

Life cycle versus systematic placement: phylogenetic and cytogenetic studies in annual *Artemisia* (Asteraceae, Anthemideae)

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Abstract: *Artemisia* L. comprises ca. 20 annual species out of ca. 500, which are distributed mainly across the Old World grades, accounting for multiple independent acquisitions of annual habit throughout the evolutionary history of the genus. This makes *Artemisia* an interesting subject for the study of plant life cycle evolution in a phylogenetic context and its consequences at genomic level. The main aim of this phylogenetic analysis was to circumscribe 17 of the annual representatives within the major lineages of *Artemisia*. Genome size has been assessed and ancestral values reconstructed on the tree. Fluorescent in situ hybridisation (FISH) has also been performed to characterise the physical distribution of ribosomal DNA loci. Our results show that annual *Artemisia* have been especially misassigned at subgeneric level and confirm that they are phylogenetically restricted to basal grades, while absent from the derived ones. Annuals display great diversity of genomic traits; however, although most of them show genome downsizing with respect to their most recent common ancestors, no apparent correlation exists between this trait, the number of rDNA sites, and the phylogenetic placement. Nonetheless, such diversity suggests that higher rates of genome restructuring may have been key in governing genome evolution in annual species.

Key words: Ancestral trait reconstruction, C-value, ETS, fluorescent in situ hybridisation, flow cytometry, genome size, ITS, phylogenetic classification

1. Introduction

Artemisia L. is one of the largest genera of the Anthemideae, with ca. 500 species distributed widely across the northern hemisphere; only a few of these extend to South America and North Africa (Funk et al., 2009; Ling et al., 2011). From the early molecular studies by Kornkven et al. (1999) to the present, several approaches aimed to understand the evolutionary relationships of the genus have been published (Vallès et al., 2003; Sanz et al., 2008; Riggins and Seigler, 2012), some focusing on specific complexes (Pellicer et al., 2010a; Garcia et al., 2011; Pellicer et al., 2011; Hobbs and Baldwin, 2013). These works have helped to distil and establish new boundaries for specific subgeneric circumscriptions (Garcia et al., 2011; Riggins and Seigler, 2012; Hobbs and Baldwin, 2013) but have also evidenced taxonomic controversy between traditional classification and molecular compatibilities. Certainly, some of these conflicts are clearly illustrated by the

intricate relationships between subgenera *Absinthium* and *Artemisia*, where the taxonomic utility of morphological characters to differentiate between groups (i.e. floral traits) might have been compromised. Recently, a new lineage accommodating the so-called subgenus *Pacifica* Hobbs & Baldwin has been described (Hobbs and Baldwin, 2013), which includes the Hawaiian endemics and their Asian congener *A. chinensis* L., formerly segregated as an independent monotypic genus (*Crossostephium chinense* (L.) Makino). Notwithstanding, a major in depth re-classification (combining molecular and traditional data of the genus) has not yet been proposed aside from the above-mentioned, and subgenera *Artemisia*, *Absinthium* (Mill.) Less., *Dracunculus* (Besser) Rydb., *Seriphidium* Besser ex Less., and *Tridentatae* (Rydb.) McArthur are still widely used in their traditional circumscription.

Most representatives of the genus are perennial herbs and subshrubs, and some of them reach a relatively high

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degree of woodiness (e.g., subg. *Tridentatae*). Only ca. 20 species are known to be annual or biennial (Poljakov, 1961; Ling et al., 2011). Annual representatives in *Artemisia* are scattered amongst the different subgenera, with the exception of the North American *Tridentatae*, where no endemic annual has been described to date. This suggests that multiple independent acquisitions of annual habit have occurred throughout the evolutionary history of the genus. However, this point has never been specifically tackled before in depth, which makes *Artemisia* an interesting case subject to study the evolution of the plant life cycle in a phylogenetic context.

Environments predicted to favour annual versus perennial life histories are those with low survival of parents and high survival of seedlings (Silvertown and Charlesworth, 2001). Indeed, the distribution of annual and perennial habits within the phylogenies suggests that shifts in life habit may have occurred several times during plant evolution (e.g., *Nemesia* Vent.; Datson and Murray, 2008) but could also be the result of an adaptation triggered by environmental changes (Fiz et al., 2002). In fact, these authors hypothesised that the shift towards annual life history in *Bellis* L. was probably the result of an adaptation to the dry conditions following the Messinian crisis and related to establishment of summer drought in the Mediterranean basin. This is in agreement with the findings of Datson and Murray (2008), who concluded that annual species occur in regions with lower and seasonal rainfall, suggesting that the development of annual forms has allowed the spread into drier environments.

Annual species present different adaptive mechanisms than perennial, and due to their brief life cycle a different genome organisation can be also expected (Bennett and Leitch, 2005). One of the traits that raises the interest of scientists is plant genome size (GS) and its correlation to different aspects of species ecology and biology (Leitch and Bennett, 2007; Pellicer et al., 2013). Among these, a potential correlation between GS and life cycle has been suggested (Bennett, 1972). Indeed, bearing in mind that annual plants develop much faster, it is reasonable to expect them to be characterised by relatively small genomes. However, some authors have also suggested approaching this correlation with caution since many annuals are selfing, and auto-compatibility may have been associated with decreasing GS (Albach and Greilhuber, 2004).

The consequences of a shift in life cycle in relationship to perennial counterparts can also be approached by analysing potential genome restructuring that may have taken place during evolution. Fluorescent in situ hybridisation (FISH) of ribosomal DNA is an interesting approach as it identifies changes in the number, size, and

distribution of repetitive DNA loci in the chromosomes (Schwarzacher and Heslop-Harrison, 2000). Ribosomal RNA genes (35S, also named 18S-5.8S-26S and 5S) are arranged into tandem repeats and may be helpful for understanding evolutionary relationships between closely related species, as is the case in *Artemisia* (Torrell et al., 2003; Hoshi et al., 2006; Pellicer et al., 2010a, 2013). Nevertheless, very few annual species have been characterised cytogenetically to date in the genus, and data are only available for the widespread, Asian-originated *A. annua* L. and the South American endemic *A. magellanica* Sch. Bip. (Torrell et al., 2003; Hoshi et al., 2006; Pellicer et al., 2010a).

Here we used phylogenetic tools to construct an evolutionary framework that represents an essential backbone for testing model-based approaches for tracking trait evolution. In addition, molecular cytogenetic techniques were employed to further understand the implications of life cycle in the systematics and evolution of *Artemisia*. The specific goals pursued in this study were: (i) to circumscribe the annual representatives in the genus along the phylogeny in order to (ii) provide a cytogenetic characterisation of diploid annual species by means of GS assessments and FISH of 35S and 5S rRNA genes and (iii) to discuss the cytogenetic profiles and potential genomic reorganisation in light of life cycle and phylogenetic context.

2. Materials and methods

2.1 Plant materials

Leaf tissue and actively growing root tips of 12 annual species were obtained from cypselae collected in the field and germinated in petri dishes or from potted plants. Note that data from an additional 5 annual species were used from previous published works (see Table 1). Information about the populations studied, vouchers (deposited in the herbaria BCN, Centre for Research on Plant Biodiversity, University of Barcelona and LE, Botanical Institute “Komarov” of the Russian Academy of Sciences), and collectors are listed in Table S1 (on the journal’s website).

2.2. DNA extraction, PCR, sequence editing, and phylogenetic analyses

Most DNA sequences used to build a phylogenetic framework were downloaded from GenBank (many of them from our previous studies; see Table S2 (on the journal’s website) for accession numbers). New sequences were generated for *A. anethifolia* Weber ex Stechm., *A. anethoides* Mattf., *A. blepharolepis* Bunge, *A. jacutica* Drob., and *A. macrocephala* Jacq. ex Besser. DNA was extracted following the CTAB method of Doyle and Doyle (1987). The nuclear nrDNA ITS and ETS regions were amplified

by PCR and sequenced using the same conditions as described in Sanz et al. (2008). Nucleotide sequences were assembled and edited using BioEdit v. 7.0.9 (Hall, 1999). Alignments were made separately for each region with ClustalW (Thompson et al., 1994) using default settings implemented in BioEdit, and gaps were manually adjusted.

