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From the flowers to the cells: a trait-based and cytogenomic study of Macaronesian *Aeonium* genus (Crassulaceae)

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Based on the current dissertation was made an oral presentation in an international congress showing the main results and three publications are being prepared.

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Brilhante *et al.* The biggest plant radiation of Macaronesia – *Aeonium*: positive correlations between genome size variation and their conservation status (*In prep*)

Brilhante *et al*. Identification of biodiversity hotspots within Macaronesian Islands: centres of *Aeonium* species richness and ende-mism as a case-study (*In prep*)

These provisional titles correspond to a publication in preparation to be submitted to international peer – reviewed journals in the field of Plant Biology.

According to the pronounced on paragraph 2, art.8, Decree-Law 388/70, the author of this dissertation declares that he was an intervenient on the conception and execution of the fieldwork, in the collection and analysis of the data, and in the writing of present manuscript.

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Abstract

Macaronesian region comprises five archipelagos (i.e., Azores, Madeira, Selvagens, Canary Islands and Cabo Verde) in the Northeastern Atlantic Ocean. Volcanic in nature, all archipelagos reach high altitudes even in relatively small areas and harbor an extremely rich endemic flora, including notable examples of plant radiation, such as the genus *Aeonium* (ca. 41 taxa), *Sonchus* (ca. 31 taxa) and *Echium* (ca. 27 taxa). Considered a biodiversity hotspot, Macaronesia is recognized as a model for studying the evolutionary processes involved in flora diversification.

Aeonium genus (Crassulaceae) includes about 41 taxa and is considered the most speciose radiation of Macaronesia, occupying many contrasting habitat types in the Canary Islands, Madeira and Cabo Verde. The great species richness and the wide range of characters observed in *Aeonium* species make it a model of study par excellence.

This dissertation aimed to study the reproductive traits and cytogenomic variation patterns of *Aeonium* genus in order to understand the characters that influence the distribution of the most speciose radiation of Macaronesian Region. For these purposes, a holistic approach was used that integrates different methods in the areas of reproductive biology **(Chapter 2)**, cytogenomics **(Chapter 3)** and conservation biology **(Chapter 4)**, based on extensive fieldwork on the islands of the Canary Islands.

The reproductive trait-based results **(Chapter 2)** showed that inflorescence height was positively correlated to flower number and geographic range size. This suggested that species that invest in taller inflorescences and more reproductive units have wider distributions and a more generalist behavior. According to the cytogenomic results **(Chapter 3)**, *Aeonium* species tend to have small genomes, being subshrubs the large-genomed ones. Comparative analysis across the genus revealed a positive correlation between 2C-values and conservation status, owning endangered species, on average, large genomes. Finally, in a conservation approach **(Chapter 4)**, most of *Aeonium* taxa occur in Tenerife in Teno Rural Park and Anaga Rural Parks, being there their hotspot of biodiversity. The representation of *Aeonium* endemics in the network of the Protected Areas is relatively high once a large area (40%) of Canary Islands is classified as protected.

Thus, by integrating the knowledge of the various approaches applied in the three chapters (2 – 4) of this dissertation, it was possible to contribute with new data to explain the distribution of *Aeonium* and detect hotspots areas of species diversity, based on inflorescence height, genome size and abiotic factors. Some species have their limited range of occurrence, among other external factors (e.g., anthropogenic action and invasive species), by the reduced inflorescence height. On the other hand, species with a larger genome do not respond to this trend and have also limited dispersal ranges.

However, further morphological, cytogenomic analyzes and more fieldwork in all Macaronesian Islands to inventory all *Aeonium* species will reinforce the results and identify the main factors leading to the large diversification of largest plant radiation of Macaronesia, contributing to the preservation of the unique biodiversity existing in these island ecosystems.

Keywords: Conservation; insular endemic plants; genome size; oceanic islands; reproductive traits;

Resumo

A região da Macaronésia compreende cinco arquipélagos (i.e., Açores, Madeira, Selvagens, Canárias e Cabo Verde), que se situam no Atlântico Norte ao longo da costa africana os 15º e os 40º de latitude. Todos os arquipélagos, de origem vulcânica, atingem altitudes acentuadas mesmo em áreas relativamente pouco extensas e albergam uma flora endémica extremamente rica, incluindo exemplos notáveis de radiações de plantas, como os géneros *Aeonium* (ca. 41 taxa), *Sonchus* (ca. 31 taxa) e *Echium* (ca. 27 taxa). Considerada um *hotspot* de biodiversidade, a Macaronésia é reconhecida como um óptimo modelo para o estudo dos processos evolutivos envolvidos na diversificação da flora extante.

O género *Aeonium* (Crassulaceae) inclui cerca de 41 taxa e é considerado a radiação mais especiosa da Macaronésia, ocupando uma grande quantidade de tipos contrastantes de habitats nos arquipélagos das Canárias, Madeira e Cabo Verde. A grande riqueza de espécies e o vasto leque de caracteres particulares observados nas espécies de *Aeonium* remetem-no como um modelo de estudo, por excelência, em ecologia, evolução, biogeografia e botânica.

A presente dissertação teve como principal objetivo estudar padrões de diversidade morfológica e citogenómica do género *Aeonium*, de modo a contribuir com novos dados para se compreender os factores que influenciam a distribuição da maior radiação de plantas da Região da Macaronésia e deste modo para a sua conservação. Para esse fim, usou-se uma abordagem holística integrando diferentes metodologias nas áreas da morfologia floral **(Capítulo 2)**, citogenómica **(Capítulo 3)** e da biologia da conservação **(Capítulo 4)**, tendo por base um extenso trabalho de campo realizados nas ilhas das Canárias.

Numa primeira abordagem, com base em caracteres morfológicos **(Capítulo 2)**, considerando o facto de a massa e tamanhos das sementes ser um caracter muito uniforme ao longo do género *Aeonium*, foi estudada a relação entre a altura das inflorescências e o alcance geográfico que as espécies conseguem alcançar. Considerou-se a altura das inflorescências como o principal agente a actuar na dispersão deste grupo de espécies. Este estudo foi baseado no pressuposto de que em contexto insular as espécies de plantas tendem a perder as suas capacidades de dispersão pela redução do investimento nas mesmas. Assim, a altura das inflorescências desde do solo até ao topo foi medido e o número de flores, o merismo e o número de inflorescências por indivíduo contabilizado para 22 espécies de *Aeonium*. O alcance geográfico para cada espécie foi calculado com base em bibliografia disponível e observações de campo. Os resultados mostraram que a altura das inflorescências está positivamente correlacionada com o número de flores ($R^2 = 0.86$) e com o alcance geográfico ($R^2 = 0.52$). Assim sugere-se que espécies que investem em inflorescências mais altas e em mais unidades reprodutivas, neste caso flores, possuem distribuições geográficas mais amplas e um comportamento mais generalista. Estes resultados permitem melhorar o conhecimento acerca das estratégias de dispersão do género *Aeonium*, assim como das suas distribuições naturais.

Considerando uma abordagem a nível celular **(Capítulo 3)**, no presente estudo, foi analisado o conteúdo nuclear de DNA para 27 taxa de *Aeonium* endémicos nos arquipélagos das Canárias, Madeira e Cabo Verde, usando a técnica de Citometria de Fluxo. Apesar do grande número de filogenética e diversidade genética (i.e., marcadores moleculares) focados na flora das Ilhas da Macaronésia, os estudos na área da citogenómica têm sido pontuais e a sua ligação a dados de raridade das espécies e respectivas categorias de ameaça nunca foi explorado até à data. Neste capítulo, foi caracterizada a diversidade citogenómica de 27 taxa de *Aeonium*, tendo por base um extenso trabalho de campo realizado nos três arquipélagos das Macaronésia onde o género se encontra distribuído, tendo sido relacionado a diversidade citogenómica com as ameaças e estatuto de conservação das espécies estudadas. Os resultados revelaram que, de modo geral, as espécies de *Aeonium* tendem a ter genomas pequenos, mas existe uma variação que vai desde os 0.98 pg (*Aeonium dodrantale*) até aos 2.54 pg (*Aeonium gorgoneum*). Verificou-se que espécies subarbustivas possuem, em média, tamanhos maiores de genoma. Uma análise comparativa através do grupo de amostras em estudo revelou uma correlação positiva entre a quantidade diploide de DNA nuclear e os estatutos de conservação da IUCN. Assim, espécies em perigo (EN) possuem, em média, maiores tamanhos de genoma. Além disso, quando testadas variáveis ambientais, uma relação positiva foi encontrada entre tamanhos de genoma e a temperatura média anual. Os dados apresentados neste estudo e a respectiva análise contribuem com uma nova visão para entender, não só os padrões de diversificação da radiação mais especiosa da Macaronésia: *Aeonium*, como também de outras radiações emblemáticas de plantas.

Tendo em conta o elevado valor de conservação que as espécies endémicas têm na Região da Macaronésia, as ameaças a que estão sujeitas implicam o conhecimento da sua distribuição para se contribuir para a proteção destas espécies unicas que nas ilhas ocupam áreas muito restritas **(Capítulo 4)**. Este último capítulo teve como principal objectivo atualizar o inventário dos taxa de *Aeonium* e investigar os padrões de distribuição, para detectar àreas de grande riqueza destas espécies dentro de cada ilha das Canárias. Foi ainda relacionando a distribuição das espécies de *Aeonium* com a rede de áreas protegidas deste arquipélago. Os resultados mostraram que a maior parte dos taxa de *Aeonium* ocorrem em Tenerife, sendo esta a maior ilha do arquipélago. Mais especificamente, a maior riqueza específica do género ocorre nos sectores nordeste e noroeste da ilha, que compreendem as florestas Laurissilva dos Parques Rurais da Anaga e Teno, respectivamente. A representatividade das espécies endémicas de *Aeonium* em áreas protegidas do domínio das Canárias é relativamente alta, uma vez que cerca de 40% da superfície do arquipélago é classificado como protegido. Neste sentido uma reavaliação das áreas protegidas deve ser requerida de modo a promover uma conservação mais efectiva da flora canária.

Assim, integrando os conhecimentos das várias abordagens aplicadas nos três capítulos (2 – 4) desta dissertação, foi possível contribuir com novos dados para explicar a distribuição das espécies de *Aeonium* e detectar *hotspots* de diversidade com base na altura das inflorescências, tamanho do genoma e factores abióticos. Algumas espécies têm a sua área de ocorrência limitada, entre outros factores externos (e.g., acção antropogénica e espécies invasoras), pela reduzida altura da inflorescência. Por outro lado, espécies com genoma maior, não respondem a esta tendência, tendo também alcances de dispersão limitados.

Contudo, novas análises morfológicas, citogenómicas e mais trabalho de campo em todas as ilhas da Macaronésia, para inventário de todas as espécies de *Aeonium*, irão permitir reforçar os resultados obtidos e identificar quais os principais factores que levaram à grande diversificação da maior radiação de plantas das ilhas da Macaronésia, contribuindo para a preservação da biodiversidade única existente nestes ecossistemas insulares.

Palavras-chave: Conservação; espécies endémicas insulares; ilhas oceânicas; tamanho do genoma; *traits* reprodutivos;

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Acronyms and Abbreviations

1C-value – Haploid Nuclear Content of DNA
2C-value – Diploid Nuclear Content of DNA
A – Santo Antão Island
AIZ – *Aeonium aizoon*
AnnPrec – Annual Mean Precipitation
AnnMTemp – Annual Mean Temperature
AUR – *Aeonium aureum*
ARB – *Aeonium arboreum*
BAS – Herbarium of the University of Basel
BR – Branching Rosette
BS – *Buxus sempervirens*
C – Gran Canaria Island
C$_3$ – C$_3$ Carbon Dioxide Fixation
CAN – *Aeonium canariense*
CAM – Crassulacean Acid Metabolism
CAS – *Aeonium castellopaivae*
CHELSA – Climatologies at high resolution for the earth's land surface areas
CIL – *Aeonium ciliatum*
CUN – *Aeonium cuneatum*
CV – Coefficient of Variation
D – Desertas Islands
DAV – *Aeonium davidbramwelii*
DEC – *Aeonium decorum*
DIP – *Aeonium diplocyclum*
DNA – Deoxyribonucleic Acid
DO – *Delairea odorata*
DOD – *Aeonium dodrantale*
E – Ethiopia
EN – Endangered
ERA – European Re-Analysis
EX – Extinct
F – Fuerteventura Island
FCM – Flow cytometry
FL – Fluorescence Pulse Integral in Linear Scale
G – La Gomera Island
GBIF – Global Biodiversity Information Facility
Georange – Geographic Range Size
GOM – *Aeonium gomerense*
GPCC – Global Precipitation Climatology Centre
H – El Hierro Island
HAW – *Aeonium haworthii*
IPA – Important Plant Area
IUCN – International Union for Conservation of Nature
K – Kenya
L – Lanzarote Island
LC – Least Concern
LISU – Herbarium of Botanical Garden of the University of Lisbon (LISU)
LISI – João de Carvalho e Vasconcellos Herbarium/ Superior Institute of Agronomy
Logflw1 – Log$_{10}$ of the Number of Flowers per Individual
Logheight1 – Log$_{10}$ of the Inflorescence Height
Loginf – Log$_{10}$ of the Number of Inflorescences per Individual
Logmerism – Log$_{10}$ of Flower Merism
M – Morocco
Ma – Madeira Island
Mbp – Megabasepairs
MJG – Botanical Museum of the University of Mainz – Herbarium
MR – Single Monocarpic Rosette
MTempColdQ – Mean Temperature of the Coldest Quarter
MTempWetQ – Mean Temperature of the Wettest Quarter
N – São Nicolau Island
NE – Not Evaluated
NT – Near Threatned
***p* –** Statistical probability significance, p-value
P – Porto Santo Island
Pa – La Palma Island
PCA – Principal Component Analysis
PER – *Aeonium percarneum*
PI – Propidium Iodide
pg – Picograms
PrecColdQ – Precipitation of the Coldest Quarter
PrecDriM – Precipitation of the Driest Month
PrecDriQ – Precipitation of the Driest Quarter
PrecWarQ – Precipitation of the Warmest Quarter
PrecWetM – Precipitation of the Wettest Month
PrecWetQ – Precipitation of the Wettest Quarter
PSE – *Aeonium pseudourbicum*
r – Correlation coefficient
R^2 – Coefficient of determination
S – Somalia
SAU – *Aeonium saundersii*
SD – Standard deviation
SED – *Aeonium sedifolium*
SIM – *Aeonium simsii*
SL – *Solanum lycopersicum*
SMI – *Aeonium smithii*
SPA – *Aeonium spathulatum*
SS – Subshrub
SSC – Side Light Scatter
T – Tenerife Island
Ta – Tanzania
V – São Vicente Island
VU – Vulnerable
Y – Yemen
U – Uganda
UND – *Aeonium undulatum*
URB – *Aeonium urbicum*
WPB – Woody Plant Buffer

Chapter 1

General introduction

1.1. Radiation, a speciation process

Radiation, *per se*, is a speciation process which leads to a diversification of characters, ecology and consequently the lineages of living organisms in a given context, namely the insular context, which is the focus of this work) **(Jorgensen & Olesen, 2001)**. In a more restricted view, adaptive radiation is a very rare and fast phenomenon in nature. It´s considered to be an ecological and phenotypic diversification of a lineage of species with capacity to explore many types of ecological niches and a diverse range of resources **(Rundell & Price, 2009)**. Morphologically and physiologically these species differ in the exploration of these different niches avoiding competition **(Givnish, 2010)**. Therefore, these differences are considered adaptations and allow the generation of biological diversity and the coexistence of closely related species **(Givnish, 2015)**. From a fundamental perspective, a common ancestor can originate species with homologous characters, through divergent evolution, considering different selective pressures. These characters allow the adaptability of the organisms to the corresponding niches **(Reece *et al.*, 2017)**.

The most paradigmatic example of adaptive radiation is the Darwin finches in the Galapagos Islands **(Darwin, 1936)**. The fourteen species of finches observed evolved through adaptive radiation. The ecological differentiation observed is through the different sizes of the beaks and respective diet **(Grant & Grant, 2011)**. Other typical examples include African rift-lake cichlids **(Muschick *et al.*, 2012)**, Caribbean Anolis lizards **(Losos *et al.*, 2009)**, and Hawaiian honeycreepers **(Lerner *et al.*, 2011)**.

Plant radiations in oceanic islands is a very common phenomenon in this type of environment. Such phenomenon can be seen when studying the floras of isolated oceanic islands because within these restricted areas numerous plant genera are provided by a large number of endemic species. Notably, Canary Islands forms the hub large radiations such as *Aeonium* Alliance (> 40 endemic taxa) Thus, radiation on islands have a great interest as plant groups radiate across a very confined geographical area, are relatively recent, and their evolutionary history can be demonstrated through the geological history of the islands **(Jorgensen & Olesen, 2001; MacArthur & Wilson, 1967).**

1.2. Oceanic islands as a natural laboratories for understanding speciation and diversification processes

Oceanic islands comprise an isolated habitat of volcanic origin thas has never been connected to the mainland. These islands are small with well-defined boundaries, considerable habitat heterogeneity, a greater number of available niches, lower competition compared to continental habitats and simplified biota **(e.g., Carlquist, 1980)**. They are called *virgin islands* from any kind of life form at the origin of its genesis, and we can assume that all the species arrived there through colonization from the mainland. After colonization, the biota had to adapt to new conditions and available habitats and evolved isolated from their continental ancestral species **(Losos & Ricklefs 2009; Whittaker & Fernández-Palácios 2007)**. According to the above-mentioned facts, a considerable increase in the number of endemic species is observed in relation to mainland.

These species, being in an island context, began radiating very recently, within a very restricted geographic area and in a very rapid way. For example, the combination of specific climatic and geological conditions in the islands creates many microclimates at single sites, that is, adaptive zones as referred by **Simpson (1944)**. This series of available niches create a radiation gradient of new species with functional characters adapted to what the same environment provides (such as food, climate, soil, etc.) and with a tendency to avoid interspecific competition. All things considered, oceanic islands are natural living laboratories, which gather the perfect conditions to study species evolution **(Carlquist, 1974; Jorgensen & Olesen, 2001; Bouchenak-Khelladi *et al.*, 2015; Givnish, 2015).**

1.3. Studying across Macaronesia

Macaronesia region hosts a rich endemic flora including notable examples of plant radiations. It comprises five volcanic archipelagos: Azores, Madeira, Salvage, Canaries, and Cabo Verde and is located in the Atlantic Ocean along the African west coast between 15º to 40ºN **(Figure 1.1.).** As a biodiversity hotspot, Macaronesia is recognized not only for its great characteristic plant biodiversity but also for its large number of endemisms **(Mansion *et al.*, 2009)**. The great exclusivity of this region allows it to be a natural model for the study of plant rate diversification and, at the same time, to understand the adaptive mechanisms of these habitats with intrinsic dynamics **(Bramwell, 1976)**.

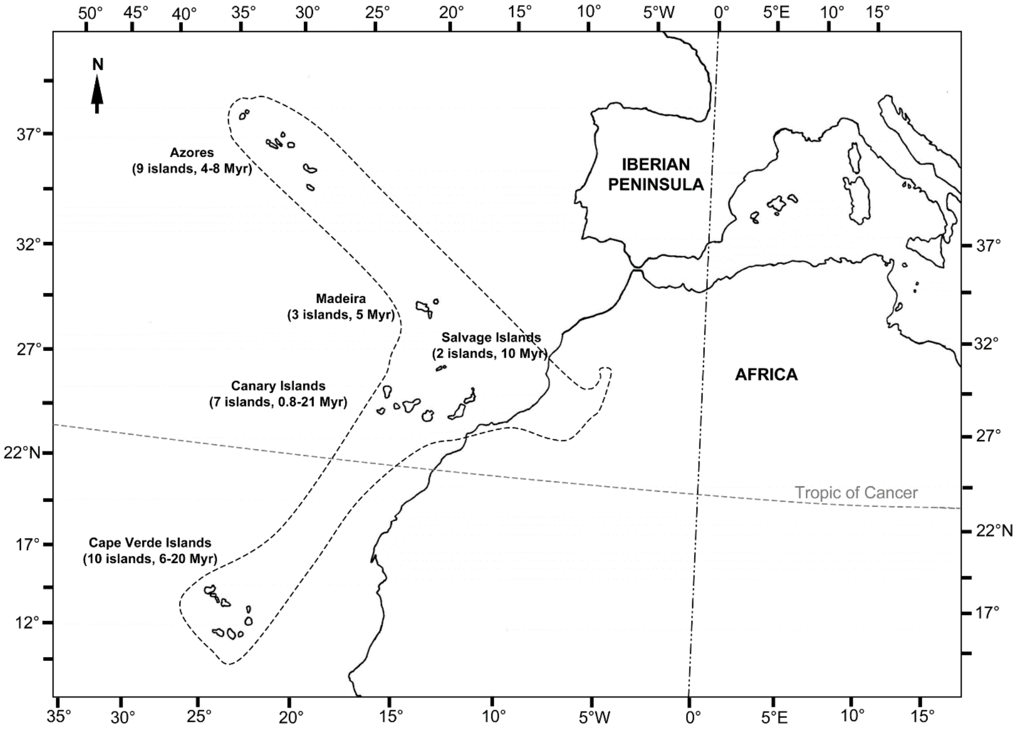


Figure 1.1. Geographical map of the Macaronesian biogeographical zone presenting the five oceanic archipelagos (Azores, Madeira, Salvage, Canaries, and Cabo Verde). **(Adapted from Kim *et al.*, 2008)**

Canary Islands, the most biodiverse archipelago of Macaronesia and the main study area of this study, form a volcanic archipelago holding a chain of seven islands in the Central Atlantic (28° 32' 11" N; 15° 43' 17" W), off the west coast of Morocco **(Figure 1.1.)**. The origin of this archipelago is somewhat controversial; however, it is favored by the classic hotspot model of **Wilson (1963)**, even with some statements against. According to this theory, the archipelago was formed sequentially in the last 20 million years, as a repercussion of the slow movement of the African Plate to the northeast on a fixed volcanic hotspot of mantelic origin **(Carracedo & Perez – Torrado, 2013)**. It is known that the oldest islands in the sequence are Fuerteventura (20.7 My), Lanzarote (15.4 My), Gran Canaria (13.9 – 16 My), Tenerife (11.6 My), La Gomera (10 – 12.5 My), La Palma (1.5 – 2 My) and El Hierro (less than 0.7 My). **(Carracedo, 1994; Del – Arco *et al.*, 2002)**.

The Canaries are situated less than 100 km off the Atlantic coast of Africa, which makes this archipelago quite reachable yo colonization from continental sources **(Cajuapé – Castells *et al.*, 2017)**. Also, the range of geological ages observed among all the islands, as mentioned above, reveals a great contributor to the co-occurrence of several taxa of the same group evolved among different eras **(Carracedo, 1994; Fernandopullé, 1976)**.

Canary archipelago suffered several radiation processes, namely in the reptiles (e.g., *Gallotia* lizards and *Tarentola* geckos), or among the vascular plant lineages (e.g., *Aeonium*, *Argyranthemum*, *Echium*, *Sideritis*, and *Sonchus*) leading to a high biological diversity. It is possible to recognize approximately 1000 species of higher plants, of which near 680 are endemisms. This great number of endemisms is related to the geology and climate (subtropical), that is, the influence of northeast cold humid trade winds and dry winds from Sahara desert. This combined with the large range of altitudes (up to 3718 m in Tenerife) originates a great number of ecological zones **(Juan, 2000; Kim et *al.*, 2008; Lems, 1960; Whittaker & Fernández-Palacios, 2007).**

In the mountain islands, it is possible distinguish six ecological zones, from the coast to the top of each island, according to the island slope **(see Figure 1.2.) (Del-Arco Aguilar *et al.*, 2010; Fernández-Palacios *et al.*, 2008; gobiernodecanarias.org)**:

(1) **The sub-desert coastal scrub (*tabaibas*):** [0 to 700 m high] has great similarities with the closest continental African ecosystems. The predominance of endemic xerophytic shrubs of the genus *Euphorbia* is observed, which are adapted to drought, strong winds and constant solar radiation.
(2) **Thermophile forests:** [200 to 600 m high] are the ecosystems most similar to the Mediterranean ones. In this type of ecosystem, rain and temperatures are moderate. Occur species such as *Juniperus turbinata*, *Dracaena draco* and *Phoenix canariensis*.
(3) **Laurel forest:** [500 to 1000 m high] is called a subtropical ecosystem, where a dense forest of large trees of the tertiary period (in the Miocene and Pliocene) is inserted. Can be found here species of ferns, lichens, laurel, willows and beeches.
(4) **Pine forest:** [800 to 2000 m high] forest with predominance of *Pinus canariensis*.
(5) **Summit scrub:** [2000 to 2700 m high] vegetation dominated by endemic shrub legumes.
(6) **Peak ecosystem:** [from 2700 m high] exclusively represented on the highest slopes of the Teide volcano in Tenerife. It is a dry environment with high temperatures. In this ecosystem is characteristically found the endemic *Viola cheiranthifolia*.

