

# Time-calibrated phylogenies reveal mediterranean and pre-mediterranean origin of the thermophilous vegetation of the Canary Islands

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Running title: *Origin of thermophilous plants from the Canary Islands*

## Summary

- **Background and Aims** The Canary Islands have strong floristic affinities with the Mediterranean Basin. One of the most characteristic and diverse vegetation belt of the archipelago is the thermophilous woodland (between 200 and 900 m). This thermophilous plant community consists of many non-endemic species shared with the Mediterranean Floristic Region together with Canarian endemic species. Consequently, phytogeographic studies have historically proposed the hypothesis of a origin of the Canarian thermophilous species following the establishment of the summer-dry mediterranean climate in the Mediterranean Basin around 2.8 million years ago.
- **Methods** Time-calibrated phylogenies for 39 plant groups including Canarian thermophilous species were primarily analysed to infer colonization times. In particular, we used 26 previously-published phylogenies together with 13 time-calibrated phylogenies (including newly generated plastid and nuclear DNA sequence data) to assess whether the time interval between stem and crown ages of Canarian thermophilous lineages postdates 2.8 Ma. For lineages postdating this time threshold, we additionally conducted ancestral area reconstructions to infer the potential source area for colonization.
- **Key Results** A total of 43 Canarian thermophilous lineages were identified from 39 plant groups. Both mediterranean (16) and pre-mediterranean (9) plant lineages were found. However, we failed to determine the temporal origin for 18 lineages because a stem-crown time interval overlaps with the 2.8 Ma threshold. The spatial origin of thermophilous lineages was also heterogeneous, including ancestral areas from the Mediterranean Basin (nine) and other regions (six).
- **Conclusions** Our findings reveal an unexpectedly heterogeneous origin of the Canarian thermophilous species in terms of colonization times and mainland

1 source areas. A substantial proportion of the lineages arrived in the Canaries  
2 before the summer-dry climate was established on the Mediterranean Basin. The  
3 complex temporal and geographical origin of Canarian thermophilous species  
4 challenges the view of the Canary Islands (and Madeira) as a subregion within the  
5 Mediterranean Floristic Region.

6 **Keywords:** thermophilous woodland, Canary Islands, Mediterranean Floristic Region,  
7 colonization times, stem age, crown age, ancestral area, extinction

## INTRODUCTION

Oceanic islands emerge lifeless from the seafloor and are usually separated from continents by wide stretches of sea, which means that all of their terrestrial plants have their origin in other landmasses. The Canary archipelago is formed by seven volcanic islands situated c. 100 km off the Saharan coast (north-western Africa). Since their emergence (last 21 million years), plant lineages have colonized the islands from the mainland, grouping together into six main vegetation belts: coastal vegetation, xerophytic shrubland, thermophilous woodland, laurel forest (*laurisilva*), pine woodland, and alpine legume scrub (del Arco Aguilar and Rodríguez-Delgado 2018). Interestingly, the temporal origin of these floral elements is not well understood. Despite the proximity of the islands to the current Saharo-Arabian Floristic Region (Takhtajan 1986), only some elements of the coastal vegetation and xerophytic shrubland of the Canarian flora are linked to this region (Rivas-Martínez 2009). Iconic tree species of the laurel forest have been traditionally considered both subtropical in origin and surviving representatives of a once more widely distributed Tertiary-Tethyan flora (Bramwell 1976, Mai 1995); a more heterogeneous origin has been lately suggested by Kondrakov et al. 2015. Irrespective of some floristic elements connecting the Canary Islands with floristic regions of the Sahara-Arabia, East/South Africa and America (Quézel 1978, Grehan 2016), most phytogeographers agree that the Canarian flora has a predominant connection with the Mediterranean Floristic Region (MFR).

Since the 18th century, phytogeographers have been discussing whether plants of the Canaries and other Macaronesian archipelagos of the Atlantic Ocean form an independent floristic region (Engler 1879, Sunding 1979, Takhtajan 1986, Bolòs 1996) or a subregion within the MFR (Meusel 1965, Lobin 1982, Rivas-Martínez 2009, del Arco Aguilar and Rodríguez-Delgado 2018). On the one hand, high species endemism (c. 35 %) and numerous floristic elements linked to several continental floras are considered

strong arguments to accept a high-level phylogeographic classification (Macaronesian Floristic Region). On the other hand, a high percentage of Mediterranean elements and lineages are in turn understood as a clear criterion to propose a lower-level phylogeographic classification (Canarian-Madeiran subregion of the MFR). The Mediterranean element is pervasive in the six vegetation belts of the Canaries in such a way that plant lineages exhibiting traits common in mediterranean climates or having their centers of diversity in the Mediterranean Basin are particularly abundant in the pine woodland and the thermophilous woodland (Francisco-Ortega et al. 2002, Rivas-Martínez 2009, White et al. 2020, Albaladejo et al. 2021). Indeed, the thermophilous vegetation is the plant community most associated with the MFR, including some arboreal and shrubby communities named in Spanish after their dominant tree species: “sabinares” (*Juniperus turbinata* subsp. *canariensis* (Guyot & Mathou) Rivas Mart., Wildpret & P.Pérez), “almacigares” (*Pistacia atlantica* Desf.), “lentiscales” (*Pistacia lentiscus* L.), “acebuchales” (*Olea europaea* subsp. *guanchica* P.Vargas, J.Hess, Muñoz Garm. & Kadereit) (Fernández-Palacios et al. 2008, Nezadal and Welss 2009). The last three tree species have sclerophyllous leaves with flexural stiffness and strength (Schimper 1903, Read and Sanson 2003), which characterize the vegetation of the MFR (Rundel et al. 2016, Vargas 2020). All these sources of evidence suggest that phylogeographic origin of Canarian plant lineages may be predominantly associated with the MFR. If so, the question remains as to whether phylogenetic relationships and divergence times of a significant number of species from the thermophilous woodland can provide evidence of a temporal mediterranean origin, i.e., following the establishment of the mediterranean climate across the Mediterranean Basin (Carine et al. 2004, Fernández-Palacios et al. 2008, Vargas 2020).

The establishment of the mediterranean climate across southern Europe and northern Africa 2.8 million years ago (Ma) provides the opportunity to consider a relatively well-defined temporal framework. This temporal threshold (2.8 Ma) is based on

independent evidence from palaeoceanographic (Hernández-Molina et al. 2014), palaeoclimatic (Hernández-Molina et al. 2014, Grant et al. 2022), palaeobotanical (Bocquet and Kiefer 1978, Suc 1984, Palmarev 1989; Tzedakis 2007, Postigo et al. 2009, Jiménez-Moreno et al. 2010) and phylogenetic (Fiz-Palacios and Valcárcel 2013 and references within it) evidence that support a ‘sharp’ climatic event. In particular, the establishment of the Mediterranean climate brought about a significant environmental change because of the concurrence of a drought period during the warm season. Interestingly, this threshold is close to the limit between the Pliocene and Pleistocene (2.6 Ma), as recognised by the International Chronostratigraphic Chart (<https://stratigraphy.org/chart>). Such a temporal threshold has been used in multiple studies to categorise Mediterranean floristic elements according to their temporal origin (paleo-mediterranean vs. neo-mediterranean; Herrera 1992, Peñuelas et al. 2001, Verdú and al. 2003). In addition, it has been used to test specific hypotheses such as the timing of colonisation of the Canary Islands by certain lineages (Vargas 2007; Salvo et al. 2010), diversification rate shifts (Fiz-Palacios and Valcárcel 2013) and karyotypic changes (Escudero et al. 2018) in combination with time-calibrated phylogenies.

