 1.24 pg in diploid *T. cygnorum* (sect. *Australasica*) to 2C = 7.39 pg in tetraploid *T. tricolor* (sect. *Suavia*). On the other hand, estimates of GC content in investigated dataset varied only 1.2-fold. Moreover, the GC content together with the genome size values distinguished the (putatively) evolutionary basal taxa from the derived ones. Finally, the sequence analysis of ITS region in genomes of four sexual and five apomictic dandelion taxa revealed limited variation (with 2-8 variants) in sexuals but conversely high variation (with 20-36 variants) in apomicts.

Although the results of the thesis have revealed a number of new findings in case of the structure and variability of genomes of sexual and apomictic dandelions across different sections, this topic has not yet been completely exhausted and elucidated due to mentioned largeness and complexity of the genus. Conversely, future karyological studies can build on our methodology and results. They can use them as a „starting point“ for further molecular cytogenetic analyses of 45S and 5S rDNA in *Taraxacum* in order to better understand the complex reticulate evolution of this genus.

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Analysis of karyological variability in *Taraxacum*

Summary of the Ph.D. Thesis

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The summary of the Ph.D. thesis was sent for distribution on …………….. .

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The Ph.D. thesis is available in the Library of the Biological Departments of Faculty of Science at Palacký University Olomouc , Šlechtitelů 27, Olomouc.

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1 INTRODUCTION

Taraxacum F. H. Wiggers (dandelions) is a species-rich genus from family Asteraceae (subfamily Cichorioideae). It comprises to date about 2800 described (micro)species of dandelions grouped into approximately 60 sections (putative evolutionary groups; Stevens 2001; Kilian *et al.* 2009; Záveská Drábková *et al.* 2009; Kirschner *et al.* 2015; Mandel *et al.* 2017). It is well-known for its worldwide distribution and taxonomic complexity, which is caused by reticulate evolution including sexual diploid and apomictic polyploid species (Kirschner & Štěpánek 1996; Mártonfiová *et al.* 2007; Majeský *et al.* 2017).

Apomixis (asexual reproduction through seeds) in this genus is a type of meiotic diplospory, known as *Taraxacum* type (Asker & Jerling 1992). It is controlled by three dominant loci (for meiotic diplospory, parthenogenetic embryo development and autonomous development of endosperm), where the locus for meiotic diplospory is located on at least one of the NOR-chromosomes (Ozias-Akins & Van Dijk 2007; Vijverberg & Van Dijk 2007; Vašut *et al.* 2014).

The basic chromosome number, which is common in dandelion genomes across all *Taraxacum* sections, is eight (Mogie & Richards 1983). In general, the individual metaphase chromosomes in dandelion karyotypes are relatively small (c. 1-4 μm; e.g. Singh *et al.* 1974; Kirschner *et al.* 1994; Baeza *et al.* 2013; Gedik *et al.* 2014; Sato *et al.* 2007, 2011, 2012, 2014, 2015, 2019; Gürdal & Özhatay 2018). Dandelion karyotypes are most often highly symmetrical, with a predominance of metacentric chromosomes (e.g. Richards 1972; Kirschner *et al.* 1994; Baeza *et al.* 2013; Sato *et al.* 2007, 2011, 2012, 2014, 2015, 2019; Gürdal & Özhatay 2018) and the total number of SAT-chromosomes in a complement is diverse across various dandelion sections (e.g. Den Nijs *et al.* 1978; Mogie & Richards 1983; Krahulcová 1993; Kirschner *et al.* 1994; Gürdal & Özhatay 2018).

To date, plenty of karyological studies have been published (e.g. recently published Kula *et al.* 2013, Gedik *et al.* 2014, Sato *et al.* 2014, 2015, 2019; Wolanin & Musiał 2017, Gürdal *et al.* 2018, Gürdal & Özhatay

2018) to determinate the total chromosome number in many *Taraxacum* species of various sections. However, due to a large number of known taxa in this genus, the amount of this data is still insufficient. Conversely, other characteristics of karyotypes, such as length and morphology of individual chromosomes, are known to date only for several selected dandelion species (e.g. Sato *et al.* 2007, 2011, 2012, 2014, 2015, 2019; Baeza *et al.* 2013; Gedik *et al.* 2014; Gürdal & Özhatay 2018) and so far no study has been published on the distribution of both the 5S and 45S rDNA on metaphase chromosomes across the genus *Taraxacum*.

