D. R. Agius, J. Čížková, J. P. Ebejer, L. Fresta, S. Lanfranco, J. Doležel & R. Farrugia

Genome size estimation of three endemic plant taxa from Malta

Abstract

Agius, D. R., Čížková, J., Ebejer, J. P., Fresta, L., Lanfranco, S., Doležel, J. & Farrugia, R.: Genome size estimation of three endemic plant taxa from Malta. — Fl. Medit. 33: 215-224. 2023. — ISSN: 1120-4052 printed, 2240-4538 online.

The Maltese archipelago has several endemic species adapted to an arid and hot climate. Due to its limited land area (316 km^2) and high human population density most of these endemics are endangered or critically so. Few genomic studies have been carried out on this flora to date. The purpose of the present study was to estimate genome sizes (1C-values) of three of these endemic taxa using flow cytometry. The genome size of *Cheirolophus crassifolius*, was found to be 0.98 pg. This is the highest recorded value in this genus and does not fit published values and trends. The genome size of the octoploid *Sedum album* subsp. *rupimelitense* was found to be 1.05 pg and that of *Anthyllis hermanniae* subsp. *melitensis* 0.52 pg.

Key words: endemism, island biodiversity, genome size, *Sedum*, *Cheirolophus*, *Anthyllis*.

Introduction

The Mediterranean basin is a hotspot of plant biodiversity (Myers & al. 2000). Sixty percent of the native plant species are endemics to the region with most being restricted to a single well-defined geographical area. It has also been observed that in the Mediterranean region, endemic species tend to occur on rocky habitats, on steeper slopes and in open vegetation rather than woodland (Thompson 2020). The Maltese archipelago, being central to this biodiversity hotspot, has a flora rich in rare endemic and relict species. This is also attributed to its geomorphology, edaphic factors, climatic fluctuations over the past eons and past geological activity amongst others (Cassar & al. 2008; Brullo & al. 2020).

Malta is one of the most densely populated countries. This creates strong pressures that are reshaping the distribution of the endemic taxa and changing the natural landscape. Conflicting land uses are resulting in loss of habitat for the endemics, with most of them becoming extremely rare and critically endangered. Many of these species are relevant to phylogenomic studies of the major taxa (Rokas & Abbot 2009; Brullo & al. 2020). A restricted and fragmented habitat is also resulting in loss of biodiversity within these taxa. This genetic diversity is currently of scientific interest since it confers adaptability to the

extreme climatic conditions found locally (Scheben & al. 2016). It can provide insight into these characteristics in view of the worldwide need of crops adapted to such climates, which are becoming more prevalent in wider geographical areas due to climate change (Rajpal & al. 2023). Genomic studies will address these issues including the conservation of this diversity (Theissinger & al. 2023). Of special interest are the three endemic and threatened taxa *Cheirolophus crassifolius* (Bertol.) Susanna, the Maltese rock-centaury, which is the islands' own national plant, *Sedum album* L. subsp. *rupimelitense* Mifsud, Stephenson & Thiede and *Anthyllis hermanniae* L. subsp. *melitensis* Brullo & Giusso del Galdo.

Cheirolophus crassifolius (*Asteraceae*: *Cardueae*: *Centaureinae*), a rupestral endemic of the Maltese islands, is considered a relict species of the preglacial circum-Mediterranean distribution of this genus (Susanna & al. 1999). Genome size has been recorded for twenty-four out of the twenty-seven species in this genus (Garnatje & al. 2007; Hidalgo & al. 2017). Garnatje & al. (2007) reported a statistically significant difference between mean 2C nuclear DNA contents of continental (1.58 pg) and insular (1.38 pg) *Cheirolophus* species. The smaller genome size of the insular species was attributed to selection pressures linked to speciation in restricted space or due to founder effects. The genome size of *C. crassifolius* was not reported in this study. Both chloroplast and nuclear DNA sequences have shown that *C. crassifolius* forms the most basal lineage in this genus (Hidalgo & al. 2017). This Maltese endemic is designated as critically endangered (Stevens & Lanfranco 2006).

