

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

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## 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 temporal 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 thermophilous species were analysed to infer colonization times. In particular, we used 26 previously-published phylogenies as well as 13 time-calibrated phylogenies including newly generated plastid and nuclear DNA sequence data to assess whether the time interval between stem and crown ages postdates the 2.8 Ma threshold.
- **Key Results** A total of 43 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.
- **Conclusions** Our findings reveal an unexpectedly heterogeneous origin of the Canarian thermophilous species in terms of colonization times. A substantial proportion of the lineages arrived in the Canaries before the summer-dry climate was established on the Mediterranean Basin. The temporal origin of Canarian thermophilous species both before and after the establishment of the

- 1 mediterranean climate challenges the view of the Canary Islands (and Madeira)
- 2 as a subregion within the Mediterranean Floristic Region.
- 3 **Keywords:** thermophilous woodland, Canary Islands, Mediterranean Floristic Region,
- 4 colonization times, stem age, crown age, 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 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 of subtropical origin and surviving representatives of a once more widely distributed Tertiary-Tethyan flora (Bramwell 1976, Mai 1995), but a more heterogeneous origin is pointed to in Kondraskov 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 phytogeographic 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 phytogeographic classification (Canarian-Madeiran subregion of the MFR). While the Mediterranean element is pervasive in the six vegetation belts of the Canaries, plant lineages exhibiting traits common in mediterranean climates or that have 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 several arboreal and shrubby communities named in Spanish after their dominant tree species: “sabinares” (*Juniperus turbinata* subsp. *canariensis*), “almacigares” (*Pistacia atlantica*), “lentiscales” (*Pistacia lentiscus*), “acebuchales” (*Olea europaea* subsp. *guanchica*) (Fernández-Palacios et al. 2008, Nezadal and Welss 2009). The last three tree species are sclerophyllous, having leaves with flexural stiffness and strength (Schimper 1903, Read and Sanson 2003) and currently found in the MFR (Rundel et al. 2016, Vargas 2020). The phytogeographic origin of Canarian plant lineages may therefore 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 around the Mediterranean sea (Carine et al. 2004, Fernández-Palacios et al. 2008, Vargas 2020).

The spatial boundaries of the MFR with the Eurosiberian Floristic Region to the north (Europe) and the Saharo-Arabian Floristic Region to the south (Africa) have been fluctuating over the last few millions of years as a result of climate changes (Suc 1984, Suc et al. 2018). Despite frequent fluctuations, the establishment of the mediterranean

1 climate across southern Europe and northern Africa in the last 2.8 million years provides  
2 the opportunity to fix a relatively well-defined temporal framework. This permits  
3 estimation of the origin of mediterranean plant lineages using time-calibrated  
4 phylogenies. The use of phylogenetic analyses based on DNA sequences to estimate the  
5 timing of evolutionary events has become a basic tool in biogeography (Sanmartín 2014).  
6 Time can be measured in absolute units when the tree is calibrated with fossils,  
7 phylogeny-based secondary calibrations and/or biogeographic events (Forest 2009, Ho  
8 and Phillips 2009; see Hipsley and Müller 2014 for alternative calibration approaches). In  
9 particular, relaxed-clock Bayesian methods can elucidate a prior distribution on the age of  
10 a node, taking into account the uncertainty associated with tree topology, branch length  
11 and calibrations (Drummond et al. 2006, Yang and Rannala 2006). This method can be  
12 applied to a large number of plant groups, thus helping describe general patterns  
13 (Pokorný et al. 2015, Vargas et al. 2018). Applying time-calibrated phylogenetic  
14 approaches to investigate divergence of Canarian lineages from their continental relatives  
15 may provide valuable insight into the very complex patterns of relationships between the  
16 Canary Islands and continental floras (Carine et al. 2004, Kondrakov et al. 2015,  
17 Caujapé-Castells et al. 2017, Valente et al. 2017).

18 Palaeoclimatic, floristic and phytogeographic studies lead us to examine the  
19 hypothesis of an origin of Canarian thermophilous species following the establishment of  
20 the mediterranean climate across the Mediterranean Basin. To test this hypothesis, we  
21 analyzed new phylogenetic datasets produced for this study, together with previously  
22 published time-calibrated phylogenies to investigate whether ancestral thermophilous  
23 plant lineages had a temporal origin since the mediterranean climate became established  
24 around 2.8 million years ago (Suc et al. 2018).

