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

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- **Background and Aims** The Canary Islands have strong floristic affinities with the Mediterranean Basin. One of the most characteristic and diverse vegetation belts of the archipelago is the thermophilous woodland (between 200 and 900 m.a.s.l.). 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 an 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 new 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 source areas. A substantial proportion of the lineages arrived in the Canaries before the summer-dry climate was established in the Mediterranean Basin. The complex temporal and geographic origin of Canarian thermophilous species challenges the view of the Canary Islands (and Madeira) as a subregion within the Mediterranean Floristic Region.

Key words: Thermophilous woodland, Canary Islands, Mediterranean Floristic Region, 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 ~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 and Rodríguez-Delgado, 2018). Interestingly, the 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 or a subregion within the MFR (del Arco and Rodríguez-Delgado, 2018). On the one hand, high species endemicity (~35 %) and numerous floristic elements linked to several continental floras are considered strong arguments to accept a high-level phytogeographic classification (Macaronesian Floristic Region; Engler, 1879; Sunding, 1979; Takhtajan, 1986; Bolòs, 1996). 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 phytogeographic classification (Canarian–Madeiran subregion of the MFR; Meusel, 1965; Lobin, 1982; Rivas-Martínez, 2009; del Arco and Rodríguez-Delgado, 2018). 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 centres of diversity in the Mediterranean Basin are particularly abundant in the pine woodland and 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*), ‘almacigares’ (*Pistacia atlantica*), ‘lenticales’ (*Pistacia lentiscus*) and ‘acebuchales’ (*Olea europaea* subsp. *guanchica*) (Fernández-Palacios *et al.*, 2008; Nezádal 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 the phytogeographic 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 *et al.*, 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) information that supports 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 boundary between the Pliocene and Pleistocene (2.6 Ma), as recognized by the International Chronostratigraphic Chart (<https://stratigraphy.org/chart>). Such a temporal threshold has been used in multiple studies to categorize Mediterranean floristic elements according to their temporal origin (paleo-mediterranean versus neo-mediterranean; Herrera, 1992; Peñuelas *et al.*, 2001; Verdú *et al.*, 2003). In addition, it has been used to test specific hypotheses such as the timing of colonization 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 posterior 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 (e.g. Pokorný *et al.*, 2015; Vargas *et al.*, 2018). The time-calibrated phylogenies obtained from Bayesian relaxed-clock methods are also used as input for inferring the ancestral areas from which colonization may have occurred (e.g. Albaladejo *et al.*, 2021). These ancestral area reconstruction analyses give new possibilities for quantitative analysis in island biogeography (Lamm and Redelings, 2009). Thus, applying time-calibrated phylogenetic approaches to investigate the divergence of Canarian lineages from their continental relatives may provide valuable insight into the very complex patterns of relationships between the Canary Islands and continental floras (Carine *et al.*, 2004; Kondrakov *et al.*, 2015; Caujapé-Castells *et al.*, 2017; Valente *et al.*, 2017).

Palaeoclimatic, floristic and phytogeographic studies led us to examine the hypothesis of an origin of Canarian thermophilous species following the establishment of the mediterranean climate across the Mediterranean Basin 2.8 Ma. To test this hypothesis, we firstly analysed the phylogenetic relationships of thermophilous Canarian lineages and their sister groups using improved phylogenetic datasets in terms of extended taxonomic and geographic sampling. Secondly, we evaluated whether ancestral thermophilous plant lineages had a temporal origin since the mediterranean climate became established around 2.8 Ma (Suc *et al.*, 2018), based on both newly generated and previously published time-calibrated phylogenies. Thirdly, we implemented ancestral area reconstruction analyses to assess whether colonization of thermophilous Canarian lineages had a potential source area for colonization in the Mediterranean Basin or another geographic area. In sum, we are quantifying the degree to which there is a spatio-temporal biogeographic link between the Canarian flora and the MFR.

MATERIALS AND METHODS

Study area

The thermophilous woodland is a vegetation type characteristic of the Canary Islands. It occurs between the xerophytic vegetation and the laurel forest on the windward slopes (~200–500 m.a.s.l.) and between the xerophytic vegetation and the pine woodland on the leeward slopes (~300–900 m.a.s.l.) (del Arco *et al.*, 2006; Fernández-Palacios *et al.*, 2008). It has a mediterranean-type climate 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 southern Europe and northern Africa (particularly *Juniperus turbinata*, *Pistacia atlantica*, *Pistacia lentiscus*, *Olea europaea*), which may be accompanied by endemic trees (*Gymnosporia cassinoides*, *Dracaena draco*, *Phoenix canariensis*, *Sideroxylon canariense*, *Visnea mocanera*). 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 islands, who mostly settled around 2000 years ago, and a more severe deforestation has taken 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 (7 Canarian endemics, 5 Macaronesian endemics, 4 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 estimates; 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 taken directly from the literature (Supplementary Data Table S1). A total of 39 thermophilous plant groups (i.e. genera, rarely family) were analysed.

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 provided directly 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 these 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 being made for Mediterranean Iberia and north-western 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 in 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 the Qiagen DNeasy Plant Mini Kit according to the manufacturer's protocol. Sixteen different DNA regions were amplified and sequenced based on previously published studies for each plant group (Supplementary Data Table S2). In particular, we analysed two nuclear regions (ETS and ITS), one mitochondrial region (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). We used Geneious 2021.2.2 (<https://www.geneious.com>) to edit chromatograms, aligned sequences with the MUSCLE algorithm (Edgar, 2004) and concatenated 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, Supplementary Data 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

TABLE 1. List of the 16 Canarian thermophilous plant species for which colonization times were newly inferred for this study. Taxonomic rank, DNA regions and bibliographic references of the DNA sequence datasets used are indicated, as well as the calibrated approach and the bibliographic reference on which the strategy for calculating divergence times is based on. The last column indicates the reasons why the original datasets had to be improved, namely: (1) low support for monophyletic groups formed by 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 estimates; and (5) need for technical improvement in terms of new methods or new calibration points. DNA regions marked in bold indicate the regions for which we have provided new sequences in this study (more details on the samples included by genus and species in *Supplementary Data Table S2*)