Sedum album subsp. *rupimelitense* is a rupestral endemic, which is also critically endangered (Mifsud & al. 2015). *S. album* reverts to CAM photosynthesis under drought conditions, making it of prime interest in agricultural productivity research (Wai & al. 2019). Cytologically it is heterogenous with a base chromosome number of 17 from which a polyploid series is derived. The documented genome size for a diploid *S. album* is 0.15 pg ('t Hart 1991). Wai & al. (2019) reported genome size of 0.62 pg for a tetraploid individual. The Maltese subspecies, which reproduces asexually, has $2n = 8x = 136$ but undocumented genome size (Mifsud & al. 2015).

Anthyllis hermanniae L. species complex is divided in several disjunct and mostly isolated populations in Asian countries and the north-eastern Mediterranean. This complex was recently revised, to include several subspecies which are considered schizoendemics. *A. hermanniae* subsp. *melitensis*, has been consequently identified as a Maltese endemic. It is given the status of endangered in the IUCN Red List (Brullo & Giusso del Galdo 2006). The members of this genus are typically diploid with $2n = 2x = 12, 14$ (Goldblatt 2007). The genome size of *A. hermanniae* and its Maltese subspecies have not been estimated yet.

In the past fifty years, genome size estimation has enabled a multitude of investigations into biological patterns and processes (Kron & al. 2007). The genome size is the amount of DNA (C-value) in the haploid gametic nucleus (Doležel 2005), quantified in picograms (pg) or megabase pairs (Mbp), where 1 pg of nuclear DNA amounts to a length of 978 Mbp (Doležel & al. 2003). Genome size estimation by flow cytometry (FCM) is the gold standard of techniques for this metric (Al-Qurainy & al. 2021). FCM is not destructive which is critical when studying endangered species. Genome size estimation is one of the initial steps in genomic studies (Rhie & al. 2021). Its importance is evident in the fact that online databases documenting C-values have seen a steady input of data in the past decades.

Bioinformatics based methods for genome size estimation are becoming increasingly popular, although their level of accuracy, especially in highly repetitive plant genomes, is still to be investigated (Pellicer & Leitch 2020).

The main purpose of this work is to estimate the genome size of the three endemic, and threatened taxa from the islands of Malta, *C. crassifolius, S. album* subsp. *rupimelitense* and *A. hermanniae* subsp. *melitensis* using FCM.

Materials and Methods

Plant material collection. - Leaves from five different individuals of each taxon were used in this investigation. Table 1 lists the sites of specimen collection. In the case of *Anthyllis hermanniae* subsp. *melitensis* and *Cheirolophus crassifolius,* one sample was from a plant cultivated on the premises of the local Plant Protection Directorate at Lija, Malta. Collection and shipment of samples were done according to permit EP 1288/22 issued by the Environmental and Resource Authority of Malta. Photographic records were kept of the plants, together with their geographic coordinates. Each plant was tagged (with a reference number attached to the stem of the plant using a PVC wrap) for future reference.

Sample preparation and cytometric measurements. - The FCM was carried out at the Centre of Plant Structural and Functional Genomics (Institute of Experimental Botany, Czech Republic) using a Sysmex CyFlow® Space flow cytometer (Partec GmbH, Göttingen, Germany) installed with the software FloMax and equipped with a 532 nm green high-grade solid-state laser.

All leaves chosen for this analysis were young and visibly free from infection by parasites. The internal standard and isolation buffer were chosen so that the coefficient of variation (CV) was kept to a minimum (Loureiro & al. 2007). Mechanical isolation of the plants' nuclei was carried out according to Galbraith & al. (1983). This involved chopping the sandwiched leaf material from the studied plant and internal reference standard together, in isolation buffer, using sharp razor blades in glass Petri dishes. The homogenate was filtered through a double layer of 50 µm nylon mesh (Silk & Progress, CR) and stained with the fluorochrome propidium iodide (PI) at a concentration of 50 mg/mL and supplemented with 50 mg/mL RNase.

Table 1. Site of specimen collection for each species (N/A: not applicable).

