Patterns of colonization and diversification within Macaronesian Apiaceae lineages: Ecological and Cytogenomic approach

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Abstract

The Macaronesian region comprises five archipelagos (i.e., Azores, Madeira, Selvagens, Canary Islands and Cabo Verde) and harbours a rich endemic flora, approximately 900 vascular plant species. For this reason, it is considered a biodiversity hotspot and an ideal model to study evolutionary processes involved in flora diversification. The Apiaceae family is very relevant in agriculture and includes *taxa* such as the carrot *Daucus carota* subsp. *sativus* and new emerging crops such as the sea fennel *Crithmum maritimum*. Seventy Apiaceae *taxa* occur in the Macaronesia (including naturalized *taxa*), of which 35 are archipelago-endemic species. This thesis aimed to study two lineages of the Apiaceae family with different speciation models (i.e., cladogenesis and anagenesis), using cytogenomic data, complemented with ecological and morphological information. The cladogenesis speciation model is represented by the *Daucus* complex (*Daucus* – *Melanoselinum* – *Monizia* – *Tornabenea*), while *Crithmum maritimum* (a monotypic genus) represents an anagenesis speciation model. The study of different species belonging to the Daucinae subtribe revealed that the taxon is a good predictor of genome size, however, only at a species level, as it is unable to clearly discriminate the *Daucus carota* subspecies. Although from a monotypic genus, *Crithmum maritimum* presented a high cytogenomic variation. To determine which environmental factors, better explain the observed cytogenomic variation, we calculated different generalized linear models. Revealing a tendency for an increase in GS along the coast, from south to north, in association with lower temperatures, higher precipitation and lower precipitation seasonality. However, this gradient might result from historical phylogeographical events associated with previous dispersal and extinction of local populations. Finally, both studies seem to support the tendency of smaller genomes in islands comparing to mainland. Overall, this thesis provides new cytogenomic data that can contribute to understand the relationships among native Apiaceae species from Macaronesia and mainland Portugal, and how genome size shapes their evolution. Nonetheless, further morphological, and molecular data will help to clarify the radiation patterns and low-level taxonomies among Macaronesian native *taxa* and support informed decisions for the conservation of plant genetic resources in the Macaronesian Islands.

Keywords: Apiaceae, Diversification, DNA Flow Cytometry, Endemism, Macaronesia

Resumo

A região da Macaronésia compreende cinco arquipélagos (i.e., Açores, Madeira, Selvagens, Canárias e Cabo Verde) e alberga uma flora endémica bastante diversa, que inclui aproximadamente 900 espécies de plantas vasculares. Como tal, é considerado um *hotspot* de biodiversidade, sendo um modelo ideal para o estudo de processos evolutivos. A família das Apiaceae tem grande importância na agricultura e inclui espécies como a cenoura *Daucus carota* subsp. *sativus* e novas cultivares como o funcho marítimo *Crithmum maritimum*. Existem 70 espécies de Apiaceae na Macaronésia (espécies naturalizadas incluídas), das quais 35 são endémicas dos arquipélagos. Esta dissertação tem como objectivo estudar duas linhagens da família das Apiaceae que apresentam diferentes métodos de especiação (i.e., cladogénese e anagénese). O modelo de especiação por cladogénese é representado pelo complexo dos *Daucus* (*Daucus* – *Melanoselinum* – *Monizia* – *Tornabenea*), enquanto *Crithmum maritimum* (pertencente a um género monotípico) representa o modelo de especiação por anagénese. Para este estudo utilizámos dados citogenómicos, complementados com dados ecológicos e morfológicos. O uso das diferentes espécies pertencentes à subtribo Daucinae revelou que o taxon é um bom preditor do tamanho do genoma mas apenas ao nível da espécie, pois não consegue discriminar as diferentes subespécies de *Daucus carota*. Apesar de ser de um género monotípico, *Crithmum maritimum* apresentou uma grande variabilidade citogenómica. Para determinar quais os factores ambientais que melhor explicam a variação citogenómica observada, calculou-se diferentes modelos lineares, que revelaram uma tendência para o aumento do genoma de sul para norte, associada a temperaturas mais baixas, maiores precipitações e menor sazonalidade de precipitação. No entanto, este gradiente pode resultar de eventos filogeográficos históricos, associados a eventos de dispersão e extinção de populações locais. Finalmente, ambos os estudos confirmam a tendência de menores genomas em ilhas comparando com continentes. No geral, esta tese fornece novas dados citogenómicos que podem contribuir para a compreensão das relações entre as espécies nativas das Apiaceae da Macaronésia e de Portugal continental, e a forma como o tamanho do genoma estrutura a sua evolução. Contudo, novos dados morfológicos e moleculares irão não só ajudar a clarificar os padrões de diversificação, como contribuir para decisões informadas sobre a conservação dos recursos genéticos vegetais existentes nas ilhas da Macaronésia.

Palavras-chave: Apiaceae, Citometria de fluxo ADN, Diversificação, Endemismo, Macaronésia

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List of abbreviations

A – African territory

AIC – Akaike's Information Criterion

Az – Azores

AZB – Herbarium of the University of the Azores

Ca – Canary archipelago

CV – Cabo Verde

CVa – Coefficient of variation

D – Desertas

End – Endemic

Eu – European territory

F – Fogo

Fa – Faial

Fl – Flores

FCM – Flow cytometry

GS – Genome size

GLM – General linear model

I – Introduced

IUCN – International Union for Conservation of Nature

LISC – Herbarium of the Instituto de Investigação Científica Tropical from University of Lisbon

LISI – Herbarium of Instituto Superior de Agronomia from University of Lisbon

Lu – Mainland Portugal

Ma – Madeira archipelago

Mac – Endemic from Macaronesia

N – Native

PCA – Principal component analysis

Pi – Pico

pg – Picograms

PMCMR – Pairwise multiple comparison of mean ranks package

S – Santiago

Se – Selvagens archipelago

SA (Az) – Santa Maria

SA (CV) – Santo Antão

SN – São Nicolau

SV – São Vicente

TE – Transposable elements

UMAD – Herbarium of the University of Madeira

WPB – Woody plant buffer

Chapter 1

General introduction

1.1 Oceanic islands: The Macaronesia region as a study model

Oceanic islands can result from volcanoes rising above the water surface, close to the mid-ocean ridges, or where lithospheric plates converge (Whittaker & Fernández-Palacios, 2007). Oceanic islands have never been connected to continental landmasses and are depleted of life at their genesis (Gillespie, 2007). Therefore, it can be assumed that all species arrived there from the mainland or nearby islands. Some of the colonizing taxa will speciate, originating endemic species at a rate that will depend on the interplay of isolation and time (Shaw & Gillespie, 2016).

Although islands represent only 5% of Earth’s land surface, they host 25% of the world’s species of vascular plants (Kreft *et al.*, 2008; Kier *et al.*, 2009). On the Atlantic Ocean there are some well-recognized hotspot archipelagos, such as Madeira or Canary Islands (**Figure 1.1.**), which together with Azores, Selvagens, and Cabo Verde Islands, are commonly grouped as Macaronesian region. These islands are included in the Mediterranean basin biodiversity hotspot and characterized by high levels of endemism, with approximately 6520 endemic terrestrial *taxa* (Fernández-Palacios, 2011), of which 900 are endemic vascular plants (Whittaker & Fernández-Palacios, 2007).

Macaronesia is considered by several authors as a biogeographical entity based on the similarities of fauna and flora shared among the five archipelagos (Whittaker *et al.*, 2008; Fernández-Palacios, 2011). However, the validity and coherence of the Macaronesia region is a theme of discussion (e.g., Beyhl *et al.*, 1995; Hazevoet, 1995; Rivas-Martínez, 2009; Freitas *et al.*, 2019). Rivas-Martínez (2009) focused on the vegetation of each of its archipelagos and proposed that the Azores should be considered a biogeographic province of the Eurosiberian region, Madeira and Canary Islands should be included in the Mediterranean region, and that Cabo Verde subprovince should be included in the Sahelo-Sudanic biogeographic region.

Despite general acceptance of the biogeographical region of Macaronesia to include the Azores, Madeira, Selvagens, Canary and Cabo Verde archipelagos, the origin and geographical affinities of some lineages have generated controversy, particularly among the marine taxa (Freitas *et al.*, 2019). Nevertheless, endemic species that occur in mountain areas of Cabo Verde are more related to other Macaronesian species, namely from Canary and Madeira archipelagos. Among these species, are some of the biggest plant radiations in Macaronesia (e.g., *Aeonium* Webb & Berthel.; *Echium* L.; *Euphorbia* L.; *Micromeria* Benth.; *Sonchus* L.; *Tolpis* Adans.) and phylogenies published during the last two decades, suggest that the colonization of the Macaronesian Islands appears to be a recent event having taken place, for most of the plant lineages, in the Canary Islands from which the Cabo Verde archipelago should have been colonized (Romeiras *et al.* 2019). Also in Macaronesia, several endemic Apiaceae species occur, and among them 10 endemic taxa of the subtribe Daucinae, which from an economical point of view is an important group of species, because it includes the cultivated carrot (*Daucus carota* subsp. *sativus*) (Wojewódzka *et al.,* 2019).

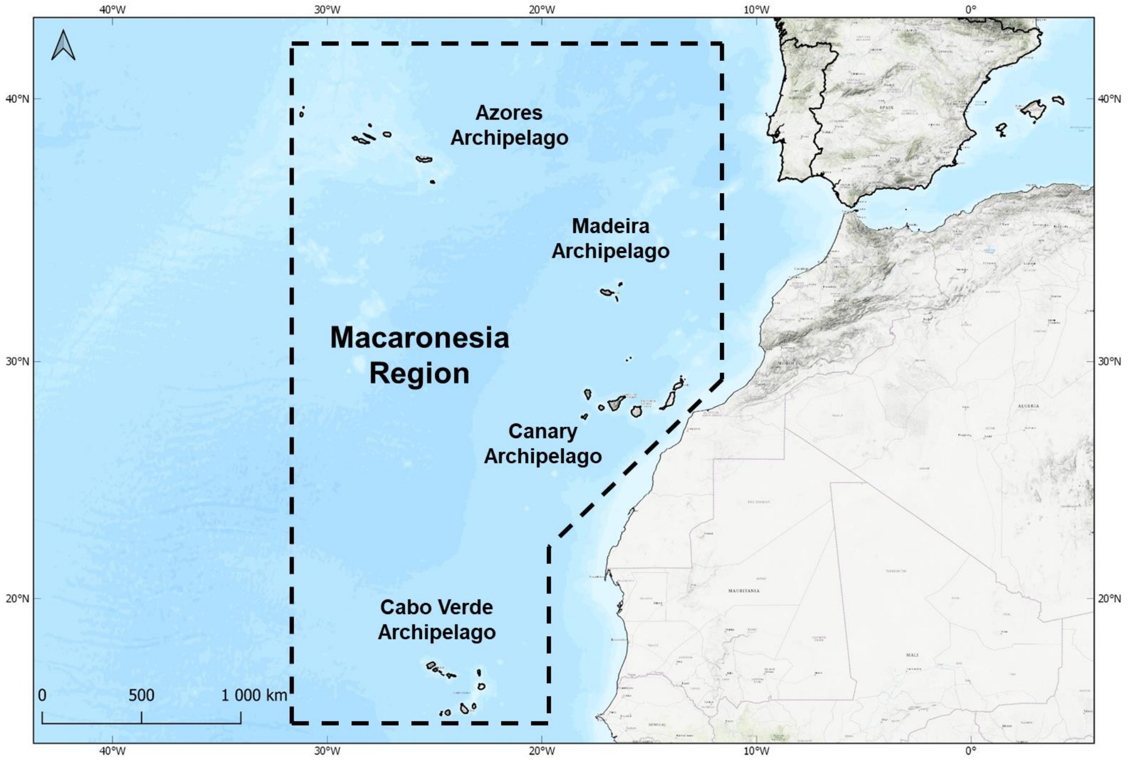


Figure 1.1: Map of the location of the Macaronesia region, the study area.

1.2 Apiaceae family

The Apiaceae family (Umbelliferae) includes 428 genera and *circa* 3500 species, including herbs and, less frequently, shrubs and trees (Mabberley, 2017). The most characteristic morphological aspect of this family is the inflorescence, the umbel, that can be simple or compound; the fruit is a schizocarp consisting of two fused carpels that upon maturation separate into two mericarps with one seed each (Mabberley, 2017). It is nearly worldwide distributed, but is most diverse in areas of temperate climate, such as Eurasia and North America, and is quite rare in tropical humid regions (Reduron, 2020). It occurs in a variety of habitats, from sea level to alpine zones (Watson *et al.,* 2001). The Apiaceae family appears to have originated in Australasia (region including Australia, Tasmania, New Zealand, New Guinea, New Caledonia, and several island groups) during the late Cretaceous/ early Eocene (Nicolas & Plunkett, 2014).

The Apiaceae are greatly important in agriculture, namely *taxa* such as the carrot (*Daucus carota* subsp. *sativus*), celery (*Apium graveolens*), parsley (*Petroselinum crispum*), fennel (*Foeniculum vulgare*), and many others. The local communities tend to use further Apiaceae species, found in the native flora. For example, during the human colonization of the Madeira island, *Monizia edulis* used to be cooked and eaten as a digestive, the leaves of *Crithmum maritimum* were used as diuretic and stomachic (Rivera & Obon, 1995), and *Tornabenea bischoffii* was used to treat discomforts related to the skin (Ortet *et al.*, 2011).

In Macaronesia, 70 Apiaceae taxa occur (including naturalized ones, **Table 1.1.**), of which 35 are endemic to the archipelago and two are shared between Madeira and the Canary Islands (*Drusa glandulosa* and *Bupleurum salicifolium* subsp. *salicifolium*). The archipelago with the highest number of endemic species is the Canary Islands with 18 *taxa*, followed by Madeira with seven, and Azores and Cabo Verde with six. The reason for such a diversity of endemic *taxa* is that the original colonizers speciated in a unique insular environment isolated from its congeners (Sulloway, 1982), resulting in lineages that diversified into several new *taxa* and others that did not.

Table 1.1. Apiaceae taxa that occur in the Macaronesia region and their status in each archipelago (Az: Azores; Ma: Madeira; Se: Selvagens; Ca: Canary; CV: Cabo Verde; I: Introduced; N: Native; End: Endemic; Mac: Endemic from Macaronesia)

Taxon	**Status**				
	Az	**Ma**	**Se**	**Ca**	**CV**
Ammi majus	I	N	N	N	
Ammi procerum				N	
Ammi seubertianum	End				
Ammi trifoliatum	End				
Ammi visnaga	I	N		N	
Ammodaucus nanocarpus				N	
Anethum graveolens	I			I	I
Angelica lignescens	End				
Anthriscus caucalis		I		I	
Apium graveolens	I	N		I	I
Apium nodiflorum	N	N		N	
Apium repens				N	
Astydamia latifolia			N	N	
Bifora testiculata				I	
Bunim brevifolium		End			
Bupleurum handiense				End	
Bupleurum lancifolium		N		I	
Bupleurum salicifolium* subsp. *aciphyllum				End	
Bupleurum salicifolium* subsp. *salicifolium		Mac		Mac	
Bupleurum semicompositum				N	
Capnophyllum peregrinum				I	I
Chareophyllum azoricum	End				
Conium maculatum	I	I		I	
Coriandrum sativum	I	I		I	I
Crithmum maritimum	N	N		N	
Cryptotaenia elegans				End	
Cyclospermum leptophyllum	I	I		I	I

Daucus carota **subsp.** ***azoricus***	End			
Daucus carota **subp.** ***carota***		N		
Daucus carota **subsp.** ***hispidus***		N		
Daucus carota **subsp.** ***maritimum***	I			
Daucus carota **subsp.** ***maximus***			N	
Daucus durieua			I	
Drusa glandulosa		Mac	Mac	
Ferula lancerottensis			End	
Ferula latipinna			End	
Ferula linkii			End	
Foeniculum vulgare	I	N	N	I
Melanoselinum decipiens		End		
Monizia edulis		End		
Oenanthe divaricata		End		
Petroselinum crispum	I	I	I	I
Peucedanum lowei		End		
Pimpinella anagodendron			End	
Pimpinella cumbrae			End	
Pimpinella dendrotragium			End	
Pimpinella junoniae			End	
Pimpinella rupicola			End	
Pimpinella villosa	I			
Ridolfa segetum	I		I	
Sanicula azorica	End			
Scandix pecten-veneris		N	N	
Seseli webbii			End	
Smyrnium olusatrum			I	
Tinguarra cervariaefolia			End	
Todaroa aurea **subsp.** ***aurea***			End	
Todaroa aurea **subsp.** ***suaveolens***			End	
Todaroa montana			End	
Torilis arvensis **subsp.** ***arvensis***	I	N	N	
Torilis arvensis **subsp.** ***neglecta***	I	N	N	
Torilis arvensis **subsp.** ***purpurea***		N	N	
Torilis elongata			N	
Torilis leptophylla			N	
Torilis nodosa		N	N	
Torilis webbii			N	
Tornabenea annua				End
Tornabenea bischoffii				End
Tornabena humilis				End
Tornabenea insularis				End
Tornabena ribeirensis				End
Tornabenea tenuissima				End

The Daucinae subtribe encompasses umbellifers that have fruits with prominent secondary ridges projecting into wings or spines. It includes *genera* such as *Cuminum*, *Daucus*, *Laser*, *Thapsia* and the Macaronesian endemic *genera Melanoselinum*, *Monizia* and *Tornabenea*. The first phylogenetics studies carried out in the Laserpitieae and Caucalideae revealed that these tribes were polyphyletic and that species from both tribes grouped to form the *Daucus* clade (Downie & Katz-Downie, 1996; Plunkett *et al*., 1996). This means that some nodes in the Daucinae tree proved to be extremely problematic and remained unresolved, particularly among the Macaronesian endemic *taxa*. In the Macaronesia, 10 endemic *taxa* of this subtribe occur (i.e., *Daucus carota* subsp. *azoricus*, *Cryptotaenia elegans*, *Melanoselinum decipiens*, *Monizia edulis*, *Tornabenea annua*, *Tornabenea bischoffii*, *Tornabenea humilis*, *Tornabenea insularis*, *Tornabenea ribeirensis*, *Tornabenea tenuissima*), which have been subjected to several phylogenetic studies (Banasiak *et al.*, 2016; Frankiewicz *et al.*, 2020; Wojewódzka *et al.*, 2019), leading to the reappraisal of the endemic Macaronesian *taxa* and their inclusion in the *Daucus* genus. This genus includes 43 accepted species (sub-species not included) and is widespread in the northern hemisphere (POWO, 2019).

On the other hand, the Apiaceae family also includes *taxa* that did not diversify, such as *Crithmum maritimum*, a monospecific genus (Meot-Duros & Magne, 2009) with a very wide distribution, occurring along the European Atlantic coasts, Azores, Madeira, and Canary Islands archipelagos, the Mediterranean and Black Sea coasts, northwest Africa, and West Asia (Crawford & Stuessy, 1997). Therefore, the Macaronesian native Apiaceae *taxa* are an ideal model to understand which factors are more important to trigger speciation along the process of plant evolution.

1.3 Flow cytometry

A comprehensive understanding of plant genome sizes is still largely missing, due to the relatively small number of species examined to date (*circa* 12,273 species, Pellicer & Leitch, 2020). The flow cytometry (FCM) technique is an effective tool to estimate DNA content of nuclei (Galbraith *et al.*, 2021). It processes large numbers of particles, providing information about cellular composition of tissues and individuals, as well as the composition of populations and communities (Kron *et al.*, 2007). For this fact, there is an increasing demand for C-values (i.e., haploid nuclear genome sizes) as phylogenetic (e.g., Leitch *et al.*, 1998) and taxonomic markers (e.g., Chumová *et al.* 2015). For example, a study was able to support the currently defined species based on the combination of life cycle and genome size (Chumová *et al.*, 2015). C-values can also be used as ecological indicators. For example, in *Helianthus* spp. (Asteraceae) negative correlations between genome size (GS) and temperature seasonality were found (Qiu *et al.*, 2019), and in *Zea mays* (Poaceae) GS was found to negatively correlate with altitude (Díez *et al.*, 2013). Regarding Macaronesian endemic species, the GS of *Crambe* (Brassicaceae) and *Sonchus* (Asteraceae) increases with altitude and rainfall and decreases with temperature (Suda *et al.*, 2003; Suda *et al.*, 2005). Therefore, the potential of FCM to approach intraspecific and interspecific variation in recently evolved species is of interest and deserves further study. Furthermore, when integrated with ecological studies, FCM can play an important role in elucidating patterns of diversification in hotspot insular regions, such as the Macaronesian region.

1.4 General objectives

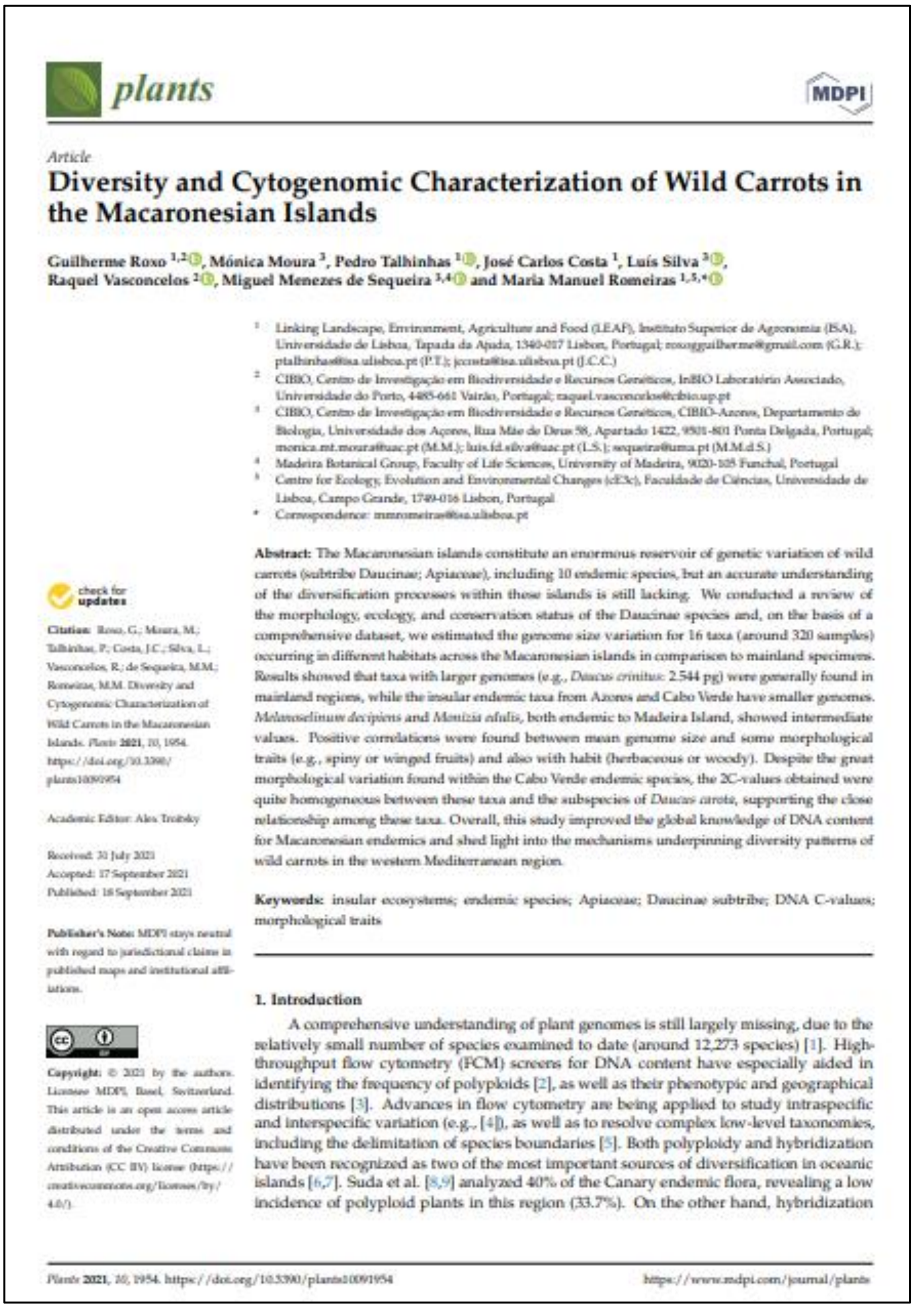
This thesis is organised as follows: a general introduction, two research articles, general conclusions, and supplementary material. Both articles report cytogenomic approaches to study the diversification of the Macaronesian native Apiaceae taxa. The low resolution of the phylogeny outlined the difficulty of an accurate understanding of the systematics and the relationships within some Macaronesian groups. Therefore, cytogenomic approaches can be highly relevant to understand both the diversification of groups (cladogenesis) and the lineages which did not diversify (anagenesis) but present a high cytogenomic heterogeneity.

The first article, already published, focuses on the Daucinae subtribe, which diversified across all the Macaronesian islands, aiming to review and clarify the taxonomy of the Daucinae taxa native to Macaronesia, based on ecological, morphological and cytogenomic data. This information is of utter importance because most of these crop wild relatives (CWR) are endemic to these island ecosystems, and their presence and conservation are likely to be threatened by climate changes.

The second article, in preparation, focuses on *Crithmum maritimum*, a monospecific genus with a wide distribution across the Mediterranean region and present in all the Macaronesia archipelagos except Cabo Verde. The main objective was to understand the high cytogenomic variation across the different populations, and if it correlates with ecological variables.

Chapter 2

Manuscript I



plants

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Article

Diversity and Cytogenomic Characterization of Wild Carrots in the Macaronesian Islands

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Abstract: The Macaronesian islands constitute an enormous reservoir of genetic variation of wild carrots (subtribe Daucinae; Apiaceae), including 10 endemic species, but an accurate understanding of the diversification processes within these islands is still lacking. We conducted a review of the morphology, ecology, and conservation status of the Daucinae species and, on the basis of a comprehensive dataset, we estimated the genome size variation for 16 taxa (around 320 samples) occurring in different habitats across the Macaronesian islands in comparison to mainland specimens. Results showed that taxa with larger genomes (e.g., *Daucus crinitus*: 2.544 pg) were generally found in mainland regions, while the insular endemic taxa from Azores and Cabo Verde have smaller genomes. *Melanoselinum decipiens* and *Monizia edulis*, both endemic to Madeira Island, showed intermediate values. Positive correlations were found between mean genome size and some morphological traits (e.g., spiny or winged fruits) and also with habit (herbaceous or woody). Despite the great morphological variation found within the Cabo Verde endemic species, the 2C-values obtained were quite homogeneous between these taxa and the subspecies of *Daucus carota*, supporting the close relationship among these taxa. Overall, this study improved the global knowledge of DNA content for Macaronesian endemics and shed light into the mechanisms underpinning diversity patterns of wild carrots in the western Mediterranean region.

Keywords: insular ecosystems; endemic species; Apiaceae; Daucinae subtribe; DNA C-values; morphological traits

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1. Introduction

A comprehensive understanding of plant genomes is still largely missing, due to the relatively small number of species examined to date (around 12,273 species) [1]. High-throughput flow cytometry (FCM) screens for DNA content have especially aided in identifying the frequency of polyploids [2], as well as their phenotypic and geographical distributions [3]. Advances in flow cytometry are being applied to study intraspecific and interspecific variation (e.g., [4]), as well as to resolve complex low-level taxonomies, including the delimitation of species boundaries [5]. Both polyploidy and hybridization have been recognized as two of the most important sources of diversification in oceanic islands [6,7]. Suda et al. [8,9] analyzed 40% of the Canary endemic flora, revealing a low incidence of polyploid plants in this region (33.7%). On the other hand, hybridization

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Diversity and cytogenomic characterization of wild carrots in the Macaronesian Islands

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Abstract

Macaronesian Islands constitute an enormous reservoir of genetic variation of wild carrots (subtribe Daucinae; Apiaceae), including 10 endemic species, but an accurate understanding of the diversification processes within these islands is still lacking. This paper conducts a review of the morphology, ecology, and conservation status of the Daucinae species and based on a comprehensive dataset, we estimate the genome size variation for 16 taxa (ca. 320 samples) occurring in different habitats across the Macaronesian Islands by comparison to mainland specimens. Results show that taxa with larger genomes (e.g., *Daucus crinitus*: 2.544 pg) were generally found in mainland regions, while the insular endemic taxa from Azores and Cabo Verde have smaller genomes. *Melanoselinum decipiens* and *Monizia edulis*, both endemic in Madeira Island, showed intermediate values. Positive correlations were found between mean genome size and some morphological traits (e.g., spiny, or winged fruits) and also with habit (herbaceous or woody). Despite the great morphological variation found within the Cabo Verde endemic species, the 2C-values obtained were quite homogeneous between these taxa and the subspecies of *Daucus carota*, supporting a close relationship among these taxa. Overall, this study improved the global knowledge of DNA content for Macaronesian endemics and shed light into the mechanisms underpinning diversity patterns of wild carrots in Western Mediterranean Region.