Phylogenetic reconstructions using Bayesian inference (BI) were carried out with MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003). The most appropriate nucleotide substitution models for each partition were chosen under the Akaike information criterion (AIC) with MrModeltest (v.2.; Nylander, 2004). The GTR + I + G model was selected for the ITS dataset, GTR + G for the ETS, and GTR + I + G for the concatenated matrix. For each analysis 4 Markov chains were run simultaneously for 20×10^6 generations and sampled every 1000 generations. The MCMC sampling was considered sufficient as the effective sample size (ESS) was >200 in each case after evaluation in Tracer v.1.5 (Rambaut and Drummond, 2007). Data from the first 5×10^6 generations were discarded as the burn-in period in each analysis, and the remaining trees were used to construct a 50% majority-rule consensus tree. Posterior probabilities (PP) of nodes were calculated from the pooled samples.

2.3. Genome size estimations

Genome size was estimated by propidium iodide (PI) flow cytometry following the one-step protocol. Briefly, leaf tissue of the target samples was chopped in 600 μL of LB01 isolation buffer (Doležel et al., 1989) with a razor blade, together with the chosen internal standard, and supplemented with 100 $\mu\text{g}/\text{mL}$ of ribonuclease A (RNase A, Boehringer). Five specimens per population were processed, and 2 independent samples were extracted per individual. Samples were subsequently stained with PI to a final concentration of 60 $\mu\text{g}/\text{mL}$ (Sigma-Aldrich Química), kept on ice for 20 min, and measured in an Epics XL flow cytometer (Coulter Corporation). Further technical details regarding the procedure can be found in Pellicer et al. (2010b). Measurements were carried out at the Scientific and Technological Centers, University of Barcelona.

2.4. Reconstruction of ancestral genome size

A sample of 500 post-burn trees from the initial BI was taken to reconstruct the ancestral GS of selected nodes using BayesTraits v.2 (<http://www.evolution.rdg.ac.uk/BayesTraits.html>). In order to avoid the effect of polyploidy in the estimated nuclear DNA contents, the original tree files were pruned using BayesTrees v.1.3 (www.evolution.rdg.ac.uk/BayesTrees.html) to restrict the sampling to diploid taxa of known GS. Genome size data (1Cx-values) were log-transformed in order to ensure a normal

distribution (Kolmogorov–Smirnov test, $P = 0.213$) prior to analysis. The best fitted model for analysis of continuously varying characters (i.e. random walk versus directional) was selected by conducting BayesFactor tests using the logarithm of the harmonic mean estimated from 5 separate runs under the MCMC option [settings: sampling every 500 generations, 20×10^6 iterations, burn-in of 1×10^6 iterations, and estimating scaling parameters (δ , κ , and λ)]. Parameter values were inspected with Tracer v.1.5 to ensure they were stationary. The random walk model was favoured in all runs and, therefore, selected. The posterior distribution of the scaling parameters generated was used as model-setting for the second phase of the analysis in which we estimated the GS of selected internal nodes by using the add MRCA command. Ancestral GS reconstruction was also conducted using unordered maximum parsimony (MP) as implemented for continuous characters in Mesquite v.2.73 software (Maddison and Maddison, 2007) using the 50% consensus tree as the input tree file.

2.5. Probe labelling and fluorescent in situ hybridisation (FISH)

Accumulation of metaphase chromosomes in root tips for protoplast preparation, probe labelling, and FISH was conducted following the protocol described in Pellicer et al. (2013). Briefly, the 35S rDNA was detected using the clone pTa71, a 9 kb *EcoRI* fragment isolated from *Triticum aestivum* L., which contains the 18S-5.8S-26S genes and the intergenic spacer region (Gerlach and Bedbrook, 1979). The 5S rDNA probe was directly obtained by PCR from *Artemisia princeps* Pamp. The 35S and 5S probes were labelled with avidin-FITC BioNick labelling system (Invitrogen) and digoxigenin-dUTP (Roche Diagnostics), respectively, following the manufacturer's instructions.

Slide preparations were incubated in 100 $\mu\text{g mL}^{-1}$ of DNase-free RNase in $2 \times \text{SSC}$ for 1 h at 37 °C in a wet chamber, washed once in $2 \times \text{SSC}$ (pH 7) for 10 min with slow shaking and then 10 min in $1 \times \text{PBS}$ (pH 7.4), treated with 4% paraformaldehyde in $1 \times \text{PBS}$ for 10 min, denatured at 72 °C with 70% deionised formamide in $2 \times \text{SSC}$ for 1.5 min, dehydrated through an ethanol series (70%, 90%, 100%), and air dried. Probes were denatured by boiling for 10 min, and 15–20 μL was loaded onto each slide. The preparations were denatured for 5 min at 75 °C then quickly cooled to 37 °C and left to hybridise overnight using a Hybaid Omnislide thermal cycler. Post-hybridisation stringency washes were done with agitation as follows: 2 washes in $4 \times \text{SSC}$ at 42 °C for 10 min followed by 1 wash in $2 \times \text{SSC}$ (with 0.2% Triton X-100) at room temperature. For 5S detection, the slides

were treated with 1% (w/v) bovine serum albumin (BSA) in $2 \times$ SSC with 0.2% Triton X-100 for 45 min at 37 °C and then incubated for 1.5 h at 37 °C in $20 \mu\text{g mL}^{-1}$ of anti-digoxigenin-rhodamine Fab fragments (Roche Diagnostics) in the same buffer. Slides were washed twice for 10 min in $2 \times$ SSC with 0.2% Triton X-100 at 42 °C, once in $2 \times$ SSC at room temperature for 5 min, once in distilled water at room temperature for 5 min, and then dehydrated for 5 min each in a series of 70%, 90%, and 100% ethanol. Counterstaining was done with Vectashield (Vector Laboratories), a mounting medium containing 500 ng mL^{-1} of DAPI (4',6-diamidino-2-phenylindole).

3. Results

3.1. Phylogenetic placement of annual *Artemisia*

The nrDNA original set included 234 *Artemisia* representatives selected to provide a good representation of all subgenera and main lineages, of which 17 annual species were analysed together for the first time in a phylogenetic reconstruction of the genus. The concatenated aligned data set consisted of 821 characters, excluding the 5.8S gene, which was not used for phylogenetic analysis since it was missing in several of the sequences downloaded from GenBank (see Table S2). The 50% consensus phylogram corresponding to the analysis of both nuclear datasets is depicted in Figure 1. Preliminary analyses in individual regions (data not shown) did not reveal robust incongruence, mainly because of a lack of resolution in the nodes involved, and there was no significant impact on the overall topology of the tree. Concerning the origin of annual species, according to our phylogenetic reconstruction, annual habit arose in at least 7 independent episodes throughout the evolutionary history of the genus (Figure 1). The distribution pattern of annual species confirmed that these taxa were concentrated in the earlier branches of the tree, whilst the core of the subgenera *Artemisia*, *Pacifica*, *Seriphidium*, and *Tridentatae*, which are late-branched, completely lacked annual representatives (Figure 1). The phylogenetic results highlighted a close relationship between the first subgenus to diverge, *Dracunculus*, and 7 annual taxa. Four of them (*A. edgeworthii* N.P.Balakr., *A. pectinata* Pall., *A. pewzowii* C.Winkl., and *A. scoparia* Waldst.) were embedded in the core of subgenus *Dracunculus*, consistent with their traditional subgeneric assignment. By contrast, *A. biennis* Willd., *A. magellanica*, and *A. palustris* L. were placed sister to the subgenus *Dracunculus* despite their traditional assignment to the subgenus *Artemisia*. Four other annual species (*A. jacutica*, *A. leucodes*, *A. macrocephala*, and *A. sieversiana* Ehrh. ex Willd.) were located in the core of the subgenus *Absinthium*, whilst the remaining annual species

(*A. anethifolia*, *A. anethoides*, *A. annua*, *A. apiacea* Hance, *A. blepharolepis*, and *A. tournefortiana*) were segregated from the main grade of any subgenera. It is striking, however, that only 7 out of the 17 annual species revealed congruence between phylogenetic placements and their traditional subgeneric classification. Furthermore, it is worth highlighting the absence of annual taxa reported in the main grade of the subgenus *Artemisia*, as well as in the core of subgenus *Seriphidium*, although in this case there was a tight relationship between the latter and the annual species *A. anethoides*, *A. anethifolia* (subgenus *Absinthium*), *A. annua*, and *A. apiacea* (subgenus *Artemisia*).

