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First genome size assessments in *Carduncellus* and its related genera *Femeniasia* and *Phonus* (Asteraceae, Cardueae), with data on 21 taxa

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Abstract

First genome size assessments in *Carduncellus* and its related genera *Femeniasia* and *Phonus* (Asteraceae, Cardueae), with data on 21 taxa.— Genome size of 18 species of the genus *Carduncellus*, two species of the related genus *Phonus* and the monotypic genus *Femeniasia* (*F. balearica*) has been assessed by flow cytometry for the first time. Ploidy levels were assigned using genome size data together with previously reported chromosome counts. A phylogenetic framework was built to visualize how cytogenetic traits distributed across taxa. The results confirmed three ploidy levels (2*x*, 4*x* and 6*x*), with a predominance of diploids. The 2C values ranged from 3.24 pg in *Carduncellus calvus* to 11.16 pg in *C. eriocephalus*, whereas monoploid genome size (1C*x*) ranged from 1.29 pg in *C. duvauxii* (4*x*) to 2.30 pg in *Phonus rhiphaeus* (2*x*). The mean 1C*x* for tetraploids was lower than for diploids. For each ploidy level, genome size values of *Carduncellus*, *Femeniasia* and *Phonus* were found to be higher than those of *Carthamus*. This result is consistent with a trend frequently observed in plants, of higher genome sizes in long life cycle taxa compared to short-lived relatives.

Key words: 2C-values; *Carthamus*-*Carduncellus* complex; DNA amount; life-cycle; polyploidy; ploidy level.

Resumen

Primeras medidas del tamaño del genoma en *Carduncellus* y los géneros afines *Femeniasia* y *Phonus* (*Asteraceae*, *Cardueae*), con datos para 21 táxones.— El tamaño del genoma de 18 especies del género *Carduncellus*, dos especies de los géneros relacionados, *Phonus* y el género monotípico *Femeniasia* (*F. balearica*) ha sido medido por primera vez mediante citometría de flujo. Los niveles de ploidía se asignaron utilizando datos de tamaño del genoma junto con los recuentos de cromosomas previamente reportados. Se construyó un marco filogenético para visualizar la distribución de las características citogenéticas de los táxones. Los resultados confirmaron tres niveles de ploidía (2*x*, 4*x* y 6*x*), con un predominio de los táxones diploides. Los valores de 2C oscilaron entre 3,24 pg en *Carduncellus calvus* y 11,16 pg en *C. eriocephalus*, mientras que el tamaño del genoma monoploide (1C*x*) osciló entre 1,29 pg en *C. duvauxii* (4*x*) y 2,30 pg en *Phonus rhiphaeus* (2*x*). La media de los valores 1C*x* para los tetraploides fue menor que para los diploides. Los valores de tamaño del genoma de *Carduncellus*, *Femeniasia* y *Phonus* fueron más elevados que los de *Carthamus* dentro del mismo nivel de ploidía. Este resultado concuerda con una tendencia frecuentemente observada en plantas en la que los táxones con ciclos de vida largos presentan tamaños del genoma más elevados que los táxones relacionados que poseen ciclos de vida cortos.

Palabras clave: cantidad de ADN; ciclo vital; complejo *Carthamus*-*Carduncellus*; nivel de ploidía; poliploidía; valores 2C.

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INTRODUCTION

Genome size has been revealed as a powerful tool for studying allopolyploidy and hybridization in the genus *Carthamus* L. (Garnatje *et al*., 2006) and evolutionary processes in the Asteraceae as a whole (e.g. Torrell & Vallès, 2001; Bureš *et al*., 2004; Bancheva & Greilhuber, 2006; Garcia *et al*., 2006, 2008; Suda *et al*., 2007; Hidalgo *et al*., 2008, 2017; Pellicer *et al*., 2010; Trávníček *et al*., 2013; Pegoraro *et al.*, 2020; Vitales *et al*., 2020).