The use of phylogenetic analyses based on DNA sequences to estimate the timing of evolutionary events has become a basic tool in biogeography (Sanmartín 2014). Time can be measured in absolute units when the tree is calibrated with fossils, phylogeny-based secondary calibrations and/or biogeographic events (Forest 2009, Ho and Phillips 2009; see Hipsley and Müller 2014 for alternative calibration approaches). In particular, relaxed-clock Bayesian methods can elucidate a prior distribution on the age of a node, taking into account the uncertainty associated with tree topology, branch length and calibrations (Drummond et al. 2006, Yang and Rannala 2006). This method can be applied to a large number of plant groups, thus helping describe general patterns (Pokorný et al. 2015, Vargas et al. 2018). The time-calibrated phylogenies obtained from Bayesian relaxed-clock

1 methods are often used as input for inferring the ancestral areas from which colonisation  
2 may have occurred (e.g. Albaladejo et al 2021). These ancestral area reconstruction  
3 analyses give new possibilities for quantitative analysis in island biogeography (Lamm and  
4 Redelings, 2009). Thus, applying time-calibrated phylogenetic approaches to investigate  
5 divergence of Canarian lineages from their continental relatives may provide valuable  
6 insight into the very complex patterns of relationships between the Canary Islands and  
7 continental floras (Carine et al. 2004, Kondrakov et al. 2015, Caujapé-Castells et al. 2017,  
8 Valente et al. 2017).

9 Palaeoclimatic, floristic and phytogeographic studies lead us to examine the  
10 hypothesis of an origin of Canarian thermophilous species following the establishment of  
11 the mediterranean climate across the Mediterranean Basin 2.8 Ma. To test this hypothesis,  
12 we firstly analyzed the phylogenetic relationships of thermophilous Canarian lineages and  
13 their sister groups using improved phylogenetic datasets in terms of extended taxonomic  
14 and geographical sampling. Secondly, we evaluated whether ancestral thermophilous plant  
15 lineages had a temporal origin since the mediterranean climate became established around  
16 2.8 Ma (Suc et al. 2018) based on the both newly generated and previously published time-  
17 calibrated phylogenies. Thirdly, we implemented ancestral area reconstruction analyses to  
18 assess whether colonisation of thermophilous canarian lineages had a potential source area  
19 for colonization in the Mediterranean Basin or another geographic area. In sum, we are  
20 quantifying at what degree there is a spatio-temporal biogeographic link between the  
21 Canarian Flora and the MFR.

## 22 MATERIALS AND METHODS

### 23 *Study area*

24 The thermophilous woodland is a vegetation type characteristic of the Canary Islands. It  
25 occurs between the xerophytic vegetation and the laurel forest on the windward slopes

(ca. 200-500 m) and between the xerophytic vegetation and the pine woodland on the leeward slopes (ca. 300-900 m) (del Arco et al. 2006, Fernández-Palacios et al. 2008). Its climate fits into mediterranean conditions with annual rainfall between 250 and 450 mm, mostly occurring in winter, and with average temperature between 15° and 19° C, depending on elevation. The thermophilous woodland is defined by some communities dominated by tree species also found in the southern Europe and northern Africa (particularly *Juniperus turbinata*, *Pistacia atlantica*, *Pistacia lentiscus*, *Olea europaea*), which may be accompanied by endemic trees (*Gymnosporia cassinoides* (L'Hér.) Masf., *Dracaena draco* L., *Phoenix canariensis* H.Wildpret, *Sideroxylon canariense* Leyens, Lobin & A.Santos, *Visnea mocanera* L.f.). In addition, a high number of shrub and herb species characterize this vegetation belt (Fernández-Palacios et al. 2008). Unfortunately, this vegetation type is extremely rare in a pristine state because its trees have been eliminated by anthropogenic activity. Chronologically, African human groups colonized the seven the islands, who mostly settled around 2000 years ago, and then a more severe deforestation took place since colonization by European groups (Fernández-Palacios et al. 2008). As a result, currently only 11 % of the original thermophilous woodland is estimated to remain (Fernández-Palacios et al. 2008, Castilla-Beltrán et al. 2021).

#### *Sampling strategy and DNA sequencing*

In this study, stem and crown ages were inferred by extending DNA sequence datasets of 13 previously published phylogenies, which included a total of 16 thermophilous species (seven Canarian endemics, five Macaronesian endemics, four non-endemic natives) occurring in the Canary Islands. These previous datasets are heterogeneous and the published phylogenies share some of the following weak points: (1) low support for monophyletic groups including Canarian species; (2) poor sampling of potential mainland sister groups (species/populations); (3) poor sampling of species or populations from the



Canary Islands; (4) lack of divergence-time estimations; and (5) need for technical improvement in terms of new methods or calibration points (Table 1). Additionally, stem and crown ages for 26 plant groups with species occurring in the Canarian thermophilous vegetation belt were directly taken from the literature (Supplementary Data Table S1). A total of 39 thermophilous plant groups (i.e., genera, rarely family) were analyzed.

To generate new data on divergence times for 16 thermophilous species, we first obtained 13 DNA sequence datasets from the most comprehensive phylogenetic studies at the genus level (occasionally at family level). Alignments were directly provided by authors of the studies or obtained from the GenBank or TreeBase databases. GenBank accession numbers and geographic origin for all downloaded sequences are available in each publication referenced in Table 1. Second, we extended taxonomic and/or population sampling of those DNA sequence datasets to infer stem and crown nodes for the Canarian species or populations. The new samples were obtained from three sources: field trips, herbarium specimens, and colleagues who contributed with field samples (Supplementary Data Table S2). We tried to sample at least one individual per island where the taxon is present depending on available material and accessibility of populations. We additionally sampled at least one individual from the most closely-related species based on taxonomic information when not available in the DNA sequence database. For non-endemic taxa, we also sampled individuals from at least two populations from nearby continental areas. Many plants of the Canary Islands have a Palearctic origin (Sunding 1979, Carine et al. 2004), and thus we focused our sampling on the archipelagos and mainland regions of Africa and Europe that are close to the Canary Islands, with particular effort on Mediterranean Iberia and northwestern Africa (Valente et al. 2017). Overall, we newly generated 262 DNA sequences from 104 samples of 33 taxa (16 representative thermophilous species and 17 close relatives) and 16 different DNA regions, which were included into the published DNA sequence datasets.

Sample information for all specimens and GenBank accession numbers for all new DNA sequences are provided in Supplementary Data Table S2.

DNA was extracted from dried leaves using QIAGEN DNeasy Plant Mini Kit according to the manufacturer's protocol. The sixteen different DNA regions were amplified and sequenced based on previously published studies for each plant group (Supplementary Data Table S2). In particular, we analyzed two nuclear (ETS and ITS), one mitochondrial (PHYC) and 13 plastid DNA regions (*matK*, *psbA-trnH*, *psbB1-psbB2*, *rbcL*, *rpl16*, *rpl32-trnL*, *rps4*, *trnD-trnT*, *trnK*, *trnL-trnF*, *trnL-trnF*, *trnS-trnG*, *trnV*). Primers and PCR cycles applied for each plant group are included in Supplementary Data Table S3. Amplified products were sequenced by standard Sanger sequencing at Macrogen Europe ([www.macrogen.com](http://www.macrogen.com)). We used Geneious 2021.2.2 (<https://www.geneious.com>) to edit chromatograms, align sequences with the MUSCLE algorithm (Edgar 2004) and concatenate DNA regions (Table 1). The resulting alignments were checked visually and corrected where the algorithm failed to identify gaps.

