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DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

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# Genome size of invasive and non-invasive succulent species

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Bioquímica, realizada sob a orientação científica do Professor Doutor João Loureiro (Universidade de Coimbra) e da Doutora Sílvia Castro (Universidade de Coimbra)

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2015

*“Challenges are good because they produce persistence, and persistence produces character, and character, hope”*

*Countless people encouraged and supported me in the good journey of University.*

*To you, my tutors, mom & brother, academic family and family in Christ,*

*a big thank you.*

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# Abstract

Invasiveness risk assessment and control strategies have been performed based on the study of plant traits that differ between invasive and non-invasive species. In the last decades, genome size started to be incorporated into this overall picture, and in several studies the possession of small genomes has been proposed to favor invasiveness. Despite invasiveness is a multivariate and complex puzzle, genome size has been considered one of the top eight best predictors of invasiveness, with small genomes being correlated with other traits that are also associated with invasiveness, such as minimum generation time, seed traits, relative growth rate of seedlings, specific leaf area, stomatal size and density, water and nutrient use and photosynthetic efficiency. Considering all this, the objective of this study was to evaluate if there was any correlation between genome size and invasiveness in a large data set of Cactaceae species. Moreover, other correlations with seed traits were explored to evaluate their usefulness in predicting invasiveness in this family. Therefore, the nuclear DNA content of the 191 species of Cactaceae, of which 41 are invasive, was estimated using propidium iodide based flow cytometry. Also, seed size and seed weight was calculated for the great majority of the species.

Results from this study demonstrated no significant relationship between genome size and invasiveness, and genome size is also not an explanatory variable of seed weight or seed size. However, invasive species tended to have larger and heavier seed sizes which opens the possibility to this trait's incorporation into risk invasiveness assessment strategies, besides the invasiveness traits already proposed for this family.



Keywords: Genome size, Flow cytometry, Cactaceae, Invasiveness, seed traits

## Resumo

Analises do risco de invasão e estratégias de controlo do mesmo têm sido delineadas com base no estudo de características fenotípicas que diferem entre espécies invasoras e não-invasoras. Nas ultimas decadas, o tamanho do genoma começou a ser incorporado neste quadro global, e em vários estudos, um tamanho de genoma mais pequeno foi proposto como um factor que favorece o caracter invasor. Nesse sentido, apesar do processo de invasão ser um processo complexo e onde entram em conta multivariaveis, o tamanho do genoma tem sido considerado um dos oito melhores caracteres previsores de invasão, com os genomas pequenos a serem correlacionados com outras caracteristicas associadas à invasão, como o tempo minimo de geração, caracteristicas das sementes, crescimento relativo, *ratio* de superficie da folha, densidade e tamanho dos estomas, eficiência fotossintética e do uso da água e nutrientes. Considerando tudo isto, o objectivo deste estudo foi avaliar se havia alguma correlação entre o tamanho de genoma e o carácter invasor num conjunto alargado de espécies da família Cactaceae. Adicionalmente, explorar características das sementes, e correlações possiveis com o tamanho do genoma, para avaliar a sua utilidade na previsão do caracter invasor. Para isso, foi efectuada uma análise do conteúdo em ADN de 191 espécies diferentes de Cactáceas, das quais 41 são invasoras, através da tecnica de citometria de fluxo com iodeto de propidio. O tamanho e peso das sementes foi também medido para a grande maioria das espécies.

Os resultados do presente estudo demonstraram não haver uma relação significativa entre o tamanho do genoma e o carácter invasor, e que o tamanho do genoma também não é explicativo da variação do peso e tamanho das sementes. Contudo, as espécies invasoras tendem a ter sementes maiores e mais pesadas, facto que abre a possíbilidade para as caracteristicas da semente serem incorporadas em estratégias de análise de risco, para além dos já anteriormente propostos para esta família.



Palavras-chave: tamanho do genoma, citometria de fluxo, Cactaceae, Invasão, caracteristicas das semente

# Introduction

# Introduction

The cell encompasses the majority of its genetic material in the nucleus, and genome size is one of its intrinsic properties. Amongst different groups of living organisms there are massive variations in terms of genome size. In angiosperms alone it varies more than 2,300-fold, from 64 Mbp (*Genlisea aurea*, Greilhuber et al. (2006) to approximately 150,000 Mbp (*Paris japonica*, Pellicer et al. 2010).

There are several mechanisms responsible for genome size variation, such as polyploidy (Comai 2005), hybridization (Baack et al. 2005), higher rate of nucleotide deletion over insertion through illegitimate recombination or through unequal intra-strand homologous recombination, and transposon amplification (Bennetzen et al. 2005).

Additionally, some theories pointed out that genome size might be involved in the scaling of living organisms, influencing its characteristics from cellular to organismal level by setting thresholds within which the genes can operate – the nucleotypic effect (Bennett 1971). All of the above reasons have made possible to use, genome size to predict correlations between this character and a given phenotype, ecological or physiological trait.

### *Methods for genome size estimation*

In the literature, plant genome size estimation has been accomplished with a series of different techniques. Amongst the most used are Feulgen microdensitometry and flow cytometry (Greilhuber 2008).

Feulgen microdensitometry is a technique in which the isolated nuclei are stained with a specific dye in a microscopic slide and analyzed for the amount of absorbed light

(Greilhuber 2005). This enables the posterior storage of the samples, as well as, the visual observation of particles, cell by cell, to assert the quality of the procedure before measurement. However, a major drawback of this technique is it is time consuming when considering studies of large populations. Moreover, the sensitivity of the technique makes it prone for erroneous measures when protocols are suboptimal (Greilhuber 2005; Greilhuber 2008).

Technological advancements have enabled the application of flow cytometry (FCM) to estimate genome size of plants. This technique is based on the quantitative staining of isolated nuclei with a DNA specific fluorochrome, which are then excited by a laser beam. Fluorescence emission is measured, thus allowing the estimate the amount of DNA the sample contains, in contrast to a standard with a known nuclear DNA content (Galbraith et al. 1983). Advantages of this technique are the ability to measure DNA amounts to the smallest differences amongst individuals, with relative speed, following fairly simple protocols and resulting in highly accurate estimates. Despite many applications require fresh material, flow cytometry is nowadays the best technique for genome size analysis in plants (Dolezel et al. 2007). Recently, one of the few drawbacks of flow cytometry, i.e., the lack of possibility to visualize the particles, has been circumvented with new instruments that combines the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery and functional insights of microscopy (e.g., FlowSight, ImageStream$^X$ Mark II from Amnis).

Regarding studies with plants, the technique has its best results using fresh young leaf tissue. Other materials such as seeds and herbarium collections can also be used, despite herbarium samples providing poorer results the older the sample is (Kolár et al. 2012; Suda & Trávnícek 2006).

# Introduction

To address the variation in genome size in plants, a database that compiles the available information was established, i.e., the DNA C-values database harbored by the Royal Botanical Gardens of Kew (Bennett & Leitch 2012). In its entries the database contains, among other groups, values for 7135 angiosperms (2% of all angiosperms) and 340 gymnosperms (33%). The majority of its nuclear DNA estimate entries were obtained using flow cytometry, while the oldest estimates were obtained by Feulgen microdensitometry.

Research regarding the broad impacts of genome size on plant traits has to consider large geographic scales and sampling across a wide range of taxonomic entities, thus, such databases are of great importance. Nonetheless, it is important to be mindful results were obtained in different laboratories, using different techniques and practices, and should therefore be regarded critically (Dolezel et al. 1998; Dolezel et al. 2007).

### *Plant invasions*

Invasiveness has been proposed as a multi-stage process. Plants or their propagules are first to be introduced, overcoming several major geographical barriers (Richardson et al. 2000). This process is many times initiated with their transportation to a new location via human mediated activities (Vitousek et al. 1997) and then facilitated by one or more new factors, such as the presence or absence of natural enemies, mutualists, competitors and abiotic factors in comparison with the native area (Mitchell et al. 2006). In this multistage process, naturalization is referred to as the process through which plants overcome biotic and abiotic obstacles to survive and reproduce. Invasion can be

mentioned when reproductive offspring are produced and spread to areas distant from their introduction sites (Richardson et al. 2000; Colautti & Macisaac 2004).

Invasive plants reduce the abundance and biodiversity of the communities in which they install themselves, leading eventually to the extinction of native species (Mack 2000; Sala et al. 2000). This is mostly due to their ability to modify the properties of ecosystems including carbon sequestration, nutrient cycles, hydrology, fire regimes and interaction with pollinators (Vitousek et al. 1996; Mack 2000; Ferrero et al. 2013; Levine et al. 2003). Furthermore, invasive species can also function as vectors for diseases or produce allergenic substances, making them a health threat, as well (Pimentel et al. 2001; Belmonte & Vilà 2004). Invasive plants also have impacts at the socio-economical level, as in agricultural, tourism and recreation activities (Vilà et al. 2009; Pyšek & Richardson 2010; Pimentel et al. 2000; Pimentel et al. 2005). Excluding the health costs, it is estimated that, for only 13% of all the invasive species/plants documented in Europe, 12.5 billion dollars are spent in Europe every year with the invasive species problematic, and 336 billion dollars annually when considering the United States, United Kingdom, Australia, India, Brazil and South Africa (Kettunen et al. 2009; Vilà et al. 2009; Pimentel et al. 2001).

Considering all the impacts of invasive species, it became urgent to develop protocols to detect potential invasive species in early stages after introduction or even before introduction. Several researchers are thus studying plant traits that might be related with invasion in order to predict their potential for it (eg. Pysek & Richardson 2007).

The effect of genome size on plants distribution is hardly direct, but several hints, given by different studies, have provided us with a better understanding of its overall usability as an invasiveness determinant. For instance, a study performed with more than 3,500 angiosperms revealed that invasive weedy species had smaller genomes (Chen et al.

2010). Other specific studies performed in weedy versus non-weedy genera, revealed a similar trend (Kuester et al. 2014; Leitch et al. 1998). Pandit et al. (2014) provided yet another global analysis with 890 invasive plant species, which again pointed towards smaller genomes being associated with invasiveness. Furthermore studies performed with genus such as *Artemisia, Briza* and even invasive seaweeds such as *Caulerpa* spp. demonstrated a correspondence between invasiveness and a small genome size (Garcia et al. 2008; Rejmánek 1996; Varela-Álvarez et al. 2012). Considering all this, and not surprisingly, small genome size was suggested to be one of the top eight best predictors for invasiveness (Rejmánek 1996).

Local studies provided results that equally demonstrated a small genome was an important trait in the establishment of plants outside the native range, such as naturalization, but was not necessarily relevant when it came to large spread processes. An example is the analysis of the Czech flora which compared 93 alien naturalized species of 32 families with their non-invading congeneric and confamilial species. Naturalized plants presented smaller genomes than congeners not known to be naturalized or invasive elsewhere in the world. Also, in confamilials, naturalized alien species had smaller genomes than non-invading ones. Finally, there was no difference between invasive and naturalized plants regarding genome size (Kubešová et al. 2010).

However, when genomes are smaller than a given threshold, which is the case of the genus *Acacia,* the presence of a small DNA is not necessarily linked to invasiveness (Gallagher et al. 2011).