Together with *Carthamus*, the genera *Carduncellus* Adans., *Femeniasia* Susanna and *Phonus* Hill constitute the *Carthamus-Carduncellus* complex (Vilatersana *et al*., 2000*a*; Vilatersana, 2002). *Carthamus* comprises 18 annual species growing in disturbed habitats of the eastern part of Mediterranean basin and western Asia. Sister to the genus *Carthamus*, the clade comprising the genera *Carduncellus* (*ca*. 26 species, from North Africa and the Iberian Peninsula), *Femeniasia* (one species from Menorca, Balearic Islands) and *Phonus* (two species from North Africa and Iberian Peninsula) is made of perennial species which grow in few disturbed habitats (Vilatersana *et al*., 2000*a*; López González, 2012). However, generic circumscription in the *Carthamus-Carduncellus* complex is still a matter of debate. In the last two decades, different taxonomic treatments resulted in the recognition of (i) the four genera *Carthamus*, *Carduncellus*, *Femeniasia* and *Phonus*, the treatment we followed in this study (Vilatersana *et al*., 2000*a*), (ii) an expanded genus *Carthamus* encompassing the three other genera (Greuter, 2003), and (iii) two genera, *Carthamus* and *Carduncellus*, the delimitation of the latter being extended to include *Femeniasia* and *Phonus* (López González, 2012).

In addition to their distinct life cycles and habitat preference, the genera of the *Carthamus-Carduncellus* complex also have different karyological and cytogenetic profiles (Vilatersana *et al.*, 2000*b*). The evolution of *Carthamus* involved descending dysploidy (x = 12, 11, 10) and polyploidy (2x, 4x, 6x; Vilatersana *et al*., 2000*b*, 2007; Garnatje *et al*., 2006). This genus presents 2C values from 2.26 to 7.46 pg and monoploid genome sizes (1Cx) from 1.13 to 1.53 pg (Garnatje *et al*., 2006). Genome size of allopolyploids was found to be the sum of their parental species, or slightly inferior (Garnatje *et al*., 2006). The clade constituted by *Carduncellus*, *Femeniasia* and *Phonus* presents a constant base chromosome number of x = 12, with 2x cytotypes for *Femeniasia* and *Phonus*, and 2x to 6x cytotypes for *Carduncellus* (Vilatersana *et al*., 2000*b*; Vilatersana, 2002). In *Carduncellus*, diploids predominate especially among the endemic species occupying narrow areas, tetraploids are relatively frequent, while triploids and hexaploids are found more sporadically (López González, 1990; Vilatersana *et al*., 2000*b*; Vilatersana, 2002). B chromosomes are not rare in these species, indicating high genome dynamism (Vilatersana, 2002). No data on genome size is available so far for any taxa of the *Carduncellus-Femeniasia-Phonus* clade.

This study aims at improving our understanding of cytogenetic evolution within the *Carthamus-Carduncellus* complex. We provide the first genome size data for the genera *Carduncellus*, *Femeniasia* and *Phonus* and discuss cytotype diversity in the light of phylogenetic and ecological contexts.

MATERIALS AND METHODS

Plant material

The sampling comprises a total of 41 populations of 18 species of genus *Carduncellus*, including three subspecies, two species of *Phonus* and one population of *Femeniasia balearica* (J. J. Rodr.) Susanna. Origin, collectors and dates are shown in Table 1. Voucher specimens for each population are deposited in the herbarium BC (Botanical Institute of Barcelona).

Table 1. Origin, collectors and dates of the studied material. Vouchers are deposited in the herbarium BC.

