Contribution to the genome size knowledge of New World species from the Heliantheae alliance (Asteraceae)

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Abstract

This paper contributes first genome size assessments by flow cytometry for 16 species, 12 genera and three tribes from family Asteraceae, mostly belonging to the Heliantheae alliance, an assembly of 13 tribes from subfamily Asteroideae with a large majority of its species in the New World. Most genome sizes are accompanied by their own chromosome counts, confirming in most cases, although not all, previous counts for the species, and revealing possible cases of unknown dysploidy or polyploidy for certain taxa. The data contribute to the pool of knowledge on genome size and chromosome numbers in the family Asteraceae and will further allow deeper studies and a better understanding on the role of dysploidy in the evolution of the Heliantheae alliance. However, we still lack data for tribes Chaenactideae, Neurolaeneae, Polymnieae and Feddeae (the latter, monospecific) to complete the alliance representation.

Key words: chromosome counts, Compositae, C-value, flow cytometry, nuclear DNA amount, nuclear DNA content

Introduction

Genome size is the amount of nuclear DNA in an organism, and it is a very relevant biological character, with which many biotic and abiotic characters are correlated (Bennett and Leitch 2005). Swift (1950) noted the constancy of this value and proposed the term C-value (where C stands for constant), as the DNA content of the unreplicated haploid complement. The study of genome size and its variation has been receiving general attention by plant biologists since the first systematic compilations of plant genome sizes (Bennet and Smith 1976) and further by the onset of the Plant DNA C-values Database (<http://data.kew.org/cvalues/>). However, still genome size is currently known for only around 2% of angiosperms (Vallès et al. 2017).

The Asteraceae (Compositae) is one of the largest families of angiosperms containing the second largest number of described species of any plant family (24,700) after the Orchidaceae (26,470), distributed in about 1620 genera found virtually everywhere (Christenhusz et al. 2017). Given these high figures, together with their cosmopolitan distribution and the particular flower and inflorescence (capitula) features, the family has been subject to extensive studies (even creating its own discipline, the synantherology), including more recent ones based on DNA sequencing (Panero et al. 2016). One of the major outcomes of phylogenetic molecular studies of the Asteraceae is the recognition of the Heliantheae alliance (Panero 2007), an assembly of tribes within subfamily Asteroideae, comprising about 5838 species or ca. 23% of the species recognized in the family including sunflowers, eupatoriums and sneezeweds (Panero and Crozier 2016). The alliance consists of 13 tribes: Bahieae, Chaenactideae, Coreopsideae, Eupatorieae, Feddeae, Helenieae, Heliantheae, Madieae, Millerieae, Neurolaeneae, Perityleae, Polymnieae and Tageteae (Panero et al. 2016). Most of them originated in the New World and some of its species have radiated worldwide. There is

a morphological trait that nearly all members of the Heliantheae alliance share, the presence of phytomelanin in their fruits. It is an extracellular layer, very resistant to degradation which appears between the hypodermis and sclerenchyma (Pandey et al. 1989, 2014) and produced and secreted by fiber cells (De-Paula et al. 2013). It usually has diagnostic surface features occasionally used for taxonomic purposes (Robinson, 1981; Stuessy and Liu 1983).

It is considered that dysploidy has played a major role in the evolution and diversification of the alliance (Mota et al. 2016; Panero and Crozier 2016). The basic chromosome number of most of the alliance tribes is $x=19$, which is the highest of the family (whose inferred ancestral basic chromosome number, also the most common, is $x=9$; Mota et al. 2016) and it is considered secondarily derived (Semple and Watanabe 2009). Most likely, processes of dysploidy, polyploidy and subsequent diploidisation are involved in the evolution of the basic number in Asteraceae (Smith 1975; Robinson 1981), while dysploidy seems to shape this evolution particularly in the Heliantheae alliance. In this regard, while tribes Heliantheae, Helenieae, Tageteae, Millerieae, Madieae and Perityleae are $x=19$ -based, other tribes have dysploid basic numbers with respect to $x=19$, such as Coreopsideae ($x=18$), Bahieae ($x=17$), Polymnieae ($x=15$) or Eupatorieae ($x=17$), among others (Semple and Watanabe 2009).