### *Phylogenetic relationships*

We applied Bayesian Inference analysis to the 13 DNA sequence datasets to infer the phylogenetic relationships of plant groups with thermophilous species in the Canary Islands. The best-fitting substitution model for each partition (DNA region) was selected using the Akaike Information Criterion implemented in jModelTest v.0.1.1 (Posada 2008). We did not test the congruence between nuclear and plastid matrices because this was already analysed in the previous phylogenetic studies. Thus, we used the phylogenetic dataset (single or combined DNA matrix) that provided the highest phylogenetic resolution in those studies (Table 1; Figs. S1-S13). MrBayes v.3.2.6 (Ronquist et al. 2012) was used in all cases on XSEDE via the CIPRES Science Gateway (<http://www.phylo.org/>) with two simultaneous runs, each with four parallel Markov

chains (three hot and one cold), and sampled every 10,000 trees to obtain a total of 10 million generations. The first 25% of trees of each run were discarded (burn-in) and 50% majority-rule consensus trees were constructed. An additional approach using maximum likelihood was implemented in RAxML to obtain an alternative estimate of phylogenetic relationships and support values (see Supplementary Data, Methods S1).

#### *Divergence times*

To estimate divergence times of stem and crown nodes for plant groups with thermophilous species in the Canary Islands, we generated 13 new time-calibrated phylogenies using the Bayesian uncorrelated log-normal relaxed clock model in BEAST 1.84 (Drummond and Rambaut 2007). We used the same substitution models already applied for the phylogenetic reconstructions. For each analysis, we ran two independent chains of 100 million generations with a birth–death tree prior, which accounts for both speciation and extinction (Gernhard 2008). All molecular dating analyses in BEAST were performed using the computer cluster Trueno (CSIC, Madrid, Spain). We assessed convergence of chains and appropriate burn-ins with Tracer v.1.7.1 (Rambaut et al. 2018), combined runs using LogCombiner, and produced maximum clade credibility (MCC) trees with mean node heights in Tree Annotator.

Calibration points used to estimate divergence times were based on the fossil record and published secondary calibration points, depending on data availability for each plant group (Supplementary Data Figs. S14-S26). When reliable fossils were available, a lognormal prior on age estimates was used, since this distribution better represents the stratigraphic uncertainty associated with the fossil record (Ho and Phillips 2009). The offset of the lognormal distribution was set to the upper bound of the stratigraphic period where the fossil was found. In the absence of reliable fossils, only secondary calibration points taken from original papers were implemented to the corresponding deep nodes

(Table 1). Secondary calibration points were obtained from fossil-calibrated phylogenies of higher taxonomic ranks, which included samples of our study groups (e.g., typically the family to which the genus belongs to), and were assigned normal distribution priors (Ho and Phillips 2009) in the BEAST analysis including the mean and the 95 % highest posterior density (HPD) interval from those studies. A summary of time constraints used for each DNA dataset and their provenance can be found in Supplementary Data Figs. S14-S26.

#### *Colonization times: crown and stem ages*

Colonization of the Canary Islands by a lineage have taken place at a time between the stem age and the crown age of the lineage (Fig. 1; Swenson et al. 2014, García-Verdugo et al. 2019a). The stem age, representing the time of divergence between the island lineage and the mainland sister lineage, is most commonly used as an indicator of colonization time (Fig. 1A, see examples in Keeley and Funk 2011, Spalik et al. 2014, Kondrakov et al. 2015, Grover et al. 2017, Schüßler et al. 2019). However, the stem age tends to overestimate colonization time as a result of extinction or incomplete sampling of closely related mainland lineages (Fig. 1B; Mairal et al. 2015, Pillon and Buerki 2017). Alternatively, recent studies are using the crown age, which corresponds to the onset of the divergence from the most recent common ancestor of the lineage within the archipelago, and thus a proxy for the colonization time from the mainland (Fig. 1A; Pokorny et al. 2015, García-Verdugo et al. 2019a). However, the crown age may provide an underestimate of colonization time, due to extinction of island lineages or poor sampling of the archipelago (Fig. 1C; see examples of Canarian lineages potentially affected by extinction in Sanmartín et al. 2008, and García-Verdugo et al. 2019b). Therefore, the crown age represents the minimum age (lower bound) at which

colonization of the archipelago from the mainland could have occurred, whereas the stem age represents the maximum age (upper bound) of that event.

To address our working hypothesis, we compared both stem and crown ages with the 2.8 Ma threshold, which marks the establishment of mediterranean climate (Suc 1984). By considering the stem node as upper limit and the crown node as lower limit of colonization time, our approach accounts for the potential impact of lineage extinction and incomplete sampling on colonization time estimates. Based on estimates of stem and crown ages (mean and 95 % HPD intervals) from 26 previously-published and 13 newly-generated phylogenies, we classified 43 Canarian thermophilous lineages into three categories: (1) mediterranean lineages, when stem and thus crown ages postdated the 2.8 Ma threshold (i. e., both the stem and the crown node are mediterranean in time); (2) pre-mediterranean lineages, when crown and thus stem ages predated the 2.8 Ma threshold (i. e., both the stem and the crown ages are pre-mediterranean); and (3) undetermined lineages, for those groups with stem ages falling any time before this threshold (pre-mediterranean stem ages), and crown ages afterwards (mediterranean crown ages) (Fig. 2).

In the context of this hypothesis, the term “mediterranean” is referred to a window of colonization of the Canary Islands after the establishment of the mediterranean climate (temporal origin) in the Mediterranean Basin. This does not necessarily imply that the lineages colonized the thermophilous belt directly from the Mediterranean Basin.. Indeed, the closest mainland taxa for a few of the Canarian lineages are not from the Mediterranean Basin according to the literature (Supplementary Data Table S1). Although both temporal and spatial origins would have ideally to be fulfilled for a lineage to be considered "mediterranean", we classify taxa according to temporal origin rather than geographic origin to address our working hypothesis because the temporal

framework allows us to integrate stem age and crown age estimates together and thus partially circumvent the impact of poor sampling, low phylogenetic resolution, extinction and fluctuant limits of the MFR over time (see Discussion).

Nodes could not be unambiguously designated as mediterranean or pre-mediterranean when their 95 % HPD intervals spanned the 2.8 Ma threshold (Fig. 2). For these nodes, a complementary approach was taken (Fig. 3). Marginal probability distributions of divergence times for such nodes were extracted from 100,000 trees using TreeStat v.1.8.4 (Rambaut and Drummond 2016). Then, we used the percentage of occurrence of node ages before or after the 2.8 Ma threshold, following the approach of Vargas et al. (2014). In other words, a node was considered mediterranean when the majority of the marginal probability distribution of the node age postdated the establishment of the mediterranean climate (2.8 Ma), and as pre-mediterranean when the majority of the marginal probability distribution of the node age predated such a threshold (Fig. 3). We chose a 50 % threshold to include the majority of probability distributions (Vargas et al. 2014). For the previously published time-calibrated phylogenies we took a different approach, in which the "mediterranean" or "pre-mediterranean" status of nodes was estimated based on mean values of node ages given by the authors.

In some cases, low phylogenetic resolution and the tree topology hinder estimation of stem and crown ages. For instance, this can happen when posterior probability (PP) of nodes is low ( $< 0.90$ ), or when multiple representatives of Canarian lineages are placed at distant positions in the tree with low support. These scenarios make it difficult to distinguish between incomplete lineage sorting and multiple colonization events. In such cases, a most inclusive approach was taken, in which we considered the time estimate of the most recent common ancestor (TMRCA) of island and mainland lineages to be an upper bound. Since this TMRCA is equivalent to the stem age (when relationships are

resolved), it also represents the maximum age at which the lineage may have colonized the archipelago (Valente et al. 2017).

### *Ancestral Area Reconstructions*

To reconstruct the ancestral distribution range of the lineages with thermophilous species in the Canary Islands (both endemics and non-endemics natives), we employed a model-based maximum-likelihood approach for ancestral area optimization: the dispersal-extinction-cladogenesis (DEC) model implemented in the BioGeoBEARS R package (Matzke, 2013). For the sake of brevity and addressing our working hypothesis, biogeographical reconstructions were conducted using only three geographic areas (A = Mediterranean Basin, B = Macaronesia, C = Other regions) allowing ancestors to be present in a maximum of three areas. We set symmetric dispersal between areas, and constant dispersal rates through time. The analyses were run using the MCC tree of the 13 newly inferred time-calibrated phylogenies (see Materials and Methods, Divergence times) after pruning tips in multi-sampled species in order to represent each species with a single terminal branch. We estimated whether colonisation occurred from the Mediterranean Basin (A, AB, AC, ABC) or from other geographic areas (C, BC) by considering the most likely ancestral distribution range recovered for the stem node of the Canarian lineages (or in the TMRCA when PP of stem nodes were lower than 0.90). As a result, we interpreted that any ancestral range shared by the Mediterranean Basin and the Canary islands indicates an origin from the Mediterranean Basin.