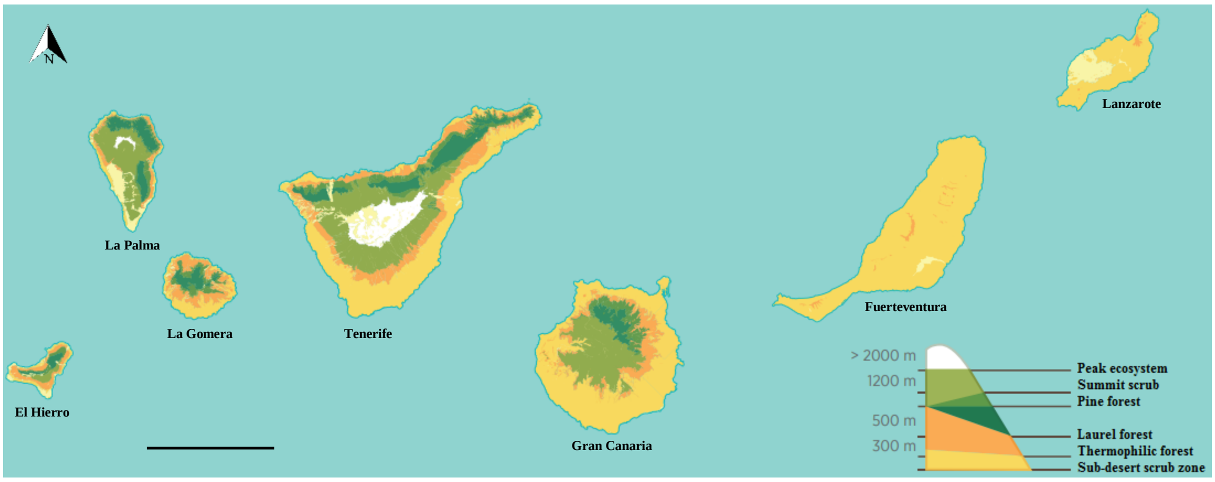


Figure 1.2. Bioclimatic altitudes and potential natural Vegetation on Canary Islands. Scale Bar = 50 km. **(Adapted from: www3.gobiernodecanarias.org)**

1.4. *Aeonium* as study model

Aeonium Webb & Berthel. is a rosette-forming genus of the succulent family Crassulaceae with approximately 40 species distributed in the three Macaronesian archipelagos (i.e., Canaries, Madeira, and Cabo Verde), East Africa, Yemen, and Morocco **(details on species names aare provided in Table 1.1.)**. Based on molecular phylogenetic studies, it is assumed that the genus arose in the Canarian Archipelago and only after dispersed to Madeira, Cabo Verde, and Africa. Together with *Aichryson* Webb & Berthel. and *Monanthes* Haw. constitutes the *Aeonium* alliance, a monophyletic clade of Crassulaceae family **(Vam Ham & 't Hart, 1998; Mort *et al.*, 2001)**.

This genus is provided by a huge immense morphological, ecological and physiological variation being composed by several woody growth-form types including large, monocarpic rosette trees, highly branched shrubs, and single rosettes. The inflorescences vary from big dome-shaped inflorescences with thousands of flowers (e.g. *Aeonium urbicum*) to flat-topped ones with a small number of flowers (e.g. *Aeonium sedifolium*). The flowers are polymerous with a range of several colors (yellow, white, green, pink and dark red) and a great variation in merism, that is, the number of parts in a flower (from 5 to 35-merous). In relation to physiology, there are species with C_3, CAM or Intermediate photosynthesis pathways. Ecologically, in Canary Islands where is mainly distributed, *Aeonium* species colonized a lot of vegetation zones, occupying a wide range of habitat types, from humid Laurel forests to xeric volcanic subcoastal shrubland. The greatest part of the species are single - island endemics and each one appears in a very restricted ecological niche. In addition, most of the species occur in sympatry or parapatry and natural hybrids are observed **(Esfeld, 2009; Jorgensen & Olesen, 2001; Liu, 1989; Mort *et al.*, 2007).** According to the huge morphological variation and the large variety of niches exploited by different species observed in *Aeonium*, it is assumed by various authors, that there was an adaptive radiation process **(Lems 1960; Voggenreiter 1974)**.

Aeonium species are distributed across all the islands of Canaries (as well as some islands of Madeira and Cabo Verde) allowing comparisons and inferences between all the islands; have a great morphological and ecological variation occupying a large spectrum of vegetation zones in a very heterogeneous. The above-mentioned facts make this genus an ideal model to test several theories of evolutionary biology.

Table 1.1. List of *Aeonium* Webb & Berthel. species described according to their accepted classifications **(Bañares 2015; Eggi & Hartmann, 2002)**.

Insular species			Growth-Form
Archipelago	**Islands**	**Current classification**	
Madeira	Ma, D, P	*Aeonium glandulosum* (Aiton) Webb & Berthel.	MR
	Ma, D	*Aeonium glutinosum* (Aiton) Webb & Berthel.	SS
Canary Islands	L, F	*Aeonium balsamiferum* Webb & Berthel.	SS
	L	*Aeonium lancerottense* (Praeger) Praeger	SS
	C	*Aeonium arboreum* ssp. *arboreum* (L.) Webb & Berthel.	SS
	C	*Aeonium canariense* ssp. *virgineum* (Webb ex Christ) Bañares	BR
	C	*Aeonium percarneum* (R. P. Murray) Pit. & Proust.	SS
	C	*Aeonium simsii* (Sweet) Stearn.	BR
	C	*Aeonium undulatum* Webb & Berthel.	SS
	T	*Aeonium aizoon* (Bolle) T.H.M.Mes	BR
	T, Pa, H	*Aeonium arboreum* ssp. *holochrysum* (H.Y.Liu) Bañares	SS
	C, T	*Aeonium aureum* (C.Sm. ex Hornem.) T.H.M.Mes	BR
	T	*Aeonium canariense* ssp. *canariense* (L.) Webb & Berthel.	BR
	T	*Aeonium ciliatum* (Willd.) Webb & Berthel.	SS
	T	*Aeonium cuneatum* Webb & Berthel.	BR
	T	*Aeonium dodrantale* (Willd.) T.H.M.Mes	BR
	T	*Aeonium haworthii* Salm – Dyck ex Webb & Berthel.	SS
	T	*Aeonium lindleyi* ssp. *lindleyi* Webb & Berthel.	SS
	T	*Aeonium pseudourbicum* Bañares	MR
	T, G, Pa	*Aeonium sedifolium* (Webb ex Bolle) Pit. & Proust.	SS
	C, T, G, Pa, H	*Aeonium spathulatum* (Hornem.) Praeger	SS
	T	*Aeonium smithii* (Sims) Webb & Berthel.	SS
	T	*Aeonium tabuliforme* (Haworth) Webb & Berthel.	MR
	T	*Aeonium urbicum* var. *urbicum* (C.Sm. ex Hornem.) Webb & Berthel.	MR
	T	*Aeonium urbicum* var. *meridionale* Bañares	MR
	T	*Aeonium volkerii* Hernández & Bañares	SS
	G	*Aeonium appendiculatum* Bañares	MR
	G	*Aeonium arboreum* subsp. *holochrysum* var. *rubrolineatum* (Svent.) H.Y.Liu	SS
	G	*Aeonium canariense* subsp. *latifolium* (Burchard) Bañares	BR
	G	*Aeonium castellopaivae* Bolle	SS
	T, G	*Aeonium decorum* var. *decorum* Webb ex Bolle	SS
	G	*Aeonium decorum* var. *alucense* Bañares & M.V. Marrero	SS
	G	*Aeonium gomerense* (Praeger) Praeger	SS
	G	*Aeonium lindleyi* ssp. *viscatum* (Bolle) Bañares	SS
	G	*Aeonium saundersii* Bolle	SS
	Pa	*Aeonium canariense* ssp. *christhii* (Burchard) Bañares	BR
	Pa	*Aeonium davidbramwelii* H.Y.Liu	SS
	G, Pa, H	*Aeonium diplocyclum* (Webb ex Bolle) T.H.M.Mes	BR
	Pa	*Aeonium goochiae* (Christ.) Praeger	SS
	Pa	*Aeonim nobile* (Praeger) Praeger	MR
	Pa, H	*Aeonium hierrense* (R. P. Murray) Pit. & Proust.	MR
	H	*Aeonium valverdense* (Praeger) Praeger	SS
Cabo Verde	A, N, V	*Aeonium gorgoneum* J. A. Schmidt	SS

Continental species			
Continent	**Region**	**Current classification**	
Africa	M	*Aeonium korneliuslemsii* H.-Y. Liu	SS
	E, K, Ta	*Aeonium stuessyi* H.-Y. Liu	SS
Africa; Asia	Y, E, S, K, U	*Aeonium leucoblepharum* Webb ex A. Richard	SS

Ma: Madeira; D: Desertas; P: Porto Santo; L: Lanzarote; F: Fuerteventura; C: Gran Canaria; T: Tenerife; G: La Gomera; Pa: La Palma; H: El Hierro; A: Santo Antão; N: São Nicolau; V: São Vicente; M: Morocco; E: Ethiopia; K: Kenya; Ta: Tanzania; Y: Yemen; S: Somalia; U: Uganda; SS: Subshrub; BR: Branching rosette; MR: Single monocarpic rosette;

1.5. Objectives

The main goal of this thesis is to study the diversity patterns, by a trait – based and cytogenomic approach, among the Macaronesian *Aeonium* species (with a special focus in the Canary Islands where this genus is more widespread) and contribute with new data for further conservation of the biggest plant radiation within Macaronesian Islands. Specifically, it will be:

1) Test whether *Aeonium* species that invest in taller inflorescences and more reproductive units have wider distributions and a more generalist behavior, relating the inflorescence height with species natural distributions.

2) Determine the cytogenomic diversity of *Aeonium* species and relevance of considering cytogenomic diversity in nature conservation.

3) Assess the patterns of diversity of the genus *Aeonium* in Canary Islands and contribute with new data to identify areas of high importance for biodiversity, particularly in terms of species richness.

The study made in this thesis is presented in three chapters: The inflorescence height as an approach to explain the *Aeonium* species distribution in the Canary Islands (Chapter 2); The biggest plant radiation of Macaronesia – *Aeonium*: positive correlations between genome size variation and their conservation status (Chapter 3) and Identification of biodiversity hotspots within Macaronesian Islands: centers of *Aeonium* species richness and endemism as a case-study (Chapter 4).

Chapter 2 focuses on the following hypothesis: *Aeonium species with taller inflorescence can disperse at wider distributions and so, are more generalist than species with smaller inflorescence*. Testing this assumption involves the measuring of several *Aeonium* inflorescences belonging a different kind of ecological niches and functional traits. This hypothesis is based in the relation between inflorescence characteristics and species distribution along the island, as well as, their endemicity. Species with longer inflorescences may be able to disperse along large distances and, possibly, to other islands representing more generalist species.

Chapter 3 focuses on the following hypothesis: *threatened Aeonium species have, on average, a larger genome than their congeners*. Testing this hypothesis requires a genome size estimation by flow cytometry techniques and it correlation with conservation status. Involves the assessment of nuclear DNA content for several *Aeonium* taxa belonging to a different Macaronesian islands. This assumption is based in the relation between large genome sizes and low rates of growth. Species with greater genome sizes tends to have small effective and fragmented populations and, thus, higher likelihood of extinction.

Chapter 4 focuses on the detection of diversity hotspots for the Macaronesian endemic *Aeonium* genus. Involves the collection of occurrence data from fieldwork, scientific collections and the large spectrum of available bibliography. This study includes the spatial detection of the areas with the higher *Aeonium* taxa richness and their relationship to ecological zones and protected areas.

Through this comprehensive approach, the aim is to better understand distributional, cytogenomic and conservation patterns of *Aeonium* genus in the Macaronesian Islands.

Chapter 2

The inflorescence height as an approach to explain the *Aeonium* species distribution in the Canary Islands

Abstract

The genus *Aeonium* (Crassulaceae) constitutes the most speciose radiation of Macaronesian Islands, where it diversified into nearby 40 endemic species. Comprise a rosette-forming plant genus that has diversified mainly in the Canary Islands into different life-forms and survival strategies, occupying several habitat types. Insular species tend to lose their dispersal abilities by reducing dispersal investment, but this process poorly understood. *Aeonium* seed mass and size are very conserved features among all species, hence, the current study focused on inflorescence height as the major character that driving seed dispersal in this genus. In the present work, it was measured the inflorescence height and counted the number of flowers, merism and inflorescence number per individual for 22 *Aeonium* species. For the analysis of the species distribution, geographic range size was estimated by field observations and literature descriptions. The results showed that inflorescence height was positively correlated to flower number ($R^2 = 0.86$) and geographic range size ($R^2 = 0.52$). This suggests that species that invest in taller inflorescences and more reproductive units have wider distributions and a more generalist behavior. These findings enhance our knowledge of *Aeonium* dispersal strategies and improve our understanding about their natural distributions.

Keywords: Macaronesia; dispersal strategies; oceanic islands; occurrence area; reproductive traits;

2.1. Introduction

Dispersal, i.e., the movement of seeds or other propagules (e.g., vegetative reproduction) away from the mother plant, is a key factor in plant reproduction and establishment (i.e., life cycle) **(Levin et *al.*, 2003)**. This process determines the species' distribution, the persistence and maintenance of natural populations in their habitats and the potential recruitment area **(Nathan & Muller-Landau, 2000; Nathan, 2006; Traveset *et al.*, 2014)**. For example, species with limited seed dispersal ability tend to undergo shrinkage of their geographical range and, in extreme cases, extinction **(Matlack & Monde, 2004)**. Understanding the processes and traits associated with the spatial dynamics of species and how their populations are spatially structured is a challenging ecological and evolutionary approach **(Sutherland *et al.*, 2013; Thompson *et al.*, 2011; Zhang *et al.*, 2018)**. Several studies have contributed to enlighten the relationship between the species dispersal distance and their seed dispersal ability **(e.g., Ackerman *et al.* 1996; Murray *et al.*, 2002; Tremlová K. & Münzbergová Z., 2007)**.

Assessing seed dispersal distance of plant species is a complex task and, therefore, there is a great lack of available data. Some researchers predicted seed dispersal distance based on field measurements and mathematical models, but these approaches are less representative because they consider only a few number of individuals **(Vittoz & Engler, 2007)**. Thus, biologists commonly use morphological traits as a proxy to predict seed dispersal distance, since their evaluation is quite easy and there are several plant trait databases available **(e.g., Perez-Harguindeguy *et al.*, 2003; Kattgle *et al.*, 2011).** The most common characteristics used as seed dispersal proxies are dispersal structures, seed mass and plant height **(e.g., Thompson *et al.*, 2011; 2017)**.

According to **Thomson *et al.* (2011)** the maximum plant height alone is better than seed mass as a predictor of species dispersal distances, not only for the good correlation but also because height is an easier trait to collect in field. Regardless of the dispersal syndromes, dispersal investment, and dispersal structure mass, a positive relationship exists between mean dispersal distance and plant height, that is, on average taller plants can disperse their seeds to further distances. The best explanation for the above-mentioned can be the fact that plant height enhances the visibility to dispersal vectors and seed release heights **(Muñoz *et al.*, 2017; Tackenberg *et al.*, 2003)**. Besides that, plant height is a very important trait in an ecological context because it is correlated with species lifetime and longevity, seed mass (i.e., supposedly taller plants have a great annual seed production, therefore, it may increase the dispersal distance; **Moles *et al.*, 2004**), capacity to compete for light, seed bank longevity and the success in establishment **(Moles & Westoby, 2004; Moles *et al.*, 2009)**.

Particularly, on islands, there are reports of a decrease in dispersal investment in plants, with the loss of seed dispersal abilities. This fact occurs, presumably, to avoid the fall out of seeds into the sea **(Carlquist, 1966a; 1966b; 1974; Cody & Overton, 1966; Kavanagh & Burns, 2014)**. This reduction of dispersal abilities can be overtaken by morphological traits enabling the populations maintenance **(Vazacova & Münzbergová, 2014a)**. In this way, once that insular species develop large individual sizes endowed with a woody growth forms (e.g., *Aeonium*; **Jorgensen & Olesen, 2001**; *Echium*; **Bohle *et al.*, 1996**; *Sonchus*; **Kim *et al.*, 1996**) and, consequently, taller species, it is expected that the plant height is an optimal estimator of dispersal distance **(Thomson *et al.*, 2017)**.

The leaf-succulent rosette-forming *Aeonium* genus Webb & Berthel. (Crassulaceae; Sempervivoideae) is an exceptional example of islands radiation, being the most speciose in Macaronesia with ca. 44 taxa (i.e., 41 in Canary Islands, two in Madeira, and one in Cabo Verde) and only three species are outside of Macaronesia (i.e., one in Morocco and two in East Africa and Asia). **(Jorgensen & Olesen, 2001; Mort *et al.*, 2007)**. Presumably, the group diversified as a result of just one colonization event from Africa **(Thiv *et al.*, 2010)**. The explosive diversification into approximately 41 endemic taxa, mainly single island endemics, is characterized by a huge diversity of growth-forms **(Figure 2.1.)** such as single monocarpic rosettes, branching rosettes, small and large subshrubs, physiological features, and ecological niches, occupying almost all the vegetation zones of Canary archipelago **(Lems, 1960; Liu, 1989; Lösch, 1990; Mes *et al.*, 1996)**. Although the variation of growth form is one of the most prominent features, the variation along the reproductive units (i.e., inflorescences, flowers, fruits, and seeds) must also be considered **(Bañares, 2015; Esfeld *et al.*, 2009; Liu, 1989)**.

Figure 2.1. ***Aeonium*** **growth – forms.** **(A)** *Aeonium urbicum* var. *meridionale* as single monocarpic rosettes (MR); **(B)** *Aeonium dodrantale* as a **b**ranching rosettes (BR); and **(C)** *Aeonium arboreum* ssp. *holochrysum* as Subshurb (SS). (Photos: **(A – B)** Miguel Brilhante; **(C)** Patrícia dos Santos; Tenerife, May 2019)

Generally, *Aeonium* inflorescences are terminal cymose panicle with basal determinate flowering sequencing and a variable number of flowers and/or branching orders. The flowers are included in the general type of crassulacean flowers with radial symmetry, hypogynous flowers, a differentiated calyx and corolla (yellow, reddish or white), two whorls of stamens and one whorl of carpels. At the base of each carpel, some species, with exception of *Greenovia* section **(Bañares, 2015)**, exhibits a white or greenish – white nectariferous glands as a feature for pollination **(Liu, 1989; Jorgensen & Olesen, 2001; Eggli & Hartmann, 2002)**. Additionally, fruits are non-fused follicles and respective seeds are ellipsoidal, small (0.4–0.6 mm long) and light (0.02– 0.04 mg) without dispersal structures, being a very uniform character among all species **(Lems, 1960; Liu, 1989)**. Considering the lack of seed specialized traits, the most important dispersal agent of *Aeonium* is presumably the gravity besides some occasional effects of wind **(Harter *et al.*, 2015; Vazačová & Münzbergová, 2014b)**.

The Canary Islands holds the greatest number of *Aeonium* species, some with more restricted ecological preferences (specialists) and others with more ubiquitous distributions (generalists). Reproductive traits are intrinsically associated with the plants' life-form. Some *Aeonium* species are perennial monocarpic with single rosettes, growing for several years until flowering and die. Polycarpic species are typically subshrubs provided by various rosettes, setting flower several times during their lifetime. This implies different investments between vegetative and reproductive traits **(Bañares, 2015; Jorgensen & Olesen, 2001; Liu, 1989)**. As mentioned before, *Aeonium* seed mass and size is a very conserved feature among all species **(Liu, 1989)**, therefore, this study will be focused on flower number and inflorescence height as the major characters that drive seed dispersal in this genus. Considering the above-mentioned, this study aims to test whether *Aeonium* species that invest in taller inflorescences and more reproductive units have wider distributions and a more generalist behavior, relating the inflorescence height with species natural distributions. Additionally, to increase the knowledge about *Aeonium* genus, the reproductive trait differentiation among species is also studied.

2.2.Methodology

2.2.1. *Study area*

Canary Islands have a volcanic origin and comprise seven major islands aged between 0.7 My (El Hierro) and 20.7 My (Fuerteventura). This area is located between 27'37º–29'25ºN and 13'20º–18'10º W in the NE sector of the Atlantic Ocean **(See Figure 1.1.)**. Tenerife is the biggest of all Macaronesian islands with 2034 km^2 of area and 3718 m of altitude (**Pérez *et al.*, 2003**). On its west side has La Gomera, La Palma, and El Hierro; and on its east side has Gran Canaria, Fuerteventura, and Lanzarote. The North and South border of the island is defined by the Atlantic Ocean **(Del-Arco Aguilar *et al.*, 2010)**. The archipelago is located about 100 km from Fuerteventura to Morocco and 1300 km from the Iberian Peninsula **(Juan *et al.*, 2000)**. The climate is generally subtropical Mediterranean **(Del-Arco *et al.*, 2010)**, being influenced by the north-eastern trade winds and the refreshing cool waves of the Gulf currents. It is moderate, temperate and very homogeneous throughout the year. There are no periods of very cold or periods of strong heat. Average temperatures are generally 18°C in the Winter and 25°C in the Summer **(González Morales, 2000)**.

The surprising array of plant species (ca. 2100 species with 20% of endemic species; **Arechavaleta *et al.*, 2010**) in the Canary Islands has emerged as a result of the very particular environmental conditions, in other words, the islands' varied topography combined with climatic conditions leads to a huge variety of microclimates along the spatial gradient. Thus, it is possible to observe a huge diversity of natural habitats such as: semi-desert coastal scrub, thermophilic, laurel and pine forest, summit scrub and alpine zone **(Fernández-Palacios & Whittaker, 2008)**.

2.2.2. *Sampling*

Fieldwork was carried out between February and June 2019 on Tenerife, La Palma, Gran Canaria, and La Gomera because it's the islands with the greatest diversity of *Aeonium* species **(Figure 2.2.)**. Twenty-two species of *Aeonium* were studied. For each species, a minimum of 3 healthiest flowering individuals (n = 132), in full bloom, were sampled in a healthy, well-accessible and randomly selected population. For each individual, maximum inflorescence height were measured, in cm, and the number of inflorescences per individual, number of flowers per inflorescence and flower merism were recorded. The maximum inflorescence height was measured as the distance from the ground level to the top of the inflorescence, that is, until the level of the upper flower **(Perez-Harguindeguy *et al.*, 2016)**. The number of inflorescences per individual was recorded by counting the inflorescences from the current year representing the annual offspring. To count the number of flowers, all the inflorescences per sampled individual were collected and the number of flowers was integrally counted. Flower merism was obtained by counting the number of carpels per flower for 9 flowers per inflorescence (3 from the base, 3 from the middle, and 3 from the top of inflorescence) **(See Appendix I)**.