Knowledge on genome size in the Asteraceae, and particularly in the Heliantheae alliance, is relatively limited. There is a web-based database aimed to assemble known nuclear DNA amounts in the family, the genome size in the Asteraceae database (www.asteraceagenomesize.com; Garnatje et al. 2011), which is currently being updated (Garcia et al., in preparation). Currently data are available for about 5% of the species in the family (Garcia et al., 2014), of which about 500 belong to the tribes of the Heliantheae alliance. The purpose of the present work is to contribute to the pool of

genome sizes in these tribes and in some species of the closely related Astereae tribe, mainly in taxa native to the New World. We accompany the genome size measurements with chromosome counts whenever possible, and we discuss genome size and chromosome numbers in the context of known data of these groups.

Materials and Methods

Plant materials were collected in the field from natural populations. Vouchers, containing the complete information on each population, have been deposited in the herbarium of the University of Texas at Austin (TEX). For genome size measurements, fresh leaves were collected from seedlings grown from the harvested cypselae. For chromosome counts, root tip meristems were obtained from cypselae sowed in wet filter paper in Petri dishes.

For chromosome counts root tips were pre-treated with 0.05% aqueous colchicine at room temperature for 3 h, fixed and preserved in absolute ethanol 1-2 h, hydrolysed in 1M HCl at 60°C for 2-7 min, stained with 1% acetoorcein at room temperature for 2-24 h, and squashed in a drop of 45% acetic acid – glycerol (9:1). The slides were observed with a Zeiss Axioplan microscope, and the best metaphase plates were photographed with a Zeiss AxioCam HRm camera.

Genome size was assessed by flow cytometry. *Petunia hybrida* Vilm. ‘PxPc6’ and *Pisum sativum* L. ‘Express long’ (2C=2.85 pg and 8.37 pg, respectively; Marie and Brown 1993) were used as internal standards. Seeds of the standards were provided by the Institut des Sciences du Végétal, CNRS, Gif-sur-Yvette (France). The leaf tissue of five individuals per studied population was chopped up together using a razor blade

with the leaf tissue of the internal standard in 1200 µl of LB01 isolation buffer (Doležal et al. 1989), supplemented with 100 µg/ml of ribonuclease A (RNase A, Boehringer). For each individual, one sample was extracted, filtered and measured twice. The nuclei suspension was stained with 40 µl of propidium iodide (1 mg/ml) (Sigma-Aldrich Química, SA, Madrid, Spain), kept on ice for 5 min and measured in a CyAn™ ADP cytometer (Beckman-Coulter Life Sciences). Measurements were made at the Centres Científics i Tecnològics (Universitat de Barcelona). The instrument was set up with the standard configuration: excitation of the sample was done using a 488 nm laser. Forward scatter, side scatter and red (613/20 nm) fluorescence for propidium iodide were acquired. The total nuclear DNA content (2C) was calculated by multiplying the known DNA content of the standard with the quotient between the peak positions (mode) of the target species and the standard in the histogram of fluorescence intensities. This is done by assuming that there is a linear correlation between the fluorescent signals from the stained nuclei of the unknown specimen, the known internal standard and the DNA amount (Doležal 1991). A minimum of 8000 particles were measured in each run.

Information for the analysis and discussion of results on previously reported chromosome counts has been extracted from the Chromosome Counts Database (<http://ccdb.tau.ac.il/home/>, Rice et al. 2015) and from the Index to Chromosome Numbers in Asteraceae (www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceae_e). In the same line, previous genome size estimates have been found both in the Plant DNA C-values database (data.kew.org/cvalues/) and in the Genome Size in the Asteraceae Database (Garnatje et al. 2011, www.asteraceagenomesize.com).