3.2. Nuclear DNA contents and ancestral GS reconstruction

Estimated nuclear DNA contents (2C-values) for the target species are summarised in Table 1 along with previously published reports in *A. annua*, *A. leucodes*, *A. magellanica*, *A. scoparia*, and *A. tournefortiana* (Torrell and Vallès, 2001; Garcia et al., 2004; Pellicer et al., 2010b). Genome sizes varied ca. 4.7-fold, with the small genome of *A. anethoides* ($2C = 3.28 \text{ pg}$) at the lower end and the large genome of *A. leucodes* ($2C = 15.38 \text{ pg}$; Garcia et al., 2004) at the opposite end of the scale. Nuclear DNA contents of the species studied here and those from diploid perennial relatives were superimposed onto a sub-sampled phylogeny to illustrate and reconstruct the dynamics of this parameter within an evolutionary context (Figures 2a and 2b). For those species with more than 1 value available, mean 2C-values were used for subsequent analyses.

The scaling parameter values obtained were as follows: mean κ value = 1.32 (95% confidence interval (CI) ± 0.013), indicating that GS evolved faster in longer branches. Mean $\delta = 0.86$ (CI ± 0.006), evidencing a rate of GS evolution close to constant, although probably early evolution of the group contributed more to trait diversification. Finally, $\lambda = 0.94$ (CI ± 0.001) indicated that GS evolution in *Artemisia* was strongly influenced by phylogenetic signal. As stated in the M & M, these parameters were incorporated into the evolutionary model to reconstruct the most recent common ancestors (MRCAs) of selected nodes of interest (i.e. those defining clades with annual taxa involved (see Figure 2a)), which are also summarised in Table 2. The GS of MRCAs reconstructed using both MP and BI approaches were very similar, showing consistency in the patterns of GS displayed. With the exception of *A. blepharolepis* and *A. leucodes*, the GS of all annual taxa studied were smaller than the reconstructed value for the corresponding MRCA. Due to computing limitations, the MRCA of node 3 (Figure 2a) selected to evaluate the dynamics of *A. blepharolepis* was reconstructed only under MP (Table 2).

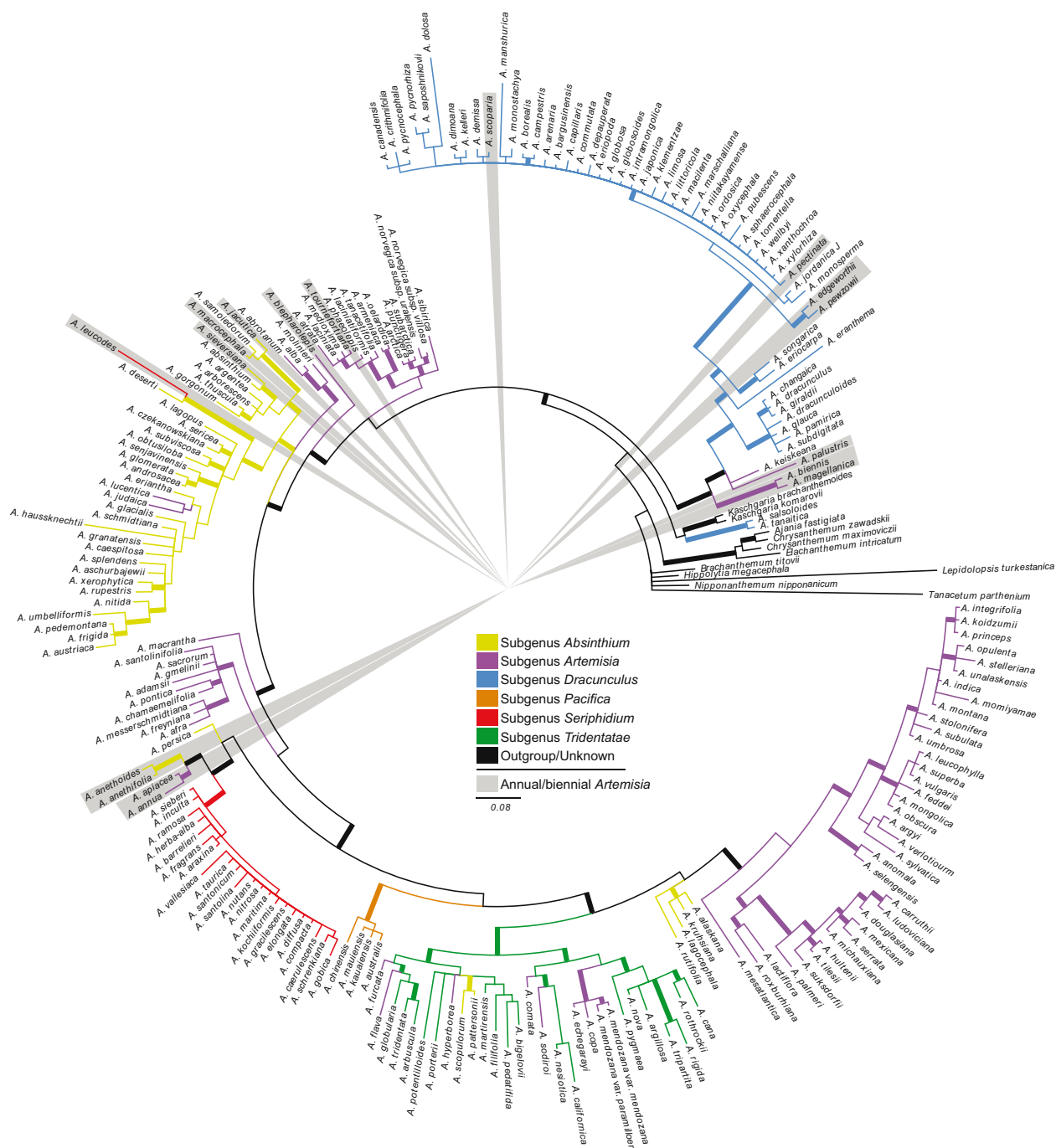


Figure 1. The 50% majority-rule consensus tree from Bayesian inference of the concatenated ITS and ETS dataset. Bold branches indicate nodes with posterior probability values $\geq 95\%$. A traditional subgeneric classification of *Artemisia*, including recent updates from Hobbs and Baldwin (2013), is depicted. Phylogenetic placement of annual taxa is highlighted in grey.

3.3. Physical mapping of 35S and 5S rDNA loci

All the species studied here were found to be diploid, most of them displaying $x = 9$ as the basic chromosome number, with the exception of *A. anethifolia*, *A. anethoides*, and *A. scoparia*, which were $x = 8$ -based. The number of rDNA

loci (35S and 5S) observed in each species is displayed in Table 1. As for the GS data, previous results published on annual species *A. annua* and *A. magellanica* (Hoshi et al., 2006; Pellicer et al., 2010a) were also indicated in the table. Images of the FISH signals on metaphase chromosomes

Table 1. Karyological data, nuclear DNA amounts, and fluorescent in situ hybridisation results in 35S (18S-5.8S-26S) and 5S rDNA loci.