Keywords: Apiaceae, Daucinae subtribe, DNA C-value, Endemic species, Insular ecosystems, Morphological traits

2.1. Introduction

A comprehensive understanding of plant genomes is still largely missing, due to the relatively small number of species examined to date (ca. 12,273 species) (Pellicer & Leitch, 2020). High-throughput flow cytometry (FCM) screens for DNA content have especially aided in identifying the frequency of polyploids (Galbraith *et al.*, 2021), as well as their phenotypic and geographical distributions (Laport & Ng, 2017). Advances in flow cytometry are being applied to study intraspecific and interspecific variation (e.g., Lopes *et al.*, 2021), and to resolve complex low-level taxonomies, including the delimitation of species boundaries (Husband *et al.*, 2013). Both polyploidy and hybridization have been recognized as two of the most important sources of diversification in oceanic islands (Wood *et al.*, 2009; Curto *et al.*, 2018). Suda *et al.*, (2003, 2005), analyzed 40% of the Canary endemic flora, revealing a low incidence of polyploid plants in this region (33.7%). On the other hand, hybridization appears to be common in most species-rich and rapidly diversifying groups (Whitney *et al.*, 2010), in fact some studies indicate that after multi-introductions, hybridization plays a greater role in diversification than the progressive accumulation of genetic variation through time (Kerbs *et al.*, 2017). In addition, the number of endemic species per genus was found to be negatively correlated with genus-average genome size, regarding insular lineages that radiated (i.e., cladogenesis) on the Hawaii and Marquesas archipelagos (Kapralov & Filatov, 2011)

The northeastern Atlantic Macaronesia Region (i.e., Azores, Madeira, Selvagens, Canary and Cabo Verde archipelagos) shows high levels of plant endemism (ca. 900 endemic taxa), with several range-restricted species occurring in different habitats (Whittaker & Fernández-Palacios, 2007). These islands have a remarkable genetic diversity (García-Verdugo *et al.*, 2015) regarding Crop Wild Relatives (CWR), which are related to globally important crops (Romeiras *et al.*, 2016; Rocha *et al.*, 2021). In Apiaceae, the Daucinae subtribe, has an important economic value (Rong *et al.*, 2010) since it includes the widely consumed carrot (*Daucus carota* subsp. *sativus*). Regarding its CWR, Macaronesia harbors 10 Macaronesian endemic taxa from five different genera, the Azorean *Daucus carota* subsp. *azoricus*, *Melanoselinum decipiens* and *Monizia edulis* from Madeira, *Tornabenea* the only endemic genus from Cabo Verde, which radiated in six different taxa, *Tornabenea annua*, *Tornabenea bischoffii*, *Tornabenea humilis*, *Tornabenea insularis*, *Tornabenea ribeirensis*, and *Tornabenea tenuissima* (**see Figure 2.1**) and *Cryptotaenia elegans* from the Canary Islands.

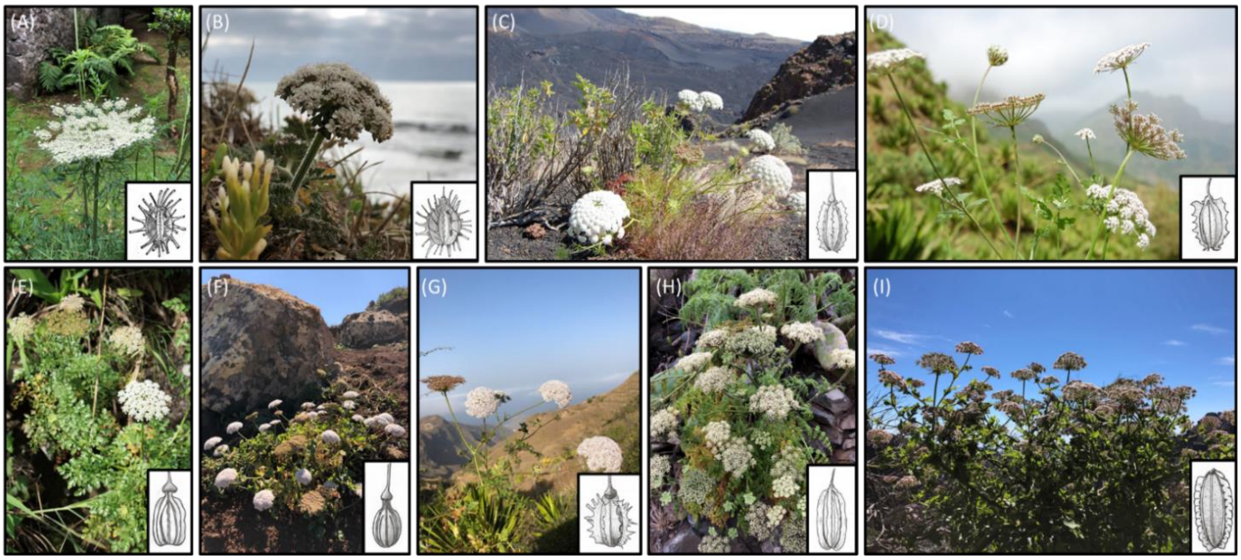

Figure 2.1. Daucinae subtribe endemic species from Macaronesia and mainland Portugal. Drawings of the fruits of each taxon are displayed. (A) *Daucus carota* subsp. *azoricus*; (B) *Daucus carota* subsp. *halophilus*; (C) *Tornabenea tenuissima*; (D) *Tornabenea annua*; (E) *Tornabenea ribeirensis*; (F) *Tornabenea bischoffii*; (G) *Tornabenea insularis*; (H) *Monizia edulis*; and (I) *Melanoselinum decipiens*.

Daucus carota subsp. *azoricus* was described by Franco (1971). Due to infraspecific phenotypical diversity, there is uncertainty on distribution and diagnostic characters. A recent genomic study did not support the Azorean *Daucus carota* as a distinct taxon. Arbizu *et al.* (2016) and Frankiewicz *et al.* (2020) suggested that the plants were dispersed by settlers. A molecular genetics study including a thorough archipelago-wide sampling is currently underway to better clarify the taxonomy of the Azorean *Daucus* (Moura, personal comm.).

The *Melanoselinum* genus was described by Hoffmann (1814) to accommodate a specimen of unknown origin growing in the botanical garden of Hannover. In 1829, Richard Lowe found it along the Ribeira de São Jorge, Madeira Island (Lowe, 1838). Since then, various authors, have given accounts of the diversity and taxonomy of this genus, which is currently accepted as a monotypic genus (Chevalier, 1935; Press & Short, 1994; Jardim & Menezes de Sequeira, 2008).

In 1855, Richard Lowe found *Monizia edulis* plants growing in a sea cliff on the east side of Deserta Grande (Madeira archipelago). In 1856, Lowe (1856) described this new taxon, and reported it (Lowe, 1869) in the Selvagens archipelago (Selvagem Grande) based on observations by the owner of those islands at that time, Constantino Cabral de Noronha. No further reports occurred, and Jardim & Menezes de Sequeira

(2008) considered it extinct in Selvagens. It also occurs on Porto Santo (Madeira archipelago), restricted to Ilhéu de Cima (Carvalho *et al.*, 2013).

Undescribed specimens of *Tornabenea* were first collected by Vogel in 1841. In 1849, Parlatore, with material from a single species (*Tornabenea insularis*), established a new tribe with a monospecific genus, *Tetrapleura* (Hooker *et al.*, 1849a). The name was a homonym and renamed to *Tornabenea* by Parlatore (Hooker *et al.*, 1849b). In 1851, J. A. Schmidt collected material later described as *Tornabenea bischoffii* and *Tornabenea hirta* (Schmidt, 1852). These were considered synonyms by Lobin & Zizka (1987), while Martins (1996), included both taxa in the "Cabo Verde Flora" as synonyms of *Tornabenea insularis*. The number of accepted species in the genus has recently varied from three, Martins (1996) (*Tornabenea annua*, *Tornabenea insularis* and *Tornabenea tenuissima*), to five, Brochmann *et al.* (1997), by including *Tornabenea bischoffii* and *Tornabenea humilis* and, finally, to six, Sánchez-Pinto *et al.* (2005), by including *Tornabenea ribeirensis*.

Despite the publication of several phylogenetic studies to resolve the evolutionary relationships among the major clades of Daucinae subtribe (e.g., Downie *et al.*, 2000; Downie *et al.*, 2001; Spalik & Downie, 2007; Iorizzo *et al.*, 2013; Spooner *et al.*, 2013), some nodes in the Daucinae tree have proved to be extremely problematic and have remained unresolved, particularly among the Macaronesian endemic taxa. Recent studies including the analysis of wood anatomy, life form, life span and reproductive strategies, led to a reappraisal of the endemic genera *Melanoselinum*, *Monizia* and *Tornabenea*, and their inclusion in the *Daucus* genus (Frankiewicz *et al.*, 2020; Spooner *et al.*, 2013; Banasiak *et al.*, 2016). Also, using Genotyping-by-Sequencing, Martínez-Flores *et al.* (2020) provided evidence that at least some *Tornabenea* species should be included within the *Daucus carota* complex, constituting a remarkable case of allopatric speciation in the Cabo Verde Islands with total or rarely almost total reduction of mericarp spines. However, only two species (i.e., *Tornabenea annua* and *Tornabenea tenuissima*) were evaluated by the authors. Therefore, relationships within Macaronesian Daucinae taxa are still poorly understood and a good field-sampling is needed, along with fossil data to produce a robust phylogeny (Romeiras *et al.*, 2019). Nevertheless, Góis-Marques *et al.* (2019) recently described the oldest macrofossil of a wild carrot and the first fossil of a plant displaying insular woodiness (i.e., *Melanoselinum decipiens*), providing a calibration for future Apiaceae phylogenies.

The low resolution of the phylogeny within the Macaronesian group outlined the difficulty of an accurate comprehension of the systematics and the relationships within the Daucinae subtribe. Thus, unraveling the variations in genome size (also known as C-value) within this subtribe, can be highly relevant to understand its diversification in the Macaronesian region. Although the meaning of the variation in the C-value is still not completely understood, it is known that the variation can be correlated to non-coding DNA, more specifically transposable elements (Gregory, 2005). Several life-history traits have been found to correlate with genome size through the associated effects of nuclear DNA content on cell size (Hessen & Pearson, 2009). Similarly, significant associations between genome size and conservation status suggest that threatened species are more prone to be associated with larger genomes (Brilhante *et al.*, 2021).

Native plants from Macaronesian Islands are significantly underrepresented in the existing genome size database (Pellicer & Leitch, 2020) but show a remarkable range of genome size (Suda *et al.*, 2005). Regarding the endemic taxa of the Daucinae subtribe, only two species have been analyzed so far, *Daucus carota* subsp. *azoricus* (2C-values = 1.64 ± 0.02 pg, Nowicka *et al.*, 2016) and *Cryptotaenia elegans* (2C-values = 0.94 ± 0.01 pg, Suda *et al.*, 2005). Moreover, the counting of chromosomes has also been used extensively as an important phylogenetic character in the context of cytotaxonomy (Guerra, 2008), helping in the definition of different taxa.

Within the Macaronesian Islands, molecular studies with CWR have been focused on establishing their taxonomic status and depicting phylogenetic relationships (e.g., Romeiras *et al.*, 2016; Dias *et al.*, 2018). However, few studies have been carried out using cytogenomic data, complemented with data on the morphology and ecology of CWR species. The present study aimed to review of the morphology, ecology (including associated plant communities), and conservation status of the Daucinae species, and based on a comprehensive dataset, we estimated the genome size variation for 16 taxa, which occur in different habitats across the Macaronesian Islands by comparison to mainland specimens. Such data are needed because most of these CWR are endemic species within these island ecosystems, and their presence and conservation are likely to be threatened by ongoing climate change.

2.2. Materials and methods

2.2.1. Study area

Taxa from the Daucinae subtribe occur in all Macaronesian archipelagos. However, our sampling focused on Azores, Madeira, and Cabo Verde, where the main genera of this subtribe occur (i.e., *Daucus* L., *Melanoselinum* Hoffm., *Monizia* Lowe. and *Tornabenea* Parl.) (**Figure 2.2**). The Azores archipelago consists of nine islands, is the northernmost one of Macaronesia and is located approximately 1300 km west of mainland Portugal. It is composed of an eastern group of islands (São Miguel and Santa Maria), a central group (Faial, São Jorge, Graciosa, Pico, and Terceira) and a western group (Flores and Corvo). The Madeira archipelago comprises Madeira, the largest island of the archipelago, Porto Santo and its six islets, and Desertas (Bugio, Deserta Grande and Ilhéu Chão). Cabo Verde includes ten main islands and several islets, grouped into two main sets according to the prevailing NE winds: the windward islands – Santo Antão, São Vicente, Santa Luzia, São Nicolau, Sal and Boavista –, and the Leeward islands – Maio, Santiago, Fogo, and Brava.

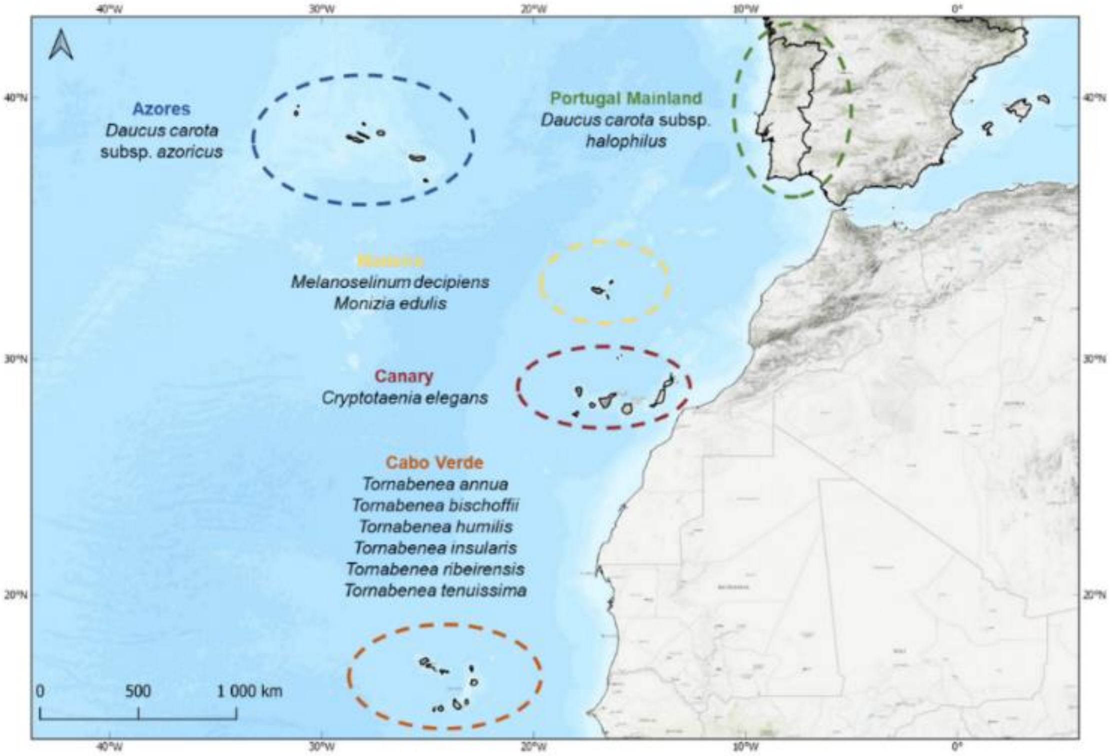

Figure 2.2. Distribution of the target endemic species in Macaronesia and mainland Portugal.

2.2.2. Studied Macaronesian endemics and sampling

In the Macaronesia, 10 endemic species occur, of which eight were included in the study: *Daucus carota* L. subsp. *azoricus* Franco; *Melanoselinum decipiens*; *Monizia edulis*; *Tornabenea annua*, *Tornabenea bischoffii*, *Tornabenea insularis*, *Tornabenea ribeirensis*, and *Tornabenea tenuissima* (**see Figure 2.1**). The data presented in **Table 2.1** and **Table 2.2** were obtained from a thourough bibliographic revision, which included the protologues and a morphometric revision of the type specimens, whenever deemed possible.

For cytogenomic studies, specimens of the Daucinae subtribe were collected during several field surveys in the Macaronesia archipelagos from 2017 to 2020. Fieldwork took place in four islands of Azores (Faial, Flores, Pico, and Santa Maria), two islands of Madeira (Madeira and Deserta Grande) and five islands of Cabo Verde (Santiago, Santo Antão, São Nicolau, São Vicente, and Fogo). Additionally, specimens from mainland Portugal (Beja, Faro, Leiria, Lisboa, and Setúbal) were also sampled. A total of 16 native taxa (from 48 different populations) were sampled for the study (**Supplementary Data Table 2.S4**). Furthermore, from each population a minimum of three specimens was collected. The samples were preserved in wet tissue paper wrapped in aluminium foil and zip-locked bags at 5 °C, and posted to the laboratory, C-DNA data were obtained after a maximum period of three days.

Vouchers from the majority of the samples were collected and deposited at the Herbarium of Instituto Superior de Agronomia, University of Lisbon (LISI), at the Herbarium of the University of Madeira (UMAD) and at the Herbarium of the University of the Azores (AZB).

2.2.3. Data collection

A database for the sampled species was assembled, including information for each taxon on (i) geographical distribution, (ii) accepted scientific names according to Banasik *et al.* (2016), Martínez-Flores (2016), and Wojewódzka *et al.* (2019) (iii) habit, (iv) habitat (herbaceous or woody) (v) conservation status, (vi) number of chromosome and (vii) Raunkiaer classification (**Table 2.S1**) (Raunkiaer, 1934).

Information on the geographical distribution (inside Macaronesia and worldwide) was extracted from the most recent checklists (Sánchez-Pinto *et al.*, 2005; Silva *et al.*, 2005; Jardim & Menezes de Sequeira, 2008; Menezes de Sequeira, 2011).

Information on the habit and morphology of all sampled taxa were obtained from floras of each archipelago (Press & Short, 1994; Martins, 1996; Brochmann *et al.*, 1997; Franco & Rocha-Afonso, 1984; Castroviejo, 2003) and included the following morphological traits: (i) leaves, (ii) inflorescence, (iii) bracts, (iv) bracteoles, (v) fruits, and (vi) secondary ribs of the fruit (spiny vs winged).

Drawings (**see details in Figure 2.1**) were made based on photographs and on herbarium specimens housed in LISI and Herbarium of the Instituto de Investigação Científica Tropical from University of Lisbon (LISC). Ecological data including sinecological data is based on Mucina *et al.* (2016) and Rivas-Martinez *et al.* (2017). The chromosome number of the various taxa was obtained consulting Bramwell & Murray (1972), Borgen (1974), Zizka (1986), Dalgaard (1994), Grosso *et al.* (2008), Nowicka *et al.* (2016), and Spooner (2019). The conservation status of the sampled species was obtained following the International Union for Conservation of Nature (IUCN) Red List of threatened species criteria (IUCN, 2020), Corvelo (2010) and Romeiras *et al.* (2016).

2.2.4. Cytogenomic analysis

Nuclear DNA content was estimated using flow cytometry (FCM). Preparation of suspensions of intact nuclei for analysis was performed following the method of Galbraith *et al.* (1983). 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 MgCl2, 1% Triton X-100, Na2EDTA 2 mM, NaCl 86 mM, sodium metabisulfite 20 mM, PVP-10 a 1%, pH 7,5; Loureiro *et al.*, 2007). The nuclear suspension was sieved through a nylon mesh with 30 µm to remove large debris. The obtained nuclei were stained with 25 µg mL-1 of propidium iodide (PI; Sigma-Aldrich, USA). To estimate the nuclear DNA content, the use of a reference standard of known genome size is required. The following standards were employed: *Solanum lycopersicum* L. (2C = 1.96 pg; Doležel *et al.*,1992), *Raphanus sativus* L. (2C = 1.11 pg; Doležel *et al.*,1992), and *Rhamnus alaternus* L. (2C = 0.68 pg; Carvalho *et al.*, 2018). Furthermore, the acquisition of numeric data and fluorescence graphs was made by Sysmex FloMax software v2.4d (Sysmex, Görlitz, Germany), as described by Guilengue *et al.* (2020). From the analyzed samples, the diploid quantity of DNA (in pg, per nucleus) was estimated using the formula:

$$\text{Nuclear DNA Content (pg)} = \frac{\text{Sample G1 Peak Mean } \times \text{Genome size of Reference Standard}}{\text{Reference Standard G1 Peak Mean}}$$

2.2.5. Statistical analysis

Statistical analyses and descriptive statistics were performed using R software (R Core team, 2019). Descriptive statistics were calculated for each species, namely mean and standard deviation (SD) of the genome size (2C-values, pg). For the data on 2C-values, descriptive analyses were performed using the boxplot statistical algorithm. Comparisons between genome size values and: i) Habit (woody vs herbaceous); ii) Raunkiaer classification (chamaephyte vs hemicryptophte vs terophyte) (see Table 2.S1); iii) Distribution (archipelago vs mainland); and iv) Morphology of secondary ribs (spiny vs winged). Group comparisons were implemented using non-parametric tests since genome size data were not Gaussian ($p<0.05$ with the Shapiro–Wilk test; Shapiro & Wilk, 1965) even after using Box-Cox Transformation (Box & Cox, 1964) or other conventional transformation techniques (Zar, 2010). Thus, we opted for the Mann-Whitney test for two group comparisons and for the Kruskal-Wallis test for comparisons of more than two groups, using the respective functions in R. Since the Kruskal-Wallis test indicated the rejection of the null hypothesis, we applied a non-parametric multiple comparison test (Conover & Iman, 1979; Siegel & Castellan, 1988) using the function posthoc.kruskal.conover.test of the “The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR) R package” (Pohlert, 2014), including Bonferroni-type adjustment of p-values, and which aims to ensure a relatively high level of statistical power (i.e., reduction in the probability of committing a Type II Error).

We calculated different Gaussian Generalized Linear Models (GLMs), to determine what factors could better explain the observed 2C-values: a Null model was used as a benchmark; an Habitat model (island vs mainland specimens); an Habit model (woody vs herbaceous); a Raunkiaer classification model (chamaephyte vs hemicryptophyte vs terophyte); a Fruit type model (specimens with spiny vs wing secondary ribs); a Full model including all the previous factors, as well as other models resulting from its simplification; and a taxon model, comparing all the included taxa. The implementation followed Ávila *et al.* (2018) and Parelho *et al.* (2021) (see references therein), using the glm function of R. The best models were selected based on the maximum likelihood approach using AIC (Shapiro & Wilk, 1965). The model with the lowest AIC and the highest R^2 was considered to best fit the data. The R package “mass” was used to evaluate GLMs. Although the data failed to comply with normality, the calculated GLMs included all the samples in the analysis. Thus, according to the central limit theorem, when independent random variables are added, their properly normalized sum tends toward a normal distribution even if the original variables themselves are not normally distributed. That is, with a large sample size (i.e., more than 100 observations in this case) the mean tends to a normal distribution, even if the underlying data are not Gaussian (Kwak & Kim, 2017). Since the GLMs model de mean of the distribution, we considered their application as correct in this context.

2.3. Results

2.3.1. Reappraisal of morphology, ecology and conservation status of Daucinae in Macaronesia

A total of 16 taxa were sampled (**Table 2.1**), nine of them with endemic status: one from the Azores (*Daucus carota* subsp. *azoricus*), two from Madeira Island (*Melanoselinum decipiens* and *Monizia edulis*), five from Cabo Verde (*Tornabenea annua*, *Tornabenea bischoffii*, *Tornabenea insularis*, *Tornabenea ribeirensis*, and *Tornabenea tenuissima*) and one from mainland Portugal (*Daucus carota* subsp. *halophilus*) (**see Figure 2.1**).

Table 2.1. Geographical distribution, native status in the Macaronesia, Conservation status (i.e. LC, Least Concern; NE, Not Evaluated; DD, Data Deficient; EN, Endangered; and CR, Critically Endangered; (see references in Material and Methods) and ecology of the study taxa of Daucinae subtribe based on bibliographic revision.

Taxa	Geographical distribution	Native status in Macaronesia	Conserva-tion status	Ecology
Daucus carota L. subsp. *azoricus* Franco	Azores	Endemic	LC	Sea rocks, cliffs, pastures, uncultivated or cultivated lands, roadsides. From 0-800m
Daucus carota L. subsp. *carota*	Europa, Asia, Siberia, N. Africa	Native	NE	Ruderal
Daucus carota subsp. *gummifer* (Syme) Hook.f.	Europe	-	NE	Coastal cliffs and dunes and uncultivated lands, on sandy soil
Daucus carota L. subsp. *halophilus* (Brot.) A. Pujadas	Mainland Portugal	-	DD	Coastal cliffs and coastal upland areas
Daucus carota L. subsp. *maximus* (Desf.) Ball	Mediterranean; Asia	Native	NE	Ruderal
Daucus carota L. subsp. *sativus* (Hoffm.) Arcang.	Widely cultivated	-	NE	Cultivated
Daucus crinitus Desf.	Africa; Europe	-	LC	Grassland, shrubland
Daucus muricatus (L.) L.	Africa; Europe	Naturalized	LC	Grassland, shrubland
Melanoselinum decipiens (Schrad. & J.C.Wendl.) Hoffm.	Madeira	Endemic	NE	Shady rocks and banks in Laurel forests
Monizia edulis Lowe	Madeira	Endemic	CR	Mainly found on fissures in cliffs
Pseudorlaya pumila (L.) Grande	Mediterranean; Canaries	Native	NE	Marine sands, mainly primary dune
Tornabenea annua Bég.	Cabo Verde	Endemic	EN	Southern hygrophyte restricted to montane areas on Santiago
Tornabenea bischoffii J.A. Schmidt	Cabo Verde	Endemic	EN	Northern hygrophyte restricted to montane areas on Santo Antão
Tornabenea insularis (Parl. Ex Webb) Parl. Ex Webb	Cabo Verde	Endemic	EN	Sub-humid montane areas above 600m of S. Vicente, S. Nicolau, and Brava
Tornabenea ribeirensis Schmidt & Lobin	Cabo Verde	Endemic	CR	Restricted to shady and seasonally damp bottoms of valleys of very few *Ribeiras* in the North of S. Nicolau
Tornabenea tenuissima (A. Chev.) A. Hansen & Sunding	Cabo Verde	Endemic	CR	Restricted to sub-humid montane areas; mainly above 1200m on Fogo

Most of the endemic species sampled in the Macaronesia are single-island endemics except *Daucus carota* subsp. *azoricus*, which occurs in all the Azores Islands, *Monizia edulis*, in all the Madeira sub-archipelagos and *Tornabenea insularis*, the only *Tornabenea* species occurring in more than one island of Cabo Verde (Brava, São Nicolau and São Vicente).

Regarding the ecological data (**Table 2.1**), *Daucus carota* subsp. *azoricus* is a coastal taxon that occurs in sea rocks, cliffs, pastures, uncultivated or cultivated lands, and roadsides. *Melanoselinum decipiens* and *Monizia edulis*, both endemic species from Madeira, occupy different ecological zones, that is, shady and rocky places of clearances of the Laurel Forest and fissures in cliffs, respectively. *Tornabenea* species occupy similar ecological zones on Cabo Verde Islands, being five of the six species single-island endemics (the exception being *Tornabenea insularis*). Only *Tornabenea humilis* is found in lowland/coastal areas below 500 m altitude at Fogo, whereas the other five species are restricted to sub-humid habitats of the mountain areas above 600 m.

The conservation status of the sampled species revealed that six of them (38%) are threatened: three (19%) are Critically Endangered and the other three (19%) are Endangered (**Table 2.1**). Among the threatened species, five occur in Cabo Verde (i.e., *Tornabenea* genus) and the sixth, *Monizia edulis*, occurs in Madeira. The archipelago of Cabo Verde is the one presenting the highest number of threatened species of the Daucinae subtribe.

The Daucinae subtribe is mostly composed of hemicryptophyte taxa, namely: *Daucus carota* subsp. *azoricus*, *Daucus carota* subsp. *carota*, *Daucus carota* subsp*. gummifer*, *Daucus carota* subsp*. halophilus*, *Daucus carota* subsp. *maximus*, *Daucus carota* subsp. *sativus*, *Daucus crinitus*, *Tornabenea annua*, *Tornabenea insularis*, and *Tornabenea ribeirensis*. Moreover, the chamaephyte habit is only seen in the endemic Macaronesian species (**Table 2.1**). Similarly, the winged secondary ribs are traits only present in the endemic species of the Macaronesia (**Table 2.2**).