Genome size correlation with plant traits associated with invasiveness has also been addressed in several studies (Suda et al. 2014). Genome size has been positively correlated with minimum generation time. This is expected, as plants with larger amounts of genetic material will take longer to duplicate than plants with smaller sets of DNA.

# Introduction

Smaller genomes, and therefore, a shorter minimum generation time are favorable for invasiveness in plant species. However plant genome size has demonstrated to correlate in opposite ways with various plant physiology traits consistently associated with invasiveness (Table 1).

**Table 1:** Plant traits consistently associated with invasiveness and their relationships with genome size, adapted from Suda et al. (2014)

| Plant trait | State favoring invasiveness | Relationship with genome size |
|---|---|---|
| **Plant defense chemistry** | High | Unknown |
| **Resistance to herbivory** | High | Unknown |
| **Water and nutrient use efficiency** | High | Negative |
| **Seed mass** | Low | Positive |
| **Seed number** | High | Negative |
| **(Seedling) growth rate** | Fast | Negative |
| **Specific leaf area** | High | Negative in gymnosperms, positive in angiosperms |
| **Photosynthetic rate** | High | Negative in gymnosperms, none in angiosperms |
| **Flowering period** | Long | Unknown |
| **Minimum generation time** | Short | Positive |
| **Stature** | Tall | None to weakly negative |

For example, studies in the *Pinus* genus revealed that genome size had a strong positive correlation with seed mass. Still, other traits such as specific leaf area, leaf area ratio and relative growth rates showed negative correlation with genome size (Grotkopp et al. 2004). Also, while, genome size is negatively associated with seed mass, the opposite trend is observed with seed number; in any case, both traits promote invasiveness. Moreover, the relationship of genome size with other plant traits is either ambiguous or unknown (Table 1).

Thus, in invasiveness prediction, genome size is just one facet to be considered in a complex puzzle in which several factors may play a role (Suda et al. 2014).

### *Study system*

Succulent plants are plants that store pronounced amounts of water in their tissues, which confers them the ability to survive in water deficient environments. A series of other characteristics allows them to thrive in water deficit, such as a shallow root system for the rapid uptake of water, a waxy tick cuticle to minimize water losses, and CAM photosynthesis that allows the plant to uptake $CO_2$ during the night to further minimize water losses (Arakaki et al. 2011). Some of the most prominent succulent plant families regarding species-richness and ecological importance are the Aizoaceae, Euphorbiaceae, Crassulaceae, Aloaceae, Agavaceae, Apocynaceae-Asclepiadoideae, and Cactaceae (Arakaki et al. 2011).

The Cactaceae family includes around 1850 species with various shapes, sizes and agglomerates of spines arising from the areoles – highly specialized axillary or lateral short shoots or buds or branches, unique to the family (Novoa et al. 2014). Cactuses are

native of arid desertic regions, and the bulge of their diversity can be found in north-eastern Mexico, Bolivia, Andes, Argentina and south-eastern Brazil (Novoa et al. 2014). Cactaceae species were brought by the Europeans from America in the 15$^{th}$ century due to ornamental purposes, becoming part of the collections and gardens of that time (Anderson 2001). From there on, cactuses spread to other locations following horticultural trade industries that favourited these species due to their drought tolerance. Nowadays, cactuses are suitable to be found virtually in almost all habitats on Earth (Novoa et al. 2014).

Despite it is estimated that only 3% of the Cactaceae species are invasive in the present, this family includes some of the most important alien species worldwide, some of which with great potential for expansion to new areas (Novoa et al. 2014). The main invaded areas are the Iberian Peninsula (mostly Spain), Australia and South Africa. The genus *Mammillaria* is the most sold, with a globose growth form, but no invasive behavior has been observed so far, and the genura *Opuntia* and *Cylindropuntia* contain the most cultivated, introduced and invasive species of the family. Many new species of cactuses are joining the status of invasive species. *Cereus hexagonus* constitutes an example of a species of a very recent introduction (last year) in the list of national invasive species of South Africa (Novoa et al. 2014).

A thorough assessment of Cactaceae species invasiveness risk for each species is still to be performed. Despite of the high contribution of human mediated activities to the species dissemination, factors such as growth form (vegetative reproduction through cuttings), size of native area and climatic match between native and recipient locations, have demonstrated to be relevant determinants of invasiveness (Novoa et al. 2014).

# Introduction

### *Objectives*

The main objective of this study was to perform a thorough flow cytometry based genome size analysis of a collection of 191 Cactaceae species, including a good percentage of invasive species, and observe if there is any correlation between genome size and invasiveness. Plant traits such as seed size and seed weight were also measured to evaluate possible correlations between these traits, genome size and invasiveness. All species analyzed are sold as ornamentals, of which, 41 are listed as invasive worldwide according to the definition of Richardson et al. (2000).

Studies with such a large data set for a given family, with both invasive and non-invasive species at a worldwide scale, are very rare, thus offering unique opportunities to evaluate the role of genome size to predict invasiveness. Furthermore, this family is largely underrepresented in the Kew Gardens C-value database, with only 48 species with recorded genome size, and not always obtained with the method that is considered, nowadays, more accurate, i.e., flow cytometry

# Materials and Methods

# Materials and Methods

### *Plant material*

For the present study, a collection of 293 seeds from different genera, plus 11 living adult plants were kindly provided by Ana Novoa (Centre for Invasion Research at the Stellenbosch University, South Africa), who obtained the plant material from Imzaadex (Netherlands), Cactus adventures (Spain) and Koehres kaktus (Germany). Seeds were conditioned in individual hermetic plastic bags, labeled and stored at room temperature. According with the information available in the literature (Novoa et al. 2014), the status of each taxa (invasive/non-invasive) was recorded. Additionally, living specimens of 11 taxa were obtained by mail and transplanted to pots (Ø16cm) filled with regular commercial soil.

Seeds were germinated in plastic cuvettes (4.5x4.5x4.5cm) filled with commercial soil or with a mixture of sand and soil in the proportions 1:2, respectively. Immediately after sowing, cuvettes were placed on trays with a thin layer of water to promote germination. Trays were conditioned in a greenhouse at 26°C with a photoperiod of 16/8h (light/dark). When seedlings emerged they were transplanted to larger pots (Ø8-10cm) .

### *Seed parameters*

In order to explore possible relationships between some seed traits and genome size, seed weight and seed size were recorded for each analysed taxa.

In most cases, seed weight was measured as the average of 3 to 5 seeds using a precision digital scale (Radwag AS-110/C/2, RADWAG USA L.L.C, FL, USA), and due to the very low weight of the seeds, a larger number of seeds was used to calculate the

average weight. Still, for some taxa, due to the small size of their seed, it was impossible to obtain the seed weight even using a pool of seeds, namely the species *Parodia microsperma.*

For seed size, 1-3 seeds of each taxa were photographed along with a scale (Appendix 2) using a Canon 600D camera coupled to a binocular microscope (Leica M80, Leica Microsystems, Wetzlar, Germany). Seed size measurements were performed using Image J (Schneider et al. 2012). When more than one estimate was obtained, seed size was represented as the average length value of the seeds measured.

***Genome size analysis***

Genome size estimates were made using flow cytometric (FCM) analyses of nuclei isolated from plant tissues. Primary reference standard selected was *Solanum licopersicum*, against which other standards were calibrated, namely *Bellis perennis* (*Solanum lycopersicon*, S.l., 2C = 1.96 pg, Doležel et al. 1992, primary reference standard; *Bellis perennis*, B.p., 2C = 3.57 pg, secondary reference standard).

Genome size measurements were obtained using isolated nuclei following the chopping method described by Galbraith et al. (1983), with some modifications. Initial analyses were performed using the grown seedlings or adult plants, but these revealed to possess a large amount of mucilaginous compounds that hampered the isolation of nuclei in good conditions, and thus, further FCM analyses. As an alternative, root tissues and seeds were tested. Seeds revealed to provide histograms with a high quality and were used to assess genome sizes. Nuclear suspensions were prepared using ideally one seed,

although, in taxa with small seeds, more seeds from the same individual had to be pooled and chopped simultaneously. Seed coat was removed and the seed was placed in a Petri dish together with approximately 50 mg of leaf material of the internal reference standard and 1 ml of the WPB Buffer (0.2 M Tris.HCl, 4mM $MgCl_2.6H_2O$, 1% Triton X-100, 2mM EDTA $Na_2.2H_2O$, 86mM NaCl, 10mM metabissulfite, 1% PVP-10, pH adjusted to 7.5 and stored at 4 ºC; (Loureiro et al. 2007)). Chopping intensities were adjusted to provide a similar number of sample and standard nuclei. Nuclear suspensions were then filtered through a 50 µm nylon filter into a sample tube, and stained with 50 mg/mL propidium iodide (PI, Fluka, Buchs, Germany). Also, 50 mg/mL of RNAse (Fluka, Buchs, Germany) were added to destroy RNA and prevent staining of double stranded RNA.

Samples were kept at room temperature and analysed within a 10 minute period in a Cyflow Partec flow cytometer (Partec GmbH, Görlitz, Germany) equipped with 532 nm green solid-state laser, operating at 30 mW. For a given taxon the amplifier system was set to a constant voltage and gain, throughout whole the estimates. To ensure further sample quality, each day and prior to analysis, the instrument stability and linearity was verified using fluorescent beads (Partec GmbH, Görlitz, Germany). Samples were run when baseline CV values of the fluorescent beads were bellow 3%.

The results were acquired using the FloMax software (v. 2.4d) in the form of six graphics: fluorescence pulse integral in linear scale (FL); forward scatter (FS) *vs.* side scatter (SS), both in logarithmic (log) scale; FL *vs.* time; FL *vs.* fluorescence pulse height; FL vs. FS in log scale and FL *vs.* SS in log scale.

Polygon regions were defined either in the FL vs. FS or in the FL *vs.* SS cytograms and further applied to the other graphics. This enabled to remove debris and improve

sample's quality. Mean fluorescence values and CV value of the fluorescence of both sample and standard were obtained for at least 1300 nuclei in each $G_0/G_1$ peak, whenever possible, given sample flow speed and sample amount. Samples collected presented CV values below 5%, or else they were discarded and prepared again to try to achieve a better quality. For some taxa, due to the high levels of mucilage, this 5% CV value threshold was not attained, even after repeated measurements; still, even in those occasions the CV values did not surpass the 6%.

For each taxa, up to 6 genome size estimates were obtained in different days to account for variation generated by the cytometer.

The holoploid genome size in pg (2C; complete genome size, *sensu* Greilhuber et al. (2005) of each individual was estimated according with the following formula:

$$2C\ nuclear\ DNA\ content\ (pg) = \frac{sample\ species\ G1\ peak\ FL}{standard\ G1\ peak\ FL}\ x\ nuclear\ DNA\ content\ of\ the\ standard$$

The obtained values were compared with previous genome size available at the plant DNA C-values database of the Royal Botanical Gardens of Kew (Bennett & Leitch 2012) as compiled in Appendix 1.

***Statistical analysis***

Descriptive statistics of genome size estimates, seed size and seed weight were calculated for each species (mean, standard deviation of the mean, minimum and maximum values) using Microsoft Excel 2013.

# Materials and Methods

Differences in genome size, seed size and seed weight between invasive and non-invasive species were assessed using a non-parametric Mann-Whitney Rank Sum Test (normality and homoscedasticity was not achieved even after data transformation), using Sigmaplot 12.5 (Systat Software, San Jose, CA) and SPSS Statistics 19 (IBM Corp., Armonk, NY).