Taxon	Sample No. (No. of technical replicates)	GPS coordinates		Locality Name
		Latitude	Longitude	
Cheirolophus crassifolius	C1 (6), C2 (6), C4 (3), C5 (3)	35°50'47.6"N	14°23'36.3"E	Dingli Cliffs
	C3 (6)	N/A	N/A	
Sedum album subsp. *rupimelitense*	S1 (5), S2 (4), S3 (6), S4 (3), S5 (3)	35°50'47.6"N	14°23'36.2"E	Dingli Cliffs
Anthyllis hermanniae subsp. *melitensis*	A1 (6), A2 (6), A4 (3), A5 (3)	35°50'47.6"N	14°23'36.3"E	Dingli Cliffs
	A3 (6)	N/A	N/A	

Woody plant buffer (Loureiro & al. 2007) was found to be the optimal buffer for *A. hermanniae* subsp. *melitensis*. In this case the internal standard used was *Solanum lycopersicum* L. cv. Stupické with a 2C DNA content of 1.96 pg (Doležel & al. 2007).

CyStain PI OxProtect Kit (Sysmex, United Kingdom), used according to manufacturer's instructions, was found to be the optimal system for the genome size estimation of *C. crassifolius* and *S. album* subsp. *rupimelitense*. The internal standards used were *Glycine max* Merr. cv. Polanka with a 2C DNA content of 2.50 pg and *Zea mays* L. cv. CE-777 with a 2C genome size of 5.43 pg respectively (Doležel & al. 2007). A minimum of five thousand events were analysed for each run. To improve accuracy and precision, the genome sizes were determined for each species as the mean of five individual specimens and several technical replicates (Table 1) to enable the standard error of the mean to be calculated. The technical replicates were spread out on different days.

The obtained histograms were visualised using FlowJo software (Version 10.8.2, Treestar, Ashland, OR, United States). The sample 2C DNA content was calculated according to the formula:

$$\text{2C DNA content of sample} = \frac{\text{MFI of sample G1 peak}}{\text{MFI of standard G1 peak}} \times \text{standard 2C DNA content}$$

Where MFI is the mean fluorescence intensity, the standards used were as specified above for each sample plant and the 2C DNA content is given in picograms (pg).

A one-way Analysis of Variance (ANOVA) was performed to compare the effect of each individual replicate (per taxon) on estimated genome size measured using FCM. The ANOVA test was implemented in R (version 4.3.0).

Results and Discussion

The results obtained from flow cytometric analysis of PI-stained nuclei are summarised in Table 2. Figure 1 illustrates representative histograms of the genome size investigations. All three genomes in this study are on the smaller range in the plant kingdom (Pellicer & Leitch 2020). In our design we collected five individual plants for each of the three species: *C. crassifolius, S. album* subsp. *rupimelitense* and *A. hermanniae* subsp. *melitensis*. The number of technical replicates per individual specimen ranges from three to six (Table 1). The ANOVA test reported no statistically significant difference between the individual specimens for each species using an α-threshold of 0.05. F-values, P-values and degrees of freedom for each species are reported in Table 3.

We report that the 2C-value for *Cheirolophus crassifolius* is 1.95 pg, equivalent to a genome size of 0.98 pg (954 Mbp). This value is higher than a previously reported one of 0.9 pg, for the same species, by Hidalgo & al. (2017) which was estimated using a single specimen from the Orto Botanico of the Università degli Studi di Palermo in Italy. The value reported in the present study is the highest recorded for this genus in the Genome Size in *Asteraceae* Database (Release 3.0) (Garnatje & al. 2011). It also does not fall within the range of genome size variation documented for insular members of this genus by Garnatje & al. (2007). It is even higher than the mean value for continental members of this genus reported in the same work, hence it does not fit the hypothesis, based on statistically significant results, that insular members have a smaller genome size than continental members in this genus. It also implies that there is an incremental increase in genome

Table 2. Flow cytometry results for the three endemic taxa.

Taxon	2C-value (pg ± SD)	Ploidy level	1C-value (pg)	1C (pg)	Estimated Genome size (1C, Mbp)	Monoploid genome size (1Cx, Mbp)
Cheirolophus crassifolius	1.95 ± 0.06	2x	0.98	0.98	954	954
Sedum album subsp. *rupimelitense*	2.11 ± 0.04	8x	1.05	0.26	1027	257
Anthyllis hermanniae subsp. *melitensis*	1.04 ± 0.03	2x	0.52	0.52	509	509

Table 3. Results of ANOVA tests between individual speci-
mens for the three species.