Table 2.2 Diagnostic characters of the sampled species of Daucinae subtribe, regarding habit, leaves, inflorescence, bracts, bracteoles, fruits and secondary ribs based on bibliography (see references in Material and Methods).

Taxa	Habit	Leaves	Inflorescence	Bracts	Bracteoles	Fruits	Secondary ribs
Daucus carota* subsp. *azoricus	Annual or biennial, up to 70 cm. Herbaceous	Hispid, 2–3 pinnate	Large terminal umbel, up to 9 cm diameter	6–11 pinnatisect. Filiform lobes	7–9 simple. Linear lobes	2-4 mm, cylindrical	Spiny
Daucus carota* subsp. *carota	Perennial, up to 110 cm. Herbaceous	Basal, 1–3(4) pinnate oblong to lanceolate, while uper leaves, 1-3 pinnate are linnear to lanceolate	(1.5)3–7(11) cm diameter, becoming strongly contracted in fruit	7–9 pinnatisect. Sublinear or filiform lobes	6–9 simple. Linear lobes	1.8–3.2 x 1-1.8 mm, ellipticals, purplish or light brown	Spiny
Daucus carota* subsp. *gummifer	Perennial, up to 50 cm. Herbaceous	Basal, (1)2–3(4) pinnate. Uper leaves similar to basal ones	(1.5)3–6(10) cm diameter, convex to sub–hemispherical, slightly contracted in fruit	7–10 shorter than the rays. Linear to lanceolate lobes	7–9 with a trifid apex. Lanceolate	1.8–3.0 x 1.3-2.5 mm, oblong to ovoid, brown.	Spiny
Daucus carota* subsp. *halophilus	Perennial, up to 25 cm. Herbaceous	Basal leaves 1–2 (3) pinnate. Uper leaves similar to basal ones, 1 – 2 pinnate	(3) 4–12 cm diameter hemispherical and slightly contracted in fruit	8–10 pinnatisect. Ovoid lobes	7–8 simple, trifid apex. Ovoid to widely lanceolate	2 -3.5 x 1.5-2.5 mm, ovoid to elliptical, purplish, or brown	Spiny
Daucus carota* subsp. *maximus	Perennial up to 220 cm. Herbaceous	Basal, (1)2–3 pinnate ovate to oblong. Upper similar to basal ones 1-2 pinnate	12–23 cm diameter, becoming strongly contracted in fruit	10–13 pinnatisect. Linear or filiform lobes	6–10 simple. Short and linear lobes	1.5–2.5 x 1-2 mm, ellipsoid - oblong, sometimes subspherical	Spiny
Daucus carota* subsp. *sativus	Perennial, up to 78 cm. Herbaceous	Basal, 3–4 pinnate, largely petiolate. Upper leaves 2(3) pinnate.	5–10 cm diameter, slightly convex	(8)10–13 pinnatisect. Long and linear lobes	7–9 simple. Linear to lanceolate lobes	3–3.5 x 1.2-2.0 mm, oblong, brown	Spiny
Daucus crinitus	Perennial, up to 115 cm	Basal, 3–4 pinnate, sessile or subsessile segments. Upper leaves similar to basal, 1-3 pinnate	Convex, doesn´t contract in fruit	5–10 simple, pinnatisect to trifid. Linear to lanceolate lobes	5–9 simple. Lobes linear to lanceolate	4–7(9) mm, elliptical and sometimes oblong	Spiny
Daucus muricatus	Annual up to 105 cm. Herbaceous	Basal leaves (2)3–4 pinnate, hispid. Upper leaves similar but slighty smaller	Long peduncle. Slightly convex. Sterile central flower absent	(4)6–10 pinnatisect. Linear or setaceous lobes	4–9 simple. Linear lobes	5–8(10) m, elliptical	Spiny
Melanoselinum decipiens	Tall rosetted perennial monocarpic, up to 3 m. Woody	Large triangular, up to 60 cm	50–90 cm diameter, terminal above leaf crown	10–20, 20–30 mm. Leafy	As long as the pedicels	12–14 mm, oblong, pubescent, blackish	Winged

Monizia edulis	Long lived perennial, up to 1m. Woody	Yellowish–green, glossy, triangular in out line	Paniculate, 20–25 rays in each umbel	Lanceolate or linear, puberulent, fringed at margin	Lanceolate or linear, puberulent, fringed at margin	10–14 x 5-7 mm, oblong to ellipsoid, pubescent, pale coloured when ripe	Winged
Pseudorlaya pumila	Annual up to 30 cm. Herbaceous	2–3 pinnate, hispid	3–7 unequal rays	2–5 linear to pinnatisect	3–5 similar to the bracts but smaller	(5.5)7.5–12 x 3.5-10 mm, having the spines in the dorsal and lateral ribs different sizes	Spiny
Tornabenea annua	Annual or biennial, up to 80 cm. Herbaceous	Up to 35 cm, 2–3 pinnate, 3–6 pairs of pinnae	More or less flat, up to 7.5 cm diameter	7–8, entire rarely somewhat bi or trifid. Inconspicuous	7–8. Inconspicuous	3.5 mm, strongly compressed dorsally	Spiny
Tornabenea bischoffii	Stout perennial up to 1.5 m. Woody	Up to 35 (–50) cm, 2 (–3) pinnate, 7 pairs of pinnae	Hemispherical, nearly spherical when fruiting, up to 9 cm diameter	10–15, up to 3 cm long. pinnately divided.	Trifid, bifid or entire	Up to 2 mm long, only slightly compressed dorsally	Winged
Tornabenea insularis	Stout perennial up to 90 cm. Woody	Up to 30 cm, sub coriaceous to delicate, 1–2 pinnate, 3–6 pairs of pinnae	Flat to hemispherical, up to 9 cm diameter	4–13, up to 2.8 cm long, pinnately divided. In fruiting slightly deflexed	7–9 narrow, trifid, bifid or entire	2 mm, long elliptical in dorsoventral view	Spiny
Tornabenea ribeirensis	Annual or biennial, up to 80 cm. Herbaceous	Thin light green, lamina deltoid to obovate in outline, 3–4 pairs of pinnae	Umbels with up to 25 rays, upward direct bristles, spread, and slightly constricted when fruiting	3–10 cm long, mainly undivided, rarely one bi or trifid	Inconspicuous. 2–3 mm long. Undivided	2.5 mm, compressed dorsally	Winged
Tornabenea tenuissima	Stout perennial up to 1 m high. Woody	Up to 40 cm, 2–3 pinnate, up to 6 (–7) pairs of pinnae. Segments very narrow, filiform	Hemispherical, nearly spherical when fruiting, up to 9 cm diameter	Up to 10, 2 cm long. Pinnately divided, segments slender and narrow	Trifid, bifid, or rarely entire	Reddish brown, up to 3.9 mm, compressed dorsally	Spiny

In the Daucinae subtribe the morphological characters such as the leaves, fruits and umbels are usually used to recognize the infratribal taxa (Franco, 1971). For the identification of some subspecies of *Daucus carota*, the degree of contraction in the fruiting umbels is a key trait; the presence and absence of a sterile flower in the center of the umbel is also a character that helps to distinguish between *Daucus carota* s.l. and *Daucus muricatus*. Moreover, the leaves of *Daucus crinitus* are very relevant for the identification of this taxon, as they exhibit a sessile or subsessile segment which is not observed in the other *Daucus* species (see Material & Methods). Traits such as bracts, bracteoles and fruits are also very useful to distinguish genera, namely between the genus *Daucus* and the related endemic Macaronesian genera (i.e., *Melanoselinum*, *Monizia* and Tornabenea). *Daucus* is characterized by fruits with spiny secondary ribs and pinnate bracts. In contrast, *Melanoselinum* taxa exhibit winged secondary ribs that are serrated and irregularly cut bracts. *Monizia* fruits have swollen and corky ribs, as well as bracts and bracteoles with fringed margins (**see Table 2.2**). The genus *Tornabenea* is more similar to *Daucus*, but fruits are compressed dorsally in the former. Some taxa also present winged secondary ribs (*Tornabenea bischoffii* and *Tornabenea ribeirensis*) opposing to the spiny secondary ribs in *Daucus*. Still and all, the *Tornabenea* taxa that presented spiny secondary ribs, have spines that are less differentiated from the spines of *Daucus* (**see Figure 2.1**).

2.3.2. Cytogenomic characterization

The cytogenomic results obtained are summarized in **Table 2.3**, which shows the mean 2C-values in picograms (pg) with standard deviations (SD), coefficient of variation (%) for the 16 analyzed species, along with 2C-values estimates evaluated by Nowicka *et al.* (2016). Genome size determinations based on flow cytometry produced histograms of fluorescence of G0/G1 peaks (**Figure 2.3**), and the coefficient of variation (CVa) values ranged from 2.31% to 5.99% (mean 4.12%) for the analyzed specimens (**Table 2.3**).

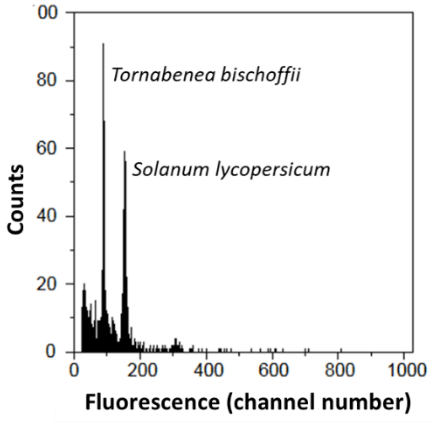

Figure 2.3. Flow cytometry output: Flow cytometric histogram of relative fluorescence intensities from propidium iodide stained *Tornabenea bischoffii* nuclei using *Solanum lycopersicum* (2C-values =1.96 pg) as an internal reference standard.

Estimations for sixteen species within the Daucinae subtribe are presented (**Table 2.3**), of which eight had already been previously estimated by Nowicka *et al.* (2016): the values obtained in both studies are very similar, with average differences of 11%, ranging between 26% for *Daucus carota* subsp. *maximus* and 4% for *Daucus carota* subsp. *halophilus*. The genome size of the analyzed species was quite homogenous (1.349 ± 0.442 pg), namely on *Tornabenea* genus (1.235 ± 0.016 pg), ranging from 1.214 ± 0.026 pg in *Tornabenea ribeirensis* to 1.259 ± 0.211 pg in *Tornabenea bischoffii*. Values were the smallest for *Pseudorlaya pumila* (**Table 2.3**). Moreover, no differences were found between the *Tornabenea* specimens and *Daucus carota* specimens, with the exception for subspecies *Daucus carota* subsp. *carota*. *Melanoselinum decipiens* and *Monizia edulis*, both from Madeira, showed intermediate values, also differing significantly from all the remaining taxa. *Daucus crinitus* and *Daucus muricatus* specimens from mainland Portugal showed the highest values, which were significantly different from those for all the remaining taxa (**Figure 2.4**).

Table 2.3. Cytogenomic results obtained for 16 taxa. Average 2C-values in picograms with standard deviation (SD), group (the same letter(s) indicates species within the genus that are not significantly different), sample coefficient of variation in percentage (CVa), previous 2C-value estimates in pictograms based on a previous study (Nowicka *et al.*, 2016), and the origin of the collected specimens (Az, Azores; CV, Cabo Verde; Lu, mainland Portugal; Ma, Madeira). The number of chromosomes was retrieved from the following literature: Bramwell & Murray (1972); Borgen (1974); Zizka (1986); Dalgaard (1994); Grosso *et al.* (2008); Nowicka *et al.* (2016); and Spooner (2019).

Taxa	**2C-values ± SD (pg)**	**Group**	**Sample CV (%)**	**Previous 2C-value (pg)**	**Chromossome number**	**Origin**
Daucus carota subsp. *azoricus*	1.167 ± 0.024	**abc**	4.470	1.064	18	Az
Daucus carota subsp. *carota*	1.093 ± 0.085	**a**	4.298	0.989	18	Lu
Daucus carota subsp. *gummifer*	1.173 ± 0.036	**ab**	4.821	1.016	18	Lu
Daucus carota subsp. *halophilus*	1.205 ± 0.028	**bcd**	4.624	1.154	18	Lu
Daucus carota subsp. *maximus*	1.244 ± 0.062	**e**	4.897	0.920	18	Lu
Daucus carota subsp. *sativus*	1.087 ± 0.021	**abc**	4.984	0.960	18	Lu
Daucus crinitus	2.544 ± 0.102	**g**	3.655	2.403	22	Lu
Daucus muricatus	2.135 ± 0.040	**g**	2.314	2.036	20	Lu
Melanoselinum decipiens	1.591 ± 0.046	**f**	3.431	-	22	Ma
Monizia edulis	1.940 ± 0.081	**g**	2.310	-	22	Ma
Pseudorlaya pumila	0.847 ± 0.031	**-**	5.990	-	26	Lu
Tornabenea annua	1.235 ± 0.018	**de**	3.781	-	18	CV
Tornabenea bischoffii	1.259 ± 0.211	**abc**	3.309	-	22	CV
Tornabenea insularis	1.223 ± 0.031	**de**	3.668	-	18	CV
Tornabenea ribeirensis	1.214 ± 0.026	**cde**	4.016	-	-	CV
Tornabenea tenuissima	1.248 ± 0.012	**def**	5.370	-	16	CV

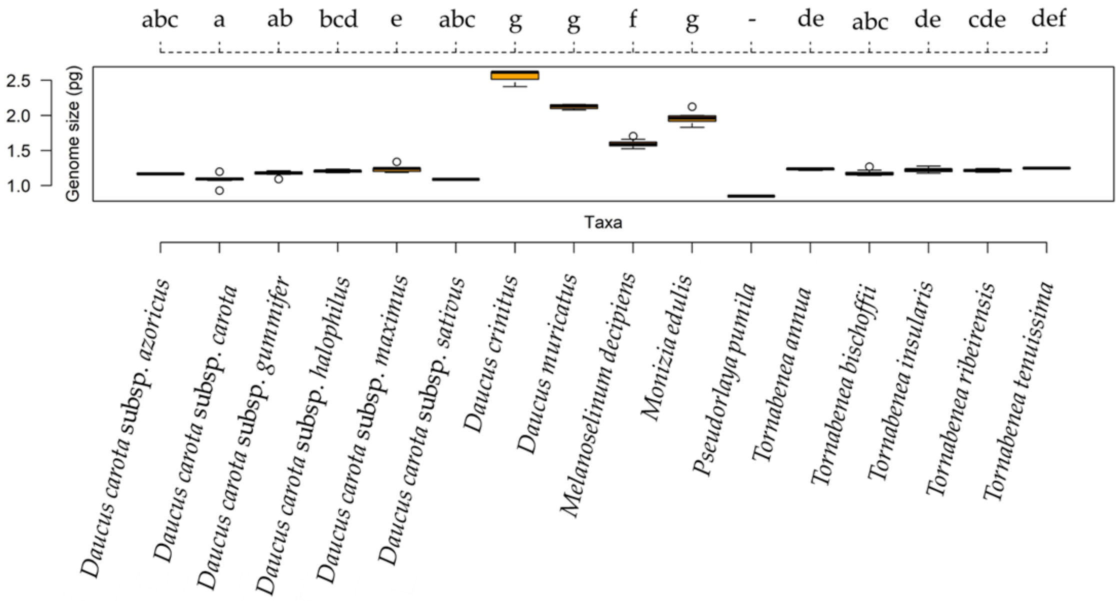

Figure 2.4 Boxplot diagram showing the genome size variation across the 16 taxa from the Daucinae subtribe. The orange box represents the 25th, 50th (median) and 75th percentiles, while whiskers represent the 10th and 90th percentiles with minimum and maximum observations. The dots represent the outliers. Different letters indicate group of taxa with significant differences.

The results of the application of different GLMs (**Table 2.4**) showed that the most explanatory factor was the taxon, with the lowest Akaike's An Information Criterion (AIC) and highest adjusted R^2, what was also confirmed using the Kruskal-Wallis test (Chi-squared= 29.061, df= 3, p= 2.174e-06).

Table 2.4. General Linear Model results for all explanatory variables included in the analysis. The models were ranked by its Akaike's An Information Criterion (AIC) and adjusted R^2 values.

General Linear Model	**AIC**	**R^2**
Taxon	-387.044	0.9851364
Region + Raunkiaer classification + Habit	63.587	0.3459922
Full	63.587	0.3459922
Region + Raunkiaer classification	63.845	0.3325822
Raunkiaer classification	76.666	0.2203145
Region	79.530	0.2159600
Fruit secondary ribs	89.827	0.1188375
Habit	98.585	0.0548866
Null	103.543	-
Habitat	105.587	0.0004328

The genome size variation for the studied Daucinae taxa according to fruit secondary ribs (**Figure 2.5A**); habit (**Figure 2.5B**); Raunkiaer classification (Raunkiaer, 1934) (**Figure 2.5C**) (**see Table 2.S1**) and regions (**Figure 2.5D**) is presented in **Figure 2.5.** With some exceptions, the genomes of herbaceous taxa were smaller than those from woody taxa, with a similar pattern between taxa with spiny and winged fruits. The exceptions are obvious in **Figures 2.5A** and **Figure 2.5B**. Regarding herbaceous taxa, two mainland species appear with very high values, therefore originating a high level of heterogeneity, justifying the low adjustment of the respective GLM (**Table 2.4**). Likewise, concerning taxa with spiny fruits, the same taxa from mainland originate a high level of heterogeneity, justifying the low adjustment of the respective GLM (**Table 2.4**).

When considering Raunkiaer life-forms (**see Table 2.S1**), terophytes tended to show the highest values and hemicryptophytes the lowest. However, one of the therophytes showed the lowest value and one of the hemicryptophytes showed the highest, therefore originating a high level of heterogeneity (**Figure 2.5C**), justifying the low adjustment of the respective GLM (**Table 2.4**). When considering the different regions, the Azores (1.167 ± 0.006 pg) and Cabo Verde (1.238 ± 0.059 pg) showed the lowest mean values, followed by the specimens from mainland Portugal (1.531 ± 0.483 pg) and Madeira (1.617 ± 0.183 pg; **Figure 2.5D**). However, it should be noted that specimens from mainland Portugal included taxa with a wide range of values, from the lowest to the highest, as mentioned above when comparing the results by taxon. As also seen above, the two endemic taxa from Madeira showed intermediate results, while the smallest values for that archipelago were associated with specimens from *Daucus carota* subsp. *carota*. This heterogeneity justifies the low adjustment of the respective GLM (**Table 2.4**).

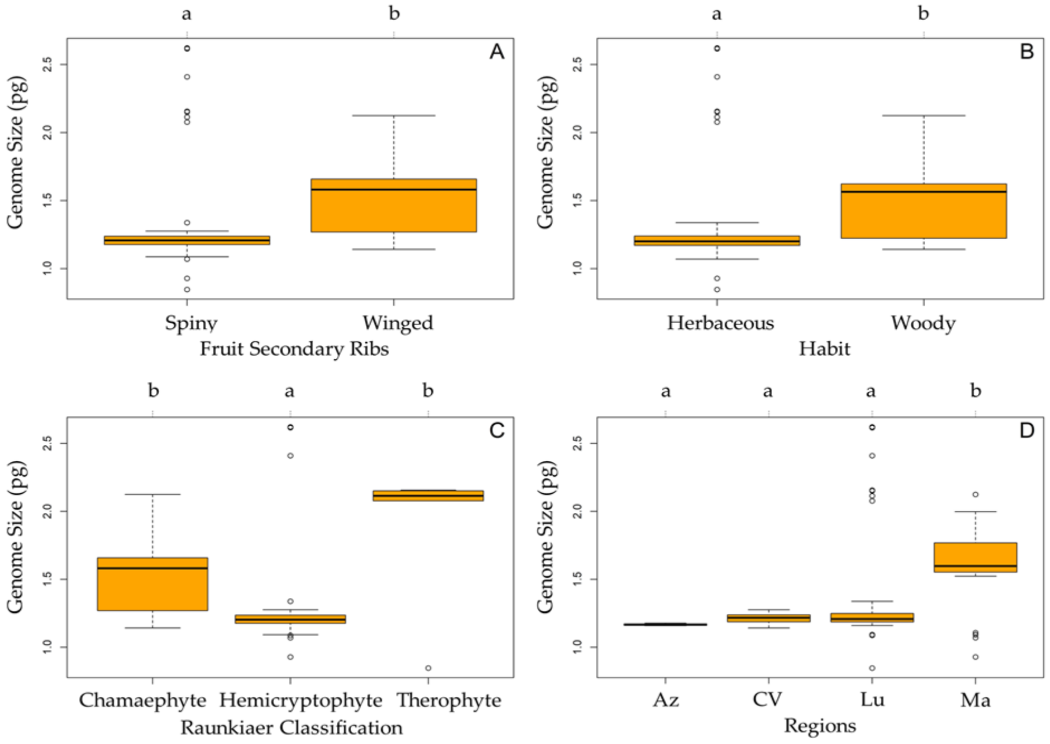

Figure 2.5. Boxplot diagram showing the genome size variation for the studied Daucinae taxa according to (A) morphology of the secondary ribs (winged or spiny); (B) habit (herbaceous or woody); (C) Raunkiaer classification (chamaephyte, hemicryptophyte, or terophyte) and (D) regions (Az, Azores; CV, Cabo Verde; Lu, Portugal mainland; and Ma, Madeira). The orange box represents the 25th, 50th (median) and 75th percentiles, while whiskers represent the 10th and 90th percentiles with minimum and maximum observations. The dots represent the outliers. Different letters indicate significant differences between groups (p<0.05).

2.4. Discussion

The Daucinae subtribe comprises taxa of high economic importance such as *Daucus carota* subsp. *sativus* (cultivated carrot), and its CWR represent invaluable genetic resources, with genes that can improve traits such as productivity and resilience in agriculture (Martínez-Flores *et al.*, 2020). Our study provides new cytogenomic data, for eight endemic taxa from Azores, Madeira and Cabo Verde archipelago, which can contribute to understand the relationship of the Daucinae subtribe within the Macaronesian Islands, where several threatened taxa occur. Overall, with the previous estimates stored at the Angiosperm DNA C-values database (Pellicer & Leitch, 2020), only one Macaronesian endemic taxon from the subtribe Daucinae is missing (*Tornabenea humillis*, endemic in Fogo Island, Cabo Verde).

In general, the species with the biggest genomes occur in mainland Portugal (*Daucus crinitus* and *D. muricatus*) and the insular taxa presented lower mean 2C-values compared to the continental ones. This tendency towards small genomes in islands has been observed in Macaronesia (Suda *et al.*, 2003, 2005) and in Hawaiian and Marquesas archipelagos (Kapralov & Filatov, 2011). Even though the Azores and Cabo Verde presented lower mean 2C-values than the mainland Portugal, and this could support the above-mentioned hypothesis, it is important to note that continental regions also presented the taxon with the lowest genome size (i.e., *Pseudorlaya pumila*) and Madeira presented endemic taxa with larger genome sizes than continental taxa (i.e., *Melanoselinum decipiens* and *Monizia edulis*).

According to our data, 2C-values are quite homogeneous across the *Daucus carota* complex (including *Tornabenea* species) supporting the close relationship among the taxa, previously mentioned by several authors (Banasiak *et al.*, 2016; Wojewódzka *et al.*, 2019; Frankiewicz *et al.*, 2020; Martínez-Flores *et al.*, 2020). Although the use of genome size as a taxonomical marker has been widely used with other plant lineages (e.g., Lopes *et al.*, 2021; Chumová *et al.*, 2015), *Daucus carota* complex is considered one of the taxonomically most difficult group within Apiaceae family and our 2C-values were unable to clearly discriminate the *Daucus carota* subspecies. The high outcrossing rate (ca. 96%) in wild carrot implies that high frequencies of gene flow may occur among the different subspecies (Rong *et al.*, 2010). In addition, pollen of wild carrots could be dispersed by insects over a long distance, and cultivated and wild carrot are fully interfertile, often overlap in flowering time, and hybrids may sometimes have high fertility and viability. While gene flow within *Daucus carota* complex appear extensive, more work is still needed (e.g., integrative genomic and morphological studies) to clearly discriminate population structure within the *Daucus carota* complex, as well as using additional samples from more diverse geographic origins, to provide future support for recognizing some species and geographically defined subspecies (Arbizu *et al.*, 2016; Martínez-Flores, 2020).

Particularly, the molecular relationships between some *Tornabenea* species and *Daucus carota s.l.* have been difficult to established and recent phylogenetic studies (Banasiak *et al.*, 2016; Wojewódzka *et al.*, 2019; Frankiewicz *et al.*, 2020; Martínez-Flores *et al.*, 2020), provided all evidence that at least some *Tornabenea* species should be included within the *Daucus carota* complex. As stated above, our results also revealed very little cytogenomic differentiation between *Tornabenea* and *Daucus carota* specimens, confirming the need for a clarification of the taxonomy of *Tornabenea* species (e.g., Martins, 1996). Moreover, the ecological data, including information on plant communities (**see Table 2.1, Table 2.S2, and Table 2.S3**), the currently recognized *Tornabenea* taxa are single-island endemics (except *Tornabenea insularis*), or ecologically isolated when occurring in the same island. For instance, *Tornabenea tenuissima* is restricted to sub-humid montane areas mainly above 1200m on Fogo, while *Tornabenea humilis* is found in lowland/coastal areas below 500m altitude in this island (**see Table 2.1**). Also, the rare *Tornabenea ribeirensis* is restricted to shady and seasonally damp valleys of very few streams in the North side of São Nicolau (Schmidt & Lobin, 1999) whereas *Tornabenea insularis* is found in open, non-sheltered and sometimes extremely exposed habitats and in a different plant communities alliance (**see Tables 2.S2 and Table 2.S3**). Directional selection into diverse island environments, is reported to many of the well-known spectacular examples of adaptive radiation, such as *Echium* species in the Southern Cabo Verde Islands in which two lines of speciation occur (*Echium vulcanorum* and *Echium hypertropicum*), each driven by selection within markedly different ecological zones (Romeiras *et al.*, 2011). The same pattern can be seen in Madeira Island with *Melanoselinum decipiens* and *Monizia edulis*, in which both occur in a phytosociological very distinct communities (**see Table 2.S2 and Table 2.S3**), with the former occurring in forest clearances and the latter in succulent-rich scrub volcanic rock substrates and walls.

The morphology of the secondary ribs might also provide some indication of the recent colonization and radiation of the *Tornabenea* genus (Martins, 1996; Schmidt & Lobin, 1999). The presence of winged secondary ribs in some taxa is likely to be a reversal evolution and linked to insular habitats as proposed by Wojewódzka *et al.* (2019). In fact, such trait is only seen in insular taxa (*Monizia edulis*, *Melanoselinum decipiens*, *Tornabenea bischoffii* and *Tornabenea ribeirensis*). Spiny ribs are seen as an advantageous trait for epizoochorous dispersion and might have been lost due to the general absence of native terrestrial mammals in volcanic islands, which were never connected to the mainland (Wojewódzka *et al.*, 2019). This loss of dispersal abilities through epizoochory is a common feature in oceanic islands (Gillespie, 2007). On oceanic islands, there is usually a reversal evolution from spines to wings, as this feature is ideal for wind dispersal, as well as an increment of the fruit size (Gillespie, 2007). Alternatively, this characteristic may have been lost by drift. Altogether, we can hypothesize that the island taxa with winged secondary ribs are older than the ones with spiny secondary ribs. Our results showed that taxa with spiny ribs have on average significantly smaller genomes than taxa with winged secondary ribs. The larger genome of taxa with winged secondary ribs might be related with the increase of mericarp size in insular taxa. In fact, such a positive correlation between seed mass and genome size has been shown by various authors (Caceres *et al.*, 1998; Lopes *et al.*, 2021).

Insular woodiness and a perennial life cycle are a key evolutionary innovation that drives radiations in insular systems (Nürk *et al.*, 2019). Moreover, it is the lineages that developed secondary woodiness that diversified more in the Macaronesia (Carine *et al.*, 2010). Interestingly, chamaephyte taxa presents the highest mean 2C-values when compared with hemicryptophytes and terophytes. Nonetheless, Beaulieu *et al.* (2010) noted that mean genome sizes in the Fabaceae family were significantly smaller in woody than in herbaceous species and presented a smaller variation.