Box plots with mean and standard deviation of the mean of genome size, seed size and seed weight between invasive and non-invasive species were computed using Graphpad 6.01 (Prism); similar box plots were calculated for particular genera containing both invasive and non-invasive species. Scatter plots, histograms and linear regressions between genome size and seed weight and seed size considering invasive and non-invasive species were also drawn using Graphpad 6.01.

# Results

# Results

The Royal Botanical Garden's genome size database (Bennett & Leitch 2012) comprises entries for 48 Cactaceae species (Appendix 1).

In total, the genome size of 191 succulent species was analyzed, of which 177 were estimations new to the Royal Botanical Garden's genome size database. This number increases to 182 if considering only the estimates acquired by flow cytometry (Table 2). Thus, this study contributes for an increase of 3.7x in the number of available entries, more than 78% of the current entries.

The available genome size measurements in the literature recorded a minimum genome size of 1.59 pg for *Leptocereus quadricostatus*, and a maximum value for *Mammilaria rodhanta* 13.25 pg (Appendix 1). In comparison, the lowest genome size recorded was for *Cylindropuntia leptocaulis*, an invasive species with genome size of 2.37 pg, and the largest for *Espostoa guentheri*, a non-invasive species, with a genome size of 10.51 pg (Table 2). Therefore the genome size range observed in this study was similar to the one previously described in the literature, that is, no genome sizes were recorded for the categories for large or very large genome sizes (2C ≥ 28 pg *sensu* Leitch et al. (1998)) The general quality of the samples was good, with most CV values under 5% (Table 2), and in most samples, little debris or secondary metabolites affecting the visibility of the nuclei observation (Figure 1).

**Table 2**: Nuclear DNA estimates for all Cactaceae species studied in this Thesis. Table provides the average nuclear DNA content obtained by FCM for each species, given in picograms (GS, pg), standard deviation (SD), coefficient of variation of the mean fluorescence obtained for each species (CV, %), minimum and maximum genome size value obtained (Min Gs and Max GS, respectively), number of samples analyzed for each species (n) seed size (mm) and seed weight (mg). Previous estimate values are also provide when available (Yes indicates first estimate; when a previous value is available, the value is provided in pictograms; * marks previous estimates obtained by Feulgen microdensitometry). In cases where more than one previous estimate was attained, an average of existent values was represented. Tables are presented for both invasive and non-invasive species, and for both groups, the total row represents the average values for the whole class.