## RESULTS

### *Newly inferred phylogenetic relationships*

Our phylogenetic reconstructions based on Bayesian Inference using MrBayes (Figs. S1-S13) and Maximum Likelihood using RAXML (results not shown) are in accordance with previous published phylogenies regarding the topological relationships for the

thermophilous species occurring in the Canary Islands. However, the phylogenetic support obtained for temporal divergence using BEAST was higher in most cases (Figs. S14-S26). We following describe well supported clades obtained in BEAST.

Most of the species analyzed formed well-supported monophyletic groups (Figs. 4-6, Supplementary Data Table S1). However, we could not confirm the monophyly of Canarian populations of non-endemic species (i.e., *Ephedra fragilis* Desf., *Juniperus turbinata*, *Pistacia atlantica*, *Pistacia lentiscus*) as well as the endemics *Asparagus umbellatus* Link and *Thesium retamoides* (A.Santos) J.C.Manning & F.Forest (Figs. 4A-4E, Fig. 5C, Fig. 6D, Supplementary Data Table S1). Similarly, sister-group relationships had high statistical support (above 0.90 PP), except for the non-endemic species and for the Macaronesian endemic species *Dracunculus canariensis* Kunth (Figs. 4A-4E, Fig. 6F, Supplementary Data Table S1).

The following phylogenetic relationships within the Canarian groups or between the Canarian species and continental sister groups were documented for the first time: (i) placement of *Asparagus scoparius* Lowe within the clade of *Asparagus nesiotus* Svent. and *Asparagus plocamoides* Webb ex Svent., and of *Asparagus umbellatus* within the clade of *Asparagus fallax* Svent. and *Asparagus arborescens* Willd. ex Schult. & Schult.f.; (ii) close relationship of the two Canarian species of *Gymnosporia* (Wight & Arn.) Hook.f. (*Gymnosporia cassinoides* (L'Hér.) Masf. and *Gymnosporia cryptopetala* Reyes-Bet. & A.Santos), which are sisters to the Madeira endemic *Gymnosporia dryandrii* (Lowe) Masf.; (iii) *Chrysojasminum odoratissimum* (L.) Banfi sister to the mainland clade of *Chrysojasminum parkeri* (Dunn) Banfi, *Chrysojasminum humile* (L.) Banfi, *Chrysojasminum bignoniaceum* (Wall. ex G.Don) Banfi and *Chrysojasminum fruticans* (L.) Banfi; and (iv) the two Canarian species of *Thesium* L. (*Thesium retamoides*



and *Thesium subsucculentum* (Kämmer) J.C.Manning & F.Forest) sister to the mainland  
*Thesium mauritanicum* Batt. (Figs 4-6).

As a result, 16 independent Canary Island colonization events were inferred for  
the 13 plant groups with thermophilous species analyzed in this study: 10 genera with a  
single colonization and two with more than one colonization (three of *Pistacia* L. and two  
of *Asparagus* Tourn. ex L.) (Figs. 4-6, Supplementary Data Table S4). In addition, some  
lineages displayed cladogenesis in the Canary Islands, including species from other  
vegetation zones: *Asparagus* lineage I (*A. umbellatus* in the thermophilous woodland, *A.*  
*arborescens* in the xerophytic shrubland and *A. fallax* in the laurel forest), *Asparagus*  
lineage II (*A. scoparius* in the thermophilous woodland, *A. nesiotus* in xerophytic  
shrubland and *A. plocamoides* in the pine forest), *Gymnosporia* (*G. cassinoides* and *G.*  
*cryptopetala* in the thermophilous woodland), and *Thesium* (*T. retamoides* in  
thermophilous woodland and *T. subsucculentum* in the xerophytic shrubland) (Figs. 4-  
6). For these cases (i.e., lineages that diversified in different vegetation zones of the  
Canary Islands), stem and crown ages considered to categorize lineages as mediterranean  
or pre-mediterranean were those corresponding to the entire Canarian lineage (including  
thermophilous and non-thermophilous species).

#### *Colonization times*

The estimated mean crown ages of the 16 Canarian lineages newly analyzed in this study  
ranged from 1.07 Ma within *Myrsine excelsa* D.Don to 7.99 Ma within *Sideroxylon*  
*canariense*, while mean stem ages ranged from 1.15 Ma for *Pistacia atlantica* to 37.73  
Ma for *Sideroxylon canariense* (Figs. 4-6; Supplementary Data Figs. S14-S26, Table S4).  
These age ranges include five cases in which the TMRCA had to be used: *Asparagus*  
lineage II, *Ephedra fragilis*, *Juniperus turbinata* subsp. *canariensis*, *Pistacia atlantica*  
and *Pistacia lentiscus* lineage II. Considering the complete list of 43 lineages (including

those with previously published time-calibrated phylogenies), we identified 16 mediterranean lineages, nine pre-mediterranean lineages and 18 undetermined lineages (Table 2, Supplementary Data Table S1). We following describe these results in detail.

#### Mediterranean lineages (stem ages < 2.8 Ma)

The newly generated phylogenies allowed identification of five thermophilous plant lineages as mediterranean with respect to their colonization times, as their stem ages postdated the 2.8 Ma threshold: *Asparagus* lineage I, *Asparagus* lineage II, *Ephedra fragilis*, *Pistacia atlantica* and *Pistacia lentiscus* lineage II (Fig. 4). Most stem ages accumulated a marginal posterior distribution clearly after the 2.8 Ma threshold, so they were assigned as mediterranean with high statistical support (Supplementary Data Table S4). The highest uncertainty was estimated for the stem nodes of *Asparagus* lineage II, in which only 61 % of the stem age distribution fell in the last 2.8 Ma. Additional studies taken from the literature provided 11 more plant lineages as mediterranean. As a result, a total of 16 of the 43 thermophilous lineages display a clear cut mediterranean origin (from youngest to oldest stem ages; Fig. 7): *Globularia* lineage (0.30 Ma), *Smilax aspera* L. (0.30), *Cistus monspeliensis* L. (0.50), *Brachypodium arbuscula* Gay ex Knoch (0.80), *Erysimum* lineage (0.80), *Pistacia atlantica* (1.15, Fig. 4C), *Ephedra fragilis* (1.29, Fig. 4E), *Convolvulus* lineage II (1.50), *Asparagus* lineage II (1.60, Fig. 4B), *Solanum* lineage (1.70), *Helianthemum* Mill. sect. *Helianthemum* (1.82), *Argyranthemum* lineage (2.20), *Olea europaea* subsp. *guanchica* (2.60), *Malva canariensis* M.F.Ray (2.78) and *Asparagus* lineage I (2.80, Fig. 4A). Although the mean stem age of *Asparagus* lineage I clearly overlaps the mediterranean threshold, the posterior distribution of trees showed that more than 60 % of the stem age distribution and 96 % of crown age distribution in the mediterranean climate period.