2 AIMS OF THE THESIS

The main aim of the presented Ph.D. thesis was to provide new perspectives on the structure and variability of sexual and apomictic dandelion genomes across various *Taraxacum* sections using standard karyological method or modern molecular cytogenetic technique and to put these data to broader context of cytogenetic and molecular analyses.

At the centre of interest were the following specific aims:

- To establish the chromosome number, ploidy level and to estimate the genome size for 28 selected species of *Taraxacum* sect. *Taraxacum.*
- To compare the karyotypes with fluorescently labelled 45S and 5S rDNA loci between sexual and apomictic dandelions within one or more evaluated *Taraxacum* sections.
  - To determine the chromosome number, the karyotype formula, the number and localization of 45S and 5S rDNA loci on mitotic metaphase chromosomes and to create haploid idiogram for each evaluated *Taraxacum* taxa.
  - To detect whether the number of satellite chromosomes corresponds to the ploidy level and the loci for 45S rDNA on metaphase chromosomes are distributed only in the NOR in each evaluated dandelion karyotype.
  - To reveal whether a pattern of the distribution of 45S and 5S rDNA loci on metaphase chromosomes is associated with the reproduction strategy of studied *Taraxacum* taxa (i.e. allogamy, autogamy and apomixis).
  - To test whether there is a correlation between the number of 45S and 5S rDNA loci and the estimations of genome size or GC base content in evaluated *Taraxacum* taxa.
  - To bring new insights into the complex evolution in *Taraxacum* genomes by studying their dynamic changes in rDNA.

3 MATERIAL AND METHODS

This section contains only a detailed description of the methods that have been performed by myself, other cytogenetic and molecular methods are delineated in individual publications.

3.1 Plant material

In the first publication, we studied a total of 28 *Taraxacum* species belonging to *Taraxacum* sect. *Taraxacum*. Plants and achenes of the investigated species were collected in natural habitats of several localities of Central Europe (AT, CZ, DE, HU, IT and SK; country codes according to ISO 3166-1 alpha-2). The studied plant material was documented by herbarium specimens and is deposited in the herbarium of the Department of Botany, Palacký University in Olomouc, Czech Republic (OL). All studied species were apomictic (agamospermous); thus, maternal plants and offspring plants (grown from seeds) were taxonomically (genetically) identical. For karyological analyses, achenes were sown in Petri dishes containing 1% agar solution and germinated at room temperature. Fresh young leaves for flow cytometric analyses were collected from juvenile plants cultivated in a greenhouse at the Department of Botany, Faculty of Science, Palacký University in Olomouc.

In the second paper, we studied 38 *Taraxacum* taxa belonging to 17 different sections (putative evolutionary groups). The taxon selection was made in order to completely cover natural distribution range of the genus (Europe, Asia, Africa, Australia, and America), and putative contrasting evolutionary age (ancestral, precursor and derived; see Kirschner *et al.*, 2015). Investigated sample-set includes 13 diploid sexual taxa (native to five continents) and 25 polyploids (triploid and tetraploid) apomictic taxa (native to Europe and Asia). Besides the investigation of rDNA loci among different evolutionary lineages (represented by different sections), the sample-set was “fine-tuned” to allow also the investigation of variation within the sections. This was achieved by including 8+1 and 5+1 taxa from the two of the most common and widespread dandelion groups

in Europe: i.e., *T.* sect. *Erythrosperma* (lesser dandelions, clade II according to Kirschner *et al.*, 2015) and *T.* sect. *Taraxacum* (*T. officinale* group, clade VI according to Kirschner *et al.*, 2015).