Taxon (code)	**Origin, collectors and date**
***Carduncellus caeruleus* (L.) C. Presl. (1)**	Spain, Málaga: Tolox, *Garcia-Jacas, Susanna 1610 & Vilatersana*, 22.VI.1996
***Carduncellus caeruleus* (2)**	Morocco, Fes: Oued Zloul valley near Ahermoumou, *Garnatje, Susanna 1801 & Vilatersana*, 18.VI.1997
***Carduncellus caeruleus* (3)**	Spain, Córdoba: between Jauja and Puente Genil, *Vilatersana 59*, 8.IV.1998
***Carduncellus calvus* Boiss. & Reut.**	Morocco, Tahanaout, El Fellah, *Romo 14025 & Vilatersana*, 17.VI.2006
***Carduncellus catrouxii* Emb.**	Morocco, Mgoun area: Ouzighimt-Tal, *Finckh & Staudinger 859*, 1.VII.2002 (Herbarium Hamburgense)
***Carduncellus cuatrecasasii* G. López (1)**	Spain, Jaén: Sierra del Ahillo, *Sanz & Vilatersana 506,* 12.VI.2005
***Carduncellus cuatrecasasii* (2)**	Spain, Jaén: Sierra de Segura, *Sanz & Vilatersana 527,* 18.VI.2005
***Carduncellus dianius* Webb. (1)**	Spain, Valencia: Xàbia, Cap de Sant Antoni, *Garcia-Jacas, Susanna 1479 & Vilatersana*, 17.VI.1995
***Carduncellus dianius* (2)**	Spain, Balearic Islands, Eivissa: Cala Ximena, *Garnatje & Vilatersana 402*, 15.IV.2004
***Carduncellus duvauxii* Batt. et Trab.**	Morocco, Ujdah: Bouarfa, *Garnatje, Susanna 1779 & Vilatersana*, 16.VI.1997
***Carduncellus eriocephalus* Boiss.**	Morocco, Ujdah: Bouanane, *Garnatje, Susanna 1785 & Vilatersana*, 16.VI.1997
***Carduncellus fruticosus* (Maire) Hanelt**	Morocco, Ouarzazate: River Todrha, *Benedí, G. Montserrat & J. M. Montserrat 2407,* 5.VI.1980
***Carduncellus hispanicus* Boiss. ex DC. subsp. *araneosus* (Boiss. & Reut.) G. López**	Spain, Toledo: between Huertas de Valdecábanos and Cabañas de Yepes, *Sanz & Vilatersana 529,* 18.VI.2005
Carduncellus hispanicus* subsp. *hispanicus	Spain, Almería: Sierra de Gádor, *Sanz & Vilatersana 486,* 8.VI.2005
***Carduncellus hispanicus* subsp. *intercedens* (Degen & Hervier) G. López (1)**	Spain, Alacant: Serra de Bèrnia, *Garnatje & Vilatersana 450,* 22.VI.2005
***Carduncellus hispanicus* subsp. *intercedens* (2)**	Spain, Murcia: Sierra de la Muela, *Garnatje & Vilatersana 460*, 24.VI.2005
***Carduncellus hispanicus* subsp. *intercedens* (3)**	Spain, Granada: Sierra de Baza, *Sanz & Vilatersana 516*, 15.VII.2005
***Carduncellus lucens* Ball (1)**	Morocco, Oikaimeden, *Romo 13929 & Vilatersana*, 13.VI.2006
***Carduncellus lucens* (2)**	Morocco, Oikaimeden, *Romo 13930 & Vilatersana,* 13.VI.2006
***Carduncellus lucens* (3)**	Morocco: Oukaimeden, *Romo 13927 & Vilatersana*, 12.VI.2006
***Carduncellus mareoticus* (Del.) Hanelt (1)**	Egypt, Alexandria: road Alexandria-Marsah Matruh, *Susanna 1860 & Vilatersana,* 22.VI.1996
***Carduncellus mareoticus* (2)**	Egypt, Alexandria: El Amiriya, *Susanna 1850 & Vilatersana*, 7.VI.1998
***Carduncellus mareoticus* (3)**	Egypt, Alexandria: New Bourg-el-Arab, *Susanna 1846 & Vilatersana*, 7.VI.1998

Table 1. Origin, collectors and dates of the studied material. Vouchers are deposited in the herbarium BC. (cont.)