Species	F-value	P-value	Degrees of Freedom
Cheirolophus crassifolius	1.738	0.183	4
Sedum album subsp. *rupimelitense*	1.760	0.186	4
Anthyllis hermanniae subsp. *melitensis*	0.795	0.543	4

size in *C. crassifolius* when compared to the Mediterranean ancestral species *C. uliginosus*. This also does not fit the finding that there is a smaller genome size in the derived species within *Cheirolophus* (Garnatje & al. 2007). This might be interpreted as confirmation of basal position of this species within this genus which has a trend of genome size reduction in the derived species as showed by Hidalgo & al. (2017). Further genomic studies within this genus are required to shed light on the reasons for this larger genome size.

Sedum album subsp. *rupimelitense* was found to have an unreduced nucleus with a DNA content of 2.11 pg, amounting to a holoploid genome size of 1.05 pg (1,027 Mbp). The monoploid genome size (1Cx) is 0.26 pg equivalent to 254 Mbp of DNA. The 1Cx value, is a quantitative measure of the amount of DNA in the basic set of chromosomes. It is calculated by dividing the 2C-value by the ploidy level (Leitch & Bennett 2004). The value of 1Cx for *S. album* documented here is higher than the 1Cx value documented by 't Hart (1991) for a diploid *S. album*. This is counter to the literature finding that 1Cx values tend to decrease with increased ploidy (Leitch & Bennett 2004). However, 't Hart (1991) does not specify the method by which genome size was estimated (van Prooijen-Knegt & al. 1980; Doležel & al. 1998). This general trend, showing a decrease in 1Cx values with increasing ploidy is attributed to the predominance of deletion over insertion mutations, gene fractionation and unequal recombination which eliminates retrotransposon sequences (Bennetzen 2002). Wai & al (2019) reported a holoploid genome size of 0.62 pg and a monoploid genome size of 0.31 pg for a tetraploid *S. album* while k-mer analysis gave a monoploid genome size of 256 Mbp (equivalent to 0.26 pg of DNA) for the tetraploid. Our results are more concordant with the results published by Wai & al. (2019) for *S. album*. Further studies into the genome size of this taxon are required. The second minor peak in the histogram of *S. album* subsp. *rupimelitense* (Fig. 1B) represents the G2 nuclei of this species and as expected its position shows doubling in the DNA amount of the G1 nuclei.