Species	2n ¹	2C ± SD (pg) ²	1Cx ³ (pg)	1Cx (Mbp) ⁴	rDNA signals	
					35S	5S
<i>A. annua</i> *	18	3.50 ± 0.03	1.75	1711.50	2	2
<i>A. anethifolia</i>	16	4.18 ± 0.01	2.09	2044.02	6	6
<i>A. anethoides</i>	16	3.28 ± 0.02	1.64	1608.81	6	6
<i>A. biennis</i> (pop. 1)	18	6.50 ± 0.03	3.25	3178.50	2	2
<i>A. biennis</i> (pop. 2)	18	6.24 ± 0.04	3.12	3056.25	-	-
<i>A. blepharolepis</i>	18	9.98 ± 0.06	4.99	4880.22	2	2
<i>A. jacutica</i> (pop. 1)	18	4.86 ± 0.02	2.43	2376.54	6	6
<i>A. jacutica</i> (pop. 2)	18	4.82 ± 0.03	2.41	2356.98	-	-
<i>A. leucodes</i> *	18	15.38 ± 0.21	7.69	7784.88	8	8
<i>A. macrocephala</i>	18	5.04 ± 0.03	2.52	2464.56	6	6
<i>A. magellanica</i> *	18	6.18 ± 0.16	3.09	3022.02	2	2
<i>A. palustris</i> (pop. 1)	18	5.16 ± 0.05	2.58	2523.24	4	4
<i>A. palustris</i> (pop. 2)	18	5.28 ± 0.03	2.64	2586.81	-	-
<i>A. pectinata</i> (pop. 1)	18	4.98 ± 0.04	2.49	2435.22	-	-
<i>A. pectinata</i> (pop. 2)	18	4.92 ± 0.02	2.46	2410.77	2	2
<i>A. scoparia</i> *	16	3.54 ± 0.02	1.77	1731.06	10	10
<i>A. sieversiana</i> (pop. 1)	18	6.12 ± 0.06	3.06	2992.68	4	4
<i>A. sieversiana</i> (pop. 2)	18	6.16 ± 0.03	3.08	3017.13	-	-
<i>A. tournefortiana</i> *	18	6.68 ± 0.06	3.34	3266.52	4	4

Note: ¹Chromosome counts from Vallès et al. (2001) are confirmed in the present study. ²Nuclear DNA content. ³1Cx = monoploid genome size. ⁴1pg = 978 Mbp (Doležel et al., 2003). (*indicates data from previous works used for statistical analysis (Torrell and Vallès, 2001; Garcia et al., 2004; Hoshi et al., 2006; Pellicer et al., 2010a, 2010b)). Species listed as in Table S1.

are presented in Figure 2c. Ribosomal DNA loci in the species studied revealed a colocalised pattern of both 35S and 5S rDNA regions, all them located in the distal ends or in satellites of chromosomes, in agreement with the findings of Torrell et al. (2001).

4. Discussion

4.1. Phylogenetic circumscription of annual histories in *Artemisia*

The addition of extra annual members in this study did not produce significant topology conflicts among the major *Artemisia* lineages, and the results largely agreed with previous studies in terms of overall topology and placement of annuals (Sanz et al., 2008; Garcia et al., 2011; Pellicer et al., 2011; Riggins and Seigler, 2012; Hobbs and Baldwin, 2013). Our results confirmed that annuals

do not distribute randomly throughout the phylogeny. Instead, they were restricted phylogenetically to basal grades and absent from derived ones (subgenus *Pacifica*, core *Tridentatae*, and core *Artemisia*). Furthermore, a biogeographical pattern was also evidenced. The origin of annual taxa was mostly restricted to the Old World (Figure 2b), with occurrences in the New World derived only from migrations of widespread annual taxa (e.g., *A. biennis* and *A. annua*) towards the Americas, likely following Beringian routes (Riggins and Seigler, 2012). Certainly, according to Pellicer et al. (2010a), the unique South American endemic *A. magellanica* is intimately related to the relatively widespread *A. biennis*, which occurs in North America and Eurasia; hence, a potential speciation by isolation from an ancestral *A. biennis*-like taxon could have been at the origin of the former. Within

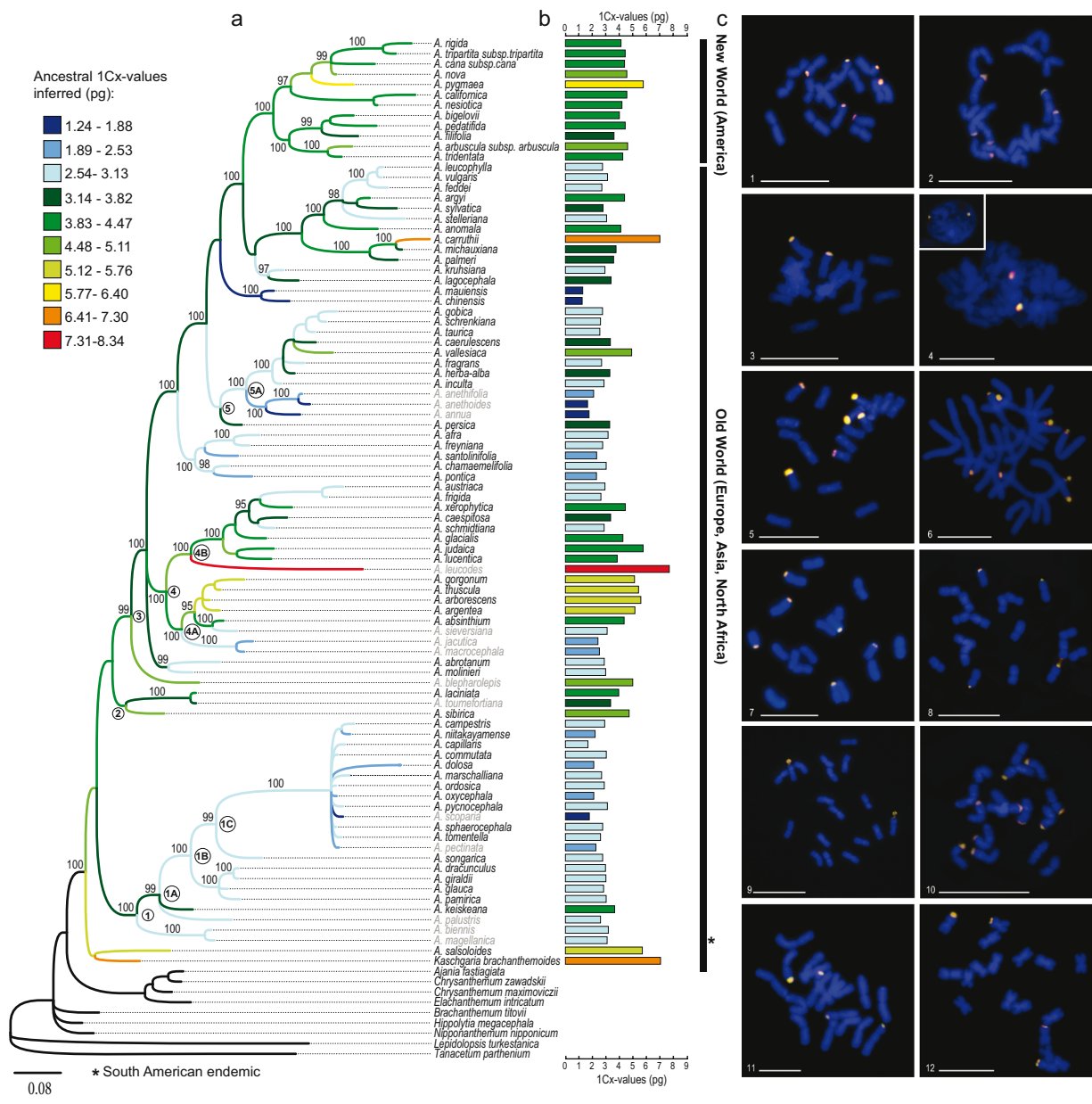


Figure 2. (a) The 50% majority-rule consensus tree from Bayesian inference of the concatenated ITS and ETS dataset pruned to include only diploid representatives of known GS (posterior probabilities are provided when $\geq 95\%$). Ancestral GS reconstruction under MP is depicted along branches and under BI for selected nodes (inferred values on node are included in Table 2). Annual taxa along the phylogeny are highlighted in grey. (b) Superimposed genome size data (1C-values) from diploid representatives in *Artemisia*. (c) Fluorescent in situ hybridisation of rDNA loci 35S (18S-5.8S-26S) and 5S on somatic metaphase protoplasts in annual *Artemisia*: 1. *A. anethifolia*, 2. *A. anethoides*, 3. *A. biennis*, 4. *A. blepharolepis*, 5. *A. jacutica*, 6. *A. leucodes*, 7. *A. macrocephala*, 8. *A. palustris*, 9. *A. pectinata*, 10. *A. scoparia*, 11. *A. sieversiana*, 12. *A. tournefortiana*. Scale bars = 10 μm .