Canarian thermophilous species	Plant group analysed	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> *	Genus <i>Asparagus</i> (Asparagaceae)	PHYC, trnH-psbA, trnD-T, ndhF	Norup <i>et al.</i> , 2015	Secondary calibration (Fig. S14)	Chen <i>et al.</i> , 2013	1, 3, 4
<i>Asparagus umbellatus</i> *						
<i>Bosea yervamora</i> **	Family Amaranthaceae	matK	Di Vincenzo <i>et al.</i> , 2017	Fossil and secondary calibration (Fig. S15)	Di Vincenzo <i>et al.</i> , 2017	2, 3
<i>Bryonia verrucosa</i> **	Genus <i>Bryonia</i> (Cucurbitaceae)	ITS, LFY, trnL, trnL-trnF, psbA-trnH, trnH2, trnR-atpA	Volz and Renner, 2008	Secondary calibration (Fig. S16)	Schaefer <i>et al.</i> , 2009	5
<i>Chrysojasminum odoratissimum</i> *	Genus <i>Chrysojasminum</i> (Oleaceae)	ITS, matK, trnL-F and trnH-psbA	Jeyarani <i>et al.</i> , 2018	Secondary calibration (Fig. S17)	Vargas <i>et al.</i> , 2014	2, 3, 4
<i>Dracunculus canariensis</i> *	Genus <i>Arum</i> (Araceae)	trnL, trnF, matK, trnK, rbcL, rps16	Mansion <i>et al.</i> , 2008	Fossil and secondary calibration (Fig. S18)	Mansion <i>et al.</i> , 2008; Nauheimer <i>et al.</i> , 2012	3, 5
<i>Ephedra fragilis</i>	Genus <i>Ephedra</i> (Ephedraceae)	26S, 18S, ITS, atpB, rbcL, matK, rps4, psbA-trnH, trnL, trnL-trnF	Ickert-Bond <i>et al.</i> , 2009	Fossil calibration (Fig. S19)	Ickert-Bond <i>et al.</i> , 2009	2, 3
<i>Gymnosporia cryptopetala</i> **	Genus <i>Gymnosporia</i> (Celastraceae)	ITS	Oberprieler <i>et al.</i> , 2017	Secondary calibration (Fig. S20)	Davis <i>et al.</i> , 2005	3, 5
<i>Gymnosporia cassinoides</i> **						
<i>Juniperus turbinata</i> subsp. <i>canariensis</i>	Genus <i>Juniperus</i> (Cupressaceae)	ITS, matK, rbcL, trnL-trnF, rps4, trnS-trnG, trnD-trnT, trnV, petB-petD and psbB1-psbB2	Mao <i>et al.</i> , 2010	Fossil calibration (Fig. S21)	Mao <i>et al.</i> , 2010	2, 3
<i>Heberdenia excelsa</i> *	Genus <i>Myrsine</i> (Primulaceae)	ITS, ETS	Appelhans <i>et al.</i> , 2020	Secondary calibration (Fig. S22)	Rose <i>et al.</i> , 2018	3, 4
<i>Pistacia atlantica</i>	Genus <i>Pistacia</i> (Anacardiaceae)	ITS, ETS	Xie <i>et al.</i> , 2014	Secondary calibration (Fig. S23)	Xie <i>et al.</i> , 2014	2, 3
<i>Pistacia lentiscus</i>						
<i>Rhamnus crenulata</i> **	Genus <i>Rhamnus</i> (Rhamnaceae)	ITS, trnL-trnF	Bolmgren and Oxelman, 2004	Secondary calibration (Fig. S24)	Onstein <i>et al.</i> , 2015	2, 3
<i>Sideroxylon canariense</i> **	Genus <i>Sideroxylon</i> (Sapotaceae)	ITS, trnH-psbA	Stride <i>et al.</i> , 2014	Fossil calibration (Fig. S25)	Stride <i>et al.</i> , 2014	3
<i>Thesium retamoides</i> **	Genus <i>Thesium</i> (Santalaceae)	ITS, matK, rpl32-trnL, trnL-trnF	Zhigila <i>et al.</i> , 2020	Secondary calibration (Fig. S26)	Moore <i>et al.</i> , 2010	3, 4

*Macaronesian endemics; **Canarian endemics. Species with no asterisks are non-endemic natives.

additional approach using maximum likelihood was implemented in RAxML to obtain an alternative estimate of phylogenetic relationships and support values (*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), 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

Colonizations 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 more 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 have used 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 minimum time for the colonization from the mainland (Fig. 1A; Pokorný *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 Canary 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 the mediterranean climate (Suc, 1984). By considering the stem node as the upper limit and the crown node as the 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 Canary 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’ refers 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 Canary 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 first 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 section).

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 the posterior probability (PP) of nodes is low (<0.90), or when multiple representatives of Canary lineages are placed at distant positions in the tree with low support. These reconstructions 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