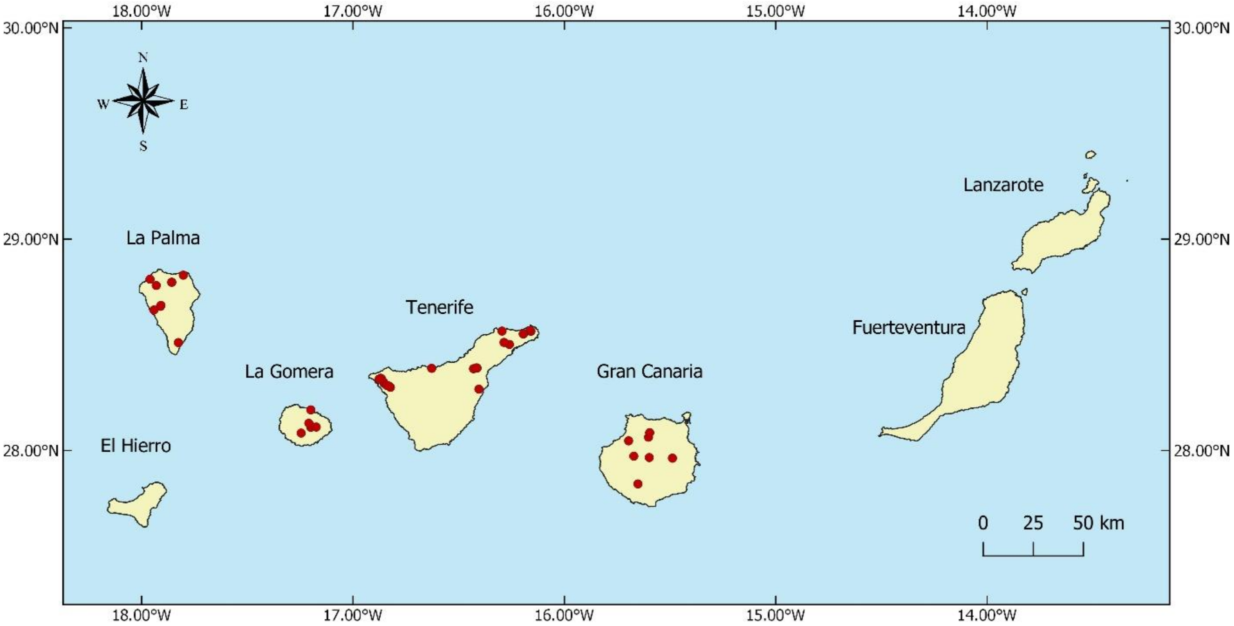


Figure 2.2. Sampling points (red dots) at La Palma, La Gomera, Tenerife and Gran Canaria. Scale Bar = 50 km. At each point can occur several *Aeonium* species **(QGIS Development Team, 2019)**.

2.2.3. *Geographic range size estimation*

Geographic range size was estimated in km^2 by counting the number of 5 km grid squares with at least one occurrence of each species in Canary Islands. Distribution data were collected from **Bañares (2015)** because it is the most recent and complete bibliography about canarian *Aeonium* genus, and from field observations.

2.2.4. *Environmental data*

The 19 bioclimatic variables (precipitation and temperature; **Appendix II**) used in current work were extracted from CHELSA dataset version 1.2 (Climatologies at High Resolution for the Earth's Land Surface Areas, available at https://chelsa-climate.org/) **(Karger *et al.*, 2017)**. CHELSA provides 19 bioclimatic variables at high resolution (30 arcsec) representing the average global climatic data from 1979 to 2013. This dataset uses a quasi-mechanistic statistical downscaling of the ERA (European Re-Analysis) interim global circulation model with a GPCC (Global Precipitation Climatology Centre) bias correction **(Karger *et al.*, 2017)**.

2.2.5. *Statistical analysis*

Maximum inflorescence height, number of inflorescences, number of flowers and merism data were $\log_{10}$-transformed before analyses. All the analyses were performed using R v.3.6.1 **(R Core Team, 2019)**. To investigate the distribution of *Aeonium* species according to the reproductive morphology of the genus and understand how different the species are in terms of morphological traits, a Principal Component Analysis (PCA) based on the correlation matrix was performed using the vegan package **(Oksanen *et al.*, 2019)**. In order to test which environmental variables explain the plot bivariate relationships, the *envfit* function of vegan package was used **(Oksanen *et al.*, 2019)**. Results of PCA were visualized with the biplot function. In order to clarify the relationships between traits and environmental variables shown in PCA, a Heatmap illustrating Pearson correlation coefficients was made using *melt* and *ggplot* functions of reshape2 and ggplot2 packages, respectively **(Wickham, 2007; 2016)**.

To test the relationship between plant inflorescence height and the number of flowers and geographic range size, at a first step, boxplots and bar plot were done to show trait variation across the species and understand their relations. As a second step, using a single linear regressiom model, correlation plots were made with *ggscatter* function of ggpubr package **(Kassambara, 2019)**. The model of the relationship between inflorescence height and the geographical range was performed both with all the sampled species (n=132), and also after excluding the species without significant relationship between the two variables (n=110).

2.3. Results

2.3.1. *Reproductive trait differentiation among species*

The PCA **(Figure 2.3)** including *Aeonium* species distribution according to their reproductive traits, that is, $\log_{10}$ of maximum inflorescence height, number of inflorescences, number of flowers per individual and merism, show a general view of trait variation within the genus. The trait correlation at the genus level is well explained by the first two axes of PCA, PC1 (59.29%) and PC2 (27.66%). Additionally, the environmental vectors fitted as a passive variable, give us its relationship with species distribution across PCA. Overall, the shown PCA reflects three trait continua with trade-offs: one from species with high merism, other from species with a great number of flowers, and another from species with more inflorescences.

The heatmap presents the heatmap based on the Pearson correlation matrix of the 15 variables (4 morphological traits and 11 environmental variables) under study, is present in **Figure 2.4.** The weak correlations between variables are displayed in white, while stronger negative and positive correlations are shown in blue and red, respectively. This approach reflects and proves the relationships highlighted in the plotted bivariate relationships of **Figure 2.3.**

According to **Figure 2.3.** and **Figure 2.4.**, associated with a big merism are the species from *Greenovia* section **(Bañares 2015)**, all branching rosettes, *Aeonium aizoon*, *Aeonium aureum*, *Aeonium diplocyclum*, and *Aeonium dodrantale*. In this case, there are a smaller number of flowers per individual with a great number of carpels per flower. This observation elucidates a trade-off between these two traits. It is possible to see that the merism increase with precipitation variables, being the precipitation of the coldest quarter the most positively correlated; and decrease with the increment of the mean temperature of the wettest quarter which is the most negatively correlated variable.

Considering the number of inflorescences per individual, as expectable, subshrub species (i.e., *Aeonium arboreum*, *Aeonium castellopaivae*, *Aeonium decorum*, *Aeonium haworthii*, *Aeonium percarneum*, *Aeonium saundersii*, *Aeonium sedifolium*, *Aeonium smithii*, and *Aeonium spathulatum*) and the branching rosette *Aeonium simsii*, are the species endowed with the greatest number. A negative correlation between the number of inflorescences and merism is found. It is also observed a negative association between species with more inflorescences and the precipitation of the driest quarter.

The number of flowers per individual and maximum inflorescence height increase in the same way. So, it can be assumed that taller species produce more flowers by the strong positive correlation found. The taller species with a great number of flowers comprise the following species: the subshrubs *Aeonium arboreum*, *Aeonium percarneum,* and *Aeonium davidbramwellii,* and the monocarpic rosette *Aeonium urbicum*. As it is possible to see, the number of flowers per individual and maximum inflorescence height increase contrarily with annual precipitation, precipitation of the wettest month, precipitation of the wettest quarter and precipitation of the coldest quarter. On the other hand, stands out a positive correlation with the annual mean temperature, mean temperature of the wettest quarter, mean temperature of the coldest quarter and also with the geographic range size. Precipitation of warmest quarter is positively correlated only with maximum inflorescence height.

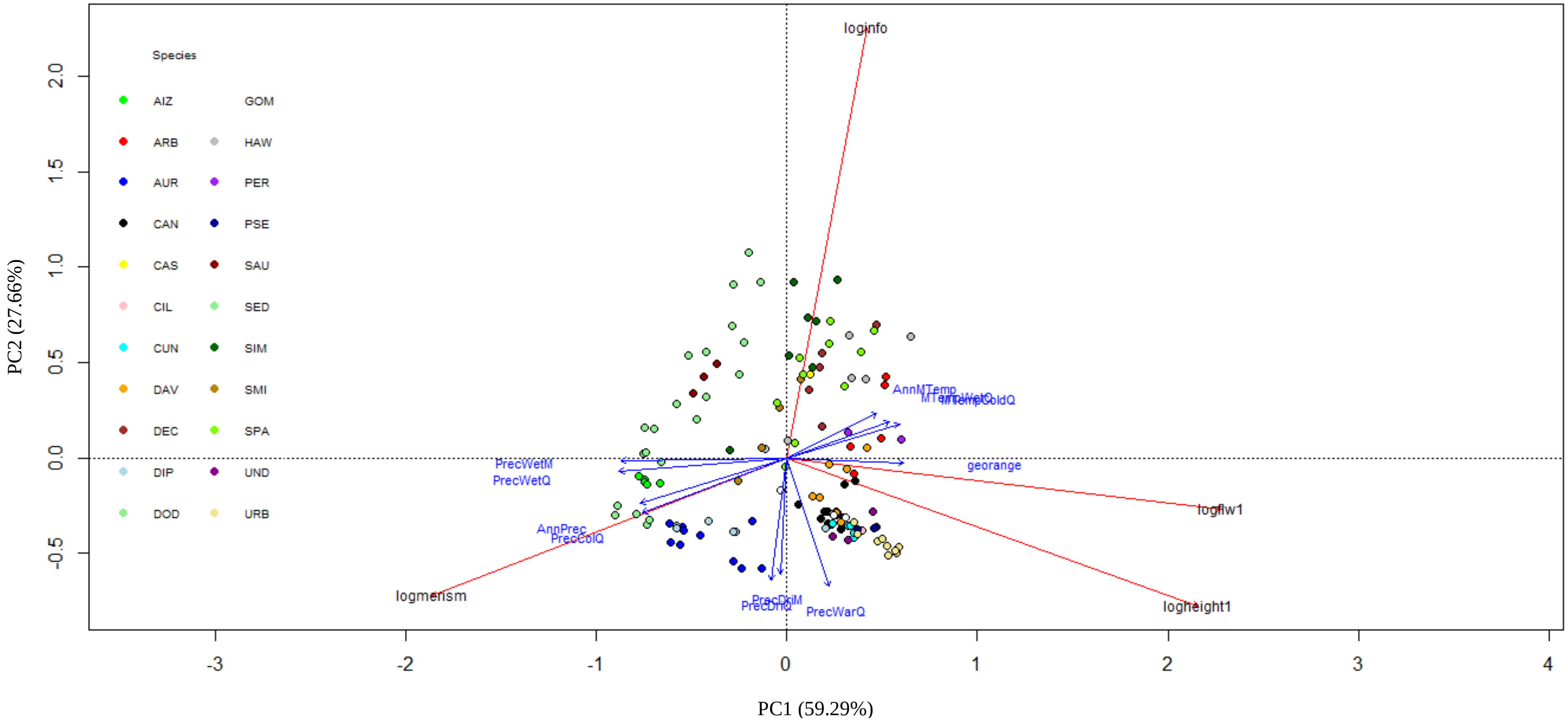
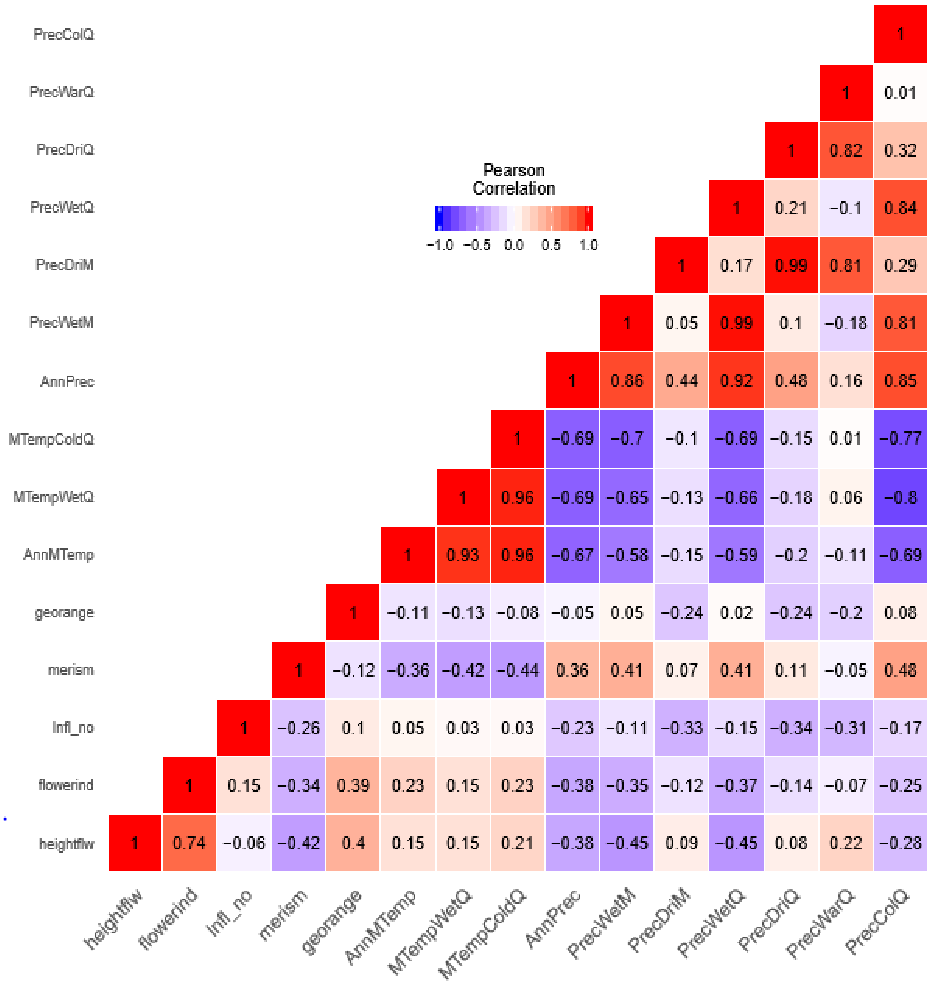


Figure 2.3. Principal component analysis based on the trait values measured and environmental data for the 22 *Aeonium* species. The colored dots (n = 132) represents the sampled *Aeonium* individual according to their morphological traits at logarithmic scale ($\log_{10}$). The red arrows show the contribution of each trait (number of inflorescences, inflorescence height, number of flowers per individual and merism) to explain the data patterns. The blue arrows show the fitted environmental vector for data explanation (p = 0.001). The arrow lengths show differences in variance explained relative to each other. See Appendix 1 and 2 for the description of traits and environmental variables, respectively. As shown, the variance of *Aeonium* species are explained by the first and second axes was 59.29% and 27.66%, respectively. See Appendix I and II for the description of traits and environmental variables, respectively. Species legend: AIZ: *Aeonium aizoon*; ARB: *Aeonium arboreum*; AUR: *Aeonium aureum*; CAN: *Aeonium canariense*; CAS: *Aeonium castellopaivae*: CIL: *Aeonium ciliatum*; CUN: *Aeonium cuneatum*; DAV: *Aeonium davidbramwellii*; DEC: *Aeonium decorum*; DIP: *Aeonium diplocyclum*; DOD: *Aeonium dodrantale*; GOM: *Aeonium gomerense*; HAW: *Aeonium haworthii*; PER: *Aeonium percarneum*; PSE: *Aeonium pseudourbicum*; SAU: *Aeonium saundersii*; SED: *Aeonium sedifolium*; SIM: *Aeonium simsii*; SMI: *Aeonium smithii*; SPA: *Aeonium spathulatum*; UND: *Aeonium undulatum*; URB: *Aeonium urbicum*;



	heightflw	flowerind	Infl_no	merism	georange	AnnMTemp	MTempWetQ	MTempColdQ	AnnPrec	PrecWetM	PrecDriM	PrecWetQ	PrecDriQ	PrecWarQ	PrecColQ
PrecColQ															1
PrecWarQ														1	0.01
PrecDriQ													1	0.82	0.32
PrecWetQ												1	0.21	-0.1	0.84
PrecDriM											1	0.17	0.99	0.81	0.29
PrecWetM										1	0.05	0.99	0.1	-0.18	0.81
AnnPrec									1	0.86	0.44	0.92	0.48	0.16	0.85
MTempColdQ								1	-0.69	-0.7	-0.1	-0.69	-0.15	0.01	-0.77
MTempWetQ							1	0.96	-0.69	-0.65	-0.13	-0.66	-0.18	0.06	-0.8
AnnMTemp						1	0.93	0.96	-0.67	-0.58	-0.15	-0.59	-0.2	-0.11	-0.69
georange					1	-0.11	-0.13	-0.08	-0.05	0.05	-0.24	0.02	-0.24	-0.2	0.08
merism				1	-0.12	-0.36	-0.42	-0.44	0.36	0.41	0.07	0.41	0.11	-0.05	0.48
Infl_no			1	-0.26	0.1	0.05	0.03	0.03	-0.23	-0.11	-0.33	-0.15	-0.34	-0.31	-0.17
flowerind		1	0.15	-0.34	0.39	0.23	0.15	0.23	-0.38	-0.35	-0.12	-0.37	-0.14	-0.07	-0.25
heightflw	1	0.74	-0.06	-0.42	0.4	0.15	0.15	0.21	-0.38	-0.45	0.09	-0.45	0.08	0.22	-0.28

Figure 2.4. Visualization of the Pearson correlation matrix of plot-level trait means of all 4 traits (maximum inflorescence height, number of flowers per individual, number of inflorescence per individual and merism) in the entire dataset (n = 132 individuals) with 11 environmental variables (geographic range size, annual mean temperature, mean temperature of wettest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation of warmest quarter, and precipitation of coldest quarter). Positive correlations are shown in red and negative ones in blue color. As the correlation value moves away from absolute 0 the color intensity increases.

2.3.2. *Cross-species relationships between inflorescence height, number of flowers and geographic range size*

Boxplots shown in **Figure 2.5.A – B** reveals the variation within the species of the number of flowers and maximum inflorescence height. Relating to number of flowers per inflorescence (n = 132; median = 648; range = [6 – 13920]**; Figure 2.5.A**), the species with the greatest number includes the subshrubs *Aeonium arboreum* (6206.90 ± 46322.61 cm), *Aeonium davidbramwellii* (2820.25 ± 2741.51 cm), *Aeonium percarneum* (5652.75 ± 4730.19 cm), and the monocarpic rosette *Aeonium urbicum* (7365.89 ± 2713.52 cm). According to this species, it can be seen a great interspecific variation across sampled individuals. The species with a small number of flowers include the branching rosettes *Aeonium aizoon* (20.00 ± 5.24 cm), *Aeonium dodrantale* (22.30 ± 6.31 cm), the subshrubs *Aeonium saundersii* (46.00 ± 21.63 cm) and *Aeonium sedifolium* (53.97 ± 46.05 cm). These species are consistent in terms of number of flowers and, according to **Figure 2.3.** their merism is relatively bigger than other species with more flowers.

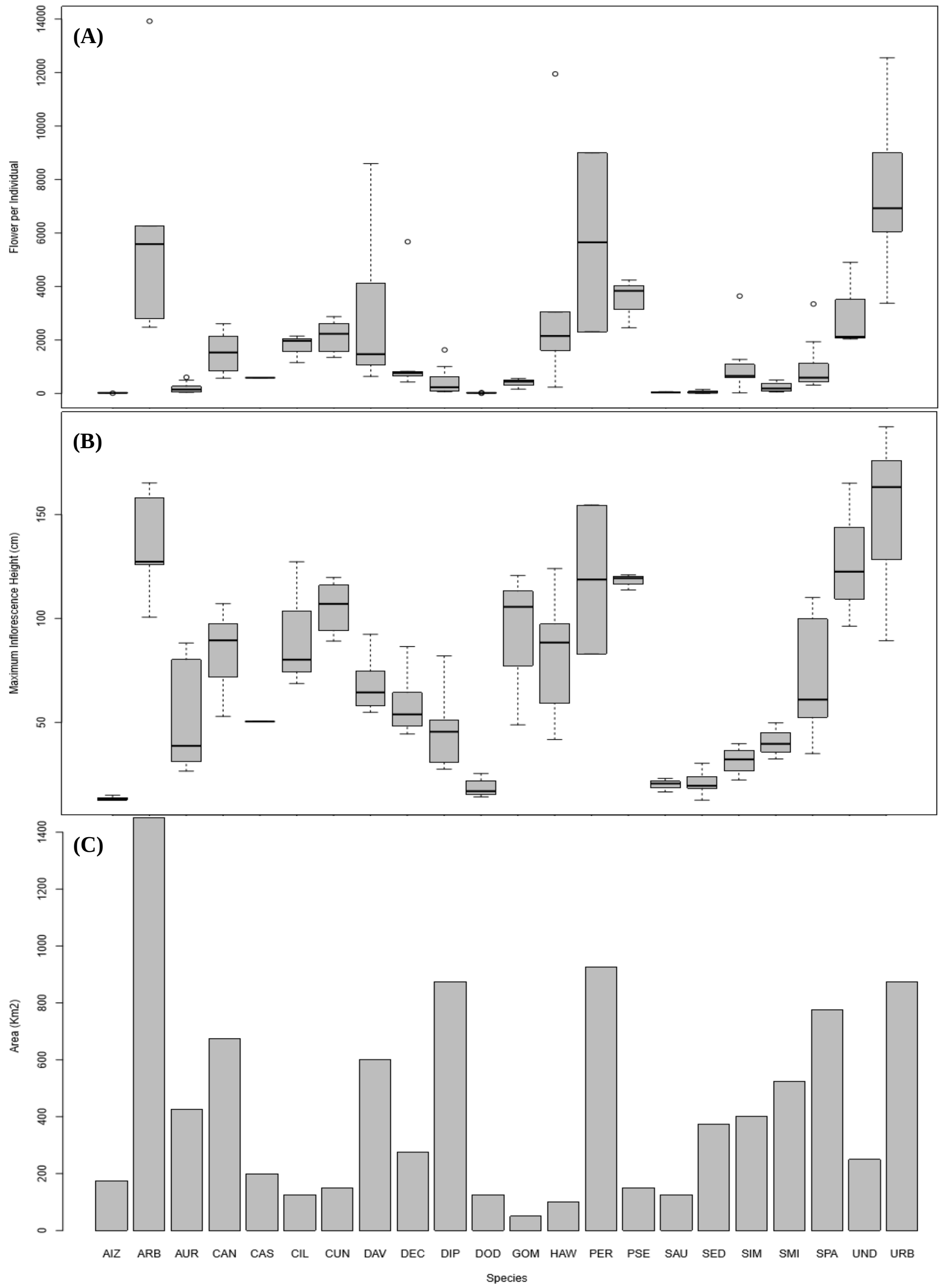


Figure 2.5. Boxplot and bar plot diagrams showing the intraspecific and interspecific variation across 22 Aeonium species in relation to: (A) Number of flowers per individual (n = 132; median = 648; range = [6 – 13920]); **(B)** Maximum inflorescence height (cm) (n = 132; median = 59.05; range = [12.60 – 192.20])**.** The grey box represents the 25th, 50th (median) and 75th percentiles, while whiskers represent 10th and 90th percentiles with minimum and maximum observations. The black dots represent the outliers; and **(C)** Geographic range size (km^2) (n=132; median = 400; range = [50 – 1450]). Species legend: AIZ: *Aeonium aizoon*; ARB: *Aeonium arboreum*; AUR: *Aeonium aureum*; CAN: *Aeonium canariense*; CAS: *Aeonium castellopaivae*: CIL: *Aeonium ciliatum*; CUN: *Aeonium cuneatum*; DAV: *Aeonium davidbramwellii*; DEC: *Aeonium decorum*; DIP: *Aeonium diplocyclum*; DOD: *Aeonium dodrantale*; GOM: *Aeonium gomerense*; HAW: *Aeonium haworthii*; PER: *Aeonium percarneum*; PSE: *Aeonium pseudourbicum*; SAU: *Aeonium saundersii*; SED: *Aeonium sedifolium*; SIM: *Aeonium simsii*; SMI: *Aeonium smithii*; SPA: *Aeonium spathulatum*; UND: *Aeonium undulatum*; URB: *Aeonium urbicum*.

For maximum inflorescence height (cm) (n = 132; median = 59.05; range = [12.60 – 192.20]; **Figure 2.5.B**), it is show that higher species include the subshrubs *Aeonium arboreum* (135.41 ± 26.26 cm), *Aeonium percarneum* (118.73 ± 50.66 cm), and *Aeonium undulatum* (127.97 ± 34.72 cm), and the monocarpic rosette *Aeonium urbicum* (148.68 ± 37.24 cm) with a broadly variation along of each species. The shorter species include, as seen for number of flowers, the branching rosettes *Aeonium aizoon* (13.44 ± 0.96 cm) and *Aeonium dodrantale* (18.20 ± 3.79 cm), and the subshrubs *Aeonium saundersii* (20.08 ± 3.30 cm), and *Aeonium sedifolium* (20.77 ± 5.08 cm) with a narrow variation range observed for each species.