## MATERIAL AND METHODS

### *Study area*

Thermophilous woodland is the potential vegetation (i.e., plant community occurring in the absence of human intervention) in the Canary Islands 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 characterized 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 are accompanied by endemic trees (*Gymnosporia cassinoides*, *Dracaena draco*, *Phoenix canariensis*, *Sideroxylon canariense*, *Visnea mocanera*). In addition, a high number of shrub and herb species are found in 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 firstly by the earlier African human inhabitants of the islands, who mostly settled around 2000 years ago, and then more severely 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*

The spatial uncertainty of the geographical boundaries of the MFR in the past makes the reconstruction of ancestral areas a weak approach to evaluate the Mediterranean origin of the Canarian thermophilous species. This is because geographic areas in the

Mediterranean Basin may have not had that climate at the time of colonization of the Canaries. In contrast, a mediterranean temporal origin of the thermophilous plants can be more adequately assessed from divergence times of stem and crown nodes of representative Canarian species (Fernández-Palacios et al. 2008, del Arco Aguilar and Rodríguez-Delgado 2018). 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) present in the Canary Islands. These datasets are heterogeneous but 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



1 samples (Supplementary Data Table S2). We sampled individuals from as many islands  
2 as possible, as well as from the most closely-related species based on available  
3 phylogenetic and taxonomic information. For non-endemic taxa, we also sampled  
4 population(s) from nearby continental areas. Many plants of the Canary Islands have a  
5 Palearctic origin (Sunding 1979, Carine et al. 2004), and thus we focused our sampling  
6 on the archipelagos and mainland regions of Africa and Europe that are close to the  
7 Canary Islands, with particular effort on Mediterranean Iberia and northwestern Africa  
8 (Valente et al. 2017). Overall, we generated DNA sequences from 104 samples of 16  
9 representative thermophilous species and 17 close relatives, which were then included  
10 into the published DNA sequence datasets. Sample information for all specimens and  
11 GenBank accession numbers for all new DNA sequences are provided in  
12 Supplementary Data Table S2.

13 DNA was extracted from dried leaves using QIAGEN DNeasy Plant Mini Kit  
14 according to the manufacturer's protocol. Different DNA regions were amplified and  
15 sequenced for each plant group, based on the regions used in previously published  
16 studies (Supplementary Data Table S2). We analyzed a total of 16 DNA nuclear and  
17 plastid regions. Primers and PCR cycles applied for each plant group are included in  
18 Supplementary Data Table S3. Amplified products were sequenced by standard Sanger  
19 sequencing at Macrogen Europe ([www.macrogen.com](http://www.macrogen.com)). We used Geneious 2011.2.2  
20 (<https://www.geneious.com>) to edit chromatograms, align sequences with the MUSCLE  
21 algorithm (Edgar 2004) and concatenate DNA regions (Table 1). The resulting  
22 alignments were checked visually and corrected where the algorithm failed to identify  
23 gaps. In total, this process generated 254 new sequences from 33 species.

1 *Phylogenetic relationships and divergence times*

2 To estimate phylogenetic relationships and divergence times of stem and crown nodes  
3 for plant groups with thermophilous species in the Canary Islands, we generated 13 new  
4 time-calibrated phylogenies using the Bayesian uncorrelated log-normal relaxed clock  
5 model in BEAST 1.84 (Drummond and Rambaut 2007). The best-fitting substitution  
6 model for each partition (DNA region) was selected using the Akaike Information  
7 Criterion implemented in jModelTest v.0.1.1 (Posada 2008). For each analysis, we ran  
8 two independent chains of 100 million generations with a birth–death tree prior, which  
9 accounts for both speciation and extinction (Gernhard 2008). All molecular dating  
10 analyses in BEAST were performed using the computer cluster Trueno (CSIC, Madrid,  
11 Spain). We assessed convergence of chains and appropriate burn-ins with Tracer v.1.7.1  
12 (Rambaut et al. 2018), combined runs using LogCombiner, and produced maximum  
13 clade credibility (MCC) trees with mean node heights in Tree Annotator.

14 Calibration points used to estimate divergence times were based on the fossil  
15 record and published secondary calibration points, depending on data availability for  
16 each group (Supplementary Data Figs. S1-S13). When reliable fossils were available, a  
17 lognormal prior on age estimates was used, since this distribution better represents the  
18 stratigraphic uncertainty associated with the fossil record (Ho and Phillips 2009). The  
19 offset of the lognormal distribution was set to the upper bound of the stratigraphic  
20 period where the fossil was found. In the absence of reliable fossils, only secondary  
21 calibration points taken from original papers were applied to the corresponding deep  
22 nodes (Table 1). Secondary calibration points were obtained from fossil-calibrated  
23 phylogenies of higher taxonomic ranks, which included samples of our study groups  
24 (e.g., typically the family to which the genus belongs), and were assigned normal  
25 distribution priors (Ho and Phillips 2009) in the BEAST analysis including the mean  
26 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. S1-S13.