Taxon (code)	**Origin, collectors and date**
***Carduncellus mitissimus* DC.**	Spain, Navarra: between Burgui and Navascués, *Carretero & Vilatersana 72*, 7.VII.2000
***Carduncellus monspelliensium* All. (1)**	Spain, Tarragona: Serra del Monsant, *Vilatersana 18*, 23.IX.1995.
***Carduncellus monspelliensium* (2)**	Spain, Soria: Montejo de Tiermes, *Garcia-Jacas & Susanna 2223*, 27.VII.2001
***Carduncellus monspelliensium* (3)**	Spain, Tarragona: Morera del Montsant, *Vilatersana 17*, 18.X.1997
***Carduncellus monspelliensium* (4)**	Spain, Tarragona: Santa Coloma de Queralt*, Garcia-Jacas, Susanna & Vilatersana 10*, 10.VI.1995
***Carduncellus monspelliensium* (5)**	Spain, Tarragona: Sant Magí de Brufaganya, *Del Rey & Vilatersana 702,* 8.VIII.2006
***Carduncellus pectinatus* DC.**	Morocco: Azerzou, between Tanourdi and Ait-Mouli, *Romo 14028 & Vilatersana,* 17.VI.2006
***Carduncellus pinnatus* (Desf.) DC.**	Morocco: Ouarzazate, Tizi n'Tichka Pass, 2169 m, *Romo 13910 & Vilatersana*, 12.VI.2006
***Carduncellus pomelianus* Batt. (1)**	Morocco, Middle Atlas: near Djebel Amjoud, *J. Molero, J. M. Montserrat 6787& L. Sáez*, 24.VII.2000
***Carduncellus pomelianus* (2)**	Morocco: Boumia, *Romo 14060 & Vilatersana*, 18.VI.2006
***Carduncellus reboudianus* Batt. (1)**	Morocco, Ksar es Souk: Tizi n'Talrhem, *Garnatje, Susanna 1788 & Vilatersana*, 17.VI.1997
***Carduncellus reboudianus* (2)**	Morocco: between Ait Toughach and Zaida, *Romo 14031 & Vilatersana*, 17.VI.2006
***Carduncellus rhaponticoides* Coss. & Dur. (1)**	Morocco, Oukaïmeden: Col du Tizrag, *G. López 8958 & F. Muñoz Garmendia*, 11.VII.1984
***Carduncellus rhaponticoides* (2)**	Morocco: between Oualeger and the Zad pass, *Romo 14054 & Vilatersana*, 17.VI.2006
***Femeniasia balearica* (J. J. Rodr.) Susanna**	Spain, Balearic Islands, Menorca: Mongofre Vell, *J. M. Montserrat 2802*, 5.VII.1991
***Phonus arborescens* (L.) G. López (1)**	Spain, Almería: Sierra de Gádor near Félix, *J. M. Montserrat*, 27.VII.1990
***Phonus arborescens* (2)**	Spain, Almería: between Roquetas and Félix, *Garcia-Jacas, Susanna 1611 & Vilatersana,* 24.VI.1996
***Phonus rhiphaeus* (Font Quer & Pau) G. López**	Morocco, Al Hoceima: Tleta Oued Laou between Tarerha and Azenti, *J. M. Montserrat 4360, Pallàs & Veny,* 23.VI.1993

DNA content assessment

Fresh young leaves of the plants studied were co-chopped using a razor blade with an internal standard in the proportions 2:1 in 1200 µl of LB01 buffer (Doležel *et al*., 1989) with 0.5% of Triton X-100 and supplemented with 100 µg/ml ribonuclease A (RNase A, Boehringer, Meylan, France) in a plastic Petri dish. *Pisum sativum* L. 'Express Long' (2C = 8.37 pg) and *Petunia hybrida* Vilm. 'PxPc6' (2C = 2.85 pg) were used as internal standards and were first analysed separately in 600 µl of LB01 buffer to locate their peak positions. Nuclei were filtered through a 70-µm nylon filter in order to eliminate cell debris before the addition of 36 µl of propidium iodide (1 mg/ml, solution in water; Invitrogen Eugene, Oregon, USA). Samples were kept on ice before measurement. For each population (Table 1), two samples of each individual were prepared and measured independently. Fluorescence analysis was carried out using an Epics XL flow cytometer (Coulter Corporation, Hialeah, Florida, USA) at the Centres Científics i Tecnològics de la Universitat de Barcelona with the standard configuration as described in Garnatje *et al.* (2006). Acquisition was stopped at 8000 nuclei. The DNA content was calculated for 10 of the aforementioned runs, assuming a linear correlation between the fluorescence signals (of the stained nuclei) and DNA amount. Mean and standard deviations were calculated for 2C values of each population based on five individuals.