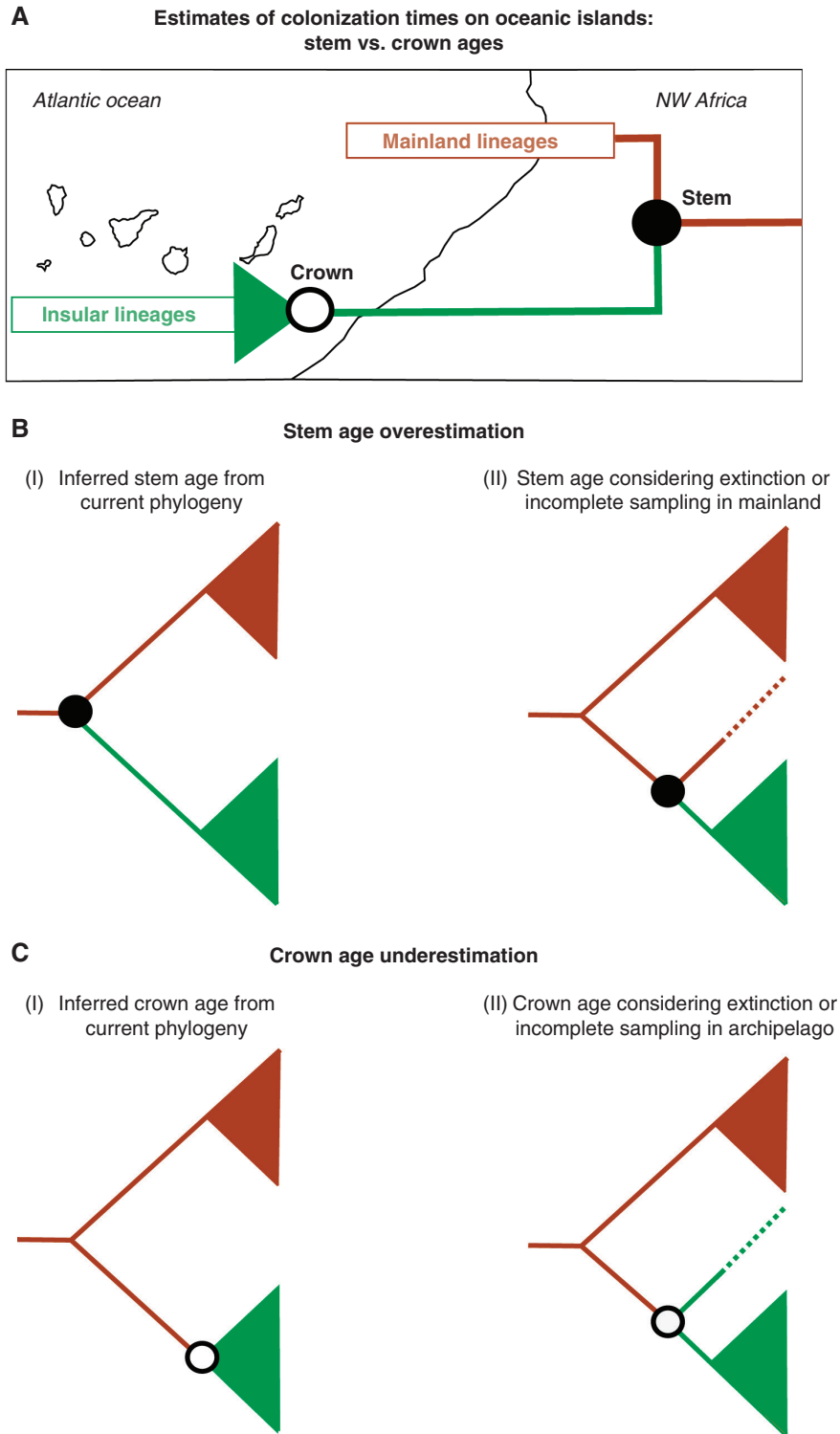


FIG. 1. Two approaches typically used to infer colonization times on oceanic island: stem (solid circles) and crown (open circles) ages. Brown lines represent lineages with mainland distribution while green lines represent lineages with insular distribution. Dashed lines represent extinct or unsampled lineages. (A) Graphical representation of stem and crown ages of a hypothetical island lineage (modified from García-Verdugo *et al.*, 2019a). (B) Impact of considering extinction or incomplete taxon sampling of the mainland ancestor on stem age estimates. (C) Impact of considering extinction or incomplete taxon sampling of the archipelago ancestor on crown age estimates.

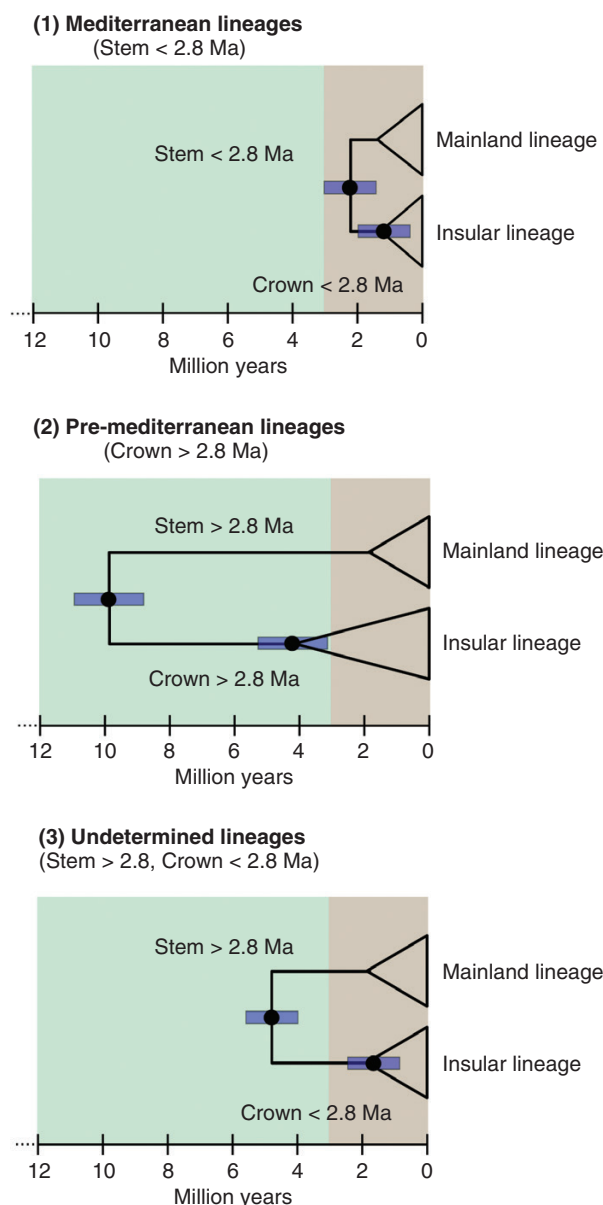


FIG. 2. Classification of lineages according to divergence times for the Canary thermophilous plant community, following the methodology proposed in this paper. (1) Mediterranean lineages when stem and thus crown ages postdate the 2.8-Ma threshold; (2) pre-mediterranean lineages when crown and thus stem 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. For these cases, 95 % posterior credibility intervals (blue bars) do not span the 2.8-Ma threshold, and therefore nodes can be unambiguously designated as mediterranean or pre-mediterranean.

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-endemic 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, biogeographic 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 symmetrical 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 colonization 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 for the TMRCA when the PP of stem nodes was <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 (Supplementary Data Figs S1–S13) and maximum likelihood using RAXML (results not shown) are in accordance with previously 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 (Supplementary Data Figs S14–S26). For this reason, we selected the phylogenetic trees obtained in BEAST to describe the phylogenetic relationships and the statistical support values of the nodes.