An additional approach using a maximum likelihood analysis was performed to obtain an alternative estimate of phylogenetic relationships and support values (see Supplementary Data, Methods S1).

#### *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 million year (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 (both the stem and the crown node are mediterranean); (2) pre-mediterranean lineages, when crown and thus stem ages predated the 2.8 Ma threshold (both the stem and the crown ages are pre-mediterranean); and (3) undetermined lineages, for those groups with stem ages falling 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” does not thus refer to the geographical origin but 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 in the literature (Supplementary Data Table S1) are not from the Mediterranean Basin. Even when they are, poorly supported sister-group relationships prevent hypothesizing the closest relatives of several lineages, and therefore their biogeographic origin.

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 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 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 probabilities of nodes are low, 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).

## RESULTS

### *Newly inferred phylogenetic relationships*

Our phylogenetic reconstructions are in accordance with previous published phylogenies regarding the topological relationships for the thermophilous species occurring in the Canary Islands (Table 1). 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*, *Juniperus turbinata*, *Pistacia atlantica*, *Pistacia lentiscus*) *Asparagus umbellatus* and *Thesium retamoides* (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* (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* within the clade of *A. nesioties*-*A. plocamoides*, and of *Asparagus umbellatus* within the clade of *A. fallax*-*A. arborescens*; (ii) close relationship of the two Canarian species of *Gymnosporia* (*G. cassinoides*-*G. cryptopetala*), which are sisters to the Madeira endemic *G. dryandrii*; (iii) *Chrysojasminum odoratissimum* sister to the mainland clade of *C. parkeri*-*C. humile*-*C. bignoniaceum*-*C. fruticans*; and (iv) the two Canarian species of *Thesium* (*T. retamoides* and *T. subsucculentum*) sister to the mainland *T. mauritanicum* (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* 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. nesiotetes* 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).

#### *Mediterranean and pre-mediterranean 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* to 7.99 Ma within *Sideroxylon canariense* (Supplementary Data Table S4). Likewise, mean stem ages ranged from 1.15 Ma for *Pistacia atlantica* to 37.73 Ma for *Sideroxylon canariense*. 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 (Figs. 4-6, 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. 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 revealed a further 11 plant lineages as mediterranean. As a result, a total of 16 of the 43 thermophilous lineages display a clear mediterranean origin (from youngest to oldest stem ages; Fig. 7): *Globularia* lineage (0.30 Ma), *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 mean stem age of *Asparagus* lineage I clearly overlaps the mediterranean threshold, the posterior distribution of trees shows that more than 60 % of the stem age distribution and 96 % of crown age distribution in the mediterranean climate period. We therefore consider the probability of the lineage having colonized the thermophilous belt in mediterranean times to be high.

#### 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. Crown ages of 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* (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, *Myrsine excelsa*, *Juniperus turbinata* subsp. *canariensis*, *Pistacia lentiscus* lineage I and *Rhamnus crenulata*. 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. 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* (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), *Visnea mocanera* (mean stem: 27.00; mean crown: 2.50).

## 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 other 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 origin both predating (pre-mediterranean) and postdating (mediterranean) the 2.8 Ma threshold considered for the establishment time of the

1 mediterranean climate (Table 2). In fact, some typical elements of the thermophilous  
2 woodland are likely much older than previously thought. Taking all these results  
3 together, the thermophilous vegetation appears as a complex assemblage of species with  
4 a heterogeneous origin in terms of colonization times (Figs. 7, 8; Supplementary Data  
5 Table S1).

6 *Unexpected combination of pre-mediterranean and mediterranean elements in the*  
7 *thermophilous plant community*

8 As expected by the hypothesis of a mediterranean temporal origin for the Canarian  
9 thermophilous species, a considerable number of lineages (16) are estimated to have  
10 colonized the archipelago after the establishment of the mediterranean climate (2.8 Ma).  
11 Data from meteorological stations (<https://www.acanmet.org/>) between 200 and 600  
12 m.a.s.l. and climatic variables used for species distribution modeling of thermophilous  
13 plants (Coello et al. 2020) indicate that current conditions in the Mediterranean Basin  
14 are similar to those of the thermophilous vegetation belt in the Canaries (Rivas-  
15 Martínez 2009). Since the late Pliocene (2.8 Ma), a progressive summer aridification of  
16 southern Europe and northern Africa may have produced wider distributions and  
17 abundances of plant elements of the Mediterranean Floristic Region. There is thus a  
18 higher likelihood of dispersal and colonization to neighboring territories such as the  
19 Canarian archipelago (Meusel 1965, Sunding 1979). That is why trees with one of the  
20 most characteristic mediterranean-type syndromes (sclerophylly, a trait displayed  
21 mostly in leaves) may have found ideal conditions in the Canaries in the last 2.8 million  
22 years (Axelrod 1975, Verdú et al. 2003, Rundel et al. 2016, Vargas et al. 2018). Our  
23 results partly support this prediction for the sclerophyllous *Olea europaea*, *Pistacia*  
24 *atlantica* and *Pistacia lentiscus* lineage II, but provide undetermined results for the  
25 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, and so did not help support a mediterranean temporal origin.