Phylogenetic framework

The nuclear ribosomal dataset includes ITS1 and ITS2 regions for 23 species, including two outgroups. ITS sequences of 17 species were available from GenBank and four taxa were sequenced for this study following the same protocol as described in Barres *et al.* (2013). *Carthamus glaucus* M. Bieb. and *C. oxyacantha* M. Bieb. were chosen as outgroup based on published phylogeny by Vilatersana *et al.* (2000*a*). DNA sequences were edited with Chromas v2.6.4 (Technelysium PTy, Tewantin, Queensland, Australia) and Bioedit v7.0.9 (Hall, 1999) and aligned visually. The aligned matrix is available from the corresponding author. The General Time Reversible model (GTR + G) was chosen for ITS nrDNA dataset based on AIC criterion implemented in jModeltest v2.1.2 (Darriba *et al*., 2012). Markov Chain Monte Carlo (MCMC) analysis was carried out in MrBayes v3.2.6 (Ronquist *et al*., 2012) for 2,000,000 generation sampling every 100 generations. The first 25% of the trees were discarded as the 'burn-in' period, after confirming that the average standard deviation of the split frequencies was <0.01, and the potential scale reduction factor approached 1.0 for all parameters. The remaining samples were pooled to construct a 50% majority rule consensus tree. The resulting summary trees were visualised in Figtree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree).

Bar plots showing the distribution of genome size values across the taxa of the phylogenetic inference were generated with the package phytools (Revell, 2012; implemented in R v3.2.2; R Core Team, 2016). A graph illustrating the distribution of mean genome size values for the species of the *Carthamus*-*Carduncellus* complex at different ploidy levels was generated with the package ggplot2 (Wickham, 2016; implemented in R) using the new genome size assessments together with the previously published data of Garnatje *et al.* (2006).

Statistical analyses

One-way ANOVA was carried out in order to test the 1C*x* differences between ploidy levels and between *Carthamus* and *Carduncellus* lineages. The analyses were performed with XLSTAT 2018.7 (Addinsoft Inc.).

RESULTS AND DISCUSSION

Nuclear DNA amount (2C in pg and 1C in Mbp), chromosome numbers counted for the same populations (Vilatersana *et al*., 2000*b*), ploidy levels and 1C*x* values (pg) are shown in Table 2 and Fig. 1. The GenBank accession numbers for new sequences are included in Table 3. To our knowledge and according to recently updated Asteraceae and plant genome size databases (respectively https://www.asteraceaegenomesize.com, Vitales *et al*., 2019; and https://cvalues.science.kew.org, Pellicer & Leitch, 2020, both accessed August 11, 2020), these are the first nuclear DNA amount assessments for the 21 species and three genera studied.

Table 2. Nuclear DNA content and other karyological characteristics of the studied species.