Overall, this study improved the global knowledge of DNA content for Macaronesian endemics and shed light into the mechanisms underpinning diversity patterns of wild carrots in Western Mediterranean Region. Despite the relatively coherent results revealed by the cytogenomic analyses, based on ca. 320 field-collected samples, further efforts should be performed to increase the plant collection, namely of *Tornabenea humilis* from Fogo Island and of *Cryptotaenia elegans* from Canary Islands.

Although the Macaronesian Islands harbours a rich diversity of wild carrots, it was exposed that particularly the endemic species should be protected, as ca. 40% of the studied taxa are threatened, endemic and red listed species (i.e., 20%: Critically Endangered and 20%: Endangered; **see Table 2.1**). Presently, it is widely recognized that only the conservation of these populations in their habitats (in-situ conservation), will ensure the continued supply of the novel genetic material, critical for future crop improvement (Monteiro *et al.*, 2018). Thus, our results revealed that the C-values are quite homogeneous across the "*Daucus-Tornabenea-Monizia-Melanoselinum*", indicates that these taxa can easily be used in crop improvement. Of these species, *Monizia edulis* endemic in Madeira archipelago and all the *Tornabenea* species should be prioritized taxa, and urgent conservation actions must be implemented, particularly, as they have a very limited geographic range (often rare and endemic taxa), and they were classified in threatened categories (**see Table 2.1**). Finally, a better understanding of how the intraspecific diversity is changing over time and space is required, and we argue that the cytogenomic analyses, can contribute with additional data that can be useful to make informed decisions for the conservation of Plant Genetic Resources in the Macaronesian Islands.

2.5. References

Arbizu, C. I., Ellison, S. L., Senalik, D., Simon, P. W., & Spooner, D. M. (2016). Genotyping-by-sequencing provides the discriminating power to investigate the subspecies of *Daucus carota* (Apiaceae). BMC evolutionary biology, 16(1): 1-16. https://doi.org/10.1186/s12862-016-0806-x

Ávila, S.P., Cordeiro, R., Madeira, P., Silva, L., Medeiros, A., Rebelo, A.C., Melo, C., Neto, A.I., Haroun, R., & Monteiro, A. (2018) Global change impacts on large-scale biogeographic patterns of marine organisms on Atlantic oceanic islands. Marine Pollution Bulletin. 126: 101–112, https://doi.org/10.1016/j.marpolbul.2017.10.087.

Banasiak, Ł., Wojewódzka, A., Baczyński, J., Reduron, J.P., Piwczyński, M., Kurzyna-Młynik, R., Gutaker, R., Czarnocka-Cieciura, A., Kosmala-Grzechnik, S., & Spalik, K. (2016) Phylogeny of Apiaceae subtribe Daucinae and the taxonomic delineation of its genera. Taxon. 65: 563–585, https://doi.org/10.12705/653.8.

Beaulieu, J.M., Smith, S.A., & Leitch, I.J. (2010) On the tempo of genome size evolution in Angiosperms. Journal of Botany. 2010, 989152, https://doi.org/10.1155/2010/989152.

Borgen, L. (1974) Chromosome numbers of Macaronesian flowering plants II. Nordic Journal of Botany. 21: 195–210.

Box, G.E.P., & Cox, D.R. (1964) An analysis of transformations, Journal of the Royal Statistical Society. Series B. 26: 211–252.

Bramwell, D., & Murray, B. (1972) A preliminary report on the cytology of some Cape Verde islands plants. Cuadernos de Botanica Canaria. 14/15: 27-29.

Brilhante, M., Roxo, G., Catarino, S., Santos, P., Reyes-Batancort, J.A., Caujapé-Castels, J., Sequeira, M.M., Talhinhas, P., & Romeiras, M.M. (2021) Diversification of Aeonium species across Macaronesian Archipelagos: Correlations between genome-size variation and their conservation status. Frontiers in Ecology and Evolution. 9: 607338, https://doi.org/10.3389/fevo.2021.607338.

Brochmann, C.R.Ø.H., Lobin, W., & Kilian, N. (1997) The endemic vascular plants of the Cape Verde Islands, W. Africa. Sommerfeltia, 24: 1–356.

Caceres, M., De Pace, C., Mugnozza, G.S., Kotsonis, P., Ceccarelli, M., & Cionini, P. (1998) Genome size variations within *Dasypyrum villosum*: Correlations with chromosomal traits, environmental factors and plant phenotypic characteristics and behaviour in reproduction. Theoretical and Applied Genetics. 96: 559–567.

Carine, M.A., Santos-Guerra, A., Guma, I.R., & Reyes-Betancort, J.A. (2010) Endemism and evolution of the Macaronesian Flora. In Beyond Cladistics, Williams, D.M.K.S., Ed., University of California Press: Oakland, CA, USA, pp. 101–124.

Carvalho, J.A., Fernandes, F.M., & Santos-Guerra, A. (2013) The vascular flora of Porto Santo: A catalogue of its islests. Boletim do Museu Municipal do Funchal. Funchal, 63: 335.

Carvalho, R., Silva, E., Loureiro, J., Ramos, A.P., & Talhinhas, P. (2018) Characterisation of Puccinia hemerocallidis causing the first outbreak of daylily rust in Europe. Revista de Ciências Agrárias. 41: 110–115, https://doi.org/10.19084/rca.17075.

Castroviejo, S. (2003) Flora ibérica: Plantas vasculares de la Península Ibérica e Islas Baleares. In Flora Ibérica, Real Jardin Botanico Consejo Superior de Investigaciones Cie: Madrid, Spain, Volume 10, pp. 1–784.

Chevalier, A. (1935) Les iles du Cap Vert. Géographie, biogéographie, agriculture. Flore de l'Archipel. Journal d'agriculture traditionnelle et de botanique, 15, 733–1090.

Chumová, Z., Krejčíková, J., Mandáková, T., Suda, J., & Trávníček, P. (2015) Evolutionary and taxonomic implications of variation in nuclear genome size: Lesson from the grass genus *Anthoxanthum* (Poaceae). PLoS ONE. 10: e0133748, https://doi.org/10.1371/journal.pone.0133748.

Conover, W.J., & Iman, R.L. (1979) On Multiple-Comparison Procedures, Technical report, Los Alamos Scientific Laboratory: Los Alamos, NM, USA.

Corvelo, R.A.F. (2010) Estatuto de Conservação das Plantas Vasculares Endémicas dos Açores Segundo os Critérios da IUCN: Implicações ao Nível do Ordenamento do Território e do Planeamento Ambiental. Ph.D. Thesis, Universidade dos Açores, Ponta Delgada, Portugal.

Curto, M., Schachtler, C., Puppo, P., & Meimberg, H. (2018). Using a new RAD-sequencing approach to study the evolution of *Micromeria* in the Canary Islands. Molecular phylogenetics and evolution, 119: 160-169. https://doi.org/10.1016/j.ympev.2017.11.005

Dalgaard, V. (1994) Checklist of chromosome numbers counted in Madeiran flowering plants, with notes on polyploidy, life form, endemism and evolution. Nordic Journal of Botany. 14: 241–255.

Dias, E.F., Kilian, N., Silva, L., Schaefer, H., Carine, M., Rudall, P.J., Santos-Guerra, A., & Moura, M. (2018) Phylogeography of the Macaronesian lettuce species *Lactuca watsoniana* and *L. palmensis* (Asteraceae). Biochemical Genetics. 56: 315–340, https://doi.org/10.1007/s10528-018-9847-8.

Doležel, J., Sgorbati, S., & Lucretti, S. (1992) Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. Physiologia Plantarum 85: 625–631, https://doi.org/10.1111/j.1399-3054.1992.tb04764.x.

Downie, S., Plunkett, G., Watson, M., Spalik, K., Katz-Downie, D., Valiejo-Roman, C., Terentieva, E., Troitsky, A., Lee, B.Y., & Lahham, J. (2001). Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data. Edinburgh Journal of Botany, 58(2): 301-330. https://doi.org/10.1017/S0960428601000658

Downie, S.R., Katz-Downie, D.S., & Spalik, K. (2000) A phylogeny of Apiaceae tribe Scandiceae: Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. American Journal of Botany. 87: 76–95. https://doi.org/10.2307/2656687

Franco, J.A. (1971) Nova Flora de Portugal (Continente e Açores): Lisboa, Portugal, Volume I.

Franco, J.d.A., & Rocha-Afonso, M. (1984) Nova Flora de Portugal (Continente e Açores): Lisboa, Portugal, Volume II.

Frankiewicz, K.E., Oskolski, A., Banasiak, Ł., Fernandes, F., Reduron, J.P., Reyes-Betancort, J.A., Szczeparska, L., Alsarraf, M., Baczyński, J., & Spalik, K. (2020). Parallel evolution of arborescent carrots (*Daucus*) in Macaronesia. American journal of botany, 107(3): 394-412. https://doi.org/10.1002/ajb2.1444

Galbraith, D., Loureiro, J., Antoniadi, I., Bainard, J., Bureš, P., Cápal, P., Castro, M., Castro, S., Čertner, M., Čertnerová, D., Chumová, Z., Doležel, J., Giorgi, D., Husband, C. B., Kolář, F., Koutecký, P., Kron, P., Leitch, L. J., Ljung, K., Lopes, S., Lučanová, M., Lucretti, S., Ma, W., Melzer, S., Molnár, I., Novák, O., Poulton, N., Skalický, V., Sliwinska, E., Šmard, P., Smith, W. T., Sun, G., Talhinhas, P., Tárnok, A., Temsch, M. E., Trávníček, P., & Urfus, T. (2021). Best practices in plant cytometry. Cytometry, 99(4): 311-317. https://doi.org/10.1002/cyto.a.24295

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

García-Verdugo, C., Sajeva, M., La Mantia, T., Harrouni, C., Msanda, F., & Caujapé-Castells, J. (2015). Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. Molecular Ecology, 24(4): 726-741. https://doi.org/10.1111/mec.13060

Gillespie, R.G. (2007) Oceanic islands: Models of diversity. Encyclopaedia of Biodiversity. 1-13.

Góis-Marques, C.A., de Nascimento, L., Fernández-Palacios, J.M., Madeira, J., & Menezes de Sequeira, M. (2019) Tracing insular woodiness in giant Daucus (sl) fruit fossils from the Early Pleistocene of Madeira Island (Portugal). Taxon. 68: 1314–1320, https://doi.org/10.1002/tax.12175.

Gregory, T.R. (2005) The C-value enigma in plants and animals: A review of parallels and an appeal for partnership. Annals of Botany. 95: 133–146, https://doi.org/10.1093/aob/mci009.

Grosso, A., Rodrigues, L., Gomes, I., Martins, E., & Teixeira, G. (2008) Preliminary data on microcharacters and chromosome number in *Tornabenea* species (Apiaceae) from Cape Verde Islands. Plant Biosystematics. 142: 87–93, https://doi.org/10.1080/11263500701872523.

Guerra, M. (2008) Chromosome numbers in plant cytotaxonomy: Concepts and implications. Cytogenetic and Genome Research. 120(3-4): 339-350, https://doi.org/10.1159/000121083.

Guilengue, N., Alves, S., Talhinhas, P., & Neves-Martins, J. (2020) Genetic and genomic diversity in a tarwi (*Lupinus mutabilis* Sweet) germplasm collection and adaptability to Mediterranean climate conditions. Agronomy. 10: 21, https://doi.org/10.3390/agronomy10010021.

Hessen, D.O., & Persson, J. (2009) Genome size as a determinant of growth and life-history traits in crustaceans. Biological Journal of the Linnean Society. 98: 393–399, https://doi.org/10.1111/j.1095-8312.2009.01285.x.

Hoffmann, G.F. (1814) Genera Plantarum Umbelliferarum Eorumque Characteres Naturales Secundum Numerum, Figuram, Situm Et Proportionem Omnium Fructificationis Partium: Accedunt Icones Et Analyses Æri Incisæ: Moscow, Russia, Volume 1.

Hooker, W.J. (1849b) Hooker's Journal of Botany and Kew Garden Miscellany; Reeve, Benham, and Reeve: London, United Kingdom, Volume 1, p. 370.

Hooker, W.J., Vogel, T., Webb, P.B., Hooker, J.D., & Bentham, G. (1849a) Niger flora: Or, An enumeration of the plants of western tropical Africa; H. Bailliere: London United Kingdom, Volume 1. pp. 131–132.

Husband, B.C., Baldwin, S.J., & Suda, J. (2013) The incidence of polyploidy in natural plant populations: Major patterns and evolutionary processes. In Plant Genome Diversity; Springer: Vienna, Austria. Volume 2. pp. 255–276.

Iorizzo, M., Senalik, D.A., Ellison, S.L., Grzebelus, D., Cavagnaro, P.F., Allender, C., Brunet, J., Spooner, D.M., Van Deynze, A., & Simon, P.W. (2013) Genetic structure and domestication of carrot (Daucus carota subsp. sativus) (Apiaceae). American Journal of Botany, 100: 930–938, https://doi.org/10.3732/ajb.1300055.

IUCN. (2020) The IUCN Red List of Threatened Species. Version 2020-1594. Available online: https://www.iucnredlist.org (accessed on 25 March 2021).

Jardim, R., & Menezes de Sequeira, M. (2008) The vascular plants (Pteridophyta and Spermatophyta) of the Madeira and Selvagens Archipelagos. In A List of the Terrestrial Fungi, Flora and Fauna of Madeira and Selvagens Archipelagos. Funchal and Angra do Heroísmo. Borges, P.A.V., Aguiar, A.M.F., Carvalho, P., Jardim, R., Melo, I., Oliveira, P., Sérgio, C., Serrano, A.R.M., Vieira, P., Eds; Direcçao regional do ambiente, Governo regional da Madeira: Direcção Regional do Ambiente da Madeira and Universidade dos Açores, Ponta Delgada, Portugal. pp. 157–178.

Kapralov, M. V., & Filatov, D. A. (2011). Does large genome size limit speciation in endemic island floras. Journal of Botany, 2011: 1-6. https://doi.org/10.1155/2011/458684

Kerbs, B., Ressler, J., Kelly, J.K., Mort, M.E., Santos-Guerra, A., Gibson, M.J., Caujapé-Castells, J., Crawford, D.J., & Hiscock, S.J. (2017). The potential role of hybridization in diversification and speciation in an insular plant lineage: insights from synthetic interspecific hybrids. AoB Plants, 9(5). https://doi.org/10.1093/aobpla/plx043

Kwak, S.G., & Kim, J.H. (2017) Central limit theorem: The cornerstone of modern statistics. Korean Journal of Anesthesiology. 70: 144–156, https://doi.org/10.4097/kjae.2017.70.2.144.

Laport, R. G., & Ng, J. (2017). Out of one, many: The biodiversity considerations of polyploidy. American Journal of Botany, 104(8): 119–1121. http://dx.doi.org/10.3732/ajb.1700190.

Lobin, W., & Zizka, G. (1987) Einteilung der Flora (Phanerogamae) der Kapverdischen Inseln nach ihrer Einwanderungsgeschichte. Courier Forschungs institut Senckenberg, 95: 127–153.

Lopes, S., Mota, L., Castro, M., Nobre, G., Novoa, A., Richardson, D.M., Loureiro, J., & Castro, S. (2021). Genome size variation in Cactaceae and its relationship with invasiveness and seed traits. Biological Invasions, 23: 1-16. https://doi.org/10.1007/s10530-021-02557-w

Loureiro, J., Rodriguez, E., Doležel, J., & Santos, C. (2007) Two new nuclear isolation buffers for plant DNA flow cytometry: A test with 37 species. Annals of Botany. 100: 875–888, https://doi.org/10.1093/aob/mcm152.

Lowe, R. (1856) Species Plantarum Maderensium quaedam Novae, vel hactenus ineditae, breviter descriptae. Trib. Thapsieae, lb. 58, 202. Gen. *Monizia*, Lowe. Hooker's. Journal of Botany. 8, 295.

Lowe, R.T. (1838) Novitiae florae maderensis: Or notes and gleanings of Madeiran botany. Transactions of the Cambridge Philosophical Society, 6, 523.

Lowe, R.T. (1869) Florulae Salvagicae Tentamen: An Essay of the Flor of the Selvagens Islands; John Van Voorst: London, UK.

Martínez-Flores, F. (2016) Sistemática del Género *Daucus* L. (Apiaceae): Implicaciones Taxonómicas y Filogenéticas. Ph.D. Thesis, Universitat d'Alacant-Universidad de Alicante, Spain.

Martínez-Flores, F., Crespo, M.B., Simon, P.W., Ruess, H., Reitsma, K., Geoffriau, E., & Spooner, D.M. (2020) Subspecies Variation of *Daucus carota* Coastal ("Gummifer") Morphotypes (Apiaceae) Using Genotyping-by-Sequencing. Systematic Botany. 45: 688–702. https://doi.org/10.1600/036364420X15935294613527

Martins, E. (1996) Flora de Cabo Verde-Plantas Vasculares Apiaceae; IICT: Lisboa, Portugal; INIDA: Praia, Cabo Verde, Volume 67.

Menezes de Sequeira, M., Espírito Santo, D., Aguiar, C., Capelo, J., & Honrado, J.J. (2011) Checklist da Flora de Portugal Continental, Açores e Madeira, Associação Lusitana de Fitossociologia: Lisboa, Portugal.

Monteiro, F., Frese, L., Castro, S., Duarte, M.C., Paulo, O.S., Loureiro, J., & Romeiras, M.M. (2018) Genetic and genomic tools to asssist sugar beet improvement: The value of the crop wild relatives. Frontiers in Plant Science. 9: 74, https://doi.org/10.3389/fpls.2018.00074.

Mucina, L., Bültmann, H., Dierßen, K., Theurillat, J.P., Raus, T., Čarni, A., Šumberová, K., Willner, W., Dengler, J., & García, R.G. (2016) Vegetation of Europe: Hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. Applied Vegetation Science. 19: 3–264, https://doi.org/10.1111/avsc.12257.

Nowicka, A., Sliwinska, E., Grzebelus, D., Baranski, R., Simon, P.W., Nothnagel, T., & Grzebelus, E. (2016) Nuclear DNA content variation within the genus *Daucus* (Apiaceae) determined by flow cytometry. Scientia Horticulturae. 209: 132–138, https://doi.org/10.1016/j.scienta.2016.06.023.

Nürk, N.M., Atchison, G.W., & Hughes, C.E. (2019) Island woodiness underpins accelerated disparification in plant radiations. New Phytologist. 224: 518–531, https://doi.org/10.1111/nph.15797.

Parelho, C., Rodrigues, A., do Carmo Barreto, M., Cruz, J.V., Rasche, F., Silva, L., & Garcia, P. (2021) Bioaccumulation and potential ecotoxicological effects of trace metals along a management intensity gradient in volcanic pasturelands. Chemosphere. 273: 128601, https://doi.org/10.1016/j.chemosphere.2020.128601.

Pellicer, J., & Leitch, I. J. (2020). The Plant DNA C-values database (release 7.1): an updated online repository of plant genome size data for comparative studies. New Phytologist, 226(2): 301-305. https://doi.org/10.1111/nph.16261

Pohlert, T. (2014) The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR). R Package. Available online: http://CRAN.R-project.org/package=PMCMR (accessed on 25 July 2021).

Press, J., Short, M. (1994) Flora of Madeira; HMSO: London, UK

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.R-project.org/ (accessed 30 March 2021).

Raunkiaer, C. (1934) The life forms of plants and statistical plant geography, being the collected papers of C. Raunkiaer.

Rivas-Martinez, S., Lousã, M., Costa, J.C., & Duarte, M.C. (2017) Geobotanical survey of Cabo Verde Islands (West Africa). International journal of geobotanical research. 17: 1–103, https://doi.org/10.5616/ijgr170001.

Rocha, V., Duarte, M. C., Catarino, S., Duarte, I., & Romeiras, M. M. (2021). Cabo Verde’s Poaceae flora: A reservoir of crop wild relatives diversity for crop improvement. Frontiers in plant science, 12: 630217. https://doi.org/10.3389/fpls.2021.630217

Romeiras, M.M., Catarino, S., Gomes, I., Fernandes, C., Costa, J.C., Caujapé-Castells, J., & Duarte, M.C. (2016) IUCN Red List assessment of the Cape Verde endemic flora: Towards a global strategy for plant conservation in Macaronesia. Botanical Journal of the Linnean Society 180: 413–425, https://doi.org/10.1111/boj.12370.

Romeiras, M.M., Paulo, O.S., Duarte, M.C., Pina-Martins, F., Cotrim, M.H., Carine, M.A., & Pais, M.S. (2011) Origin and diversification of the genus *Echium* (Boraginaceae) in the Cape Verde archipelago. Taxon. 60: 1375–1385.

Romeiras, M.M., Pena, A.R., Menezes, T., Vasconcelos, R., Monteiro, F., Paulo, O.S., & Moura, M. (2019) Shortcomings of phylogenetic studies on recent radiated insular groups: A meta-analysis using Cabo Verde biodiversity. International Journal of Molecular Sciences. 20: 2782, https://doi.org/10.3390/ijms20112782.

Romeiras, M.M., Vieira, A., Silva, D.N., Moura, M., Santos-Guerra, A., Batista, D.; Duarte, M.C., & Paulo, O. (2016). Evolutionary and biogeographic insights on the Macaronesian Beta-Patellifolia species (Amaranthaceae) from a time-scaled molecular phylogeny. PLoS One, 11(3): e0152456. https://doi.org/10.1371/journal.pone.0152456

Rong, J., Janson, S., Umehara, M., Ono, M., & Vrieling, K. (2010) Historical and contemporary gene dispersal in wild carrot (*Daucus carota* ssp. *carota*) populations. Annals of Botany. 106: 285–296.

Sánchez-Pinto, L., Rodríguez, M.L., Rodríguez, S., Martín, K., Cabrera, A., Carmen & Marrero, M. (2005) Pteridophyta, Spermatophyta. In Lista Preliminar de Especies Silvestres de Cabo Verde. Hongos, Plantas y Animales Terrestres; Arechavaleta Hernández, M., Pérez, N.Z., Gómez, M.M., Esquivel, J., Eds.; Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias: La Laguna, Spain, pp. 38–57.

Schmidt, J.A. (1852) Beiträge zur Flora der Cap Verdischen Inseln: Mit Berücksichtigung Aller bis Jetzt Daselbst Bekannten Wildwachsenden und Kultivirten Pflanzen. Nach Eigenen Untersuchungen und Mit Benutzung der Gewonnenen Resultate Anderer Reisenden Dargestellt; E. Mohr: Heidelberg, Germany.

Schmidt, K., & Lobin, W. (1999) Tornabenea ribeirensis (Apiaceae) a new species from São Nicolau, Cape Verde Islands (West Africa). Feddes Repertorium. 110: 7–11.

Shapiro, S.S., & Wilk, M.B. (1965) An analysis of variance test for normality (complete samples). Biometrika. 52: 591–611, https://doi.org/10.1093/biomet/52.3-4.591.

Siegel, S., & Castellan, N.J. (1988) Non Parametric Statistics for the Behavioural Sciences, MacGraw Hill Int.: New York, NY, USA,. pp 213–214.

Silva, L., Pinto, N., Press, B., Rumsay, F., Carine, M., Henderson, S., & Sjögren, E. (2005) Lista das plantas vasculares (Pteridophyta e Spermatophyta). In A list of the terrestrial fauna (Mollusca and Arthropoda) and flora (Bryophyta, Pteridophyta and Spermatophyta) from the Azores, Borges, P.A.V., Cunha, R., Gabriel, R., Martins, A.F., Silva, L., Vieira, V., Eds., Direcção Regional do Ambiente and Universidade dos Açores: Horta, Angra do Heroísmo and Ponta Delgada, Portugal. pp. 131–155.

Spalik, K., & Downie, S.R. (2007) Intercontinental disjunctions in *Cryptotaenia* (Apiaceae, Oenantheae): An appraisal using molecular data. Journal of Biogeography, 34: 2039–2054, https://doi.org/10.1111/j.1365-2699.2007.01752.x.

Spooner, D., Rojas, P., Bonierbale, M., Mueller, L.A., Srivastav, M., Senalik, D., & Simon, P. (2013) Molecular phylogeny of Daucus (Apiaceae). Systematic Botany. 38: 850–857, https://doi.org/10.1600/036364413X670449.

Spooner, D.M. (2019) The carrot genome. In Daucus: Taxonomy, Phylogeny, Distribution, Simon, P., Iorizzo, M., Grzebelus, D., Baranski, R., Eds., Springer: Cham, Switzerland. pp. 9–26.

Suda, J., Kyncl, T., & Jarolímová, V. (2005). Genome size variation in Macaronesian angiosperms: forty percent of the Canarian endemic flora completed. Plant Systematics and Evolution, 252(3-4): 215-238.

Suda, J., Kyncl, T., & Freiová, R. (2003). Nuclear DNA amounts in Macaronesian angiosperms. Annals of Botany, 92(1): 153–164. https://doi.org/10.1093/aob/mcg104

Wei, M. C. F., Maldaner, L. F., Ottoni, P. M. N., & Molin, J. P. (2020). Carrot Yield Mapping: A Precision Agriculture Approach Based on Machine Learning. AI, 1(2): 229-241. https://doi.org/10.3390/ai1020015

Whitney, K. D., Ahern, J. R., Campbell, L. G., Albert, L. P., & King, M. S. (2010). Patterns of hybridization in plants. Perspectives in Plant Ecology, Evolution and Systematics, 12(3): 175-182.

Whittaker, R.J., & Fernández-Palacios, J.M. (2007) Island Biogeography: Ecology, Evolution, and Conservation, 2nd ed., Oxford University Press: Oxford, UK. pp. 46–74.

Wojewódzka, A., Baczyński, J., Banasiak, Ł., Downie, S.R., Czarnocka-Cieciura, A., Gierek, M., Frankiewicz, K., & Spalik, K. (2019) Evolutionary shifts in fruit dispersal syndromes in Apiaceae tribe Scandiceae. Plant Systematics and Evolution. 305: 401–414. https://doi.org/10.1007/s00606-019-01579-1

Wood, T. E., Takebayashi, N., Barker, M. S., Mayrose, I., Greenspoon, P. B., & Rieseberg, L. H. (2009). The frequency of polyploid speciation in vascular plants. Proceedings of the national Academy of sciences, 106(33): 13875-13879. https://doi.org/10.1073/pnas.0811575106

Zar, J.H. (2010) Biostatistical Analysis, 5th ed., Prentice Hall: Pearson, USA.

Zizka, G. (1986) Chromosomenzahlungen bei einigen kapverdischen Pflanzen. Courier Forschungsinstitut Senckenberg. 81: 181–182.

2.6. Supplementary material

Table 2.S1: Raunkier classification of the sampled taxa.

Taxon	Raunkier Classification
Daucus carota L. subsp*. azoricus* Franco	Hemicryptophyte
Daucus carota L. subsp*. carota*	Hemicryptophyte
Daucus carota subsp. *gummifer* (Syme) Hook.f.	Hemicryptophyte
Daucus carota L. subsp*. halophilus* (Brot.) A. Pujadas	Hemicryptophyte
Daucus carota L. subsp. *maximus* (Desf.) Ball	Hemicryptophyte
Daucus carota L. subsp. *sativus* (Hoffm.) Arcang	Hemicryptophyte
Daucus crinitus Desf.	Hemicryptophyte
Daucus muricatus (L.) L.	Therophyte
Melanoselinum decipiens (Schrad. & J.C.Wendl.) Hoffm.	Chamaephyte or Microphanerophyte*
Monizia edulis Lowe	Chamaephyte
Pseudorlaya pumila (L.) Grande	Therophyte
Tornabenea annua Bég.	Hemicryptophyte
Tornabenea bischoffii J.A. Schmidt	Chamaephyte
Tornabenea insularis (Parl. Ex Webb) Parl. Ex Webb	Hemicryptophyte
Tornabenea ribeirensis Schmidt & Lobin	Hemicryptophyte
Tornabenea tenuissima (A. Chev.) A. Hansen & Sunding	Chamaephyte

* On the statistical analysis we only considered one of the classifications, in which we followed the classification of Frankiewicz *et al.* [20].

Table 2.S2: Phytosociological vegetation units of the sampled taxa.