| Non-invasive species | GS (pg) | SD (pg) | CV (%) | Min of GS | Max of GS | n | Seed size (mm) | Seed weight (mg) | First estimate |
|---|---|---|---|---|---|---|---|---|---|
| *Astrophytum capricorne* | 7.54 | - | 1.17 | - | - | 1 | 2.41 | 1.86 | Yes |
| *Astrophytum myriostigma* | 3.41 | 0.10 | 2.53 | 3.30 | 3.51 | 4 | 2.05 | 1.32 | Yes |
| *Astrophytum ornatum* | 3.47 | 0.09 | 1.89 | 3.39 | 3.60 | 4 | 3.03 | 1.96 | 3.66 |
| *Browningia chlorocarpa* | 2.90 | 0.07 | 2.55 | 2.84 | 2.98 | 3 | 1.08 | 0.22 | Yes |
| *Browningia pilleifera* | 2.97 | - | 3.54 | - | - | 1 | 1.00 | 0.19 | Yes |
| *Carnegiea gigantea* | 3.27 | - | 2.86 | - | - | 1 | 1.74 | 1.24 | 2.87 |
| *Cereus hexagonus* | 3.81 | - | 2.62 | - | - | 1 | 2.77 | 2.80 | Yes |
| *Cipocereus bradei* | 3.37 | 0.03 | 2.23 | 3.35 | 3.41 | 4 | 1.12 | 0.53 | Yes |
| *Cleistocactus hyalacanthus* | 7.27 | - | 1.58 | - | - | 1 | 1.22 | 0.32 | Yes |
| *Cleistocactus icosagonus* | 9.27 | - | 2.04 | - | - | 1 | 1.05 | 0.27 | Yes |
| *Cleistocactus roezlii* | 4.52 | - | 2.34 | - | - | 1 | 1.68 | 0.78 | Yes |
| *Cleistocactus tarijensis* | 3.83 | 0.11 | 2.64 | 3.70 | 3.91 | 3 | 1.10 | 0.25 | Yes |
| *Coleocephalocereus goebelianus* | 3.24 | - | 2.71 | - | - | 1 | 1.53 | 0.90 | Yes |
| *Coryphantha pallida* | 4.25 | 0.07 | 2.59 | 4.19 | 4.35 | 4 | 2.01 | 0.69 | Yes |
| *Coryphatha cornifera* | 4.54 | - | 1.60 | - | - | 1 | 1.65 | 0.48 | Yes |
| *Echinocactus grusonii* | 2.97 | 0.05 | 2.41 | 2.93 | 3.02 | 3 | 1.48 | 0.88 | 2.85 |
| *Echinopsis atacamensis* | 3.93 | - | 1.95 | - | - | 1 | 1.39 | 0.57 | Yes |
| *Echinopsis candicans* | 4.20 | - | 2.40 | - | - | 1 | 1.09 | 0.41 | Yes |
| *Echinopsis huascha* | 8.54 | - | 1.66 | - | - | 1 | 1.10 | 0.25 | Yes |
| *Echinopsis lageniformis* | 4.27 | 0.26 | 1.87 | 4.05 | 4.56 | 3 | 1.72 | 0.74 | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Echinopsis leucantha* | 4.61 | 0.20 | 1.87 | 4.35 | 4.83 | 4 | 1.52 | 0.91 | Yes |
| *Echinopsis pachanoi* | 3.93 | - | 1.84 | - | - | 1 | 1.78 | 0.82 | Yes |
| *Echinopsis peruviana* | 4.49 | - | 2.12 | - | - | 1 | 1.84 | 0.90 | Yes |
| *Echinopsis tarijensis* | 4.01 | - | 1.87 | - | - | 1 | 1.35 | 0.46 | Yes |
| *Echinopsis terscheckii* | 3.86 | - | 1.99 | - | - | 1 | 1.46 | 0.70 | Yes |
| *Eriosyce napina* | 3.18 | 0.08 | 2.32 | 3.12 | 3.29 | 5 | 1.08 | 0.35 | Yes |
| *Espostoa guentheri* | **10.60** | 0.20 | 1.41 | 10.42 | 10.81 | 3 | 1.13 | 0.40 | Yes |
| *Espostoa lanata* | 7.66 | - | 1.43 | - | - | 1 | 1.26 | 0.38 | Yes |
| *Espostoa melanostele* | 3.98 | 0.12 | 2.18 | 3.84 | 4.17 | 5 | 1.26 | 0.31 | Yes |
| *Espostoa mirabilis* | 3.71 | - | 2.08 | 3.71 | 3.71 | 1 | 1.30 | 0.32 | Yes |
| *Espostoa nana* | 7.33 | - | 1.57 | - | - | 1 | 1.39 | 0.37 | Yes |
| *Espostoa ritteri* | 6.97 | - | 1.47 | 6.97 | 6.97 | 1 | 1.33 | 0.32 | Yes |
| *Espostoa ruficeps* | 7.42 | - | 1.43 | - | - | 1 | 1.59 | 0.38 | Yes |
| *Espostoa senilis* | 3.72 | - | 2.22 | - | - | 1 | 1.72 | 0.46 | Yes |
| *Espostoopsis dybowskii* | 3.04 | 0.01 | 2.45 | 3.03 | 3.06 | 3 | 1.36 | 0.34 | Yes |
| *Facheiroa ulei* | 3.17 | 0.11 | 2.46 | 3.06 | 3.29 | 3 | 1.57 | 0.48 | Yes |
| *Ferocactus cylindraceus* | 3.45 | - | 2.64 | - | - | 1 | 2.03 | 1.68 | Yes |
| *Ferocactus emoryi* | 3.22 | 0.11 | 2.27 | 3.08 | 3.42 | 6 | 2.08 | 1.71 | Yes |
| *Ferocactus glaucescens* | 3.50 | 0.13 | 2.86 | 3.35 | 3.58 | 3 | 1.69 | 0.58 | Yes |
| *Ferocactus gracilis* | 3.27 | - | 1.80 | - | - | 1 | 1.96 | 1.44 | Yes |
| *Ferocactus hamatacanthus* | 3.43 | 0.02 | 2.45 | 3.42 | 3.45 | 2 | 1.37 | 0.62 | Yes |
| *Ferocactus histrix* | 3.45 | - | 2.60 | - | - | 1 | 1.27 | 0.32 | Yes |
| *Ferocactus latispinus* | 3.02 | 0.01 | 2.04 | 3.01 | 3.03 | 2 | 1.40 | 0.38 | Yes |
| *Ferocactus peninsulae* | 3.19 | 0.00 | 2.01 | 3.19 | 3.19 | 2 | 1.81 | 1.65 | Yes |
| *Ferocactus pilosus* | 3.69 | - | 2.09 | - | - | 1 | 1.56 | 1.66 | Yes |
| *Ferocactus wislizenii* | 3.25 | 0.05 | 1.98 | 3.17 | 3.31 | 4 | 2.41 | 1.92 | 2.80 |
| *Gymnocalycium baldianum* | 4.47 | 0.11 | 2.03 | 4.38 | 4.59 | 3 | 1.35 | 0.78 | Yes |
| *Gymnocalycium eurypleurum* | 4.27 | 0.16 | 2.73 | 4.07 | 4.45 | 4 | 0.90 | 0.44 | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Gymnocalycium monvillei* | 8.38 | - | 2.14 | - | - | 1 | 1.40 | 0.32 | Yes |
| *Gymnocalycium ochoterenae* | 4.07 | - | 2.13 | - | - | 1 | 1.24 | 0.19 | Yes |
| *Gymnocalycium pflanzii* | 6.32 | - | 0.79 | - | - | 1 | 0.67 | 0.07 | Yes |
| *Gymnocalycium quehlianum* | 4.00 | - | 1.90 | - | - | 1 | 1.09 | 0.08 | Yes |
| *Gymnocalycium saglionis* | 4.19 | 0.08 | 1.74 | 4.10 | 4.25 | 3 | 0.84 | 0.13 | Yes |
| *Gymnocalycium spegazzinii* | 4.34 | - | 2.35 | - | - | 1 | 1.00 | 0.24 | Yes |
| *Gymnocalycium stenopleurum* | 4.49 | - | 2.62 | - | - | 1 | 0.86 | 0.24 | Yes |
| *Haageocereus acranthus* | 7.69 | 0.13 | 1.05 | 7.51 | 7.80 | 6 | 1.41 | 0.51 | Yes |
| *Haageocereus pseudomelanostele* | 3.97 | 0.15 | 2.18 | 3.80 | 4.10 | 3 | 1.28 | 0.33 | Yes |
| *Haageocereus versicolor* | 4.20 | - | 1.76 | - | - | 1 | 1.21 | 0.37 | Yes |
| *Harrisia pomanensis* | 8.26 | - | 1.62 | - | - | 1 | 2.29 | 1.72 | Yes |
| *Harrisia tetracantha* | 4.00 | 0.12 | 2.17 | 3.87 | 4.09 | 3 | 1.63 | 1.94 | Yes |
| *Isolatocereus dumortieri* | 3.17 | 0.05 | 3.18 | 3.13 | 3.22 | 3 | 1.28 | 0.47 | Yes |
| *Leuchtenbergia principis* | 3.21 | 0.09 | 1.94 | 3.14 | 3.27 | 2 | 2.43 | 2.92 | Yes |
| *Mammillaria albilanata* | 3.27 | 0.19 | 2.45 | 3.08 | 3.47 | 3 | 1.06 | 0.26 | 3.15 |
| *Mammillaria backebergiana* | 3.42 | 0.08 | 1.68 | 3.36 | 3.47 | 2 | 1.00 | 0.19 | Yes |
| *Mammillaria bocasana* | 3.94 | - | 1.76 | - | - | 1 | 0.82 | 0.02 | 6.93* |
| *Mammillaria bombycina* | 3.43 | - | 1.95 | - | - | 1 | 1.01 | 0.21 | Yes |
| *Mammillaria carnea* | 3.56 | - | 0.99 | - | - | 1 | 0.92 | 0.25 | Yes |
| *Mammillaria columbiana* | 6.63 | - | 1.93 | - | - | 1 | 1.13 | 0.23 | Yes |
| *Mammillaria compressa* | 3.78 | 0.05 | 1.69 | 3.75 | 3.81 | 2 | 1.10 | 0.21 | Yes |
| *Mammillaria crinita* | 4.01 | 0.10 | 1.41 | 3.94 | 4.08 | 2 | 0.87 | 0.20 | Yes |
| *Mammillaria decipiens* | 3.84 | - | 1.81 | - | - | 1 | 0.93 | 0.17 | Yes |
| *Mammillaria densispina* | 3.58 | - | 2.20 | - | - | 1 | 1.04 | 0.24 | Yes |
| *Mammillaria discolor* | 3.43 | - | 2.03 | - | - | 1 | 1.06 | 0.21 | Yes |
| *Mammillaria duoformis* | 3.73 | - | 1.76 | - | - | 1 | 0.88 | 0.23 | Yes |
| *Mammillaria elongata* | 3.66 | - | 1.76 | - | - | 1 | 1.15 | 0.26 | Yes |
| *Mammillaria geminispina* | 3.56 | - | 1.94 | - | - | 1 | 0.99 | 0.22 | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Mammillaria glochidiata* | 4.21 | - | 2.47 | - | - | 1 | 0.90 | 0.25 | Yes |
| *Mammillaria guelzowiana* | 4.03 | - | 2.44 | - | - | 1 | 1.39 | 0.58 | Yes |
| *Mammillaria haageana* | 3.59 | - | 2.27 | - | - | 1 | 1.00 | 0.34 | 3.12 |
| *Mammillaria hahniana* | 3.68 | 0.05 | 1.90 | 3.63 | 3.73 | 3 | 1.34 | 0.23 | 9.80* |
| *Mammillaria heyderi* | 3.99 | - | 1.61 | - | - | 1 | 0.93 | 0.18 | Yes |
| *Mammillaria johnstonii* | 3.79 | - | 2.43 | - | - | 1 | 0.84 | 0.18 | Yes |
| *Mammillaria karwinskiana* | 3.75 | 0.10 | 2.49 | 3.68 | 3.83 | 2 | 0.84 | 0.13 | Yes |
| *Mammillaria magnifica* | 3.11 | - | 1.75 | - | - | 1 | 0.98 | 0.17 | Yes |
| *Mammillaria magnimamma* | 3.75 | - | 1.33 | - | - | 1 | 0.92 | 0.16 | Yes |
| *Mammillaria mammillaris* | 3.89 | - | 1.88 | - | - | 1 | 1.04 | 0.17 | Yes |
| *Mammillaria marksiana* | 3.99 | - | 1.82 | - | - | 1 | 0.70 | 0.06 | Yes |
| *Mammillaria matudae* | 3.29 | - | 2.28 | - | - | 1 | 0.93 | 0.09 | Yes |
| *Mammillaria microhelia* | 3.76 | 0.24 | 1.99 | 3.60 | 3.93 | 2 | 1.23 | 0.19 | Yes |
| *Mammillaria muehlenfordtii* | 3.96 | - | 1.82 | - | - | 1 | 0.98 | 0.21 | Yes |
| *Mammillaria mystax* | 4.19 | - | 2.83 | - | - | 1 | 1.19 | 0.18 | Yes |
| *Mammillaria nivosa* | 3.82 | 0.22 | 2.02 | 3.67 | 3.98 | 2 | 1.01 | 0.23 | Yes |
| *Mammillaria nivosa flavescens* | 3.68 | 0.20 | 2.00 | 3.46 | 3.85 | 3 | 1.24 | 0.30 | Yes |
| *Mammillaria parkinsonii* | 7.34 | - | 1.41 | - | - | 1 | 1.14 | 0.23 | Yes |
| *Mammillaria petterssonii* | 3.95 | - | 2.50 | - | - | 1 | 0.75 | 0.16 | Yes |
| *Mammillaria picta* | 3.86 | - | 1.67 | - | - | 1 | 1.21 | 0.37 | Yes |
| *Mammillaria plumosa* | 3.63 | - | 2.45 | - | - | 1 | 1.03 | 0.28 | 13.25* |
| *Mammillaria rekoi* | 3.30 | - | 2.48 | - | - | 1 | 0.82 | 0.13 | Yes |
| *Mammillaria rhodantha* | 3.99 | 0.13 | 2.90 | 3.90 | 4.14 | 3 | 0.96 | 0.17 | 13.9* |
| *Mammillaria schiedeana* | 3.93 | 0.03 | 1.51 | 3.91 | 3.96 | 2 | 1.33 | 0.48 | Yes |
| *Mammillaria schwarzii* | 4.16 | - | 2.16 | - | - | 1 | 1.20 | 0.29 | Yes |
| *Mammillaria spinosissima* | 3.46 | 0.12 | 2.02 | 3.36 | 3.59 | 3 | 0.90 | 0.14 | Yes |
| *Matucana aurantiaca* | 4.33 | - | 2.64 | - | - | 1 | 1.42 | 0.58 | Yes |
| *Matucana haynei* | 4.34 | 0.06 | 2.29 | 4.27 | 4.42 | 5 | 1.71 | 0.62 | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Matucana krahnii* | 4.30 | - | 2.23 | - | - | 1 | 2.12 | 0.32 | Yes |
| *Matucana madisoniorum* | 4.02 | 0.11 | 1.84 | 3.90 | 4.11 | 3 | 1.93 | 0.58 | Yes |
| *Matucana ritteri* | 4.53 | - | 2.01 | - | - | 1 | 1.46 | 0.39 | Yes |
| *Melocactus azureus* | 3.05 | - | 1.98 | - | - | 1 | 1.38 | 0.80 | Yes |
| *Melocactus bahiensis* | 7.27 | - | 1.98 | - | - | 1 | 1.16 | 0.49 | Yes |
| *Melocactus curvispinus* | 3.26 | 0.02 | 2.22 | 3.25 | 3.28 | 3 | 1.13 | 0.49 | Yes |
| *Melocactus matanzanus* | 6.82 | - | 1.77 | - | - | 1 | 1.30 | 0.56 | Yes |
| *Melocactus peruvianus* | 3.36 | 0.28 | 2.22 | 3.07 | 3.81 | 5 | 1.02 | 0.34 | Yes |
| *Melocactus zehntneri* | 7.08 | - | 1.14 | - | - | 1 | 1.27 | 0.58 | Yes |
| *Micranthocereus estevesii* | 3.04 | 0.10 | 2.85 | 2.98 | 3.15 | 3 | 1.78 | 0.67 | Yes |
| *Mila caespitosa* | 4.04 | 0.10 | 2.04 | 3.88 | 4.13 | 5 | 1.00 | 0.19 | Yes |
| *Neobuxbaumia polylopha* | 2.89 | 0.08 | 2.68 | 2.80 | 2.98 | 4 | 2.26 | 2.58 | 3.20 |
| *Neobuxbaumia tetetzo* | 2.71 | - | 3.03 | - | - | 1 | 2.02 | 1.12 | Yes |
| *Neoraimondia herzogiana* | 3.18 | - | 3.81 | - | - | 1 | 1.54 | 0.38 | Yes |
| *Oreocereus celsianus* | 7.49 | 0.30 | 1.66 | 7.17 | 7.84 | 4 | 2.08 | 1.84 | Yes |
| *Oreocereus leucotrichus* | 9.99 | - | 1.07 | 9.99 | 9.99 | 1 | 1.50 | 1.02 | Yes |
| *Oreocereus trollii* | 3.54 | - | 2.34 | - | - | 1 | 2.06 | 1.30 | Yes |
| *Oroya borchersii* | 4.40 | 0.10 | 2.11 | 4.28 | 4.45 | 3 | 1.59 | 1.16 | Yes |
| *Oroya peruviana* | 4.24 | 0.14 | 1.63 | 4.07 | 4.38 | 4 | 1.81 | 1.32 | Yes |
| *Pachycereus marginatus* | 5.79 | - | 2.13 | - | - | 1 | 3.35 | 4.20 | Yes |
| *Pachycereus pringlei* | 6.33 | 0.22 | 2.06 | 6.12 | 6.61 | 4 | 1.02 | 6.72 | Yes |
| *Pachycereus schottii* | 3.24 | - | 2.56 | - | - | 1 | 2.59 | 3.54 | Yes |
| *Parodia arnostiana* | 3.90 | - | 2.73 | - | - | 1 | 1.06 | 0.68 | Yes |
| *Parodia buiningii* | 3.99 | - | 2.59 | - | - | 1 | 1.38 | 0.88 | Yes |
| *Parodia chrysacanthion* | 4.07 | - | 2.91 | - | - | 1 | 0.89 | 0.08 | Yes |
| *Parodia concinna* | 4.38 | 0.15 | 2.05 | 4.27 | 4.49 | 2 | 1.12 | 0.36 | Yes |
| *Parodia haselbergii* | 3.49 | - | 2.37 | - | - | 1 | 1.03 | 0.22 | Yes |
| *Parodia horstii* | 4.51 | 0.15 | 1.82 | 4.38 | 4.68 | 3 | 1.07 | 0.26 | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Parodia leninghausii* | **2.36** | 0.04 | 2.18 | 2.34 | 2.39 | 2 | 0.98 | 0.18 | Yes |
| *Parodia microsperma* | 4.85 | 1.86 | 2.12 | 3.70 | 8.14 | 5 | 0.67 | 0.00 | Yes |
| *Parodia nothorauschii* | 8.46 | - | 1.48 | - | - | 1 | 1.26 | 0.36 | Yes |
| *Parodia ottonis* | 7.01 | - | 1.18 | - | - | 1 | 1.20 | 0.66 | Yes |
| *Parodia schumanniana* | 2.52 | 0.12 | 2.52 | 2.44 | 2.65 | 3 | 1.47 | 0.38 | Yes |
| *Parodia scopa* | 4.01 | - | 1.91 | - | - | 1 | 0.92 | 0.28 | Yes |
| *Parodia tabularis* | 8.05 | - | 2.34 | - | - | 1 | 1.15 | 0.28 | Yes |
| *Parodia warasii* | 2.44 | - | 2.49 | - | - | 1 | 1.07 | 0.19 | Yes |
| *Pilosocereus chrysostele* | 3.32 | - | 2.26 | - | - | 1 | 1.56 | 0.50 | Yes |
| *Pilosocereus fulvilanatus* | 3.46 | 0.14 | 2.12 | 3.36 | 3.56 | 2 | 1.78 | 0.58 | Yes |
| *Pilosocereus gounellei* | 3.55 | - | 1.85 | - | - | 1 | 1.94 | 1.04 | Yes |
| *Pilosocereus leucocephalus* | 7.10 | 0.07 | 1.40 | 7.05 | 7.18 | 3 | 0.00 | 1.72 | Yes |
| *Pilosocereus magnificus* | 3.75 | - | 2.75 | - | - | 1 | 1.46 | 0.45 | Yes |
| *Pilosocereus pachycladus* | 6.80 | 0.24 | 1.67 | 6.63 | 6.96 | 2 | 1.49 | 0.60 | Yes |
| *Polaskia chichipe* | 3.20 | 0.04 | 3.03 | 3.16 | 3.23 | 3 | 1.56 | 0.78 | Yes |
| *Rebutia minuscula* | 3.43 | 0.17 | 2.11 | 3.30 | 3.62 | 3 | 1.38 | 0.23 | Yes |
| *Stenocereus pruinosus* | 3.19 | 0.03 | 2.31 | 3.17 | 3.24 | 4 | 2.30 | 2.54 | Yes |
| *Stetsonia coryne* | 3.23 | 0.09 | 2.50 | 3.17 | 3.33 | 3 | 1.57 | 0.44 | Yes |
| **Total** | 4.40 | 1.63 | 2.10 | 2.36 | 10.60 | 150 | 1.38 | 0.77 | - |