#### Pre-mediterranean lineages (crown ages > 2.8 Ma)

The newly generated phylogenies allowed identification of three of the 16 thermophilous plant lineages as pre-mediterranean, as their crown ages predated the 2.8 Ma threshold: *Sideroxylon canariense*, *Chrysojasminum odoratissimum* and *Thesium* lineage (Fig. 5). Crown ages for the *Chrysojasminum odoratissimum* lineage and *Sideroxylon canariense* accumulated a marginal posterior distribution clearly before 2.8 Ma, so they were assigned as pre-mediterranean with high statistical support (Supplementary Data Table S4). The highest uncertainty was estimated for the crown node of the *Thesium* lineage, in which only 69 % of the age distribution predated 2.8 Ma. Previously published studies revealed six additional pre-mediterranean lineages. As a result, nine of the 43 thermophilous lineages displayed a pre-mediterranean origin (from youngest to oldest crown ages; Fig. 8): *Sideritis* lineage (3.30 Ma), *Echium* lineage (3.70), *Thesium* lineage (3.86), *Chrysojasminum odoratissimum* (4.79), *Euphorbia* sect. *Aphyllis* subsect. *Macaronesicae* Molero & Barres (6.92), *Sideroxylon canariense* (7.99), *Ruta* lineage (8.10), *Crambe* lineage (8.20) and *Sonchus* lineage (8.50).

#### Undetermined lineages (stem ages > 2.8 Ma, crown ages < 2.8 Ma)

The temporal origin of eight of the 16 thermophilous lineages were undetermined because their stem ages predated and their crown ages postdated the 2.8 Ma threshold: *Bosea yervamora* L., *Bryonia verrucosa* Aiton, *Dracunculus canariensis*, *Gymnosporia* lineage, *Myrsine excelsa*, *Juniperus turbinata* subsp. *canariensis*, *Pistacia lentiscus* lineage I and *Rhamnus crenulata* Aiton (Fig. 6). Most of their stem and crown ages accumulated a marginal posterior distribution clearly before and after 2.8 Ma. The highest uncertainty was recorded in the crown node of *Dracunculus canariensis*, in which only 60 % of the trees fell within the last 2.8 Ma time period (Supplementary Data Table S4). Previously published studies also revealed 10 undetermined lineages. In total, the following 18 lineages were classified as undetermined (Figs. 7, 8): *Gonospermum* lineage (mean stem:

3.10 Ma; mean crown: unknown), *Gymnosporia* lineage (mean stem: 3.78 Ma; mean crown: 1.27 Ma; Fig. 6E), *Artemisia* lineage (mean stem: 3.84; mean crown: unknown), *Rhamnus crenulata* (mean stem: 3.88; mean crown: 1.71; Fig. 6B), *Pistacia lentistus* lineage I (mean stem: 3.96; mean crown: 1.15; Fig. 4D), *Myrsine excelsa* (mean stem: 3.98; mean crown: 1.07; Fig. 6G), *Juniperus turbinata* subsp. *canariensis* (mean stem: 4.91, mean crown: unknown; Fig. 6D), *Bryonia verrucosa* (mean stem: 5.19; mean crown: 1.74; Fig. 6C), *Bosea yervamora* (mean stem: 5.69; mean crown: 1.64; Fig. 6A), *Rubia fruticosa* Aiton (mean stem: 6.69; mean crown: 2.10), *Navaea phoenicea* (Vent.) Webb & Berthel. (mean stem: 6.77; mean crown: unknown), *Anagyris latifolia* Brouss. ex Willd. (mean stem: 8.20; mean crown: 1.90), *Cheirolophus* lineage (mean stem: 8.50; mean crown: 1.70), *Hypericum canariense* L. (mean stem: 10.80; mean crown: 1.90), *Dracaena* lineage (mean stem: 11.80; mean crown: 2.30), *Dracunculus canariensis* (mean stem: 12.10; mean crown: 2.76), *Dioscorea edulis* (Lowe) Campos, Wilkin & Viruel (mean stem: 13.48; mean crown: unknown), *Visnea mocanera* (mean stem: 27.00; mean crown: 2.50).

#### *Ancestral Area Reconstructions*

Biogeographical analyses supported nine lineages with stem node ranges including the Mediterranean Basin (i.e. *Asparagus* lineage II, *Bosea yervamora*, *Bryonia verrucosa*, *Dracunculus canariensis*, *Ephedra fragilis*, *Juniperus turbinata*, *Pistacia lentiscus*, *Rhamnus crenulate* and *Thesium* lineage) and six lineages with stem node ranges not including the Mediterranean Basin (i.e. *Asparagus* lineage I, *Chrysojasminum odoratissimum*, *Gymnosporia* lineage, *Myrsine excelsa*, *Pistacia atlantica* and *Syderoxylon canariense*) (Figs. 4-6; Supplementary Data Figs. S27-S39, Table S5). No correspondence between the temporal origin of the lineages (mediterranean vs. pre-mediterranean) and the ancestral ranges (including vs. excluding the Mediterranean

Basin) was detected in three plant lineages (i.e. *Asparagus* lineage I, *Pistacia atlantica*, *Thesium* lineage).

## DISCUSSION

The thermophilous woodland is considered a relatively recent ecosystem, originated after the establishment of the mediterranean climate in the Mediterranean Basin (2.8 Ma; Fernández-Palacios et al. 2008, Rivas-Martínez 2009, del Arco Aguilar and Rodríguez-Delgado 2018). In this study, we provide new divergence time estimates (i.e., stem and crown ages) based on time-calibrated phylogenies for 16 species representative of the thermophilous Canarian vegetation, which we analyzed together with previously-published results from additional 27 thermophilous plant lineages (Figs. 7, 8). In island biogeography, colonization times are more precisely considered to have taken place sometime between the stem and crown ages of the island lineage (Swenson et al. 2014, García-Verdugo et al. 2019a). Based on this phylogenetic principle, our study suggests that the Canarian thermophilous plant community is composed of lineages with an temporal origin both predating (pre-mediterranean) and postdating (mediterranean) the 2.8 Ma threshold considered for the establishment time of the mediterranean climate (Figs. 7, 8; Table 2). In addition, biogeographic reconstruction analyses showed that six of the 16 colonisation events here inferred may have occurred from areas that did not include the Mediterranean Basin by continental ancestors (Figs. 4-6). Taking all these results together, the thermophilous vegetation appears to be a complex assemblage of species with a heterogeneous origin in terms of colonization times and geographical origins.

### *Pre-mediterranean and mediterranean elements in the thermophilous plant community*

As expected by the hypothesis of a mediterranean temporal origin for the Canarian thermophilous species, a considerable number of lineages (16) are estimated to have

colonized the archipelago after the establishment of the mediterranean climate (2.8 Ma). Data from meteorological stations (<https://www.acanmet.org/>) between 200 and 600 m.a.s.l. and climatic variables used for species distribution modeling of thermophilous plants (Coello et al. 2020) indicate that current conditions in the Mediterranean Basin are similar to those of the thermophilous vegetation belt in the Canaries (Rivas-Martínez 2009). Since the late Pliocene (2.8 Ma), a progressive summer aridification of southern Europe and northern Africa may have produced wider distribution that may have facilitated dispersal from the MFR to other areas. There is thus a higher likelihood of dispersal and colonization to neighboring territories such as the Canarian archipelago since then (Meusel 1965, Sunding 1979). That is why trees with one of the most characteristic mediterranean-type syndromes (sclerophylly, a trait displayed mostly in leaves) may have found ideal conditions in the Canaries in the last 2.8 million years (Axelrod 1975, Verdú et al. 2003, Rundel et al. 2016, Vargas et al. 2018). Our results partly support this prediction for the sclerophyllous *Olea europaea* L., *Pistacia atlantica* and *Pistacia lentiscus* lineage II, but provide undetermined results for the sclerophyllous *Rhamnus crenulata* and *Pistacia lentiscus* lineage I. Alternatively, some other tree species (e.g., *Gymnosporia* spp., *Myrsine excelsa*, *Sideroxylon canariense*, *Visnea mocanera*) with leathery leaves and thick cuticles (typically observed in sclerophyllous species) did not display evidence for an origin postdating 2.8 Ma, which did not help support a mediterranean temporal origin for all the trees characterizing the thermophilous vegetation belt.