3.2 Methods

3.2.1 Chromosome spread preparation and chromosome counting

For chromosome counts, we used mitotically active root tip meristems of dandelion seedlings. Seedlings of the investigated species with 1–2 cm long roots were collected in the morning. To obtain the desired metaphase index, the roots were pre-treated in a 2 mM solution of 8-hydroxyquinoline for two hours at room temperature and an additional two hours at 4 °C in the dark. Then, the material was fixed in Carnoy's fixative (a mixture (3:1, v/v) of absolute ethanol and acetic acid) and stored in a refrigerator (4 °C) until further processing (Hasterok & Małuszyńska 2000). For slide preparation, a combination of protocols in Hasterok & Małuszyńska (2000) and Van Baarlen *et al.* (2000) was used with the following changes for the investigated species of dandelions. Fixed root tips were washed in citrate buffer (0.01 M, pH 4.8) for 5 min and then enzymatically digested in a mixture of 0.1% cellulose, 0.1% pectolyase and 0.1% cytohelicase in the citrate buffer for 90 min at 37–40 °C. To remove trace amounts of the enzymatic mixture, the root tips were then gently washed in citrate buffer for 5 min. Only the mitotically active meristematic tissue of a root tip was cut off under a stereoscopic microscope, transferred into a drop of 50% acetic acid on a slide and covered by a coverslip. After heating the preparation to 42 °C for 1–2 min, cells were spread between a glass slide and coverslip in a drop of 50% acetic acid. The coverslip was mechanically removed by a razor blade after deep freezing in liquid nitrogen, and the slide was air dried. To increase the contrast of metaphase chromosomes for counting, the preparations were stained with DAPI in Vectashield media.

For each dandelion species, at least ten metaphases were analysed to determine the chromosome number. Well-spread metaphase images were captured using Olympus BX 60 and Axio Imager Z.2 Zeiss fluorescence

microscopes, both equipped with a CCD camera and ISIS software (Metasystems, Altlussheim, Germany).

3.2.2 Preparation and labelling of probes for FISH

Genomic DNA of *Taraxacum linearisquameum* van Soest (i.e., diploid sexual taxon of *T.* sect. *Taraxacum*, or *T. officinale* agg.) was isolated from lyophilized leaves using NucleoSpin Plant II kit following the manufacturer's recommendations and further used as template DNA for preparation of FISH probes. The 5S and 45S rDNA probes were amplified using the following specific primers:

- 5S-F: 5´-AAACGGCTACCACATCCAAG-3´
- 5S-R: 5´-CGAAGGCCAACGTAATAGGA-3´
- 18S-F: 5′-GATCCCATCAGAACTCCGAAG-3´
- 18S-R: 5´-CGGTGCTTTAGTGCTGGTATG-3´.

The PCR mix contained 25 ng of genomic DNA, 0.2 mM digoxigenin-11-dUTP or biotin-16-dUTP, 1 µM of specific primers, 1X PCR buffer with 1.5 mM MgCl2 and 2U of *Taq* DNA polymerase in a final volume of 25 µl. The amplification was performed with the following conditions: 94 °C for 5 min (1 cycle), 94 °C for 50 s, 55 °C for 50 s and 72 °C for 1 min (35 cycles) and 72 °C for 5 min (1 cycle). Finally, the quality of both probes was resolved in 1.2 % agarose gels.

3.2.3 Fluorescence in situ hybridization (FISH)

The air-dried slides of chromosome preparations were counterstained with DAPI in Vectashield media and checked using a fluorescent microscope to FISH analysis. The preparations containing the best chromosomal spreads were selected for FISH, destained from DAPI and post-fixed by following conditions at room temperature: washed in 2 x SSC (2 x 5min), 4% (v/v) formaldehyde solution (1 x 10 min), 2 x SSC (3x 4 min), dehydrated in an increasing ethanol series (70, 90 and 96% ethanol, 2 min each) and air-dried.

Hybridization mixture consisting of 50% (v/v) formamide, 10% (v/v) dextran sulfate, 2 × SSC and 200-400 ng of each labelled probe was

applied onto slides, covered with a glass coverslip and denatured at 80 °C for 3 min. The hybridization was carried out at 37 °C overnight in a humidified chamber. The digoxigenin-labelled (5S rDNA) and biotin-labelled (45S rDNA) probes were detected using anti-digoxigenin-FITC or anti-streptavidin-Cy3, respectively. The antibodies were applied in concentrations recommended by manufacturers. Finally, chromosomes were counterstained with DAPI in Vectashield media (15 μl / slide) and covered with a coverslip. Metaphase spreads were acquired with Olympus BX 60 and Axio Imager Z.2 Zeiss fluorescence microscopes, both equipped with Cooled Cube 1 camera (Metasystems, Altlussheim, Germany), appropriate optical filters and ISIS software (Metasystems).