Eurasia, Hobbs and Baldwin (2013) pointed out that annual *A. tournefortiana* and *A. scoparia* have the wider biogeographical origin area in their respective subgeneric clades. These findings in *Artemisia* were consistent with patterns found in other plant groups in which annual representatives have triggered biogeographical expansion

due to their high migration potential (e.g., Lavergne et al., 2012).

As mentioned earlier, the emergence of the annual life habit took place several times (at least 7) during the evolutionary history of the genus (Figure 1). Episodes of multiple origins of annual species have been reported

Table 2. Ancestral genome size (1Cx) inferences for the MRCAs of selected nodes inferred under parsimony and Bayesian (MCMC) approaches (node numbers are depicted in Figure 2a).

Node	Parsimony	MCMC (95% confidence interval)
1	3.744	3.572 (3.566–3.577)
1A	3.414	3.456 (3.341–3.500)
1B	3.087	3.104 (3.099–3.109)
1C	2.957	3.073 (3.067–3.078)
2	4.394	4.253 (4.245–4.278)
3	4.180	-
4	4.155	4.105 (4.099–4.110)
4A	4.242	4.146 (4.140–4.151)
4B	4.482	4.293 (4.286–4.300)
5	3.378	3.678 (3.673–3.683)
5A	2.869	2.542 (2.538–2.546)

in several genera containing both annual and perennial members, such as *Houstonia* L. (Church, 2003) and *Sidalcea* A.Gray (Andreasen and Baldwin, 2001). Fiz et al. (2002) also reported independent origins for the annual life forms derived from perennials in *Bellis*, where annuals occurred in areas with marked summer drought in contrast with perennials, which specialised in colonising wetter areas.

Riggins and Seigler (2012) revealed that many morphological characters were largely homoplastic when traced throughout the *Artemisia* phylogeny. If we apply this classification to annual taxa the scattered distribution of annual species reported here is evidence of some of these major conflicts (Figure 1). Indeed, the annual species have been especially misassigned at the subgeneric level (ca. 60%). The case of *A. blepharolepis* is even more puzzling, because its systematic position has been argued since early morphological studies, although it was never investigated in a molecular phylogeny until now. Ling et al. (2011) supported the inclusion of this taxon as a member of the subgenus *Dracunculus*. However, several authors rejected this hypothesis, instead proposing its inclusion within the subgenus *Artemisia* (e.g., Darijma, 1989). Our results evidenced the segregation of this species from the subgenus *Dracunculus* and firmly place this annual in one of the grades between the subgenera *Seriphidium* and *Absinthium* (Figure 1; PP > 95%). This was also the first attempt to locate *A. jacutica* on a molecular phylogenetic basis; however, in this case the result was congruent with its traditional classification (Figure 1).

Another interesting case of conflicting phylogenetic position is that of *A. leucodes* (subg. *Seriphidium*), which appeared embedded within 1 of the 2 main lineages of the subgenus *Absinthium* (Figure 1; PP > 95%). Vallès et al. (2003) reported its segregation from the core grade of the subgenus *Seriphidium*. Our results confirmed this finding and provided further details on its phylogenetic placement sister to *A. deserti* Krasch. The long length of the branch leading to these 2 species, in addition to the length of the *A. leucodes* branch, indicates an elevated substitution rate, which tends to evolve rapidly in annuals, as previously noted for these markers (Andreasen and Baldwin, 2001). The annual representatives of the subgenus *Dracunculus*, however, illustrated a case contrasting with the above-mentioned conflicts; all were embedded within the main clade of this subgenus (Figure 1), in agreement with the morphological classifications. Most diversity of this group diverged recently (ca. 2–3 Mya) as the result of a radiation across East Asia. Consequently, there is relatively low phylogenetic resolution in this clade (Pellicer et al., 2011). Furthermore, most species showed very low branch length along this unresolved polytomy, thereby preventing conclusive hypotheses about the possible implications of shifts in ecological preferences linked to the emergence of annual life histories in this subgenus.

4.2. Lack of correlation between cytogenetic profiles and systematic placement

Given the apparent inter- and intraspecific stasis of chromosomal features (i.e. karyotype morphometry) in *Artemisia* (see Vallès et al., 2011 for a review of the field), it is difficult to use this information to help clarify systematic relationships among related congeners. Nevertheless, on the basis of a numerical analysis of karyotypes in the genus, Matoba et al. (2007) proposed that the subgenus *Artemisia* could be more advanced. This argument could help explain the more heterogeneous pattern of rDNA loci distribution, with shifts in GS, as well as the phylogenetic placement of annual representatives out of the core clade of this subgenus.

All the studied species traditionally included in subgenus *Artemisia* exhibited the same chromosome number ($2n = 18$), but contrasting nuclear DNA contents and rDNA loci numbers (Figures 2b and 2c; Table 2). *Artemisia palustris*, *A. annua*, and *A. tournefortiana* (Figure 2c) were segregated in different lineages across the genus. These species evidenced a GS reduction with respect to their MRCAs but retained the characteristic number of rDNA loci (i.e. 4) described in perennial congeners (Torrell et al., 2003; Hoshi et al., 2006), suggesting that speciation does not necessarily imply drastic shifts in cytogenetic profiles. However, *A. blepharolepis*, *A. biennis*, and the previously studied *A. magellanica* (Pellicer et al., 2010a),

with just one rDNA locus, deviated significantly from this above-mentioned pattern. Indeed, the loss of rDNA loci number or copies might have an impact on the overall GS of an organism (Prokopowich et al., 2003). Nonetheless, in the case of *A. blepharolepis* this apparent reduction in loci number has occurred in parallel to DNA accumulation, as evidenced by the increase in GS with respect to its MRCA (1Cx = 4.180 pg). Amplification of repetitive DNA (mainly transposable elements, TEs) is widely counteracted by mechanisms stimulating genome contraction, such as recombination (see Kejnovsky et al., 2012 for a review of the subject), which acts as a driving force in maintaining the balance between TE insertion/deletion. However, at this scale, bursts of amplification in specific TE families are key to generating GS diversity, even between closely related species (Wicker et al., 2009), and may have resulted in a GS increase in *A. blepharolepis*.

The subgenus *Absinthium* also revealed heterogeneous behaviour. Annual members segregate in 2 main lineages (Figure 2a) and show unexpected patterns of rDNA loci distribution and GS. The number of ribosomal loci in *A. sieversiana* was consistent with *A. absinthium*, a perennial to which it is intimately related, but revealed evidence of a certain genome downsizing with respect to the remaining species in the clade and their MRCA (1Cx = 3.07 pg, MRCA (1Cx) = 4.24 pg). The FISH results of *A. sieversiana* contrasted with those of *A. jacutica* and *A. macrocephala*, in which an extra rDNA locus was found (Figure 2c). Several mechanisms have been invoked to explain variation in the number of loci among related species, such as structural chromosome rearrangements (Levin, 2002) and transposition (Datson and Murray, 2008). With the results presented here it would be speculative to hypothesise a potential origin of this extra locus, but it is worth highlighting the differences in fluorescence intensity, especially in *A. jacutica*. This has been found in several plant groups, including *Artemisia* (Srisuwan et al., 2006; Pellicer et al., 2013) and reflects the semi-quantitative value of FISH, indicating potential differences in gene copy number (Maluszynska and Heslop-Harrison, 1993).