Our investigations shows that the nuclear DNA content of *Anthyllis hermanniae* subsp. *melitensis* is 1.04 pg amounting to a genome size of 0.52 pg (509 Mbp). This is the third
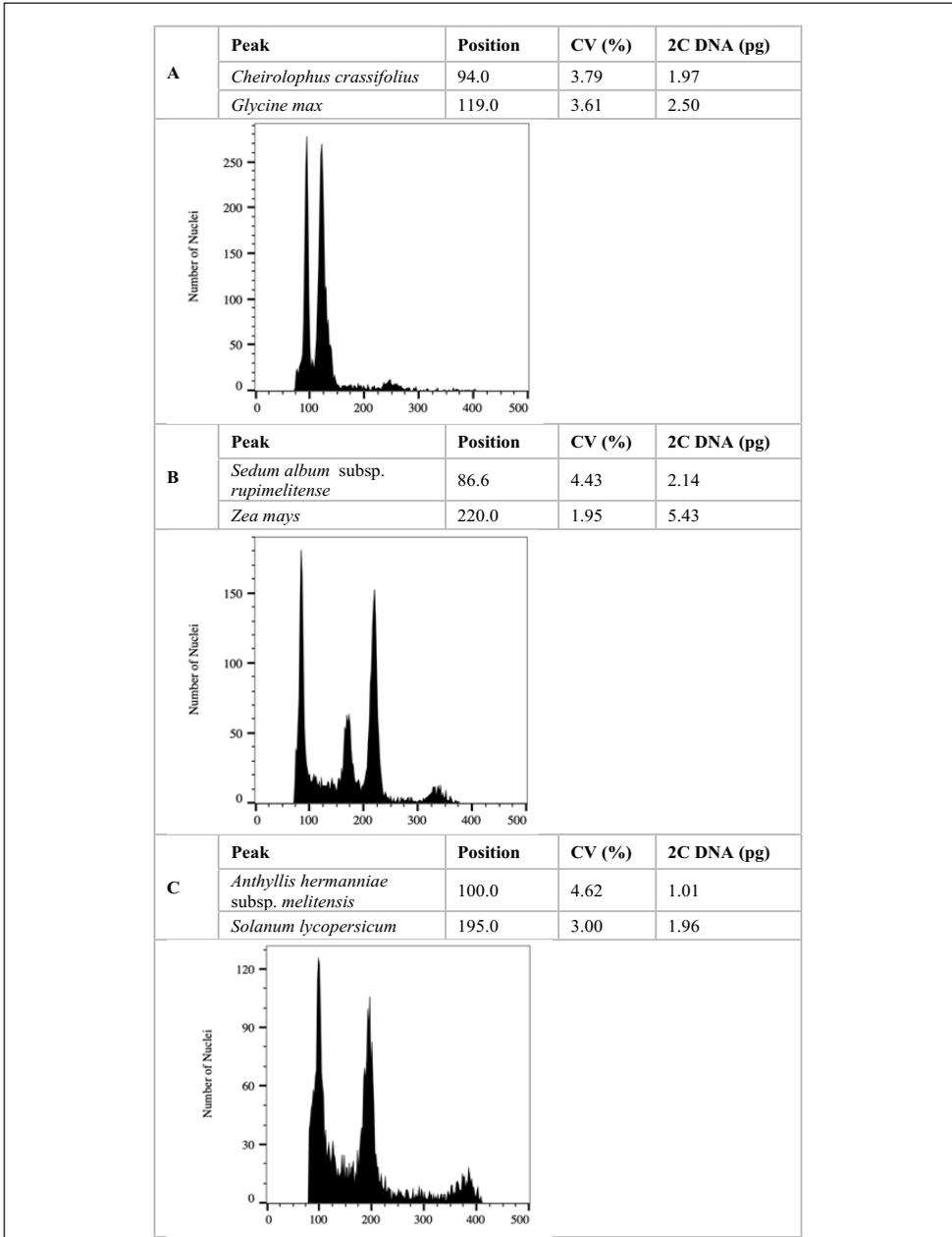
A	Peak	Position	CV (%)	2C DNA (pg)
	Cheirolophus crassifolius	94.0	3.79	1.97
	Glycine max	119.0	3.61	2.50

B	Peak	Position	CV (%)	2C DNA (pg)
	Sedum album subsp. *rupimelitense*	86.6	4.43	2.14
	Zea mays	220.0	1.95	5.43

C	Peak	Position	CV (%)	2C DNA (pg)
	Anthyllis hermanniae subsp. *melitensis*	100.0	4.62	1.01
	Solanum lycopersicum	195.0	3.00	1.96

Fig. 1. Representative histograms of flow cytometry data showing 2C DNA content of 3 taxa endemic to Malta. The x-axis shows channel as a function of relative fluorescence intensity, while the y-axis represents the number of nuclei. (A) *C. crassifolius* (left peak) and *G. max* Merr. cv. Polanka (right peak); (B) *S. album* subsp. *rupimelitense* (left peak) and *Z. mays* L. 'CE-777' (right peak). The second minor peak in the histogram, at position 173, represents the G2 nuclei of this species. (C) *A. hermanniae* subsp. *melitensis* (left peak) *S. lycopersicum* L. cv. Stupické (right peak). (CV: coefficient of variation).

documented C-value within this genus of 23 species. It falls well within the range for this genus of 0.50 – 0.67 pg DNA (Pellicer & Leitch 2020). The chromosome number within the sporophytes of this genus varies from 12 to 14 chromosomes (Goldblatt 2007). This finding supports the indication that genome size within this genus is on the smaller side within the plant kingdom.

This is the first study of nuclear genome size of Maltese endemic plant taxa. Herein we report the genome size of *C. crassifolius,* the Maltese rock-centaury, and two recently described taxa *S. album* subsp. *rupimelitense* and *A. hermanniae* subsp. *melitensis.* We determined the genome size by means of FCM. All three species inhabit Special Areas of Conservation. The small geographical area they inhabit, and anthropogenic factors make these taxa endangered, some of them critically so.

The genera *Cheirolophus*, *Anthyllis* and *Sedum* have been studied extensively ('t Hart 1991; Susanna & al. 1999; Brullo & Giusso del Galdo 2006; Garnatje & al. 2007; Pellicer & Leitch 2020). However, most of these works do not include the Maltese endemics making the available knowledge incomplete. The results presented here shed some additional light into the evolutionary history of these genera and can also be used in further studies including studies into the characteristics that give them adaptability to the extreme local hot and dry environment, which is becoming more prevalent worldwide.