Most of the thermophilous plants analysed formed well-supported monophyletic species (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*, *Juniperus turbinata*, *Pistacia atlantica*, *Pistacia lentiscus*) as well as the endemics *Asparagus umbellatus* and *Thesium retamoides* (Figs 4A–E, 5C and 6D, Supplementary Data Table S1). Similarly, sister-group relationships had high statistical support (>0.90 PP), except for the non-endemic species and for the Macaronesian endemic species *Dracunculus canariensis* (Figs 4A–E and 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: (1) placement of *Asparagus scoparius* within the clade of *Asparagus nesiotis* and *Asparagus plocamoides*, and placement of *Asparagus umbellatus* within the clade of *Asparagus fallax* and *Asparagus arborescens*; (2) close relationship of the two Canarian species of *Gymnosporia* (*G. cassinoides* and *G. cryptopetala*, which are sisters to the Madeira endemic *G. dryandrii*); (3) *Chrysojasminum odoratissimum*, sister to the mainland clade of *Chrysojasminum parkeri*, *Chrysojasminum humile*, *Chrysojasminum bignoniaceum* and *Chrysojasminum*

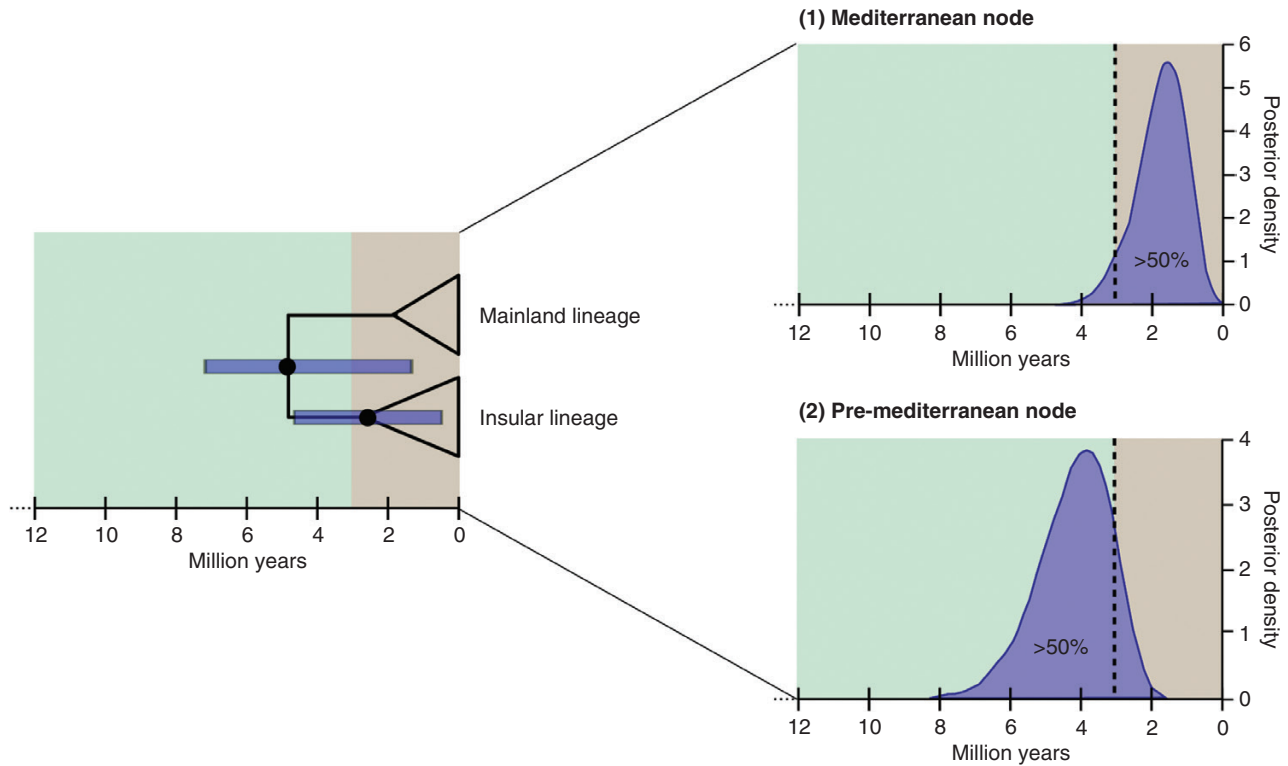


FIG. 3. Assignment of mediterranean versus pre-mediterranean nodes when the 95 % posterior credibility interval (blue bars) spans the 2.8-Ma threshold: (1) mediterranean nodes, when most of the posterior distribution of trees (>50 %) provide node ages younger than 2.8 Ma; and (2) pre-mediterranean nodes, when most of the trees (>50 %) provide node ages older than 2.8 Ma.

fruticans; and (4) the two Canarian species of *Thesium* (*Thesium retamoides* and *Thesium subsucculentum*) sister to the mainland *Thesium mauritanicum* (Figs 4–6).

As a result of our phylogenetic reconstructions, 16 independent Canary Island colonization events were inferred for the 13 plant groups with thermophilous species analysed: ten genera with a single colonization and two with more than one colonization (three of *Pistacia* and two of *Asparagus*) (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. nesioties* 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 or Macaronesian lineage (including thermophilous and non-thermophilous species).

Colonization times

The estimated mean crown ages of the 16 Canarian lineages newly analysed in this study ranged from 1.07 Ma within

Heberdenia excels 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, Supplementary Data 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).

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 node 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 (Ma) (Fig. 7): *Globularia* lineage