Artemisia scoparia and *A. pectinata* were the 2 annual diploid members of the subgenus *Dracunculus* studied from a cytogenetic standpoint (Figure 2). Previous research in the genus (Garcia et al., 2004) reported a polyploid population of *A. pectinata* ($2n = 36$, $2C = 10.56$ pg). Polyploidy in annual *Artemisia* is very scarce, with only a single tetraploid population in *A. sieversiana* reported to date (Korobkov and Kotseruba, 2003). The lack of polyploids in annual representatives prevented us from making inferences regarding the dynamics of genome size evolution across this group. Nonetheless, either the recent diversification of the subgenus *Dracunculus* (2–3 Mya),

to which this species belongs, or an autopolyploid origin could be behind the almost proportional increase of DNA content between the 2 cytotypes of this species ($2C = 4.92$ pg/ $2x$, $2C = 10.56$ pg/ $4x$).

When we compared these diploid annuals with their perennial relatives we observed that, without any apparent correlation, annual taxa deviated from the overall cytogenetic profiles in perennials. *A. scoparia* revealed a significant GS reduction (1Cx = 1.77 pg, MRCA (1Cx) = 2.957 pg) coupled with an increase in rDNA sites, which contrasts with the single rDNA locus of *A. pectinata*. The GS reduction found in *A. scoparia* may have been favoured by the ecology of this species. Indeed, the population included in the present work was collected in an intermittently dry river bed. Reductions in total chromosome length and, hence, in overall GS, have been observed in plant groups growing in unstable habitats comprising semidesert regions or seasonally xeric areas, which have to complete their cycle faster than groups inhabiting less stressful environments (Watanabe et al., 1999).

Finally, *A. leucodes* illustrates the largest GS increase with respect to its MRCA (ca. 2-fold) of any diploid annual or perennial member of *Artemisia*, coupled with a considerably higher number of rDNA sites than its perennial counterparts. Although most of the annuals studied here revealed the opposite trend, with genome downsizing predominant in their evolution, Garnatje et al. (2004) reported a GS increase similar to that of *A. leucodes* in the annual *Siebera pungens* J.Gay (Cardueae), with respect to its perennial relatives, and Hidalgo et al. (2008) found an important increase in the number of rDNA sites with respect to the perennial relatives in an annual representative of the *Rhaponticum* Vaill. group, although coupled with an exceptional decrease in GS.

5. Concluding remarks

Annual *Artemisia* taxa are phylogenetically restricted across the genus, although they display great diversity among studied traits (i.e. GS and rDNA loci) suggesting that there is no primary pattern of evolution. This is mainly evidenced by the lack of apparent correlation between these traits, phylogenetic placement of annuals, and the picture found in their close perennial relatives. Given these findings, such diversity (as previously reported by Vallès et al. (2013) for GS) suggests that higher rates of genome restructuring are key to governing genome evolution in annual species.

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Supplementary information:**Table S1.** Information about the origins, collectors, and vouchers of the species studied in this work.

SPECIES	ORIGIN, COLLECTORS, AND VOUCHERS
<i>A. anethifolia</i> Weber ex Stechm.	Russia, Republic of Buryatia, Selenge raion: path from Selenduma to Shanan, bottom of a dried lake, 18.ix.2005, A.A. Korobkov (LE-Korobkov 06-22)
<i>A. anethoides</i> Mattf.	Mongolia, Selenge aimag: Shaamar sum, 3 km west of the sum, Buureg Tolgoi hills, near river Okhon, 700 m, 9.ix.2004, Sh. Darijmaa, Sh. Tsooj, J. Vallès & E. Yatamsuren (BCN 23790)
<i>A. biennis</i> Willd. (1)	Canada, Mississauga: University campus, on a road margin. 22.x.2008, J. Pellicer (BCN)
<i>A. biennis</i> Willd. (2)	United States of America, Utah: Uinta National Forest, Santaquim canyon, on ruderal soils. 31.viii.2008, S. Garcia, E.D. McArthur, S.C. Sanderson & J. Vallès (BCN SC28)
<i>A. blepharolepis</i> Bunge	Mongolia, Umnu (South) Gobi aimag: Bulgan sum, 1 km north of the sum, desert steppe, 26.viii.2004, Sh. Darijmaa, D. Samjid, Sh. Tsooj & J. Vallès (BCN 34490)
<i>A. jacutica</i> Drob. (1)	Russia, Sakha Republic (Yakutya): Ust-Aldans camp, near the village of Oner, ruderal, 10.ix.2005, V.N. Zakharova (LE 06-31)
<i>A. jacutica</i> Drob (2)	Russia, Republic of Buriatya: Eravnin district, between lakes Bolshoe Eravnoe and Maloe Eravnoe, 4.x.2007, A.A. Korobkov (LE)
<i>A. leucodes</i> Schrenk.	Uzbekistan, Dgizak: near lake Aidarkul, 1 km from Issikul, semi-desert, 8xi1999, L. Kapustina, F. Khassanov, A. Susanna S2064 & J. Vallès (BCN 11631)
<i>A. macrocephala</i> Jacq. ex Besser	Mongolia, Uvur Khangai aimag: Arvaykheer city, ruderal in streets, 30.viii.2004, Sh. Darijmaa, Sh. Tsooj & J.Vallès (BCN 23801)
<i>A. palustris</i> L. (1)	Mongolia, Uvur-Khangai aimag: Khotont sum, 10 km east, margins of cultivated fields in steppe area, 26.viii.2004, Sh. Darijmaa, Sh. Tsooj & J. Vallès (BCN 34847)
<i>A. palustris</i> L. (2)	Russia, Republic of Buriatya: district of Selenge. Near Bilyutai, 18.ix.2005, A.A. Korobkov (LE 06-20)
<i>A. pectinata</i> Pall. (1)	People's Republic of China, Inner Mongolia: Ulanqab province, Dörnböt /Siziwang qi, 29.viii.2007, B. Liu, R. Cao & J. Vallès (BCN)
<i>A. pectinata</i> Pall. (1)	Mongolia, Umnu (South) Gobi aimag: 10 km S of Bulgan sum, 1.ix.2004, Sh. Darijmaa, D. Samjid, Sh. Tsooj & J. Vallès (BCN Mong. 54)
<i>A. scoparia</i> Waldst. & Kit.	Uzbekistan, Karakalpakstan: Sultanuizdag mountains, near the road from Gazli to Nukus, 79 km from Nukus, dry river bed, 400 m, 3xi1999, L. Kapustina, F. Khassanov, A. Susanna S2044 & J. Vallès (BCN 11628)
<i>A. sieversiana</i> Ehrh. ex Willd. (1)	Russia, Chitin oblast, Kyr raion: northern part of Onon-Baldzhin mountain system, southern slope, valley of a small river, 24.viii.2005, A.A. Korobkov (LE-Korobkov 06-24)
<i>A. sieversiana</i> Ehrh. ex Willd. (2)	Mongolia, Ulaan Baatar, within the city, 7.ix.2004, Sh. Darijmaa, Sh. Tsooj & J. Vallès (BCN-Mong.80)
<i>A. tournefortiana</i> Reichenb.	Uzbekistan, Karakalpakstan: 25 km from Muynak, near the road to Nukus; banks of a channel, 100 m, 4.xi.1999, L. Kapustina, F. Khassanov, A. Susanna S2047, J. Vallès & M. Nizamitdin, (BCN 11630)

Table S2. GenBank accessions and 2C-values used from bibliography of taxa included in Figure 2.