Acknowledgements

References

Al-Qurainy, F., Gaafar, A. -R. Z., Khan, S., Nadeem, M., Alshameri, A. M., Tarroum, M., Alansi, S., Almarri, N. B. & Alfarraj, N. S. 2021: Estimation of genome size in the endemic species *Reseda pentagyna* and the locally rare species *Reseda lutea* using comparative analyses of flow cytometry and k-mer approaches. – Plants **10:** 1362. https://doi.org/10.3390/plants10071362

Bennetzen, J. L. 2002: Mechanisms and rates of genome expansion and contraction in flowering plants. – Genetica **115:** 29-36. https://doi.org/10.1023/A:1016015913350

Brullo, S. & Giusso del Galdo, G. 2006: Taxonomic Remarks on the *Anthyllis hermanniae* L. (*Fabaceae, Faboideae*) Species Complex of the Mediterranean flora. – Novon **16:** 304-314.

—, Brullo, C., Cambria, S. & Giusso del Galdo, G. 2020: The Vegetation of the Maltese Islands. – Cham. https://doi.org/10.1007/978-3-030-34525-9

Cassar, L. F., Conrad, E. & Schembri, P. J. 2008: The Maltese Archipelago. – Pp. 297-322 in: Vogiatzakis, I., Pungetti, G. & Mannion, A. M. (eds), Mediterranean Island Landscapes. – Dordrecht. https://doi.org/10.1007/978-1-4020-5064-0_13

Doležel, J. 2005: Plant DNA flow cytometry and estimation of nuclear genome size. – Ann. Bot. **95:** 99-110. https://doi.org/10.1093/aob/mci005

Doležel, J., Greilhuber, J. & Suda, J. 2007: Flow cytometry with plant cells: analysis of genes, chromosomes and genomes. – Weinheim.

—, Bartoš, J., Voglmayr, H. & Greilhuber, J. 2003: Letter to the editor: Nuclear DNA content and genome size of trout and Human – Cytometry **51A:** 127-128. https://doi.org/10.1002/cyto.a.10013

—, Greilhuber, J., Lucretti, S., Meister, A., Lysák, M. A., Nardi, L. & Obermayer, R. 1998: Plant genome size estimation by flow cytometry: inter-laboratory comparison. – Ann. Bot. **82:** 17-26. https://doi.org/10.1093/oxfordjournals.aob.a010312

Galbraith, D. W., Harkins, K. R., Maddox, J. M., Ayres, N. M., Sharma, D. P. & Firoozabady, E. 1983: Rapid flow cytometric analysis of the cell cycle in intact plant tissues. – Science **220:** 1049-1051. https://doi.org/10.1126/science.220.4601.1049

Garnatje, T., Garcia, S. & Canela, M. Á. 2007: Genome size variation from a phylogenetic perspective in the genus *Cheirolophus* Cass. (*Asteraceae*): biogeographic implications. – Pl. Syst. Evol. **264:** 117-134. https://doi.org/10.1007/s00606-006-0489-7

—, Canela, M. Á., Garcia, S., Hidalgo, O., Pellicer, J., Sánchez-Jiménez, I., Siljak-Yakovlev, S., Vitales, D. & Vallès, J. 2011: GSAD: A genome size in the *Asteraceae* database. – Cytometry **79A:** 401-404. https://doi.org/10.1002/cyto.a.21056

Goldblatt, P. 2007: The index to plant chromosome numbers - past and future. – Taxon **56:** 984-986. https://doi.org/10.2307/25065898

Hidalgo, O., Vitales, D., Vallès, J., Garnatje, T., Siljak-Yakovlev, S., Leitch, I. J. & Pellicer, J. 2017: Cytogenetic insights into an oceanic island radiation: The dramatic evolution of pre-existing traits in *Cheirolophus* (*Asteraceae*: *Cardueae: Centaureinae*). – Taxon **66:** 146-157. https://doi.org/10.12705/661.8