Taxon	**2C ± SD (pg)[1]**	**1C (Mbp)[2]**	**1Cx (pg)[3]**	**2*n*[4]**	***x*[5]**	**Standard**
Carduncellus caeruleus (1)	6.48 ± 0.04	3169	1.62	48	4	*Petunia*
Carduncellus caeruleus (2)	6.29 ± 0.04	3076	1.57	48	4	*Petunia*
Carduncellus caeruleus (3)	6.32 ± 0.16	3091	1.58	-	4*	*Petunia*
Carduncellus calvus	3.24 ± 0.21	1584	1.62	-	2*	*Petunia*
Carduncellus catrouxii	7.07 ± 0.09	3457	1.77	-	4*	*Petunia*
Carduncellus cuatrecasasii (1)	4.24 ± 0.15	2073	2.12	-	2*	*Petunia*
Carduncellus cuatrecasasii (2)	3.32 ± 0.03	1624	1.66	-	2*	*Petunia*
Carduncellus dianius (1)	3.45 ± 0.01	1687	1.73	24	2	*Petunia*
Carduncellus dianius (2)	3.43 ± 0.02	1677	1.72	-	2*	*Pisum*
Carduncellus duvauxii	5.15*	2518	1.29	48	4	*Petunia*
Carduncellus eriocephalus	11.16 ± 0.41	5457	1.86	72	6	*Pisum*
Carduncellus fruticosus	4.28 ± 0.05	2093	2.14	24	2	*Petunia*
Carduncellus hispanicus subsp. *araneosus*	3.50 ± 0.13	1712	1.75	24	2	*Petunia*
Carduncellus hispanicus subsp. *hispanicus*	4.33 ± 0.30	2177	2.17	24	2	*Petunia*
Carduncellus hispanicus subsp. *intercedens* (1)	7.00 ± 0.05	3423	1.75	-	4*	*Pisum*
Carduncellus hispanicus subsp. *intercedens* (2)	7.31 ± 0.29	3575	1.83	-	4*	*Pisum*
Carduncellus hispanicus subsp. *intercedens* (3)	7.09 ± 0.23	3467	1.77	-	4*	*Petunia*
Carduncellus lucens (1)	3.56 ± 0.09	1741	1.78	-	2*	*Pisum*
Carduncellus lucens (2)	3.27 ± 0.19	1599	1.64	-	2*	*Pisum*
Carduncellus lucens (3)	3.27*	1599	1.64	-	2*	*Petunia*
Carduncellus mareoticus (1)	4.40 ± 0.06	2152	2.20	-	2*	*Petunia*
Carduncellus mareoticus (2)	4.56*	2230	2.28	24	2	*Pisum*
Carduncellus mareoticus (3)	4.52 ± 0.09	2210	2.26	-	2*	*Petunia*
Carduncellus mitissimus	3.59 ± 0.02	1756	1.80	-	2*	*Petunia*
Carduncellus monspelliensium (1)	7.38*	3609	1.85	48	4	*Petunia*
Carduncellus monspelliensium (2)	6.94 ± 0.11	3394	1.74	-	4*	*Petunia*
Carduncellus monspelliensium (3)	7.14*	3492	1.79	48	4	*Petunia*
Carduncellus monspelliensium (4)	7.22 ± 0.13	3531	1.81	-	4*	*Petunia*
Carduncellus monspelliensium (5)	7.74 ± 0.77	3638	1.94	-	4*	*Petunia*
Carduncellus pectinatus	3.43 ± 0.18	1677	1.72	-	2*	*Pisum*
Carduncellus pinnatus	4.22 ± 0.56	2064	2.11	-	2*	*Petunia*
Carduncellus pomelianus (1)	3.41 ± 0.06	1668	1.71	-	2*	*Petunia*
Carduncellus pomelianus (2)	3.54 ± 0.16	1731	1.77	-	2*	*Pisum*
Carduncellus reboudianus (1)	6.55 ± 0.12	3203	1.64	48	4	*Petunia*
Carduncellus reboudianus (2)	6.71 ± 0.16	3281	1.68	-	4*	*Pisum*
Carduncellus rhaponticoides (1)	6.79 ± 0.12	3320	1.70	-	4*	*Petunia*
Carduncellus rhaponticoides (2)	6.96 ± 0.18	3403	1.74	-	4*	*Pisum*
Femeniasia balearica	3.84 ± 0.03	1878	1.92	24	2	*Petunia*
Phonus arborescens (1)	4.54 ± 0.07	2220	2.27	24	2	*Petunia*
Phonus arborescens (2)	4.56 ± 0.04	2230	2.28	-	2*	*Petunia*
Phonus rhiphaeus	4.60 ± 0.06	2249	2.30	24	2	*Petunia*

[1] Holoploid genome size (2C) values in pg with standard deviation. Asterisk indicates when a single measurement has been done.
[2] Holoploid genome size (1C) values in Mbp. 1 pg = 978 Mbp (Doležel *et al.*, 2003).
[3] Monoploid genome size (1C*x*) values in pg.
[4] Chromosome counts from Vilatersana *et al.* (2000*b*) corresponding to the same accessions measured for genome size.
[5] Ploidy levels. Asterisk indicates when chromosome counts from other accessions than the one measured for genome size were used to infer the ploidy level.