The relatively old stem and crown ages inferred for some plant lineages clearly indicate a pre-mediterranean origin for a considerable number of the thermophilous species (nine of 43 lineages). Indeed, the following plants appear to have already been present in the Canary Islands before the establishment of the mediterranean climate: *Sideritis* lineage, *Echium* lineage, *Thesium* lineage, *Chrysojasminum odoratissimum*,

*Euphorbia* sect. *Aphyllis* subsect. *Macaronesicae*, *Sideroxylon canariense*, *Ruta* lineage, *Crambe* lineage and *Sonchus* lineage (Fig. 7, Table 2). Three non-mutually exclusive hypotheses can be put forward to explain the presence of pre-mediterranean lineages in the current Canarian thermophilous plant community: (i) a direct colonization from the mainland to pre-existent thermophilous vegetation in pre-mediterranean times, (ii) an indirect colonization from other Canarian vegetation types followed by a more recent species differentiation in the thermophilous vegetation and (iii) an old colonisation into non-thermophilous vegetation (on the islands) in pre-mediterranean times and long-term adaptation to thermophilous conditions *in situ* in pre-mediterranean or mediterranean times. The first hypothesis is supported by the fossil record. In particular, fossils of sclerophyllous leaves related to Miocene relicts have been found on the island of Gran Canaria (Anderson et al. 2009). These macrofossils moreover share characteristics with certain representative species of the present-day thermophilous scrub vegetation (e.g., *Cistus* L., *Gymnosporia*, *Euphorbia* L.). However, detailed anatomical investigation of fossil material is required for correct phylogenetic placement (Anderson et al. 2009). Molecular phylogenetic reconstructions and the fossil record are congruent with a Canarian palaeo-flora adapted to semi-arid conditions, scattered over dry slopes and canyons (*barrancos*) in the Canary Islands during pre-mediterranean times. This would be followed by geographical expansion of the species when the climate became more favorable. Indeed, an evolutionary process in which lineages adapted to pre-mediterranean conditions in relatively small, xeric pockets became dominant when mediterranean-like conditions were expanded has already been proposed for plants of the Mediterranean Basin (Barrón et al. 2010, Vargas et al. 2018). The second and third hypotheses are congruent with a pattern of high differentiation into species in different vegetation belts and ecological shifts into thermophilous conditions. This includes some speciation events associated with colonization of the thermophilous woodland, as

documented in most of the evolutionary radiations of Canarian plants (e.g., *Sideritis* lineage, *Echium* lineage, *Euphorbia* sect. *Aphyllis* subsect. *Macaronesicae*, *Crambe* lineage, *Sonchus* lineage; see Supplementary Data Table S1). In any case, these three patterns of evolution could have been operating to ultimately merge into this particularly rich flora.

#### *Impact of extinction on the inferred temporal origin of the thermophilous lineages*

The time of origin (mediterranean vs. pre-mediterranean) of 25 out of 43 thermophilous lineages was successfully assigned using the approach proposed in this study, whereas 18 of them remained undetermined due to long temporal gaps between stem and crown ages spanning the 2.8 Ma threshold. These gaps entail a high uncertainty for the inference of colonization times (Cano et al. 2018, García-Verdugo et al. 2019a). The most striking case among those analyzed herein is *Dracunculus canariensis*, for which the difference between stem and crown ages was around 10 million years. An extreme case obtained from the literature is *Visnea mocanera*, which shows a difference between stem and crown ages of ca. 25 million years. Long stem-to-crown intervals have been previously found for other Macaronesian groups (e.g., *Cicer* L., *Campylanthus* Roth), an observation related to high extinction rates by recent studies (Antonelli and Sanmartín 2011, Nagalingum et al. 2011, Pokorny et al. 2015).

High extinction rates in mainland ancestral lineages as a result of abrupt climatic and geological changes (e.g., formation of the Sahara desert, Pleistocene climatic oscillations) has been the most commonly accepted explanation for the temporal gaps between stem and crown ages of Macaronesian lineages (Thiv et al. 2010, Kondraskov et al. 2015). Indeed, the Rand flora (i.e., lineages that exhibit a Canarian-eastern African disjunction) is primarily explained by widespread extinction of central-western Africa lineages rather than long-distance dispersal (Mairal et al. 2015, Pokorny et al. 2015). The



extinction of lineages across the mainland may have been more pronounced in Canarian lineages of older temporal origin (i.e. pre-mediterranean and undetermined). Potential extinction coupled with the spatial uncertainty of the geographical boundaries of the MFR in the past (Suc 1984, Suc et al. 2018) makes the reconstruction of ancestral areas a suboptimal approach to evaluate the Mediterranean origin of the Canarian thermophilous species, and their results thus should be taken with caution.

García-Verdugo et al. (2019a) proposed that crown ages may be a more suitable measurement for the time of island colonization than stem ages based on the idea that stem ages are subject to higher temporal and spatial uncertainty as a result of mainland extinction. However, the analysis of crown ages suffers from the same problem of extinction and under-sampling, thus biasing the results to more recent times. The effect of island extinction in crown age estimates may be particularly pronounced in the thermophilous woodland because of multiple causes: (i) erosion and subsidence of flat, oldest islands (Fuerteventura, Lanzarote), which may previously have harbored large areas with this vegetation type (Fernández-Palacios et al. 2008, Martín Osorio et al. 2011); (ii) geological dynamics (eruptions, earthquakes, mega-landslides) (Carracedo et al. 2001, García-Olivares et al. 2017); and most importantly (iii) human land use and destruction of original vegetation (only 11 % currently preserved) between 200 and 600 m.a.s.l. (Fernández-Palacios et al. 2008, Castilla-Beltrán et al. 2021). This extreme reduction of populations, continuing even in our lifetimes, makes thermophilous vegetation the most threatened ecosystem in the Canary Islands (Castilla-Beltrán et al. 2021). This is illustrated by the few remaining individuals of non-endemic species in the thermophilous vegetation belt (*Pistacia lentiscus*, *Ephedra fragilis*), many critically endangered species (e.g., *Anagyris latifolia*, *Crambe scoparia* Svent., *Cheirolophus duranii* (Burchard) Holub, *Dracaena tamaranae* Marrero Rodr., R.S.Almeira & M.González-Martin, *Echium handiense* Svent., *Gymnosporia cryptopetala*,

*Helianthemum gonzalezferreri* Marrero Rodr., *Helianthemum bramwelliorum* Marrero  
Rodr., *Solanum lidii* Sunding, *Solanum vespertilio* Aiton subsp. *vespertilio*, *Thesium*  
*retamoides*, *Thesium canariense* (Stearn) J.C.Manning & F.Forest) and even several  
species considered already extinct (e.g., *Helianthemum aguloi* Marrero Rodr. & R.Mesa,  
*Thesium psilotocladum* Svent.) (Moreno 2010).

## Conclusions

The approach proposed here, in which stem and crown ages are evaluated together with  
respect to a clear-cut threshold (2.8 Ma for the establishment of the mediterranean  
climate), helps test the hypotheses of temporal origins of evolutionary events even in  
scenarios with dramatic lineage extinction. In particular, our results provide strong  
evidence for a heterogeneous temporal origin of the thermophilous woodland in the  
Canary Islands, which harbors elements of both recent (mediterranean) and ancient  
Tethyan-Tertiary (pre-mediterranean) origins (Table 2). In addition, several of these  
colonisation events do not show ancestral areas in the Mediterranean Basin, even in some  
lineages postdating the establishment of the Mediterranean climate (e.g. *Asparagus*  
lineage I) (Figs. 4-6). This suggests a sequential history of species colonization and  
assemblage in the current thermophilous plant community of the Canaries, including a  
previously underestimated pre-mediterranean origin. A similar pattern has been identified  
for the laurel forest and xerophytic shrubland (Kondraskov et al. 2015, Sun et al. 2016,  
Salvo et al. 2010). Given that the thermophilous vegetation contains the highest number  
of lineages previously considered of mediterranean origin (Rivas-Martínez 2009, del  
Arco Aguilar and Rodríguez-Delgado 2018; but see Bolòs 1996), we hypothesize that the  
other five main vegetation belts contain an even lower number of mediterranean-like  
lineages (Vargas 2020). If this hypothesis was confirmed, the long-lasting view of  
Macaronesia as a subregion within the MFR would be seriously challenged. Different

temporal and geographical origins resulted in a great deal of diversity of the Canarian flora in general, and the thermophilous plant community in particular. Interestingly, this is the most threatened Canarian vegetation belt (Fernández-Palacios et al. 2008; del Arco Aguilar and Rodríguez-Delgado 2018). The endangered status of a few relictual patches requires urgent prioritization for conservation and restoration at the regional, national and international levels.

