Impact of dysploidy and polyploidy on the diversification of high mountain *Artemisia* (Asteraceae) and allies

Gemma Mas de Xaxars[1], Teresa Garnatje[2], Jaume Pellicer[3], Sonja Siljak-Yakovlev[4], Joan Vallès[1], Sònia Garcia[1]*

[1] Laboratori de Botànica – Unitat associada CSIC, Facultat de Farmàcia, Universitat de Barcelona, Avinguda Joan XXIII s/n, 08028 Barcelona, Catalonia, Spain.

[2] Institut Botànic de Barcelona (IBB-CSIC-ICUB), Passeig del Migdia s/n, Parc de Montjuïc, 08038 Barcelona, Catalonia, Spain.

[3] Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK.

[4] Laboratoire d'Evolution et Systématique, Université Paris Sud, UMR8079 CNRS-UPS-AgroParis-Tech, 91405 Orsay Cedex, France.

*Corresponding author: soniagarcia@ub.edu

Abstract

Molecular cytogenetics and the study of genome size have been used to understand evolutionary and systematic relationships in many species. However, this approach has seldom been applied to alpine plants. A group of dysploid-polyploid high mountain *Artemisia* species, distributed from the European Sierra Nevada to Central Asian mountains, through the Pyrenees, the Alps and the Caucasus, is a good model to consider changes at the genome and chromosome levels. These small perennial *Artemisia*, found frequently in isolated populations, present highly disjunct distributions. Some are considered rare or even endangered. Here we show results for nine species and 31 populations, including genome size (2C-values), fluorochrome banding and fluorescent *in situ* hybridisation (FISH) of ribosomal RNA genes (rDNA). Significant intraspecific genome size variation is found in certain populations of *A. eriantha* and *A. umbelliformis*, but without taxonomic significance due to the absence of morphological or ecological differentiation. The number and position of GC-rich DNA bands is mostly coincidental with rDNA although there is an expansion of GC-rich heterochromatin in centromeres in some taxa. Ancestral character states have been reconstructed and *x*=9 is inferred as the likely ancestral base number, while the dysploid *x*=8 has appeared repeatedly during the evolution of *Artemisia*. On the basis of cytological observations, Robertsonian translocations are proposed for the appearance of dysploidy in the genus. A remarkable presence of *x*=8-based species has been detected in the clade including high mountain species, which highlights the important role of dysploidy in the diversification of high mountain *Artemisia*. Conversely, polyploidy, though present in the alpine species, is more common in the rest of the genus, particularly in arctic species. Hypotheses on the mechanisms underpinning the relative abundance of dysploids and scarcity of polyploids in high mountain *Artemisia* are discussed.

Key words: alpine plants, base chromosome number, chromomycin A_3, fluorescent *in situ* hybridisation, genome size, molecular cytogenetics, ribosomal RNA genes, Robertsonian translocation

Introduction

High mountain communities are systems that endure harsh conditions, including extreme cold and fluctuating temperatures, exposure to snow, ice and strong winds, poor and rocky soils, high levels of UV radiation and little oxygen availability. Moreover, elevation gradients allow large environmental changes in short spatial distances (Nieto Feliner 2011). All these factors promote local adaptations which, coupled with processes such as long term isolation and secondary contacts, may lead to an increased biodiversity at high altitudes (Nagy and Grabherr 2009). Distribution areas of cold-adapted species were fragmented and restricted to higher elevations during interglacial periods, which created the current pattern of vicariant taxa between mountain tops in the Mediterranean Basin (Nieto Feliner 2014). In this regard, multiple glacial refugia have been identified in mountain ranges across Europe and North Africa, both in plants (Španiel et al. 2011) and animals (Canestrelli and Nascetti 2008).

Polyploidy and genome size studies in high mountain plants: state of the art

Several studies have hypothesised that high mountain plants undergo polyploidy more frequently (Löve and Löve 1949; Petit and Thompson 1999). It is thought that the ranges for polyploid species would include more extreme environments, such as high altitudes and latitudes, than their diploid relatives (Levin 2002; Weiss-Schneeweiss et al. 2013). However, the pattern stating the prevalence of polyploid taxa in alpine environments is still controversial. Besides, dysploidy (i.e. change in basic chromosome number, usually by fusion or fission, without a significant gain or loss of genetic material) has also contributed to the diversification of several alpine groups such as *Phyteuma* (Schneeweiss et al. 2013), *Saxifraga* (Mas de Xaxars et al. 2015) or *Leontopodium* (Russell et al. 2013) and, together with polyploidy, it is another important mechanism of karyotype evolution.

The C-value (i.e. genome size or the total amount of DNA in the unreplicated haploid or gametic nucleus) has been used in many taxonomic, systematic and evolutionary studies in a range of plant groups. Although genome size has been frequently correlated with environmental, phenotypic or ecological variables (see a review in Greilhuber and Leitch 2013), it has rarely been investigated in the context of high mountain communities. Albach and Greilhuber (2004) found significantly higher DNA amounts in alpine species from *Veronica*, while Mráz et al. (2009) found negative correlations with altitude and latitude in alpine populations of *Hieracium alpinum* L. Loureiro et al. (2013) explored genome size variation, beyond polyploidy incidence, in plants from two Spanish mountain ranges, finding no significant genome size differences or correlates. However, they stated that large genomes were difficult to find in high mountain environments.

Artemisia L. *and its high mountain species*

Artemisia comprises about 500 species with a circumpolar and northern hemisphere distribution, with only a few species in South America and Africa (Tkach et al. 2008). The fossil pollen record places the onset of differentiation of *Artemisia* back to the Early Miocene, about 19.8 Ma (Sanz et al. 2011). The main speciation and diversification centre of the genus is Central Asia, with secondary centres located in the Irano-Turanian and Mediterranean regions and in western North America (Vallès et al. 2011). *Artemisia* species are mostly perennial and particularly diverse in mountains, grasslands and semidesertic areas (Ling et al. 2006). The traditional classification of the genus, which relies on capitulum characters, is partially incongruent to lineages identified in molecular studies (Sanz et al. 2008). Nevertheless, the classical subgenera [*Artemisia*, *Absinthium* (Mill.) Less., *Dracunculus* (Besser) Rydb., *Seriphidium* Besser ex Less., and *Tridentatae* (Rydb.) McArthur] are still widely used for the circumscription of the genus.

Within *Artemisia* two main basic chromosome numbers are recognized, *x*=9 and *x*=8, the former being the most common. Abundant karyological and cytogenetic information is available for the genus (for a review see Vallès et al. 2011). Polyploidy is common in the genus, ploidy levels have been found up to 16*x* and many species exist in polyploid series. Genome size variation has also been widely explored in *Artemisia*, which has become one of the best known genera in the family from this point of view.

A suitable model for studying the implications of genome size variation, dysploidy and polyploidy in the diversity and evolution of high mountain plants is found in a group of alpine *Artemisia*. This group is distributed from Sierra Nevada (Iberian Peninsula) to the Central Asian Mountains, through the Pyrenees, the Alps and the Caucasus. Some of the species associated with high elevations are currently considered as rare or endangered, such as *Artemisia granatensis*, endemic to Sierra Nevada. These small herbaceous perennials are usually found in isolated populations growing on siliceous rocky ledges or limestone substrates, often in crevices, at elevations between 1500 and 3400 m above sea level. Some taxa, such as *A. eriantha* and *A. umbelliformis*, show a highly disjunct distribution, reflecting the geographical complexity of the Pyrenees and the Alps (Sanz et al. 2014).

In the present study we aim to explore genome size variation, ploidy and base chromosome number changes, and heterochromatin and rDNA loci evolution in alpine *Artemisia* and considering a phylogenetic context. Given the wealth of information currently available on the genus, we have the opportunity to address genome dynamics and evolutionary factors in this group of alpine plants. Our findings will contribute further evidence to shape the genomic/cytogenetic characteristics of alpine species. To fulfil these goals, two

main questions have been specifically addressed: (1) is there any significant difference in basic chromosome number, genome size, heterochromatic or rDNA distribution patterns in the high mountain *Artemisia* with respect to their lower altitude counterparts?, and (2) are polyploidy and/or dysploidy more frequent in this group than in other non-high mountain *Artemisia,* and what is their biological significance? Additionally, and taking advantage of all available phylogenetic, cytogenetic and genome size information, we will infer the ancestral state of the studied features in this group and in the genus, and we will discuss the prevalence of ancestral states in the present-day high mountain *Artemisia*.

Materials and methods

Plant material. As part of an ongoing project focusing on the biogeography of two of the most abundant alpine *Artemisia* species, *A. eriantha* and *A. umbelliformis* (Sanz et al. 2014, Sanz et al. unpublished), samples of these as well as closely related species were collected to perform cytogenetic and genome size assays. All specimens were gathered from natural sites except one population of *A. eriantha* obtained via Index Seminum from the Alpine Botanical Garden of Lautaret (Hautes-Alpes, France), which had been formerly collected near the Col du Lautaret at 2300 m above sea level. Achenes were germinated in pots and cultivated in the greenhouse at the Faculty of Pharmacy, Universitat de Barcelona (UB). Vouchers have been deposited in the herbarium BCN, of the Centre de Documentació de Biodiversitat Vegetal, UB. Table 1 shows the populations studied. Figure 1 indicates the geographical locations of the populations assessed across the high mountain ranges. Seeds of *Pisum sativum* 'Express Long' and *Petunia hybrida* 'PxPc6' used as internal standard for flow cytometry measurements, were obtained from the Institut des Sciences du Végétal (CNRS, Gif-sur-Yvette, France). Root tip meristems were obtained from seedlings produced by germinating achenes on wet filter paper in Petri dishes in the dark at room temperature.