| Invasive species | GS (pg) | SD (pg) | CV (%) | Min of GS | Max of GS | n | Seed size (mm) | Seed weight (mg) | First estimate |
|---|---|---|---|---|---|---|---|---|---|
| *Acanthocereus tetragonus* | 8.81 | 0.09 | 1.06 | 8.74 | 8.87 | 2 | 2.41 | 1.86 | Yes |
| *Austrocylindropuntia cylindrica* | 2.57 | - | 2.97 | - | - | 1 | - | - | Yes |
| *Austrocylindropuntia pubescens* | 2.89 | 0.12 | 2.29 | 2.81 | 3.03 | 3 | - | - | Yes |
| *Cereus hildmannianus* | 3.90 | 0.10 | 2.30 | 3.80 | 4.00 | 3 | 2.55 | 2.32 | Yes |
| *Cereus jamaracu* | 4.27 | 0.11 | 2.20 | 4.15 | 4.37 | 3 | 2.91 | 4.54 | Yes |
| *Cereus peruvianus monstruosus* | 4.03 | 0.10 | 1.91 | 3.91 | 4.11 | 4 | 2.41 | 1.98 | Yes |
| *Cylindropuntia imbricata* | 4.11 | 0.23 | 2.01 | 3.89 | 4.35 | 3 | 4.21 | 15.80 | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Cylindropuntia kleiniae* | 3.97 | 0.03 | 1.84 | 3.94 | 3.99 | 2 | 4.51 | 15.16 | Yes |
| *Cylindropuntia leptocaulis* | 2.37 | 0.02 | 2.74 | 2.35 | 2.40 | 4 | 4.17 | 8.58 | Yes |
| *Cylindropuntia rosea* | 2.97 | 0.16 | 3.21 | 2.78 | 3.09 | 3 | - | - | Yes |
| *Cylindropuntia spinosior* | 2.37 | - | 1.90 | - | - | 1 | 3.77 | 8.79 | Yes |
| *Echinopsis chamaecereus* | 4.14 | 0.02 | 2.11 | 4.12 | 4.16 | 4 | 1.30 | 0.45 | Yes |
| *Echinopsis schickendantzii* | 8.35 | 0.25 | 1.60 | 8.09 | 8.59 | 3 | 1.39 | - | Yes |
| *Echinopsis spachiana* | 8.17 | 0.20 | 1.57 | 7.91 | 8.36 | 4 | 1.47 | 0.48 | Yes |
| *Harrisia martinii* | 8.21 | 0.20 | 1.77 | 8.01 | 8.42 | 3 | 2.52 | 2.08 | Yes |
| *Hylocereus undatus* | 3.73 | 0.16 | 2.25 | 3.56 | 3.92 | 4 | 2.32 | 1.48 | Yes |
| *Myrtillocactus geometrizans* | 2.99 | 0.09 | 2.72 | 2.91 | 3.08 | 3 | 1.61 | 0.53 | Yes |
| *Nopalea cochenillifera* | 5.52 | 2.29 | 3.05 | 3.90 | 7.14 | 2 | - | - | 1.96 |
| *Opuntia aurantiaca* | 3.68 | 0.03 | 2.22 | 3.64 | 3.71 | 3 | - | - | Yes |
| *Opuntia chlorotica* | 2.39 | 0.02 | 2.65 | 2.36 | 2.41 | 3 | 3.84 | 14.66 | Yes |
| *Opuntia dillenii* | 5.14 | 0.09 | 1.78 | 5.07 | 5.27 | 4 | 3.88 | 15.52 | 4.55 |
| *Opuntia elata* | 3.91 | 0.10 | 1.99 | 3.84 | 3.97 | 2 | 5.08 | 28.90 | Yes |
| *Opuntia elatior* | 4.31 | 0.09 | 2.42 | 4.22 | 4.40 | 3 | 3.59 | 11.28 | Yes |
| *Opuntia engelmannii* | 6.51 | 0.15 | 1.52 | 6.42 | 6.73 | 4 | 3.45 | 7.22 | Yes |
| *Opuntia ficus-indica* | 8.23 | 0.19 | 1.36 | 8.07 | 8.54 | 5 | 4.70 | 12.40 | Yes |
| *Opuntia huajuapensis* | 3.94 | 0.03 | 1.84 | 3.91 | 3.97 | 3 | 3.66 | 13.50 | Yes |
| *Opuntia humifusa* | 4.02 | 0.05 | 2.83 | 3.98 | 4.05 | 2 | - | - | Yes |
| *Opuntia macrorhiza* | 4.66 | 0.09 | 2.14 | 4.57 | 4.76 | 5 | 4.14 | 14.06 | Yes |
| *Opuntia microdasys* | 3.98 | 0.07 | 1.97 | 3.88 | 4.05 | 4 | 3.18 | 7.37 | 4.47 |
| *Opuntia phaeacantha* | 6.50 | 0.14 | 1.61 | 6.39 | 6.70 | 4 | 4.31 | 25.12 | Yes |
| *Opuntia robusta* | 4.52 | 0.08 | 2.17 | 4.46 | 4.61 | 3 | 2.80 | 15.52 | Yes |
| *Opuntia spinulifera* | 3.95 | 0.00 | 1.99 | 3.95 | 3.95 | 2 | 3.30 | 9.70 | Yes |
| *Opuntia streptacantha* | 5.55 | 0.12 | 1.49 | 5.44 | 5.71 | 4 | - | - | Yes |
| *Opuntia stricta* | 6.17 | 0.11 | 1.74 | 6.03 | 6.23 | 3 | 5.74 | 31.34 | Yes |
| *Opuntia tomentosa* | 8.23 | 0.20 | 1.58 | 8.05 | 8.50 | 4 | 5.27 | 32.20 | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Opuntia tuna* | 6.00 | 0.08 | 2.04 | 5.90 | 6.12 | 5 | 4.10 | 98.60 | Yes |
| *Pereskia aculeata* | 2.42 | 0.06 | 1.89 | 2.37 | 2.46 | 2 | 5.06 | 12.16 | Yes |
| *Samonopuntia macdonaldiae* | 3.98 | - | 2.52 | - | - | 1 | - | - | Yes |
| *Samonopuntia salmiana* | 3.65 | - | 3.39 | - | - | 1 | - | - | Yes |
| *Tephrocactus articulatus* | 9.77 | 0.69 | 1.48 | 9.28 | 10.26 | 2 | 5.47 | 17.50 | Yes |
| *Trichocereus schikendantzii* | 4.06 | - | 2.65 | - | - | 1 | - | - | Yes |
| **Total** | 4.85 | 2.00 | 2.12 | 2.37 | 9.77 | 41 | 3.52 | 14.37 | Yes |