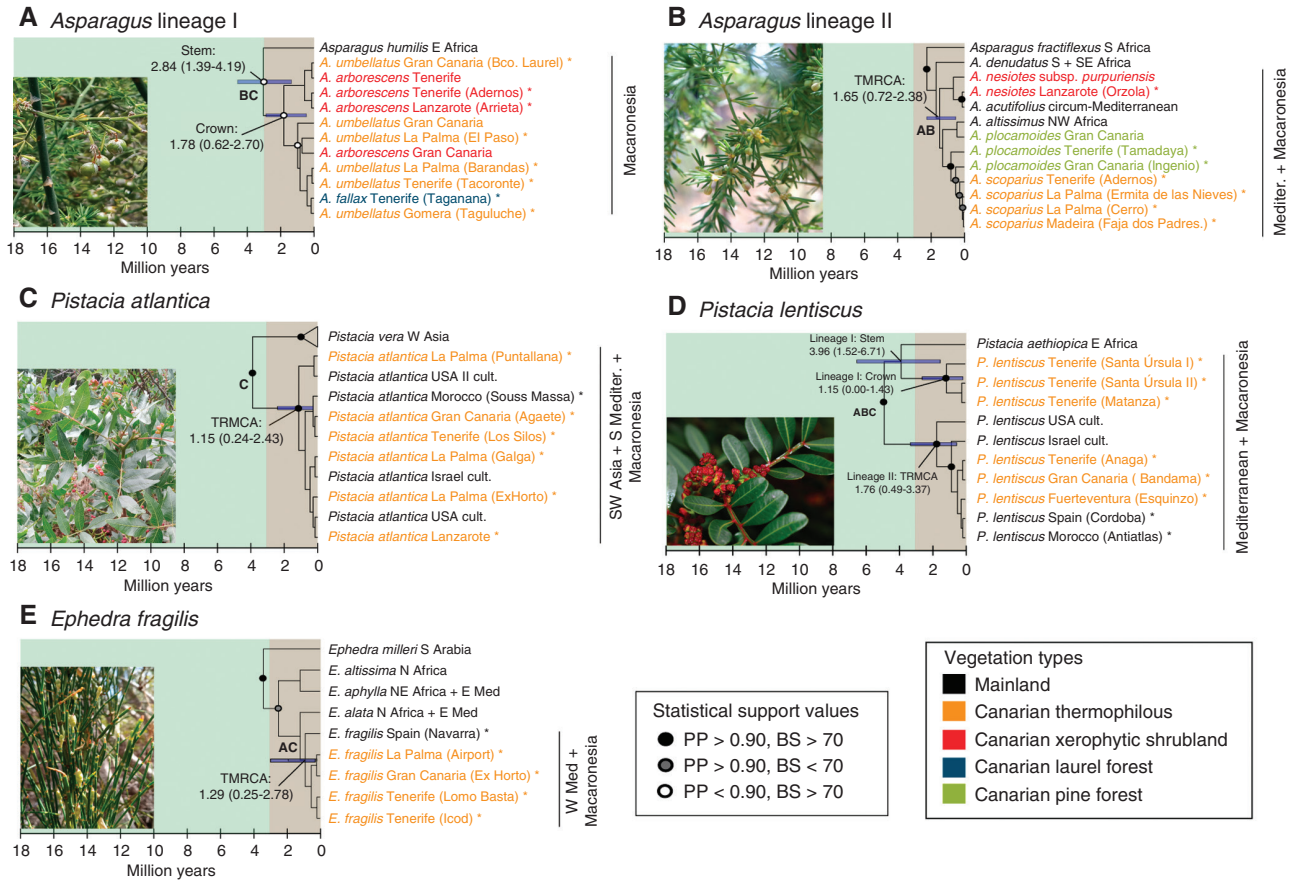


FIG. 4. The five thermophilous lineages of mediterranean origin (stem and therefore crown ages postdate 2.8 Ma) and related Canary and mainland species from the 13 time-calibrated phylogenies inferred in this study using BEAST. Mean stem and crown ages and 95 % posterior credibility intervals (blue bars 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, bootstrap (BS) support value < 70]. Circles at the nodes represent phylogenetic relationships with high posterior Bayesian probability (PP) and/or BS support values (see Statistical support values legend). Colour of plant names indicates vegetation type. Asterisks represent new samples included in the present study. The letters close to the stem node or TMRCA of the Canary lineages represent the ancestral ranges inferred for that node by the ancestral area reconstruction analysis and correspond to the following geographic areas: A, Mediterranean Basin; B, Macaronesia; C, other regions (several letters imply ranges that include multiple areas).

(0.30), *Smilax aspera* (0.30), *Cistus monspeliensis* (0.50), *Brachypodium arbuscula* (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* sect. *Helianthemum* (1.82), *Argyranthemum* lineage (2.20), *Olea europaea* subsp. *guanchica* (2.60), *Malva canariensis* (2.78) and *Asparagus* lineage I (2.80, Fig. 4A)]. Although the stem age estimates of *Asparagus* lineage I clearly overlaps the mediterranean threshold, >60 % of the stem age distribution and 96 % of crown age distribution fell within 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 *C. odoratissimum* and *S. 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 (Ma) (Fig. 8): *Sideritis* lineage (3.30), *Echium* lineage (3.70), *Thesium* lineage (3.86), *Chrysojasminum odoratissimum* (4.79), *Euphorbia* sect. *Aphyllis* subsect. *Macaronesicae* (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*, *Bryonia verrucosa*, *Dracunculus canariensis*, *Gymnosporia* lineage, *Heberdenia excelsa*, *Juniperus turbinata* subsp. *canariensis*, *Pistacia lentiscus* lineage I and *Rhamnus crenulata* (Fig. 6). Most of their stem and crown ages