DNA content assessments. DNA 2C-values of the selected species were estimated using flow cytometry. As outlined above, *P. sativum* and *P. hybrida* (2C = 8.37 and 2.85 pg, respectively; Marie and Brown 1993) were used as internal standards. We have analysed 29 populations of 8 species, with special emphasis on *A. eriantha* (12 populations) and *A. umbelliformis* (10 populations), because these are two of the most widely distributed species of this group. Young healthy leaf tissues from the species to be studied and the calibration standard were placed together in a plastic Petri dish and chopped in LB01 buffer (Doležel et al. 1989) with a razor blade. The suspension of nuclei was filtered through a nylon mesh with a pore size of 70

153 µm and stained for 20 min with propidium iodide (60 µg/mL; Sigma-Aldrich, Madrid, Spain)
154 and supplemented with 100 µg/mL ribonuclease A (Boehringer, Meylan, France). More details
155 on the methodology can be found in Garcia et al. (2006). Mean values and standard deviations
156 were calculated based on the results for five individuals per population. Results on genome
157 size have been submitted to the GSAD database (www.asteraceaegenomesize.com).
158
159 ***Chromosome preparations.*** Root tips were pre-treated with 0.05% aqueous colchicine, at
160 room temperature, for 2.5-4 hours. The material was fixed in absolute ethanol and glacial
161 acetic acid (3:1) and then stored at 4°C. Protoplast preparations for fluorochrome banding and
162 fluorescent *in situ* hybridisation (FISH) were carried out using the air-drying technique of Geber
163 and Schweizer (1987), with some modifications as explained in Garcia et al. (2007).
164
165 ***Fluorochrome banding and fluorescent* in situ *hybridisation.*** In order to reveal GC-rich DNA
166 bands, chromomycin A_3 was used, following the protocol in Garcia et al. (2007). DNA
167 hybridisation was carried out also following Garcia et al. (2007) with minor changes: the 18S-
168 5.8S-26S rDNA probe was obtained from the clone pTa71 (Gerlach and Bedbrook 1979) and
169 was labelled with direct Cy3 -red- (GE Healthcare, Buckinghamshire, UK); the 5S rDNA probe
170 was obtained from the clone pTa794 (Gerlach and Dyer 1980) and labelled with digoxigenin-
171 11-dUTP -green- (Boehringer, Meylan, France). The preparations were counterstained with
172 Vectashield (Vector Laboratories, Burlingame, CA, USA), a mounting medium containing DAPI.
173 FISH preparations were observed with an epifluorescent Zeiss Axiophot microscope with
174 different combinations of Zeiss excitation and emission filter sets (01, 07 and 15). Hybridisation
175 signals were analysed and photographed using the highly sensitive CCD camera (Princeton
176 Instruments, Trenton, NJ, USA), and an image analyser software (Metavue, version 4.6,
177 Molecular Devices Corporation, Sunnyvale, CA, USA). Results on number and position of rDNA
178 loci for each of the populations studied have been submitted to the Plant rDNA database
179 (www.plantrdnadatabase.com).
180
181 ***Phylogenetic analyses and reconstruction of character evolution.*** Beyond the high mountain
182 *Artemisia*, the sampling strategy for the phylogenetic analysis includes several complementary
183 species to survey representative taxa across the different recognized subgenera in *Artemisia*,
184 bearing in mind availability of previous cytogenetic and genome size (2C-values) data. Species
185 of *Argyranthemum*, *Nipponanthemum* and *Tanacetum* were selected as outgroups based on
186 phylogenetic results of Sanz et al. (2008) and Zhao et al. (2010), and the availability of
187 complete cytogenetic and genome size information. The nuclear ITS1+ITS2 (470 bp) and the

ETS sequences (374 bp), downloaded from GenBank (Supplementary Materials S1 and S2), and the newly generated ETS sequence for *A. genipi* (KT954132), were manually edited and concatenated using BioEdit v. 7.1.3.0 (Hall 1999). For phylogenetic analyses, only the 3' end of the ETS region was used due to the existence of subrepeats and indels causing alignment problems. Bayesian phylogenetic analysis was performed in MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003) using the SYM+G model previously determined from jModeltest v. 2.1.3 (Darriba et al. 2012) under the Akaike information criterion (Akaike 1979). The Markov chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PPs). Four consecutive MCMC computations were run for 2,000,000 generations, with tree sampling every 100 generations. The first 1000 samples were discarded as the burn-in period. PPs were estimated through the construction of a 50% majority rule consensus tree.

The ancestral character reconstructions were performed with Mesquite v. 3.02 software (Maddison and Maddison 2015). All taxa included in these analyses were diploid in order to avoid the bias of polyploidy in the estimated nuclear DNA contents and number of rDNA sites. The 50% majority rule consensus tree resulting from Bayesian Inference analysis was used as the input tree file (after pruning polyploid taxa). Basic chromosome numbers (x=8 and x=9) and the number of rDNA sites were transformed into categorical data (discrete and ordered). Ancestral states using the "trace character history" function were then inferred using maximum likelihood under the Mk1 model, in which all changes are equally probable, following the same approach as Vaio et al. (2013). For reconstructing the ancestral holoploid genome sizes (2C), we selected squared-change maximum parsimony as implemented for continuous characters in Mesquite. This approach has been widely used to reconstruct ancestral genome size states is many plant groups (e.g. Burleigh et al. 2012).

Statistical analyses. Analyses of regression and one-way ANOVA (or alternatively the Kruskal-Wallis test), Shapiro-Wilk test for normality and Barlett's test for equality of variances were performed with RStudio, v.0.98.1078, a user interface for R (www.rstudio.com). Additionally, to analyse variation of the basic chromosome number, holoploid genome sizes, number of chromomycin A_3 positive (CMA+) bands and of rDNA sites in a phylogenetic context, the phylogenetically based generalised least squares (PGLS) algorithm, as implemented in the *nlme* package for R (Version 3.1-118), was used (Pinheiro et al. 2015). Data on holoploid genome size and ribosomal DNA loci for the complementary and outgroup species were extracted from the Genome Size in the Asteraceae Database (www.asteraceaegenomesize.com) and the Plant rDNA database (www.plantrdnadatabase.com).

Results

Genus *Artemisia* is well known from the molecular cytogenetic perspective, yet the large subgenus *Absinthium* and, in particular, the group of taxa inhabiting high mountains, had not been the focus of any cytogenetic research so far. With the present study, known genome size and molecular cytogenetic profiles of *Artemisia* span up to 32% and 10.4% of the accepted taxa, respectively (531 taxa according to The Plant List Version 1.1 http://www.theplantlist.org, accessed March 13th, 2015).

Phylogenetic placement of high mountain Artemisia

The nuclear ribosomal DNA original dataset included 73 *Artemisia* species selected to provide a good representation of all subgenera (Vallès et al. 2011). The concatenated aligned dataset consisted of 844 characters (bp), excluding the 5.8S gene. According to our phylogenetic reconstruction (Figure 2 and Supplementary Material S2), all high mountain taxa, except *A. atrata*, are grouped in a strongly supported clade (PP=100%) that also includes other members of subgenera *Absinthium* and *Artemisia*. For simplicity, we have named this group 'high mountain and allies' (HMA clade hereafter). When polyploid species are pruned from the tree, 52 species are retained and the HMA clade still retains statistical support (PP=99%).

Basic chromosome numbers

Our sample contains representatives of the main basic numbers of the genus (*x*=8 and *x*=9) and from the diploid to the hexaploid level. For both the genus and the HMA clade *x*=9 has been reconstructed as the ancestral chromosome number, while *x*=8 has appeared independently in the three main clades of the phylogeny (Figure 2). In particular, *x*=8 has arisen repeatedly in the HMA clade, with 50% of its members having this basic chromosome number. Overall, comparing the number of *x*=8 species with the number of *x*=9 species, the incidence of the former is significantly higher in this clade than elsewhere in the genus ($F_{2,59}$=5.397, p=0.007).

Genome size variation and relationship with dysploid karyotypes

Table 1 provides holoploid genome size results (2C-values) obtained in the species studied, and a plot illustrating mean genome sizes of *x*=8 and *x*=9 based *Artemisia* is presented in Supplementary Material S3. Results range from 5.6 pg for the diploid *A. genipi* to 18.4 pg for the hexaploid *A. nitida*, which means a global 3.28-fold range, with a 1.59-fold at the diploid

level. The low HPCV (half peak coefficient of variation corresponding to ten samples of five different individuals) mean value (3.22%) indicates good quality of the flow cytometric assessments. Intraspecific (interpopulation) differences have been detected in cases in which several populations were assessed, reaching up to 11.26% in diploid *A. eriantha* and 8.39% in the tetraploid *A. umbelliformis*. Within these species, several populations (those usually with values at the limits of the range) had significantly different genome sizes ($F_{11,40}$=5.882, p<0.0001 for the populations of *A. eriantha* and $F_{9,40}$=10.963, p<0.0001 for the populations of *A. umbelliformis*). Two populations of each for *A. glacialis* and *A. granatensis* were also measured, but differences were small, 1.48% and 2.42%, respectively.

Using the phylogenetically generalized least squares method (PGLS) we have observed that holoploid genome size (2C) is significantly and positively correlated with ploidy level ($t_{2,47}$=8.345, p=0.0039) and chromosome number ($t_{2,47}$=8.358, p=0.023), as expected, while monoploid genome size (1C*x*) decreases significantly with both ($t_{2,47}$=-1.680, p=0.0000; $t_{2,47}$=-1.631, p=0.0000, respectively), indicating a certain degree of genome downsizing. There is an approximate genome size reduction of 13% and 20% at the 4*x* and 6*x* levels, respectively.

Fluorochrome banding (GC-rich heterochromatin) and rDNA loci

Results of chromomycin A_3 (CMA+) banding, indicative of GC-rich bands and rDNA FISH are presented in Figure 3 and 4. The number of CMA+ signals in one population of *A. eriantha* and *A. genipi* is four, while in another population of *A. eriantha* it is eight, all located at terminal position and overlapping with rDNA sites. *Artemisia umbelliformis* presents six terminal sites, although one faint centromeric signal is also visible. *Artemisia melanolepis* chromosomes bear five to six terminal sites, while the closely related tetraploid *A. splendens* displays eight CMA+ signals. The remaining species display much more CMA+ signals, some of them being particularly abundant in pericentromeric position. This is the case for *A. glacialis*, showing up to 20 CMA+ signals out of which 18 are pericentromeric, and *A. assoana* with 16 to 18 visible CMA+ bands, out of which at least 12 are pericentromeric. *Artemisia nitida* shows *c*. 50 CMA+ bands although few are pericentromeric in this case. DAPI after FISH has resulted in signals at both chromosome ends in most chromosomes of *A. glacialis* and *A. assoana*, and in some of *A. nitida*.

For rDNA loci, our results (Table 2) show a homogenized L-type organisation of ribosomal RNA genes, in which all rDNA sites, in all species, present overlapping 5S and 35S signals, thus both rRNA genes colocalise in the same loci. Perfect coincidence between the number of rDNA sites and the number of CMA+ bands is only seen, however, in *A. eriantha* (Figure 4), *A. genipi* (Figure 3 c,d) and in *A. splendens* (Figure 3 m, n), while in another *A*

eriantha (Figure 3 a, b, corresponding to the Lautaret population) and in *A. umbelliformis* (Figure 3 o, p) not all rDNA sites are located in CMA+ regions, and in *A. melanolepis*, *A. glacialis*, *A. nitida* and *A. assoana* there are more CMA+ bands than rDNA sites –in the last three cases many more (Figure 3 g, I, k). The number of sites ranges from four to eight at the diploid, six to eight at the tetraploid, and nine at the hexaploid levels. The position of signals is invariably terminal and its number is constant within a population except for *A. eriantha* and *A. umbelliformis*, in which individuals presenting six and eight rDNA sites have been found; in one *A. eriantha* a small and unpaired chromosome shows an rDNA site at each end (Figure 4). As for genome size, the number of rDNA sites is positively correlated with chromosome number ($F_{1,62}$=82.06, p<0.0001) and ploidy level ($F_{1,62}$=79.18, p<0.0001), and both for the whole genus and for the HMA clade (the distribution of ploidy levels in *Artemisia* is presented in Figure 5). Also, there is a significant ($F_{1,46}$=4.767, p=0.0341) reduction of the number of rDNA sites per chromosome complement with increasing ploidy. Considering data from all the diploid and tetraploid *Artemisia* known until present (mean 2C=2.63 vs. 2C=1.93 in 2*x* and 4*x*, respectively), 26.6% overall loci loss has been found. The number of sites per monoploid genome in *Artemisia* is gradually reduced at higher ploidy levels: hexaploids present a 33.5% reduction in number of loci per monoploid genome with respect to the diploids, and the highest polyploids (decaploids) have 35.36% less loci than the diploids. Finally, several species show considerable site size differences, such as *A. assoana* (Figure 3l) and *A. nitida* (Figure 4j). Besides, a detached rDNA site is observed in *A. glacialis* (Figure 3h).