In relation to geographic range size (km^2) (n=132; median = 400; range = [50 – 1450]; **Figure 2.5C**), the species with a greater area of occurrence are *Aeonium arboreum* (1450 km^2), *Aeonium diplocyclum* (925 km^2), *Aeonium percarneum* (925 km^2), *Aeonium spathulatum* (775 km^2), and *Aeonium urbicum* (825 km^2). Species with a shorter area of distribution includes: *Aeonium aizoon* (175 km^2), *Aeonium ciliatum* (125 km^2), *Aeonium cuneatum* (150 km^2), *Aeonium dodrantale* (125 km^2), *Aeonium gomerense* (50 km^2), *Aeonium haworthii* (100 km^2), *Aeonium pseudourbicum* (150 km^2), and *Aeonium saundersii* (125 km^2). Altogether, species with more flowers per individual tend to be taller and more prone to have a large geographic range size of distribution.

Based on **Figure 2.5.** and identical analyses on subsets of the full data (n=132 individuals), the species without relationship between inflorescence height and geographic range size was removed from dataset to improve the correlation models. This reduction of dataset by removing the 6 specialists' species with restricted habitat (i.e., *Aeonium ciliatum*, *Aeonium cuneatum*, *Aeonium gomerense*, *Aeonium haworthii*, and *Aeonium pseudourbicum*; n=22 individuals) increased the explanation for the correlation between inflorescence height and geographic range size, and between number of flowers per individual and geographic range size. The correlation between number of flowers per individual and inflorescence height was the same for 16 and 22 species.

As predicted, was found a significant positive relationship between maximum inflorescence height and the number of flowers per individual ($R^2 = 0.83$; $P < 0.001$; n= 110 individuals; **Figure 2.6.A**). Thus, species with taller inflorescences had a greater number of flowers. There was a positive correlation between maximum inflorescence height and geographic range size ($R^2 = 0.52$; $P < 0.001$; n= 110 individuals; **Figure 2.6.B**). Species that have greater investment in taller inflorescences tend to have a great geographic range size than shorter species. It was also found a positive tendency between the number of flowers per individual and geographic range size ($R^2 = 0.45$; $P < 0.001$; n= 110 individuals; **Figure 2.6.C**). Species with more flowers tend to have a greater geographic range size, that is, a larger occurrence area.

2.4. Discussion

The current study englobes exclusive data of four reproductive traits for 22 *Aeonium* taxa endemic to Canary Islands (i.e., Gran Canaria, Tenerife, La Gomera and La Palma) including: *Aeonium aizoon*, *Aeonium arboreum* ssp. *holochrysum*, *Aeonium aureum*, *Aeonium canariense* ssp. *canariense*, *Aeonium canariense* ssp. *latifolium*, *Aeonium canariense* ssp. *virgineum*, *Aeonium castello-paivae*, *Aeonium ciliatum*, *Aeonium cuneatum*, *Aeonium davidbramwelli*, *Aeonium decorum*, *Aeonium diplocyclum*, *Aeonium dodrantale*, *Aeonium gomerense*, *Aeonium haworthii*, *Aeonium percarneum*, *Aeonium saundersii*, *Aeonium sedifolium*, *Aeonium simssii*, *Aeonium smithii*, *Aeonium undulatum*, and

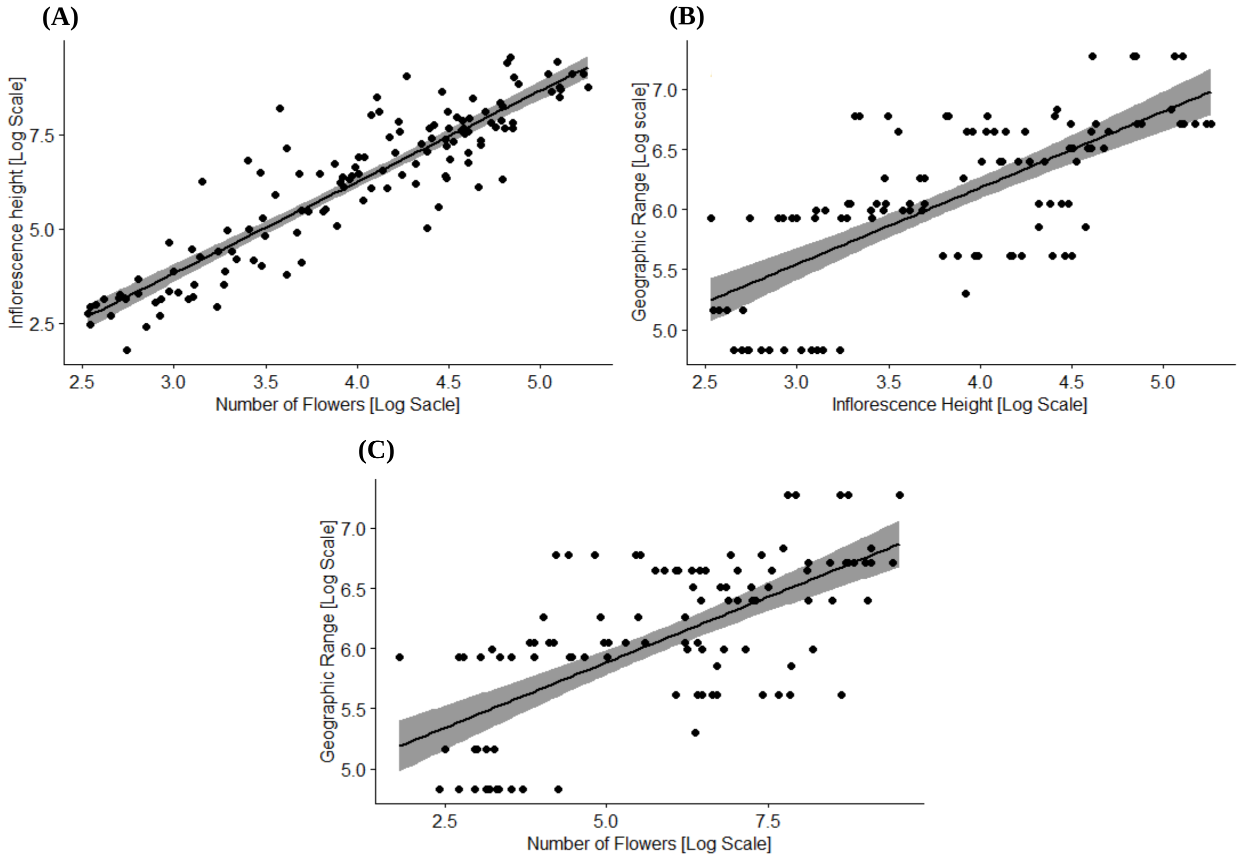


Figure 2.6. Relationships between $\log_{10}$ maximum inflorescence height (cm) and **(A)** Number of flowers (n = 110 individuals) and **(B)** geographic range (n = 110 individuals), and between **(C)** number of flowers and geographic range size (n = 110 individuals). Solid lines represent the linear regression models for inflorescence height and number of flowers, and inflorescence height and geographic range models. Grey shading indicates the 95% confidence interval. Black dots represent each sampled individual for 16 *Aeonium* species (species that have no relationship between inflorescence height and geographical range was removed; **See Appendix IV**).

Aeonium urbicum **(e.g., Figure 2.7.)**. These taxa include widespread species called as generalists and habitat-restricted species as specialists.

Seed dispersal in *Aeonium* genus and, also insular systems, is still poorly understood, however, it is known that seed is a very conserved trait within the whole genus. Seeds have a uniform size and mass, are light and without adaptations to any kind of dispersal agents **(Lems, 1960; Liu, 1989)**. Thus, it is supposed that dispersal is performed by gravity with some occasional help of the wind **(Harter *et al.*, 2015)**. Taking account the above mentioned, it was explored a possible use of inflorescence height as a proxy to explain the *Aeonium* species distribution range. Additionally, the differentiation among species according to their reproductive traits was studied, taking account that all the species were originated within insular conditions. The observed trait differences reflect their evolutionary history and an adaptation to environments in which they are currently occurring.

Trait correlations at the species level were relatively well captured by the first two axes of a principal component analysis (PCA). The PCA of plot – level trait means **(Figure 2.3.)** reflects three main functional continua on which species trait values converge: one from great merism, few numbered of flowers species and inflorescences; other for species with more inflorescences per individual, mainly characterized by subshrubs; and the last one from taller species with a great number of flowers, mainly being species of generalist distributions across the islands, that is, endowed with a large geographic range size. Specifically, the first continuum shows that *Aeonium* species with a great merism, mainly belonging to the distinct *Greenovia* section **(Bañares, 2015)**, have a bigger number of carpels per flower than remaining species. In this sense, once having a low number of flowers combined with a high merism, the number of seeds per individual increase. It is shown a trade-off between merism and number of flowers. **(Figure 2.4.)**. On the other continuum, species with more inflorescences per individual are mainly subshrubs and flowering several times per life cycle, being polycarpic species. Finally, the third continuum, being the most relevant in the context of this work, presents species with taller inflorescences and a greater number of flowers. In this way, it appears the species with the ability to disperse to further distances and, therefore, endowed with larger geographic range sizes. As shown in **Figures 2.3.** and **Figure 2.4.**, despite weak, the number of flowers is negatively correlated with precipitation variables and positively with temperature variables. *Aeonium* species with more flowers per individual are monocarpic species canalizing all energy to flowering. According to **Jorgensen & Olesen (2001)**, *Aeonium* monocarpic species are associated with arid and disturbed sites, as lava flows or abandoned fields. As it possible to see in **Figure 2.5.A**, the monocarpic *Aeonium urbicum* has the greatest number of flowers per individual. It can be assumed this trait as a colonizer strategy getting considerable extensions of geographic area.

According to the present results, the most important finding is that inflorescence height is a good predictor of *Aeonium* species distribution across the Canary Islands. Species with taller inflorescences have, on average, larger geographic range sizes **(Figure 2.6.B)**, that is, it can be assumed that taller species have large dispersal distances. Accounting this clear positive relationship, the correspondent model presented in **Figure 2.6.B** was improved by removing the habitat – restricted species to laurel forest (R^2 increased from 0.09 to 0.52; **See Appendix IX**). These species have very restricted occurrence areas due to specific adaptations to this kind of habitat **(Bañares, 2015)** and this can explain the fact that they do not respond to the mentioned relationship. Coherent to these findings, **Thomson *et al.* (2011; 2017)** shows that plant height is a better proxy to assess dispersal distances than seed mass.

As expected, it was found a very strong positive relationship between number of flowers and inflorescence height. Taller inflorescences have a great number of flowers and supposedly produce more seeds **(Figure 2.6.A)**. Thus, plants with more flowers per individual tend to have also a large geographic range size **(Figure 2.6.C)**. Based on **Moles & Leishman (2008)**, plant height can be a predictor of the amount of annual seed production, that is, taller species tend to produce more seeds per year, and tall species have longer life-cycles than shorter ones. This trait also improves the abilities to capture light **(Falster & Westoby, 2005)** and, therefore, great photosynthetic rates and energy are provided. In addition, taller species, in this case, taller inflorescences, seeds are released from great heights, increasing the chances of a greater dispersal distance **(Travis *et al.*, 2010)**. Contrarily to the present results, **Bossuyt & Honnay (2006)** suggest that species with long life-cycles require lesser dispersal capacity investment that short-lived species to avoid extinction.

In the insular context, species tend to lose their dispersal abilities by reducing dispersal investment. However, these kinds of species evolve taller woody growth forms **(e.g., Carlquist, 1974)**. Taking into account the present results and being easy to assess in the field, inflorescence height from the ground or plant maximum height as described by **Thomson *et al.* (2011)**, might be the best surrogate of dispersal distance in *Aeonium* genus attain larger geographic range sizes. Nevertheless, further work is still needed increasing the sampling for the whole genus and for more individuals per taxa. Future directions for research include the counting of the number of seeds per flowers to relate the inflorescence height and seed productions. Presumably taller inflorescences produce a greater amount of seeds than shorter ones.

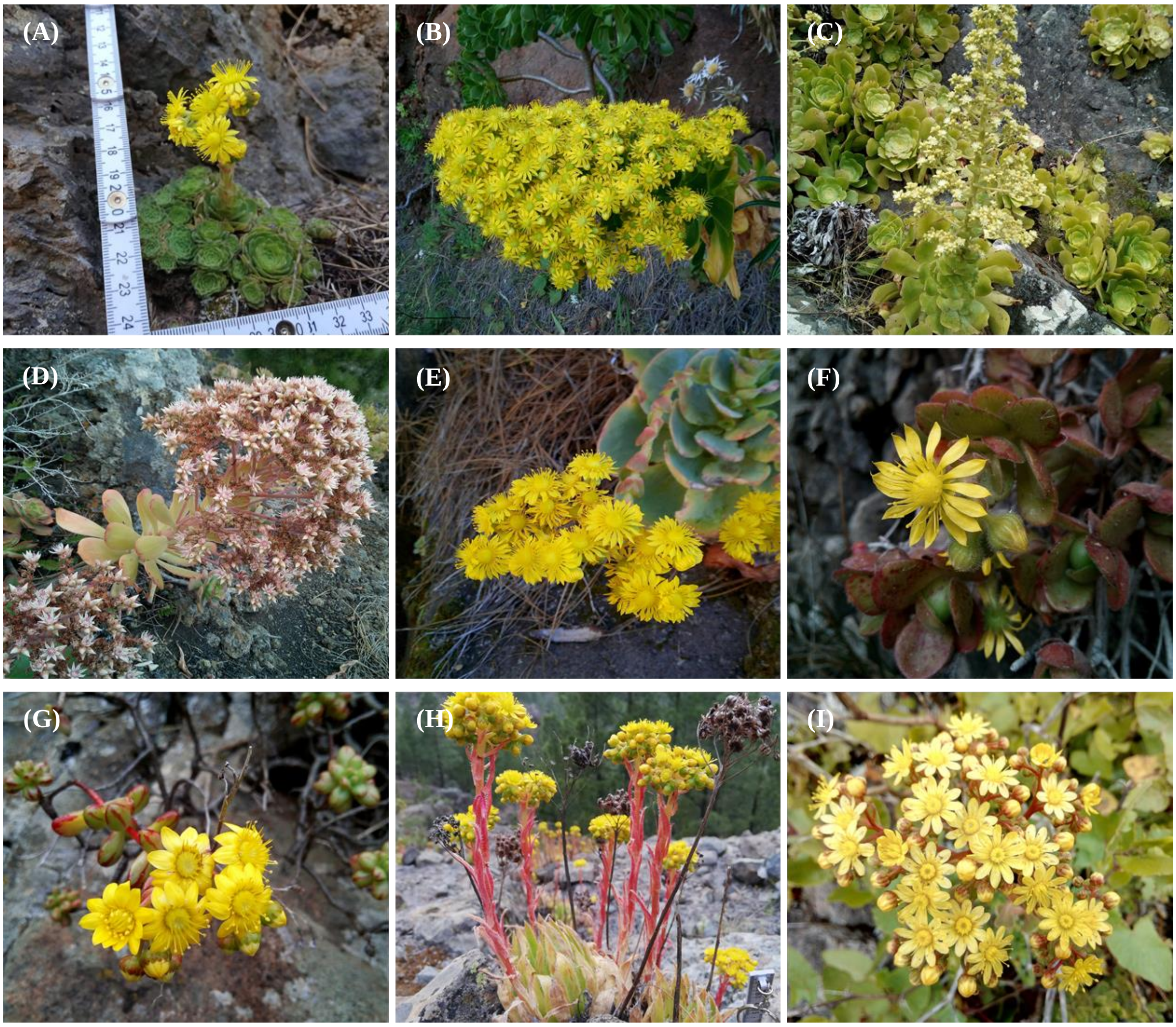


Figure 2.7. Examples of *Aeonium* inflorescences. **(A)** *Aeonium aizoon*; **(B)** *Aeonium arboreum* subsp. *holochrysum*; **(C)** *Aeonium canariense* subsp. *canariense*; **(D)** *Aeonium davidbramwelii*; **(E)** *Aeonium diplocyclum*; **(F)** *Aeonium saundersii*; **(G)** *Aeonium sedifolium*; **(H)** *Aeonium simsii*; **(I)** *Aeonium spathulatum*. (Photos: **(A)** Patrícia dos Santos; Tenerife, June 2019; **(B)** Miguel Brilhante; Tenerife, February 2019**; (C)** Miguel Brilhante; Tenerife, June 2019; **(D - E)** Miguel Brilhante; La Palma, April 2019**; (F)** Miguel Brilhante; La Gomera, May 2019; **(H)** Patrícia dos Santos, Gran Canaria, April 2019**; (I)** Miguel Brilhante; Tenerife, April 2019).

Chapter 3

The biggest plant radiation of Macaronesia – *Aeonium*: positive correlations between genome size variation and their conservation status

Abstract

Aeonium genus consists of nearby 41 endemic species to Macaronesia that occupy diverse habitats types and vary in vegetative and reproductive morphology. In the current study, it was generated a comprehensive database of nuclear DNA content estimation for 27 *Aeonium* taxa using flow cytometric approaches. The variability across the genus was examined and a relationship between 2C – values and conservation status performed. Results demonstrated that, overall, *Aeonium* species tend to have small genomes, ranged from 0.98 pg (*A. dodrantale*) to 2.54 pg (*A. gorgoneum*). Subshrubs species have, on average, large genome size. Comparative analysis across the genus revealed a positive correlation between 2C-values and conservation status, owning endangered species, on average, large genomes. It was also found a positive association between genome sizes and annual mean temperature. The data presented here, and correspondent analysis contribute to new insight to understand the diversification patterns of the best speciose radiation of Macaronesia.

Keywords: Cytogenomics; endemism; genome size; oceanic islands; threatened species;

3.1.Introduction

Macaronesian Region belongs to the Mediterranean Basin Hotspot, which comprises one of the 36 worldwide biodiversity hotspots **(Myers *et al.*, 2000** and updates at **http://www.biodiversityhotspots.org)**. Macaronesia englobes five volcanic archipelagos (i.e., Azores, Madeira, Selvages, Canaries, and Cabo Verde) and is located in the eastern Atlantic Ocean on both sides of the Tropic of Cancer **(Figure 1.1.)**. Due to its geomorphological context, that is, the proximity to mainland (100 km from Fuerteventura to Morocco), the broad range of altitudes (3718 m in Tenerife) and ages (from approximately 0.7 My in El Hierro to 20.7 My in Fuerteventura), these oceanic islands have a great number of habitat types with contrasting ecological conditions **(Carracedo *et al.*, 2002; Juan *et al.*, 2000)**. They were never connected to the mainland and were colonized by long-distance dispersal **(Vargas, 2007)**. This contributes to an enormous floristic diversity, high level of endemic taxa (ca. 900 taxa), and a great prevalence of woodiness (whereas the continental congeners are annual or biannual herbs) **(Bramwell, 1976; Caujapé-Castells *et al.*, 2010)**, highlighting several large evolutionary radiations (e.g., *Aeonium* alliance [4 genera, ca. 61 species], *Echium* [27 species], *Sideritis* [subgenus *Marrubiastrum*, 23 species], *Crambe* [section *Dendrocrambe*, 14 species] and *Sonchus* alliance [6 genera, ca. 31 species]) **(Kim *et al.*, 2008)**.

The biodiversity of Macaronesian islands has become one of the most threatened (e.g., 41% of Canaries' and 27% of Madeira's flora are endangered; **Médail & Quézel, 1997**) due to loss or habitat alterations caused by the restricted geographical and threats that are subjected (e.g. human impact, invasive species, overexploitation of resources, climate change). The combination of the above-mentioned with the high endemicity index makes this region very important in terms of conservation **(Caujapé-Castells *et al.*, 2010; Whittaker & Fernández-Palacios, 2007)**.

Genome size has been widely used as a term to indicate different things (e.g., amount of DNA in an unreplicated monoploid chromosome set (n) or in a polyploid nucleus where the DNA has been replicated) in the field of cytogenetic. In order to stabilize this concept, **Greilhuber *et al.* 2005**, created the following terminology: C-value or 'Holoploid genome size' refers to the amount of DNA in the whole chromosome complement of the nucleus with *n* chromosomes, regardless of the degree of generative polyploidy. On the other hand, the Cx-value or 'Monoploid genome size' represents the quantity of DNA in one chromosome set of an organism. The use of genome size data is an approach in active growth for several research areas, like taxonomy, systematics, ecology, and evolutionary biology **(Kron *et al.*, 2007)**; for example, was found a correlation between seed weight **(Chung *et al.*, 1998)**, plant phenology, sensitivity to frost, mean temperature and genome size **(Grime & Mowforth, 1982; Grime *et al.*, 1985; MacGillivray & Grime 1995; Suda *et al.*, 2003; 2005)**.

In recent years, the available data comprises estimates for 7058 angiosperms **(Leitch *et al.*, 2019)**. Across the group of angiosperms, the size of the nuclear genome varies greatly **(e.g., Pellicer *et al.*, 2018)**, ranging from 1C = 0.065 pg in *Genlisea aurea* (Lentibulariaceae; **Greilhuber *et al.*, 2006**) to 1C=152.23 pg in *Paris japonica* (Melanthiaceae; **Pellicer *et al.*, 2010**). This variation is reported to be caused predominantly through polyploidy and amplification of non-coding repetitive DNA, especially retrotransposons. These expansion processes are compensated by mechanisms that result in a decrease in genome size. Despite this is poorly understood, it is supposed that the latter is based on recombinational phenomena, such as homologous or illegitimate recombination **(Grover & Wendel, 2010; Pellicer *et al*, 2018; Qiu *et al.*, 2019)**.

When examining the genome size variation across Macaronesian endemic plants, it is clear the limited range of 1C-values, from 0.19 pg to 9.52 pg **(Leitch *et al.*, 2019; Suda *et al.*, 2003, 2005)**. Most of the species have a relatively small amount of DNA, therefore, DNA shrinkage might be evolutionarily advantageous in an insular context, taking into account the strong selective pressures acting in Macaronesia **(Suda *et al.*, 2005)**. Supposedly larger genomes are maladaptive due to the fact DNA replication is slow and consequently the cell cycle and the individual development; it is not favored by natural selection because it restricts plant growth and it is associated with small effective populations **(Bennet & Leitch, 2005, 2011; Knight *et al.*, 2005; Lynch & Conery, 2003)**. In this way, **Vinogradov (2003)** showed that threatened plants, with declining populations, own on average larger genomes and slower rates of diversification than their congeners. Then, the surplus of noncoding DNA seems to be clearly correlated with the likelihood of extinction.

Aeonium Webb. & Berthel. (Crassulaceae, Sempervivoideae), the most speciose Macaronesian radiation, with 44 insular taxa of which 41 on the Canary Islands, 2 on Madeira, and 1 on Cabo Verde, the remaining species occur in Morocco (1 species) and East Africa (2 species) **(Table 1.1.)**. *Aeonium* comprises a perennial succulent and rosette-forming genus endowed with a huge morphological **(Figure 3.1.)**, ecological and physiological variation among the several species **(Bañares, 2015; Jorgensen & Olesen, 2001; Liu, 1989).** Cytogenomic studies of this genus are restricted to 14 species, for which it was estimated the genome size (C-value) by the flow cytometry **(Hanson *et al*., 2001; Suda *et al.*, 2005; Zonneveld *et al.*, 2005)**. According to this data, the C-values across *Aeonium* species vary from 0.51 pg (*Aeonium ciliatum*; **Suda *et al.*, 2005**) to 4.25 pg (*Aeonium nobile*; **Zonneveld *et* al., 2005**), being the majority of the species have a very small C-value (defined as less or equal to 1.40 pg).

Having *Aeonium* genus as a study model, at first instance, the present study aims to increase the knowledge about the variation in genome size among angiosperms of Macaronesian Islands. The main goal of this study is to test whether the genome size variation is related to the conservation status of *Aeonium* genus. Thus, the central hypothesis of this work is: threatened *Aeonium* species have, on average, a larger genome than their congeners with small genome.

Figure 3.1. *Aeonium* morphological variation. (A) *Aeonium urbicum* var. *urbicum*; **(B)** *Aeonium dodrantale*; and **(C)** *Aeonium canariense* subsp. *canariense*; **(D)** *Aeonium cuneatum*; **(E)** *Aeonium nobile*; and **(C)** *Aeonium haworthii*. (Photos: **(A – F)** Miguel Brilhante; Tenerife, May 2019).