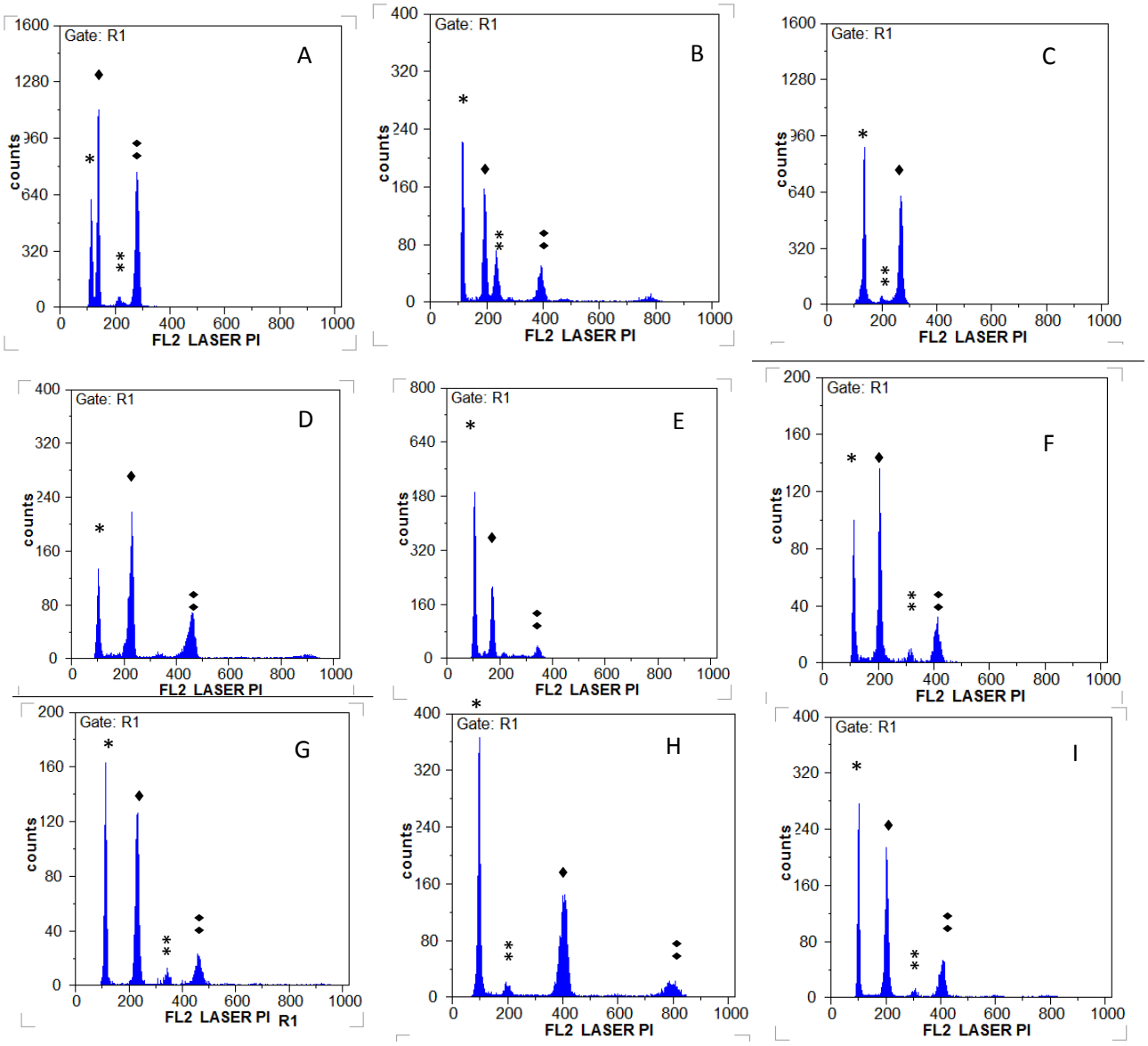


**Figure 1**: Fluorescence histograms of isolated nuclei from different Cactaceae species, stained with propidium iodide and analyzed with flow cytometry in the present study. In the graphics, reference standard, *Solanum lycopersicum* is denoted by * and ** above the respective $G_{0/1}$ and $G_2$ peaks, respectively. Likewise, sample's $G_{0/1}$ and $G_2$ peaks are marked with ♦ and ♦♦, respectively. (A) *Solanum licopersicum* and *Opuntia chlorotica* (B) *Solanum lycopersicum* and *Gymnocalycium baldianum* (C) *Solanum lycopersicum* and *Cylindropuntia imbricata* (D) *Solanum lycopersicum* and *Oroya peruviana* (E) *Solanum lycopersicum* and *Stetsonia coryne* (F) *Solanum lycopersicum* and *Mammillaria haageana* (G) *Solanum lycopersicum* and *Harrisia tetracantha* (H) *Solanum lycopersicum* and *Echinopsis spachiana* (I) *Solanum lycopersicum* and *Echinopsis atacamensis*.

# Results

***Does genome size differ between invasive and non-invasive species?***

When considering only invasive species, the smallest genome size recorded in this study was of 2.37 pg for *Cylindropuntia leptocaulis*, and the largest was of 9.77 pg, for *Tephrocactus articulates*, conferring to this group a 4.12-fold variation in genome size. As for non-invasive species, the smallest genome size was of 2.36 pg for *Parodia leninghausii*, and the largest was for *Espostoa guenteri*, with 10.6 pg, accounting for a variation of 4.49-fold in this group alone. Thus, variation in genome size estimates for invasive and non-invasive species group was very similar.

Box plots for both invasive and non-invasive groups were drawn (Figure 2), allowing for visual comparison of medians and media between invasive and non-invasive groups. The differences in the median genome size values between invasive and non-invasive species was not great enough to exclude the possibility that the difference was due to random sampling variability; thus, no statistically significant differences between the two groups was observed ($U = 2573$; $p = 0.157$).

Both invasive and non-invasive species showed the highest percentage of species in the ranges of genome size between 2 and 5 pg (Figure 3), further illustrating the lack of differences between the two groups for this trait. The percentage of invasive species surpasses the non-invasives in the categories of [2-3[, [5,7[, [8-10[, and were inferior to non-invasives in all other categories, with no representatives in the categories [7-8[ and [10-11[. No species were recorded for genome sizes between [0-2[ .

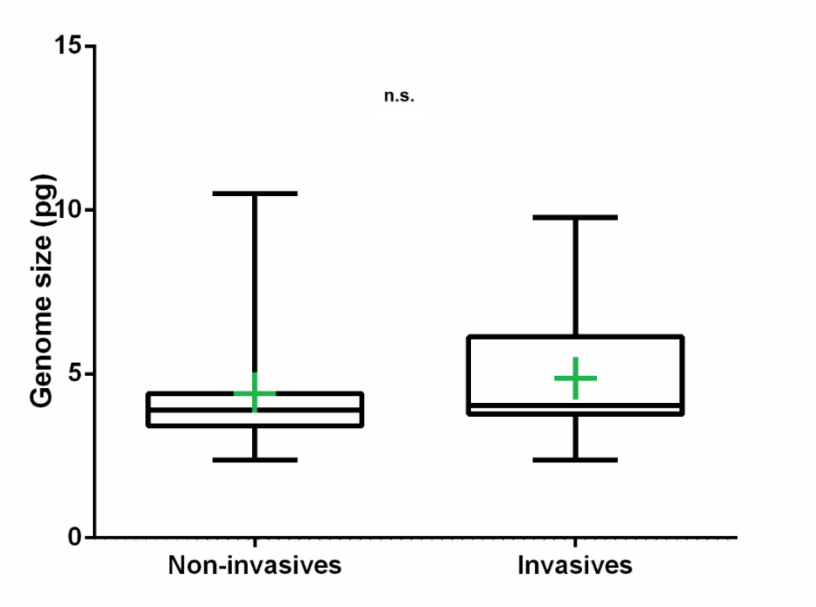


**Figure 2**: Genome size comparison between invasive and non-invasive species. Boxes extend from the 25% and 75% percentiles and whiskers the minimum and maximum values of genome size for each group. Medians are depicted as a horizontal line within the box and the media as a plus symbol. Statistical differences were not observed between both groups ($p > 0.05$)

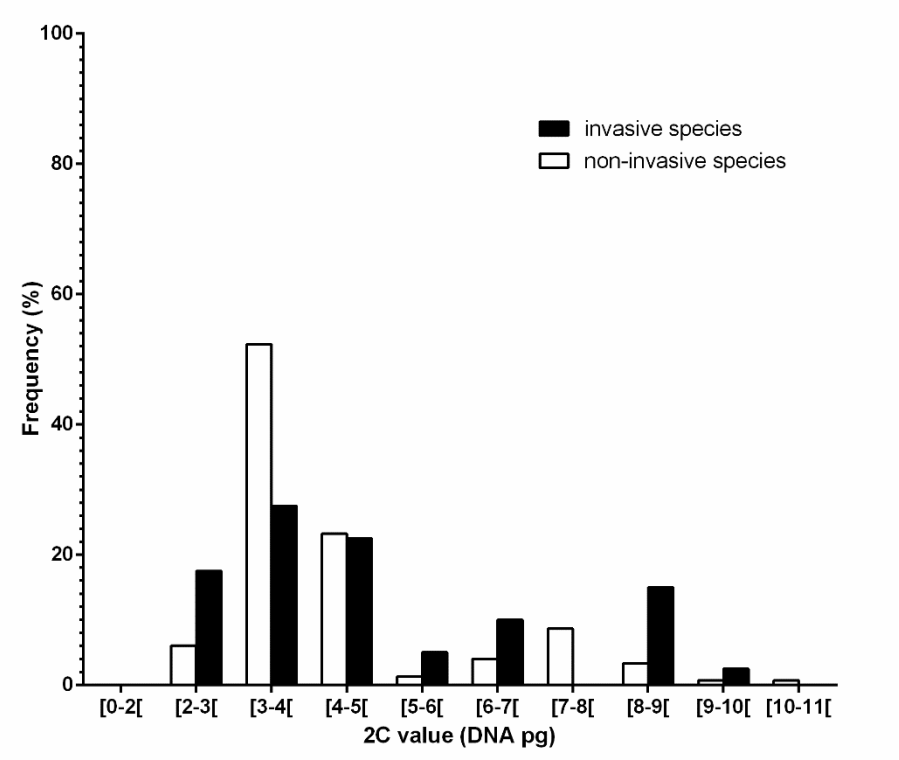


**Figure 3:** Comparison of genome size values obtained by FCM in the present work. Frequencies of distribution were calculated for all 151 invasive (Black bars) and 40 non-invasive species (white bars).

# Results

Genera containing both invasive and non-invasive species were analyzed in more detail (Figure 4). A statistical analyses of these species through a student t-test revealed no significant differences between invasive and non-invasive congenerics (t = - 2.16, *p* = 0.054). The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability.

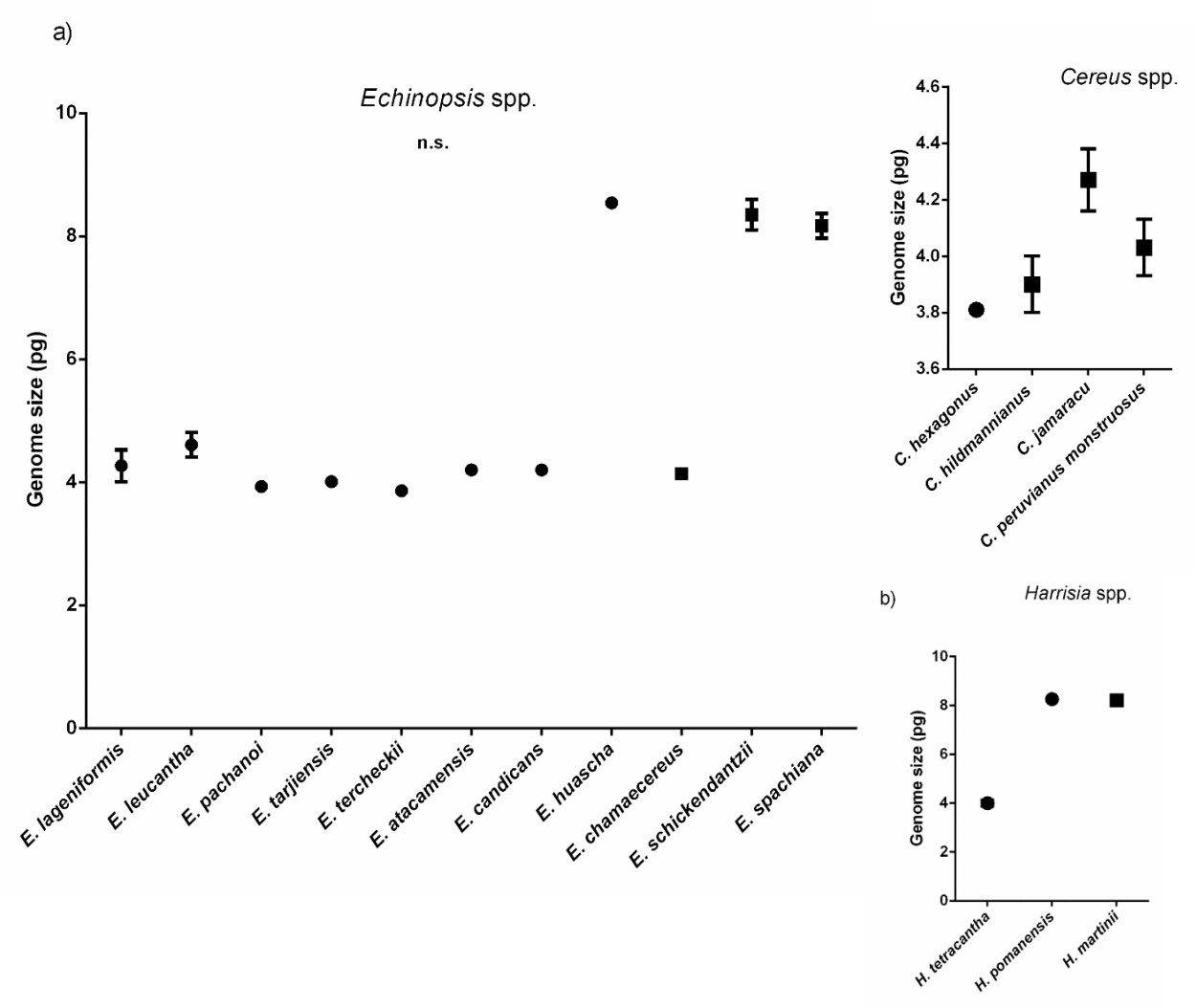


**Figure 4:** Genome size comparison between invasive (squares) and non-invasive (circles) congeneric species of the genus *Echinopsis* (a), *Harrisia* (b) and Cereus (c). When several estimates were obtained, values are given as mean and standard deviations (SD). *Echinopsis* species demonstrated no statistical significant differences between invasives and non-invasives (t = -2.16, *p* > 0.05).