3.2.4 Karyotype analysis

At least five mitotic metaphase spreads per each investigated taxa (obtained at 100x magnification) were analysed to obtain a dataset of the following chromosome features: the length of short (S) and long (L) chromosome arm, the total chromosome length (TL), centromeric index (CI), arm ratio (r) and the distance of FISH signals from the centromere. The individual chromosomes were classified according to centromeric index as metacentric (m: 50% ≥ CI > 37.5%), submetacentric (sm: 37.5% ≥ CI > 25%) and subtelocentric (st: 25% ≥ CI > 12.5%) chromosomes, followed the nomenclature of Levan *et al.* (1964). The degree of karyotype asymmetry for each dandelion species was estimated using the formulas for the interchromosomal asymmetry index A2 (Figure 1; Zarco 1986) describing heterogeneity among chromosome sizes in a complement and coefficient of intrachromosomal asymmetry A (Figure 2; Watanabe *et al.* 1999), quantifying the relative differences in the centromere position among chromosomes of a complement.

$$\boldsymbol{A_2} = \frac{s}{\bar{x}}$$

Figure 1. The formula for calculating the interchromosomal asymmetry index A2 is calculated as the ratio of sample standard deviation to the total average of the chromosome lengths in the complement (adapted from Zarco 1986).

$$\mathbf{A} = \frac{\sum_{i=1}^{n} (B_i - b_i / B_i + b_i)}{n}$$

Figure 2. The formula for calculating the intrachromosomal asymmetry index A. This index results from the sum of the ratio between the differences in the long arm length (B_i) and the short arm length (b_i) of each chromosome and the sum of the lengths of the long and short arms of each chromosome ($B_i + b_i$). The sum is divided by the haploid chromosome number (n; Watanabe *et al.* 1999; Medeiros-Neto *et al.* 2017).

3.2.5 Construction of idiograms

For the preparation of haploid idiogram per each investigated taxon, morphological features of individual chromosomes, and positions of 5S and 45S rDNA sites were measured using ISIS software (Metasystems). Chromosomes of individual metaphase were first divided into a total of 8 groups based on the total length of chromosomes, their similar arm ratio and FISH signals distribution. Subsequently, these eight chromosome groups were arranged in descending order based on the mean values of total chromosome lengths and identified either only by numbers or moreover by small letters. Numbers ranging from 1 to 8 indicated the order of individual chromosome groups, while letters represented individual variants in the distribution of the 5S and 45S rDNA loci on morphologically similar chromosomes throughout the karyotype. The resulting haploid idiogram for each taxon was prepared in DRAWID software version 0.26 (Kirov *et al.*, 2017) and Adobe Photoshop CC software based on average values determined from five measurements on different metaphase spreads.

4 PUBLICATIONS INCLUDED IN THE THESIS

4.1 New chromosome counts and genome size estimates for 28 species of *Taraxacum* sect. *Taraxacum*

Macháčková P, Majeský Ľ, Hroneš M, Hřibová E, Trávníček B, Vašut RJ

Comparative Cytogenetics, 2018, 12(3): 403–420
doi: 10.3897/CompCytogen.v12i3.27307