Results

A summary of the results obtained is presented in Table 1. We have assessed genome size for 19 species, being the first estimate for 16. All studied taxa belong to the subfamily Asteroideae, 15 to the Heliantheae alliance and five to tribe Astereae. Genome size data (2C) ranged from 2.16 pg (*Heliopsis helianthoides*) to 19.07 pg (*Lindheimera texana*). The average half peak coefficient of variation (HPCV) of samples was 3.67% and fluorescence histograms were of average good quality, and occasionally incipient 4C peaks were found for some populations (Figure 1). Out of the 19 species surveyed, 11 show small ($2.8 < 2C \leq 7$ pg), five show intermediate ($7 < 2C < 28$ pg) and four present very small ($2C \leq 2.8$ pg) genome size, according to categories established by Leitch et al. (1998) and Soltis et al. (2003). Whenever possible (in ten out of 19 cases), chromosome numbers have been provided in order to accompany genome size data (Figure 2). Chromosome numbers range from $2n=16$ (*Lindheimera texana*) to $2n=36$ (*Pericome caudata*).

Discussion

According to the last update of the Genome Size in the Asteraceae Database (Garnatje et al. 2011; Garcia et al. in preparation) the present work contributes new genome size data for 16 species, 12 genera and 3 tribes from family Asteraceae (Table 1). 75% of the genome sizes obtained in this work are considered small or very small, which fits the general trend of angiosperms to reduced genome sizes (Leitch et al. 1998). Within the available data for Asteraceae (Garnatje et al. 2011; Garcia et al. in preparation), 54% of values are considered small or very small, and the median ($2C=6.6$ pg) is also small.

New data for tribes

We contribute first assessments for tribes Bahieae and Perityleae, and the second one for tribe Helenieae. Tribe Bahieae comprises 20 genera and approximately 83 species (Panero and Crozier 2016), distributed in the south east of North America and in certain areas of South America. Some species have colonized areas in tropical Africa and in the islands of South Pacific. We have assessed genome size in its species *Palafoxia callosa*, a member of a small North American endemic genus with 11 species, all annual herbaceous. Particularly, *P. callosa* is also found naturalized in Hawaii. Our data confirm the previous counts of $2n=20$ (Figure 2H) for the species.

Regarding Perityleae, this tribe inhabits North and Central America, although some genera have colonized Peru and Chile. It consists of six genera and around 85 species. *Pericome* is a small genus with two species (Powell 1973), out of which *Pericome caudata* is a common plant native to southwestern United States and northern Mexico. We have confirmed the tetraploid level for the studied population ($2n=36$, Figure 2I), the most common for the species.

Tribe Helenieae holds 13 genera and about 115 species, also distributed exclusively in North America, particularly in the southwest and north of Mexico (Panero and Crozier 2016). From this tribe we have assessed *Hymenoxis hoopesi* (native to the west of USA) and *Gaillardia pulchella* (native to Northern Mexico and the southern and central USA; however its cultivars and hybrids have ornamental use so it is a very widespread species. Also, chromosome counts confirm previous data for the species ($2n=30$, Figure

2C and 2n=34, Figure 2B, respectively), which are found in the Heliantheae alliance, despite the base chromosome number in tribe Helenieae being $x=19$.

Out of the 13 tribes taking part of the Heliantheae alliance, this work raises to nine the number of tribes with at least one genome size assessment. We still lack data for tribes Neurolaeneae (four genera and approximately 150 species), Chaenactideae (three genera and around 25 species), Polymnieae (one genus and 4 species) and for the monotypic Feddeae, with only one species (*Feddea cubensis* Urb.) endemic to Cuba (numbers of species from Panero and Crozier 2016).

New data for genera

At the genus level, and beyond those already discussed, the work contributes new genome size data for six genera: *Engelmannia*, *Iva*, *Lindheimera*, *Tetragonotheca*, *Viguiera* and *Verbesina*. All of them are endemic to North America although some species closely related to *Viguiera* have radiated extensively in South America (Schilling and Panero 2011). Together with genome size, we have obtained chromosome counts for all the studied species of these genera, with the exception of *Tetragonotheca texana* and *Verbesina virginica*. The former species belongs to tribe Millerieae, for which about 80 species have known genome sizes, ranging from 2C=0.98-11.5 pg (average 2C=5.19 pg). The result obtained for *T. texana* (2C=8.55 pg) falls within this range. *Verbesina virginica* belongs to tribe Heliantheae and its genome size (2C=4.41 pg) falls within the wide range of tribe Heliantheae (2C=2.14-30.54 pg), one of the largest tribes in the family with about 300 genera and more than 3000 species.