Taxa	Phytosociological vegetation
***Daucus carota* L. subsp. *azoricus* Franco**	*Euphorbio azoricae – Festucion petraeae*
	Tolpido succulentae – Agrostion congestiflorae
	Holco rigidi – Brachypodietum gaditanae
Daucus carota* L. subsp. *carota	*Artemisietea vulgaris*
***Daucus carota* subsp. *gummifer* (Syme) Hook.f.**	*Crithmo – Armerietalia*
***Daucus carota* L. subsp. *halophilus* (Brot.) A. Pujadas**	*Crithmo maritimi – Daucion halophili*
	Scrophulario sublyratae – Lavateretum arboreae
***Daucus carota* L. subsp. *maximus* (Desf.) Ball**	*Onorpodion castellani*
***Daucus crinitus* Desf.**	*Hyparrhenion hirtae*
***Daucus muricatus* (L.) L.**	*Hordeion leporini*
***Melanoselinum decipiens* (Schrad. & J.C.Wendl.) Hoffm.**	*Euphorbion melliferae*
	Isoplexido sceptri – Euphorbietum melliferae
***Monizia edulis* Lowe**	*Sinapidendro angustifolii – Aeonion glutinosi*
	Monizia edulis community
***Pseudorlaya pumila* (L.) Grande**	*Vulpietalia*
***Tornabenea annua* Bég.**	*Globulario amygdalifoliae – Periplocion chevalieri*
	Echio hypertropici – Euphorbietum tuckeyanae
	Dichrostachyo platycarpae – Acacietum caboverdeanae
	Heteropogonetum melanocarpi
***Tornabenea bischoffii* J.A. Schmidt**	*Globulario amygdalifoliae – Periplocion chevalieri*
	Loto latifolii – Artemisietum gogonei
	Melanoselino bischoffii – Globarietum amygdalifoliae
	Dichanthio foveolati – Heteropogonetum contorti
***Tornabenea insularis* (Parl. ex Webb) Parl. ex Webb**	*Globulario amygdalifoliae – Periplocion chevalieri*
	Cocculo penduli – Sarcostemmetea daltonii
	Aeonio gorgonei – Sarcostemmetum daltonii
	Echio stenosiphonis – Euphorbietum tuckeyanae
	Tetraeno waterlotii – Sarcostemmetum daltonii
	Launaeo thalassicae – Euphorbietum tuckeyanae
	Asterisco smithii – Euphorbietum tuckeyanae
	Forsskaoleo procridifoliae – Ficetum gnaphalocarpae
	Campanulo bravensis – Launaetum thalassicae
***Tornabenea ribeirensis* Schmidt & Lobin**	*Dichrostachyo platycarpae – Acacietalia caboverdeanae*
	Fico gnaphalocarpae – Acacion caboverdeanae
***Tornabenea tenuissima* (A. Chev.) A. Hansen & Sunding**	*Globulario amygdalifoliae – Periplocion chevalieri*
	Echietum vulcanori
	Erysimo caboverdeanae – Periplocetum chevalieri

Table 2.S3: Description of the phytosociological vegetation units of the sampled taxa

Phytosociological Vegetation Unit	Description
***Aeonio gorgonei – Sarcostemmetum daltonii* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Climactical and edapho-xerophilous dwarf shrub savanna community rich in succulent plants well-developed on S. Nicolau and São Vicente Islands on skeletal leptosols ancient altered volcanic rocky, in tropical desertic, thermo-tropical arid bioclimate, often affected by the trade winds from the north, especially in winter.
***Artemisietea vulgaris* Lohmeyer, Preising & Tüxen in Tüxen 1950 ex von Rochow 1951**	Pioneer and ruderal sunny vegetation composed by perennial and tall biennial forbs, grasses, and thistles. These communities prosper in deep soils enriched in nitrogen (due to man or cattle action). In Mediterranean pluviseasonal and temperate bioclimate. Holarctic distribution, neophytes in all tropical areas.
***Asterisco smithii – Euphorbietum tuckeyanae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Climactical closed shrub savanna community growing on coluvisols and leptic andosols.
***Campanulo bravensis – Launaetum thalassicae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Thermotropical arid to semiarid chasmophytic association, occurs on volcanic rocks of Brava Island
***Cocculo penduli – Sarcostemmetea daltonii* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Micro-shrubland often succulent and deciduous micro-woodland open savanna of Cabo Verde, mostly with aridic tropical bioclimate; occurring in desertic, xeric, infra-thermo-meso to lower supratropical ultrahyperarid to upper dry, strongly euhyperoceanic bioclimate; growing on arenosols, andosols, leptosols (lithic, skeletic, hyperskeletic) or fluvi-regosols and sometimes on little altered volcanic materials (lava and tephra).
***Crithmo – Armerietalia* Géhu & Géhu-Frank 1984**	Atlantic chasmophytic aerohaline pioneer communities of sea cliffs.
***Crithmo maritimi – Daucion halophili* Rivas-Martínez, Lousã, T.E. Díaz, Fernández-González & J.C. Costa 1990**	Rupicolous dwarf-herb vegetation of salt-sprayed cliffs of the southwestern Iberian Peninsula and Northern Morocco
***Dichanthio foveolati – Heteropogonetum contorti* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Xeromorphic perennial grassland savanna, growing on leptic andosols, in upper thermotropical to lower mesotropical and from lower semiarid to dry bioclimates.
***Dichrostachyo platycarpae – Acacietalia caboverdeanae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Deciduous micro-woodland climactic savanna (*Fico gnaphalocarpae-Acacion caboverdeanae*), growing on lithosols, arenosols, andosols and tephras, including the edaphohygrophilous coastal palm groves (*Phoenicion atlanticae*) and temporary wet tamarisk thickets (*Tamaricion senegalensis*), occurring in infra-thermotropical and low mesotropical upper arid to dry bioclimates, occasionally in lower subhumid; when in arid bioclimates seems to be necessary a shallow temporary hydromorphism on soils.
***Dichrostachyo platycarpae – Acacietum caboverdeanae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Climactic phanerophytic deciduous microwoodland savanna community, growing on andosols and leptosols, on Santiago Island. It occurs in upper infra and thermotropical, upper arid, semiarid, and lower dry bioclimate
***Echietum vulcanori* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Shrub climactical open savanna community dominated and well characterized by the local endemic *Echium vulcanorum*.
***Cocculo penduli – Sarcostemmetea daltonii* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Micro-shrubland often succulent and deciduous micro-woodland open savanna of Cabo Verde, mostly with aridic tropical bioclimate; occurring in desertic, xeric, infra-thermo-meso to lower supratropical ultrahyperarid to upper dry, strongly euhyperoceanic bioclimate; growing on arenosols, andosols, leptosols (lithic, skeletic, hyperskeletic) or fluvi-regosols and sometimes on little altered volcanic materials (lava and tephra).
***Crithmo – Armerietalia* Géhu & Géhu-Frank 1984**	Atlantic chasmophytic aerohaline pioneer communities of sea cliffs.
***Echio hypertropici – Euphorbietum tuckeyanae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Edaphoxerophytic and climatical tall, crowded shrub savanna community of Serra da Malagueta (Santiago Island), growing on andosols and coluvisols, in tropical xeric and occasionally some years pluviseasonal, thermotropical, semiarid to dry euhyperoceanic bioclimate.
***Echio stenosiphonis – Euphorbietum tuckeyanae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Climactical shrub savanna community, growing on leptic andosols near the summit of de São Vicente Island, in topographical tropical cloudy, thermotropical, upper arid, euhyperoceanic bioclimate.
***Erysimo caboverdeanae – Periplocetum chevalieri* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Climactical and edaphoxerophillous tall shrub savanna community, occurring on leptosols and leptic andosols in Fogo Island, in tropical xeric, thermo-mesotropical, semiarid to upper dry, euhyperoceanic bioclimate.
***Euphorbio azoricae – Festucion petraeae* Lüpnitz 1976**	Vegetation of salt-sprayed coastal cliffs of the Azores. Termomesotemperate levels.
***Euphorbion melliferae* Capelo, J.C. Costa, Jardim, Sequeira, Aguiar & Lousã 2003**	Microphyllous caulirosulate communities with woody habit and large leaves, proper interrupt the forest canopy.

***Fico gnaphalocarpae – Acacion caboverdeanae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Deciduous open micro-woodlands savanna, climactical, edaphoxerophilous or seasonally hygrophilous with fleeting superficial temporal hydromorphy; growing in upper infra and thermotropical arid to dry bioclimates; developed on lithic or regosolic soils.
***Forsskaoleo procridifoliae – Ficetum gnaphalocarpae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Micro-mesophanerophytic woodland savanna community, growing on seasonal temporary moist shallow soils, on plains and torrents with fleeting superficial temporary hydromorphy
***Globulario amygdalifoliae – Periplocion chevalieri* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Desertic and xeric climactical tropical and edaphoxerophilous tall shrub savanna community; in thermo-mesotropical, occasionally lower supratropical, from upper arid to upper dry, locally some years low subhumid (Fogo Island); developed on leptosols, andosols, colluvial and tephric soils.
***Heteropogonetum melanocarpi* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Xeromorphic perennial short grassland savanna, occurring in thermo to lower mesotropical semiarid to dry bioclimates, in Santiago and Fogo Islands, growing on leptic andosols
***Holco rigidi – Brachypodietum gaditanae* Aguiar & F. Prieto in F. Prieto, Aguiar & Dias 2012**	Thermotemperate or thermomediterranean mesoxerophilous grassland of *Brachypodium gaditanum*, in less disturbed habitats co-dominated by *Holcus rigidus*. Its habitats are small platforms in rock outcrops with soil accumulations provided by solifluction and earth flows.
***Hordeion leporini* Br.-Bl. in Br.-Bl., Gajewski, Wraber & Walas 1936 corr. O. Bolòs 1962**	Mediterranean ruderal winter-annual grasslands
***Hyparrhenion hirtae* Br.-Bl., Rozeira & P.Silva 1956**	Grassland rich in perennial tall grasses, deep soils of rocky/ clayey cliffs, abandoned fields with some nitrification in infra to mesomediterranean semiarid to subhumid bioclimate; Mediterranean distribution.
***Isoplexido sceptri – Euphorbietum melliferae* Capelo, J.C. Costa, Jardim, Sequeira, Aguiar & Lousã 2003**	Microphyllous caulirosulate community, endemic from Madeira Island, on rocky basaltic walls, in mesotemperate to low supratemperate, humid to hiper-humid, in laurissilva clearances or places submitted to landslides.
***Launaeo thalassicae – Euphorbietum tuckeyanae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Edaphoxerophilous closed shrub savanna community, on leptosols and basalt on rocks and near vertical walls exposed to moist and wet winds.
***Loto latifolii – Artemisietum gogonei* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Climactical closed shrub savanna community, growing on leptics andosols, in upper thermotropical and lower mesotropical, semiarid to lower dry, euhyperoceanic bioclimate in Santo Antão Island.
***Melanoselino bischoffii – Globarietum amygdalifoliae* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Edaphoxerophilous nanophanerophytic short savanna community, growing on leptosols and basalt walls exposed to moist and wet winds, in tropical pluviseasonal, thermotropical, dry, euhyperoceanic bioclimate, in Santo Antão Island.
***Monizia edulis* community**	In vertical basaltic walls in the road for Curral das Freiras, alongside with other taxa either from moister and higher areas as well as from drier and lowest parts of the island.
***Onorpodion castellani* Br.-Bl. & O. Bolòs 1958 corr. Rivas-Martínez, T.E. Díaz, Fernández-González, Izco, Loidi, Lousã & Penas 2002**	Ruderal indifferent edaphic thistles communities, in abandoned fields and crops on amended deep soils, with a short period of hydromorphy, in infra -supra -mediterranean bioclimate with Mediterranean West Iberian, Baetic and Maghrebi distribution.
***Scrophulario sublyratae – Lavateretum arboreae* J.C. Costa, Capelo, Neto, Arsénio & Lousã 2012**	Ornitocoprophilous, nitrophilous and aerohaline association; islands reefs and coastal cliffs with rough sea, in the Sadensean-Portuguese Subprovince frontier; indifferent edaphic, in granites, syenites, and limestones in Mediterranean pluviseasonal -oceanic, euhyperoceanic, lower mesomediterranean to upper thermomediterranean, dry bioclimate.
***Sinapidendro angustifolii – Aeonion glutinosi* Capelo, J.C. Costa, Lousã, Fontinha, Jardim, Sequeira & Rivas-Martínez 2000**	Madeiran chomophytic and chasmophytic succulent-rich scrub on volcanic rock substrates and walls.
***Tetraeno waterlotii – Sarcostemmetum daltonii* Rivas-Martínez, Lousã, J.C. Costa & M.C. Duarte 2017**	Climactical and edafoxerophilous close dwarf shrub savanna community, occurring in Brava Island on leptosols in lower thermotropical arid, euhyperoceanic bioclimate.
***Tolpido succulentae – Agrostion congestiflorae* Aguiar & F. Prieto in F. Prieto, Aguiar & Dias 2012**	Thermomediterranean, occasionally thermoremperate, perennial graminoid communities of cliffs and landslide scarps.
***Vulpietalia* Pignatti 1953**	Mediterranean and Ibero-Atlantic ephemeral therophytic vegetation on coastal sand dunes under influence of salt spray.

Table 2.S4: Taxa of the sampled Daucinae tribes and their sampling locations (A, African Territory, Eu, European Territory; Az, Azores; CV, Cabo Verde; EU, Lu, mainland Portugal; Ma, Madeira; D, Desertas; F, Fogo; Fa, Faial; Fl, Flores; MA, Madeira Island; Pi, Pico; S, Santiago; SA (Az), Santa Maria; SA (CV), Santo Antão; SN, São Nicolau; SV, São Vicente).

Taxa	**Location**
Daucus carota subsp. *azoricus*	EU, Lu, Az: Fa, Horta, Castelo Branco
	EU, Lu, Az: Fl, Santa Cruz das Flores
	EU, Lu, Az: Pi, Madalena, Areia Larga
	EU, Lu, Az: SA, Vila Do Porto, São Pedro
Daucus carota subsp. *carota*	EU, Lu, Mainland: Lisboa, Lisboa, Tapada da Ajuda
Daucus carota subsp. *gummifer*	EU, Lu, Mainland: Leiria, Alcobaça, Praia da Polvoeira
	EU, Lu, Mainland: Leiria, Nazaré, Praia do Norte
	EU, Lu, Mainland: Leiria, Peniche, Papôa
	EU, Lu, Mainland: Leiria, Pombal, Carriço
Daucus carota subsp. *halophilus*	EU, Lu, Mainland: Beja, Odemira, Almograve
	EU, Lu, Mainland: Beja, Odemira, Cabo Sardão
	EU, Lu, Mainland: Setúbal, Sines, Porto Covo
	EU, Lu, Mainland: Faro, Vila do Bispo, Cabo de São Vicente
	EU, Lu, Mainland: Lisboa, Cascais, Praia do Guincho
	EU, Lu, Mainland: Beja, Odemira, Praia do Carvalhal
Daucus carota subsp. *maximus*	EU, Lu, Mainland: Faro, Loulé, Monte da Charneca
	EU, Lu, Mainland: Faro, Loulé, Benafim Grande
	EU, Lu, Mainland: Faro, Vila do Bispo, Cabo de São Vicente
Daucus carota subsp. *sativus*	EU, Lu, Mainland: Lisboa, Lisboa, Tapada da Ajuda
Daucus crinitus	EU, Lu, Mainland: Setúbal, Setúbal, Serra da Arrábida, El Carmen
	EU, Lu, Mainland: Setúbal, Setúbal, Portinho da Arrábida
Daucus muricatus	EU, Lu, Mainland: Faro, São Brás de Alportel
	EU, Lu, Mainland: Lisboa, Lisboa, Tapada da Ajuda
Melanoselinum decipiens	EU, Lu, Ma: MA, Ponta de Sol, Paul Serra, Levada 25 Fontes
	EU, Lu, Ma: MA, Santana, Caldeirão Verde
	EU, Lu, Ma: MA, Santana, Pico Ruivo
	EU, Lu, Ma: MA, Porto Moniz, Ribeira da Janela
	EU, Lu, Ma: MA, São Vicente, Lameiros, Rota do Cal
	EU, Lu, Ma: MA, São Vicente
Monizia edulis	EU, Lu, Ma: D, Deserta Grande
	EU, Lu, Ma: MA, Câmara de Lobos, Curral das Freiras
	EU, Lu, Ma: MA, Câmara de Lobos, Cabo Girão
Pseudorlaya pumila	EU, Lu, Mainland: Faro, Vila do Bispo, Cabo de São Vicente
Tornabenea annua	A, CV: S
	A, CV: S, Tarrafal Serra da Malagueta
Tornabenea bischoffii	A, CV: SA, Paúl, Cova
	A, CV: SA, Ribeira Grande, Corda
	A, CV: SA, Ribeira Grande, Estraga
	A, CV: SA
Tornabenea insularis	A, CV: SN, Vila da Ribeira Brava, Monte da Sentinha
	A, CV: SN, Monte Gordo
	A, CV: SV, Monte Verde
Tornabenea ribeirensis	A, CV: SN, Tarrafal, Assumada de Mancebo
Tornabenea tenuissima	A, CV: F

Chapter 3

Manuscript II

New clues on cytogenomic differences between islands and continents: a case study with *Crithmum maritimum*

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Abstract

Angiosperms present an astonishing diversity of genome sizes (GS), that can vary intra- or interspecifically. Several studies on insular taxa reported that these lineages of plants tend to have smaller genomes when compared with their continental congeners. However, few studies have focused on the factors shaping this variation. In this study, we analyze the genome variation of insular and continental populations of sea fennel or rock samphire *Crithmum maritimum*. We present a comprehensive GS dataset of 114 populations of mainland and insular Portuguese populations of *C. maritimum*. Flow cytometry cytogenomic GS values showed a considerable intraspecific variation spanning from less than 4.1 pg in the Azores and Madeira to over 5.0 pg in the north of mainland Portugal. In the latter, there was a tendency for an increase in GS along the coast, from south to north, in association with lower temperatures, higher precipitation and lower precipitation seasonality. However, this gradient might be the result of historical phylogeographical events associated with previous dispersal and extinction of local populations. The results indicate that a taxonomic revision of the genus is required and provide evidence that conservation strategies must be set in place to protect the putatively distinct populations.

Keywords: DNA flow cytometry; genome-climate interactions; genomic trait; intraspecific variation; population studies

3.1 Introduction

A genome is the haploid set of chromossomes in each cell, and it constitutes a biological level of organization, with a unique evolutionary history that results in an inherited genome size (GS; Gregory, 2005). The diploid amount of nuclear DNA can be obtained through Flow Cytometry (FCM), which is an easy and reliable technique for isolating and count nuclei from solid tissues (Galbraith *et al.*, 1983). Genome sizes (GS) vary dramatically among plant species (e.g., Pellicer *et al.*, 2018), spanning 1C-values from 0.065 picograms (pg) in *Genlisea aurea* A.St.-Hill. (Lentibulariaceae, Fleischmann *et al.*, 2014) to 152.23 pg in *Paris japonica* Franch (Trilliaceae, Pellicer *et al.*, 2010). Intraspecific genome size variation has also been reported (Gregory, 2001), for example a variation of 16% has been reported for *Brassica rapa* (Boutte et al., 2020), for *Sinningia speciosa* a genome size variation of 21 – 26 % was also found in a group of wild specimens (Zaitli & Pierce, 2010).

This variation of GS can be due to an expansion or contraction (Qiu *et al.*, 2019). Expansion of GS can be due to polyploidization (Wood *et al.*, 2009) and/ or amplification of transposable elements (TEs; Hawkins *et al.*, 2008), the latter being facilitated by recombination processes (Schubert & Vu, 2016). Apart from the impact that the GS has on the structure of the genome, it can also be significant at the ecological and evolutionary level (Biémont, 2008), since it correlates with several phenotypic traits, such as flowering time, flower size, seed mass and photosynthetic rate (e.g., Meagher & Vassiliadis, 2005; Beaulieu *et al*., 2007a, b, 2008). Furthermore, it also correlates with environmental variables, namely altitude, latitude, and temperature (e.g., Knight *et al.*, 2005, Suda *et al.*, 2005). However, some authors have proposed that the observed instraspecific C-value variation can be due to the presence of more than one entity within a species. The taxa *Lachnagrostis lyallii* and *Deyeuxia avenoides,* presented a genome size variation of 1.9-fold in the former and 1.2-fold in the latter, moreover, they are considered highly variable species, which led to Murray (2005) proposing a more detailed study in order to access their taxonomy.

It seems that GS is shaped by natural selection, nonetheless, the opposite (i.e., drift) has also been proposed to explain GS variation (Oliver *et al.*, 2007; Whitney *et al.*, 2010). To test if GS undergoes selection, population-level analyses are ideal (Díez *et al*., 2013). However, up to date, most of the studies were only performed at interspecific scales (Díez *et al*., 2013). The sea fennel or rock samphire *Crithmum maritimum* L. (Apiaceae), is a monospecific taxon (Castroviejo, 2003; Meot-Duros & Magné, 2009). Therefore, is a potential model species to test possible environmental correlations with the GS variation. This species is a facultative halophyte that grows in rocky sea cliffs and occasionally in sands and gravel (**Figure 3.1**). It has a very wide distribution, occurring along the European Atlantic coasts, the Azores, Madeira, and Canaries archipelagos, the Mediterranean and Black Sea coast, northwest Africa, and West Asia, with its distribution limited by temperature (Crawford, 1982, Castroviejo, 2003). However, due to climate change, its distribution is currently expanding northwards (Metzing & Gerlach, 2001). It is an aromatic herb with therapeutic healing properties known since ancient times, it was mentioned by Hippocrates in the 4th century B.C to soothe vesical pains (Pline, 1957), and by sailors who ate its fresh leaves to prevent scurvy (Baytop, 1984).

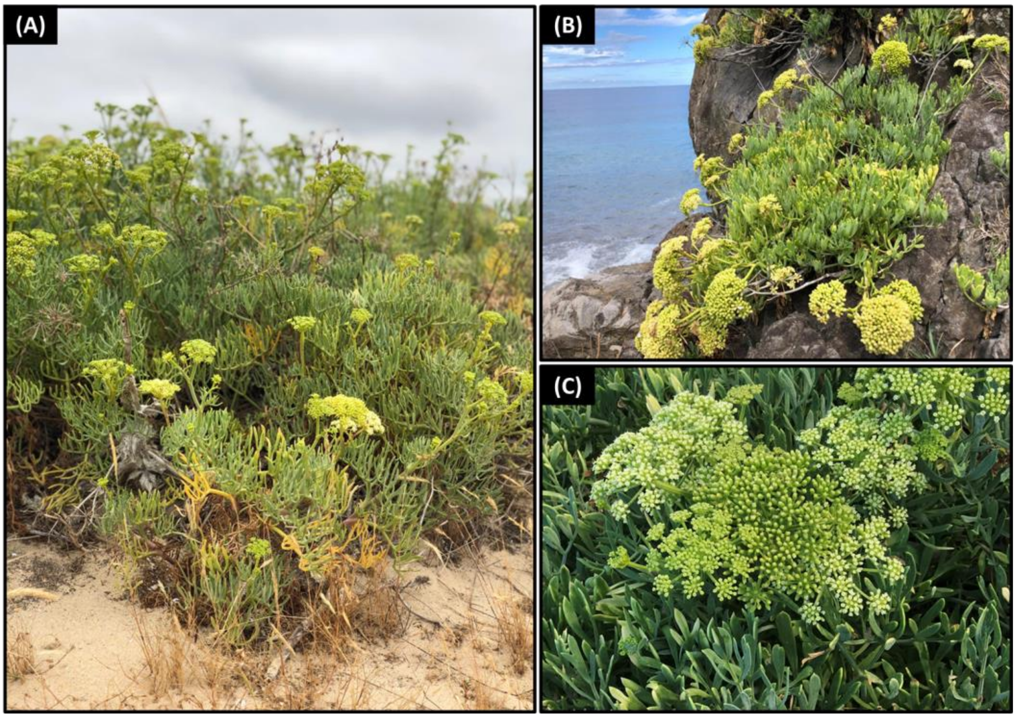


Figure 3.1 Plants of *Crithmum maritimum* **(A)** growing in sands in Castelo Branco; **(B)** growing in coastal rocky cliffs in São Jorge, Madeira island; and **(C)** detail of its inflorescences, specimen from Paúl do Mar, Madeira island.

Over the centuries, the use of this plant has decreased, but in the 21st century more studies have been published suggesting its potential as an emerging crop (e.g., Renna, 2018). In fact, its leaves hold high contents of carotenoids, flavonoids, vitamin C, and bioactive substances (Özcan, 2001).

Recent studies identified high levels of both genetic (Latron *et al.*, 2018; 2020) and phytochemical differentiation among and within *Crithmum maritimum* populations (e.g., Katsouri *et al*., 2001; Maleš *et al*., 2001; Kulišić-Bilušić *et al*., 2010). However, cytogenomically *C. maritimum* was analyzed only from one location (i.e., Strunjan saltpan in Slovenia), resulting in an estimation of a nuclear DNA amount (2C-value) of 4.38 pg (Koce *et al.*, 2008).

The present study aims to investigate the cytogenomic variation of *C. maritimum* at the population-level, and if it correlates with environmental variables. Namely, we intend to determine the amount of variation of GS among populations, and if an association with a geographical gradient or with environmental variables exists. Additionally, we discuss our results from an evolutionary and taxonomic point of view, in accordance with current theories of genomic mutations, and provide some conservation considerations regarding the analyzed populations.

3.2 Material and methods

3.2.1 Sampling

Specimens of *Crithmum maritimum* were collected for cytogenomic studies during several field surveys (2017 – 2020), in mainland Portugal and the archipelagos of the Azores, Berlengas and Madeira. Particularly, fieldwork took place on six Azorean islands (Corvo, Faial, Pico, Santa Maria, São Jorge, São Miguel), three Madeiran islands (Deserta Grande, Madeira, Porto Santo) and on one island of the Berlengas (Berlenga Grande). A total of 114 populations was sampled (**Figure 3.2; Supplementary Data Table 3.S1**). From each population, a minimum of three specimens was collected. For each site, we recorded geographical coordinates and altitude with a GPS. Each sample was collected and preserved in wet tissue paper, wrapped in aluminium foil and zip-lock bags, then preserved at 5°C, and posted to the laboratory.

Vouchers from most of the samples (at least one per population) were collected and deposited at Herbarium of Instituto Superior de Agronomia, University of Lisbon (LISI), at the Herbarium of the University of Madeira (UMAD) and at the Herbarium of the University of the Azores (AZB).

3.2.2 Environmental data

The 19 climatic variables used in the present study (**Supplementary Table 3.S2**) 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). The geographical coordinates were extracted using a GPS.

Figure 3.2. Bubble map plot showing the gradient of genome size across the studied areas. The genome size (in picograms, pg) per sampled populations is mapped according to the colour of the bubbles.

3.2.3 Cytogenomic analysis

Nuclear DNA content was estimated using FCM. Preparation of suspensions of intact nuclei for analysis was performed following the method of Galbraith *et al.* (1983). 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 MgCl2, 1% Triton X-100, Na2EDTA 2 mM, NaCl 86 mM, sodium metabisulfite 20 mM, PVP-10 a 1%, pH 7,5; Loureiro *et al*., 2007). The nuclear suspension was sieved using a nylon mesh with 30 µm to remove large debris. Then, nuclei were stained with 25 µg ml-1 of propidium iodide (PI; Sigma-Aldrich, USA). To estimate the nuclear DNA content, it is required the use of a reference standard of known GS. The following standard was employed: *Solanum lycopersicum* L. (2C = 1.96 pg; Doležel *et al*., 1992). The acquisition of numeric data and fluorescence graphs was made by Sysmex FloMax software v2.4d (Sysmex, Görlitz, Germany), as described by Guilengue *et al*. (2020). The histograms for each sample were recorded and the C-values were calculated with the following formula:

$$\text{Nuclear DNA Content (pg)} = \frac{\text{Sample G1 Peak Mean } \times \text{GS of Reference Standard}}{\text{Reference Standard G1 Peak Mean}}$$

3.2.4 Statistical analysis

Statistical analyses and descriptive statistics were performed using RStudio v1.3.1093 software (R Core Team, 2020). Descriptive statistics were calculated for each population, namely mean and standard deviation (SD) of the genome size (2C-values). For the data on 2C-values, descriptive analyses were performed using boxplots. Comparisons between GS values and: i) among populations; ii) island/ mainland populations; and iii) among mainland districts and archipelagos. Group comparisons were implemented using non-parametric tests since GS data were not following a Gaussian distribution ($p<0.05$ with the Shapiro–Wilk test; Shapiro & Wilk,1965) even after using Box-Cox transformation (Box & Cox, 1964) or other conventional transformation techniques (Zar, 2010). Thus, we opted for the Mann-Whitney test for two group comparisons and for the Kruskal-Wallis test for comparisons of more than two groups, using the respective functions in R. When the Kruskal-Wallis test indicated the rejection of the null hypothesis, we applied a non-parametric multiple comparison test (Siegel & Castellan, 1988; Conover & Iman, 1979) using the function posthoc.kruskal.conover.test of the “The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR) R package (Pohlert, 2014), including Bonferroni-type adjustment of p-values, and which aims to ensure a relatively high level of statistical power (i.e., reduction in the probability of committing a type II error).