Reconstruction of ancestral characters

The reconstruction of ancestral holoploid genome size onto the phylogeny (Figure 2), based on diploid taxa, infers a holoploid genome size between 6.92 pg and 8.13 pg as the likely ancestral estimates both for the genus and for the HMA clade, in which ups and downs with respect to their putative common ancestors have occurred during the evolution of the genus. There has been an increase in the number of rDNA sites in 14 cases (45.16%, three of them within the HMA clade, two within the high mountain) and a decrease in only two cases (6.45%); the remaining 15 (48.39%) retain the alleged ancestral number of signals.

Discussion

Intraspecific genome size variation

Research on genome size variation within the species level has a long tradition, from the first reports in flax (Evans et al. 1966). Nevertheless, the extent of true intraspecific variation is still

a source of debate. Critical reassessments of such variations have proved them to be artefacts in some cases, having identified technical problems, methodological errors or presence of endogenous staining inhibitors as likely causes (Greilhuber 2005). However, many well-documented examples, based on accurate assessments, do suggest the existence of genuine intraspecific variation in nuclear DNA content (e.g. Suda et al. 2007; Trávniček et al. 2013).

With regards to the high mountain *Artemisia*, intraspecific genome size variation has been evaluated in *A. eriantha* (11.26%) and *A. umbelliformis* (8.39%), the most widely distributed species through the European mountains. The percentages here reported reflect a certain degree of variation, and interpopulational differences are significant in some cases in both species. Other studies on intraspecific variation across a distribution range performed in *Artemisia* have detected similar percentages: 8.8% for 17 populations in the diploid *A. arborescens* (Garcia et al. 2006) and 9.22% for 45 populations in the hexaploid *A. crithmifolia* (Pellicer et al. 2009). Yet it is difficult to set a cut-off point from which a given percentage of variation should be considered important. Intraspecific genome size variations have been related to microevolutionary differentiations and could be taxonomically significant, especially if there is some degree of morphological or ecological differentiation (Murray 2005). Neither *A. eriantha* nor *A. umbelliformis* show a clear morphological, geographical or ecological pattern to correlate with this variation; however, the detected differences are probably genuine. Possible sources of true intraspecific genome size variation that could explain the detected differences include the presence of aneuploidy or B-chromosomes, introgression or hybridisation processes (Morgan-Richards et al. 2004) or changes in repetitive DNA content (Trávniček et al. 2013).

Polyploidy is associated with genome downsizing and loci loss, but not necessarily with loss of GC-rich heterochromatin

Most studies have found genome downsizing on polyploids in multiple occasions, as it is the case with the alpine *Artemisia*. Recently, Meudt et al. (2015) found not only significant genome downsizing across *Veronica* but also a link with the diversification success of polyploid lineages. However, in some cases as in *Nicotiana* (Leitch et al. 2008, Renny-Byfield et al. 2011), despite the most common response to polyploidy is still genome downsizing, unchanged genome sizes and even genome upsizing have also been found linked to polyploidy processes in certain species.

Besides, the loci loss detected in these high mountain polyploids fits well with previous findings, particularly at the highest ploidies. Globally, most diploid *Artemisia* have four rDNA sites (58%) while some have six (26%), eight (13%), and only in two cases nine and 10 sites

(www.plantrdnadatabase.com, accessed March 20th 2015). Regarding tetraploids, *Artemisia* species mostly show eight sites, consistent with our new findings for *A. splendens* and one population of *A. umbelliformis*. As with genome downsizing, loci loss is commonly observed across ploidy levels and seems to be a global response to polyploidy, however, others have found directly proportional increase (Bareka et al. 2012) or even loci gain (Hasterok et al. 2006). Factors such as qualitative and quantitative differences in transposable element (TE) composition of genomes, possible hybrid origins, and the amount of time elapsed after the polyploidisation event, may play a role on the extent of genome size loss after polyploidisation.

The amplification of certain GC-rich repetitive DNA families specific to certain species could explain the differential pattern observed in those species showing considerably more CMA+ signals, such as *A. nitida*. A similar finding was reported for the tetraploid *A. argilosa* (Garcia et al. 2007) and more recently, Olanj et al. (2015) found a striking number of CMA+ signals in *Tanacetum*, a genus closely related to *Artemisia* (reaching up to 66 at the diploid level, 2n=18). The observed abundance of pericentromeric CMA+ signals in certain species (*A. glacialis* and *A. assoana*) while most are (sub)terminal, would also point to other kinds of repetitive GC-rich DNA amplifying differently in these taxa. Contrasting to genome downsizing and loss of rDNA loci, there is no clear pattern with respect to the pattern of change of CMA+ bands with increasing ploidy: a given species can have very different profiles, and in some cases tetraploids have less CMA+ bands than diploids. As shown previously (Garcia et al. 2007, Olanj et al. 2015), the underpinning evolutionary dynamics of GC-rich DNA is particularly elusive.

Reconstruction of ancestral characters and* Artemisia *cytogenetic features

Studies that address the evolution of cytogenetic traits considering a phylogenetic and temporal perspective are not common and there is no clear pattern in the direction of their changes (Leitch et al. 2008). During *Artemisia*'s evolution, both genome size and number of rDNA loci have undergone ups and downs, as others have previously found (Burleigh et al. 2012), this showing the dynamic nature of plant genomes. The ancestral-like condition of four rDNA sites in *Artemisia* is consistent with the most frequently found numbers of rDNA sites in angiosperms (www.plantrdnadatabase.com). The (sub)terminal position is also the most common, both in *Artemisia* and in angiosperms as a whole, consistently with evolutionary constraints favouring the terminal position for rDNA (Roa and Guerra 2012). Holoploid genome size has mostly remained stable or increased during *Artemisia*'s evolution: the reconstruction of ancestral holoploid genome size onto the phylogeny (Figure 2) infers a value between 6.92 and 8.13 as the likely ancestral estimates both for the genus and for the HMA clade. This is

considered intermediate (7≤2C<28 pg) according to the genome size categories of plants (Leitch et al. 2005). In angiosperms, the ancestral genome size has been reconstructed as very small (2C≤2.8 pg) with several independent increases and decreases taking place (Bennett and Leitch 2005).

***Low incidence of polyploid taxa in the high mountain* Artemisia**

Polyploidy in the HMA clade is not particularly abundant when compared to the whole genus. In *Artemisia*, 44% of species are only diploid, 30% only exclusively polyploid, while 26% are known both at diploid and polyploid levels (Vallès et al. 2011). However, in the HMA clade, while 73.08% are only diploid, 19.23% are exclusively polyploid and 7.69% are known both at diploid and polyploid levels (Figure 5). Other few high mountain species not belonging to this clade are also diploid, such as *A. atrata*. Nie et al. (2005) only detected 22% of polyploid taxa across the flora of the Hengduan Mountains (Qing-Hai Tibet Plateau). More recently, Loureiro et al. (2013) found 23% of polyploids across two Spanish high mountain ranges, showing that polyploidisation is probably not essential in determining species adaptation to such environments. Conversely, Love and Love (1967) and Morton (1993) found a high rate of polyploids in the alpine zone of Mt. Washington and in the Cameroon Mountains, respectively. Likewise, Vamosi and McEwen (2013) detected an increase of polyploids in high elevations of British Columbia. However, it has been suggested that a high genome size (product of polyploidisation) would not be selected in alpine environments, in principle (Baack 2004). This could have promoted a lower frequency of alpine polyploids in medium-large holoploid genome size genera such as *Artemisia*. Interestingly, as Tkach et al. (2008) pointed out, we confirmed a higher frequency of polyploids in arctic *Artemisia* species (Figure 5). This suggests that polyploidy ranges, and their increase with latitude, could be more related with their ability to colonize new habitats, such as newly deglaciated environments (Brochmann et al. 2004), rather than with a greater cold-hardiness or higher altitudes (Stebbins 1971; te Beest et al. 2012).

Descending dysploidy by recurrent Robertsonian translocations

A pioneer study by Kawatani and Ohno (1964) suggested descending dysploidy in *Artemisia* on the grounds of the much larger proportion of *x*=9-based taxa. Dysploidy has contributed to species differentiation in many genera, occurring once or repeatedly (Blöch et al. 2009; Schneeweiss et al. 2013). As pointed out previously, within the HMA clade there is a significant number of *x*=8 (dysploid) species (12 out of 26 species) in a mostly *x*=9 based genus, tribe and

family (Schweizer and Ehrendorfer 1983). Within *Artemisia*, *x*=8-based species are around 14% of the total whose chromosome number is known (Vallès et al. 2011). Yet in the HMA clade this grows to 46%. Our ancestral state reconstruction points to the fact that *x*=9 is the ancestral condition in the genus, as in family Asteraceae, although Semple and Watanabe (2009) hypothesised *x*=10 was the ancestral base number in tribe *Anthemideae* (to which *Artemisia* belongs). This would support a hypothesis of downwards dysploidy within the tribe. Descending dysploidy has been related to plants adapted to extreme habitats in some Asteraceae genera (Garnatje et al. 2004) and this might be linked to *x*=8 being over-represented in the HMA clade.