Abstract

The species-rich and widespread genus *Taraxacum* F. H. Wiggers, 1780 (Asteraceae subfamily Cichorioideae) is one of the most taxonomically complex plant genera in the world, mainly due to its combination of different sexual and asexual reproduction strategies. Polyploidy is usually confined to apomictic microspecies, varying from 3x to 6x (rarely 10x). In this study, we focused on *Taraxacum* sect. *Taraxacum* (= *T.* sect. *Ruderalia*; *T. officinale* group), i.e., the largest group within the genus. We counted chromosome numbers and measured the DNA content for species sampled in Central Europe, mainly in Czechia. The chromosome number of the 28 species (*T. aberrans* Hagendijk, Soest & Zevenbergen, 1974*, T. atroviride* Štěpánek & Trávníček, 2008*, T. atrox* Kirschner & Štěpánek, 1997*, T. baeckiiforme* Sahlin, 1971*, T. chrysophaenum* Railonsala, 1957*, T. coartatum* G.E. Haglund, 1942*, T. corynodes* G.E. Haglund, 1943*, T. crassum* H. Øllgaard & Trávníček, 2003*, T. deltoidifrons* H. Øllgaard, 2003*, T. diastematicum* Marklund, 1940*, T. gesticulans* H. Øllgaard, 1978*, T. glossodon* Sonck & H. Øllgaard, 1999, *T. guttigestans* H. Øllgaard in Kirschner & Štěpánek, 1992*, T. huelphersianum* G.E. Haglund, 1935, *T. ingens* Palmgren, 1910, *T. jugiferum* H. Øllgaard, 2003*, T. laticordatum* Marklund, 1938*, T. lojoense* H. Lindberg, 1944 (= *T. debrayi* Hagendijk, Soest & Zevenbergen, 1972, *T. lippertianum* Sahlin, 1979), *T. lucidifrons* Trávníček, ineditus*, T. obtusifrons* Marklund, 1938*, T. ochrochlorum* G.E. Haglund, 1942*, T. ohlsenii* G.E.

Haglund, 1936*, T. perdubium* Trávníček, ineditus*, T. praestabile* Railonsala, 1962*, T. sepulcrilobum* Trávníček, ineditus*, T. sertatum* Kirschner, H. Øllgaard & Štěpánek, 1997*, T. subhuelphersianum* M.P. Christiansen, 1971, *T. valens* Marklund, 1938) is 2n = 3x = 24. The DNA content ranged from 2C = 2.60 pg (*T. atrox*) to 2C = 2.86 pg (*T. perdubium*), with an average value of 2C = 2.72 pg. Chromosome numbers are reported for the first time for 26 species (all but *T. diastematicum* and *T. obtusifrons*), and genome size estimates for 26 species are now published for the first time.

Keywords: Asteraceae, chromosome number, flow cytometry, karyology, *Taraxacum officinale*

4.2 New insights into rDNA variation in apomictic and sexual *Taraxacum* (Asteraceae)

Macháčková P, Majeský Ľ, Hroneš M, Bílková L, Hřibová E,

Vašut RJ

submitted to Annals of Botany

Abstract

Background and Aims Apomictic genera have a complex evolutionary history, mostly including reticulate hybridization, and variation in reproduction modes. In sexually reproducing taxa, evolutionary events leading to their origin may be quickly masked through the homogenization of tandemly organized arrays of nrDNA. In contrast, restriction from the functional meiosis considerably hampers the gene conversion in polyploid apomictic taxa, leaving footprints of past hybridization events in their genomes. Rapid chromosomal rearrangements and transposable elements (TEs) play an important role in shaping the genome diversity. *Taraxacum*, a genus with apomictic taxa, has still hazy and considerably complex reticulate evolutionary history; earlier investigation of rDNA sequences proved the complex pattern of the evolution, but without direct evidence for particular processes.

Methods The distribution and number of rDNA loci were investigated in 38 *Taraxacum* taxa (covering different reproduction modes, geographic regions, and putative phylogenetic groups) using FISH. Additionally, genome size and GC content were measured and tested for correlation with karyotype patterns. The ITS1-5.8S-ITS2 region of four sexual and five apomictic taxa was sequenced to investigate inter- and intra-individual variation.

Key Results Most of the studied species considerably differ in chromosome position of loci and karyotype patterns, but conserved in the number of 45S and 5S rDNA loci with 1:2 ratio per haploid genome. Six species differ

in the number of rDNA loci, the most deviating *T. eriopodum* has 4:2 ratio of rDNA loci per haploid genome. Genome size (2C) varies 6-fold and together with GC-content, partly distinguishes the evolutionary basal taxa from the derived ones. Sexual taxa showed limited variation in the sequenced region (with 2-8 variants), but apomictic taxa varied significantly (with 20-36 variants).

Conclusions Extensive reticulate evolution in *Taraxacum* and consequent phenomena like genome repatterning, non-effective concerted evolution, are likely causing dynamic nature of *Taraxacum* karyotypes, as well as the large variation in genome size and rDNA sequences.