Our count for *Viguiera dentata* ($2n=34$, Figure 2K) is consistent with previous reports, and its genome size ($2C=2.79$ pg) also falls within the range of tribe Heliantheae. Similarly, our data confirm previous counts of *Iva annua* ($2n=34$, Figure 2D), *Engelmannia peristenia* ($2n=18$, Figure 2A), and *Lindheimera texana* ($2n=16$, Figure 2G). The large chromosomes of the latter two are consistent with their remarkable higher genome size as compared to others.

New data at specific level

At the species level, new data are given for *Ageratina havanensis* and for two ploidy levels of *Liatris*, both belonging to tribe Eupatorieae. The genome size range known for this tribe is $2C=0.79-7.2$ pg and the average $2C=3$ pg, being results are consistent with these. For the former genus, genome size was only available for *Ageratina altissima* (L.) King & H.E.Robins. ($2C= 2.2-2.4$ pg, $2n=34$). *Ageratina* comprises approximately 330 species and it is found in temperate North American areas, particularly in Mexico where 150 of its species are endemic (Clewett and Wooten 1971). Our value ($2C=4,10$ pg) almost duplicates previous data for *A. altissima*, expanding its genome size range, which is presumably wider given the number of species contained. The chromosome spreads obtained for the studied population were not good enough to determine the exact count (data not shown) but *ca.* $2n=34$ chromosomes were seen, as previous reports for the species. Therefore we presume that this increased genome size with respect to previous data for the genus is probably showing the interspecific genome size variation within the genus at the same ploidy level rather than a tetraploid level (which could also be assumed for the almost double genome size) for the species, still not detected until present.

Genus *Liatris* comprises about 40 species with also exclusive North American distribution. From the point of view of genome size assessments, only one species was known up to date, *L. aspera* ($2C=3.4-3.6$ pg, $2n=20$) (Bai et al. 2012). Our work contributes data for the species *L. punctata* at two ploidy levels: $2C=2.74$ pg and 3.53 pg, for $2n=16$ (Figure 2E) and $2n=28$ (Figure 2F), respectively, coexisting in the same population. The only diploid chromosome number known for the species is $2n=20$ therefore a process of descending dysploidy (from $2n=20$ to $2n=16$) could explain the difference in the diploid level for *L. punctata*, as described previously in many Asteraceae (Garnatje et al. 2004; Mas de Xaxars et al. 2016). It seems that the genus *Liatris* may be karyologically very dynamic since ploidy levels up to the hexaploid and dysploid populations are well documented. In this regard, triploidy and also subsequent descending disploidy might be the responsible of the second karyotype (from a diploid $2n=20$ to $2n=30$ and then to $2n=28$); although uncommon, triploid ploidy levels are occasionally detected in plants (see Garcia-Jacas and Garnatje, 2011, for examples within the *Asteraceae*), as well as descending disploidy. Since $2n=30$ karyotypes have been already found for *L. punctata* (Darlington and Wylie 1955), the finding of $2n=28$ could be explained from such processes.

Other Asteraceae and previously reported values

The survey performed also includes some species from tribe Astereae (within subfamily Asteroideae), from genera *Baccharis* and *Solidago*, both mostly native to North America, although largely present in other areas as well (such as, for instance, South America and Europe). The results obtained are within the genome size range and average known for the tribe ($0.47-21.43$ and 3.70 pg, respectively). *Baccharis* is one of

the largest genera in the family, with about 350-450 species (Sundberg et al. 2006). Genome size data were available for *Baccharis* only for four species, and we contribute here those from one more species, *B. neglecta* ($2C=4.54$ pg), which is within the genome size range already known for the genus ($2C=4.78-6.72$ pg). Genus *Solidago* comprises about one hundred species, of which genome size data are available for a dozen only. We contribute new data for *S. altissima* and *S. simplex*, and we also confirm previous genome size assessments for *S. canadensis* and *S. flexicaulis* (and the chromosome count for the latter at $2n=36$, Figure 2J). The results for the two new *Solidago* are within the generic genome size range already known.