Few works have shown in detail the specific process leading to a decrease in basic chromosome number. However, a likely case of Robertsonian (Rb) translocation that could account for the descending dysploidy has been found in *A. eriantha* (Figure 4). In fact, similar processes had already been suggested for *Artemisia* on the basis of observed centromeric fragility in a large chromosome pair (Vallès and Siljak-Yakovlev 1997). Likewise, a Rb translocation could have been involved in the appearance of *x*=17 in species such as *A. umbelliformis*. Given the distribution of *x*=8 taxa across *Artemisia*'s phylogeny, Rb translocations may have occurred independently several times in the genus (notwithstanding any other mechanisms promoting descending dysploidy). It is possible, however, that the chromosomes involved in the translocation are not the same in all instances where *x*=8 appears (i.e. different translocations affecting different chromosomes). We hypothesise that this may be the case in *Artemisia* since, (1) a notably larger chromosome pair is seen in several x=8 species from the HMA clade (Figure 4, e, f, g, h, k, l, arrows) while this is rarely observed in the other *x*=8 species outside this clade and (2) holoploid genome size of *x*=8 species from the HMA clade is significantly higher than that of the other *x*=8 species outside this clade (Supplementary Material S3) which could be related to their specific translocation process (involving different chromosomes, which would explain the observed size differences, and perhaps different genomic processes which somehow could be related with the genome size differences detected). Therefore, the fact that only species from the HMA clade share these features (i.e. large chromosome pair and increase genome size) indicates that the event leading to *x*=8 in this group would be common and may differ from other emergences of the dysploid karyotype in other groups, leading to size differences in the translocation chromosomes and genome size accumulation.

Conclusions

This work shows that *Artemisia* species from high mountain habitats do not present, at first sight, particular karyological features as compared with other alpine plant groups or other non-high mountain *Artemisia*. However, we do detect a high frequency of dysploids in the clade containing alpine *Artemisia*. Besides, polyploids are remarkably less abundant in the high mountains than in the rest of the genus. Thus, it seems that evolutionary processes in high mountain *Artemisia* have been less influenced by polyploidy and more affected by dysploidy and other mechanisms involving genome size increase, such as, for example, mobilisation of transposable elements or amplification of repetitive DNA, which still remain to be proved in these taxa. As previously reported for high mountain species from genus *Phyteuma* (Schneeweiss et al. 2013), descending dysploidy could be associated with lineage diversification in alpine *Artemisia*.

Acknowledgments and funding

This work was supported by the Ministerio de Economía y Competividad from the Government of Spain (CGL2010-22234-C02-01,02/BOS and CGL2013-49097-C2-2-P), the Generalitat de Catalunya, government of Catalonia ("Ajuts a grups de recerca consolidats", 2009SGR0439 and 2014SGR514) and the IRBIO (Institut de Recerca de la Biodiversitat). GMdX benefited from an ADR grant from Universitat de Barcelona and SG from a Juan de la Cierva postdoctoral contract of the Ministerio de Economía y Competividad from the Government of Spain. We acknowledge Cristina Roquet, María Sanz and Roser Vilatersana for their help in collecting plant material. Ricard Àlvarez, Jaume Comas, Chari González and Sonia Ruiz are acknowledged for their assistance in flow cytometric analyses, and Spencer Brown for supplying seeds of the internal standards used. We also thank Odile Robin with technical assistance at FISH slide preparation, Samuel Pyke for English proofreading and three anonymous reviewers for helpful comments on earlier versions of the manuscript.

494 **References**

495 Akaike H (1979) A Bayesian extension of the minimum AIC procedure of autoregressive model
496 fitting. Biometrika 66:237–242

497 Albach DC, Greilhuber J (2004) Genome size variation and evolution in *Veronica*. Ann Bot
498 94:897–911

499 Baack EJ (2004) Cytotype segregation on regional and microgeographic scales in snow
500 buttercups (*Ranunculus adoneus*: Ranunculaceae). Am J Bot 91:1783–1788

501 Bareka P, Siljak-Yakovlev S, Kamari G (2012) Molecular cytogenetics of *Bellevalia*
502 (Hyacinthaceae) species occurring in Greece. Plant Syst Evol 298:421–430

503 Blöch C, Weiss-Schneeweiss H, Schneeweiss GM, Barfussa MHJ, Rebernig CA, Villaseñor JC,
504 Stuessy TF (2009) Molecular phylogenetic analyses of nuclear and plastid DNA sequences
505 support dysploid and polyploid chromosome number changes and reticulate evolution in
506 the diversification of *Melampodium* (Millerieae, Asteraceae). Mol Phylogenet Evol
507 53:220–233

508 Brochmann C, Brysting A, Alsos IG, Borgen L, Grundt HH, Scheen AC, Elven R (2004) Polyploidy
509 in arctic plants. Biol J Linn Soc 82:521–536

510 Burleigh JG, Barbazuk WB, Davis JM, Morse AM, Soltis PS (2012) Exploring diversification and
511 genome size evolution in extant gymnosperms through phylogenetic synthesis. J Bot
512 2012

513 Canestrelli D, Nascetti G (2008) Phylogeography of the pool frog *Rana* (*Pelophylax*) *lessonae* in
514 the Italian peninsula and Sicily: multiple refugia, glacial expansions and nuclear–
515 mitochondrial discordance. J Biogeogr 35:1923–1936

516 Darriba D, Taboada G, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics
517 and parallel computing. Nat Methods 9:772

518 Doležel J, Binarova P, Lucretti S (1989) Analysis of nuclear DNA content in plant cells by flow
519 cytometry. Biol Plant 31:113–120

520 Doležel J, Bartoš J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of
521 trout and human. Cytom Part A 51:127–8

522 Evans GM, Durrant A, Rees H (1966) Associated nuclear changes in the induction of flax
523 genotrophs. Nature 212:697–699

524 Garcia S, Sanz M, Garnatje T, Kreitschitz A, McArthur ED, Vallès J (2004) Variation of DNA
525 amount in 47 populations of the subtribe *Artemisiinae* and related taxa (Asteraceae,
526 Anthemideae): karyological, ecological, and systematic implications. Genome 47:1004–14

527 Garcia S, Garnatje T, Twibell JD, Vallès J (2006) Genome size variation in the *Artemisia*
528 *arborescens* complex (Asteraceae, Anthemideae) and its cultivars. Genome 49:244–253

529 Garcia S, Garnatje T, Hidalgo O, McArthur DE, Siljak-Yakovlev S, Vallès J (2007) Extensive
530 ribosomal DNA (18S-5.8S-26S and 5S) colocalisation in the North American endemic
531 sagebrushes (subgenus *Tridentatae*, *Artemisia*, Asteraceae) revealed by FISH. Plant Syst
532 Evol 267:79–92

533 Garnatje T, Vallès J, Vilatersana R, Garcia-Jacas N, Susanna A, Siljak-Yakovlev S (2004)
534 Molecular cytogenetics of *Xeranthemum* L. and related genera (Asteraceae, Cardueae).
535 Plant Biol (Stuttg) 6:140–6

536 Geber G, Schweizer D (1987) Cytochemical heterochromatin differentiation in *Sinapis alba*
537 (Cruciferae) using a simple air-drying technique for producing chromosome spreads.
538 Plant Syst Evol 158:97–106

539 Gerlach W, Bedbrook J (1979) Cloning and characterisation of ribosomal RNA genes from
540 wheat and barley. Nucleic Acids Res 7:1869–1885

541 Gerlach W, Dyer T (1980) Sequence organisation of the repeating units in the nucleus of wheat
542 which contain 5S rRNA genes. Nucleic Acids Res 8:4851–4865

543 Greilhuber J (2005) Intraspecific variation in genome size in angiosperms: identifying its
544 existence. Ann Bot 95:91–98

545 Greilhuber J, Leitch IJ (2013) Genome size and the phenotype. In: Greilhuber J, Doležel J,
546 Wendel JF (eds). Plant Genome Diversity Springer Vienna. 2:323–344

547 Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis
548 program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

549 Hasterok R, Wolny E, Hosiawa M, Kowalczyk M, Kulak-Ksiazczyk S, Ksiazczyk T, Heneen WK,
550 Maluszynska J (2006) Comparative analysis of rDNA distribution in chromosomes of
551 various species of Brassicaceae. Ann Bot 97:205–216

552 Kawatani T, Ohno T (1964) Chromosome numbers in *Artemisia*. Bull Natl Inst Hyg Sci 82:183–
553 193

554 Leitch IJ, Hanson L, Lim KY, Kovarik A, Chase MW, Clarkson JJ, Leitch AR (2008) The ups and
555 downs of genome size evolution in polyploid species of *Nicotiana* (Solanaceae). Ann Bot
556 101:805–814

557 Levin DA (2002) The role of chromosomal change in plant evolution. Oxford University Press.
558 New York

559 Ling YR, Humphries CJ, Shultz L, Flora of China Editorial Committee (2006) The Genus *Artemisia*
560 L. Flora of China, 20:1151–1259

561 Loureiro J, Castro M, Cerca de Oliveira J, Mota L, Torices R (2013) Genome size variation and
562 polyploidy incidence in the alpine flora from Spain. Anales Jard Bot Madrid 70:39–47

563 Löve Á, Löve D (1949) The geobotanical significance of polyploidy. I. Polyploidy and latitude.
564 Port Acta Biol Ser A 273–352

565 Löve Á, Löve D (1967) Polyploidy and altitude: Mt. Washington. Biol Zentralbl Suppl 307–312

566 Maddison W, Maddison D (2015) Mesquite: a modular system for evolutionary analysis.
567 Version 2.75. http://mesquiteproject.org

568 Marie D, Brown SC (1993) A cytometric exercise in plant DNA histograms, with 2C values for 70
569 species. Biol Cell 78:41–51

570 Mas de Xaxars G, García-Fernández A, Barnola P, Martín J, Mercadé A, Vallès J, Vargas P, Vigo J,
571 Garnatje T (2015) Phylogenetic and cytogenetic studies reveal hybrid speciation in
572 *Saxifraga* subsect. *Triplinervium* (Saxifragaceae). J Syst Evol 53:53–65

573 Meudt HM, Rojas-Andrés BM, Prebble JM, Low E, Garnock-Jones PJ, Albach DC (2015) Is
574 genome downsizing associated with diversification in polyploid lineages of *Veronica*? Bot
575 J Linn Soc 178:243–266

576 Morgan-Richards M, Trewick SA, Chapman HM, Krahulcová A (2004) Interspecific hybridization
577 among *Hieracium* species in New Zealand: evidence from flow cytometry. Heredity
578 93:34–42

579 Morton J (1993) Chromosome numbers and polyploidy in the flora of Cameroons Mountain.
580 Op Bot 121:159–172

581 Mráz P, Chrtek J, Šingliarová B (2009) Geographical parthenogenesis, genome size variation
582 and pollen production in the arctic-alpine species *Hieracium alpinum*. Bot Helv 119:41–51

583 Murray BG (2005) When does intraspecific C-value variation become taxonomically significant?
584 Ann Bot 95:119–125

585 Nagy L, Grabherr G (2009) The biology of alpine habitats. Oxford University Press. New York

586 Nie Z, Wen J, Gu Z, Boufford DE, Sun H (2005) Polyploidy in the flora of the Hengduan
587 Mountains hotspot, southwestern China. Ann Missouri Bot Gard 92:275–306