3.2.Methodology

3.2.1. *Plant material*

Sampling on Canaries, Madeira and Cabo Verde took place from February and June 2019. The origin of the samples of twenty-seven *Aeonium* species and subspecies studied in this work is presented in **Table 3.1.** Three fresh leaves per species, from different individuals, were collected from each location between the Canaries, Madeira, and Cabo Verde Islands. Herbarium vouchers are deposited in the Herbarium of Botanical Garden of the University of Lisbon (LISU), João de Carvalho e Vasconcellos

Herbarium of Superior Institute of Agronomy (LISI), Botanical Museum of the University of Mainz – Herbarium (MJG), and Herbarium of the University of Basel (BAS).

3.2.2. *Nuclear DNA content estimation*

Nuclear DNA content was estimated performing a propidium iodide flow cytometry (FCM) using three different standards in order to choose the most suitable: *Buxus sempervirens* L. (2C = 1.63 pg; **Talhinhas *et al.*, unpublished**), *Delairea odorata* Lem. (2C = 6.11 pg; **Talhinhas *et al.*, unpublished**), *Solanum lycopersicum* L. (2C = 1.96 pg; **Doležel *et al.*, 1992**) **(Table 3.2.)**. The fresh young leaves were chopped with a razor blade in a Petri dish containing 1 ml of Woody Plant Buffer (WPB 0,2 M Tris-HCl, 4 mM $MgCl_2$, 1% Triton X-100, Na_2EDTA 2 mM, NaCl 86 mM, sodium metabisulfite 20 mM, PVP-10 a 1%, pH 7,5; **Loureiro *et al.*, 2007**). A nuclear suspension obtained was filtered with 30 µm nylon filter and a solution 0,25 $\mu g/ml^{-1}$ of propidium iodide (PI; Sigma-Aldrich, EUA) was added for DNA staining ((by excitation with a green laser [532 nm]). The resulting mixture was analyzed in a flow cytometer CyFlow Space (Sysmex-Partec GmbH, Görlitz, Germany). The acquisition of numeric data and fluorescence graphs was made by Partec FloMax software v2.4d (Partec GmbH, Görlitz, Germany), according to **Tavares *et al.* (2014)**. From the analyzed sample, the diploid quantity of DNA in pg, per nucleus, was estimated using the formula:

$$\textbf{2C nuclear DNA content} = \frac{\textbf{Sample G1 peak mean} \times \textbf{Genome size of reference standart}}{\textbf{Reference standart G1 peak mean}}$$

The DNA content in number of base pairs was obtained by the following conversion factor: 1 pg = 978 Mbp **(Doležel & Bartoš, 2005).**

3.2.3. *Statistical analyses*

Statistical analyses were performed using R **(R Core Team, 2019)**. Descriptive statistics were calculated for each taxon studied namely, mean and standard deviation (SD) of the holoploid genome size (2C, pg). Normality was tested by applying a Shapiro-Wilk test with the function *shapiro.test* which revealed that genome size values and conservation status were not normally distributed. Comparisons among genome size values and conservation status were done with the Kruskal Wallis test (function *Kruskal.test*) and a subsequent *post-hoc* test after Dunn, using *dunn.test* function (package dunn.test; **Dinno & Dinno, 2017**). The significance level for all statistical tests was set to the 95% confidence interval.

To understand the relationship between genome size and locations, sections and growth – forms boxplots were done. Relationships between 2C – Values and annual mean temperature **(**CHELSA; **Karger *et al.*, 2017)** and geographic range size **(See Methods: Chapter 2)** were made by Spearman rank correlation coefficients with *ggscatter* function of GGPUBR package **(Kassambara, 2019)**.

Table 3.1. Taxa and locations of *Aeonium* Webb. & Berthel. populations studied.

Species	Locality
Aeonium aizoon (Bolle) T.H.M.Mes	Spain, Canary Islands: Tenerife, Corona Forestal Natural Park
Aeonium appendiculatum A. Bañares	Spain, Canary Islands: La Gomera, Imada
Aeonium arboreum ssp. *holochrysum* var. *holochrysum* (H.Y.Liu) Bañares	Spain, Canary Islands: Tenerife, Guimar
Aeonium arboreum ssp. *holochrysum* var. *rubrolineatum* (Svent.) H.Y.Liu	Spain, Canary Islands: La Gomera, Imada
Aeonium aureum (C.Sm. ex Hornem.) T.H.M.Mes	Spain, Canary Islands: Tenerife, Cañadas del Teide
Aeonium canariense ssp. *canariense* (L.) Webb & Berthel.	Spain, Canary Islands: Tenerife, Anaga Rural Park
Aeonium canariense ssp. *latifolium* (Burchard) Bañares	Spain, Canary Islands: La Gomera, Garojonay Nacional Park
Aeonium castello-paivae Bolle	Spain, Canary Islands: La Gomera, Hermígua
Aeonium ciliatum (Willd.) Webb & Berthel.	Spain, Canary Islands: Tenerife, Anaga Rural Park
Aeonium cuneatum Webb & Berthel.	Spain, Canary Islands: Tenerife, Anaga Rural Park
Aeonium decorum Webb ex Bolle	Spain, Canary Islands: La Gomera, La Laja
Aeonium dodrantale (Willd.) T.H.M.Mes	Spain, Canary Islands: Tenerife, Las Mercedes
Aeonium glandulosum (Aiton) Webb & Berthel.	Portugal, Madeira Archipelago: Madeira, Machico
Aeonium glutinosum (Aiton) Webb & Berthel.	Portugal, Madeira Archipelago: Madeira, São Vicente
Aeonium gomerense (Praeger) Praeger	Spain, Canary Islands: La Gomera, Garojonay Nacional Park
Aeonium gorgoneum J.A.Schmidt	Cabo Verde Archipelago: Santo Antão Island
Aeonium haworthii Salm – Dyck ex Webb & Berthel.	Spain, Canary Islands: Tenerife, Teno Rural Park
Aeonium lindleyi ssp. *lindleyi* Webb & Berthel.	Spain, Canary Islands: Tenerife, Anaga Rural Park
Aeonium lindleyi ssp. *viscatum* (Bolle) Bañares	Spain, Canary Islands: La Gomera, Hermígua
Aeonium saundersii Bolle	Spain, Canary Islands: La Gomera, La Laja
Aeonium sedifolium (Webb ex Bolle) Pit. & Proust.	Spain, Canary Islands: Tenerife, Barranco de Masca
Aeonium simsii (Sweet) Stearn	Spain, Canary Islands: Gran Canaria, Tejeda
Aeonium smithii (Sims) Webb & Berthel.	Spain, Canary Islands: Tenerife, Corona Forestal Natural Park
Aeonium spathulatum (Hornem.) Praeger	Spain, Canary Islands: Tenerife, Cañadas del Teide
Aeonium tabuliforme (Haw.) Webb & Berthel.	Spain, Canary Islands: Tenerife, Taganana
Aeonium urbicum (C.Sm. ex Hornem.) Webb & Berthel.	Spain, Canary Islands: Tenerife, Las Mercedes
Aeonium volkerii E.Hern. & Bañares	Spain, Canary Islands: Tenerife, Chamorga

3.3.Results

3.3.1. *Genome size variation across Aeonium species*

A total of 24 samples are collected in Canaries (15 in Tenerife, eight in La Gomera, and one in Gran Canaria), two in Madeira and one in Cabo Verde. It was possible to analyze the genome size variation for these species. After all the analytical and bioinformatics procedures, of the final dataset of 27 samples **(Table 3.2.)** 11 were previously analyzed by **Suda *et al.* (2005)**.

In **Table 3.2.** it is shown the means of 2C-value with standard deviation (SD), 1C-value in picograms and megabasepairs, sample coefficient of variation (CV), standard coefficient of variation (CV), internal standard used, growth-form, section and conservation status for 27 *Aeonium* taxa (including species and subspecies) from Macaronesia. All the sampled species/subspecies are endemic to the Macaronesian Region, more specifically, 24 are endemic to Canary Islands (which of 20 are single island endemics), two to Madeira, and one to Cabo Verde.

Genome size determinations based on flow-cytometric techniques produced histograms of fluorescence peaks with the coefficient of variation (CV) values ranged from 1.95% to 10.52% (mean 3.59%) for analyzed *Aeonium* taxa, and 3.17% to 7.18% (mean 4.84%) for internal standards. **Figure 3.2.** illustrates flow histograms presenting final genome estimation. According to **Tavares *et al*. (2014)**, in order to eliminate partial nuclei and other types of debris, polygonal regions in dot-plots of SSC (side light scatter) vs. FL (fluorescence pulse integral in linear scale) was done to present more consistent nuclei in terms of size and shape. This approach improved CV values of DNA fluorescence peaks and, consequently, the quality of histograms yielded for the analyses of genome sizes.

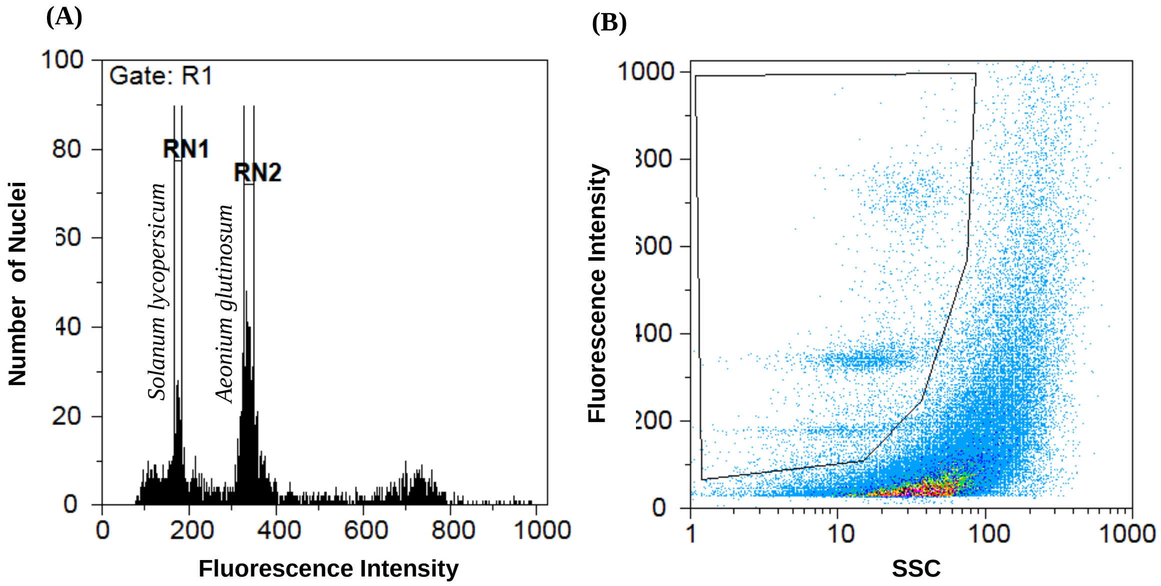


Figure 3.2. Flow cytometry outputs: (A) Flow cytometric histogram of relative fluorescence intensities from propidium iodide-stained *Aeonium glutinosum* (Aiton) Webb & Berthel nuclei using *Solanum lycopersicum* L. (2C-value = 1.96 pg DNA) as an internal reference standard. **(B)** Dot-plot of SSC vs. FL presenting the gating made to exclude as much as possible partial nuclei and other types of debris.

The mean 2C nuclear DNA content for the *Aeonium* sampled species/subspecies set are ranged from 0.98 pg in *Aeonium dodrantale* (Tenerife) to 2.54 pg in *Aeonium gorgoneum* (Cabo Verde). The genome size of *Aeonium* species is quite uniform (mean 1.36 ± 0.436 pg**)** with some exceptions as *Aeonium gorgoneum* **(Figure 3.3.(A – C))**, *Aeonium haworthii*, *Aeonium simsii*, *Aeonium volkerii*, and Aeonium gomerense **(Figure 3.3.(D – F)).** According to C-values size classes defined by **Leitch *et al.* (1998)**, all the species are endowed with a very small 1C-values (less than 1.40 pg). The medium and big genomes are totally absent within this genus.

In the present study, it is presented the genome size estimations for 27 *Aeonium* species, of which 11 had been previously evaluated: *Aeonium arboreum* ssp. *holochrysum* var. *holochrysum; Aeonium canariense* ssp. *canariense; Aeonium ciliatum; Aeonium haworthii; Aeonium lindleyi* ssp. *lindleyi; Aeonium sedifolium; Aeonium simsii; Aeonium smithii; Aeonium spathulatum; Aeonium tabuliforme* and *Aeonium urbicum.* From these 11 former estimations, only two are not consistent with the present results, more exactly for *Aeonium haworthii* and *Aeonium simsii*. Interestingly, according to both results, they are of the species with the largest genomes in the Canary archipelago.

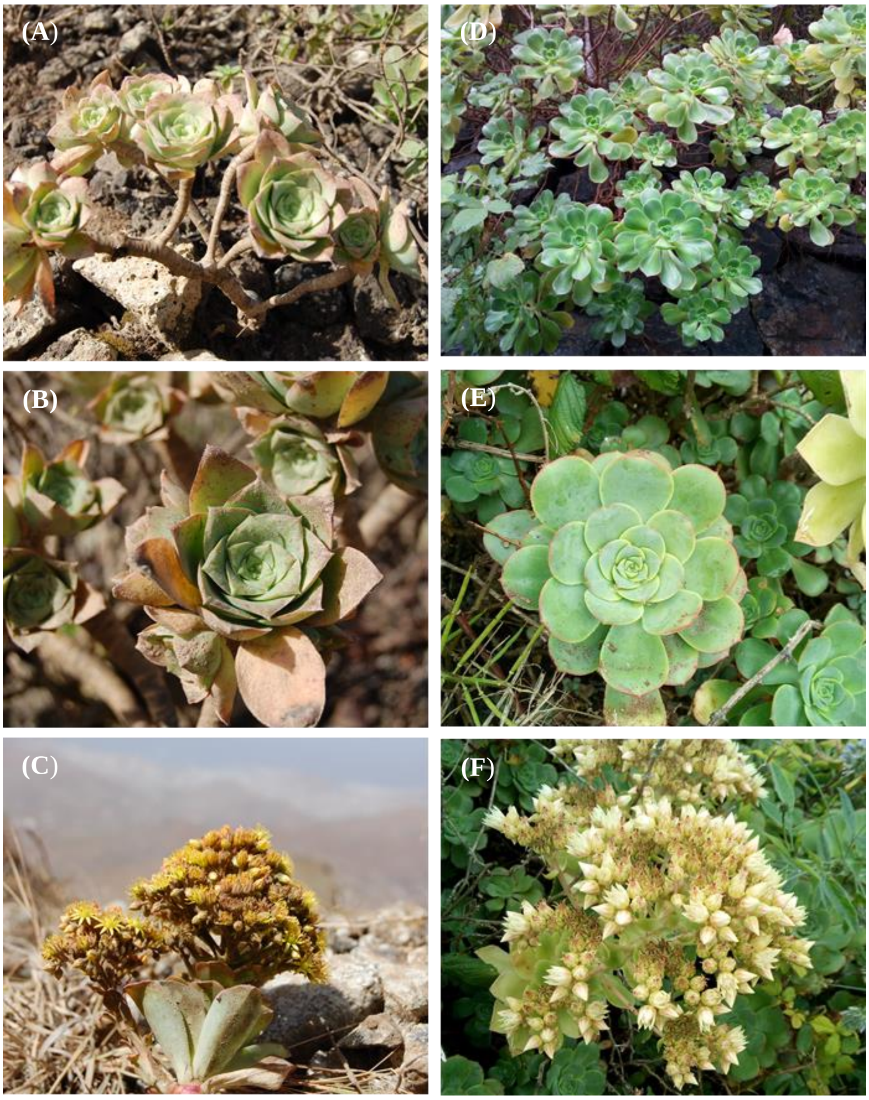


Figure 3.3. The most threatened *Aeonium* species. *Aeonium gorgoneum*: **(A)** Habit; **(B)** Rosette; and **(C)** Inflorescence; *Aeonium gomerense*: **(D)** Habit; **(E)** Rosette and **(C)** Inflorescence. (Photos: **(A – C)** Maria Romeiras; São Antão, October 2016; **(D - F)** Miguel Brilhante; La Gomera, May 2019)

Table 3.2. 2C-value with standard deviation (SD), 1C-value in picograms and megabasepairs, sample coefficient of variation, standard coefficient of variation, internal standard used, growth-form, section and conservation status of 27 *Aeonium* (Crassulaceae) species from Macaronesia.

Species	2C-Value ± SD (pg)	1C-Value (pg)	1C-Value (Mbp[1])	Sample CV (%)	Standard CV (%)	Previous 2C – Value data (pg)	Internal Standard[2]	Growth-form[3,4]	Section[5]	Conservation Status[6,7]
Aeonium aizoon (Bolle) T.H.M.Mes	1.06 ± 0.002	0.53	515	4.09	2.87		BS	BR	*Greenovia*	VU
Aeonium appendiculatum Bañares	1.09 ± 0.037	0.55	534	6.82	5.71		BS	MR	*Leuconium*	NE
Aeonium arboreum ssp. *holochrysum* var. *holochrysum* (H.Y.Liu) Bañares	1.37 ± 0.049	0.68	667	5.02	4.46	1.22[a]	SL	SS	*Aeonium*	NE
Aeonium arboreum ssp. *holochrysum* var. *rubrolineatum* (Svent.) H.Y.Liu	1.26 ± 0.013	0.63	617	5.07	3.52		SL	SS	*Aeonium*	NE
Aeonium aureum (C.Sm. ex Hornem.) T.H.M.Mes	0.99 ± 0.012	0.50	484	4.27	3.62		BS	BR	*Greenovia*	NE
Aeonium canariense subsp. *canariense* (L.) Webb & Berthel.	1.15 ± 0.015	0.57	560	4.80	3.52	1.08[a]	BS	BR	*Canariensia*	NE
Aeonium canariense subsp. *latifolium* (Burchard) Bañares	1.23 ± 0.003	0.61	599	5.76	4.15		BS	BR	*Canariensia*	NE
Aeonium castello-paivae Bolle	1.12 ± 0.012	0.56	545	5.64	4.79		BS	SS	*Leuconium*	NE
Aeonium ciliatum (Willd.) Webb & Berthel.	1.07 ± 0.021	0.53	521	6.08	4.79	1.02[a]	BS	SS	*Leuconium*	NE
Aeonium cuneatum Webb & Berthel.	1.10 ± 0.009	0.55	539	5.82	3.85		BS	BR	*Canariensia*	VU
Aeonium decorum Webb ex Bolle	1.11 ± 0.007	0.56	543	4.71	4.08		BS	SS	*Leuconium*	NE
Aeonium dodrantale (Willd.) T.H.M.Mes	0.98 ± 0.014	0.49	480	5.68	3.93		SL	BR	*Greenovia*	VU
Aeonium glandulosum (Aiton) Webb & Berthel.	1.08 ± 0.023	0.54	527	4.01	3.72		SL	MR	*Canariensia*	NE

Table 3.2. *(Continued)*

Aeonium glutinosum (Aiton) Webb & Berthel.	1.07 ± 0.033	0.53	520	5.70	1.81		SL	SS	*Petrothamnium*	NE
Aeonium gomerense (Praeger) Praeger	1.60 ± 0.026	0.80	782	4.33	4.43		SL	SS	*Leuconium*	EN
Aeonium gorgoneum J.A.Schmidt	2.54 ± 0.024	1.27	1238	4.36	4.48		SL	SS	*Aeonium*	EN
Aeonium haworthii Webb & Berthel.	2.18 ± 0.058	1.09	1063	3.65	4.22	1.55[b]	BS	SS	*Leuconium*	VU
Aeonium lindleyi subsp. *lindleyi* Webb & Berthel.	1.37 ± 0.005	0.69	670	3.87	3.99	1.36[a]	BS	SS	*Goochiae*	NE
Aeonium lindleyi subsp. *viscatum* (Bolle) Bañares	1.45 ± 0.009	0.73	710	3.85	2.76		SL	SS	*Goochiae*	NE
Aeonium saundersii Bolle	1.20 ± 0.019	0.60	584	4.04	3.66		BS	SS	*Petrothamnium*	VU
Aeonium sedifolium (Webb ex Bolle) Pit. & Proust.	1.32 ± 0.014	0.66	644	3.34	2.06	1.35[a]	SL	SS	*Petrothamnium*	NE
Aeonium simsii (Sweet) Stearn	2.36 ± 0.093	1.18	1150	4.80	3.20	4.33[c]	DO	BR	*Aeonium*	NE
Aeonium smithii (Sims) Webb & Berthel.	1.14 ± 0.024	0.57	558	4.17	3.27	1.03[a]	SL	SS	*Chrysocome*	NE
Aeonium spathulatum (Hornem.) Praeger	1.24 ± 0.013	0.62	604	4.52	3.26	1.10[a]	BS	SS	*Chrysocome*	NE
Aeonium tabuliforme (Haw.) Webb & Berthel.	1.14 ± 0.013	0.57	558	4.47	3.84	1.12[a]	BS	MR	*Canariensia*	NE
Aeonium urbicum Bañares	1.14 ± 0.016	0.57	557	6.69	3.83	1.06[a]	BS	MR	*Leuconium*	NE
Aeonium volkerii E.Hern. & Bañares	2.19 ± 0.025	1.10	1069	3.54	3.72		BS	SS	*Leuconium*	VU

Notes:

[1] 1 pg = 978 Mbp;

[2] BS: *Buxus sempervirens* L.; DO: *Delairea odorata* Lem.; SL: *Solanum lycopersicum* L;

[3] All the species are perennial

[4] SS: Subshrub; BR: Branching rosette; MR: Single monocarpic rosette;

[5] Bañares (2015);

[6] Bañares (2015); IUCN (2019);

[7] NE: Not Evaluated; VU: Vulnerable; EN: Endangered.

[a] Suda et *al.* (2005); [b] Hanson et *al.* (2001); [c] Zonneveld et *al.* (2005);

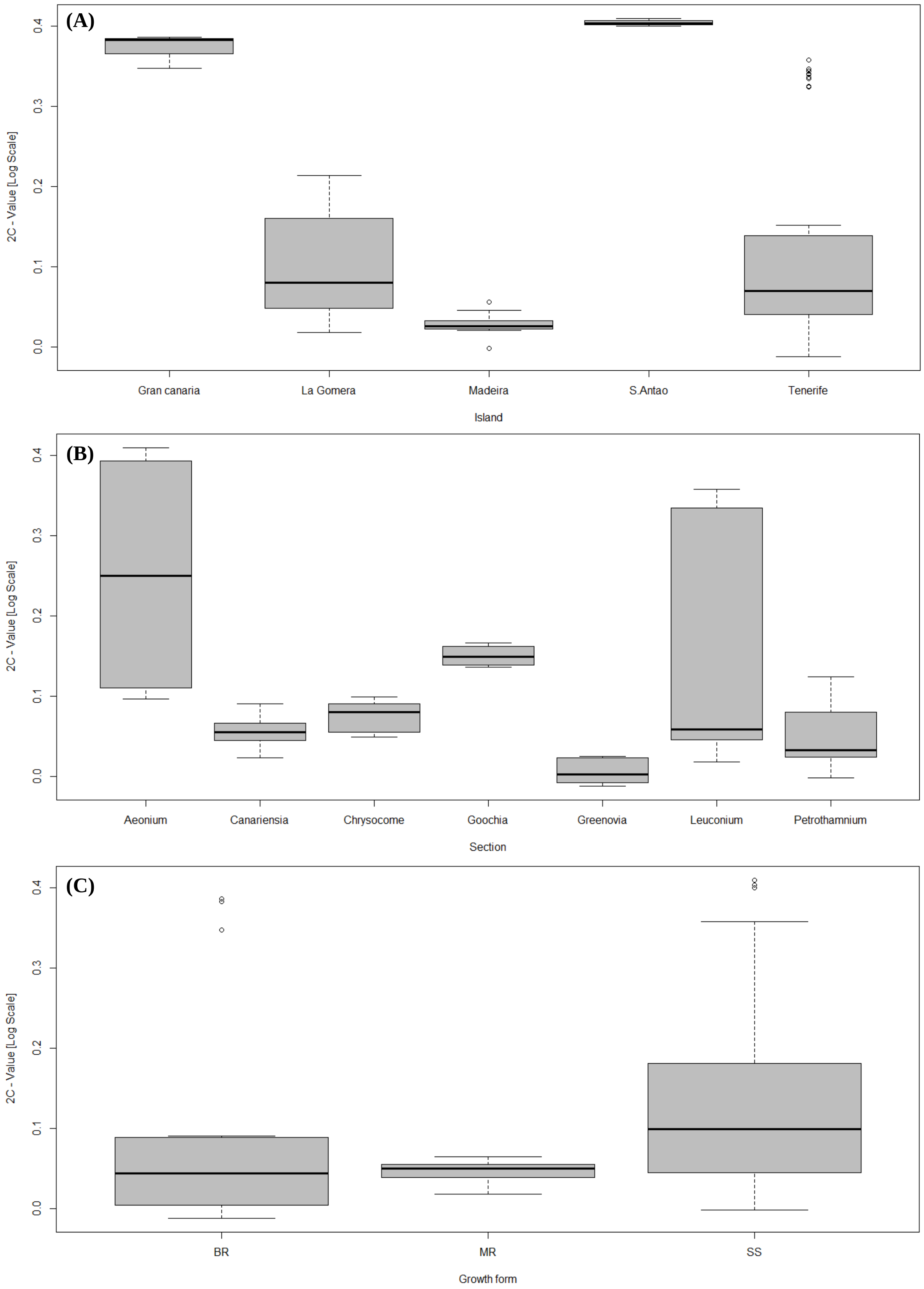


Figure 3.4. Boxplot diagram showing the $\log_{10}$ genome size variation across 27 *Aeonium* species according to: (A) Location; **(B)** Section; and **(C)** Growth-form (Legend: BR: Branching rosette; MR: Monocarpic rosette; SS: Subshrub)**.** The grey box represents the 25th, 50th (median) and 75th percentiles, while whiskers represent the 10th and 90th percentiles with minimum and maximum observations. The black dots represent the outliers.