#### SUPPLEMENTARY DATA

Supplementary data are available at <https://academic.oup.com/aob> and consist of the following. Figs. S1-S13. Majority rule consensus trees of the 13 plant groups analysed in this study resulting from applying Bayesian Inference in MrBayes and using previously published phylogenetic datasets (see Table 1) in combination with newly generated sequences (see Table S2). Figs. S14-S26. Time-calibrated phylogenies of the 13 plant groups analysed in this study obtained in BEAST using previously published phylogenetic datasets (see Table 1) in combination with newly generated sequences (see Table S2). Figs. S27-S39. Biogeographic reconstructions of ancestral ranges of the 13 plant groups analysed in this study resulting from applying dispersal–extinction–cladogenesis (DEC) analyses using the BioGeoBEARS R package. Table S1. Information extracted from the literature and obtained in this study for the 43 plant lineages including thermophilous species from the Canary Islands (Macaronesia) for hypothesis testing. Table S2. Studied taxa and their corresponding collection code, voucher information, island (archipelado or country), locality, collection date, collector's name (leg), DNA sequenced regions and GenBank accession numbers. Table S3. Primers and PCR cycles used for sequencing DNA regions of the 13 plant groups analyzed in this study (genera, seldom families). Table S4. Detailed information (phylogenetic relationships and colonization times) of the 16 lineages with thermophilous species recovered by BEAST

analyses of the 13 plant groups of this study. Table S5. Results of the ancestral area reconstruction under the dispersal-extinction-cladogenesis (DEC) analyses performed on the 13 newly generated time-calibrated phylogenies including thermophilous species in the Canary Islands analysed in this study. Methods S1. Details of the maximum likelihood phylogenetic analyses performed in this study.

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1    **Table 1.** List of the 16 Canarian thermophilous plant species for which colonization times were newly-inferred for this study. Taxonomic rank, DNA  
2    regions and bibliographic references of the DNA sequence datasets used are detailed, as well as calibration approach and bibliographic reference on  
3    which the strategy for calculating divergence times is based. The last column indicates the reasons why the original datasets had to be improved,  
4    namely: (1) low support for monophyletic groups formed by Canarian species; (2) poor sampling of potential mainland sister groups  
5    (species/populations); (3) poor sampling of species or populations from the Canary Islands; (4) lack of divergence-time estimations; and (5) need for  
6    technical improvement in terms of new methods or new calibration points. DNA regions marked in bold represent the regions for which we have  
7    provided new sequences in this study (more details on the samples included by genus and species in Table S2). \* Macaronesian endemics; \*\* Canarian  
8    endemics. Species with no asterisks are non-endemic natives.

Canarian thermophilous species	Plant group analyzed	DNA regions from published phylogenies	Reference for published DNA sequence dataset	Calibration approach	Reference for calibration points	Reasons for dataset improvement
<i>Asparagus scoparius</i> Lowe*	Genus <i>Asparagus</i> Tourn. ex L. (Asparagaceae)	<b>PHYC, <i>trnH-psbA</i>, <i>trnD-T</i>, <i>ndhF</i></b>	Norup et al. 2015	Secondary calibration (Fig. S14)	Chen et al. 2013	1, 3, 4
<i>Asparagus umbellatus</i> Link*						
<i>Bosea yervamora</i> L.**	Family Amaranthaceae	<b><i>matK</i></b>	Di Vincenzo et al. 2017	Fossil and secondary calibration (Fig. S15)	Di Vincenzo et al. 2017	2, 3
<i>Bryonia verrucosa</i> Aiton**	Genus <i>Bryonia</i> L. (Cucurbitaceae)	ITS, LFY, <i>trnL</i> , <i>trnL-trnF</i> , <i>psbA-trnH</i> , <i>trnH2</i> , <i>trnR-<i>atpA</i></i>	Volz and Renner 2008	Secondary calibration (Fig. S16)	Schaefer et al. 2009	5
<i>Chrysojasminum odoratissimum</i> (L.) Banfi*	Genus <i>Chrysojasminum</i> Banfi (Oleaceae)	<b>ITS, <i>matK</i>, <i>trnL-F</i> and <i>trnH-psbA</i></b>	Jeyarani et al. 2018	Secondary calibration (Fig. S17)	Vargas et al. 2014	2, 3, 4
<i>Dracunculus canariensis</i> Kunth *	Genus <i>Arum</i> L. (Araceae)	<i>trnL</i> , <i>trnF</i> , <b><i>matK</i>, <i>trnK</i>, <i>rbcL</i>, <i>rps16</i></b>	Mansion et al. 2008	Fossil and secondary calibration (Fig. S18)	Mansion et al. 2008, Nauheimer et al. 2012	3, 5
<i>Ephedra fragilis</i> Desf.	Genus <i>Ephedra</i> Tourn. ex L. (Ephedraceae)	26S, 18S, <b>ITS, <i>atpB</i>, <i>rbcL</i>, <i>matK</i>, <i>rps4</i>, <i>psbA-trnH</i>, <i>trnL</i>, <i>trnL-trnF</i></b>	Ickert-Bond et al. 2009	Fossil calibration (Fig. S19)	Ickert-Bond et al. 2009	2, 3
<i>Gymnosporia cryptopetala</i> Reyes-Bet. & A.Santos**	Genus <i>Gymnosporia</i> (Wight & Arn.) Hook.f. (Celastraceae)	<b>ITS</b>	Oberprieler et al. 2017	Secondary calibration (Fig. S20)	Davis et al. 2005	3, 5
<i>Gymnosporia cassinoides</i> (L'Hér.) Masf. **						
<i>Juniperus turbinata</i> subsp. <i>canariensis</i> (Guyot & Mathou) Rivas Mart., Wildpret & P.Pérez	Genus <i>Juniperus</i> L. (Cupressaceae)	<b>ITS, <i>matK</i>, <i>rbcL</i>, <i>trnL-trnF</i>, <i>rps4</i>, <i>trnS-trnG</i>, <i>trnD-trnT</i>, <i>trnV</i>, <i>petB-petD</i> and <i>psbB1-psbB2</i></b>	Mao et al. 2010	Fossil calibration (Fig. S21)	Mao et al. 2010	2, 3
<i>Myrsine excelsa</i> D.Don*	Genus <i>Myrsine</i> L. (Primulaceae)	<b>ITS, ETS</b>	Appelhans et al. 2020	Secondary calibration (Fig. S22)	Rose et al. 2018	3, 4
<i>Pistacia atlantica</i> Desf.	Genus <i>Pistacia</i> L. (Anacardiaceae)	<b>ITS, ETS</b>	Xie et al. 2014	Secondary calibration (Fig. S23)	Xie et al. 2014	2, 3
<i>Pistacia lentiscus</i> L.						
<i>Rhamnus crenulata</i> Aiton **	Genus <i>Rhamnus</i> L. (Rhamnaceae)	<b>ITS, <i>trnL-trnF</i></b>	Bolmgren and Oxelman 2004	Secondary calibration (Fig. S24)	Onstein et al. 2015	2, 3
<i>Sideroxylon canariense</i> Leyens, Lobin & A.Santos**	Genus <i>Sideroxylon</i> L. (Sapotaceae)	<b>ITS, <i>trnH-psbA</i></b>	Stride et al. 2014	Fossil calibration (Fig. S25)	Stride et al. 2014	3
<i>Thesium retamoides</i> (A.Santos) J.C.Manning & F.Forest**	Genus <i>Thesium</i> L. (Santalaceae)	<b>ITS, <i>matK</i>, <i>rpl32-trnL</i>, <i>trnL-trnF</i></b>	Zhigila et al. 2020	Secondary calibration (Fig. S26)	Moore et al. 2010	3, 4