# Results

***Do seed traits differ between invasive and non-invasive species?***

Invasive species demonstrated significantly bigger seeds than their non-invasive counterparts (U = 243.0 $p < 0.001$; Figure 5a). A similar significant difference was observed for seed weight (U = 230.5 $p < 0.001$) (Figure 5b). The variation of seed weight was also higher in invasive species than in non-invasive ones (Figure 5).

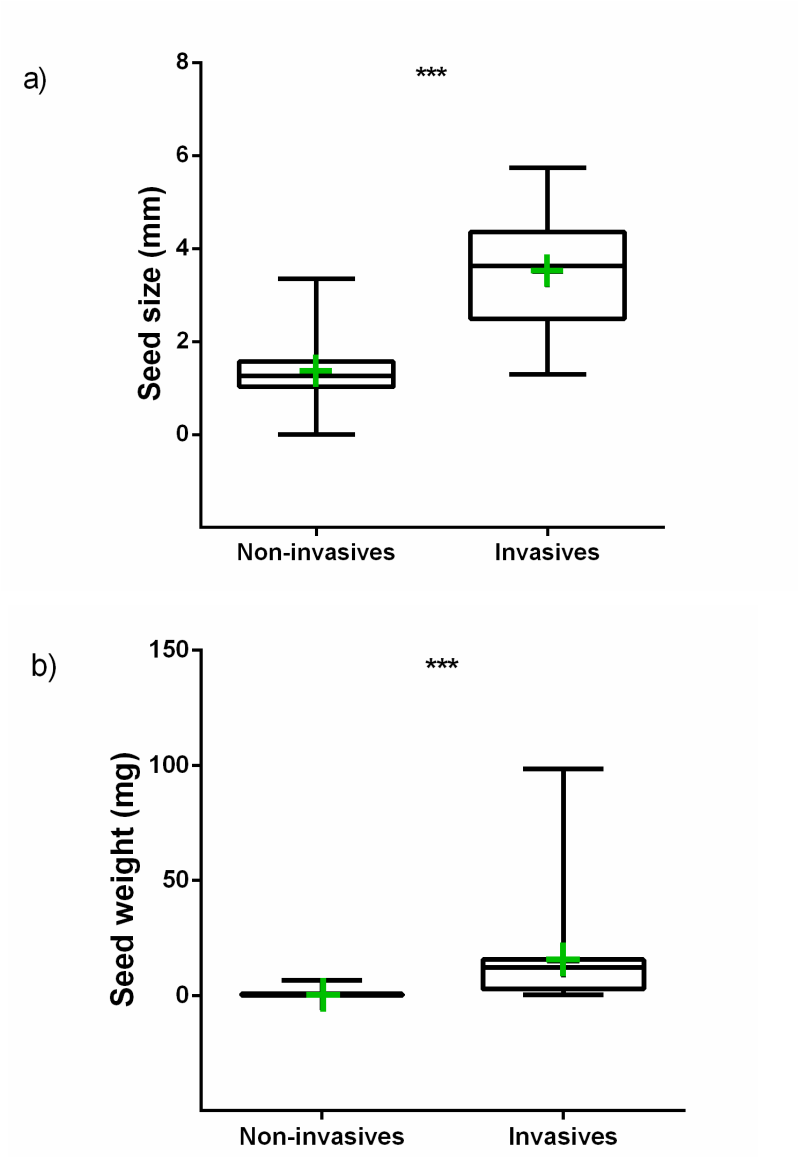


**Figure 5:** Seed size (a) and seed weight (b) comparison between invasive and non-invasive species group. Boxes extend from the 25% and 75% percentiles and whiskers the minimum and maximum values. Medians are depicted as a horizontal line within the box and the media as a plus symbol. Statistical differences were attained between groups both for seed size and seed weight parameters (U = 243 and U = 230 for a) and b) respectively, ($p < 0.001$ for both).

# Results

***How much of the seed size and seed weight is explained by the species genome size?***

Genome size and seed size did not demonstrate any significant correlation for both invasive ($R^2$ = 0.05, $p$ = 0.72) and non-invasive species ($R^2$ = 0.003, $p$ = 0.51) (Figure 6a and c). The same pattern was observed between genome size and seed weight for invasive ($R^2$ = 0.016, $p$ = 0.52) and non-invasive species ($R^2$ = 0.002, $p$ = 0.6) (Figure 6b and d).

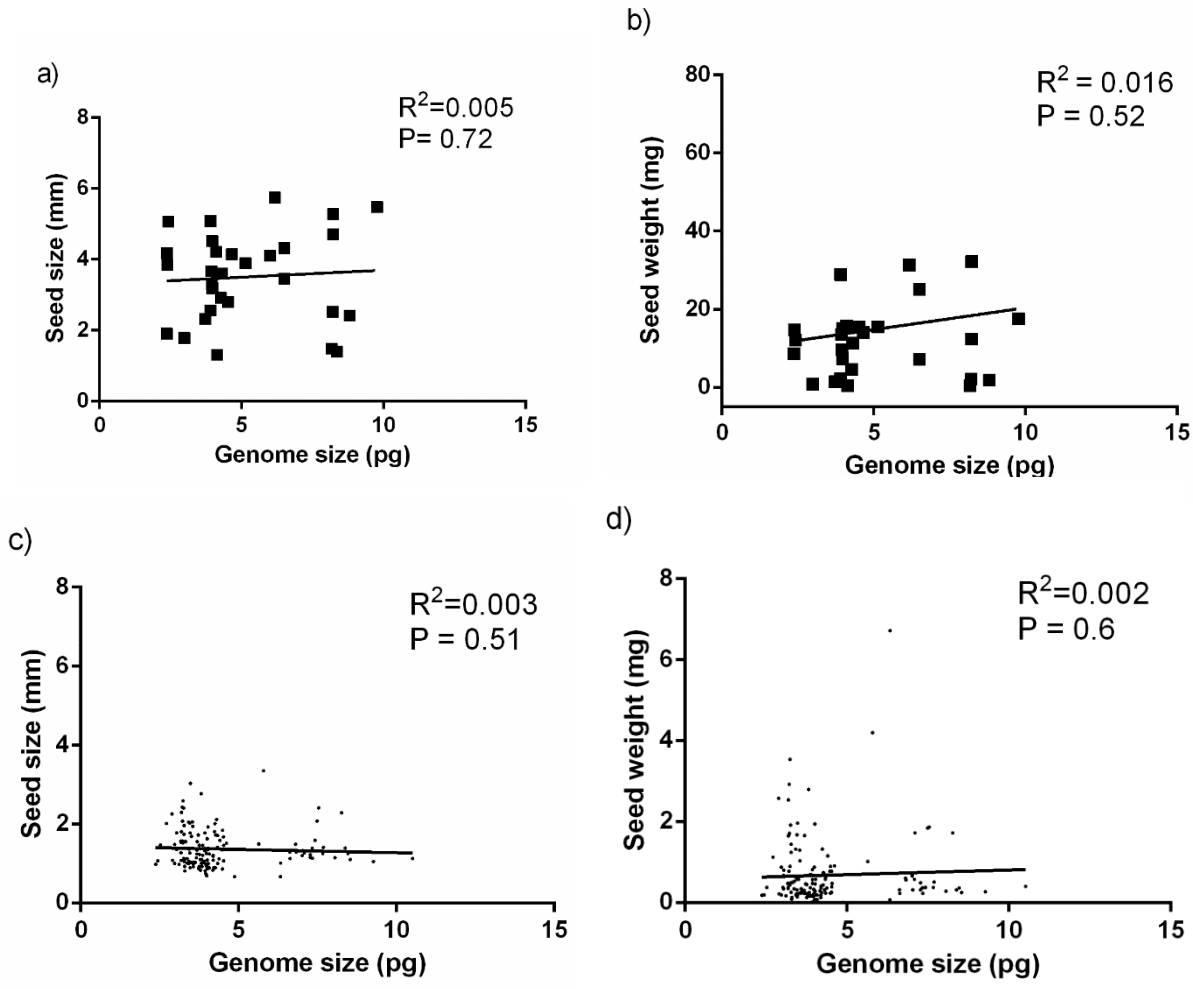


**Figure 6:** Regressions for the seed size vs. genome size [a) and c)] and seed weight vs. genome size [b) and d)] for both invasive species (squares/upper section graphs) and non-invasive species (dots/bottom graphs). $R^2$ and P values are also provided in each graph.

# Discussion

# Discussion

Invasive plant species have severe impacts on the economy, biodiversity and even human health since every year in Europe alone, billions of euros are spent to control and mitigate their negative impacts, which might be translated for example, in the decrease of agricultural overall productivity rates, production of allergenic substances for humans and even species extinction (Kettunen et al. 2009; Vilà et al. 2009; Pimentel et al. 2001; Belmonte & Vilà 2004; Mack 2000; Sala et al. 2000). Several plant traits have been used to predict invasiveness, such as minimum generation time, seed mass, stature, seedling growth rate, genome size, water and nutrient use efficiency, seed number, specific leaf area, photosynthetic rate, flowering period, resistance to herbivory and plant chemical defenses (Pyšek & Richardson 2007). Genome size has been proposed as one of the top eight best predictors of invasiveness (Rejmánek 1996). Indeed, several studies correlated genome sizes with invasiveness (eg. Chen, Guo, & Yin, 2010; Garcia et al., 2008; Kubešová, Moravcova, Suda, Jarošík, & Pyšek, 2010; Pandit, White, & Pocock, 2014; Varela-Álvarez et al., 2012), as well as with other invasive determinants such as minimum generation time (Beaulieu et al. 2007) seed characteristics (Grotkopp et al. 2004; Beaulieu et al. 2007) relative growth rate of seedlings, specific leaf area, stomatal size and density, nutrient and water consumption and even life cycle strategy (Suda et al. 2014).

If a correlation between Cactaceae genome size and invasiveness and/or invasiveness determinants were demonstrated, this could be additional traits used in the prediction of invasiveness for this family.

# Discussion

### *Genome size and invasiveness*

Several studies performed so far suggested a correlation between small genomes and invasiveness. This has been observed in several weedy invasive genera (i.e. several species of the genera *Polygonum, Chenopodium, Centaurea and Malva*) (Chen et al. 2010; Kuester et al. 2014), as well as, in global comparisons of more than 800 angiosperms using the available datasets of genome size (Pandit et al. 2014). It was suggested that small genomes can confer several adaptive advantages to plants. For example, because smaller genomes are quicker to replicate, they present a shorter minimum generation time, which is considered an important trait for invasiveness (Suda et al. 2014). Moreover, the general trend continues to associate small genomes with a wider array of possible phenotypes, because small genomes do not possess a developmental constraint, limiting of trait options (Bennett 1971; Knight et al. 2005).