We calculated two different Gaussian Generalized Linear Models (GLMs), to determine what factors could better explain the observed 2C-values, one including island and mainland populations and a second model which only included mainland populations: both included a Null model was used as a benchmark. The model with both island and mainland populations included a district model (among mainland districts and archipelagos), an island model (comparing islands with mainland populations) a Bioclimate model (including the principal components extracted from a Principal Component Analysis, PCA, applied to the 19 bioclimatic variables, BIOCLIM), and a full model including all the previous factors, as well as other models resulting from its simplification. The model with only the mainland populations included a district model (with only mainland districts), a latitude model, a longitude model, and altitude model, a BIOCLIM one (including the principal components extracted from a PCA applied to the 19 bioclimatic variables) and a full model including all the previous factors, as well as other models resulting from its simplification. To extract the principal components of the bioclim variables we used the vegan package for R and followed the Kaiser – Guttman and broken stick model criteria to determine the number of components to retain, that is, those with eigenvalues above the mean eigenvalue and the broken stick model (see

Borcard *et al*., 2011). The implementation of the GLMs followed Ávila *et al*. (2018) and Parelho *et al.* (2020) (see references therein), using the glm function of R. The best models were selected based on the maximum likelihood approach using Akaike's Information Criterion (AIC, Zar, 2010). The model with the lowest AIC and the highest R^2 was considered to best fit the data. The R package "mass" was used to evaluate GLMs. Although the raw data failed to comply with normality, the calculated GLMs included all the samples in the analysis. Thus, according to the central limit theorem, when independent random variables are added, their properly normalized sum tends toward a normal distribution even if the original variables themselves are not normally distributed. That is, with a large sample size (i.e., more than 100 observations in this case) the mean tends to a normal distribution, even if the underlying data are not Gaussian (Kwak & Kim, 2017). Since the GLMs model the mean of the distribution, we considered their application as correct in this context.

3.3 Results

3.3.1 Genome size variation among populations

The cytogenomic results are summarized in (**Supplementary Table 3.S2**) that shows the mean 2C-values in picograms (pg) with standard deviation (SD), and sample coefficient of variation (%) of the 114 analyzed *Crithmum maritimum* populations across mainland Portugal, Azores, Berlengas and Madeira. Determinations of GS based on FCM produced histograms of fluorescence of G0/G1 peaks, the coefficient of variation (CVa) values ranged from 0.840 to 6.928% (mean= 2.445%). A high cytogenomic diversity across the different populations was observed (4.710 ± 0.294 pg), with mainland populations presenting the largest GS and variabilities (4.859 ± 0.148 pg) compared to the island populations (4.332 ± 0.198 pg; **Figure 3.3**).

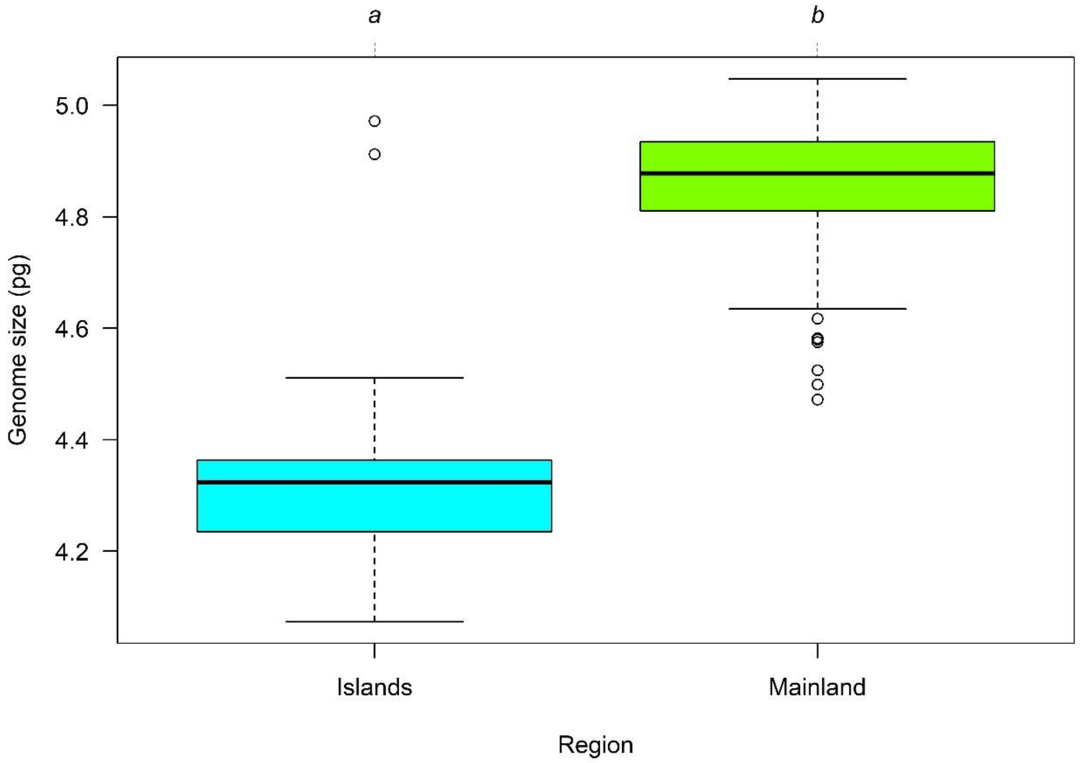


Fig. 3.3 Boxplot diagram showing the genome size variation in *Crithmum maritimum* sampled populations from insular and mainland Portugal. The plot represents the four quartiles; the dots represent the outliers. Different letters indicate a significant difference (*p*<0.05) according to the Mann-Whiteny test (W= 118, *p*= 2.704e-15). The outliers in the Islands group correspond to the Berlengas Islands.

The Azorean population presented the smallest mean 2C-values (4.217 ± 0.093 pg), followed by the Madeira population (4.348 ± 0.107 pg) and with the largest the Berlengas population (4.938 ± 0.077 pg; **Figure 3.4**). In the mainland, a geographical gradient can be observed, with the northern populations presenting larger genomes in comparison to the southern ones (**Figure 3.2**). Overall, Fajã das Achadas da Cruz in Madeira Island presented the smallest genome (4.074 ± 0.079 pg), and Praia dos Barcos, Porto, in the mainland, the largest one (5.047 ± 0.145 pg; **Table 3.S3**; **Figure 3.4**).

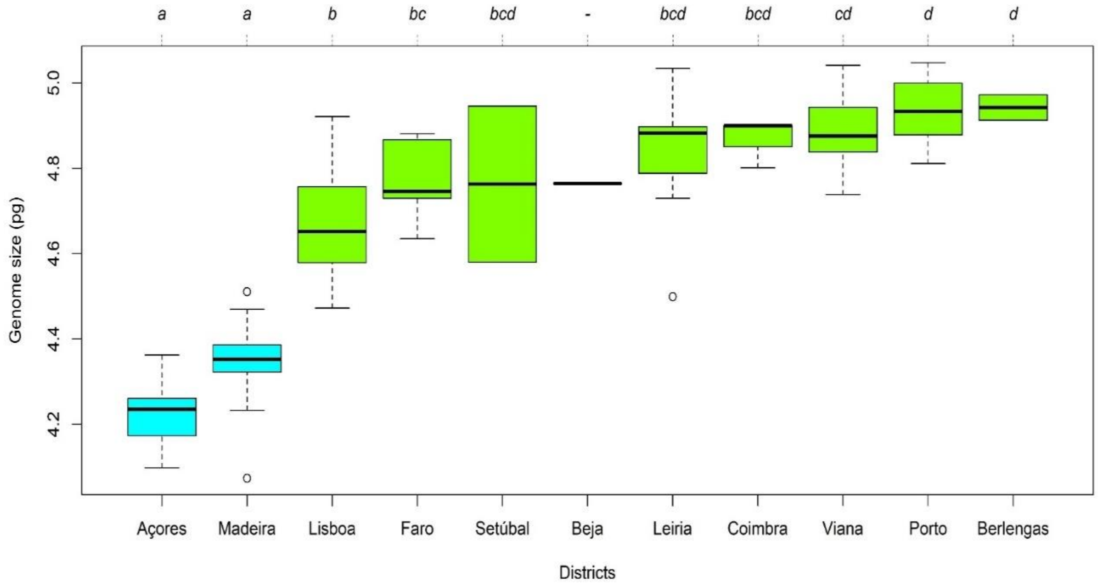


Fig. 3.4. Boxplot diagram showing the genome size variation in *Crithmum maritimum* sampled populations from insular (blue) and mainland (green) Portugal. The plot represents the four quartiles; the dots represent the outliers. Different letters indicate significant differences (*p*<0.05) according to the results of a non-parametric multiple comparison test applied after the Kruskal-Wallis test (Chi-squared= 87.334, df= 10, *p*= 1.806e-14). The Berlengas Islands are represented in green, since the respective followed the pattern found for mainland Portugal.

3.3.2 Genome size and environmental correlates

When considering the whole data set, the best GLM model corresponded to what we defined as districts (**Table 3.1**), separating Azores, Madeira and Berlengas from the districts in the mainland, which is in agreement with the non-parametric analysis shown in **Figure 3.3** and **Figure 3.4.** Although the differences between islands and the mainland were significant (**Figure 3.3**), this model explained less information than the model discriminating the islands but also the mainland districts (**Figure 3.4**). The latter model allowed to clearly separate Azores and Madeira from the mainland populations, but also incorporated the considerable variation found among the latter. Regarding the bioclimatic variables, we retained three main components characterizing the climate to be found at all included populations (**Table 3.2, Supplementary Figure 3.S1 A**). PC1 was mostly associated with high temperatures, particularly during the coldest/wettest part of the year, with low values for temperature variation and with higher precipitation in the warmest part of the year. PC2 was associated with high temperatures, low precipitation seasonality, and high precipitation. PC3 was associated with low precipitation on the driest part of the year but with high precipitation during the wettest/coldest part of the year and with high precipitation seasonality. The three retained components explained 51.2%, 26.2% and 13.8% of the variation in the data, respectively. The addition of the bioclimatic variables in the GLMs (i.e., District + BIOCLIM) also revealed to be an adequate GLM model (**Table 3.1**). However, since it did not show any relevant improvement in terms of AIC or R^2 values when compared to the model that only included the districts, it can be inferred that this information seemed to be redundant. In fact, it mostly identified the geographic differences between mainland regions on one side, and Madeira and Azores, on the other.

Table. 3.1 Results of the application of Gaussian generalized linear models to the *Crithmum maritimum* genome size data for all the samples and for mainland data only. The null model included the intercept only and is used as benchmark. Models with lowest AICc (Akaike's Information Criterion, corrected) and highest R^2 (adjusted determination coefficient) correspond to the best fit (less information loss; models shaded in grey). Models in bold include significant effects. BIOCLIM correspond to the three (all data) or two (mainland data) principal components extracted from the 19 bioclimatic variables. For mainland, some of the models only included the first (BIOCLIM[1]) or the second (BIOCLIM[2]) principal components.

Data	Models	AICc	R^2
All	**District**	**-192.6851**	**0.8960113**
	District+BIOCLIM	**-190.8943**	**0.9013585**
	BIOCLIM	-155.9616	0.8340893
	Islands+BIOCLIM	-155.5200	0.8366710
	Islands	-105.6529	0.7320499
	Null	42.3698	-
Mainland	**District+Longitude+Latitude+BIOCLIM[2]**	**-129.7283**	**0.5741775**
	District+Longitude+Latitude+BIOCLIM	-127.6926	0.5789454
	District+Longitude+Latitude+BIOCLIM[1]	-127.3333	0.5607247
	Longitude+BIOCLIM	**-123.5665**	**0.4169917**
	BIOCLIM	**-121.1520**	**0.3802646**
	District	-119.0079	0.4556500
	Altitude+BIOCLIM	-118.9623	0.3810677
	Latitude+BIOCLIM	-118.9017	0.3805799
	Latitude	**-118.1657**	**0.336854**
	District+BIOCLIM	-116.5723	0.4760385
	Longitude	**-111.1755**	**0.2738357**
	Null	-88.70380	-
	Altitude	-88.56547	0.0259979

When considering mainland data only, the fine information provided by geographic location (i.e., District, Longitude, Latitude) and climate (i.e., the two main climatic components extracted from the bioclimatic data, (**see Table 3.2,**

Supplementary Figure 3.S1 B), provided the best GLM model (**Table 3.1**). PC1 was associated with high temperatures, low precipitation, and high precipitation seasonality. PC2 was associated with low temperature variation, low temperature in the driest/ warmest part of the year, high temperature in the coldest/wettest part of the year. The two retained components explained 60.7% and 32.7% of the variation in the data, respectively. Latitude and longitude provided significant but relatively low fit models, while altitude provided a non-significant model (**Table 3.1**). Although a model including the two main climatic factors was also significant, it originated a lower fit to the data the model including mainland district, longitude, latitude and the second main climatic component. This hints to a geographic effect, associated with a climatic gradient that partly explained the variation in GS. This corresponded to a gradient from south to north (with GS increasing in that direction, Spearman correlation, r=0.46, p<0.001) and from east to west (with GS decreasing in that direction, Spearman correlation, r=0.42, p< 0.001). The correlation of GS with the second main climatic component was negative (Spearman correlation, r=-0.28, p=0.01481) meaning that larger values of GS would be found at places with high temperature variation, high temperature in the driest/warmest part of the year, and low temperature in the coldest/wettest part of the year (**see Table 3.2**). The correlation of GS with the first main climatic component was negative (Spearman correlation, r=- 0.44, p<0.001) meaning that larger values would be found at places with low temperatures, high precipitation, and low precipitation seasonality, reinforcing the possibility of a positive gradient of GS from southern to northern Portugal.

Table. 3.2 Scores of each environmental variable for each of the first main principal components (PC) extracted, including all data and only mainland data, respectively.

Variable		**All data**			**Mainland**	
Description	**Code**	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**
Annual Mean Temperature	BIO01	1.2055	-0.9658	0.02266	1.3358	0.16801
Mean Diurnal Range	BIO02	-1.4892	0.1808	0.32628	0.1413	-1.40061
Isothermality	BIO03	-1.4867	0.1874	0.38696	0.2999	-1.30236
Temperature Seasonality	BIO04	-1.4565	0.3530	0.03263	-0.2891	-1.36900
Max Temperature of Warmest Month	BIO05	-0.9998	-0.7736	0.17144	0.8519	-1.08714
Min Temperature of Coldest Month	BIO06	1.4318	-0.5967	-0.14422	0.8354	1.10800
Temperature Annual Range	BIO07	-1.4903	0.2437	0.17180	-0.0134	-1.41329
Mean Temperature of Wettest Quarter	BIO08	1.2873	-0.7814	-0.20812	1.1371	0.75212
Mean Temperature of Driest Quarter	BIO09	0.1153	-1.0839	0.84831	1.0554	-0.73100
Mean Temperature of Warmest Quarter	BIO10	0.7162	-1.1097	-0.06649	1.0554	-0.73100
Mean Temperature of Coldest Quarter	BIO11	1.3715	-0.7382	-0.08218	1.1388	0.77556
Annual Precipitation	BIO12	0.9689	1.0162	0.64521	-1.3763	0.06476
Precipitation of Wettest Month	BIO13	0.9832	0.8671	0.83583	-1.3488	0.12659
Precipitation of Driest Month	BIO14	0.5668	1.0051	-0.89236	-1.3800	0.13383
Precipitation Seasonality	BIO15	-0.1833	-0.7656	1.17066	1.3159	0.38142
Precipitation of Wettest Quarter	BIO16	0.9683	0.8956	0.82172	-1.3543	0.14575
Precipitation of Driest Quarter	BIO17	0.7114	1.0949	-0.67493	-1.3882	0.08775
Precipitation of Warmest Quarter	BIO18	1.4198	0.4623	0.16872	-1.3882	0.08775
Precipitation of Coldest Quarter	BIO19	0.8290	0.9152	0.92190	-1.3383	-0.10320

3.4 Discussion

The use of flow cytometry (FCM) in population studies has expanded over the years (Loureiro *et al.,* 2010). However, most studies have focused on the level of polyploid across the different populations (Padilla-Garcia *et al*., 2018) or the interspecific variation in closely related taxa (Ochatt *et al*., 2017; Kosiński *et al*., 2019; Erst *et al*., 2020). Our study was designed to investigate the intraspecific variation of *Crithmum maritimum* and if it relates to geographical and climatic variables. For this purpose, we chose a species that was monotypic and widely distributed along the coastline, making it possible to have a thorough sampling across the coastline of the Portuguese territory and assuming that all variation is intraspecific. Additionally, our sampling allowed to compare mainland population lineages with insular ones.

The 2C-values of the *Crithmum maritimum* presented a considerable variation among populations, in fact, the present findings seem to be consistent with research by Latron *et al.* (2018, 2020) that by using nuclear microsatellites observed a high genetic differentiation between populations. Moreover, our results revealed that the island populations presented smaller genome sizes and less variation (except the Berlengas), when compared to mainland ones. This tendency towards smaller genome size in the islands has been observed by Suda *et al.* (2003, 2005) in the Canary Islands, and by Kapralov and Filatov (2011) in the Hawaiian and Marquesas archipelagos. The latter authors proposed two theories for these observations: i) genome size miniaturization during or after its colonization, and ii) predominance of colonizers with small genomes. Furthermore, and corroborating with both theories, Guingard *et al*., (2016) showed that organisms with smaller genomes use fewer resources, having a selective advantage under insular environments (Kraaijeveld, 2010). Nonetheless, this observation of small genomes on islands did not hold for Berlengas, probably due to its close proximity (circa 10 km) to mainland (Romão, 2009). This proximity could have resulted in a higher colonization rate by mainland individuals, when compared to Azores and Madeira, which are, respectively, at 1570 km and 978 km from the Portuguese coast (Fernández-Palacios *et al*., 2010). Hence, it can be assumed that, for *C. maritimum*, the most probable hypothesis for the smaller genomes on the island populations, is the downsizing of genome size during or after its colonization, because the Berlengas populations presented the largest mean 2C-values.

The relationships between genome size and both bioclimatic and geographic variables have been widely studied among various groups of plants over time (e.g., Bottini *et al.,* 2000; Suda *et al*., 2003, 2005; Díez *et al*., 2013; Brilhante *et al*., 2021). Thitherto, during the various studies carried out, no universal consistency was reached between them. In our study, relationships were estimated, for the first time, between genome size of *C. maritimum* and the 22 predictor variables involving 19 enviromental variables and three geographic variables. It should be noted that geographic variables can be a proxy or involve a complex of several climatic variables that are correlated to them (De Frenne *et al.*, 2013).

The data collection of 114 populations across the Portuguese territory revealed that the best model corresponded to what we defined as districts, separating Azores, Madeira and Berlengas from the districts in the mainland. Climatic information seems to be redundant, since they mostly identify the geographic differences between mainland regions on one side, and Madeira and Azores, on the other. Therefore, a geographic element, related to island isolation seems to be important in shaping the genome size, more specifically in miniaturizing the genome size of island population. In this sense, as mentioned by several authors, the genome size tends to be smaller on islands compared to continental parts due to the insular selective pressures (Kapralov & Filatov., 2011; Suda *et al*., 2003, 2005). As a matter of fact, smaller genomes are advantageous, since it reduces genetic instability (Suda *et al*., 2005).

When only considering the mainland populations, the climate, and the geographic position, can partly explain the genome size differences. In fact, larger genome sizes were found in populations with high temperature variation, high temperature in the driest/ warmest part of the year, low temperature in the coldest/ wettest part of the year but also in places with low temperatures, high precipitation, and low precipitation seasonality. In other words, genome size increased from south to north and from east to west. In fact, in prokaryotes it was already seen that a greater variability of the environment resulted in genomes with a larger number of genes (Bentkowski, 2015). However, it is important to note that these observations should be taken into consideration when extrapolating to other taxa, as a matter of fact for longitude and latitude, negative (e.g., Bottini *et al*., 2000; Díez *et al*., 2013;) and positive (e.g., Chrtek *et al*., 2009; Basak *et al*., 2019) correlations have been observed.

Crithmum maritimum populations presented a considerable variation pattern of 2C-values. This variability seems to be shaped by both geographical and bioclimatic variables, that is, some environmental factors such as the characteristics of growing seasons may be responsible for the presented genome size signature. Moreover, the observed pattern between genome size and geographic location was not continuous along the Portuguese coast. A possible reason for this might be the efficient seed dispersal by hydrochory (Favre-Bac *et al*., 2016), which agrees with Latron *et al*. (2018, 2020) who also observed a lack of spatial trends in the genetic diversity of *C. maritimum*. These genomic variations may be the result of drift or of selection. However, in the case of continental populations, the latter appears to be the main evolutionary force in action, whistle in the case of the more distinct insular populations, drift, more specifically, founder effect, may have played an important role in the mutations linked to genome size alteration (Blommaert, 2020).

The distinction observed in insular populations, and also to some extent, in the mainland ones, suggest that a thorough taxonomic revision should be considered as well as additional molecular population genetics and genomic data.

The distinction observed between populations suggest that protection measures should be taken in consideration as it may contain still undescribed endemic taxa. A thorough inventory of populations and their current number of effectives should be considered, particularly in the insular populations, since the coastal habitat is one of the most intensively disturbed and fragile ecosystems in both archipelagos. Moreover, species that have evolved in relatively stable environments, such as island populations, may face the greatest risk of extinction under global climate change as genome streamlining genetically constrains even more their ability to adapt to the new environmental conditions (Bentkowski, 2015).

3.5 Conclusions

Our study revealed that *C. maritimum*, as a monotypic genus widely distributed across the Mediterranean region, is a good model to study relationships between genome size and environmental variables, since it shows several strong correlations among them. Overall, we present for the first time a comparative study between genome size and environmental variables in *C. maritimum*. Here, we emphasize that smaller genome sizes are more advantageous in insular environments compared to mainland ones. Further studies will enable the understanding of the mechanisms of genome size evolution and evaluate the merit of re-circumscribing *Crithmum* as a polytypic genus.

3.6 References

Ávila, S.P., Cordeiro, R., Madeira, P., Silva, L., Medeiros, A., Rebelo, A.C., Melo, C., Neto, A.I., Haroun, R., & Monteiro, A. (2018) Global change impacts on large-scale biogeographic patterns of marine organisms on Atlantic oceanic islands. Marine Pollution Bulletin. 126: 101–112, https://doi.org/10.1016/j.marpolbul.2017.10.087.

Basak, S., Sun, X., Wang, G., & Yang, Y. (2019). Genome Size Unaffected by Variation in Morphological Traits, Temperature, and Precipitation in Turnip. Applied Sciences. 9(2): 253. https://doi.org/10.3390/app9020253

Baytop, T. (1984). Treatment with plants in Turkey. Istanbul University. Published, 3255. Istanbul, Turkey.

Beaulieu, J. M., Leitch, I. J., & Knight, C. A. (2007a). Genome size evolution in relation to leaf strategy and metabolic rates revisited. Annals of Botany. 99(3): 495-505. https://doi.org/10.1093/aob/mcl271

Beaulieu, J. M., Leitch, I. J., Patel, S., Pendharkar, A., & Knight, C. A. (2008). Genome size is a strong predictor of cell size and stomatal density in angiosperms. New Phytologist. 179(4): 975-986. https://doi.org/10.1111/j.1469-8137.2008.02528.x

Beaulieu, J. M., Moles, A. T., Leitch, I. J., Bennett, M. D., Dickie, J. B., & Knight, C. A. (2007b). Correlated evolution of genome size and seed mass. New Phytologist. 173(2): 422-437. https://doi.org/10.1111/j.1469-8137.2006.01919.x

Bentkowski, P., Oosterhout, C. V., Mock, T. (2015) A Model of Genome Size Evolution for Prokaryotes in Stable and Fluctuating Environments, Genome Biology and Evolution. Genome Biology and Evolution. 7(8): 2344–2351. https://doi.org/10.1093/gbe/evv148

Biémont, C. (2008) Genome size evolution: Within-species variation in genome size. Heredity. 101: 297–298. https://doi.org/10.1038/hdy.2008.80

Blommaert, J. (2020). Genome size evolution: Towards new model systems for old questions. Proceedings of the Royal Society B. 287(1933): 20201441. https://doi.org/10.1098/rspb.2020.1441

Borcard, D., Gillet, F., & Legendre, P. (2011). Numerical ecologywith R. Springer, New YorkBouchard in M, Pothier D, Gauthier S (2008) Fire return intervals and tree species succession in the North Shore region of eastern Quebec. Canadian Journal of Forest Research, 38: 16211633.

Bottini, M. C. J., Greizerstein, E. J., Aulicino, M. B., & Poggio, L. (2000). Relationships among genome size, environmental conditions, and geographical distribution in natural populations of NW Patagonian species of *Berberis* L. (Berberidaceae). Annals of Botany. 86(3): 565-573.

Boutte, J., Maillet, L., Chaussepied, T., Letort, S., Aury, J. M., Belser, C., Boideau, F., Brunet, A., Coriton, O., Deniot, G., Falentin, C., Huteau, V., Lodé-Taburel, M., Morice, J., Trotoux, G., Chèvre, A. M., Rousseau-Gueutin, M., & de Carvalho, J. F. (2020). Genome size variation and comparative genomics reveal intraspecific diversity in Brassica rapa. Frontiers in plant science, 11: 577536. https://doi.org/10.3389/fpls.2020.577536

Box, G.E.P., & Cox, D.R. (1964) An analysis of transformations, Journal of the Royal Statistical Society. Series B. 26: 211–252.

Brilhante, M., Roxo, G., Catarino, S., Santos, P. d., Reyes-Betancort, J. A., Caujapé-Castells, J., Sequeira, M. M., Talhinhas, P., & Romeiras, M. M. (2021) Diversification of Aeonium Species Across Macaronesian Archipelagos: Correlations Between Genome-Size Variation and Their Conservation Status. Frontiers Ecology and Evolution. 9:607338. doi: https://doi.org/10.3389/fevo.2021.607338

Castroviejo, S. (2003) Flora ibérica: Plantas vasculares de la Península Ibérica e Islas Baleares. In Flora Ibérica, Real Jardin Botanico Consejo Superior de Investigaciones Cie: Madrid, Spain, Volume 10, pp. 1–784.

Chrtek Jr, J., Zahradníček, J., Krak, K., & Fehrer, J. (2009). Genome size in Hieracium subgenus Hieracium (Asteraceae) is strongly correlated with major phylogenetic groups. Annals of Botany. 104(1): 161-178. https://doi.org/10.1093/aob/mcp107

Conover, W.J., & Iman, R. L. (1979) “On multiple-comparison procedures.” Technical report, LA – 7677 – MS. Los Alamos Scientific Laboratory.

Crawford, R. M. M., (1982) Habitat specialisation in plants of cold climates. Transactions of the Botanical Society of Edinburgh. 44: 1–11. https://doi.org/10.1080/03746608208685407

De Frenne, P., Graae, B. J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., De Kort, H., Schrijver, A. D., Diekmann, M., Eriksson, O., Gruwez, R., Hermy, M., Lenoir, J., Plue, J., Coomes, D. A., & Verheyen, K. (2013). Latitudinal gradients as natural laboratories to infer species' responses to temperature. Journal of Ecology. 101(3): 784-795. https://doi.org/10.1111/1365-2745.12074

Díez, C. M., Gaut, B. S., Meca, E., Scheinvar, E., Montes-Hernandez, S., Eguiarte, L. E., & Tenaillon, M. I. (2013). Genome size variation in wild and cultivated maize along altitudinal gradients. New Phytologist. 199(1): 264-276. https://doi.org/10.1111/nph.12247

Doležel, J., Sgorbati, S., & Lucretti, S. (1992). Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. Physiologia plantarum. 85(4): 625-631.

Erst, A. S., Sukhorukov, A. P., Mitrenina, E. Y., Skaptsov, M. V., Kostikova, V. A., Chernisheva, O. A., Troshkina, V., Kushunina, M., Krivenko, D. A., Ikeda, H., Xiang, H., & Wang, W. (2020). An integrative taxonomic approach reveals a new species of Eranthis (Ranunculaceae) in North Asia. PhytoKeys, 140: 75.

Favre-Bac, L., Mony, C., Ernoult, A., Burel, F., & Arnaud, J. F. (2016). Ditch network sustains functional connectivity and influences patterns of gene flow in an intensive agricultural landscape. Heredity (Edinb). 116: 200–212. https://doi.org/10.1038/hdy.2015.90

Fernández-Palacios, J.M. & Whittaker, R.J. (2010) El ciclo de la isla. Atlas de la biodiversidad de Canarias (ed. by J.L. Martín Esquivel), pp. 18–27. Turquesa Ediciones, Santa Cruz de Tenerife. Tenerife, Canary.