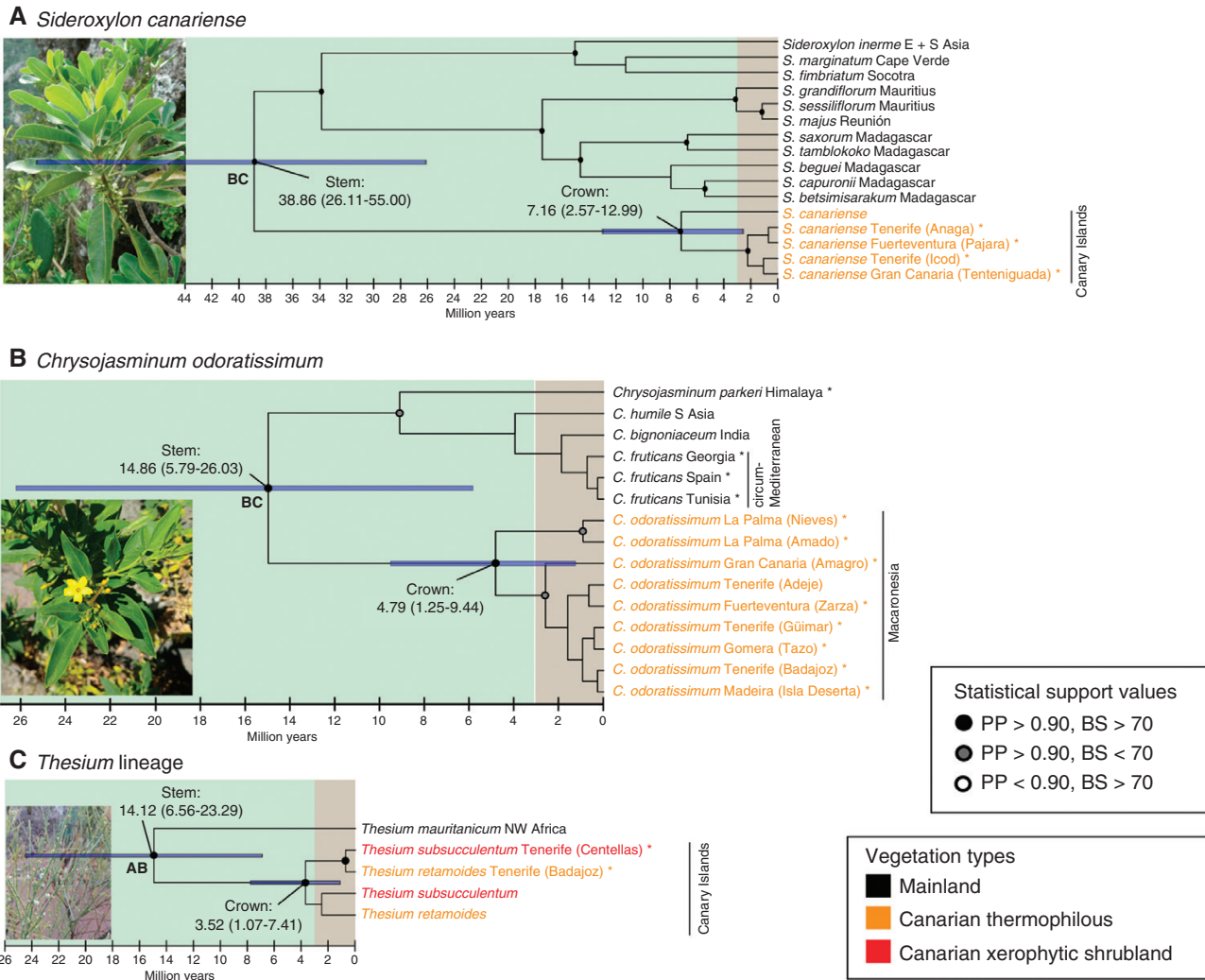


Fig. 5. The three pre-mediterranean lineages (i.e. crown and thus stem ages predate 2.8 Ma) and related Canarian and mainland species from the 13 time-calibrated phylogenies inferred in this study using BEAST. Mean stem and crown ages and 95 % posterior credibility intervals (blue bars and values in 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 Statistical support values legend). Colour of plant names indicates vegetation type. Asterisks represent new samples included in the present study. The letters close to 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 geographic areas: A, Mediterranean Basin; B, Macaronesia; C, other regions (several letters imply ranges that include multiple areas).

accumulated a marginal posterior distribution clearly before and after 2.8 Ma. The highest uncertainty was recorded in the crown node of *D. 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 ten undetermined lineages. In total, the following 18 lineages were classified as undetermined (Figs 7 and 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), *Heberdenia 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* (mean stem 6.69; mean crown 2.10), *Navaea phoenicea* (mean stem 6.77; mean crown unknown), *Anagyris latifolia* (mean stem 8.20; mean crown 1.90), *Cheirolophus* lineage (mean stem 8.50; mean crown 1.70), *Hypericum canariense* (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* (mean stem 13.48; mean crown unknown), and *Visnea mocanera* (mean stem 27.00; mean crown 2.50).

Ancestral area reconstructions

Biogeographic analyses supported nine lineages with stem node ranges including the Mediterranean Basin (i.e. *Asparagus* lineage II, *Bosea yervamora*, *Bryonia verrucosa*, *Dracunculus*