The genome size of *Eclipta prostrata* had formerly been estimated. However, our assessment, although consistent with a previous one for the same species and with the same technique ($2C=2.14$ pg; Garcia et al. 2013), is approximately 50% lower than the prime estimate for the species ($2C=3.10$ pg; Bennett et al. 1998). The latter figure was based on Feulgen cytophotometry, and authors established its chromosome number at $2n=22$; unfortunately we were unable to provide the chromosome count for our studied populations. Nevertheless, other counts for the species range from $2n=18$ to $2n=24$, but even the lowest count could not explain such a difference. This species is a widespread weed distributed in tropical and mild temperate climate areas in the world. The remarkable genome size difference detected could be related to genuine intraspecific variation or technical issues, as discussed previously.

Conclusions

The present work contributes data on genome sizes in family Asteraceae focused in the Heliantheae alliance, filling the gap in some tribes which were previously unknown

from this viewpoint. Yet as mentioned previously, four tribes from the alliance still lack a single genome size assessment and for many of them there is still only one (tribes Bahieae, Perityleae, Helenieae and Tageteae) or two (Madieae) genome sizes, despite the considerable number of species and ecological importance of some of them. For a better understanding of genome size and chromosome number evolution in this interesting group of tribes from subfamily Asteroideae, further work should focus in the completeness of tribal representation.

Finally, we have noticed that none of the species here studied presents a chromosome number compatible with the hypothesized basic chromosome number of its tribe (see Table 1), according to the review of chromosome numbers in the Asteraceae (Semple and Watanabe 2009). In most cases, our counts just confirmed what previous authors had already found. It is possible that there are several basic chromosome numbers in a tribe and even within a genus (e.g. tribe Anthemideae, also from family Asteraceae, and its genus *Artemisia*; Powell et al. 1974), so this may be a random incident. However, the inclusion of new chromosome counts and new genome size data, such as those here presented, could contribute to a more complete reconstruction on ancestral base numbers, while improving our understanding of the processes of dysploidy shaping the evolution of karyotypes in the Heliantheae alliance.

Acknowledgement

Spencer C. Brown is acknowledged for supplying internal standards for flow cytometry. We also thank Jaume Comas, Teresa Garnatje, Chari González, Màrius Mumbrú, Joan Pere Pascual and Vanessa Zurlo for collaboration in flow cytometric and karyological

experiments. We also thank an anonymous referee for helpful comments which improved the quality of the manuscript.

Disclosure statement

No potential conflict of interest is reported by the authors.

Funding

This work was supported by the Dirección General de Investigación Científica y Técnica, Government of Spain (CGL2016-75694-P) and the Generalitat de Catalunya, Government of Catalonia ("Ajuts a grups de recerca consolidats" 2014SGR514). SG benefitted from a Ramón y Cajal contract from the Government of Spain (RYC-2014-16608).

Table 1. List of the species studied, indicating its provenance data and voucher and (1) somatic chromosome number; (2) genome size (2C) in picograms (new values indicated in bold); (3) standard deviation of genome size assessment; (4) genome size (1C) in megabasepairs (Mbp); (5) half peak coefficient of variation of the problem plant; (6) category of genome size according to Leitch et al. (1998) and Soltis et al. (2003); (7) internal standard used for flow cytometry, either *Petunia hybrida* ‘PxPc6’ (*P. hybrida*) or *Pisum sativum* ‘Express Long’ (*P. sativum*) ; (8) previous chromosome counts as found in the Chromosome Counts Database (ccdb.tau.ac.il/home) and in the Index to Chromosome Numbers in Asteraceae (www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceae_e); numbers in bold are the most frequent; (9) previous genome size estimates as found in the Plant DNA C-values database (data.kew.org/cvalues) and in the Genome Size in the Asteraceae Database (www.asteraceagenomesize.com); FC=flow cytometry, PI=propidium iodide; MI=mithramycin; FE=Feulgen cytophotometry; (10) hypothesized base chromosome number according to Semple and Watanabe (2009).

Figure captions

Figure 1. Fluorescence histograms of flow cytometric genome size assessments for two species. **A.** *Iva annua* (2C=15.6 pg) using *Pisum sativum* (2C=8.37 pg) as internal standard. **B.** *Baccharis neglecta* (2C=4.54 pg) using *Petunia hybrida* (2C=2.85 pg) as internal standard. Note incipient 4C peak of *Baccharis neglecta*.