Kron, P., Suda, J. & Husband, B. C. 2007: Applications of flow cytometry to evolutionary and population biology. – Ann. Rev. Ecol. Evol. Syst. **38:** 847-876. https://doi.org/10.1146/annurev.ecolsys.38.091206.095504

Leitch, I. J. & Bennett, M. D. 2004: Genome downsizing in polyploid plants: genome downsizing in polyploids. – Biol. J. Linn. Soc. **82:** 651-663. https://doi.org/10.1111/j.1095-8312.2004.00349.x

Loureiro, J., Rodriguez, E., Dolezel, J. & Santos, C. 2007: Two new nuclear isolation buffers for plant DNA flow cytometry: A test with 37 species. – Ann. Bot. **100:** 875-888. https://doi.org/10.1093/aob/mcm152

Mifsud, S. M., Stephenson, R. & Thiede, J. 2015: *Sedum album* subsp. *rupimelitense* (*Crassulaceae*), a new vegetatively reproducing subspecies from Malta (Maltese Islands, Central Mediterranean). – Phytotaxa **227:** 135. https://doi.org/10.11646/phytotaxa.227.2.3

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. 2000: Biodiversity hotspots for conservation priorities. – Nature **403:** 853-858. https://doi.org/10.1038/35002501

Pellicer, J. & Leitch, I. J. 2020: The Plant DNA C-values database (release 7.1): an updated online repository of plant genome size data for comparative studies. – New Phytol **226:** 301-305. https://doi.org/10.1111/nph.16261

Rajpal, V. R., Singh, A., Kathpalia, R., Thakur, R. K., Khan, M., Pandey, A., Hamurcu, M. & Raina, S. N. 2023: The prospects of gene introgression from crop wild relatives into cultivated lentil for climate change mitigation. – Frontiers Pl. Sci. **14:** p.1127239. https://doi.org/10.3389/fpls.2023.1127239

Rhie, A., McCarthy, S. A., Fedrigo, O., Damas, J., Formenti, G., Koren, S., Uliano-Silva, M., Chow, W., Fungtammasan, A., Kim, J., Lee, C., Ko, B. J., Chaisson, M., Gedman, G. L., Cantin, L. J., Thibaud-Nissen, F., Haggerty, L., Bista, I., Smith, M., Haase, B., Mountcastle, J., Winkler, S., Paez, S., Howard, J., Vernes, S. C., Lama, T. M., Grutzner, F., Warren, W. C., Balakrishnan, C. N., Burt, D., George, J. M., Biegler, M. T., Iorns, D., Digby, A., Eason, D., Robertson, B., Edwards, T., Wilkinson, M., Turner, G., Meyer, A., Kautt, A. F., Franchini, P., Detrich, H. W., Svardal, H., Wagner, M., Naylor, G. J. P., Pippel, M., Malinsky, M., Mooney, M., Simbirsky, M., Hannigan, B. T., Pesout, T., Houck, M., Misuraca, A., Kingan, S. B., Hall, R., Kronenberg, Z., Sović, I., Dunn, C., Ning, Z., Hastie, A., Lee, J., Selvaraj, S., Green, R. E., Putnam, N. H., Gut, I., Ghurye, J., Garrison, E., Sims, Y., Collins, J., Pelan, S., Torrance, J., Tracey, A., Wood, J., Dagnew, R. E., Guan, D., London, S. E., Clayton, D. F., Mello, C. V., Friedrich, S. R., Lovell, P. V., Osipova, E., Al-Ajli, F. O., Secomandi, S., Kim, H., Theofanopoulou, C., Hiller, M., Zhou, Y., Harris, R. S., Makova, K. D., Medvedev, P., Hoffman, J., Masterson, P., Clark, K., Martin, F., Howe, K., Flicek, P., Walenz, B. P., Kwak, W., Clawson, H., Diekhans, M., Nassar, L., Paten, B., Kraus, R. H. S., Crawford, A. J., Gilbert, M. T. P., Zhang, G., Venkatesh, B., Murphy, R. W., Koepfli, K. -P., Shapiro, B., Johnson, W. E., Di Palma, F., Marques-Bonet, T., Teeling, E. C., Warnow, T., Graves, J. M., Ryder, O. A., Haussler, D., O'Brien, S. J., Korlach, J., Lewin, H. A., Howe, K., Myers, E. W., Durbin, R., Phillippy, A. M. & Jarvis, E. D. 2021: Towards complete and error-free genome assemblies of all vertebrate species. – Nature **592:** 737-746. https://doi.org/10.1038/s41586-021-03451-0