Fleischmann, A., Michael, T. P., Rivadavia, F., Sousa, A., Wang, W., Temsch, E. M., Greilhuber, J., Müller, K. F., & Heubl, G. (2014). Evolution of genome size and chromosome number in the carnivorous plant genus *Genlisea* (Lentibulariaceae), with a new estimate of the minimum genome size in angiosperms. Annals of Botany. 114(8): 1651-1663. https://doi.org/10.1093/aob/mcu189.

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

Gregory T. R. (2005). The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. – Annals of Botany. 95: 133–146. https://doi.org/10.1093/aob/mci009

Gregory TR. (2001). Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biology Reviews. 76: 65–101. https://doi.org/10.1017/S1464793100005595

Guignard, M. S., Nichols, R. A., Knell, R. J., Macdonald, A., Romila, C.-A., Trimmer, M., Leitch, I. J. & Leitch, A. R. (2016). Genome size and ploidy influence angiosperm species' biomass under nitrogen and phosphorus limitation. New Phytologist. 210: 1195–1206. https://doi.org/10.1111/nph.13881

Guilengue, N., Alves, S., Talhinhas, P., & Neves-Martins, J. (2020). Genetic and genomic diversity in a tarwi (*Lupinus mutabilis* Sweet) germplasm collection and adaptability to Mediterranean climate conditions. Agronomy. 10: 21.

Hawkins, J. S., Grover, C.E., & Wendel, J. F. (2008). Repeated big bangs and the expanding universe: directionality in plant genome size evolution. Plant Science. 174: 557–562. https://doi.org/10.1016/j.plantsci.2008.03.015

Kapralov, M. V., & Filatov, D. A. (2011). Does large genome size limit speciation in endemic island floras. Journal of Botany, 1-6. https://doi.org/10.1155/2011/458684

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, H.P. & Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. Scientific Data. 4: 170122.

Katsouri, E., Demetzos, C., Perdetzoglou, D., & Loukis, A. (2001). An interpopulation study of the essential oils of various parts of Crithmum maritimum L. growing in Amorgos Island, Greece. Journal of Essential Oil Research. 13(5): 303-308. https://doi.org/10.1080/10412905.2001.9712220

Knight, C. A., Molinari, N. A., & Petrov, D. A. (2005). The large genome constraint hypothesis: evolution, ecology, and phenotype. Annals of Botany. 95(1): 177-190. https://doi.org/10.1093/aob/mci011

Koce, J. D., Škondrić, S., Bačič, T., & Dermastia, M. (2008). Amounts of nuclear DNA in marine halophytes. Aquatic botany. 89(4): 385-389.

Kosiński, P., Sliwinska, E., Hilpold, A., & Boratyński, A. (2019). DNA ploidy in Salix retusa agg. only partly in line with its morphology and taxonomy. Nordic Journal of Botany, 37(7). https://doi.org/10.1111/njb.02197

Kraaijeveld, K. (2010). Genome size and species diversification. Evolutionary Biology. 37: 227–233. https://doi.org/10.1007/s11692-010-9093-4

Kulišic – bilušic, T., Blažević, I., Dejanović, B., Miloš, M. & Pifat, G. (2010). Evaluation of the antioxidant activity of essential oils from caper (Capparis Spinosa) and sea fennel (Crithmum maritimum) by different methods. Journal of Food Biochemistry. 34: 286-302. https://doi.org/10.1111/j.1745-4514.2009.00330

Kwak, S.G., & Kim, J.H. (2017) Central limit theorem: The cornerstone of modern statistics. Korean Journal of Anesthesiology. 70: 144–156, https://doi.org/10.4097/kjae.2017.70.2.144.

Latron, M., Arnaud, J. F., Ferla, H., Godé, C., & Duputié, A. (2020). Effects of contemporary shifts of range margins on patterns of genetic structure and mating system in two coastal plant species. Heredity. 124(2): 336-350.

Latron, M., Arnaud, J. F., Ferla, H., Godé, C., & Duputié, A. (2018). Polymorphic nuclear markers for coastal plant species with dynamic geographic distributions, the rock samphire (*Crithmum maritimum*) and the vulnerable dune pansy (*Viola tricolor* subsp. *curtisii*). Molecular biology reports. 45(3): 203-209.

Loureiro, J., Rodriguez, E., Doležel, J., & Santos, C. (2007). Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. Annals of botany. 100(4): 875-888.

Loureiro, J., Trávníček, P., Rauchová, J., Urfus, T., Vit, P., Štech, M., Castro, S., & Suda, J. (2010). The use of flow cytometry in the biosystematics, ecology, and population biology of homoploid plants. Preslia. 82(1): 3-21.

Maleš, Z., Blazevic, N., & Plazibat, M. (2001) Variations in the yield and composition of the essential oil of *Crithmum maritimum* L, Acta pharmaceutica (Zagreb). 51(1): 81-84.

Meagher, T. R., & Vassiliadis, C. (2005). Phenotypic impacts of repetitive DNA in flowering plants. New Phytologist. 168(1): 71-80. https://doi.org/10.1111/j.1469-8137.2005.01527.x

Meot-Duros, L., & Magné, C. (2009). Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. Plant Physiology and Biochemistry. 47(1): 37-41. https://doi.org/10.1016/j.plaphy.2008.09.006

Metzing, D., & Gerlach, A. (2001) Climate change and coastal flora. In: Walther G-R, Burga CA, Edwards PJ (eds). “Fingerprints” of climate change. Springer, Boston. USA. pp 185–202.

Murray, B. G. (2005). When does intraspecific C-value variation become taxonomically significant?. Annals of Botany, 95(1): 119-125. https://doi.org/10.1093/aob/mci007

Ochatt, S., Smykal, P., Patat-Ochatt, E., & Conreux, C. (2017). Flow cytometry measurements contribute to Pisum taxonomy. In 8. International Conference on Legume Genetics and Genomics (ICLGG) (pp. 128-p).

Oliver, M. J., Petrov, D., Ackerly, D., Falkowski, P., & Schofield, O. M. (2007). The mode and tempo of genome size evolution in eukaryotes. Genome research. 17(5): 594-601.

Özcan, M., Akgül, A., Başcr, K.H.C., Özck, T. & Tabanca, N. (2001), Essential oil composition of sea fennel (Crithmum maritimum) form Turkey. Nahrung. 45: 353-356. https://doi.org/10.1002/1521-3803(20011001)45:5<353::AID-FOOD353>3.0.CO;2 4

Padilla-Garcia, N., Rojas-Andrés, B. M., López-González, N., Castro, M., Castro, S., Loureiro, J., Albach, D. C., Machon, N., & Martinez-Ortega, M. M. (2018). The challenge of species delimitation in the diploid-polyploid complex Veronica subsection Pentasepalae. Molecular phylogenetics and evolution, 119: 196-209. https://doi.org/10.1016/j.ympev.2017.11.007

Parelho, C., Rodrigues, A., do Carmo Barreto, M., Cruz, J. V., Rasche, F., Silva, L., & Garcia, P. (2021) Bioaccumulation and potential ecotoxicological effects of trace metals along a management intensity gradient in volcanic pasturelands. Chemosphere, 273: 128601. https://doi.org/10.1016/j.chemosphere.2020.128601

Pellicer J., Fay, M. F., & Leitch, I. J. (2010). The largest eukaryotic genome of them all? Botanical Journal of the Linnean Society. 164: 10-15. https://doi.org/10.1111/j.1095-8339.2010.01072.x

Pellicer, J., Hidalgo, O., Dodsworth, S., & Leitch, I. J. (2018). Genome size diversity and its impact on the evolution of land plants. Genes. 9(2): 88. https://doi.org/10.3390/genes9020088

Pline. (1957). l' Ancien traduit par Ernaud, P. Histoire Naturelle. Les Belles Lettres, Paris, France.

Pohlert, T. (2014). The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR). R package. http://CRAN.R-project.org/package=PMCMR.

Qiu, F., Baack, E. J., Whitney, K. D., Bock, D. G., Tetreault, H. M., Rieseberg, L. H., & Ungerer, M. C. (2019). Phylogenetic trends and environmental correlates of nuclear genome size variation in *Helianthus* sunflowers. New Phytologist. 221(3): 1609-1618. https://doi.org/10.1111/nph.15465

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Renna, M. (2018). Reviewing the Prospects of Sea Fennel (Crithmum maritimum L.) as Emerging Vegetable Crop. Plants. 7(4): 92. https://doi.org/10.3390/plants7040092

Romão, J.C. (2008). Património geológico no litoral de Peniche: geomonumentos a valorizar e divulgar. II Congresso Internacional de Turismo de Leiria e Oeste, Instituto Politécnico de Leiria, Leiria

Schubert, I., & Vu, G. T. H. (2016). Genome stability and evolution: attempting a holistic view. Trends in Plant Science. 21: 749–757. https://doi.org/10.1016/j.tplants.2016.06.003

Shapiro, S.S., & Wilk, M.B. (1965) An analysis of variance test for normality (complete samples). Biometrika. 52: 591–611, https://doi.org/10.1093/biomet/52.3-4.591.

Siegel, S., & Castellan, N.J. (1988) Nonparametric statistics for the behavioural sciences. MacGraw Hill Int., New York., USA. Pp. 213-214.

Suda, J., Kyncl, T., & Freiová, R. (2003). Nuclear DNA amounts in Macaronesian angiosperms. Annals of Botany, 92(1): 153–164. https://doi.org/10.1093/aob/mcg104

Suda, J., Kyncl, T., & Jarolímová, V. (2005). Genome size variation in Macaronesian angiosperms: forty percent of the Canarian endemic flora completed. Plant Systematics and Evolution. 252(3-4): 215-238.

Whitney, K. D., Baack, E. J., Hamrick, J. L., Godt, M. J. W., Barringer, B. C., Bennett, M. D., Eckert, C. G., Goodwillie, C., Kalisz, S., Leitch, I. J., & Ross-Ibarra, J. (2010). A role for nonadaptive processes in plant genome size evolution? Evolution: International Journal of Organic Evolution. 64(7): 2097-2109. https://doi.org/10.1111/j.1558-5646.2010.00967.x

Wood, T. E., Takebayashi, N., Barker, M. S., Mayrose, I., Greenspoon, P. B., & Rieseberg, L. H. (2009). The frequency of polyploid speciation in vascular plants. Proceedings of the National Academy of Sciences USA. 106: 13875–13879. https://doi.org/10.1073/pnas.0811575106

Zaitlin, D., & Pierce, A. J. (2010). Nuclear DNA content in *Sinningia* (Gesneriaceae); intraspecific genome size variation and genome characterization in *S. speciosa*. Genome, 53(12): 1066-1082. https://doi.org/10.1139/G10-077

Zar, J.H. (2010) Biostatistical Analysis, 5th ed., Prentice Hall: Pearson, USA.

3.7. Suplementary material

Table 3.S1. Details on the sampling locations of the *Crithmum maritimum* populations.

Location	District/ Island	County	Locality
Azores	Corvo	Corvo	Corvo
Azores	Faial	Horta	Pedro Miguel
Azores	Faial	Horta	Ponta Furada
Azores	Pico	Lajes do Pico	Companhia de Cima
Azores	Pico	Madalena do Pico	Criação Velha
Azores	Pico	Madalena do Pico	Guindaste
Azores	Pico	São Roque do Pico	Lajido
Azores	Pico	Lajes do Pico	Poça das Mujas
Azores	Pico	São Roque do Pico	Ponta do Boequeirão
Azores	Pico	Madalena do Pico	Porto do Calhau
Azores	Pico	Madalena do Pico	São Matues
Azores	Santa Maria	Vila do Porto	Anjos
Azores	Santa Maria	Vila do Porto	Furna de Santana
Azores	São Jorge	Calheta	Calheta
Azores	São Miguel	Lagoa	Caloura
Berlengas	Berlenga Grande	Berlenga Grande	Forte de São João Batista
Berlengas	Berlenga Grande	Berlenga Grande	Melreu
Mainland	Coimbra	Figueira da Foz	Praia da Claridade
Mainland	Coimbra	Figueira da Foz	Praia do Cabedelo
Mainland	Coimbra	Figueira da Foz	Praia Laje do Costado
Mainland	Faro	Vila do Bispo	Cabo de São Vicente
Mainland	Faro	Vila do Bispo	Praia do Beliche
Mainland	Faro	Lagos	Praia dos Carneiros
Mainland	Faro	Portimão	Praia dos Três irmãos
Mainland	Faro	Portimão	Prainha
Mainland	Leiria	Peniche	Farol do Cabo Carvoeiro
Mainland	Leiria	Peniche	Fortaleza de Peniche
Mainland	Leiria	Caldas da Rainha	Foz do Arelho
Mainland	Leiria	Peniche	Papôa
Mainland	Leiria	Nazaré	Praia da Nazaré
Mainland	Leiria	Alcobaça	Praia da Polvoleira
Mainland	Leiria	Alcobaça	Praia de Paredes de Vitória
Mainland	Leiria	Nazaré	Praia do Norte
Mainland	Leiria	Leiria	Praia do Pedrogão
Mainland	Leiria	Alcochete	Praia do Samouco
Mainland	Leiria	Marinha Grande	Praia Vieira de Leiria
Mainland	Leiria	Alcobaça	São Martinho do Porto
Mainland	Leiria	Marinha Grande	São Pedro de Moel
Mainland	Lisboa	Sintra	Azenhas do mar
Mainland	Lisboa	Lisboa	Belém
Mainland	Lisboa	Cascais	Boca do Inferno
Mainland	Lisboa	Cascais	Guincho
Mainland	Lisboa	Oeiras	Paço de Arcos
Mainland	Beja	Vila Nova de Milfontes	Porto das Barcas
Mainland	Lisboa	Cascais	Praia da Parede
Mainland	Lisboa	Oeiras	Praia da Torre
Mainland	Lisboa	Cascais	Praia das Avencas
Mainland	Lisboa	Sintra	Praia das Maçãs
Mainland	Lisboa	Cascais	Praia de Carcavelos
Mainland	Lisboa	Cascais	São João do Estoril
Mainland	Porto	Póvoa do Varzim	Cabo de Santo André
Mainland	Porto	Póvoa do Varzim	Fortaleza da Póvoa de Varzim
Mainland	Porto	Porto	Forte de São Franisco Xavier
Mainland	Porto	Porto	Forte de São João Baptista
Mainland	Porto	Porto	Forte de São João
Mainland	Porto	Vila do Conde	Foz do Rio Ave
Mainland	Porto	Porto	Foz do Rio Douro
Mainland	Porto	Vila Nova de Gaia	Pedra do Cão
Mainland	Porto	Matosinhos	Praia Angeiras Norte
Mainland	Porto	Vila do Conde	Praia Azul Norte
Mainland	Porto	Matosinhos	Praia Cabo do Mundo
Mainland	Porto	Vila do Conde	Praia Castro de São Paio
Mainland	Porto	Matosinhos	Praia da Agudela
Mainland	Porto	Vila do Conde	Praia da Árvore

Mainland	Porto	Vila do Conde	Praia da Laderça
Mainland	Porto	Matosinhos	Praia da Memória
Mainland	Porto	Matosinhos	Praia da Senhora da Boa Nova
Mainland	Porto	Póvoa do Varzim	Praia de Aver-o-Mar
Mainland	Porto	Vila do Conde	Praia de Mindelo
Mainland	Porto	Vila Nova de Gaia	Praia de Salgueiros
Mainland	Porto	Vila Nova de Gaia	Praia de Valadares Norte
Mainland	Porto	Vila do Conde	Praia de Vila Chã
Mainland	Porto	Póvoa do Varzim	Praia do Fragosinho
Mainland	Porto	Matosinhos	Praia do Funtão
Mainland	Porto	Matosinhos	Praia dos Barcos
Mainland	Setúbal	Alcácer do Sal	Praia da Comporta
Mainland	Setúbal	Grândola	Praia do Carvalhal
Mainland	Viana do Castelo	Caminha	Caminha
Mainland	Viana do Castelo	Caminha	Capela de Santo Isidoro
Mainland	Viana do Castelo	Viana do Castelo	Forte da Areosa
Mainland	Viana do Castelo	Caminha	Forte da Lagarteira
Mainland	Viana Do Castelo	Caminha	Foz do Minho
Mainland	Viana do Castelo	Vila Nova de Foz Côa	Marina de Seixas
Mainland	Viana do Castelo	Viana do Castelo	Moinho do Vento do Canto Marinho
Mainland	Viana do Castelo	Caminha	Praia da Âncora
Mainland	Viana do Castelo	Viana do Castelo	Praia da Ínsua
Mainland	Viana do Castelo	Viana do Castelo	Praia de Fornelos e Promontório de Montedor
Mainland	Viana do Castelo	Caminha	Praia de Moledo
Mainland	Viana do Castelo	Caminha	Praia do Camarido
Mainland	Viana do Castelo	Viana do Castelo	Praia do Coral
Mainland	Viana do Castelo	Viana do Castelo	Praia do Lumiar
Mainland	Viana do Castelo	Viana do Castelo	Praia do Paçô
Mainland	Viana do Castelo	Caminha	Praia Forte do Cão
Mainland	Viana do Castelo	Caminha	Vila Praia de Âncora
Madeira	Desertas	Deserta Grande	Deserta Grande
Madeira	Madeira	Ponta Delgada	Estrada Antiga Ponta Delgada - São Vicente
Madeira	Madeira	Santana	Faial
Madeira	Madeira	Porto Moniz	Fajã das Achadas da Cruz
Madeira	Madeira	Funchal	Lido
Madeira	Madeira	Machico	Machico
Madeira	Madeira	Porto Moniz	Parque de Campismo do Porto Moniz
Madeira	Madeira	Calheta	Paul do Mar
Madeira	Madeira	Ponta Delgada	Piscinas da Ponta Delgada
Madeira	Madeira	Porto Moniz	Piscinas Naturais do Seixal
Madeira	Madeira	Porto Moniz	Piscinas Naturais velhas do Porto Moniz
Madeira	Madeira	Machico	Ponta de São Lourenço
Madeira	Madeira	Ponta do Sol	Ponta do Sol
Madeira	Madeira	Porto da Cruz	Porto da Cruz
Madeira	Madeira	Porto Moniz	Praia do Seixal
Madeira	Madeira	Funchal	Praia Formosa
Madeira	Madeira	Santana	Rocha de baixo
Madeira	Madeira	Santana	Ruínas de São Jorge
Madeira	Madeira	São Vicente	São Vicente
Madeira	Porto Santo	Porto Santo	Praia da Lagoa

Table 3.S2. Description of the environmental variables obtained from CHELSA (Karger *et al.*, 2017), their codes and units.

Description	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[5]	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

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

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

[3] Standard deviation of monthly temperature averages

[4] (BIO05 – BIO06)

[5] Standard deviation of monthly precipitation averages

Table 3.S3. Mean 2C-values (in picograms, pg), standard deviation (SD), and coefficient of variation (CVa, in percentage, %), of *Crithmum maritimum* populations.

Location	Mean	SD	Sample CVa (%)
(Ma) Madeira (Fajã das Achadas da cruz)	4.074	0.079	3.076
(Az) Pico (criação Velha)	4.098	0.052	3.434
(Az) Pico (Guindaste)	4.113	0.075	3.053
(AZ) Santa Maria (Anjos)	4.167	0.039	2.739
(Az) Pico (Porto do Calhau)	4.171	0.059	3.069
(AZ) Faial (Pedro Miguel)	4.175	0.100	6.337
(Az) Pico (Poça das Mujas)	4.191	0.036	3.252
(Az) Pico (Ponta do Boqueirão)	4.217	0.071	3.369
(Ma) Desertas (Deserta Grande)	4.233	0.063	0.840
(AZ) São Miguel (Caloura)	4.235	0.044	2.733
(Az) Pico (Lajido)	4.240	0.052	3.238
(AZ) Corvo	4.256	0.041	3.627
(AZ) São Jorge (Calheta)	4.258	0.090	2.370
(AZ) Pico (Companhia de Cima)	4.265	0.042	1.345
(Ma) Madeira (Ponta do Sol)	4.283	0.021	2.688
(Ma) Madeira (Praia do Seixal)	4.291	0.079	2.545
(AZ) Santa Maria (Furna de Santana)	4.315	0.025	2.270
(Ma) Madeira (Ruínas de S. Jorge)	4.321	0.081	2.725
(Ma) Madeira (Parque de Campismo do Porto Moniz)	4.323	0.070	2.239
(AZ) Faial (Ponta Furada)	4.329	0.098	6.928
(Ma) Madeira (Praia Formosa)	4.329	0.090	3.008
(Ma) Madeira (Piscinas Naturais do Seixal)	4.331	0.075	2.397
(Ma) Madeira (Porto da Cruz)	4.335	0.074	2.761
(Ma) Madeira (Lido)	4.352	0.069	2.851
(Ma) Madeira (Ponta de São Lourenço)	4.353	0.043	2.601
(Ma) Madeira (Rocha de baixo)	4.354	0.063	2.649
(Az) Pico (São Mateus)	4.362	0.448	2.609
(Ma) Madeira (Piscinas da Ponta Delgada)	4.363	0.089	2.444
(Ma) Madeira (Machico)	4.377	0.077	3.073
(Ma) Porto Santo (Praia da Lagoa)	4.378	0.109	2.535
(Ma) Madeira (Piscinas Naturais velhas do Porto Moniz)	4.393	0.064	2.281
(Ma) Madeira (Faial)	4.407	0.081	2.519
(Ma) Madeira (Estrada antiga de São Vicente)	4.434	0.125	2.349
(Ma) Madeira (São Vicente)	4.470	0.082	2.799
(Lu) Lisboa (Praia de Carcavelos)	4.472	0.077	2.766
(Lu) Leiria (Foz do Arelho)	4.499	0.120	2.779
(Ma) Madeira (Paul do Mar)	4.511	0.085	2.830
(Lu) Lisboa (Praia da Parede)	4.525	0.075	3.268
(Lu) Lisboa (São João do Estoril)	4.575	0.050	2.746
(Lu) Beja (Praia do Carvalhal)	4.580	0.079	4.830
(Lu) Lisboa (Praia das Maçãs)	4.582	0.056	2.772
(Lu) Lisboa (Azenhas do Mar)	4.618	0.162	2.618
(Lu) Faro (Praia dos Carneiros)	4.635	0.051	2.601
(Lu) Lisboa (Praia da Torre)	4.652	0.070	3.293
(Lu) Lisboa (Praia das Avencas)	4.658	0.103	2.548
(Lu) Lisboa (Boca do Inferno)	4.679	0.047	3.328
(Lu) Leiria (Praia do Pedrogão)	4.729	0.112	2.409
(Lu) Faro (Praia do Beliche)	4.729	0.044	2.839
(Lu) Viana do Castelo (Caminha)	4.738	0.228	2.295
(Lu) Faro (Prainha)	4.746	0.093	2.626
(Lu) Leiria (Praia da Nazaré)	4.764	0.066	2.629
(Lu) Beja (Porto das Barcas)	4.764	0.045	4.667
(Lu) Leiria (Praia do Norte)	4.788	0.098	2.306
(Lu) Coimbra (Praia da Claridade)	4.801	0.057	2.684
(Lu) Porto (Pedra do Cão)	4.811	0.087	2.133
(Lu) Viana do Castelo (Praia de Fornelos e Promontório de Montedor)	4.817	0.060	2.281

(Lu) Viana do Castelo (Praia da Ínsua)	4.820	0.065	1.759
(Lu) Viana do Castelo (Praia do Camarido)	4.828	0.075	2.010
(Lu) Lisboa (Guincho)	4.834	0.046	1.357
(Lu) Viana do Castelo (Praia do Paçô)	4.838	0.057	2.096
(Lu) Porto (Praia de Valadares Norte)	4.841	0.105	2.311
(Lu) Porto (Forte de São Francisco Xavier)	4.844	0.074	2.115
(Lu) Porto (Foz do Rio Ave)	4.855	0.133	2.230
(Lu) Leiria (Farol do Cabo Carvoeiro)	4.855	0.098	3.019
(lu) Porto (Praia de Aver-o-Mar)	4.857	0.072	1.670
(Lu) Viana do Castelo (Forte da Areosa)	4.857	0.066	1.987
(Lu) Viana do Castelo (Capela de Santo Isidoro)	4.864	0.083	2.375
(Lu) Lisboa (Belém)	4.865	0.038	2.557
(Lu) Faro (Praia dos Três Irmãos)	4.867	0.023	2.230
(Lu) Viana do Castelo (Praia da Âncora)	4.867	0.063	2.277
(Lu) Leiria (Praia Vieira de Leiria)	4.870	0.120	2.224
(Lu) Porto (Cabo de Santo André)	4.872	0.055	1.962
(Lu) Viana do Castelo (Praia do Lumiar)	4.875	0.087	2.343
(Lu) Porto (Praia de Salgueiros)	4.878	0.073	2.171
(Lu) Faro (Cabo de São Vicente)	4.880	0.228	2.470
(Lu) Leiria (Praia do Samouco)	4.882	0.091	2.722
(Lu) Leiria (Praia da Polvoleira)	4.884	0.128	2.033
(Lu) Leiria (São Martinho do Porto)	4.895	0.079	2.001
(Lu) Leiria (Papôa, Peniche)	4.897	0.128	2.397
(Lu) Porto (Praia Angeiras Norte)	4.899	0.143	2.207
(Lu) Coimbra (Praia Lajde do Costado)	4.899	0.129	2.294
(Lu) Coimbra (Praia do Cabedelo)	4.901	0.067	2.511
(Lu) Porto (Fortaleza da Póvoa de Varzim)	4.904	0.079	2.431
(Lu) Viana Do Castelo (Foz do Minho)	4.905	0.055	2.237
(Lu) Porto (Forte de São João Baptista)	4.908	0.141	1.987
(Lu) Leiria (Praia de Paredes de Vitória)	4.910	0.065	2.388
(Be) Berlenga Grande (Forte de São João Baptista)	4.912	0.095	2.241
(Lu) Viana do Castelo (Praia de Moledo)	4.915	0.075	2.206
(Lu) Lisboa (Paço de Arcos)	4.921	0.045	2.477
(Lu) Porto (Praia do Funtão)	4.927	0.127	2.094
(Lu) Porto (Praia Azul Norte)	4.928	0.068	2.119
(Lu) Porto (Praia Castro de São Paio)	4.933	0.094	2.298
(Lu) Porto (Foz do Rio Douro)	4.934	0.063	1.951
(Lu) Viana do Castelo (Marina de Seixas)	4.935	0.042	1.919
(Lu) Viana do Castelo (Praia Forte do Cão)	4.943	0.065	2.123
(Lu) Viana do Castelo (Moinho do Vento do Canto Marinho)	4.943	0.084	2.114
(Lu) Leiria (Fortaleza de Peniche)	4.945	0.048	2.444
(Lu) Setúbal (Praia da Comporta)	4.946	0.099	2.149
(Lu) Porto (Praia Cabo do Mundo)	4.946	0.065	2.052
(Lu) Porto (Forte de São João)	4.963	0.081	2.303
(Lu) Porto (Praia da Agudela)	4.966	0.103	1.951
(Be) Berlenga Grande (Melreu)	4.972	0.037	2.001
(Lu) Porto (Praia de Mindelo)	4.979	0.086	2.059
(Lu) Porto (Praia da Laderça)	4.999	0.097	2.100
(Lu) Porto (Praia da Senhora da Boa Nova)	5.000	0.049	2.041
(Lu) Porto (Praia do Fragosinho)	5.002	0.057	2.201
(Lu) Porto (Praia da Árvore)	5.005	0.054	2.157
(Lu) Porto (Praia da Memória)	5.008	0.076	2.410
(Lu) Porto (Praia de Vila Chã)	5.017	0.095	2.404
(Lu) Viana do Castelo (Praia do Coral)	5.022	0.070	2.475
(Lu) Viana do Castelo (Vila Praia de Âncora)	5.023	0.073	1.949
(Lu) Leiria (São Pedro de Moel)	5.034	0.156	2.424
(Lu) Viana do Castelo (Forte da Lagarteira)	5.041	0.081	2.107
(Lu) Porto (Praia dos Barcos)	5.047	0.145	2.181
Average	4.710	0.294	2.445

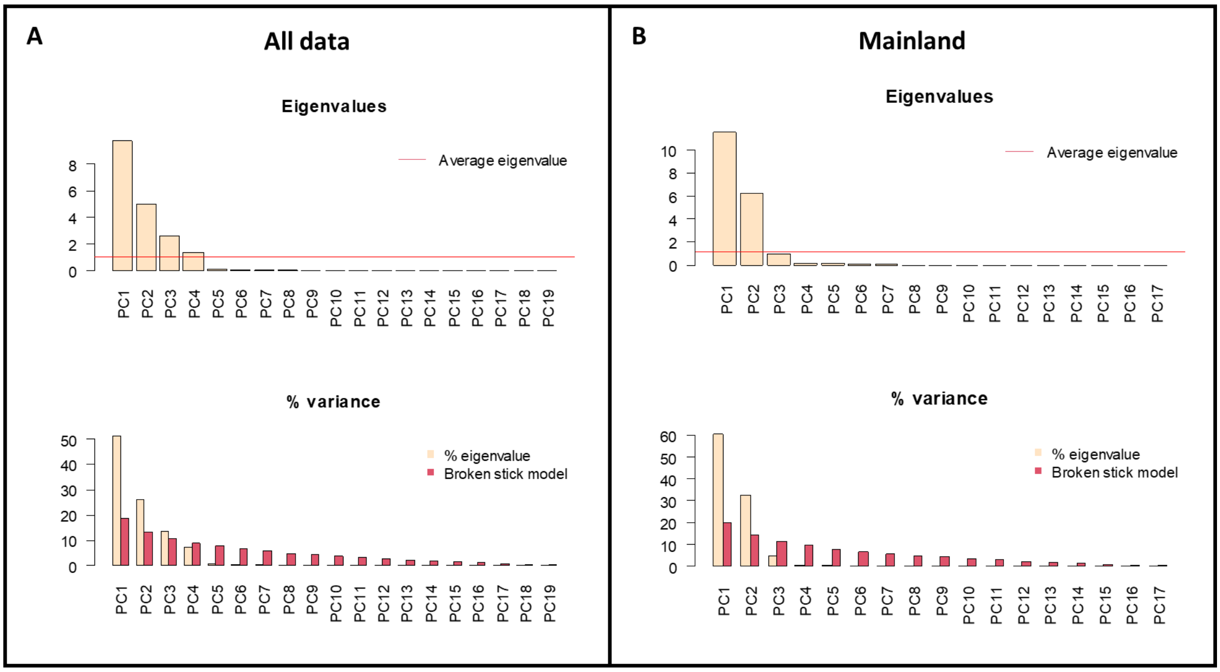


Figure 3.S1. Selection of the number of principal components to be retained in a PCA applied to the 19 bioclimatic variables, according to the Kaiser-Guttman criterion and the broken stick model for the full data including islands and mainland (**A**), and for data including mainland only (**B**), respectively. The retained components explain 51.2%, 26.2% and 13.8% (**A**) and 60.7% and 32.7% (**B**) of the variation in the data, respectively.