Figure 2. Metaphase plates of the studied species (I). **A.** *Engelmannia peristenia* (2n=18); **B.** *Gaillardia pulchella* (2n=34); **C.** *Hymenoxis hoppersi* (2n=30+B, arrow indicates B-chromosome); **D.** *Iva annua* (2n=34). **E.** *Liatris punctata* (2n=16); **F.** *L. punctata* (2n=30); **G.** *Lindheimera texana* (2n=16); **H.** *Palafoxia callosa* (2n=20); **I.** *Pericome caudata* (2n=36); **J.** *Solidago flexicaulis* (2n=36); **K.** *Viguiera dentata* (2n=34). Scale bars 5 µm. Scale bars 5 µm.

Literature

Bai C, Alverson WS, Follansbee A, Waller DM. 2012. New reports of nuclear DNA content for 407 vascular plant taxa from the United States. *Ann Bot.* 110: 1623–1629.

Bennett MD, Leitch IJ, Hanson L. 1998. DNA amounts in two samples of angiosperm weeds. *Ann Bot.* 82(suppl. 1): 121–134.

Bennett MD, Leitch IJ. 2005. Plant genome size research: A field in focus. *Ann Bot.* 95: 1–6.

Bennett MD, Smith JB. 1976. Nuclear DNA amounts in angiosperms. *Philos T Roy Soc B.* 274: 227–274.

Clewell AF, Wooten JW. 1971. A revision of *Ageratina* (Compositae: Eupatorieae) from eastern North America. *Brittonia.* 23: 123–143.

Christenhusz MJ, Fay MF, Chase MW. 2017. *Plants of the world: an illustrated encyclopedia of vascular plants.* Chicago (USA): University of Chicago Press.

Darlington CD, Wylie AP. 1955. *Chromosome atlas of flowering plants.* London: George Allen and Unwin Ltd.

De-Paula OC, Marzinek J, Trombert Oliveira DM, Rodrigues Machado S. 2013. The role of fibres and the hypodermis in Compositae melanin secretion. *Micron.* 44: 312–316.

Doležel, J. 1991. Flow cytometric analysis of nuclear DNA content in higher plants. *Phytochem Analysis* 2: 143–154.

Doležel J, Binarova P, Lucretti S. 1989. Analysis of nuclear DNA content in plant cells by flow cytometry. *Biol Plantarum.* 31: 113–120.

Garcia S, Hidalgo O, Jakovljević I, Siljak-Yakovlev S, Vigo J, Garnatje T, Vallès J. 2013. New data on genome size in 128 Asteraceae species and subspecies, with first assessments for 40 genera, three tribes and two subfamilies. *Plant Biosyst.* 147: 1219–1227.

Garcia S, Leitch IJ, Anadon-Rosell A, Canela MA, Galvez F, Garnatje T, Gras A, Hidalgo O, Johnston E, Mas de Xaxars G, Pellicer J, Siljak-Yakovlev S, Vallès J, Viales D, Bennett MD. 2013. Recent updates and developments to plant genome size databases. *Nucleic Acids Res.* 42(D1): D1159–D1166.

Garcia-Jacas N, Garnatje T. 2011. Natural triploidy in *Centaurea* and *Cheirolophus*. *Bot Helv.* 111: 25–30.

Garnatje T, Canela MA, Garcia S, Hidalgo O, Pellicer J, Sánchez-Jiménez I, Siljak-Yakovlev S, Viales D, Vallès J. 2011. GSAD: A genome size in the *Asteraceae* database. *Cytom Part A.* 79A: 401–404.

Garnatje T, Vallès J, Vilatersana R, Garcia-Jacas N, Susanna A, Siljak-Yakovlev S. 2004. Molecular cytogenetics of *Xeranthemum* L. and related genera (*Asteraceae*, *Cardueae*). *J Plant Biol.* 6: 140–146.

Index to Chromosome Numbers in Asteraceae. [accessed 2018 Feb 1].
http://www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceae_e.