588 Nieto Feliner G (2011) Southern European glacial refugia: A tale of tales. Taxon 60:365–372

589 Nieto Feliner G (2014) Patterns and processes in plant phylogeography in the Mediterranean
590 Basin. A review. Perspect Plant Ecol 16: 265–278

591 Olanj N, Garnatje T, Sonboli A, Vallès J, Garcia S (2015) The striking and unexpected
592 cytogenetic diversity of genus *Tanacetum* L. (Asteraceae): a cytometric and fluorescent *in*
593 *situ* hybridisation study of Iranian taxa. BMC Plant Biol 15:174

594 Pellicer J, Garcia S, Garnatje T, Vallès J (2009) Changes in genome size in a fragmented
595 distribution area: the case of *Artemisia crithmifolia* L. (Asteraceae, Anthemideae).
596 Caryologia 62:152–160

597 Petit C, Thompson JD (1999) Species diversity and ecological range in relation to ploidy level in
598 the flora of the Pyrenees. Evol Ecol 13:45–65

599 Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2015) nlme: Linear and Nonlinear
600 Mixed Effects Models. R package version 3.1-121, http://CRAN.R-
601 project.org/package=nlme

602 Renny-Byfield S, Chester M, Kovařík A, Le Comber SC, Grandbastien MA, Deloger M, Nichols
603 RA, Macas J, Novák J, Chase MW, Leitch AR (2011) Next generation sequencing reveals
604 genome downsizing in allotetraploid *Nicotiana tabacum*, predominantly through the
605 elimination of paternally derived repetitive DNAs. Mol Biol Evol msr112

606 Roa F, Guerra M (2012) Distribution of 45S rDNA sites in chromosomes of plants: Structural
607 and evolutionary implications. BMC Evol Biol 12:225

608 Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed
609 models. Bioinformatics 19: 1572–1574

610 Russell A, Safer S, Weiss-Schneeweiss H, Temsch E, Stuppner H, Stuessy TF, Samuel R (2013)
611 Chromosome counts and genome size of *Leontopodium* species (Asteraceae:
612 Gnaphalieae) from south-western China. Bot J Linn Soc 171:627–636

613 Sanz M, Vilatersana R, Hidalgo O, Garcia-Jacas N, Susanna A, Schneeweiss GM, Vallès J (2008)
614 Molecular phylogeny and evolution of floral characters of *Artemisia* and allies
615 (Anthemideae, Asteraceae): evidence from nrDNA ETS and ITS sequences. Taxon 57:66–
616 78

617 Sanz M, Schneeweiss GM, Vilatersana R, Vallès J (2011) Temporal origins and diversification of
618 *Artemisia* and allies (Anthemideae, Asteraceae). Collect Bot 30:7–15

619 Sanz M, Schönswetter P, Vallès J, Schneeweiss GM, Vilatersana R (2014) Southern isolation and
620 northern long-distance dispersal shaped the phylogeography of the widespread, but
621 highly disjunct, European high mountain plant *Artemisia eriantha* (Asteraceae). Bot J Linn
622 Soc 174:214–226

623 Schneeweiss GM, Pachschwöll C, Tribsch A, Schönswetter P, Barfuss MH, Esfeld K, Weiss-
624 Schneeweiss H, Thiv M (2013) Molecular phylogenetic analyses identify Alpine
625 differentiation and dysploid chromosome number changes as major forces for the
626 evolution of the European endemic *Phyteuma* (Campanulaceae). Mol Phylogenet Evol
627 69:634–652

628 Schweizer D, Ehrendorfer F (1983) Evolution of C-band patterns in Asteraceae-Anthemideae.
629 Biol Zentralbl 102:637–655

630 Semple J, Watanabe K (2009) A review of chromosome numbers in Asteraceae with
631 hypotheses on chromosome base number evolution. In: Funk VA, Susanna A, Suetssy TF,
632 Bayer RJ (eds) Systematics, evolution and biogeography of Compositae. International
633 Association for Plant Taxonomy, Viena, pp 61–72

634 Španiel S, Marhold K, Passalacqua NG, Zozomová-Lihová J (2011) Intricate variation patterns in
635 the diploid-polyploid complex of *Alyssum montanum-A. repens* (Brassicaceae) in the
636 Apennine Peninsula: evidence for long-term persistence and diversification. Am J Bot
637 98:1887–1904

638 Stebbins GL (1971) Chromosomal evolution in higher plants. Addison-Wesley. London

639 Suda J, Weiss-Schneeweiss H, Tribsch A, Schneeweiss GM, Trávníček P, Schönswetter P (2007)
640 Complex distribution patterns of di-, tetra-, and hexaploid cytotypes in the European high
641 mountain plant *Senecio carniolicus* (Asteraceae). Am J Bot 94:1391–1401

642 te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubešová M, Pyšek P (2012) The
643 more the better? The role of polyploidy in facilitating plant invasions. Ann Bot 109: 19–45

644 Tkach NV, Hoffmann MH, Röser M, Korobkov AA, von Hagen KB (2008) Parallel evolutionary
645 patterns in multiple lineages of arctic *Artemisia* L. (Asteraceae). Evolution 62:184–98

646 Torrell M, Vallès J (2001) Genome size in 21 *Artemisia* L. species (Asteraceae, Anthemideae):
647 Systematic, evolutionary, and ecological implications. Genome 44:231–238

648 Trávníček P, Kirschner J, Chudáčková H, Rooks F, Štěpánek J (2013) Substantial genome size
649 variation in *Taraxacum stenocephalum* (Asteraceae, Lactuceae). Folia Geobot 48:271–284

650 Vaio M, Gardner A, Emshwiller E, Guerra M (2013) Molecular phylogeny and chromosome
651 evolution among the creeping herbaceous *Oxalis* species of sections *Corniculatae* and
652 *Ripariae* (Oxalidaceae). Mol Phyl Evol 68: 199–211

653 Vallès J, Garcia S, Hidalgo O, Martín J, Pellicer J, Sanz M, Garnatje T (2011) Biology, genome
654 evolution, biotechnological issues and research including applied perspectives in
655 *Artemisia* (Asteraceae). Adv Bot Res 6:349–419

656 Valles J, Siljak-Yakovlev S (1997) Cytogenetic studies in the genus *Artemisia* L. (Asteraceae):
657 fluorochrome-banded karyotypes of five taxa, including the Iberian endemic species
658 *Artemisia barrelieri* Besser. Can J Bot 75: 595–606.

659 Vamosi J, McEwen J (2013) Origin, elevation, and evolutionary success of hybrids and
660 polyploids in British Columbia, Canada. Botany 91:182–188

661 Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM (2013) Evolutionary
662 consequences, constraints and potential of polyploidy in plants. Cytogenet Genome Res
663 140:137–150

664 Zhao HB, Chen FD, Chen SM, Wu GS, Guo WM (2010). Molecular phylogeny of
665 *Chrysanthemum*, *Ajania* and its allies (Anthemideae, Asteraceae) as inferred from
666 nuclear ribosomal ITS and chloroplast trnL-F IGS sequences. Plant Syst Evol 284:153–169

667

668 **Table 1**. Provenance of the populations of *Artemisia* studied. (1) Ploidy level; (2) chromosome number; (3) genome size in pg; (4) standard deviation; (5)
669 genome size in Mbp (1 pg=978 Mbp; Doležel et al. 2003); (6) monoploid genome size; (7) internal standard: *Petunia hybrida* 'PxPC6' (2C=2.85 pg), *Pisum*
670 *sativum* 'Express Long' (2C=8.37 pg). Genome size data from: [a] Garcia et al. (2004); [b] Torrell and Vallès (2001). Chromosome numbers confirmed and/or
671 extracted from the Index to Chromosome Numbers in Asteraceae (http://www.lib.kobeu.ac.jp/infolib/meta_pub/G0000003asteraceae_e) except for
672 *Artemisia melanolepis* (new count).
673

Species	Origin	LatN Long	PL[1]	2*n*[2]	2C[3] (pg)	SD[4]	2C[5] (Mbp)	1C*x*[6] (pg)	Standard[7]
A. assoana Balb.	Spain, Teruel, Puerto del Esquinazo	40º53'N 0º53'W	2	18	7.97	(0.11)	7475.86	3.98	*Petunia*
A. eriantha Ten.	Andorra, Ordino, Pic de Casamanya	42º34'N 01º33'E	2	18	6.17	(0.07)	6034.26	3.09	*Petunia*
	Bulgaria, Rila, Josifica Mountain	42º00'N 23º30'E	2	18	6.24	(0.08)	6102.72	3.12	*Petunia*
	France, Alpes-Maritimes, Pas des Ladres	44º05'N 07º24'E	2	18	6.36	(0.08)	6220.08	3.18	*Petunia*
	France, Hautes-Alpes, Col du Galibier	45º03'N 06º24'E	2	18	5.72	(0.05)	5594.16	2.86	*Petunia*
	France, Hautes-Alpes, Col du Laurichard	45º06'N 06º24'E	2	18	6.33	(0.09)	6152.76	3.17	*Petunia*
	Greece, Epirus, Epiro Ploskos	39º58'N 20º46'E	2	18	5.93	(0.22)	5799.54	2.97	*Petunia*
	Greece, Tharsis, Mount Olympus	40º05'N 22º22'E	2	18	5.83	(0.24)	5701.74	2.92	*Petunia*
	Italy, Alpi Maritime, lake Ischiator	44º18'N 07º02'E	2	18	6.11	(0.15)	5975.58	3.06	*Petunia*
	Poland, Tatra Mountains, Liliowe Turnie	49º13'N 19º54'E	2	18	5.94	(0.22)	5809.32	2.97	*Petunia*
	Rumania, Dâmbovița, Bucegi-Caraiman	45º26'N 25º27'E	2	18	6.26	(0.21)	6122.28	3.13	*Petunia*
	Spain, Huesca, Ibón de Urdiceto	42º40'N 00º15'E	2	18	6.18	(0.14)	6044.04	3.09	*Petunia*
	Rumania, Argeș, Făgăraș	45º35'N 25º39'E	2	18	6.44	(0.35)	6259.68	3.22	*Petunia*
A. genipi Weber ex. Stechm.	Italy, Valle d'Aosta, Cogne de Scaletta	45º36'N 07º21'E	2	18	5.60	(0.09)	5476.80	2.80	*Petunia*
A. glacialis L.	Italy, Valle d'Aosta, Valnontey	45º35'N 07º20'E	2	16	8.52[a]	(0.15)	8332.56	4.26	*Petunia*
	France, Alpes-Maritimes, Col de Tortisse	44º19'N 06º56'E	2	16	8.92	(0.41)	8723.76	4.46	*Petunia*