According to **Figure 3.4.A**, it is possible to see the genome sizes across the several sampled islands. Paired tests reveal significant differences across the genome size of the several islands, except for La Gomera (n= 21; median = 1.20; range = [1.04 – 1.63]) and Tenerife (n= 50; median = 1.17; range = [0.97 – 2.28]); for Gran Canaria (n= 3; median = 2.41; range = [2.23 – 2.43]) and Cabo Verde (n= 3; median = 2.53; range = [2.53 – 2.57]) ($p < 0.025$, n=92; **See Appendix V**). Cabo Verde (Santo Antão Island) has, in mean, the biggest genome size for *Aeonium* species in Macaronesia (mean 2.54 ± 0.03pg). Contrarily, on average, Madeira Islands (n= 14; median = 1.06; range = [1.00 – 1.14]) has the smallest 2C – Values (mean 1.07 ± 0.03 pg) **(Figure 3.5.)**. Overall, it is possible to differentiate the specimens collected in the several archipelagos.

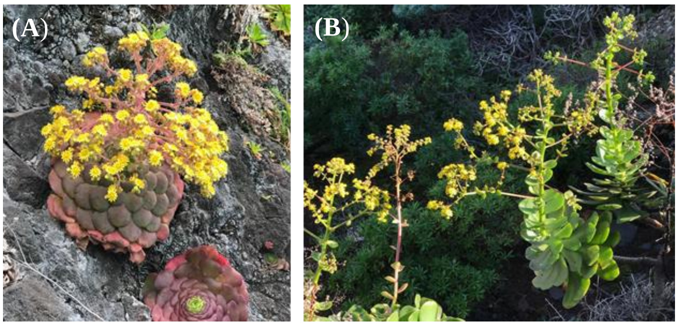


Figure 3.5. *Aeonium* species from Madeira Island. (A) *Aeonium glandulosum* and **(B)** *Aeonium glutinosum*. (Photo: Guilherme Roxo; Madeira Island, 2019)

For sectional division **(Figure 3.4.B)**, it is show that the genome size variation within sections is relatively constant. Exceptionally, the sections *Aeonium* (n= 12; median = 1.82; range = [1.25 – 2.57]) and *Leuconium* (n= 26; median = 1.14; range = [1.04 – 2.28]) have a broadly genome size variation along of each species. Species from *Greenovia* section have the lowest genome size with the lowest variation (n= 9; median = 1.01; range = [0.97 – 1.06]). Based on statistical tests, *Greenovia* section is the most distinct and conserved group in terms of genome size ($p < 0.025$, n=92; **See Appendix VI**).

According to the literature and field observations, all the sampled species are perennial with a great variation of growth – form. **Figure 3.4.C** shows the genome size variation along the growth- forms: subshrubs (n=60; median = 1.26; range = [1.00 – 2.57]), branching – rosettes (n=21; median = 1.11; range = [0.97 – 2.43]) and single monocarpic rosettes (n=11; median = 1.12; range = [1.04 – 1.16]). On average the subshrubs are endowed with the large genomes (1.44±0.46). Paired tests reveal significant differences between genome size for all the groups of growth-forms, except between branching – rosettes and single monocarpic rosettes ($p < 0.025$, n=92; **See Appendix VII**).

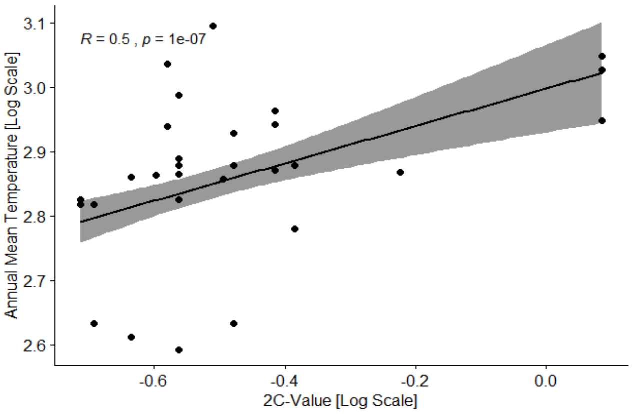


Figure 3.6. Relationships between log$_{10}$ C-value (genome size; pg) and Annual mean temperature (ºC) for 24 *Aeonium* species of Canary Islands. Solid lines represent the linear regression model for genome size and annual mean temperature models. Grey shading indicates the 95% confidence interval. R indicates the Spearman correlation coefficient. Black dots represent each sampled individual.

Correlations between 2C-Values and annual mean temperature were calculated for sampled *Aeonium* species of Tenerife and La Gomera. A positive correlation between these two variables was found (r = 0.50; P < 0.001; n= 94 individuals; **Figure 3.6.**). It shows a certain tendency of 2C-Value increase with annual mean temperature.

3.3.2. *Correlations between genome size, conservation status and geographic range size*

From the present universe of 27 *Aeonium* species analyzed 8 were found to be threatened, being 2 species endangered (*Aeonium gorgoneum* and *Aeonium gomerense*) and 6 species vulnerable (*Aeonium aizoon*, *Aeonium cuneatum*, *Aeonium dodrantale*, *Aeonium haworthii*, *Aeonium saundersii* and *Aeonium volkerii*). The other species are categorized as Not evaluated because of their abundance, they do not require concern and are supposedly Least Concern **(Bañares, 2015)**. Therefore, a conservation status assessment is required. **Figure 3.7.** presents the differences of 2C – Values within conservation categories, highlighting that endangered (mean = 2.07±0.51) species have, on average, great genome sizes. Followed by vulnerable (mean = 1.56±0.56) and least concern/not evaluated (mean = 1.23±0.28) species with lower genome sizes. *Post-hoc* Dunn test reveals significative 2C – Values differences between endangered species and both vulnerable and not evaluated/least concern but not between vulnerable and not evaluated/least concern (p < 0.025, n=92; **See Appendix VIII**).

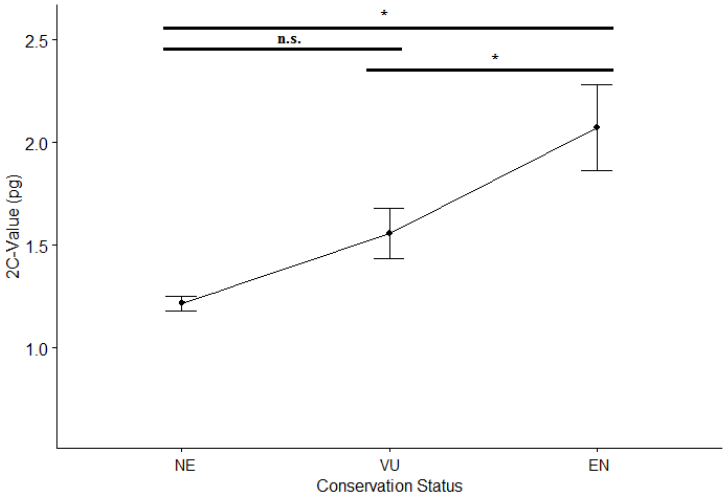


Figure 3.7. Differences in genome size among *Aeonium* species of different conservation status. Statistical significance was analyzed by Kruskal-Wallis test followed by Dunn's test. Asterisks indicate a significant difference: *P < 0.025, n.s. = not significant. Legend: NE: Not Evaluated; VU: Vulnerable; EN: Endangered.

A slightly positive relationship between genome size and the geographic range size was found (r = - 0.51; P = 0.001; n= 83 individuals; **Figure 3.8.**). Species with larger genomes tend to have a short geographic range size.

Based on identical analyses on subsets of the full data (n=92 individuals), the species without a relationship between genome size and geographic range size were removed from the dataset to improve the correlation models. This reduction of dataset by removing *Aeonium simsii* from Gran Canaria (n=3 individuals), *Aeonium aizoon* (n=3 individuals) and *Aeonium dodrantale* (n= 3 individuals) increased the explanation for the correlation between genome size and geographic range size **(See Appendix IX)**.

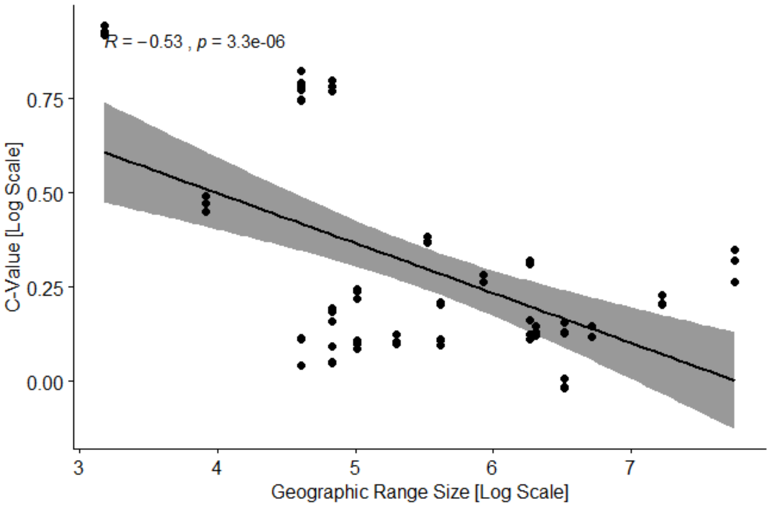


Figure 3.8. Relationships between $\log_{10}$ 2C-value (pg) and geographic range size (n = 83 individuals). Solid lines represent the linear regression model and grey shading indicates the 95% confidence interval. R indicates the Spearman correlation coefficient. Black dots represent each sampled individual for 24 *Aeonium* species.

3.4. Discussion

3.4.1. *Genome size variation across Aeonium species*

The Macaronesian flora is one of the most richness of the world comprising several plant radiations (e.g., *Aeonium, Echium,* and *Sonchus*) with a high endemicity level. However, many habitats and species are under threat or face risk of extinction **(e.g., Romeiras *et al.*, 2016)**, with limited up-to-date knowledge on genetic and genomic diversity for some group species, which still impeding implementation of holistic conservation management plans **(Romeiras *et al.*, 2016)**. Particularly, there is a low number of studies focusing on the study of cytogenomic variation in the island context of Macaronesia **(but see Suda *et al.*, 2003; 2005).**

The current study summarises new data for 16 *Aeonium* taxa endemic to the Canary Islands, Madeira, and Cabo Verde, including *Aeonium aizoon, Aeonium appendiculatum, Aeonium arboreum* ssp. *holochrysum* var. *rubrolineatum, Aeonium aureum, Aeonium canariense* ssp. *latifolium, Aeonium castello-paivae, Aeonium cuneatum, Aeonium decorum, Aeonium dodrantale, Aeonium glandulosum, Aeonium glutinosum, Aeonium gomerense, Aeonium gorgoneum, Aeonium lindleyi* ssp. *viscatum, Aeonium saundersii,* and *Aeonium volkerii* **(Table 3.2)**. Overall, with the previous estimates stored at Angiosperm DNA C-values database **(Leitch *et al.*, 2019)** 30 C-values for this Macaronesian genus are now available, missing 14 taxa. Thus, this work improved the global knowledge of nuclear DNA content, doubling the number of estimates for the biggest plant radiation of Macaronesia.

A range of 1C – values from 0.49 pg (*Aeonium dodrantale*) to 1.27 pg (*Aeonium gorgoneum*) is presented. According to genome size classes created by **Leitch *et al.* (1998)**, all the present estimation of the holoploid nuclear DNA content is included in a very small class (less than 1.40 pg). As previously evidenced by **Suda *et al.* (2005)**, under the strong selective pressures of oceanic islands, the small genome sizes tend to be advantageously favored by natural selection in order to reduce the genetic instability. Based on current results, it is coherent the predominance of small genome sizes within the insular context as mentioned above.

Comparing with the previous 2C-values estimates, all the results are compatible, except for *Aeonium haworthii* and *Aeonium simsii* that are not consistent being, in the first case, bigger and, in the second, shorter (almost half the size) **(Leitch *et al.*, 2019)**. This variation can be explained by the occurrence of different levels of polyploidy in *Aeonium simsii* **(Liu, 1989)** or by the fact that the estimation methodology being Feulgen Microdensitometry and not Propidium Iodide Flow Cytometry in *Aeonium haworthii* **(Hanson *et al.*, 2001)**.

A sectional division of *Aeonium* species reveals seven main sections **(Eggli & Hartmann, 2002; Bañares, 2015)** coherent with the present estimates of 2C-values **(Figure 3.4.B)**: Section *Aeonium* comprises a varied group of mainly woody branched subshrubs provided by leaves with glands in adaxial surface and ciliate margin, and ovoid inflorescences of yellow flowers (mean = 1.88 ± 0.60 pg; range = [1.25 – 2.57] pg). Section *Leuconium* is also a varied group comprising from woody densely to few – branched subshrubs and single monocarpic rosettes with variously reddish margins on glaucous and ciliate leaves, conical to semiglobose inflorescences, and whitish to reddish petals (mean = 1.53 ± 0.51 pg; range = [1.04 – 2.28] pg). Section *Goochiae* englobes herbaceous perennials with yellow or pink petals without nectaries (mean = 1.45 ± 0.05 pg; range = [1.37 – 1.47] pg). Section *Chrysocome* includes small densely branched subshrubs with conspicuous longitudinal brownish stripes on leaf lower face, lax inflorescences with few yellow flowers without nectaries (mean = 1.19 ± 0.06 pg; range = [1.12 – 1.26] pg). Section *Canariensia* includes large branching rosettes with pubescent leaves at margin, lax many – flowered inflorescence of pale-yellow flowers provided by nectaries (mean = 1.14 ± 0.05 pg; range = [1.05 – 1.23] pg). Section *Petrothamnium* include herbaceous perennials with flowers of yellow petals without nectaries (mean = 1.13 ± 0.11 pg; range = [1.00 – 1.33] pg). Finally, Section *Greenovia* includes branching or single monocarpic rosettes with leaves of hyaline margin, densely leafy and compact umbels of yellow 18- to 32- merous flowers (mean = 1.01 ± 0.04 pg; range = [0.97 – 1.06] pg). On average, it is possible to distinguish between the several sections, being *Greenovia* (former *Greenovia* genus; **Liu, 1989**) the most distinct and conserved section in terms of genome size content. It can be observed both the increase and decrease of 2C-values across *Aeonium* evolution.

The correlation of environmental factors and genome size has been debated for years and is of great interest to the scientific community **(e.g., Suda *et al.*, 2005)**. Macaronesian islands, more specifically the Canary Islands, comprise an ideal study model to understand the relationships between environmental factors and genome size. The great diversity of habitat types (e.g., from xerophytic to wet laurel forests and mountainous sites) with particular climatic conditions justify the above-mentioned. The current results demonstrate a positive correlation between genome size variation and annual mean temperature **(Figure 3.6.)**. This result is consistent with **Suda *et al.* (2003)** results where *Aeonium* genus is the only showed relationship with environmental variables. Aditionally, **Liu (1989)** shows that species with great ploidy levels and, therefore, large genomes are associated with more arid sites. By comparing the average nuclear DNA content for the several Macaronesian archipelagos **(Figure 3.4.A)**, it can be distinguished three groups corresponding to Cabo Verde (2.54 ± 0.03 pg), Canary Islands (1.36 ± 0.41 pg), and Madeira (1.07 ± 0.03 pg). Based on the present work, it is suggested that these differences can be explained by higher annual mean temperatures in Cabo Verde followed by the Canary Islands and Madeira with lower ones. However, further studies should be done with a more embracing sampling, that is, with a lot of replicates in several different climatic strata of the island.

Relating growth-forms with nuclear DNA contents **(Figure 3.4.C)**, it can be assumed that subshrub (SS) species have, on average, significantly large genomes than branching (BR) and monocarpic rosettes (MR). Being subshrubs a slow-growing type of plants, their larger genomes imply a more delayed DNA replication and, therefore, more slow life cycles and individual development **(Knight *et al.*, 2005)**.

3.4.2. *Correlations between genome size, conservation status and occurrence area*

The present results reveal a significant relationship between threatened species and larger genome sizes **(Figure 3.7.)**, that is, endangered species have, on average, bigger genomes than their congeners. It can be assumed linkage between large genomes and a higher likelihood of extinction. Agreeing with these observations, **Vinogradov (2003)** and **Knight *et al.* (2005)** found a negative correlation between mean genome size and taxa diversity at the genus level. This approach suggests that large genome sizes constraints the diversification capacity because it is maladaptive, that is, against natural selection. Specifically, large nuclear DNA contents are associated with a larger nucleus and very slow cell cycles. In this case, more DNA contents imply more time for their replication **(e.g., Bennett, 1977)** and, therefore, influence negatively the development and establishment of populations with considerable effective size **(Lynch & Connery, 2003)**.

As observed in **Figure 3.8.**, there is a negative correlation between nuclear DNA content and geographic range size. It is observed a tendency for species with large genomes have a short occurrence area, which is in accordance with the previous considerations. As mentioned by **Knight et *al.* (2005)**, contrary to large-genome species, the small – genome ones are found in widely variable environments. To improve this correlation was removed the following species: *Aeonium aizoon*, *Aeonium dodrantale* e *Aeonium simsii*. The two first ones have the shorter genomes of the group but have very short inflorescences, hence, they are a very restricted species in terms of geographic range size. **(See Chapter 2)** Additionally, they have a small effective population size **(Field observations)**, thus, are categorized as vulnerable **(Bañares, 2015)**. *Aeonium simsii* is a species that can experience variable ploidies (di-, tri- or tetraploid; **Liu, 1989**), suggesting their hybrid origin. Contrarily to the species with large genomes, *Aeonium simsii*, have considerable effective population sizes **(Field observations)** and it is not associated with a threatened category.

Overall, except in some cases, *Aeonium* species with larger amounts of nuclear DNA tend to have small fragmented populations of reduced effective size and therefore short geographic range size. Being the islands a highly vulnerable place to threats, such as human interference and invasive species **(Bramwell, 1990)**, the considered species are more susceptible to extinction than species with small genome sizes.

Considering the present data, despite the relatively coherent results, further efforts to increase the sampling to cover the whole *Aeonium* genus it is still necessary. The cytogenomic analysis of the remaining species may provide better consistency of this work. In addition, a conservation status assessment is required for all species as most are still in the "Not Evaluated" category.

Chapter 4

Identification of biodiversity hotspots within Macaronesian Islands: centers of *Aeonium* species richness and endemism as a case-study

Abstract

Islands are only 5% of the Earth surface and have a high level of plant endemicity. Due to threats that are subjected, and its particular features are very important in terms of conservation. The genus *Aeonium* (Crassulaceae) is the most diverse plant radiation across Macaronesia. The Canary archipelago has 41 endemic taxa with a large diversity of life forms (e.g. subshrubs, branching-rosettes and monocarpic rosettes). Considering the great diversity of species well-distributed in the Canaries, this study aims to update the inventory of the *Aeonium* in this archipelago and investigate the distribution patterns to detect biodiversity hotspots within each island and protected areas network. Results show that the greatest part *Aeonium* taxa occur in Tenerife more exactly in the northwestern Teno Rural Park and northeastern Anaga Rural Park. The representation of *Aeonium* endemics in protected areas is relatively high once a large area (40%) of the Canary Islands is classified as protected. A carefully re-assessment of protected areas classification in the Canary Islands should be required to facilitate and promote effective conservation of the unique plant diversity.

Keywords: Canary Islands; conservation; diversity patterns; species richness; protected areas;

4.1. Introduction

A comprehensive understanding of biodiversity patterns is extremely important to species conservation **(Hewitt *et al.* 2018)**, particularly on islands due to range-restricted areas and environmental changes caused by anthropogenic pressures. Despite constituting only about 5% of the worldwide land surface, islands possess one-quarter of the all known flora **(Kreft *et al.*, 2008)**. Regarding species biodiversity and conservation, much more studies are still needed to cover a larger taxonomic distribution.

The Canary Islands comprise an archipelago of seven islands occupying 7,250 km² in the Atlantic Ocean near the African Coast. The archipelago was formed sequentially in the last 20 million years, as a repercussion of the slow movement of the African Plate to the northeast on a fixed volcanic hotspot of mantelic origin **(Carracedo & Perez-Torrado, 2013)**. Spatially, it is possible to distinguish two islands groups corresponding to a sub-province of Mediterranean Region: the eastern and oldest islands (Fuerteventura and Lanzarote) and western islands (Gran Canaria, Tenerife, La Gomera, La Palma, and El Hierro) **(Rivas-Martínez, 2011)**.

The Canaries are highlighted as the region with the highest plant diversity in Macaronesia, being considered a biodiversity hotspot within the Mediterranean global diversity hotspot **(Quézel & Médail, 1995)**. Their flora is endowed with a high level of endemicity, about 607 recognized endemic taxa presenting nearly 45% of the total native flora **(Cajuapé-Castells *et al.*, 2010)**. The extremely rich flora of this archipelago appears as a result of the large range of ecological zones provided by the combination of topography and climatic conditions, that is, trade winds from the north and dry winds from Sahara Desert **(del-Arco Aguilar *et al.*, 2010)**. Along the archipelago, it is recognized mainly six ecological zones: The sub-desert coastal scrub (*tabaibas*), thermophile forests, laurel forest, pine forest, summit scrub, and the peak ecosystem. According to **Reyes-Betancort *et al.* (2008)**, despite the uneven distribution of the species, several centers of biodiversity are recognized: **(i)** Teno Rural Park (Tenerife); **(ii)** Anaga Rural Park (Tenerife); **(iii)** Corona Forestal Natural Park (Tenerife); **(iv)** Garajonay Natural

Park (La Gomera); **(v)** Tamadada Natural Park (Gran Canaria); **(vi)** Caldera de Taburiente National Park (La Palma).

From a conservation perspective, the high level of endemicity and the threats (e.g., invasive species and human interference) that these islands are subjected, make them very important in terms of conservation **(Bramwell, 1990)**. A considerable part of Canary ecosystems and species are under threat or in the risk of extinction. Specifically, approximately 30% of native plant diversity is included in the red list with some kind of threat (CR, EN or VU) **(Cajuapé-Castells *et al.*, 2010)**. For an implementation of conservation management plans and policymaking, great knowledge on biodiversity is needed (e.g., Huntley et al. 2019). Canary territory includes a network of 146 terrestrial protected areas, covering about 40% of the country **(Gobierno de Canarias, 2001)**.

On the overall, the creation and implementation of conservation actions in order to protect the native flora of the Canary Islands are urgent. It should be considered a particular concern to some species-richness endemic genera such as *Aeonium* (Crassulaceae) **(Mes, 1996)**, *Echium* (Boraginaceae) **(Bohle *et al.*, 1996)**, and *Sonchus* (Asteraceae) **(Kim *et al.*, 1996)**.

The genus *Aeonium* (Crassulaceae) is one of the most diverse plant genera of Macaronesia and shows a great diversity across the Canary Islands, being their biodiversity hotspot. This genus is the largest endemic plant radiation in the Canary Islands, including near 41 endemics **(Mort *et al.*, 2002)**. In the Canary Islands, *Aeonium* species are found in a wide variety of habitats, show a great diversity of life forms, ranging from subshrubs to branching and single monocarpic rosettes, and occur across different phytogeographical regions **(Jorgensen & Olesen, 2001; Liu, 1989)**.