Keywords: apomixis, Asteraceae, concerted evolution, FISH, GC content, genome size, hybridization, karyotype, metaphase chromosomes, rDNA, *Taraxacum.*

5 SURVEY OF RESULTS AND CONCLUSIONS

This Ph.D. thesis brought new insights into the structure and variability of sexual and apomictic dandelion genomes using standard and modern karyological methods.

The first part of the thesis was focused on section *Taraxacum* (syn. *T.* sect. *Ruderalia*, *T. officinale* agg.), which is considered to be one of the largest dandelion sections in terms of the number of its members. In this study, twenty-eight apomicts with slightly different eco-geographic preferences were selected to determine their chromosome number and ploidy level using a standard squash technique from their mitotically active root tips. The chromosome numbers of all studied dandelion species absolutely confirmed the previous assumption that apomicts of this section have a strongly prevalent triploid ploidy (2n = 3x = 24), by which they differ from the apomicts of other closely related sections (*Erythrosperma, Palustria* and *Celtica*) with known ploidies of 3x and 4x or even higher. Additionally, karyotypes of all evaluated dandelion species were described as relatively symmetrical with a predominance of small metacentric chromosomes. Similarly to the uniformity of chromosome counts, for twenty-six apomicts of this study were revealed only minor variation of DNA content in their genomes, ranged 1.08-fold from 2C = 2.60 pg in *T. atrox* to 2C = 2.86 pg in *T. perdubium*.

In the second part of the thesis, basic karyotype features along with cytogenetic mapping of 45S and 5S rDNA loci by FISH technique on mitotic metaphase chromosomes were used with the aim to reveal any common karyological features, which could be helpful for the orientation in very complex taxonomy of the genus. A database of haploid idiograms was created for straightforward comparison of a total of thirty-eight karyotypes of sexual and apomictic dandelions belonging to the seventeen different putative evolutionary groups (sections). Similarly to the previous study, most of the investigated karyotypes were relatively symmetrical, with the predominance of small to medium size metacentric chromosomes. While in twenty-four dandelion karyotypes the number of SAT-chromosomes

corresponded to ploidy level of evaluated taxa, in the remaining karyotypes, probably due to nucleolar dominance, the number of SAT-chromosomes was increased or decreased. Our study revealed the stable ratio (1:2) in the number of 45S and 5S rDNA loci per haploid level in most of the studied karyotypes and furthermore, the chromosomal positions of both rDNA loci were found separately from each other (S-type arrangement) in all investigated karyotypes. However, the karyotype pattern in chromosomal positions of rDNA loci significantly varied among studied taxa and only low similarity was observed in a few (putatively) closely related taxa (i.e., members of the same group). In general, the 5S rDNA loci were preferentially localized to the (peri)centromeric regions of the non-satellite chromosomes, whereas the 45S rDNA were predominantly detected in NOR of SAT-chromosomes. In this study, the genome size estimations for thirty-seven dandelion taxa varied 6-fold from 2C = 1.24 pg in diploid *T. cygnorum* (sect. *Australasica*) to 2C = 7.39 pg in tetraploid *T. tricolor* (sect. *Suavia*). On the other hand, estimates of GC content in investigated dataset varied only 1.2-fold. Moreover, the GC content together with the genome size values distinguished the (putatively) evolutionary basal taxa from the derived ones. Finally, the sequence analysis of ITS region in genomes of four sexual and five apomictic dandelion taxa revealed limited variation (with 2-8 variants) in sexuals but conversely high variation (with 20-36 variants) in apomicts.

Although the results of the thesis have revealed a number of new findings in case of the structure and variability of genomes of sexual and apomictic dandelions across different sections, this topic has not yet been completely exhausted and elucidated due to mentioned largeness and complexity of the genus. Conversely, future karyological studies can build on our methodology and results. They can use them as a „starting point“ for further molecular cytogenetic analyses of 45S and 5S rDNA in *Taraxacum* in order to better understand the complex reticulate evolution of this genus.