Leitch IJ, Chase MW, Bennett MD. 1998. Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. *Ann Bot* 82(suppl_1): 85–94.

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

- Mas de Xaxars G, Garnatje T, Pellicer J, Siljak-Yakovlev S, Vallès J, Garcia S. 2016. Impact of dysploidy and polyploidy on the diversification of high mountain *Artemisia* (*Asteraceae*) and allies. *Alpine Bot.* 126: 35–48.
- Mota L, Torices R, Loureiro J. 2016. The evolution of haploid chromosome numbers in the sunflower family. *Genome Biol Evol.* 8: 3516–3528.
- Pandey AK, Wilcox LW, Sack FD, Stuessy TF. 1989. Development of the phytomelanin layer in fruits of *Ageratum conyzoides* (Compositae). *Am J Bot.* 1: 739–46.
- Pandey AK, Stuessy TF, Mathur RR. Phytomelanin and systematics of the Heliantheae Alliance (Compositae). 2014. *Plant Div Evol.* 131: 145–65.
- Panero JL, Crozier BS. 2016. Macroevolutionary dynamics in the early diversification of the Asteraceae. *Mol Phylogenet Evol.* 99: 116–132.
- Panero JL. 2007. Compositae: key to the tribes of the Heliantheae alliance. In: Kadereit J, editor. Families and genera of vascular plants. Flowering Plants, Eudicots, Asterales. 8th ed. Berlin: Springer Verlag; p. 391–395.
- Panero M E, Weisberg DS, Black J, Goldstein TR, Barnes JL, Brownell H, Winner E. 2016. Failure to replicate methods caused the failure to replicate results. *J Pers Soc Psychol.* 111: e46–e54.
- Plant DNA C-values database. [accessed 2018 Jan 15]. <http://data.kew.org/cvalues/> .
- Powell AM, Kyhos DW, Raven PH. 1974. Chromosome numbers in Compositae. *Am J Bot.* 61: 909–913.

Powell AM. 1973. Taxonomy of *Pericome* (Compositae: Peritylinae). The Southwestern Naturalist. 18: 335–339.

Rice A, Glick L, Abadi S, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I. 2015. The Chromosome Counts Database (CCDB)—a community resource of plant chromosome numbers. New Phytol. 206: 19–26.

Robinson H. 1981. A revision of the tribal and subtribal limits of the Heliantheae (Asteraceae). Smithsonian Contrib. Bot. 51: 1–102.

Schilling EE, Panero JL. 2011. A revised classification of subtribe Helianthinae (Asteraceae: Heliantheae) II. Derived lineages. Bot J Linn Soc. 167: 311–331.

Semple JC, Watanabe K. 2009. A review of chromosome numbers in Asteraceae with hypotheses on chromosomal base number evolution. Systematics, Evolution, and Biogeography of Compositae. Vienna (Austria): International Association for Plant Taxonomy. 43: 61–72.

Smith EB. 1975. The chromosome numbers of North American *Coreopsis* with phyletic interpretations. Bot. Gaz. 136: 78–86.

Soltis DE, Soltis PS, Bennett MD, Leitch IJ. 2003. Evolution of genome size in the angiosperms. Am J Bot. 90: 1596–1603.

Stuessy TF, Liu HY. 1983. Anatomy of the pericarp of *Clibadium desmanthodium* and *Ichthyothere* (Compositae, Heliantheae) and systematic implications. Rhodora. 85: 213–227.

Sundberg SD, Scott D, Boggler DJ. 2006. *Baccharis*. In: Flora of North America Editorial Committee. Flora of North America: North of Mexico. Vol 20:

Magnoliophyta: Asteridae, part 7: Asteraceae. Oxford and New York (NY): Oxford University Press; p. 23–34.

Swift H. 1950. The constancy of desoxyribose nucleic acid in plant nuclei. *P Natl Acad Sci USA*. 36: 643–654.

Vallès J, Malik S, Gómez M, Siljak-Yakovlev S. 2017. Contribution to knowledge about nuclear DNA amounts in the family Asteraceae: first assessments in one genus and 12 species, with chromosome counts for three taxa. *Bot Serbica*. 41: 213–219.