A. granatensis Boiss.	Spain, Granada, Sierra Nevada 1	37º05'N 03º23'W	2	16	7.43	(0.11)	7266.54	3.72	*Petunia*
	Spain, Granada, Sierra Nevada 2	37º80'N 03º24'W	2	16	7.61	(0.15)	7442.58	3.81	*Petunia*
A. melanolepis Boiss.	Iran, Māzandarān, Elburz Mountains	36º04'N 51º44'E	2	16	8.04	(0.18)	7863.12	4.02	*Petunia*
A. nitida Bertol.	Italy, Alpi Apuane, Monte Macina	44º04'N 10º14'E	6	54	18.40	(0.14)	17259.2	3.06	*Pisum*
A. splendens Willd.	Iran, Āzarbāijān-e Gharbī, Doughron mountains	38º44'N 46º40'E	4	32	13.59[b]	(0.21)	13291.02	3.40	*Pisum*
A. umbelliformis Lam.	France, Alpes-Maritimes, Col de la Bonette	44º20'N 06º48'E	4	34	13.18	(0.19)	12899.82	3.30	*Pisum*
	France, Alpes-Maritimes, Col de Tortisse	44º18'N 06º57'E	4	34	13.03	(0.22)	12743.34	3.26	*Pisum*
	France, Hautes-Alpes, Col du Galibier	45º02'N 06º14'E	4	34	13.21	(0.08)	12919.38	3.30	*Pisum*
	France, Pyrénées-Orientales, Canigó	42º31'N 02º28'E	4	34	13.10	(0.18)	12811.8	3.28	*Pisum*
	Italy, Valle d'Aosta, Valnontey	45º35'N 07º20'E	4	34	13.40	(0.10)	13105.20	3.35	*Pisum*
	Spain, Catalonia, Girona, Noucreus	42º25'N 02º09'E	4	34	12.94	(0.42)	12650.49	3.23	*Pisum*
	Spain, Granada, Sierra Nevada 3	37º48'N 03º14'W	4	34	13.18	(0.12)	12890.04	3.30	*Pisum*
	Spain, Huesca, Collaradeta	42º44'N 00º31'W	4	34	12.40[b]	(0.25)	12127.20	3.10	*Pisum*
	Spain, Palencia, Agujas de Cardaño	43º01'N 4º43'W	4	34	13.27	(0.06)	12978.06	3.32	*Pisum*
	Switzerland, Valais, Riffelberg	45º59'N 6º45'E	4	34	13.44	(0.05)	13144.32	3.36	*Pisum*

674
675

676 **Table 2.** Summary of the results (in number of signals) of the fluorochrome banding with
677 chromomycin (CMA+) and of the fluorescent *in situ* hybridisation of rDNA. The position of
678 signals is always terminal, except for CMA+ bands in *A. glacialis* and *A. assoana*, where most
679 sites are pericentromeric. All taxa are high mountain species with the exception of *A. assoana*.
680
681

Species	**Chromosome number**	**CMA+**	**rDNA**
A. melanolepis	2*n*=2*x*=16	5 and 6	4
A. glacialis (Col de Tortisse)	2*n*=2*x*=16	20	4
A. assoana	2*n*=2*x*=16	18	6
A. eriantha (Urdiceto)	2*n*=2*x*=18	4	6
A. eriantha (Col du Lautaret)	2*n*=2*x*=18	8	8
A. genipi	2*n*=2*x*=18	4	4
A. splendens	2*n*=4*x*=32	8	8
A. umbelliformis (Noucreus)	2*n*=4*x*=34	6	6 and 8
A. nitida	2*n*=6*x*=54	*c.* 50	9

682
683
684
685
686
687
688
689
690

691 **Figure 1** Map indicating the provenance of the populations studied. Zoomed area in the upper
692 left corner corresponds to the Alps, where many of the populations studied are present.

693 **Figure 2** Ancestral state reconstruction of genome size (left) and basic chromosome number
694 and number of rDNA sites (right) for diploid *Artemisia* taxa. The HMA clade is highlighted in
695 pink and the strictly high mountain species in bold. Species for which no data are available
696 indicated in striped grey. Ancestral state reconstruction was estimated by Mesquite (v.3.02)
697 using the 50% majority-rule consensus topology obtained by Bayesian inference phylogenetic
698 analysis of the ITS1, ITS2 and ETS data sequence. The Bayesian clade-credibility values are
699 indicated as grey and black squares (posterior probability > 0.7 and > 0.95, respectively). Blue
700 bars depict genome sizes (2C values).

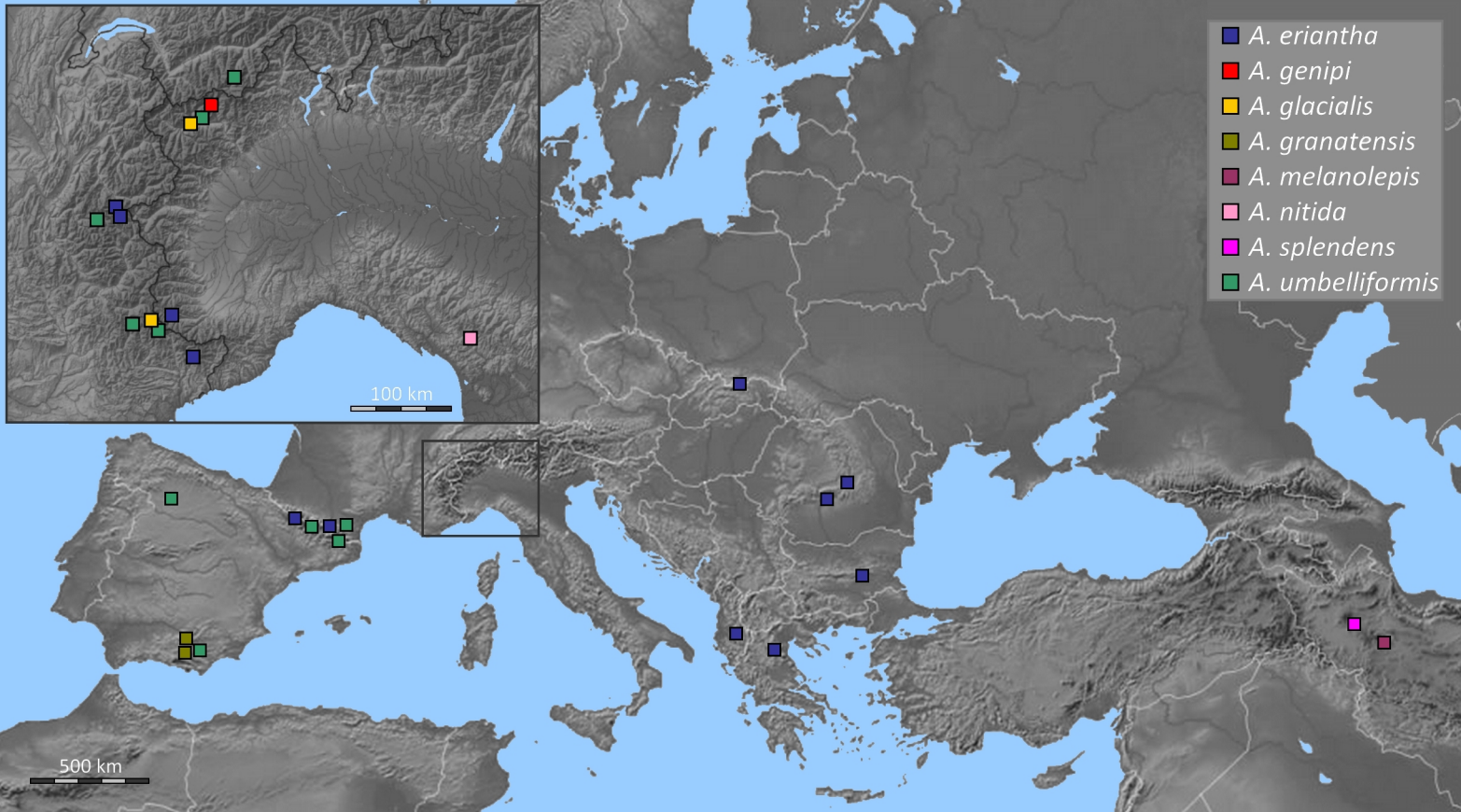
701 **Figure 3** Chromomycin A_3-positive (CMA+) and FISH pictures of metaphases of *Artemisia*
702 species from the HMA clade. (a, b) *A. eriantha,* 2*n*=18 (Urdiceto); (c, d) *A. genipi* 2*n*=18; (e, f) *A.*
703 *melanolepis* 2*n*=16; arrows in *c* indicate CMA+ signals; arrow in *e* indicates one large
704 chromosome; (g, h) *A. glacialis* 2*n*=16; arrows in *g* indicate a large chromosome pair and in *h* a
705 detached ribosomal DNA locus; most CMA+ signals are pericentromeric in *g* (i, j) *A. nitida*
706 2*n*=54; arrow in i indicates probably decondensed rDNA loci; (k, l) *A. assoana* 2*n*=16; arrows in
707 *k* and *l* indicate a large chromosome pair, and asterisks in *l* mark loci with low rDNA copy
708 number; (m, n) *A. splendens* 2*n*=32; arrows in *m* mark faint CMA+ signals; (o, p, q, r) *A.*
709 *umbelliformis* 2*n*=34; note 8 and 6 rDNA loci in pictures *p* and *r,* respectively; arrows in *q*
710 indicate faint CMA+ signals.
711
712 **Figure 4** Metaphases from *Artemisia eriantha*: regular metaphase (a, b) and metaphase with a
713 likely Robertsonian translocation (c, d). Aligned chromosomes from *b* and *d* with the
714 translocation pair underlined, and the possible mechanism of the translocation (e).
715
716 **Figure 5** Distribution of ploidy levels among different *Artemisia* groups. Data for the whole
717 genus are taken from Vallès et al. (2011). Arctic *s. str.* species are selected from Tkach et al.
718 (2008) and chromosome numbers are extracted from the Index to Chromosome Numbers in
719 Asteraceae. "n" indicates sample size.
720
721
722
723