SPECIES	GENBANK/ENA ACCESSION NUMBERS		GS (2C-value)*
	ITS	ETS	
<i>Artemisia abrotanum</i>	JX051694	JX069394	5.77
<i>A. absinthium</i>	AF079946	DQ028850	8.7
<i>A. adamsii</i>	AM398844	AM397953	
<i>A. afra</i>	JX051743	JX069431	6.32
<i>A. alaskana</i>	AM398845	AM397954	
<i>A. alba</i>	JX051695	JX069395	
<i>A. androsacea</i>	AM398846	AM397955	
<i>A. anethifolia</i>	LK391723	LK391718	4.18
<i>A. anethoides</i>	LK391724	LK391719	3.28
<i>A. annua</i>	AM398847	AM397956	3.5
<i>A. anomala</i>	JX051674	JX069377	8.2
<i>A. apiacea</i>	AM398848	AM398033	
<i>A. araxina</i>	AF045408-AF079959	DQ028870	
<i>A. arborescens</i>	AF045393-AF079945	FJ642934	11.18
<i>A. arbuscula</i>	HQ019034	HQ018992	9.22
<i>A. arctica</i>	AM398849	AM397958	
<i>A. arenaria</i>	JF326532	JF326588	
<i>A. argentea</i>	JX051696	JX069396	10.3
<i>A. argillosa</i>	HQ019037	HQ018995	8.76
<i>A. argyi</i>	FJ528302	FJ642936	
<i>A. armeniaca</i>	JX051693	JX069393	
<i>A. atrata</i>	AF504170-AF504143	FJ642937	
<i>A. aschurbajewii</i>	FJ642973-FJ643009	DQ028838	
<i>A. australis</i>	JX051753	JX069435	
<i>A. austriaca</i>	AF504171-AF504144	DQ028844	5.86
<i>A. bargusinensis</i>	JF326533	JF326589	
<i>A. barrelieri</i>	AF045410	DQ028875	
<i>A. biennis</i>	GU902817-GU902829	GU902841	6.36
<i>A. bigelovii</i>	HQ019038	HQ018996	8
<i>A. blepharolepis</i>	LK391725	LK391720	9.98
<i>A. borealis</i>	JF326534	JF326590	
<i>A. caerulescens</i>	AF045409-AF07996	DQ028872	6.66
<i>A. caespitosa</i>	AM398855	AM397957	6.7
<i>A. californica</i>	HQ019039	HQ018997	8.58

Table S2. (Continued).

<i>A. campestris</i>	JX051736	JX069426	5.82
<i>A. cana</i>	HQ019041	DQ028882	8.76
<i>A. canadensis</i>	AM398856	AM397963	
<i>A. capillaris</i>	JF326535	JF326591	3.34
<i>A. carruthii</i>	JX051722	JX069416	14
<i>A. chamaemelifolia</i>	FJ642974-FJ643010	FJ642938	6.04
<i>A. chinensis</i>	AB359701-AB359787	AB359884	2.54
<i>A. comata</i>	AM398859	AM397966	
<i>A. commutata</i>	JF326538	JF326594	6.06
<i>A. compacta</i>	AM398861	AM397968	
<i>A. copa</i>	GU902807-GU902819	GU902831	
<i>A. crithmifolia</i>	AF045399-AF079962	DQ028856	
<i>A. czekanowskiana</i>	AM398862	AM397969	
<i>A. demissa</i>	JF326539	JF326595	
<i>A. depauperata</i>	JF326540	JF326596	
<i>A. deserti</i>	HQ019043	HQ019001	
<i>A. desertorum</i>	FJ642976-FJ643012	FJ642940	
<i>A. diffusa</i>	JX051653	JX069362	
<i>A. dolosa</i>	JF326543	JF326599	4.2
<i>A. douglasiana</i>	JX051723	JX069417	
<i>A. dracunculiformis</i>	AM398865	AM397972	
<i>A. dracunculoides</i>	JF326544	JF326600	
<i>A. dracunculus</i>	JF326545	JF326601	5.94
<i>A. changaica</i>	JF326537	JF326593	
<i>A. glauca</i>	JF326550	JF326606	
<i>A. echegarayi</i>	GU902811-GU902823	GU902833	
<i>A. edgeworthii</i>	JF326547	JF326661	
<i>A. elongata</i>	JX051746	JX069432	
<i>A. eranthema</i>	AF504195-AF504168	DQ028864	
<i>A. eriantha</i>	DQ028919-DQ028906	DQ028842	
<i>A. eriocarpa</i>	AF504191-AF504164	DQ028863	
<i>A. eriopoda</i>	JF326548	JF326604	
<i>A. feddei</i>	FJ642977-FJ643013	FJ642941	5.4
<i>A. filifolia</i>	HQ019045	HQ019003	7.2
<i>A. flava</i>	AM398867	AM397974	
<i>A. fragrans</i>	AF045406-AF079957	DQ028871	5.36

Table S2. (Continued).

<i>A. freyniana</i>	AM398868	AM397975	5.52
<i>A. frigida</i>	HQ019046	HQ019004	5.26
<i>A. furcata</i>	AM398870	AM397977	
<i>A. giraldii</i>	JF326549	JF326605	6
<i>A. glacialis</i>	DQ028921-DQ028908	DQ028840	8.52
<i>A. glauca</i>	AM398871	AM397978	5.66
<i>A. globosa</i>	JF326551	JF326607	
<i>A. globosoides</i>	JF326552	JF326608	
<i>A. globularia</i>	AM398872	AM397979	
<i>A. glomerata</i>	AM398873	AM397980	
<i>A. gmelinii</i>	AM398875	AM397982	
<i>A. gobica</i>	AM398876	AM397983	5.5
<i>A. gorgonum</i>	AM398877	AM397984	10.24
<i>A. gracilescens</i>	JX051731	JX069421	
<i>A. granatensis</i>	AF045397-AF079949	DQ028841	
<i>A. haussknechtii</i>	AF504173-AF504146	DQ028837	
<i>A. herba alba</i>	AF045403-AF079954	DQ028874	6.58
<i>A. hultenii</i>	AM398878	AM397985	
<i>A. hyperborea</i>	AM398879	AM397986	
<i>A. inculta</i>	AF045405-AF079956	DQ028878	5.72
<i>A. indica</i>	JX051676	JX069379	
<i>A. integrifolia</i>	AM398880	AM397987	
<i>A. intramongolica</i>	JF326553	JF326609	
<i>A. jacutica</i>	LK391726	LK391721	4.82
<i>A. jacutica</i>	JF326554	AM397988	
<i>A. japonica</i>	JF326555	JF326610	
<i>A. jordanica</i>	AF504175-AF504148	JF326611	
<i>A. judaica</i>	X051754	DQ028848	11.52
<i>A. kauaiensis</i>	JF326556	JX069436	
<i>A. keiskeana</i>	JF326557	JF326612	7.3
<i>A. kelleri</i>	JF326558	JF326613	
<i>A. klementzae</i>	JX051747	JF326614	
<i>A. kochiiiformis</i>	AM398884	JX069433	
<i>A. koidzumii</i>	AM398885	AM398035	
<i>A. kruhsiana</i>	AM398886	AM397990	5.86
<i>A. laciniata</i>	AM398887	AM397991	7.9

Table S2. (Continued).

<i>A. laciniatiformis</i>	FJ642984-FJ643020	AM397992	
<i>A. lactiflora</i>	DQ028917-DQ028904	FJ642948	
<i>A. lagocephala</i>	AM398889	DQ028898	6.76
<i>A. lagopus</i>	FJ980353	AM397994	
<i>A. leucodes</i>	AF504176-AF504149	DQ028893	15.38
<i>A. leucophylla</i>	AM398890	AM397995	5.5
<i>A. limosa</i>	JF326561	JF326617	
<i>A. littoricola</i>	JF326562	JF326618	
<i>A. lucentica</i>	AF045390-AF079943	DQ028846	7.68
<i>A. ludoviciana</i>	HQ019048	HQ019006	
<i>A. macilenta</i>	JF326563	JF326619	
<i>A. macrantha</i>	DQ059335-DQ059336	DQ028861	
<i>A. macrocephala</i>	LK391727	LK391722	5.04
<i>A. magellanica</i>	GU902814-GU902826	GU902838	6.18
<i>A. manshurica</i>	JF326564	JF326620	
<i>A. maritima</i>	FJ642987-FJ643023	FJ642951	
<i>A. marschalliana</i>	AF504177-AF504150	JF326621	5.36
<i>A. martirensis</i>	JX051734	JX069423	
<i>A. mauiensis</i>	JX051755	JX069437	2.56
<i>A. medioxima</i>	FJ642988-FJ643024	FJ642952	
<i>A. mendozana</i> var. <i>mendozaana</i>	GU902813-GU902825	GU902837	
<i>A. mendozana</i> var. <i>paramilloensis</i>	GU902808-GU902820	GU902834	
<i>A. mesatlantica</i>	JX051749	JX069474	
<i>A. messerschmidtiana</i>	AM398894	AM397998	
<i>A. mexicana</i>	AF045414-AF079966	DQ028892	
<i>A. michauxiana</i>	AM398895	AM397999	7.5
<i>A. molinieri</i>	AF045389-AF079941	DQ028888	5.96
<i>A. momiyamae</i>	FJ642989-FJ643025	FJ642953	
<i>A. mongolica</i>	FJ642990-FJ643026	FJ642954	
<i>A. monosperma</i>	JF326565	JF326622	
<i>A. monostachya</i>	JF326566	JF326623	
<i>A. montana</i>	FJ642991-FJ643027	FJ642955	
<i>A. nesiotica</i>	HQ019049	HQ019007	8.38
<i>A. niitakayamensis</i>	FJ642992-FJ643028	FJ642956	4.4
<i>A. nitida</i>	JX051698	JX069398	
<i>A. nitrosa</i>	JX051654	JX069363	

Table S2. (Continued).