- 1 **Table 2.** List of mediterranean, pre-mediterranean and undetermined lineages (i.e. colonization events) based on the position of stem and crown
- 2 ages with respect to the 2.8-million-year-threshold (i.e. establishment of the Mediterranean climate) of the complete list of 43 lineages

Mediterranean	Pre-mediterranean	Undetermined
<i>Argyranthemum</i> lineage	<i>Chrysojasminum odoratissimum</i>	<i>Anagyris latifolia</i>
<i>Asparagus</i> lineage I	<i>Crambe</i> lineage	<i>Artemisia</i> lineage
<i>Asparagus</i> lineage II	<i>Echium</i> lineage	<i>Bosea yervamora</i>
<i>Brachypodium arbuscula</i>	<i>Euphorbia</i> sect. <i>Aphyllis</i>	<i>Bryonia verrucosa</i>
<i>Cistus monspeliensis</i>	<i>Ruta</i> lineage	<i>Cheirolophus</i> lineage
<i>Convolvulus</i> lineage II	<i>Sideritis</i> lineage	<i>Dioscorea edulis</i>
<i>Ephedra fragilis</i>	<i>Sideroxylon canariense</i>	<i>Dracaena</i> lineage
<i>Erysimum</i> lineage	<i>Sonchus</i> lineage	<i>Dracunculus canariensis</i>
<i>Globularia</i> lineage	<i>Thesium</i> lineage	<i>Gonospermum</i> lineage
<i>Helianthemum</i> lineage		<i>Gymnosporia</i> lineage
<i>Malva canariensis</i>		<i>Hypericum canariense</i>
<i>Olea europaea</i> subsp. <i>guanchica</i>		<i>Juniperus turbinata</i> subsp. <i>canariensis</i>
<i>Pistacia atlantica</i>		<i>Myrsine excelsa</i>
<i>Pistacia lentiscus</i> lineage II		<i>Navaea phoenicea</i>
<i>Smilax aspera</i>		<i>Pistacia lentiscus</i> lineage I
<i>Solanum</i> lineage		<i>Rhamnus crenulata</i>
		<i>Rubia fruticosa</i>
		<i>Visnea mocanera</i>

## FIGURE CAPTIONS

**Fig. 1.** Two approaches typically used to infer colonization times on oceanic island: stem and crown ages. Brown lines represent lineages with mainland distribution while green lines represent lineages with insular distribution. **A.** Graphical representation of stem and crown ages of a hypothetical island lineage (modified from García-Verdugo et al. 2019a). **B.** Impact of assumed extinction or incomplete taxon sampling on the mainland when inferring island colonization times based on stem-age estimates. **C.** Impact of assumed extinction or incomplete taxon sampling on the archipelago when inferring island colonization times based on crown-age estimates.

**Fig. 2.** Classification of lineages according to divergence times for the Canarian thermophilous plant community, following the methodology proposed in this paper: (1) mediterranean lineages when stem and thus crown ages postdate the 2.8 Ma threshold; (2) pre-mediterranean lineages when crown and thus stem ages predate the 2.8 Ma threshold; and (3) undetermined lineages for those groups with stem ages before and crown ages after this temporal threshold. In these examples, 95% intervals do not span the 2.8 Ma threshold, and therefore nodes can be unambiguously designated as mediterranean or pre-mediterranean.

**Fig. 3.** Assignment of mediterranean vs. pre-mediterranean nodes when the 95% HPD interval spans the 2.8 Ma threshold: (1) mediterranean nodes, when most of the posterior distribution of trees (> 50 %) provide node ages younger than 2.8 Ma, (2) pre-mediterranean nodes when most of the trees (> 50 %) provide node ages older than 2.8 Ma.

**Fig. 4.** The five thermophilous lineages of mediterranean origin (stem and therefore crown ages postdate 2.8 Ma) and related Canarian and mainland species from the 13 time-calibrated phylogenies inferred in this study using BEAST. Mean stem and crown ages

and 95% posterior credibility intervals (blue bars and values in brackets) are indicated. Divergence times of the most recent common ancestor (marked as TMRCA) are also indicated for those groups in which stem and crown nodes showed low phylogenetic support (PP<0.90, BS<70). Circles at the nodes represent phylogenetic relationships with high posterior Bayesian probability (PP) and/or bootstrap (BS) support values (see legend). Color of plant names indicates vegetation type. Asterisks represent new samples included in the present study. The letters in the stem node or MRCA of the Canarian lineages represent the ancestral ranges inferred for that node by the Ancestral Area Reconstruction Analysis, and correspond to the following geographical areas: A: Mediterranean Basin, B: Macaronesia, C: Other regions (several letters imply ranges that include multiple areas).

**Fig. 5.** The three pre-mediterranean lineages (i.e. crown and thus stem ages predate 2.8 Ma) and related Canarian and mainland species from the 13 time-calibrated phylogenies inferred in this study using BEAST. Mean stem and crown ages and 95% posterior credibility intervals (blue bars and values in brackets) are indicated next to the corresponding nodes. The circles at the nodes represent phylogenetic relationships with high posterior Bayesian probability (PP) and/or bootstrap (BS) support values (see legend). Color of plant names indicates vegetation type. Asterisks represent new samples included in the present study. The letters in the stem node of the Canarian lineages represent the ancestral ranges inferred for that node by the Ancestral Area Reconstruction Analysis, and correspond to the following geographical areas: A: Mediterranean Basin, B: Macaronesia, C: Other regions (several letters imply ranges that include multiple areas).

**Fig. 6.** The seven undetermined lineages (stem ages predating and crown ages postdating 2.8 Ma) and related Canarian and mainland species for the 13 time-calibrated phylogenies



inferred in this study using BEAST. Mean stem and crown ages and 95% posterior credibility intervals (blue bars and values in brackets) are indicated next to the corresponding nodes. Divergence times of the most recent common ancestor (marked as TMRCA) are indicated for those groups in which stem and crown nodes showed low phylogenetic support (PP<0.90, BS<70). The circles at the nodes represent phylogenetic relationships with high posterior Bayesian probability (PP) and/or bootstrap (BS) support values (see legend). Color of plant names indicates vegetation type. Asterisks represent new samples included in the present study. The letters in the stem node or MRCA of the Canarian lineages represent the ancestral ranges inferred for that node by the Ancestral Area Reconstruction Analysis, and correspond to the following geographical areas: A: Mediterranean Basin, B: Macaronesia, C: Other regions (several letters imply ranges that include multiple areas).

**Fig. 7.** Stem ages of 43 Canarian lineages including thermophilous plant species. Results from the new phylogenies obtained in this study are indicated with plant names in bold (references in Table S1). Blue bars show the 95% intervals, and mean stem ages are marked by a black line.

**Fig. 8.** Twenty-eight crown ages of Canarian thermophilous plant lineages (i.e., including more than one sample from the Canaries) of the 43 shown in Fig. 7. Results from the new phylogenies obtained in this study are indicated with plant names in bold, while results from previously published time-calibrated phylogenies have non-bold plant names (references in Table S1). Blue bars show the 95% intervals, and mean crown ages are marked by a black line.