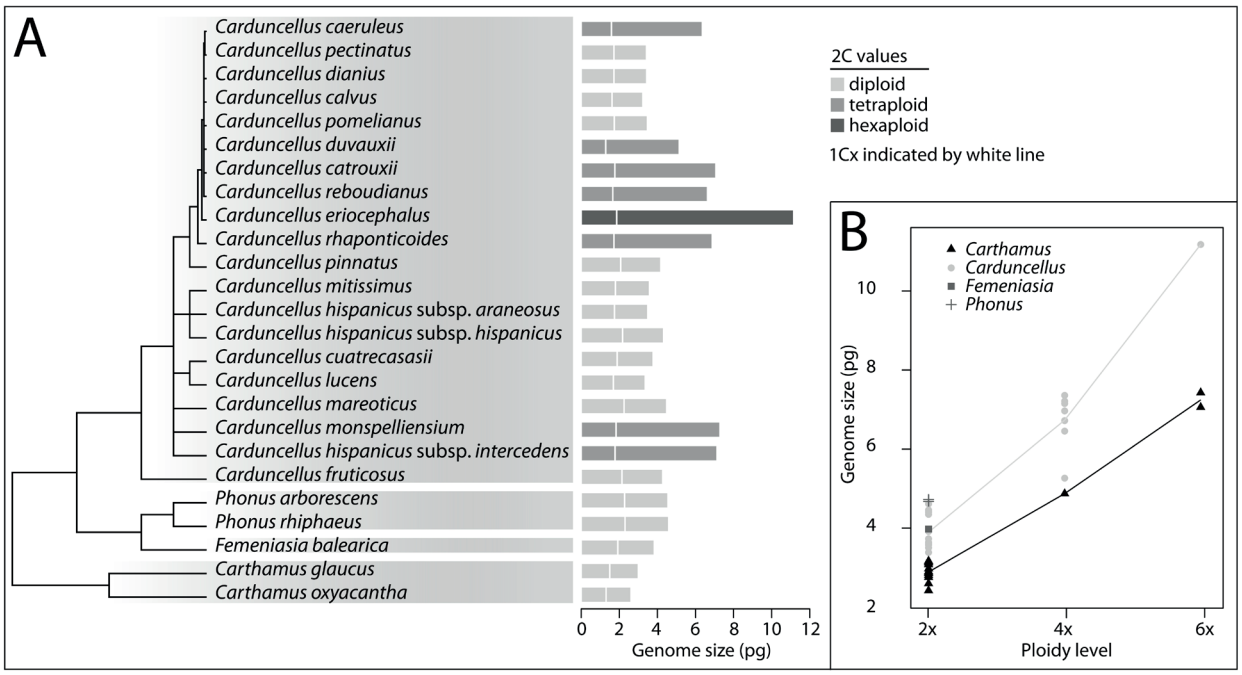

Figure 1. Distribution of genome size and ploidy levels across species of the *Carthamus*-*Carduncellus* complex: (A), molecular phylogeny of *Carduncellus* and related genera *Femeniasia* and *Phonus*. Bars represent mean 2C value per species, with 1C*x* indicated by a white line. Different colour intensities of bars depict ploidy levels; (B), distribution of mean genome size 2C values for species of the *Carthamus*-*Carduncellus* complex at diploid, tetraploid and hexaploid levels. Lines connect the mean values per genus and ploidy level. Genome size values for *Carthamus* were obtained from Garnatje *et al.* (2006).

Table 3. GenBank accession numbers for the species sequenced in this study.

Taxon	ITS1	ITS2
Carduncellus catrouxii	MW209004	MW208954
Carduncellus lucens	MW209005	MW208955
Carduncellus pectinatus	MW209007	MW208957
Carduncellus reboudianus	MW209006	MW208956

Genome size and ploidy level diversity in the *Carduncellus-Femeniasia-Phonus* clade

Genome size values (2C) range from 3.24 pg in *Carduncellus calvus* Boiss. & Reut., a diploid species endemic to Maghreb region (Morocco and Algeria), to 11.16 pg in a hexaploid accession of *C. eriocephalus* Boiss., a species showing several ploidy levels (Vilatersana *et al*., 2000*b*). This species occurs in the Maghreb region, reaching Egypt. *Femeniasia balearica*, endemic to Menorca (Balearic Islands), presents a 2C value of 3.84 pg, and *Phonus*, 2C values from 4.54 to 4.60 pg. Monoploid genome size (1C*x*) ranges from 1.29 pg in a tetraploid accession of *C. duvauxii* Batt. et Trab. (2*n* = 48; Vilatersana *et al*., 2000*b*), which has also been reported as a diploid by López González (1990), to 2.28 in *C. mareoticus* (Del.) Hanelt, a diploid species endemic from the northern part of Egypt and Libya. *Femeniasia balearica* displays a 1C*x* value of 1.92 and the 1C*x* values oscillate between 2.27 and 2.30 in the two studied species of the genus *Phonus*, all of them being diploid.