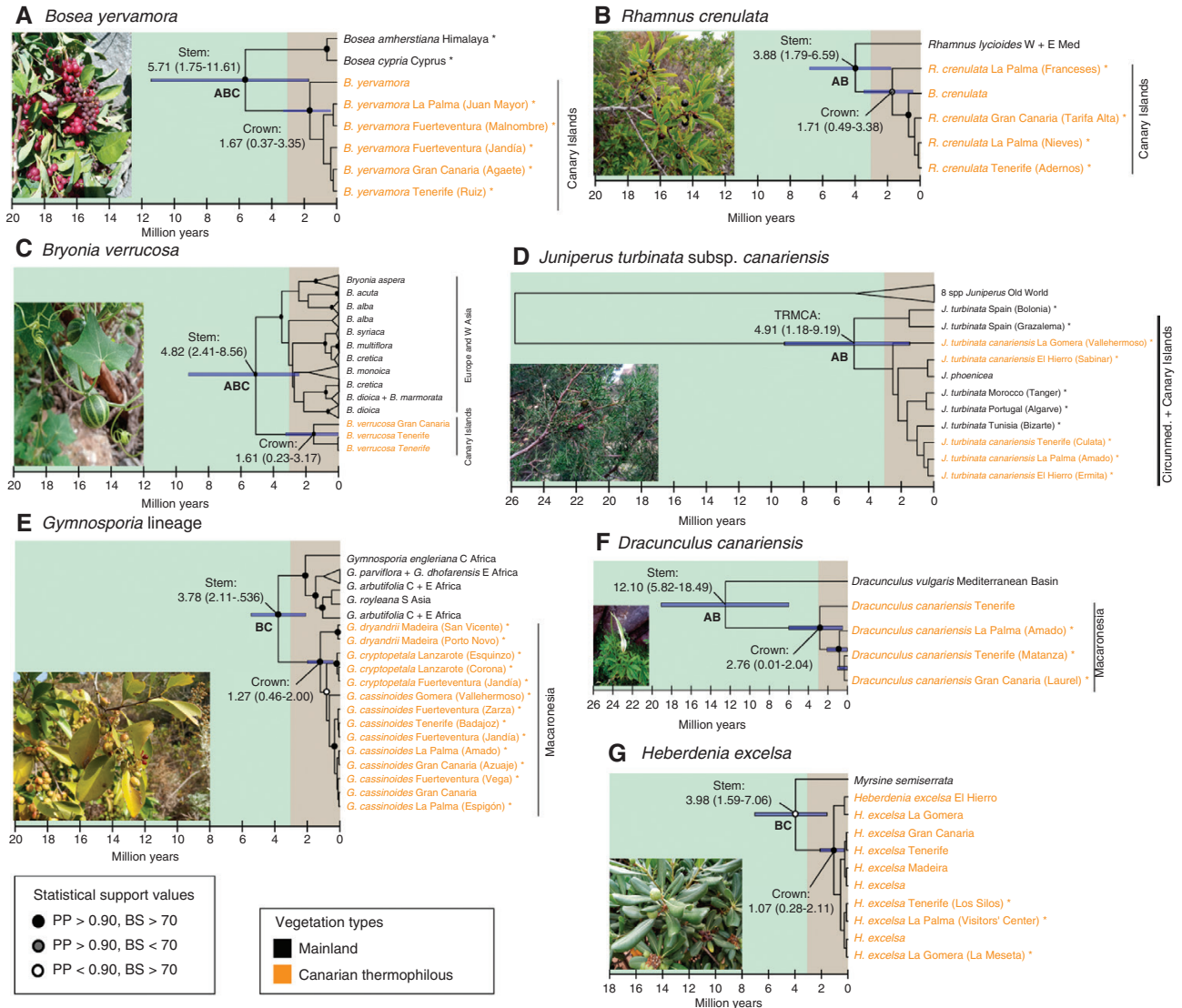


FIG. 6. The seven undetermined lineages (stem ages predating and crown ages postdating 2.8 Ma) and related Canary and mainland species for the 13 time-calibrated phylogenies inferred in this study using BEAST (with the exception of *Pistacia lentiscus* lineage I, which for simplification is included in Fig. 4D). 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 TRMCA) are indicated for those groups in which stem and crown nodes showed low phylogenetic support [PP < 0.90, bootstrap (BS) support value < 70]. The circles at the nodes represent phylogenetic relationships with high posterior Bayesian probability (PP) and/or BS support values (see Statistical support values legend). Colour of plant names indicates vegetation type. Asterisks represent new samples included in the present study. The letters in the stem node or TRMCA of the Canary lineages represent the ancestral ranges inferred for that node by the ancestral area reconstruction analysis and correspond to the following geographic areas: A, Mediterranean Basin; B, Macaronesia; C, other regions (several letters imply ranges that include multiple areas).

canariensis, *Ephedra fragilis*, *Juniperus turbinata*, *Pistacia lentiscus*, *Rhamnus crenulata* and *Thesium* lineage) and six lineages with stem node ranges not including the Mediterranean Basin (i.e. *Asparagus* lineage I, *Chrysojasminum odoratissimum*, *Gymnosporia* lineage, *Heberdenia excelsa*, *Pistacia atlantica* and *Sideroxylon canariense*) (Figs 4–6, Supplementary Data Figs S27–S39, Supplementary Data Table S5). No correspondence between the temporal origin of the lineages (Mediterranean versus pre-Mediterranean) and the ancestral ranges (including versus 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 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 Canary vegetation, which we analysed together with previously published results from an additional 27 thermophilous plant lineages (Figs 7 and 8).

TABLE 2. List of mediterranean, pre-mediterranean and undetermined lineages (i.e. colonization events) based on the position of stem and crown 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>Heberdenia 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>

In island biogeography, colonization times are more precisely considered to have taken place some time 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 a temporal origin both predating (pre-mediterranean) and postdating (mediterranean) the 2.8-Ma threshold considered for the establishment of the mediterranean climate (Figs 7 and 8; Table 2). In addition, biogeographic reconstruction analyses showed that 6 of the 16 colonization events here inferred may have occurred by continental ancestors not distributed in the Mediterranean Basin (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 geographic origins.

Pre-mediterranean and mediterranean elements in the thermophilous plant community

As predicted 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 modelling of thermophilous plants (Coello *et al.*, 2021) 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 of the mediterranean climatic conditions that may have facilitated dispersal from the MFR to other areas. There is thus a higher likelihood of dispersal and colonization to neighbouring 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 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*, *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., *Heberdenia 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 (9 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: (1) a direct colonization from the mainland to a pre-existent thermophilous vegetation in pre-mediterranean times; (2) an indirect colonization from other Canarian vegetation types followed by a more recent speciation in the thermophilous vegetation; and (3) a direct colonization into non-thermophilous Canarian vegetation and *in situ* adaptation to more recently established thermophilous conditions. 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*, *Gymnosporia*, *Euphorbia*). However, detailed anatomical investigation of fossil material is required for correct phylogenetic placement (Anderson *et al.*,