A central issue in conservation today is to identify biodiversity-rich areas. Therefore and, as *Aeonium* species is the biggest plant radiation in Macaronesia, it will be explored the phenomenon called "hotspot within-hotspot", sensu **Cañadas *et al.* (2014)** as endemic-rich areas with lesser than 3 km^2 with an exceptional concentration of endemic species, using distribution data of *Aeonium* species from the Canary Islands.

Thus, the main objective of this study is to assess the patterns of diversity of the genus *Aeonium* in the Canary Islands and contribute with new data to identify areas of high importance for biodiversity, particularly in terms of species richness. Specificall, it aims to: i) present an updated inventory of the endemic *Aeonium* taxa of Canary Islands, providing details on their taxonomy and main morphological traits; and ii) use occurrence data derived from fieldwork and online databases to compare species distribution and richness across ecological zones and protected areas in the Canary Islands to investigate congruence between species richness and ecological and biogeographical factors, and levels of protection.

4.2. Methodology

4.2.1. *Data collection*

Data for the endemic *Aeonium* taxa was obtained by means of a comprehensive review conducted through the fieldwork, and of published works and online databases. Therefore, this study was made using three main sources:

1) Field research was carried out by GPS point collection representing 21 taxa studied between February and June 2019 on Tenerife, La Palma, Gran Canaria, and La Gomera. In the course of fieldwork, botanical samples were collected for herbarium vouchers. These specimens were preserved for later identification and are kept in the Herbarium of Botanical Garden of the University of Lisbon (LISU), João de Carvalho e Vasconcellos Herbarium of Superior Institute of Agronomy (LISI), Botanical Museum of the University of Mainz – Herbarium (MJG), and Herbarium of the University of Basel (BAS).
2) A thorough investigation of the *Aeonium* data described in the literature. It was reviewed data available from the past **(e.g. Praeger, 1928; Lems, 1960; Liu, 1989; Mes, 1996)** and also more contemporary sources **(e.g. Eggli & Hartmann, 2002; Bañares, 2015; Jorgensen & Olesen, 2001; Mort *et al.*, 2002; 2007; Harter *et al.*, 2015)**.
3) Online databases, namely: i) IUCN - Red List of Threatened Species (www.iucnredlist.org) that provides useful information on each species assessed, threats and conservation actions; and ii) Global Biodiversity Information Facility (GBIF) to get distribution data (https://www.gbif.org/). All data were verified and specimens with inaccurate taxonomy or location were rejected.

Scientific names and sections were updated according to **Bañares (2015)**. Additionally, information on the conservation status of each taxon was gathered from the IUCN Red List of Threatened Species **(**https://www.iucnredlist.org/**)** and *Lista Roja 2008 de la flora vascular Española* **(Moreno, 2008)**. The final database was 2343 occurrence records belonging to 41 taxa, which served as the basis for distribution studies and hotspot identification.

4.2.2. *Statistical analysis*

Species richness maps were constructed in QGIS v.3.4.4 software **(QGIS Development Team, 2019)**. The geographic boundaries of the Canary Islands' protected areas were obtained as GIS shapefiles from the World Database of Protected Areas **(WDPA, http://www.protectedplanet.net/c/world-database-on-protected-areas)**. The areas with high conservation interest were identified based on local species richness and we overlay the maps of species occurrences with the protected areas to identify what are the species under protection. The species occurring outside the boundaries of protected areas were considered unprotected.

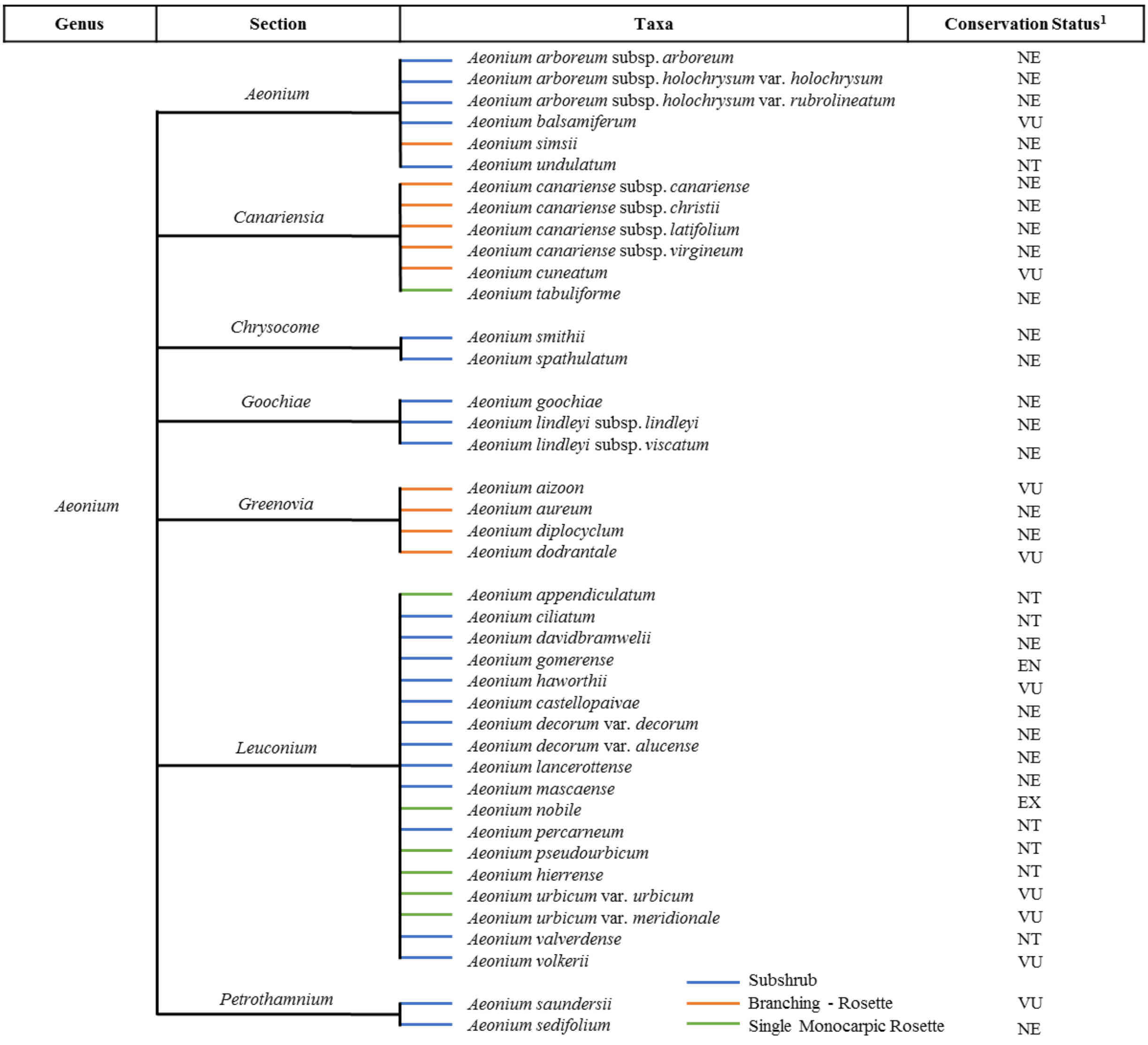
4.3.Results

4.3.1. *Diversity of Aeonium genus*

In the Canary Islands, the *Aeonium* genus comprises 41 endemic taxa (33 species, 5 subspecies and 3 varieties), being 32 single island endemic taxa and 9 multi-island endemics, sharing distribution across several islands. Additionally, six species are located outside of the Canarian archipelago: one from Cabo Verde, two from Madeira and three from Morocco and East Africa **(See Table 1.1., Chapter 1)**.

A high level of morphological diversity is recorded in the endemic species of *Aeonium* in the Canary Islands, being 59% subshrubs, 24% branching-rosettes, and only 17% are single monocarpic. Also, a relatively high infrageneric diversity is displayed, which belongs to seven sections (*Aeonium*, *Canariensia*, *Chrysocome*, *Gocchiae*, *Greenovia*, *Leuconium* and *Petrothamnium*) **(Eggli & Hartmann, 2002; Bañares, 2015) (Figure 4.1.)**.

Section *Leuconium* which contains 18 taxa, is the largest section of the genus and is broadly distributed in the Canary Archipelago. Comprises a diverse group having from densely-branched to unbranched species with large inflorescences of whitish flowers. Section *Aeonium* endowed with six taxa comprises also a varied group of mainly woody branched subshrubs and ovoid inflorescences of yellow flowers. Section *Canariensia*, with six taxa, it is recognized by the large branching rosettes with pubescent leaves and lax inflorescences of pale-yellow flowers. Section *Greenovia* has four taxa and comprises the former *Greenovia* genus. Includes branching or single monocarpic rosettes with leaves of hyaline margin, densely leafy and compact um-bels of yellow 18- to 32- merous flowers. Section *Goochiae* has three taxa and englobes herbaceous perennials with yellow or pink petals. Section Chrysocome, with two species, includes small densely branched subshrubs with longitudinal brownish stripes on leaf lower face, lax inflorescences with few yellow flowers. Finally, Section *Petrothamnium* of two species includes herbaceous perennials with flowers of yellow petals.



Genus	Section	Taxa	Conservation Status[1]
Aeonium	*Aeonium*	🟦 *Aeonium arboreum* subsp. *arboreum*	NE
		🟦 *Aeonium arboreum* subsp. *holochrysum* var. *holochrysum*	NE
		🟦 *Aeonium arboreum* subsp. *holochrysum* var. *rubrolineatum*	NE
		🟦 *Aeonium balsamiferum*	VU
		🟧 *Aeonium simsii*	NE
		🟦 *Aeonium undulatum*	NT
	Canariensia	🟧 *Aeonium canariense* subsp. *canariense*	NE
		🟧 *Aeonium canariense* subsp. *christii*	NE
		🟧 *Aeonium canariense* subsp. *latifolium*	NE
		🟧 *Aeonium canariense* subsp. *virgineum*	NE
		🟧 *Aeonium cuneatum*	VU
		🟩 *Aeonium tabuliforme*	NE
	Chrysocome	🟦 *Aeonium smithii*	NE
		🟦 *Aeonium spathulatum*	NE
	Goochiae	🟦 *Aeonium goochiae*	NE
		🟦 *Aeonium lindleyi* subsp. *lindleyi*	NE
		🟦 *Aeonium lindleyi* subsp. *viscatum*	NE
	Greenovia	🟧 *Aeonium aizoon*	VU
		🟧 *Aeonium aureum*	NE
		🟧 *Aeonium diplocyclum*	NE
		🟧 *Aeonium dodrantale*	VU
	Leuconium	🟩 *Aeonium appendiculatum*	NT
		🟦 *Aeonium ciliatum*	NT
		🟦 *Aeonium davidbramwelii*	NE
		🟦 *Aeonium gomerense*	EN
		🟦 *Aeonium haworthii*	VU
		🟦 *Aeonium castellopaivae*	NE
		🟦 *Aeonium decorum* var. *decorum*	NE
		🟦 *Aeonium decorum* var. *alucense*	NE
		🟦 *Aeonium lancerottense*	NE
		🟦 *Aeonium mascaense*	EX
		🟩 *Aeonium nobile*	NT
		🟦 *Aeonium percarneum*	NT
		🟩 *Aeonium pseudourbicum*	NT
		🟩 *Aeonium hierrense*	VU
		🟩 *Aeonium urbicum* var. *urbicum*	VU
		🟩 *Aeonium urbicum* var. *meridionale*	NT
		🟦 *Aeonium valverdense*	VU
		🟦 *Aeonium volkerii*	VU
	Petrothamnium	🟦 *Aeonium saundersii*	VU
		🟦 *Aeonium sedifolium*	NE

🟦 Subshrub
🟧 Branching - Rosette
🟩 Single Monocarpic Rosette

Figure 4.1. Infrageneric classification of *Aeonium* taxa endemic to Canary Islands. Shows the sectional division, growth – form and conservation status for each taxon. Legend: NE: Not evaluated; NT: Near threatened; VU: Vulnerable; EN: Endangered. For species distribution see **Table 1.1. (Chapter 1)**.

4.3.2. *Distribution of Aeonium species in Canary Islands*

The total number of 4 km x 4 km squares included in the analysis is 259 as shown in **Figure 4.2.** The number of *Aeonium* taxa per grid square is ranged from 1 to 7 species and the complete data matrix included a total of 2343 occurrence data. At first sight, Tenerife and La Gomera are the most richness islands in terms of *Aeonium* species richness.

According to **Figure 4.2.**, the eastern islands Lanzarote (18 squares) and Fuerteventura (7 squares) have the lowest number of *Aeonium* species. In Lanzarote, squares have the maximum of two taxa whereas in Fuerteventura have only one taxon. The western islands of the Canary Archipelago (Gran Canaria, Tenerife, La Gomera, La Palma, and El Hierro) are endowed with a greater number of *Aeonium* species than eastern ones. In Gran Canaria, 53 grid squares correspond to *Aeonium* genus occurrences. As it possible to see, the highest specific richness area is the north and center of the island owning the maximum of six taxa per square. In general, a decrease in specific richness is observed in the south sector.

The grid squares with the highest *Aeonium* richness of the whole analysis belong to Tenerife Island where it was found 93 squares of *Aeonium* taxa occurrences. Specifically, corresponds to the west of the Teno massif and the east Anaga massif, both with a minimum of seven and a maximum of 11 taxa. Also, the central-eastern zone that surrounds Teide volcano, has a considerable species richness with a maximum of eight species per square. Contrarily, the less richness of taxa was found in the south sector and in Cañadas del Teide. Overall, the large number of *Aeonium* per grid is observed in the north sector of the island.

Considering La Gomera with 24 squares of *Aeonium* presence, the areas holding the greater diversity are irregularly located within the island. However, the most prominent species – richened zone is the northeastern, comprising squares with a maximum of 10 *Aeonium* taxa. La Palma, in turn, has a uniform distribution of taxa (45 occurrence squares) along its land surface with some squares of higher species-richness. The great diversity can be found punctually on both sides of Caldera de Taburiente (i.e., the central volcano caldera) with a maximum of nine taxa per square. Specifically, Barranco de las Angustias (nine taxa) are in the western section of the caldera and is one of the greatest numbers of *Aeonium*. Also, a considerable diversity of the *Aeonium* genus is observed in the northeastern (five taxa) and southern (four taxa) of Cumbre Vieja (i.e., the mountain chain of the southern sector). Finally, El Hierro, the youngest island of Canary Islands with 19 squares of *Aeonium* occurrence, owns the highest diversity of *Aeonium* in the central-eastern sector (six taxa).

4.3.3. *Conservation of Aeonium in Canary Islands*

Considering the 41 *Aeonium* endemic taxa, 16 (39%) are listed in *Lista Roja 2008 de la flora vascular Española* being one species endangered (*Aeonium gomerense*), seven vulnerable, six near-threatened and one extinct in wild (*Aeonium mascaense*) **(Moreno, 2008)**. In relation to growth-form, 22% subshrub, 10% single monocarpic rosette and 7% branching-rosettes are extinct, threatened or near threatened **(Figure 4.1.)**. Moreover, only 3 taxa of 41 are also included in the IUCN RedList: *Aeonium balsamiferum* (VU), *Aeonium gomerense* (EN) and *Aeonium saundersii* (VU). The 25 remaining species have a not evaluated (NE) status.

The major part of the occurrences of *Aeonium* endemics was within the boundaries of protected areas, that is, the great species richness is coincident with this kind of areas **(Figure 4.3.; See Appendix X)**. Most of the taxa are found in the Anaga and Teno Rural Parks, and Corona Forestal Natural Park of

Tenerife; and Garajonay National Park of La Gomera. The Anaga Rural Park is the richness protected area owning 12 *Aeonium* species, followed by Teno Rural Park with 11 species, and Garajonay National Park and Corona Forestal with 10. Each area includes single endemic species of which was not found in other protected areas.

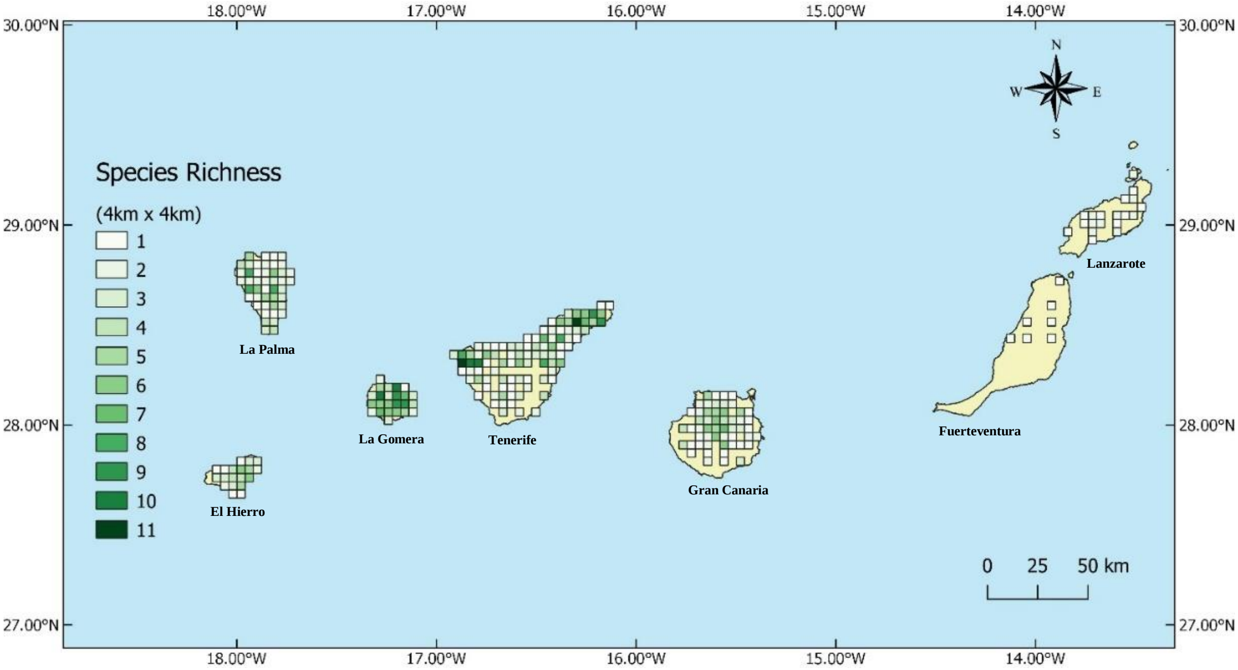


Figure 4.2. Species richness of the endemic *Aeonium* species in Canary Islands

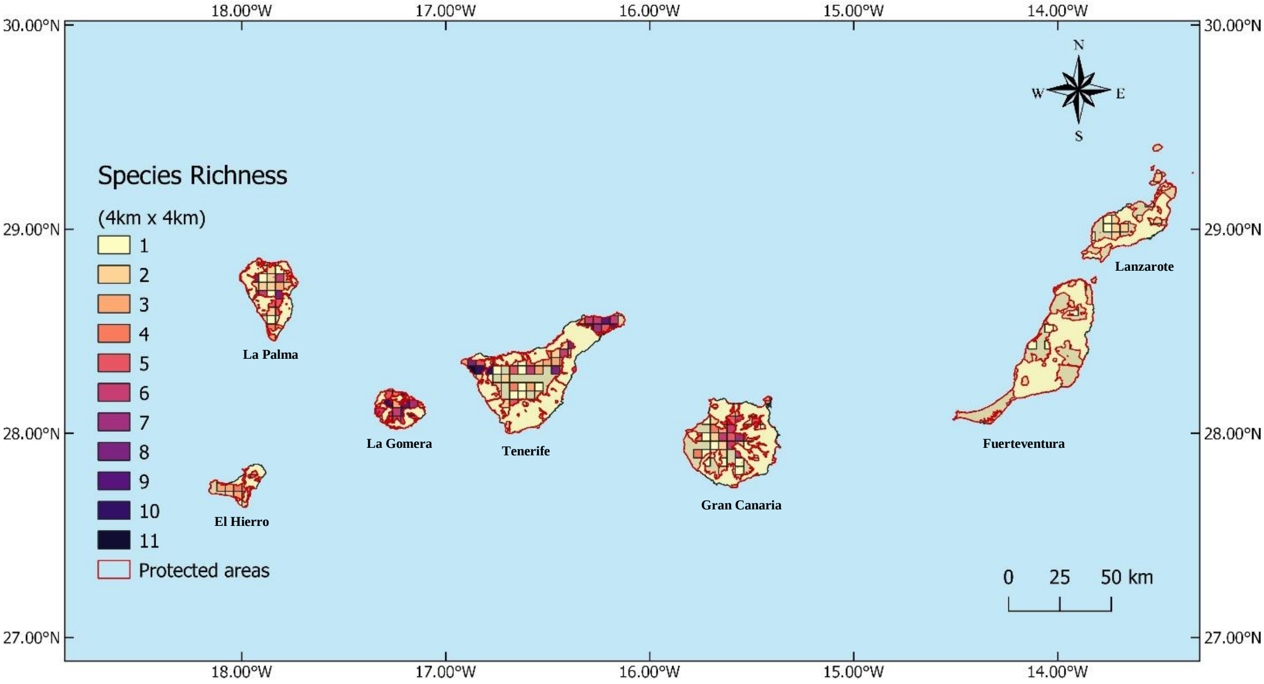


Figure 4.3. Species richness of the endemic *Aeonium* species in Canary Islands within protected areas network

4.4. Discussion

4.4.1. *Diversity, endemism and biogeography of Aeonium in Canary Islands*

To effective conservation of the biodiversity in the Canary Islands is extremely important the characterization of the endemic flora of the archipelago and its distribution patterns. The current work highlights *Aeonium* as the largest endemic plant radiation in the Canaries, with 41 endemic species, subspecies, and varieties. The analysis of biogeographical patterns shows a heterogeneous distribution of endemic *Aeonium* taxa diversity along the islands' land surface. It stands out some important biodiversity hotspots for the genus with the maximum co-occurrence of 12 species together **(Figure 4.2.)**. The presented approach, considering the hotspot identification to the *Aeonium* genus, is significant for conservation planning. This provides a tool to create conservation actions can enhance the conservation of the region's diversity.

The present results reveal that the eastern islands Lanzarote and Fuerteventura have the lowest number of *Aeonium* species presumably because of their reduced habitat diversity **(Reyes-Betancort *et al.*, 2001)** and anthropogenic impacts **(Reyes-Betancort *et al.*, 2008)**. They are the oldest islands of the archipelago and, being in an erosional stage, have lower altitudes. Furthermore, due to geographic situation, rainfall is scarce and leads to an arid environment with few wet sites at the top of the mountains **(Juan *et al.*, 2000)**: Jandia in Fuerteventura and Famara in Lanzarote. Additionally, in Lanzarote occurs *Aeonium balsamiferum* and *Aeonium lancerottense* whilst Fuerteventura occurs only *Aeonium balsamiferum* **(Bañares, 2015).**

The western islands have the highest number of *Aeonium*. Tenerife is the most *Aeonium* diverse (16 taxa), followed by La Gomera (12 taxa), La Palma (nine), Gran Canaria (seven taxa), and El Hierro (fixe taxa). In Gran Canaria, the high mountains zones in the center of the island are the focus of *Aeonium* distribution, being one of the wettest massifs of the islands. It is observed a decrease of species richness in the south, being the low altitude and scarce humidity the main responsible factors **(del-Arco *et al.*, 2010)**.

The greatest diversity of *Aeonium* genus is found in Tenerife as expectable because it is the largest island (2034 km^2) of Canary Archipelago (**Fernández-Palacios *et al.*, 2004**). It is shown that 11 taxa coexist in the same 16 km^2 square, more exactly in the facing north Teno and Anaga massifs. These regions comprise the oldest sectors of Tenerife, being the two of the three-independent paleo-islands that originate the actual single island **(Ancochea *et al.*, 1990)**. The combination of altitude, humidity and trade winds from the north explains the highest amount of *Aeonium* richness **(Carracedo & Day, 2002)**. The lower quantities of species in the southern sector of the island might be associated with the dry environment caused by the Sahara winds. In Cañadas del Teide, in turn, have their reduced species richness linked to the recent volcanic activity **(**less than 2 Mya**; Juan, 2000)**.