<i>A. norvegica</i> subsp. <i>uralensis</i>	AM398897	AM398001	
<i>A. norvegica</i> subsp. <i>villosula</i>	AM398898	AM398002	
<i>A. nova</i>	AF045412-AF079964	DQ028883	9.1
<i>A. nutans</i>	JX051661	JX069445	
<i>A. obscura</i>	AM398899	AM398003	
<i>A. obtusiloba</i>	AM398900	AM398004	
<i>A. oleandica</i>	JX051692	JX069392	
<i>A. opulenta</i>	AM398901	AM398005	
<i>A. ordosica</i>	JF326568	JF326625	5.76
<i>A. oxycephala</i>	JF326569	JF326626	4.2
<i>A. palmeri</i>	HQ019052	HQ019010	7.14
<i>A. palustris</i>	JF326570	JF326627	5.2
<i>A. pamirica</i>	JF326571	JF326628	6.04
<i>A. pattersonii</i>	JX051737	JX069425	
<i>A. pectinata</i>	DQ028927-DQ028914	DQ028895	
<i>A. pedatifida</i>	EU111672-EU111673	HQ019011	8.86
<i>A. pedemontana</i>	JX051702	JX069456	
<i>A. persica</i>	AF504179-AF504152	DQ028880	6.56
<i>A. pewzowii</i>	JF326572	JF326687	
<i>A. phaeolepis</i>	AM398905	AM398009	
<i>A. pontica</i>	FJ642993-FJ643029	FJ642957	4.6
<i>A. porterii</i>	HQ019054	HQ019012	
<i>A. potentilloides</i>	JX051760	JX069441	
<i>A. princeps</i>	AM398906	AM398037	
<i>A. pubescens</i>	JF342549	JF342552	
<i>A. punctigera</i>	AM398908	AM398011	
<i>A. pycnocephala</i>	JF326573	JF326630	6.22
<i>A. pycnorhiza</i>	JF326574	JF326631	
<i>A. pygmaea</i>	HQ019055	HQ019013	11.54
<i>A. ramosa</i>	FJ642994-FJ643030	FJ642958	
<i>A. rigida</i>	HQ019056	HQ019014	
<i>A. rothrockii</i>	HQ019057	HQ019015	8.24
<i>A. roxburghiana</i>	JX051682	JX069384	
<i>A. rupestris</i>	AM398910	AM398013	
<i>A. rutifolia</i>	AF504180-AF504153	DQ028849	
<i>A. sacrorum</i>	JX051733	JX069422	

Table S2. (Continued).

<i>A. salsoloides</i>	JF342550	JF342553	11.4
<i>A. samoiedorum</i>	AM398912	AM398015	
<i>A. santolina</i>	AF504181-AF504154	DQ028873	
<i>A. santolinifolia</i>	AF504182-AF504155	DQ028836	4.62
<i>A. santonicum</i>	JX051656	JX069364	
<i>A. saposchnikovii</i>	JF326575	JF326632	
<i>A. schmidtiana</i>	FJ642996-FJ643032	FJ642960	5.74
<i>A. schrenkiana</i>	FJ642997-FJ643033	FJ642961	5.2
<i>A. scoparia</i>	JF326576	JF326633	3.54
<i>A. scopulorum</i>	JX051709	JX069404	
<i>A. selengensis</i>	FJ642998-FJ643034	FJ642962	
<i>A. senjavinensis</i>	AM398915	AM398018	
<i>A. sericea</i>	AM398916	AM398019	
<i>A. serrata</i>	JX051706	JX069401	
<i>A. sibirica</i>	FJ643006-FJ643042	FJ642970	
<i>A. sieberi</i>	AF045407-AF079958	DQ028876	
<i>A. sieversiana</i>	AF504183-AF504156	DQ028851	6.18
<i>A. sodiroi</i>	JX051667	JX069370	
<i>A. songarica</i>	JF326577	JF326634	5.52
<i>A. sphaerocephala</i>	JF326579	JF326636	5.52
<i>A. splendens</i>	AF045396-AF079948	DQ028845	
<i>A. stelleriana</i>	DQ028918-DQ028905	DQ028896	6.1
<i>A. stolonifera</i>	FJ643000-FJ643036	FJ642964	
<i>A. subarctica</i>	AM398920	AM398023	
<i>A. subdigitata</i>	JF326580	JF326637	
<i>A. subulata</i>	FJ643001-FJ643037	FJ642965	
<i>A. subviscosa</i>	AM398921	AM398024	
<i>A. suksdorfii</i>	JX051707	JX069402	
<i>A. superba</i>	AM398922	AM398025	
<i>A. sylvatica</i>	FJ643002-FJ643038	FJ642966	5.58
<i>A. tanacetifolia</i>	AM398923	AM398026	
<i>A. tanaitica</i>	JF342551	JF342554	
<i>A. taurica</i>	FJ643003-FJ643039	FJ642967	5.1
<i>A. thuscula</i>	AM398924	AM398038	10.84
<i>A. tilesii</i>	AM398925	AM398027	
<i>A. tomentella</i>	JF326581	JF326638	5.2

Table S2. (Continued).

<i>A. tournefortiana</i>	JX051729	JX069419	6.7
<i>A. tridentata</i>	AF045411-AF079963	DQ028884	8.5
<i>A. tripartita</i>	JX051664	JX069367	8.86
<i>A. umbelliformis</i>	AF045395-AF079947	DQ028843	
<i>A. umbrosa</i>	FJ643004-FJ643040	FJ642968	
<i>A. unalaskensis</i>	AM398926	AM398028	
<i>A. vallesiaca</i>	FJ643005-FJ643041	FJ642969	9.82
<i>A. verlotiorum</i>	AF045387-AF079939	DQ028891	
<i>A. vulgaris</i>	AM398927	AM398029	6.24
<i>A. wellbyi</i>	JF326583	JF326640	
<i>A. xanthochroa</i>	JF326584	JF326641	
<i>A. xerophytica</i>	AM398929	AM398031	8.88
<i>A. xylorhiza</i>	JF326585	JF326642	
<i>Ajania fastigiata</i>	AF504142-AF504169	DQ028868	
<i>Brachanthemum titovii</i>	AF504185-AF504158	DQ028867	
<i>Chrysanthemum maximowiczii</i>	DQ028923-DQ028910	DQ028899	
<i>Chrysanthemum zawadskii</i>	DQ028924-DQ028911	DQ028901	
<i>Elachanthemum intricatum</i>	AF504186-AF504159	DQ028869	
<i>Hippolytia megacephala</i>	AF504161-AF504188	DQ028866	
<i>Kaschgaria brachanthemoides</i>	AF504189-AF504162	DQ028865	
<i>Kaschgaria komarovii</i>	DQ028925-DQ028912	DQ028902	
<i>Lepidolopsis turkestanica</i>	AF504190-AF504163	DQ028835	
<i>Nipponanthemum nipponicum</i>	DQ028926-DQ028913	DQ028834	
<i>Tanacetum parthenium</i>	AF504167-AF504194	DQ028833	

*Genome size values obtained from the GSAD-Genome Size in Asteraceae database (release 2.0), 2013.