Chapter 4

General conclusions

The amount of nuclear DNA has been a key characteristic used in various biological fields such as ecology, evolution, and biosystematics (Kron *et al.*, 2007). The present study focuses on two lineages of the Apiaceae family with different speciation models (i.e., cladogenesis and anagenesis). The cladogenesis speciation model is represented by the *Daucus* complex (*Daucus* – *Tornabenea* – *Monizia* – *Melanoselinum*), whereas *Crithmum maritimum* (a monotypic *genus*) represents an anagenesis speciation model. In this work, the cytogenomic diversity observed in both lineages was studied, using a comprehensive number of samples collected across the Macaronesian Islands and in mainland regions of Portugal.

Concerning the cytogenomic diversity of the *Daucus* complex lineage, it was possible to observe that small genomes are generally found on islands (except in the case of Madeira Archipelago). This tendency towards small genomes has been reported for other archipelagos such as Hawaii, Marquesas (Kapralov & Filatov, 2011) and Macaronesia (Suda *et al.*, 2003, 2005). According to our data, the best predictor of genome size (GS) variation is the taxon, however, only at species level, as it is unable to clearly discriminate the *Daucus carota* subspecies. Finally, the 2C-values of Cabo Verde endemics (*Tornabenea* spp.) were very similar to the *Daucus carota* subspecies, supporting the close relationship of *Daucus* and *Tornabenea genera*.

Although *Crithmum* is a monotypic genus, the results revealed an unexpectedly high cytogenomic variation, and distinct populations (or putative cryptic species) were detected relating to the geographical area (Whittall *et al*., 2004). Our results revealed a pattern of variation between genome size variation and environmental data, namely showing that GS increases from south to north and from east to west, and also larger GS are associated with temperature and precipitation variation. Nonetheless, it is important to note that isolation seems to be the most important factor in shaping GS.

Altogether, both studies seemed to support the tendency of small genomes in islands reported by several authors (Suda *et al.*, 2003, 2005, Garnatje *et al.*, 2007, Kapralov & Filatov, 2011). Moreover, and considering the mean values of both lineages, *Daucus* presented a smaller mean genome size than the *Crithmum* lineage. In addition, *Daucus* has 43 accepted species (subspecies not included) distributed worldwide, while *Crithmum* has only one accepted species, so the results are in agreement with the observations of Kapralov & Filatov (2011) who state that the number of species per *genus* is negatively correlated with genus-average genome size for island radiations in Hawaii.

In conclusion, this thesis provides new cytogenomic data that can contribute to understand the relationships among native Apiaceae species from Macaronesia and also comparing to samples collected in mainland Portugal and how GS is correlated with the diversification patterns. Therefore, using flow cytometry in further studies of morphological and molecular (i.e., molecular markers, Next Generation sequencing) data will help to clarify the radiation patterns and low-level taxonomies among Macaronesian native *taxa* and support informed decisions for the conservation of plant genetic resources in the Macaronesian Islands.

References

Banasiak, Ł., Wojewódzka, A., Baczyński, J., Reduron, J.-P., Piwczyński, M., Kurzyna-Młynik, R., Gutaker, R., Czarnocka-Cieciura, A., Kosmala-Grzechnik, S., & Spalik, K. (2016). Phylogeny of Apiaceae subtribe Daucinae and the taxonomic delineation of its genera. Taxon, 65(3): 563-585. https://doi.org/10.12705/653.8

Beyhl, F. E., Myes, B., & Ohm, P. (1995). Macaronesia: a biogeographical puzzle. Boletim do Museu Municipal do Funchal, 4: 107–113.

Chumová, Z., Krejčíková, J., Mandáková, T., Suda, J., & Trávníček, P. (2015). Evolutionary and Taxonomic Implications of Variation in Nuclear Genome Size: Lesson from the Grass Genus *Anthoxanthum* (Poaceae). PLoS One, 10(7): e0133748. https://doi.org/10.1371/journal.pone.0133748

Crawford, D. J., & Stuessy, T. F. (1997). Plant speciation on oceanic islands. In Evolution and diversification of land plants. Springer. Tokyo, Japan. pp. 249-267

Díez, C. M., Gaut, B. S., Meca, E., Scheinvar, E., Montes-Hernandez, S., Eguiarte, L. E., & Tenaillon, M. I. (2013). Genome size variation in wild and cultivated maize along altitudinal gradients. New Phytologist, 199(1): 264-276. https://doi.org/10.1111/nph.12247

Downie, S.R., Katz-Downie, D.S. & Cho, K.J. (1996). Phylogenetic analysis of Apiaceae subfamily Apioideae using nucleotide sequences from the chloroplast rpoC1 intron. Molecular Phylogeny and Evolution 6: 1–18. http://dx.doi.org/10.1006/mpev.1996.0053

Fernández-Palacios, J.M. (2010) The islands of Macaronesia Terrestrial arthropods of Macaronesia: biodiversity, ecology, and evolution (ed. by A.R.M. Serrano, P.A.V. Borges, M. Boieiro and P. Oromí) Sociedade Portuguesa de Entomología. Lisbon, Portugal. pp 11 – 39

Frankiewicz, K. E., Oskolski, A., Banasiak, Ł., Fernandes, F., Reduron, J. P., Reyes-Betancort, J. A., Szczeparska, L., Alsarraf, M., Baczyński, J., & Spalik, K. (2020). Parallel evolution of arborescent carrots (*Daucus*) in Macaronesia. American Journal of Botany, 107(3): 394-412. https://doi.org/10.1002/ajb2.1444

Freitas, R., Romeiras, M., Silva, L., Cordeiro, R., Madeira, P., González, J. A., Wirtz, P., Falcón, J. M., Brito, A., & Floeter, S. R. (2019). Restructuring of the Macaronesia biogeographic unit: A marine multi-taxon biogeographical approach. Scientific Reports, 9(1): 1-18. https://doi.org/10.1038/s41598-019-51786-6

Galbraith, D., Loureiro, J., Antoniadi, I., Bainard, J., Bureš, P., Cápal, P., Castro, M., Castro, S., Čertner, M., Čertnerová, D., Chumová, Z., Doležel, J., Giorgi, D., Husband, C. B., Kolář, F., Koutecký, P., Kron, P., Leitch, L. J., Ljung, K., Lopes, S., Lučanová, M., Lucretti, S., Ma, W., Melzer, S., Molnár, I., Novák, O., Poulton, N., Skalický, V., Sliwinska, E., Šmard, P., Smith, W. T., Sun, G., Talhinhas, P., Tárnok, A., Temsch, M. E., Trávníček, P., & Urfus, T. (2021). Best practices in plant cytometry. Cytometry, 99(4): 311-317. https://doi.org/10.1002/cyto.a.24295

Garnatje, T., Garcia, S., & Canela, M. Á. (2007). Genome size variation from a phylogenetic perspective in the genus *Cheirolophus* Cass (Asteraceae): biogeographic implications. Plant Systematics and Evolution, 264(1): 117-134. https://doi.org/10.1007/s00606-006-0489-7

Gillespie, R. G. (2007). Oceanic islands: models of diversity. Encyclopedia of biodiversity, 1-13.

Hazevoet, C. J. (1995). The birds of the Cape Verde islands. British Ornithologists' Union Check List. Tring, United Kingdom. pp 192

Kapralov, M. V., & Filatov, D. A. (2011). Does large genome size limit speciation in endemic island floras. Journal of Botany, 2011: 1-6. https://doi.org/10.1155/2011/458684

Kier, G., Kreft, H., Lee, T. M., Jetz, W., Ibisch, P. L., Nowicki, C., Mutke, J., & Barthlott, W. (2009). A global assessment of endemism and species richness across island and mainland regions. Proceedings of the national Academy of sciences, 106(23): 9322-9327. https://doi.org/10.1073/pnas.0810306106

Kreft, H., Jetz, W., Mutke, J., Kier, G., & Barthlott, W. (2008). Global diversity of island floras from a macroecological perspective. Ecology letters, 11(2): 116-127. https://doi.org/10.1111/j.1461-0248.2007.01129.x

Kron, P., Suda, J., & Husband, B. C. (2007). Applications of flow cytometry to evolutionary and population biology. Annual Review of Ecology, Evolution, and Systematics, 38: 847-876. https://doi.org/10.1146/annurev.ecolsys.38.091206.095504

Leitch, I. J., Chase, M. W., & Bennett, M. D. (1998). Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. Annals of Botany, 82(1): 85-94. https://doi.org/10.1006/anbo.1998.0783

Mabberley, D. (2017). Mabberley's Plant-Book. In Mabberley's Plant-book: A Portable dictionary of Plants, their Classification and Uses (p. I). Cambridge: Cambridge University Press.

Martínez, S. R. (2009). Ensayo geobotánico global sobre la Macaronesia. In Homenaje al Prof. Dr. Wolfredo Wildpret de la Torre (pp. 255-296). Instituto de Estudios Canarios.

Meot-Duros, L., & Magne, C. (2009). Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. Plant Physiology and Biochemistry, 47(1): 37-41. https://doi.org/10.1016/j.plaphy.2008.09.006

Nicolas, A.N. & Plunkett, G.M. (2014) Diversification times and biogeographic patterns in Apiales. The Botanical Review 80(1): 30–58. doi:10.1007/s12229-014-91324

Nunn, P. D. (1994). Oceanic islands. Blackwell, Oxford

Ortet, R., Regalado, E. L., Thomas, O. P., Pino, J. A., & Fernández, M. D. (2011). Chemical composition and antioxidant activities of the essential oil from Tornabenea bischoffii (Apiaceae). Natural product communications, 6(8): 1934578X1100600834.

Özcan, M., Akgül, A., Başcr, K., Özck, T., & Tabanca, N. (2001). Essential oil composition of sea fennel (*Crithmum maritimum*) from Turkey. Food/Nahrung, 45(5): 353-356.

Pellicer, J., & Leitch, I. J. (2020). The Plant DNA C-values database (release 7.1): an updated online repository of plant genome size data for comparative studies. New Phytologist 22(2): 301-305. https://doi.org/10.1111/nph.16261

Plunkett, G.M., Soltis, D.E. & Soltis, P.S. (1996). Evolutionary patterns in Apiaceae: Inferences based on matK sequence data. Systematics Botany 21: 477–495. http://dx.doi.org/10.2307/2419610

POWO (2019). "Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org/ Retrieved 27/09/2021.

Qiu, F., Baack, E. J., Whitney, K. D., Bock, D. G., Tetreault, H. M., Rieseberg, L. H., & Ungerer, M. C. (2019). Phylogenetic trends and environmental correlates of nuclear genome size variation in *Helianthus* sunflowers. New Phytologist, 221(3): 1609-1618. https://doi.org/10.1111/nph.15465

Reduron, J. P. (2020). Taxonomy, origin and importance of the Apiaceae family. Carrots and related Apiaceae crops, Mulhouse, France (Ed. 2), 1-8.

Renna, M. (2018). Reviewing the prospects of sea fennel (*Crithmum maritimum* L.) as emerging vegetable crop. Plants, 7(4): 92.

Rivas-Martínez, S. (2009). Ensayo geobotánico global sobre la Macaronesia. In Beltrán Tejere, E., J. Afonso Carrrillo, A. García Gallo & O. Rodríguez Delgado (Eds.): Homenaje al Professor Dr. Wolfredo Wildpret de la Torre. Instituto de Estudios Canarios. La Laguna (Tenerife, Islas Canarias). Monografia 78: 255 – 269.

Rivera, D., & Obon, C. (1995). The Ethnopharmacology of Madeira and Porto Santo Islands, a Review. Journal of Ethnopharmacology, 46(2): 73–93. https://doi.org/10.1016/0378-8741(95)01239-a

Romeiras, M. M., Pena, A. R., Menezes, T., Vasconcelos, R., Monteiro, F., Paulo, O. S., & Moura, M. (2019). Shortcomings of phylogenetic studies on recent radiated insular groups: A meta-analysis using Cabo Verde biodiversity. International journal of molecular sciences, 20(11): 2782.

Shaw, K. L., & Gillespie, R. G. (2016). Comparative phylogeography of oceanic archipelagos: Hotspots for inferences of evolutionary process. Proceedings of the National Academy of Sciences, 113(29): 7986-7993.

Suda, J., Kyncl, T., & Freiová, R. (2003). Nuclear DNA amounts in Macaronesian angiosperms. Annals of Botany, 92(1): 153-164. https://doi.org/10.1093/aob/mcg104

Suda, J., Kyncl, T., & Jarolímová, V. (2005). Genome size variation in Macaronesian angiosperms: forty percent of the Canarian endemic flora completed. Plant Systematics and Evolution, 252(3): 215-238.

Sulloway, F. J. (1982). Darwin's conversion: The Beagle voyage and its aftermath. Journal of the History of Biology, 15(3): 325-396.

Watson, M.F., Plunkett, G.M., Downie, S.R. & Lowry, P.P. II (2001) Apiales Special Issue. Presentations from XVIth International Botanical Congress, St Louis. Edinburgh Journal of Botany 58(2).

Whittaker, R. J., & Fernández-Palacios, J. M. (2007). Island biogeography: ecology, evolution, and conservation. Oxford University Press. Oxford, UK. Second edition. pp. 46-74

Whittaker, R. J., Triantis, K. A., & Ladle, R. J. (2008). A general dynamic theory of oceanic island biogeography. Journal of Biogeography, 35(6): 977-994. https://doi.org/10.1111/j.1365-2699.2008.01892.x

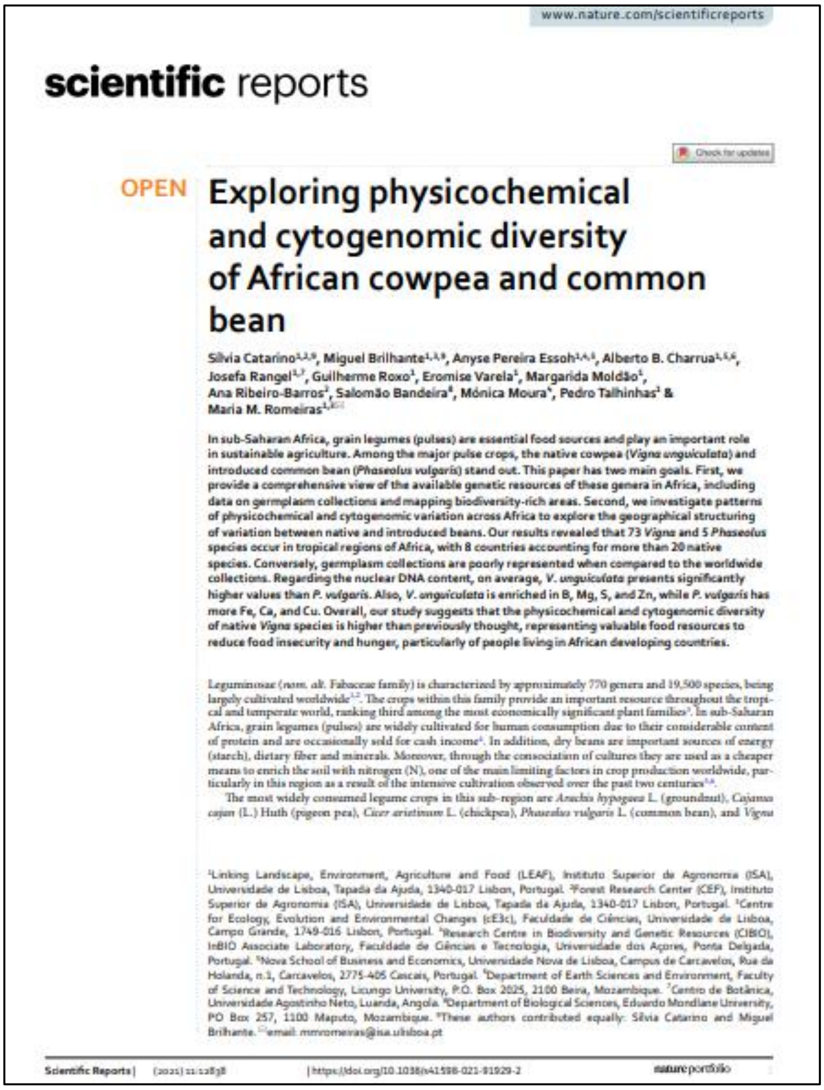
Whittall JB, Hellquist CB, Schneider EL, & Hodges SA. (2004) Cryptic species in an endangered pondweed community (*Potamogeton*, Potamogetonaceae) revealed by AFLP markers. American Journal of Botany. 91: 2022–2029. https://doi.org/10.3732/ajb.91.12.2022

Wojewódzka, A., Baczyński, J., Banasiak, Ł., Downie, S. R., Czarnocka-Cieciura, A., Gierek, M., Frankiewicz, K., & Spalik, K. (2019). Evolutionary shifts in fruit dispersal syndromes in Apiaceae tribe Scandiceae. Plant Systematics and Evolution, 305(5): 401-414. https://doi.org/10.1007/s00606-019-01579-1

5. Appendix: publications and congresses

Scientific papers:

Catarino, S., Brilhante, M., Essoh, A.P., Charrua, A. B., Rangel J., **Roxo, G.**, Varela E., Moldão, M., Ribeiro-Barros, A., Bandeira, S., Moura, M., Talhinhas, P., Romeiras, M. M. (2021) **Exploring physicochemical and cytogenomic diversity of African cowpea and common bean**. *Scientific Reports*. 11, 12838. https://doi.org/10.1038/s41598-021-91929-2 (IF: 4.379, Q1 Multidisciplinar)



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Exploring physicochemical and cytogenomic diversity of African cowpea and common bean

Sílvia Catarino[1,2,9], Miguel Brilhante[1,3,9], Anyse Pereira Essoh[1,4,5], Alberto B. Charrua[1,5,6], Josefa Rangel[1,7], Guilherme Roxo[1], Eromise Varela[1], Margarida Moldão[1], Ana Ribeiro-Barros[1], Salomão Bandeira[8], Mónica Moura[4], Pedro Talhinhas[1] & Maria M. Romeiras[1,3]✉

In sub-Saharan Africa, grain legumes (pulses) are essential food sources and play an important role in sustainable agriculture. Among the major pulse crops, the native cowpea (*Vigna unguiculata*) and introduced common bean (*Phaseolus vulgaris*) stand out. This paper has two main goals. First, we provide a comprehensive view of the available genetic resources of these genera in Africa, including data on germplasm collections and mapping biodiversity-rich areas. Second, we investigate patterns of physicochemical and cytogenomic variation across Africa to explore the geographical structuring of variation between native and introduced beans. Our results revealed that 73 *Vigna* and 5 *Phaseolus* species occur in tropical regions of Africa, with 8 countries accounting for more than 20 native species. Conversely, germplasm collections are poorly represented when compared to the worldwide collections. Regarding the nuclear DNA content, on average, *V. unguiculata* presents significantly higher values than *P. vulgaris*. Also, *V. unguiculata* is enriched in B, Mg, S, and Zn, while *P. vulgaris* has more Fe, Ca, and Cu. Overall, our study suggests that the physicochemical and cytogenomic diversity of native *Vigna* species is higher than previously thought, representing valuable food resources to reduce food insecurity and hunger, particularly of people living in African developing countries.

Leguminosae (*nom. alt.* Fabaceae family) is characterized by approximately 770 genera and 19,500 species, being largely cultivated worldwide[1,2]. The crops within this family provide an important resource throughout the tropical and temperate world, ranking third among the most economically significant plant families[3]. In sub-Saharan Africa, grain legumes (pulses) are widely cultivated for human consumption due to their considerable content of protein and are occasionally sold for cash income[4]. In addition, dry beans are important sources of energy (starch), dietary fiber and minerals. Moreover, through the consociation of cultures they are used as a cheaper means to enrich the soil with nitrogen (N), one of the main limiting factors in crop production worldwide, particularly in this region as a result of the intensive cultivation observed over the past two centuries[5,6].

The most widely consumed legume crops in this sub-region are *Arachis hypogaea* L. (groundnut), *Cajanus cajan* (L.) Huth (pigeon pea), *Cicer arietinum* L. (chickpea), *Phaseolus vulgaris* L. (common bean), and *Vigna*

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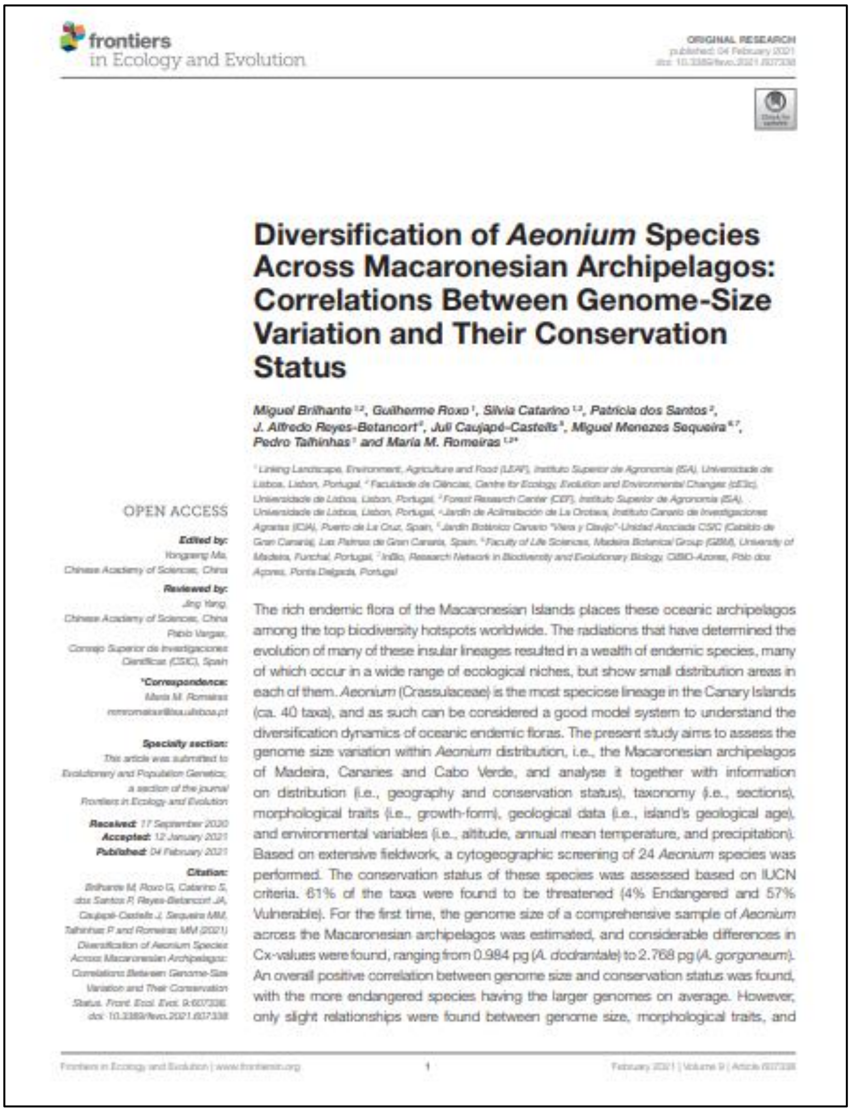

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ORIGINAL RESEARCH
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Diversification of *Aeonium* Species Across Macaronesian Archipelagos: Correlations Between Genome-Size Variation and Their Conservation Status

Miguel Brilhante[1,2], *Guilherme Roxo*[1], *Sílvia Catarino*[1,3], *Patrícia dos Santos*[2], *J. Alfredo Reyes-Betancort*[4], *Juli Caujapé-Castells*[5], *Miguel Menezes Sequeira*[6,7], *Pedro Talhinhas*[1] *and Maria M. Romeiras*[1,2*]

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The rich endemic flora of the Macaronesian Islands places these oceanic archipelagos among the top biodiversity hotspots worldwide. The radiations that have determined the evolution of many of these insular lineages resulted in a wealth of endemic species, many of which occur in a wide range of ecological niches, but show small distribution areas in each of them. *Aeonium* (Crassulaceae) is the most speciose lineage in the Canary Islands (ca. 40 taxa), and as such can be considered a good model system to understand the diversification dynamics of oceanic endemic floras. The present study aims to assess the genome size variation within *Aeonium* distribution, i.e., the Macaronesian archipelagos of Madeira, Canaries and Cabo Verde, and analyse it together with information on distribution (i.e., geography and conservation status), taxonomy (i.e., sections), morphological traits (i.e., growth-form), geological data (i.e., island's geological age), and environmental variables (i.e., altitude, annual mean temperature, and precipitation). Based on extensive fieldwork, a cytogeographic screening of 24 *Aeonium* species was performed. The conservation status of these species was assessed based on IUCN criteria. 61% of the taxa were found to be threatened (4% Endangered and 57% Vulnerable). For the first time, the genome size of a comprehensive sample of *Aeonium* across the Macaronesian archipelagos was estimated, and considerable differences in Cx-values were found, ranging from 0.984 pg (*A. dodrantale*) to 2.768 pg (*A. gorgoneum*). An overall positive correlation between genome size and conservation status was found, with the more endangered species having the larger genomes on average. However, only slight relationships were found between genome size, morphological traits, and

Talk in International congress:

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Abstract: The families Apiaceae and Brassicaceae are represented by 35 and 64 endemic taxa, respectively, in the Macaronesia region. Genome size analyses was shown to be an effective method in Taxonomy and Evolutionary Biology. Using flow cytometry, we analysed the genome size of 93 species (52 from the Apiaceae family, and 42 from the Brassicaceae family) from all the Macaronesian archipelagos and Mainland Portugal. Our results revealed a tendency towards smaller genome sizes in insular endemic species, comparing with the continental ones. Moreover, some native species (e.g. *Angelica lignescens* Reduron & Danton, *Crithmum maritimum* L., *Erysimum bicolor* (Hornem.) DC.) present some population genomic diversity. Our data also support the ancestral relationships between the genera *Daucus* L. and the endemic monotypic genus *Melanoselinum* Schrad. & J.C.Wendl., and *Monizia* Lowe from the Madeira archipelago and the endemic genera *Tornabenea* Parl. from Cabo Verde.