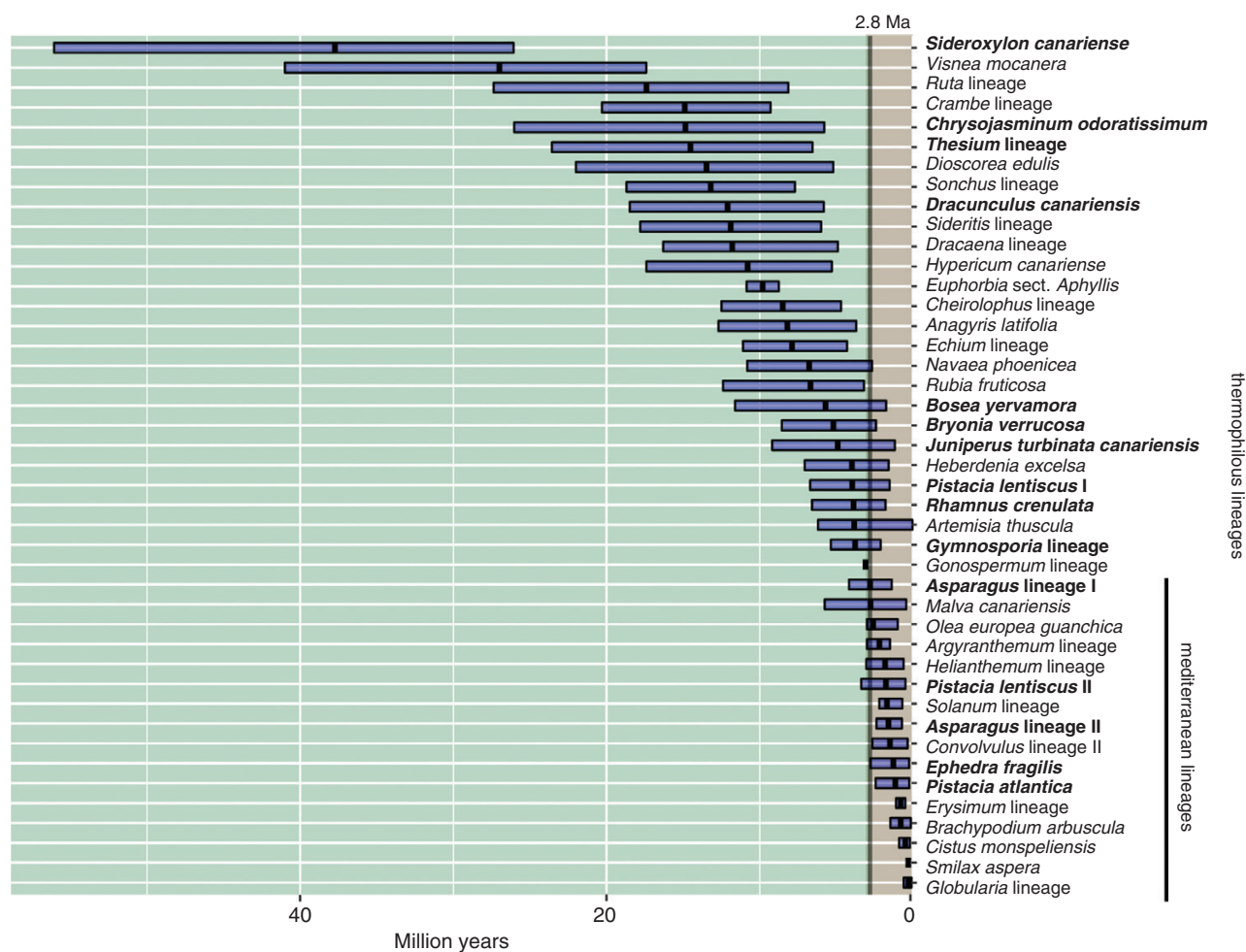


FIG. 7. Stem ages of 43 Canary lineages including thermophilous plant species. Results from the new time-calibrated phylogenies obtained in this study are indicated with plant names in bold (references in Supplementary Data Table S1). Blue bars show the 95 % posterior credibility intervals and mean stem ages are marked by a black line.

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 geographic expansion of the species when the climate became more favourable. 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 Canary 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 evolutionary processes 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 versus 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 analysed 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 25 million years. Long stem-to-crown intervals have been previously found for other Macaronesian groups (e.g. *Cicer*, *Campylanthus*), an observation related to high extinction rates according to 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

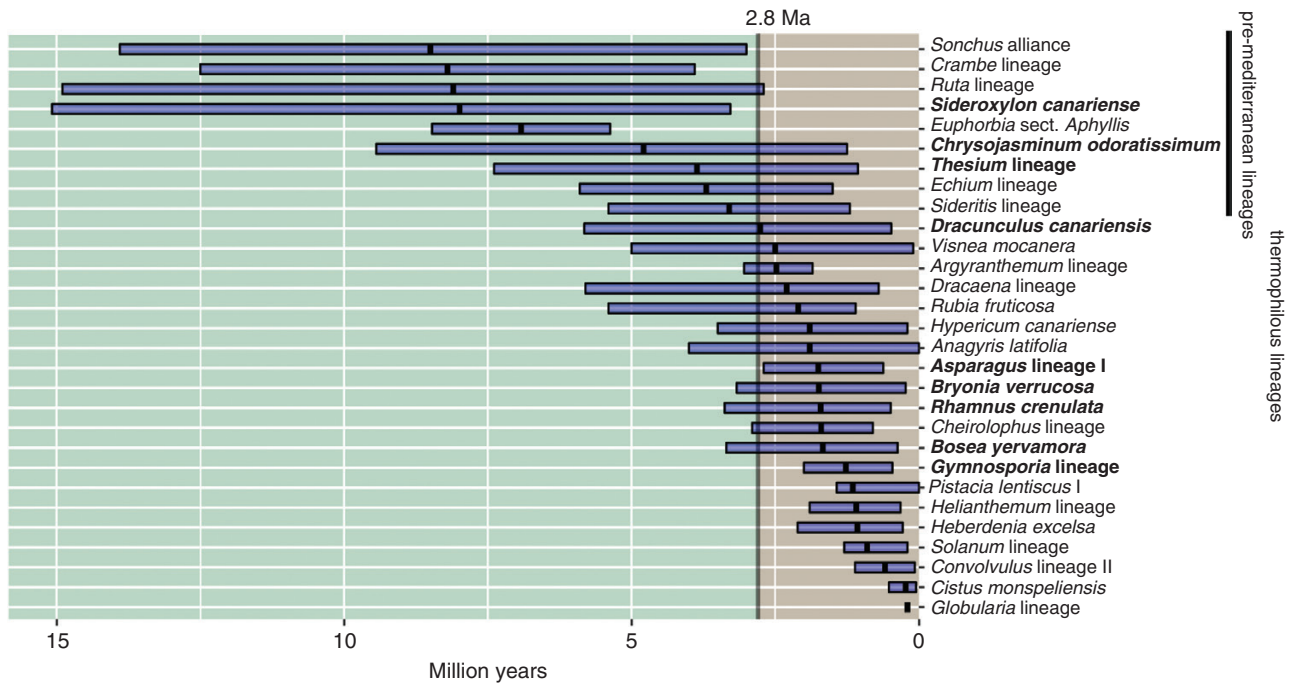


Fig. 8. Twenty-eight crown ages of Canary thermophilous plant lineages (i.e. including more than one sample from the Canaries) of the 43 shown in Fig. 7. Results from the new time-calibrated phylogenies obtained in this study are indicated with plant names in bold, while results from previously published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

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 geographic boundaries of the MFR in the past (Suc, 1984; Suc et al., 2018) makes the reconstruction of ancestral areas a suboptimal approach to evaluating 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: (1) erosion and subsidence of flat, oldest islands (Fuerteventura, Lanzarote), which may previously have harboured large areas with this vegetation type (Fernández-Palacios et al., 2008; Martín Osorio et al., 2011); (2) geological dynamics (eruptions, earthquakes, mega-landslides) (Carracedo et al., 2001; García-Olivares et al., 2017); and most importantly (3) 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*, *Cheirolophus duranii*, *Dracaena tamaranae*, *Echium handiense*, *Gymnosporia cryptopetala*, *Helianthemum gonzalezferreri*, *Helianthemum bramwelliorum*, *Solanum lidii*, *Solanum vespertilio* subsp. *vespertilio*, *Thesium retamoides*, *Thesium canariense*) and even several species considered already extinct (e.g. *Helianthemum aguloi*, *Thesium psilotocladum*) (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 harbours elements of both recent (mediterranean) and ancient Tethyan–Tertiary (pre-mediterranean) origins (Table 2). In addition, several of these colonization 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 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 geographic 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 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 online at <https://academic.oup.com/aob> and consist of the following. Figures 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 in combination with newly generated sequences. Figures S14–S26: time-calibrated phylogenies of the 13 plant groups analysed in this study obtained in BEAST using previously published phylogenetic datasets in combination with newly generated sequences. Figures S27–S39: biogeographic reconstructions of ancestral ranges of the 13 plant groups analysed in this study resulting from applying dispersal–extinction–cladogenesis 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, locality, collection date, collector's name, DNA-sequenced regions and GenBank accession numbers. Table S3: primers and PCR cycles used for sequencing DNA regions of the 13 plant groups analysed in this study. Table S4: detailed information on 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 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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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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