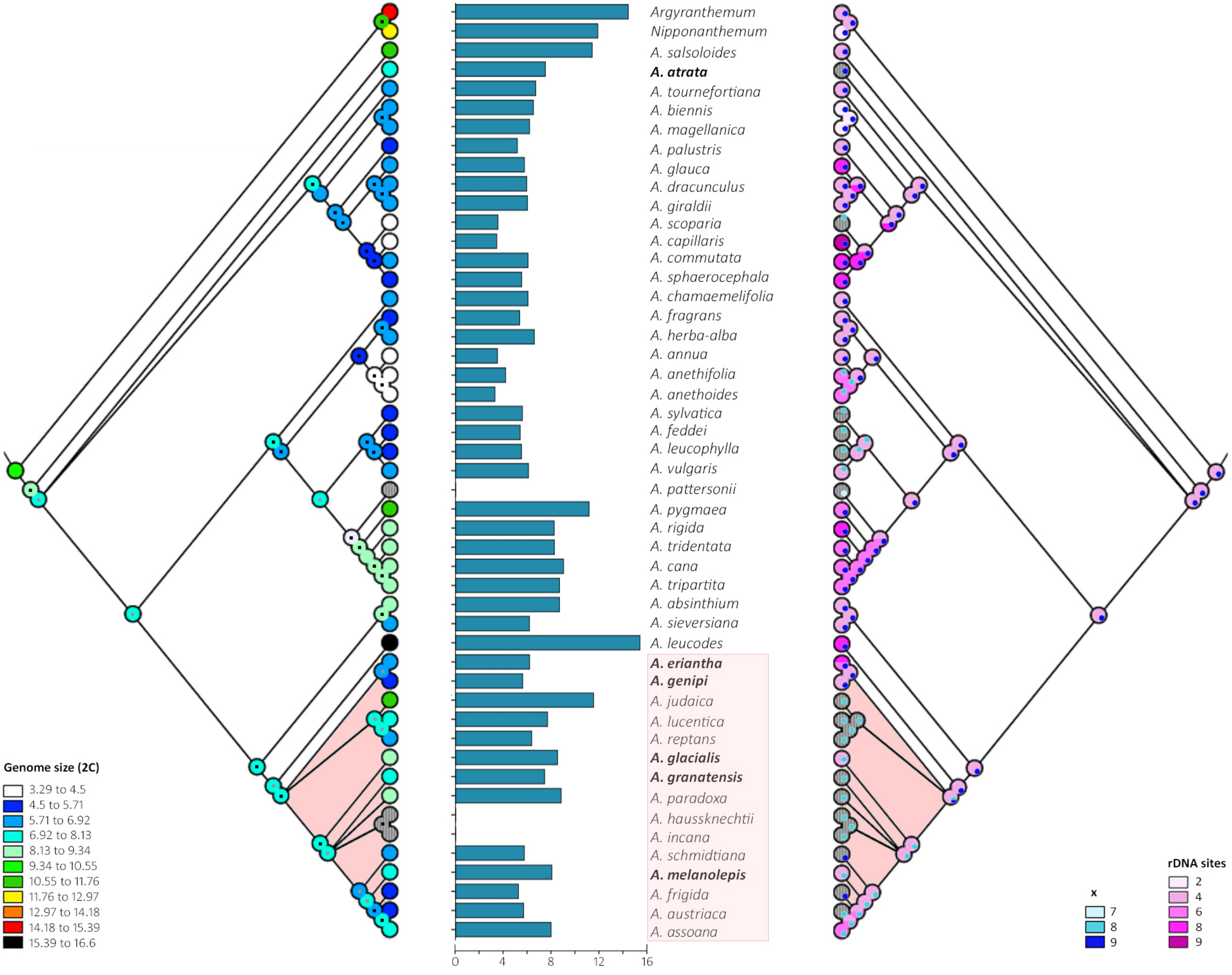
Supplementary material

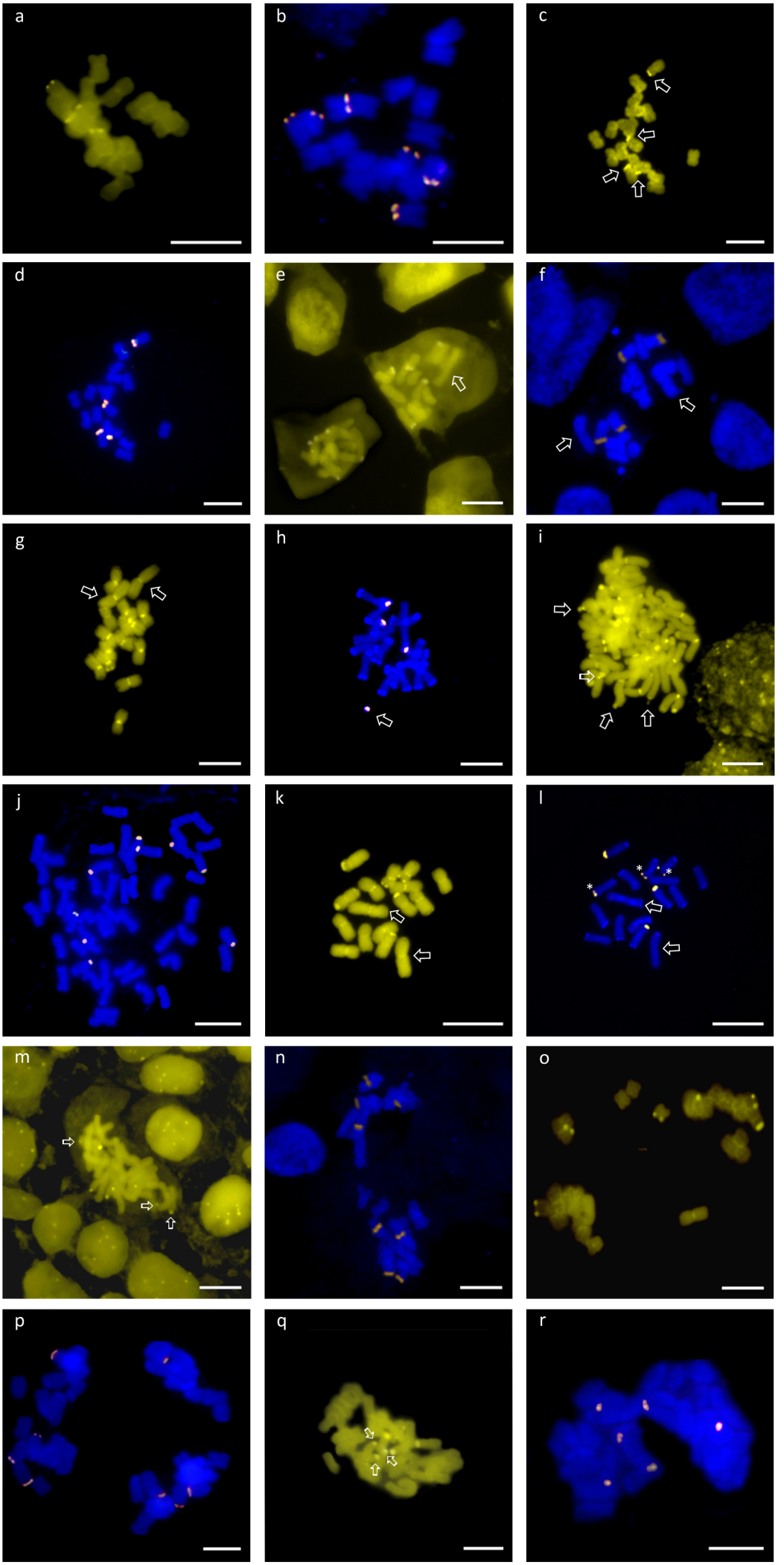
S1 List of DNA sequences used for phylogenetic analyses.

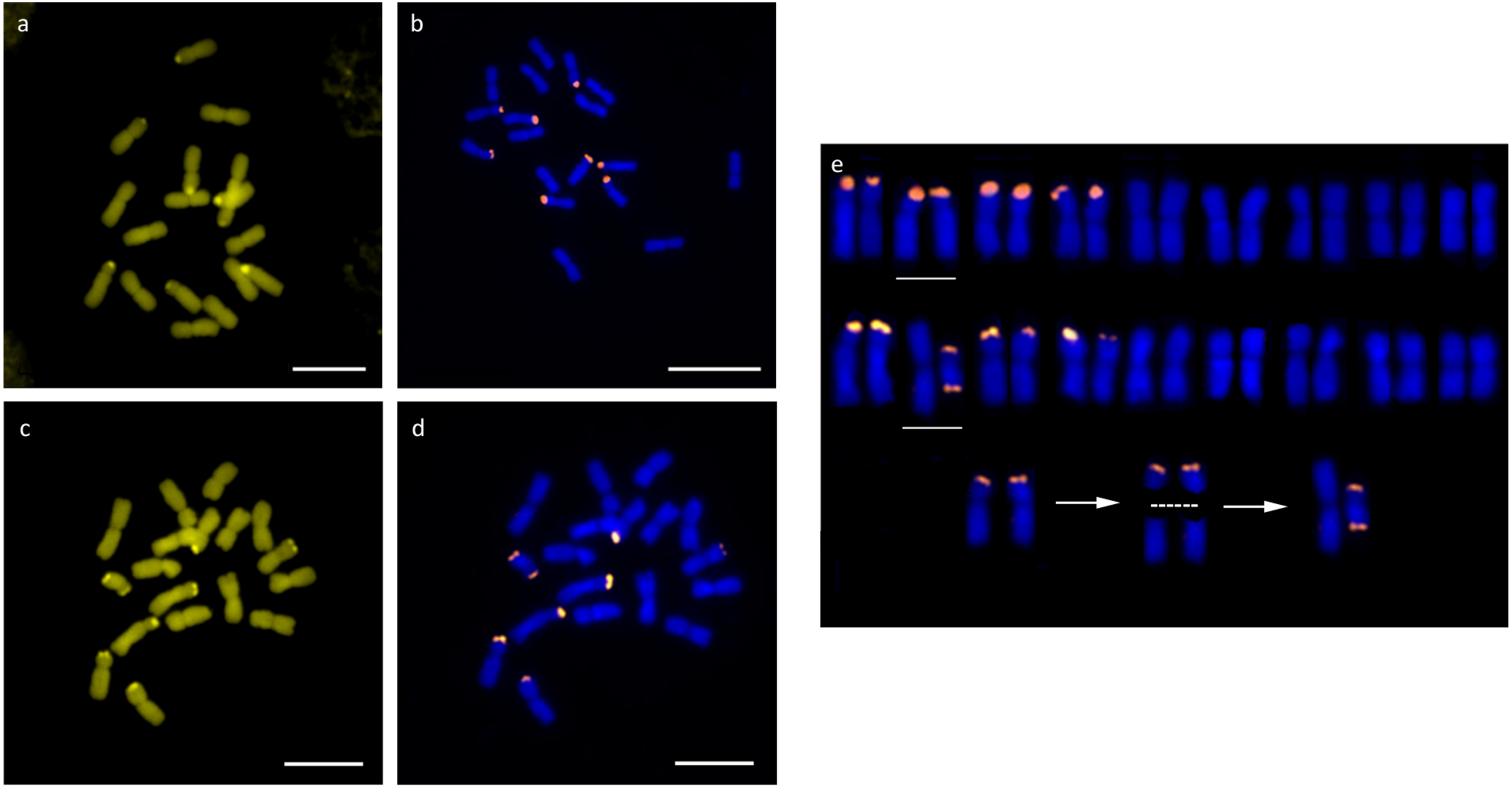
S2 Phylogenetic tree using ITS1, ITS2 and 3'ETS rDNA sequences of *Artemisia*.