The second and third most *Aeonium* richened islands, La Gomera and La Palma, own their richness associated with the north sectors. There, its have higher altitudes and are under the strong influence of the trade winds, hence, being wetter zones **(del-Arco *et al.*, 2010; Juan, 2000)**. In El Hierro, being the youngest and small island, have their number of taxa limited. The highest diversity of *Aeonium* found in the Frontera sector, that is, a relatively young region of the island characterized by recent lava flows and a slight influence of the northern humid trade wind. This provides optimal conditions to improve the richness of well-adapted *Aeonium* taxa. *Aeonium valverdense* stands out as the unique single island's endemic species of El Hierro.

4.4.2. *Conservation of Aeonium*

All the analyzed 41 endemic taxa of *Aeonium* are recorded in terrestrial protected areas. Despite this result should be regarded as preliminary and requiring further field confirmation. It indicates that the representation of plant species diversity in the Protected Areas network of Canary Islands is high because the great part of the island's surface is categorized as a protected area, being 40% of the whole archipelago **(Gobierno de Canarias, 2001)**.

Tenerife's Anaga and Teno Rural Parks stand out for their greatest endemic *Aeonium* taxa and appear to offer them potential protection. It can be considered the biodiversity hotspots of this genus. These protected areas belong to the Important Plant Areas (IPA) for Endangered plants defined by **del-Vale *et al.* (2004),** where there is a maximum of 11 endemic taxa **(See Appendix X).** It should be noted that in Anaga, *Aeonium cuneatum*, *Aeonium dodrantale*, and *Aeonium volkerii* are threatened species with a vulnerable conservation status. Teno, in turn, holds the vulnerable *Aeonium dodrantale* and *Aeonium haworthii*. Thus, the protection of the Anaga and Teno regions seems to be critical for the conservation of range-restricted *Aeonium* taxa and many other endemic plants.

Coherent with the present results, **Reyes-Betancort (2008)** and **del-Valle et *al.* (2004)** showed that Teno massif is one of the most richness area in terms of plant species in the Canary Islands. However, the present study only considers the genus *Aeonium*, thus, expanding the analysis including other groups of insular endemic plants would be very important to create conservation measures to the region’s flora. Currently, Teno and Anaga massifs are classified as a Parque Rural (i.e., managed mainly for sustainable uses of natural ecosystems) according to eight categories of the protected area recognized in the Canary Islands. Taking into account the importance of Teno and Anaga Rural Parks to endemic and threatened plant species, a re-assessment of protected area status could be done and, also, their inclusion in *Área de Sensibilidad Ecológica* (**Gobierno de Canarias, 2001).**

All things considered, there is an urgent need for creation and implementation of conservation measures in these regions with a special focus in Teno and Anaga.

Chapter 5

Final Remarks

Studies on the flora of Macaronesia are still scarce and, therefore, a greater effort is necessary to understand the patterns of diversification and preserve its threatened biodiversity. Focusing on the present work, being *Aeonium* the largest Macaronesian radiation, its careful study deserves an extreme interest.

In this work, it is presented clear evidence that inflorescence height is an approach to explain the *Aeonium* species distribution across the Canary Islands. In this sense, generalist species have taller inflorescences than range-restricted specialist ones attaining larger geographic ranges. Despite these findings, further work is still needed to prove this evidence for all the species of the genus. For this, it is necessary improvement of the sampling for the whole genus and for more individuals per taxa in different locations. Future research work englobes the count of the number of seeds or ovules per flowers to relate the seed production, inflorescence height, and geographic range size. Previously, it can be supposed that taller inflorescences produce more seeds and, hence, endowed with large geographic range size.

The current study also reveals that threatened *Aeonium* species have, on average, a larger genome than their congeners with a small genome. Thus, large 2C-values are associated with a high likelihood of extinction. Additionally, new obtained cytogenomic data can contribute to understanding the relationships among *Aeonium* species. Prospectively, a flow cytometric assessment for all the *Aeonium* species will certainly contribute to clarify the diversification patterns of closely-related species within Macaronesia. Moreover, it is necessary to increase the sampling to cover the whole genus *Aeonium* and even the closest parental to understand possible patterns of variation in genome size, and as such to understand the origin and diversification of the genus in the Canary Islands where owns its biodiversity hotspot. It would be interesting to estimate the nuclear DNA content for continental *Aeonium* species to check for genome miniaturization in an island context, as advocated by **Suda *et al.* (2003; 2005)**. A reassessment of the *Aeonium* sectional division is suggested by introducing genome size as a distinctive trait between them. Furthermore, as mentioned before, it appears that a conservation status assessment is required for all species, as most are still in the Not Evaluated (NE) category.

Altogether, integrating the last highlighted results it is possible to explain the species distribution based on inflorescence height and genome size. Some species have their occurrence area constraint by short inflorescences while others, which not respond to this approach, by large genome size.

Finally, studying the diversity patterns of the *Aeonium* genus in the Canary Islands, it was clear that the most richness island in terms of endemic species is Tenerife. Specifically, the Teno and Anaga massif in the west – northern and east – northern sector of the islands, respectively. Both regions are classified as a Rural Park, that is, are managed mainly for sustainable uses of natural ecosystems. However, considering the large number of species inherent to these areas, further studies, with a strong component of fieldwork, and a new urgent re-assessment of the status of the protected areas is needed.

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Appendices

Appendix I

Morphological traits measured for 132 individuals representing 22 *Aeonium* species between February and June 2019 in Tenerife, La Palma, Gran Canaria and La Gomera (Canary Islands).

Trait	Abbreviation	Units	Range
Inflorescence number	inf_no	inflorescence /individual	1 - 29
Inflorescence height	heightflw	cm	12.6 – 192.2
Number of flowers	flowerind	flowers/individual	6 – 13920
Merism	merism	carpels/flower	6 - 33

Appendix II

Environmental variables obtained from CHELSA **(Karger et *al.*, 2017)**. Climate data are based on averaging monthly precipitation and temperatures since 1979 to 2013.

Environmental variable	Abbreviation code	Units
Annual Mean Temperature	BIO01	°C*10
Mean Diurnal Range[1]	BIO02	°C
Isothermality[2]	BIO03	dimensionless
Temperature Seasonality[3]	BIO04	°C*100
Max Temperature of Warmest Month	BIO05	°C*10
Min Temperature of Coldest Month	BIO06	°C*10
Temperature Annual Range[4]	BIO07	°C*10
Mean Temperature of Wettest Quarter	BIO08	°C*10
Mean Temperature of Driest Quarter	BIO09	°C*10
Mean Temperature of Warmest Quarter	BIO10	°C*10
Mean Temperature of Coldest Quarter	BIO11	°C*10
Annual Precipitation	BIO12	mm/year
Precipitation of Wettest Month	BIO13	mm/month
Precipitation of Driest Month	BIO14	mm/month
Precipitation Seasonality	BIO15	coefficient of variation
Precipitation of Wettest Quarter	BIO16	mm/quarter
Precipitation of Driest Quarter	BIO17	mm/quarter
Precipitation of Warmest Quarter	BIO18	mm/quarter
Precipitation of Coldest Quarter	BIO19	mm/quarter
Geographic range size	georange	Km^2

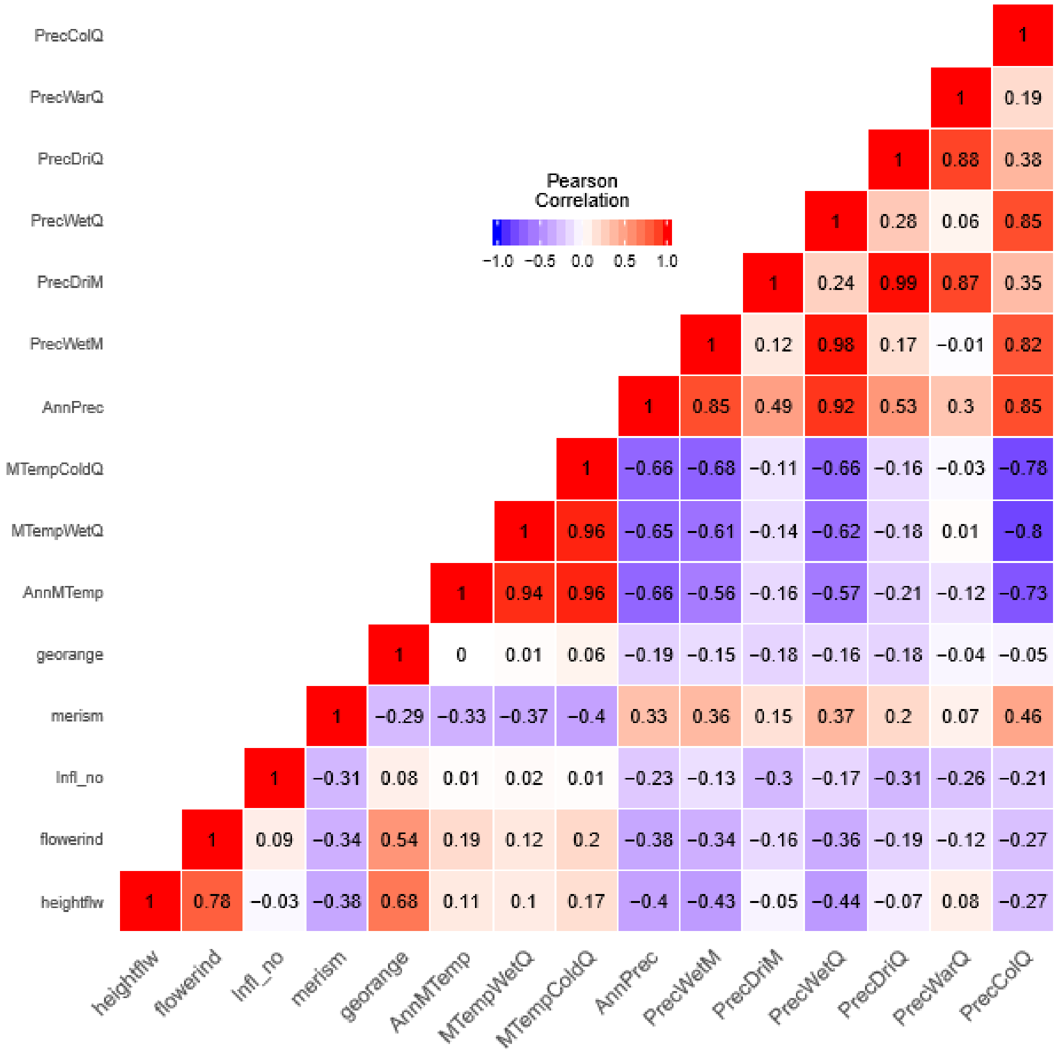
[1] Mean of monthly (Maximum temperature - Minimum temperature)

[2] (BIO02/BIO07) (*100)

[3] Standard deviation of monthly temperature averages

[4] (BIO05 – BIO06)

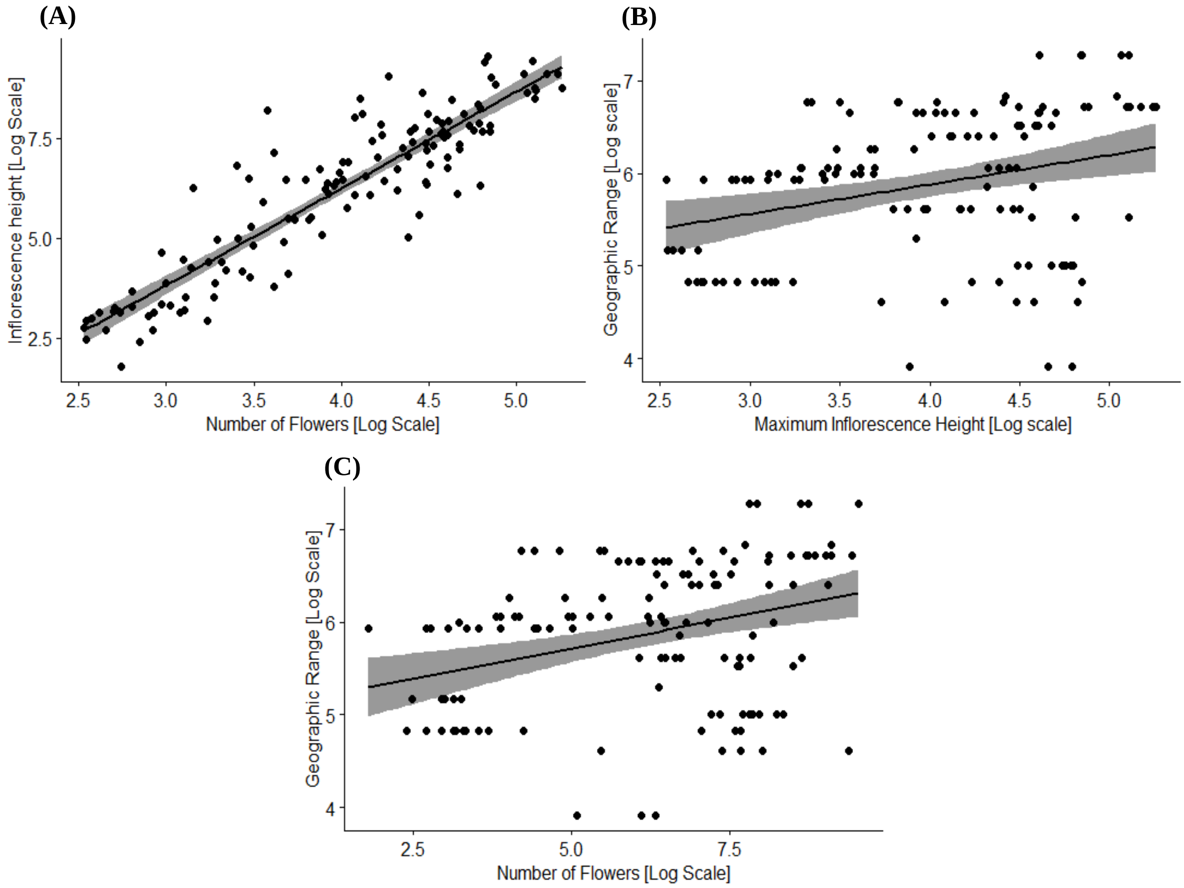
Appendix III



	heightflw	flowerind	Infl_no	merism	georange	AnnMTemp	MTempWetQ	MTempColdQ	AnnPrec	PrecWetM	PrecDriM	PrecWetQ	PrecDriQ	PrecWarQ	PrecColQ
PrecColQ															1
PrecWarQ														1	0.19
PrecDriQ													1	0.88	0.38
PrecWetQ												1	0.28	0.06	0.85
PrecDriM											1	0.24	0.99	0.87	0.35
PrecWetM										1	0.12	0.98	0.17	−0.01	0.82
AnnPrec									1	0.85	0.49	0.92	0.53	0.3	0.85
MTempColdQ								1	−0.66	−0.68	−0.11	−0.66	−0.16	−0.03	−0.78
MTempWetQ							1	0.96	−0.65	−0.61	−0.14	−0.62	−0.18	0.01	−0.8
AnnMTemp						1	0.94	0.96	−0.66	−0.56	−0.16	−0.57	−0.21	−0.12	−0.73
georange					1	0	0.01	0.06	−0.19	−0.15	−0.18	−0.16	−0.18	−0.04	−0.05
merism				1	−0.29	−0.33	−0.37	−0.4	0.33	0.36	0.15	0.37	0.2	0.07	0.46
Infl_no			1	−0.31	0.08	0.01	0.02	0.01	−0.23	−0.13	−0.3	−0.17	−0.31	−0.26	−0.21
flowerind		1	0.09	−0.34	0.54	0.19	0.12	0.2	−0.38	−0.34	−0.16	−0.36	−0.19	−0.12	−0.27
heightflw	1	0.78	−0.03	−0.38	0.68	0.11	0.1	0.17	−0.4	−0.43	−0.05	−0.44	−0.07	0.08	−0.27

Visualization of the Pearson correlation matrix of plot-level trait means of all 4 traits (maximum inflorescence height, number of flowers per individual, number of inflorescence per individual and merism) in the reduced dataset (n = 110 individuals) with 11 environmental variables (geographic range size, annual mean temperature, mean temperature of wettest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation of warmest quarter, and precipitation of coldest quarter). Positive correlations are shown in red and negative ones in blue color. As the correlation value moves away from absolute 0 the color intensity increases.

Appendix IV



Relationships between $\log_{10}$ inflorescence height (cm) and **(A)** Number of flowers ($R^2 = 0.83$; $P < 0.001$; n= 132 individuals) and **(B)** geographic range ($R^2 = 0.09$; $P < 0.001$; n= 132 individuals), and between **(C)** number of flowers and geographic range size ($R^2 = 0.11$; $P < 0.001$; n= 132 individuals). Solid lines represent the generalized linear model (GLM) results for inflorescence height and number of flowers, and inflorescence height and geographic range models. Grey shading indicates the 95% confidence interval. Black dots represent each sampled individual for 22 *Aeonium* species.

Appendix V

Post – hoc Dunn test for multiple comparison of genome sizes across the several Macaronesian Islands. Asterisks (*) shows the genome size significative differences between islands.

data: islands by samples.nucleus.pg
Kruskal-Wallis chi-squared = 29.9674, df = 4, p-value = 0

Comparison of x by group
(No adjustment)

Col Mean / Row Mean	Gran Canaria	La Gomera	Madeira	Santo Antão
La Gomera	2.307535 0.0105*			
Madeira	4.001212 0.0000*	3.250025 0.0006*		
Santo Antão	-0.154561 0.4386	-2.512001 0.0060*	-4.199572 0.0000*	
Tenerife	2.521347 0.0058*	0.286472 0.3873	-3.462203 0.0003*	2.733653 0.0031*

alpha = 0.05
Reject Ho if p <= alpha/2

Appendix VI

Post – hoc Dunn test for multiple comparison of genome sizes across the several *Aeonium* sections **(Bañares et *al.*, 2015)**. Asterisks (*) shows the genome size significative differences between sections.

data: sections by sample.nucleus.pg
Kruskal-Wallis chi-squared = 46.0507, df = 6, p-value = 0

Comparison of x by group
(No adjustment)

Col Mean Row Mean	**Aeonium**	**Canariensia**	**Chrysocome**	**Goochia**	**Greenovia**	**Leuconium**
Canariensia	3.634784 0.0001*					
Chrysocome	2.012868 0.0221*	-0.830794 0.2030				
Goochia	0.485864 0.3135	-2.411393 0.0079*	-1.322424 0.0930			
Greenovia	5.821615 0.0000*	2.749628 0.0030*	2.961138 0.0015*	4.409781 0.0000*		
Leuconium	2.556998 0.0053*	-1.589514 0.0560	-0.251846 0.4006	1.433919 0.0758	-4.330283 0.0000*	
Petrothamnium	4.392106 0.0000*	0.700756 0.2417	1.367886 0.0857	2.975739 0.0015*	-2.210178 0.0135*	2.448228 0.0072*

alpha = 0.05
Reject Ho if p <= alpha/2

Appendix VII

Post – hoc Dunn test for multiple comparison of genome sizes across the several *Aeonium* growth-forms. Asterisks (*) shows the genome size significative differences between growth – form types.

data: Growth.form by sample.nucleus.pg
Kruskal-Wallis chi-squared = 10.6798, df = 2, p-value = 0

Comparison of x by group
(No adjustment)

Col Mean / **Row Mean**	**BR**	**MR**
MR	0.097316 0.4612	
SS	-2.773271 0.0028*	-2.256018 0.0120*

alpha = 0.05
Reject Ho if p <= alpha/2

Appendix VIII

Post – hoc Dunn test for multiple comparison of genome sizes across the *Aeonium* species conservation status. Asterisks (*) shows the genome size significative differences between conservation status.

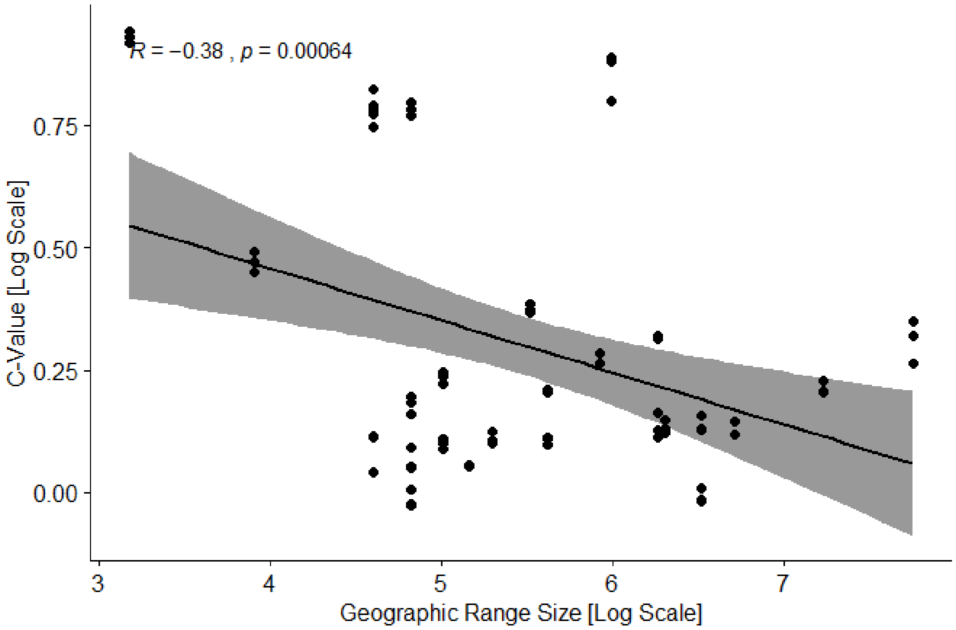
data: cons.status1 by sample.nucleus.pg
Kruskal-Wallis chi-squared = 13.4259, df = 2, p-value = 0

Comparison of x by group
(No adjustment)

Col Mean / **Row Mean**	**EN**	**LC**
LC	3.621747 0.0001*	
VU	2.755412 0.0029*	-1.076889 0.1408

alpha = 0.05
Reject Ho if p <= alpha/2

Appendix IX



Relationships between $\log_{10}$ 2C-value (pg) and geographic range size (n = 92 individuals). Solid lines represent the linear regression model and grey shading indicates the 95% confidence interval. R indicates the Spearman correlation coefficient. Black dots represent each sampled individual for 27 *Aeonium* species.

Appendix X

Aeonium species richness per protected area in Canary Islands.

Protected Areas	Number of species
Acantilados De La Culata	4
Amurga	1
Anaga	11
Archipelago De Chinijo	2
Ayagaures y Pilancones	1
Bandama	2
Barranco de Charco Hondo	3
Barranco De Guayadeque	1
Barranco De Las Angustias	6
Barranco De Ruiz	2
Barranco del Águila	2
Barranco Del Cabrito	4
Barranco Del Infierno	2
Barranco Del Jorado	2
Barrancos del Cedro y Liria	1
Benchijigua	7
Betancuria	1
Cabecera Barranco de Aguajilva	7
Campeches, Tigaiga Y Ruiz	3
Charco Del Conde	1
Chinyero	1
Corona Forestal	10
Costa De Hiscaguán	2
Costa de Majona, El Águila y Avalo	1
Cuenca de Benchijigua-Guarimiar	6
Cumbre Vieja	5
Cumbres y acantilados del norte de La Palma	8
Doramas	6
El Brezal	1
El Hierro	1
El Teide	2
Fataga	3
Frontera	6
Garajonay	1
Garajonay National Park	10
Guelguén	1
Ifonche	5
Ijuana	2
Islotes del norte de Lanzarote y Famara	2
La Caldera De Taburiente	3

La Fortaleza	1
La Geria	2
La Resbala	1
Laderas de Chío	1
Laderas de Enchereda	9
Las Cumbres	7
Las Lagunetas	8
Las Nieves	6
Las Playas	3
Lomo Del Carretón	2
Lomo Magullo	1
Los Órganos	1
Los Marteles	3
Los Volcanes	2
Macizo de Tauro II	2
Majona	7
Montaña del Cepo	5
Montañón Negro	4
Montes y cumbre de Tenerife	1
Nublo	7
Orone	9
Pijaral	3
Pilancones	1
Pinar De Garafía	1
Pino Santo	5
Reserva Marina De La Punta De La Restinga-Mar De Las Calmas	1
Risco De La Concepción	2
Riscos de Lara	1
Riscos De Tirajana	3
Roque Cano	4
Roque Nublo	3
Sabinar de La Galga	1
Siete Lomas	2
Tafira	1
Taguluche	2
Tamadaba	2
Tamanca	3
Teide National Park	8
Teno	11
Teselinde-Cabecera de Vallehermoso	5
Tibataje	5
Timanfaya	1
Timijiraque	2
Valle De Gran Rey	6
Ventejís	2
Volcanes De Teneguía	3