Rokas, A. & Abbot, P. 2009: Harnessing genomics for evolutionary insights. – Trends Ecol. Evol. **24:** 192-200. https://doi.org/10.1016/j.tree.2008.11.004

Scheben, A., Yuan, Y. & Edwards, D. 2016: Advances in genomics for adapting crops to climate change. – Curr. Pl. Biol. **6:** 2-10. https://doi.org/10.1016/j.cpb.2016.09.001

Stevens, D. & Lanfranco, E. 2006: *Cheirolophus crassifolius*. *The IUCN Red List of Threatened Species* 2006: e.T61621A12524967. https://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T61621A12524967.en [accessed 8/6/2023].

Susanna, A., Garnatje, T. & Garcia-Jacas, N. 1999: Molecular phylogeny of *Cheirolophus* (*Asteraceae:Cardueae-Centaureinae*) based on ITS sequences of nuclear ribosomal DNA. – Pl. Syst. Evol. **214:** 147-160. https://doi.org/10.1007/BF00985736

't Hart, H., 1991: Evolution and classification of the European *Sedum* species (*Crassulaceae*). – Fl. Medit. **2:** 31-61.

Theissinger, K., Fernandes, C., Formenti, G., Bista, I., Berg, P. R., Bleidorn, C., Bombarely, A., Crottini, A., Gallo, G. R., Godoy, J. A., Jentoft, S., Malukiewicz, J., Mouton, A., Oomen, R. A., Paez, S., Palsbøll, P. J., Pampoulie, C., Ruiz-López, M. J., Secomandi, S., Svardal, H., Theofanopoulou, C., de Vries, J., Waldvogel, A. -M., Zhang, G., Jarvis, E. D., Bálint, M., Ciofi, C., Waterhouse, R. M., Mazzoni, C. J. & Höglund, J. 2023: How genomics can help biodiversity conservation. – Trends Genet. **39(7):** 545-559. https://doi.org/10.1016/j.tig.2023.01.005

Thompson, J. D. 2020: Plant evolution in the Mediterranean: insights for conservation, 2nd ed. – New York.

van Prooijen-Knegt, A. C., Redi, C. A. & van der Ploeg, M. 1980: Quantitative aspects of the cytochemical Feulgen-DNA procedure studied on model systems and cell nuclei. – Histochemistry **69:** 1-17. https://doi.org/10.1007/bf00508362

Wai, C. M., Weise, S. E., Ozersky, P., Mockler, T. C., Michael, T. P. & van Buren, R. 2019: Time of day and network reprogramming during drought induced CAM photosynthesis in *Sedum album*. – PLoS Genet. **15:** e1008209. https://doi.org/10.1371/journal.pgen.1008209

Addresses of Authors:
Dolores Rita Agius[1, 2]*, Jana Čížková[3], Jean Paul Ebejer[1], Louis Fresta[4], Sandro Lanfranco[5], Jaroslav Doležel[3] & Rosienne Farrugia[1, 6]*,
[1]Centre for Molecular Medicine and Biobanking, University of Malta, Msida, MSD 2080, Malta.
[2]Ġ.F. Abela Junior College, Ġuzè Debono Square, Msida, MSD 1252, Malta.
[3]Institute of Experimental Botany of the Czech Academy of Sciences, Centre of Plant Structure and Functional Genomics, Olomouc, Czech Republic.
[4]Plant Protection Directorate, 110 Triq Annibale Preca, Lija, LJA 1915, Malta.
[5]Department of Biology, Faculty of Science, University of Malta, Msida, MSD 2080, Malta.
[6]Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta, Msida, MSD 2080, Malta.
Dolores R. Agius: dorita.agius@um.edu.mt;
Jana Čížková: cizkova@ueb.cas.cz;
Jean-Paul Ebejer: jean.p.ebejer@um.edu.mt;
Louis Fresta: louis-john.fresta@gov.mt;
Sandro Lanfranco: sandro.lanfranco@um.edu.mt;
Jaroslav Doležel: dolezel@ueb.cas.cz;
Rosienne Farrugia: rosienne.farrugia@um.edu.mt

*Corresponding author