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7 LIST OF AUTHOR'S PUBLICATIONS

7.1 Publications related to the topic of Ph.D. thesis

Macháčková P, Majeský Ľ, Hroneš M, Hřibová E, Trávníček B, Vašut RJ (2018) New chromosome counts and genome size estimates for 28 species of *Taraxacum* sect. *Taraxacum*. Comparative Cytogenetics 12(3): 403-420. https://doi.org/10.3897/CompCytogen.v12i3.27307.

Macháčková P, Majeský Ľ, Hroneš M, Bílková L, Hřibová E, Vašut RJ (subm.) New insights into rDNA variation in apomictic and sexual *Taraxacum* (Asteraceae). Annals of Botany [submitted].

7.2 Other popularizing publications

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8 SOUHRN (SUMMARY, IN CZECH)

Název práce: Studium karyologické variability v rodu *Taraxacum*

Autor: Mgr. Petra Macháčková

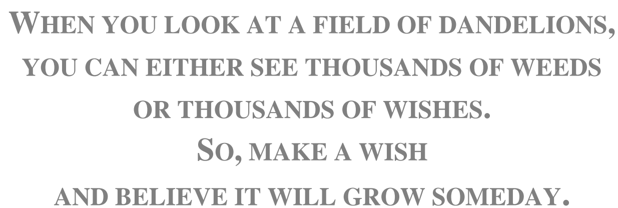
Předložená disertační práce se věnuje studiu popisu struktury a variability vybraných karyotypů sexuálních a apomiktických pampelišek reprezentující sedmnáct sekcí s různým odhadem evolučního stáří.

První část práce je zaměřena na stanovení počtu chromozomů, ploidní úrovně a velikosti genomu pro vybrané zástupce apomiktických pampelišek ze sekce *Taraxacum* (syn. sekce *Ruderalia*). Cílem této studie bylo odhalit počet chromozomů v jádrech dvaceti osmi druhů pampelišek klasickou metodou roztlakových preparátů z kořenových špiček a zjistit míru variability ve velikostech genomů u dvaceti šesti druhů pampelišek metodou průtokové cytometrie. Výsledky studie potvrdily stejný počet chromozomů a ploidní úroveň (2n = 3x = 24) u všech dvaceti osmi druhů a u zhodnocených genomů ohalily pouze malou variabilitu v obsahu DNA.

Druhá část této práce se primárně věnuje cytogenetickému mapování lokusů pro 45S a 5S rDNA na metafázních mitotických chromozomech za pomocí techniky FISH, a to v karyotypech třiceti osmi druhů sexuálních i apomiktických pampelišek, zastupujících sedmnáct sekcí. Cílem této studie bylo kromě popisu základních vlastností jednotlivých karyotypů sexuálních a apomiktických pampelišek odhalit případné společné paterny v distribuci zmíněných lokusů rDNA na mitotických metafázních chromozomech, které by mohly být nápomocné při orientaci ve velmi složité taxonomii rodu. Naše výsledky odhalily ve většině studovaných karyotypů stejný poměr (1:2) v počtu lokusů pro 45S a 5S rDNA na haploidní sadu chromozomů. Na druhou stranu však byla zjištěna značná variabilita v pozicích obou lokusů rDNA na mitotických metafázních chromozomech, a to jak při porovnání karyotypů pampelišek zastupujících blízce příbuzné sekce, stejnou sekci, tak dokonce i vrámci jednoho karyotypu. Relativně vysoká variabilita získaných výsledků nejen z karyologické studie, ale i z doplňkových metod (určení velikosti genomu, obsahu GC bází

a sekvenování ITS oblastí) je tedy pravděpodobně důsledkem složité retikulární evoluce tohoto rodu, zahrnující častou hybridizaci a polyploidizaci.

Ačkoli výsledky disertační práce přinesly řadu nových poznatků o variabilitě a struktuře karyotypů sexuálních i apomiktických pampelišek napříč různými sekcemi, přesto tato problematika i vzhledem ke zmíněné rozsáhlosti a komplikovanosti rodu nebyla zdaleka vyčerpána. Naopak použitá metodika a získané výsledky o variabilitě karyotypů vybraných druhů pampelišek mohou v budoucnu posloužit jako „stavební kámen“ pro další molekulárně cytogenetické analýzy tohoto rodu a objasnit tak další doposud nezodpovězené otázky ohledně složité evoluce tohoto rod



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