Three ploidy levels have been found (2*x*, 4*x* and 6*x*) in genus *Carduncellus*, some of which have been inferred from genome size values. Fifteen taxa are diploid, seven tetraploid and only one (*C. eriocephalus*) is a hexaploid. It is to note that a triploid

chromosome count was also reported in this same Moroccan accession of *C. eriocephalus* (Vilatersana *et al*., 2000*b*), indicating within-population cytotype diversity. In *Carduncellus*, where diploids and tetraploids predominate, the only other reports of triploidy was for *C. calvus* and of hexaploidy, for *C. caeruleus* (L.) C. Presl. and *C. pinnatus* (Desf.) DC. (López González, 1990; Rice *et al*., 2015). B chromosomes have been found in several species of *Carduncellus* and this could be one of the reasons for the wide variability in genome size found within the same ploidy level in phylogenetically closely related species, since dysploidy is not frequent in this genus (Vilatersana *et al*., 2000*b*; Vilatersana, 2002). Our results suggest a loss of DNA per basic genome in polyploids (mean 1C*x* = 1.66 pg) with respect to diploids (mean 1C*x* = 1.94 pg), a phenomenon known as genome downsizing, largely observed in plants (Leitch & Bennett, 2004), present in the family Asteraceae (e.g. Pires *et al*., 2004; Chrtek *et al*., 2009; Pellicer *et al*., 2010; Vallès *et al*., 2013). The decrease of 1C*x* values in the polyploid species has been found statistically significant by ANOVA test ($F = 7.1914$, $p = 0.0143$).

Genome size trends in the *Carthamus-Carduncellus* complex

The insular *Femeniasia* has a lower nuclear DNA content than *Phonus*, a continental sister genus with which it shares clade and chromosome number (Fig. 1, Table 2). The insularity may have played a role in the reduced genome size of a genus endemic of a Mediterranean island (Menorca) but further studies will be needed to confirm this hypothesis. Similar cases were reported in *Carthamus* (Garnatje *et al*., 2006) and *Cheirolophus* (Garnatje *et al*., 2007; Hidalgo *et al*., 2017) species, from the same tribe, as well as in other Asteraceae (Zahradníček *et al*., 2018). This phenomenon has been attributed to island colonisation pressure (Suda *et al*., 2003) and to the higher facility of naturalisation of plants with smaller genomes (Kapralov & Filatov, 2011).

Considering only the diploid taxa, the 1C*x* average was 1.33 pg for *Carthamus* and 1.85 pg for *Carduncellus-Femeniasia-Phonus* clade. Statistically significant differences in the 1C*x* values between *Carthamus* and *Carduncellus* ($F = 99.8583$, $p = 0.0000$) support the previously stated independent genomic evolution of these two lineages, although phylogenetic inferences have not yet fully resolved the relationships between *Carthamus* and the western group (*Carduncellus*, *Femeniasia* and *Phonus*; Vilatersana *et al*., 2000*a*). For each ploidy level, genome size values in the *Carduncellus-Femeniasia-Phonus* clade are consistently higher than those of *Carthamus*; indeed, their range do not even overlap (Fig. 1B). This trend of higher genome sizes in perennial taxa compared to relatives with short life cycle is frequently observed in plants (for Asteraceae, see e.g. Hidalgo *et al*., 2008; Siljak-Yakovlev *et al*., 2017; Qiu *et al*., 2019; but see Pellicer *et al*., 2014). However, as suggested by Vitales *et al*. (2019), the observed associations between genome size and life cycle in Asteraceae could be better explained by phylogenetic relatedness between taxa. Yet, more data is needed, and also analysed in an evolutionary context, to establish such an association.

CONCLUSIONS

Genome size has proved to be a valuable tool for discriminating between closely related plant groups. The observed differences in the DNA amount between the two main clades of the *Carthamus-Carduncellus* complex, the *Carthamus* genus on the one hand and *Carduncellus-Femeniasia-Phonus* clade on the other, suggest that these genera have evolved independently. In this sense, our results give support to taxonomic treatments of the *Carthamus-Carduncellus* complex that would consider at least two genera.

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