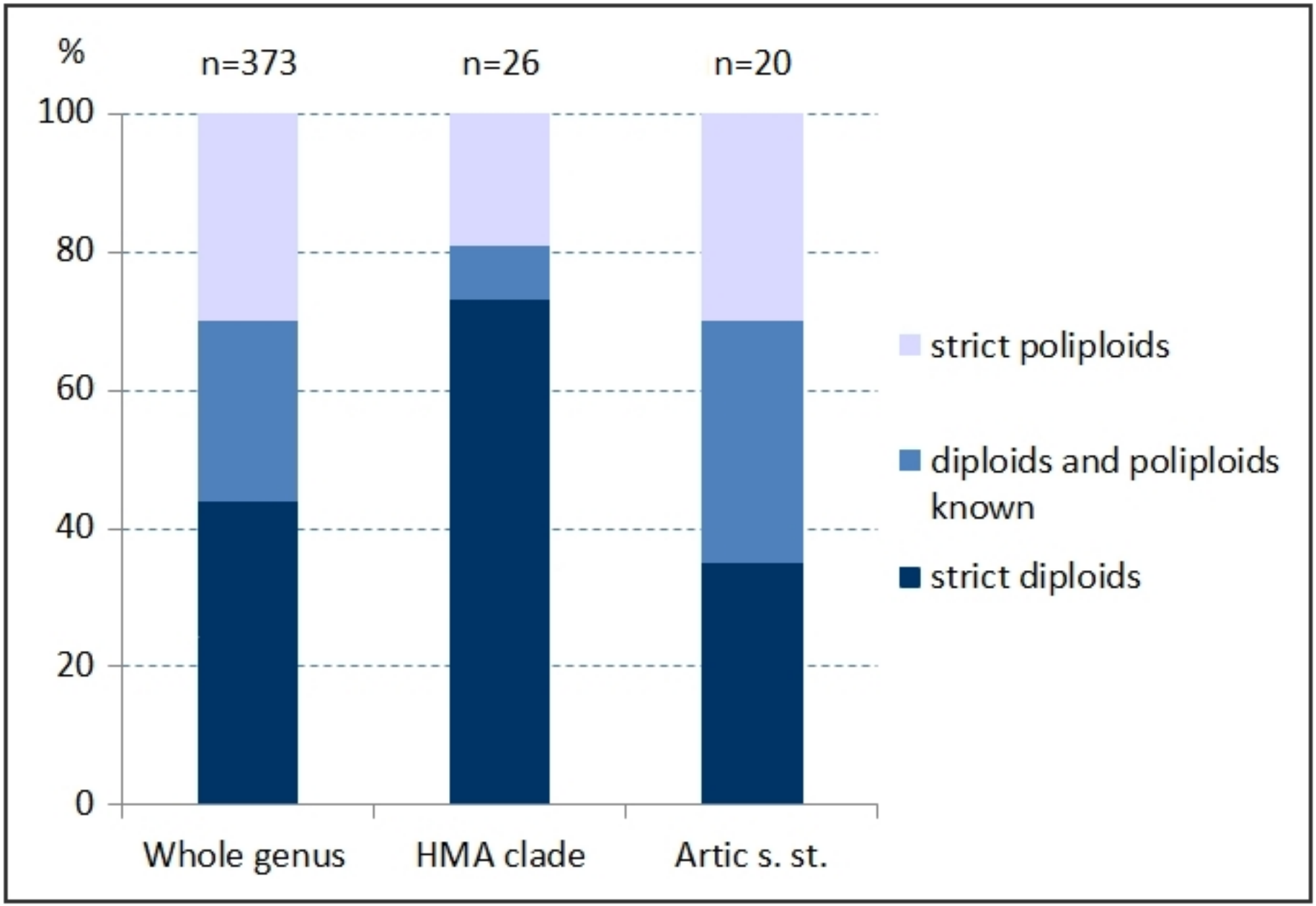
S3 Dynamite plot on mean genome sizes of *x*=8 and *x*=9 based *Artemisia* species from the HMA clade (dark blue) and from the rest of the genus (light blue). "n" indicates sample size.


A. eriantha
A. genipi
A. glacialis
A. granatensis
A. melanolepis
A. nitida
A. splendens
A. umbelliformis
100 km
500 km


Argyranthemum
Nipponanthemum
A. salsoloides
A. atrata
A. tournefortiana
A. biennis
A. magellanica
A. palustris
A. glauca
A. dracunculus
A. giraldii
A. scoparia
A. capillaris
A. commutata
A. sphaerocephala
A. chamaemelifolia
A. fragrans
A. herba-alba
A. annua
A. anethifolia
A. anethoides
A. sylvatica
A. feddei
A. leucophylla
A. vulgaris
A. pattersonii
A. pygmaea
A. rigida
A. tridentata
A. cana
A. tripartita
A. absinthium
A. sieversiana
A. leucodes
A. eriantha
A. genipi
A. judaica
A. lucentica
A. reptans
A. glacialis
A. granatensis
A. paradoxa
A. haussknechtii
A. incana
A. schmidtiana
A. melanolepis
A. frigida
A. austriaca
A. assoana
0
4
8
12
16
Genome size (2C)
3.29 to 4.5
4.5 to 5.71
5.71 to 6.92
6.92 to 8.13
8.13 to 9.34
9.34 to 10.55
10.55 to 11.76
11.76 to 12.97
12.97 to 14.18
14.18 to 15.39
15.39 to 16.6
x
7
8
9
rDNA sites
2
4
6
8
9


a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r


a
b
c
d
e

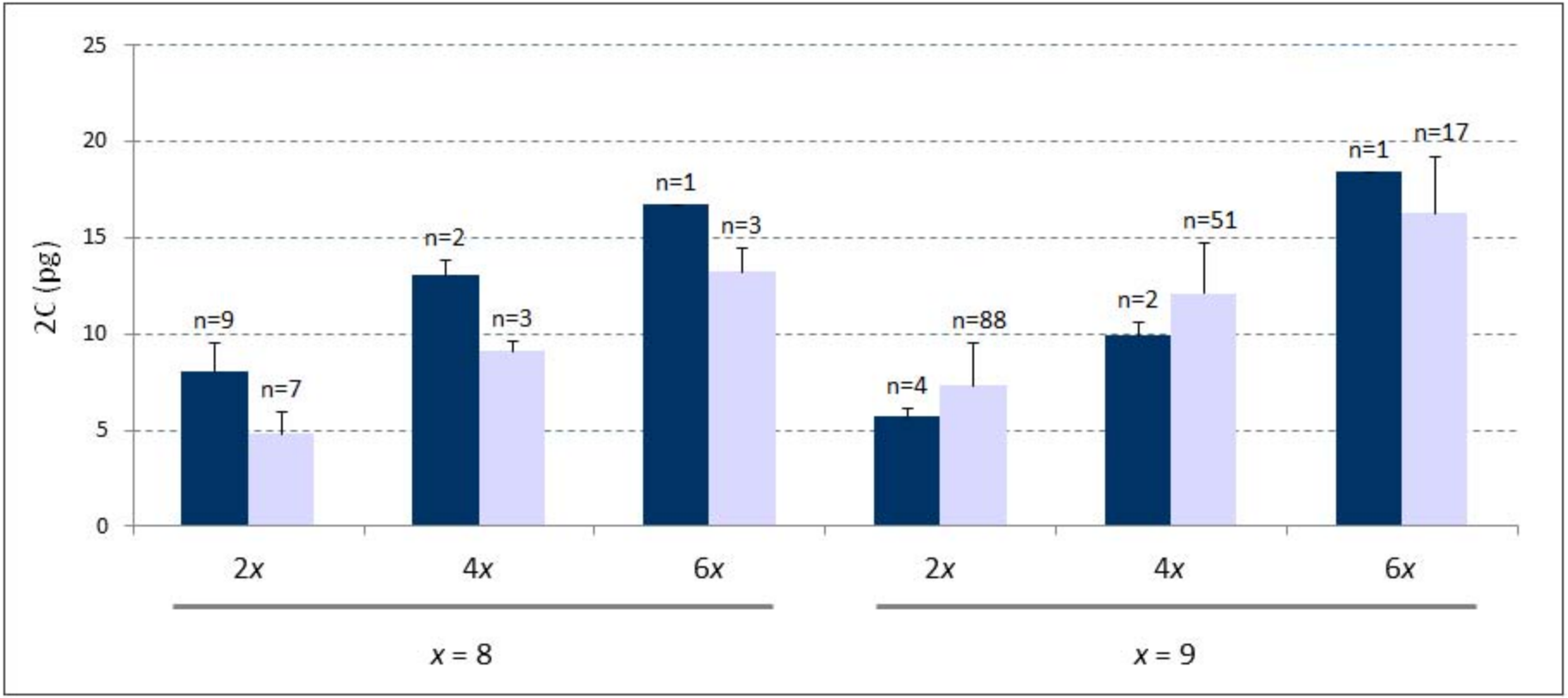

%
n=373
n=26
n=20
100
80
60
40
20
0
Whole genus
HMA clade
Artic s. st.
strict poliploids
diploids and poliploids known
strict diploids

Nipponanthemum
Argyranthemum
Tanacetum
A. salsoloides
A. biennis
A. magellanica
A. capillaris
A. commutata
A. dolosa
A. ledebouriana
A. littoricola
A. marschalliana
A. monostachya
A. pycnorhiza
A. sphaerocephala
A. scoparia
A. changaica
A. dracunculus
A. giraldii
A. glauca
A. palustris
A. tournefortiana
A. atrata
A. blepharolepis
A. anethifolia
A. anethoides
A. annua
A. barrelieri
A. fragrans
A. herba-alba
A. argilosa
A. cana ssp. *bolanderi*
A. tridentata ssp. *xericensis*
A. tripartita ssp. *rupicola*
A. tripartita ssp. *tripartita*
A. tridentata ssp. *tridentata*
A. pygmaea
A. rigida
A. bigelovii
A. pattersonii
A. feddei
A. leucophylla
A. vulgaris
A. sylvatica
A. princeps
A. stolonifera
A. verlotiorum
A. chamaemelifolia
A. macrocephala
A. absinthium
A. sieversiana
A. leucodes
A. aschurbajewii
A. austriaca
A. frigida 2
A. assoana
A. umbelliformis
A. frigida 1
A. nitida
A. melanolepis
A. splendens
A. schmidtiana
A. glacialis
A. granatensis
A. haussknechtii
A. incana
A. paradoxa
A. sericea
A. eriantha
A. genipi
A. judaica
A. lucentica
A. reptans

Artemisia subgenera
- *Absinthium*
- *Artemisia*
- *Dracunculus*
- *Seriphidium*
- *Tridentatae*

"High Mountain and allies" clade (HMA)

0.03


25
20
15
10
5
0
2C (pg)
n=9
n=7
n=2
n=3
n=1
n=3
n=4
n=88
n=2
n=51
n=1
n=17
2x
4x
6x
2x
4x
6x
x = 8
x = 9