However, contrary to this general trend, in this study with 191 species Cactaceae species, of which 41 are invasive, no significant genome size differences between invasive and non-invasive species was observed. Another study also demonstrated the lack of genome size differences between invasive and non-invasive species in *Acacia* (Gallagher et al. 2011). All *Acacia* species presented genome sizes rendered in the very small genome size category (2C < 2.8 pg) according with the nomenclature established by Leitch et al. (1998), their genome size variation was, as explained by Suda et al. (2014) beneath a given threshold of genome size where no correlations between this trait and invasiveness are found.

Similarly, because there were no Cactaceae species with large or very large genome sizes (2C ≥ 28 pg *sensu* Leitch et al. (1998), it was expected that there was no difference observed between invasive and non-invasive groups.

# Discussion

In species of the genus *Acacia,* it was further suggested that the non-significant relationship of genome size with invasiveness is explained by its lack of relationship with invasiveness determinants. In other studies, the negative correlation between genome size and invasiveness in the genus *Pinus* (Grotkopp et al. 2004) was due to secondary relationships between both traits and seed mass. However, in *Acacia* genus, no relationship between genome size and seed mass was established. Likewise, invasiveness determinant traits, among which, specific leaf area, relative growth rate, dispersal mode and native range were tested for their relationship with genome size, and invasiveness. Results demonstrated no secondary correlation, that is, if a plant trait was related to invasiveness, it wasn't correlated to genome size and vice versa.

On the contrary, in *Acacia* genera, invasiveness was better explained by plant height. Taller plants are most likely to outcompete their neighboring plants for resource availability, but there was also the effect of human interference, as these were simultaneously selected for timber trade and reforestation which not only favored taller plants but also plants with fastest growth rates (Gallagher et all, 2011). Simultaneously, the most invasive *Acacia* species were the ones that had a larger native area, which lead to the hypothesis that these species presented a broader climatic range adaptation and genetic variability (Gallagher et al, 2011).

Interestingly enough, a broad native range was one of the risk assessment factors and control strategies delineation already suggested for the Cactaceae species, as they are a strong indicator of a species potential for invasiveness (Novoa et al. 2014). It would therefore be interesting to explore further relationships between genome size and this plant trait. (For further discussion on the relationship of Cactaceae species genome size and other invasiveness determinant traits (i.e seed traits), see below)

# Discussion

Regarding congeneric comparisons, the results followed the expected trend, revealing no statistical significant differences between invasive and non-invasive species. Still, it would be interesting to increase the number of genome size estimates for invasive and non-invasive congeneric species in future studies to address if this pattern maintained.

### *Genome size and seed traits*

As previously discussed, genome size correlation with plant traits that favour invasiveness can lead to the use of genome size to predict invasiveness in a secondary way (Suda et al. 2014). Grotkopp et al (2004) provided evidence that smaller genomes were correlated with smaller seeds, and in other studies, large genome size plants were found to never be associated with small seeds (Beaulieu et al. 2007). The reason why seed characteristics can be considered as invasiveness determinants will be further adressed bellow.

In the Cactacae species analysed in the present study, no correlation between genome size and seed traits was observed. The absence of such correlation might be further evidence that the Cactaceae species, as angiosperms, with smaller genomes are prone to possess a wide variety of seed traits, as suggested by Beaulieu et al. (2007). Moreover this can be the result of inumerous selective pressures translated in different possible phenotypic displays that confer particular adaptative advantages.

# Discussion

### *Seed traits and invasiveness*

Previous studies aiming to unveil seed trait correlation with invasiveness, namely considering seed size and seed weight, demonstrated that plants could benefit from different strategies as successful invaders because invasive species have both small and large seeds (Pyšek & Richardson 2007). For example, small seeds are largely produced by short lived herbs, and correlated with increased output in the number of seeds. These seeds are easily dispersed by the wind and more persistent in the soil. On the other hand, woody species tend to have larger seeds, usually associated with attractiveness to vertebrate dispersers and better establishment rates (Pyšek & Richardson 2007).

In this study, invasive Cactaceae species were correlated to larger seed size as well as heavier seeds. Plant height and polyploidy have demonstrated to correlate with larger seeds (Beaulieu et al, 2007), and perhaps these are explanatory variables for the Cactaceae as well - Novoa et al (2014) proposes plant growth form as an invasiveness risk assessment factor. Notice how *Opuntia* and *Cylindropuntia* are amongst the most invasive genera, and posess more vertical progression, and how *Mammilaria*, a genera with no invasive species known to date, has a globose growth form and not much vertical progression.

In any case higher resource allocation to produce such type of seeds, which are often encapsulated in colorful fruits, can be a compensatory and more successful strategy due to higher attractiveness to dispersers (Pyšek & Richardson 2007). As an example, the most invasive genera (e.g., *Opuntia, Cylindropuntia*), indeed benefits from exhibiting fleshy colourful fruits, which are more likely to be subject to frugivory and subsequent zoochory.

# Conclusion

# Conclusion

***What makes Cactaceae species successful invaders then? Prospects for future studies***

The genome size analysis of the Cactaceae species in this study demonstrates that genome size is not directly correlated with invasiveness, and it is also not a good predictor of seed size and seed weight. However, the fact that no correlation was attained with one trait, does not impair correlations with other traits to be observed (Gallagher et al. 2011). Therefore, further studies could explore genome size correlation with other plant traits, in particular, determinants that were already correlated to invasiveness in this family, or that may illustrate Cactaceae species best fitness to the particular desertic environment Cactus are native of (i.e. water and nutrient use efficiency, stomata size, carbon fixation).

Meanwhile, in the particular case of Cactaceae, invasiveness prediction and risk control strategies should be formulated based primarily in other traits that already proved to be correlated to invasiveness, i.e., genus, size of the native area, climatic match and growth form (Novoa et al 2014). Indeed, according to the study lead by Novoa et al. (2014), invasive Cactaceae species have demonstrated to possess larger native areas, and belong to specific genera, such as *Opuntia* and *Cylindropuntia.* Moreover, new areas with a strong climatic match to the native area of invasive Cactaceae species are strong candidates to new invasions from these species (Richardson & Pyšek 2012; Richardson et al. 2011). The spread of Cactaceae species has further been correlated with growth form in the sense that some growth forms are better adapted for vegetative propagation from cuttings, a factor further boosted by human horticultural trade (Novoa et al. 2014).

Finally, the present study offered insight into the possible use of seed traits into invasiveness risk assessment.

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# Appendixes

# Appendix 1

Appendix 1: Royal Botanical Garden's genome size database entries for genome size measurements of Cactaceae species. The table provides the available entries ordered by genus and species (note that three species have more than one entry), estimation method (FCM:PI standing for flow cytometry with propidium iodide, and Fe for Felgen microdensitometry), Genome size (2C, pg).

| Genus | Species | Estimation method | 2C (pg) |
|---|---|---|---|
| *Aporocactus* | *flagelliformis* | Fe | 3.80 |
| *Astrophytum* | *ornatum* | FCM:PI | 3.66 |
| *Borzicactus* | *aurivillus* | Fe | 3.35 |
| *Carnegiea* | *gigantea* | FCM:PI | 2.87 |
| *Cleistocactus* | *smaragidifolius* | Fe | 3.35 |
| *Consolea* | *corallicola* | FCM:PI | 5.16 |
| *Consolea* | *falcata* | FCM:PI | 7.68 |
| *Consolea* | *macracantha* | FCM:PI | 4.88 |
| *Consolea* | *millspaughii* | FCM:PI | 4.92 |
| *Consolea* | *millspaughii* | FCM:PI | 7.67 |
| *Consolea* | *moniliformis* | FCM:PI | 5.07 |
| *Consolea* | *nashii* | FCM:PI | 5.09 |
| *Consolea* | *picardea* | FCM:PI | 4.92 |
| *Consolea* | *rubescens* | FCM:PI | 7.70 |
| *Consolea* | *rubescens* | FCM:PI | 7.93 |
| *Consolea* | *spinosissima* | FCM:PI | 5.04 |
| *Echinocactus* | *grusonii* | FCM:PI | 2.85 |
| *Escobaria* | *bella* | Fe | 3.05 |
| *Ferocactus* | *wislizenii* | FCM:PI | 2.80 |
| *Leptocereus* | *quadricostatus* | FCM:PI | **1.59** |
| *Mammillaria* | *albilanata* | FCM:PI | 3.15 |
| *Mammillaria* | *bocasana* | Fe | 4.10 |
| *Mammillaria* | *bocasana* | Fe | 9.75 |
| *Mammillaria* | *boolii* | Fe | 9.20 |
| *Mammillaria* | *crucigera* | FCM:PI | 3.21 |
| *Mammillaria* | *dixanthocentron* | FCM:PI | 3.18 |
| *Mammillaria* | *flavicentra* | FCM:PI | 3.04 |
| *Mammillaria* | *grandiflora* | Fe | 10.20 |
| *Mammillaria* | *haageana* | FCM:PI | 3.12 |
| *Mammillaria* | *hahniana* | Fe | 9.80 |
| *Mammillaria* | *huitzilopochtli* | FCM:PI | 3.12 |
| *Mammillaria* | *occidentalis* | Fe | 12.20 |
| *Mammillaria* | *plumosa* | Fe | 13.25 |
| *Mammillaria* | *rhodantha* | Fe | 13.90 |
| *Mammillaria* | *sanangelensis* | FCM:PI | 3.20 |
| *Mammillaria* | *supertexta* | FCM:PI | 3.11 |
| *Mammillaria* | *woodsii* | Fe | 3.10 |
| *Mammillaria* | *zeilmanniana* | Fe | 11.55 |

# Appendix 1

| Genus | Species | Estimation method | 2C (pg) |
|---|---|---|---|
| *Neobuxbaumia* | *polylopha* | FCM:PI | 3.20 |
| *Nopalea* | *cochenillifera* | FCM:PI | 1.96 |
| *Opuntia* | *acaulis* | FCM:PI | 7.60 |
| *Opuntia* | *dillenii* | FCM:PI | 4.55 |
| *Opuntia* | *microdasys* | FCM:PI | 4.47 |
| *Opuntia* | *violacea* | FCM:PI | 4.07 |
| *Pereskia* | *grandifolia* | FCM:PI | 1.96 |
| *Pilosocereus* | *royenii* | FCM:PI | 6.51 |
| *Pseudolobivia* | *sp.* | Fe | 3.25 |
| *Rebutia* | *albiflora* | FCM:PI | 3.81 |
| *Stenocereus* | *thurberi* | FCM:PI | 3.44 |
| *Trichocereus* | *werdermannianus* | Fe | 3.90 |
| *Weberbauerocereus* | *winterianus* | Fe | **14.20** |

# Appendix 2

**Appendix 2.:** Some of the seeds photographed with a Canon 600D camera coupled to a binocular microscope (Leica M80, Leica Microsystems, Wetzlar, Germany). A scale was placed next to the seeds so it could be converted afterwards into a digital scale used to measure the seeds using the Image J software (Schneider et al. 2012)