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). Two 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, and (ii) an indirect colonization from other Canarian vegetation types followed by a more recent species differentiation in the thermophilous vegetation. 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. These moreover share characteristics with certain representative species of the present-day thermophilous scrub vegetation (e.g., *Cistus*, *Maytenus* (= *Gymnosporia*), *Euphorbia*). 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

1 lineages adapted to pre-mediterranean conditions in relatively small, xeric pockets  
2 became dominant when mediterranean-like conditions were expanded has already been  
3 proposed for plants of the Mediterranean Basin (Barrón et al. 2010, Vargas et al. 2018).  
4 The second hypothesis is congruent with a pattern of high differentiation into species in  
5 different vegetation belts and ecological shifts into thermophilous conditions. This  
6 includes some speciation events associated with colonization of the thermophilous  
7 woodland, as occurred in most of the evolutionary radiations of Canarian plants (e.g.,  
8 *Sideritis* lineage, *Echium* lineage, *Euphorbia* sect. *Aphyllis* subsect. *Macaronesicae*,  
9 *Crambe* lineage, *Sonchus* lineage; see Supplementary Data Table S1). In any case, both  
10 patterns of evolution could have been operating to ultimately merge into this  
11 particularly rich flora.

12 All these results provide strong support for a heterogeneous temporal origin of  
13 the thermophilous woodland in the Canary Islands, which harbors elements of both  
14 recent (mediterranean) and ancient Tethyan-Tertiary (pre-mediterranean) origins  
15 (Supplementary Data Table S1). A similar pattern has been identified for the laurel  
16 forest and xerophytic shrubland (Kondraskov et al. 2015, Sun et al. 2016, Salvo et al.  
17 2010). Given that the thermophilous vegetation contains the highest number of lineages  
18 previously considered of mediterranean origin (Rivas-Martínez 2009, del Arco Aguilar  
19 and Rodríguez-Delgado 2018; but see Bolòs 1996), we hypothesize that the other five  
20 main vegetation belts contain an even lower number of mediterranean-like lineages  
21 (Vargas 2020). If this hypothesis is confirmed, the long-lasting view of Macaronesia as  
22 a subregion within the Mediterranean Floristic Region would be seriously challenged.

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

24 The time of origin (mediterranean vs. pre-mediterranean) of 25 out of 43 thermophilous  
25 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*, *Campylanthus*), 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, 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). Based on the idea that stem ages are subject to higher temporal and spatial uncertainty as a result of mainland extinction, García-Verdugo et al. (2019a) proposed that crown ages may be a more suitable measurement for the time of island colonization than stem ages. 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 the 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), and thus a high rate of population and genetic loss is expected. Indeed, we interpret considerable loss of plant diversity of the thermophilous vegetation, and thus loss of DNA-sequence variability in recent decades. 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 considered already extinct (e.g., *Helianthemum aguloi*, *Thesium psilotocladum*) (Moreno 2010).

In a nutshell, the finding of crown ages predating 2.8 Ma is a strong argument to recognize a pre-mediterranean origin of multiple plant groups that colonized the Canary Islands. However, two factors prevent us from finding more plant lineages of pre-mediterranean origin: considerable extinction of plants in one of the vegetation belts with the most intense human activities since the arrival of Europeans (Humboldt 1814) and the incomplete taxon sampling frequent in phylogenetic studies (Antonelli and Sanmartín 2011). In other words, the combination of both causes is expected to lead to an underestimated percentage of pre-mediterranean Canary Island lineages.

## Conclusions and future perspectives

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 complex history of species assemblage in the current thermophilous plant community of the Canaries, including a previously underestimated pre-mediterranean origin. However, the temporal origin of many of their lineages remains elusive, as some were classified as undetermined in this study. Thus, more data and analyses are needed to evaluate the net contribution of both mediterranean and pre-mediterranean plant species to the Canarian flora.

The use of genomic data obtained by next-generation sequencing techniques may further help discriminate undetermined lineages as a result of narrower ranges of uncertainty resulting from a larger number of informative DNA characters (Fig. 2) and a higher resolution of phylogenetic relationships in poorly-supported groups. These new genomic approaches provide well-resolved phylogenies, even for insular radiating lineages (e.g., *Argyranthemum*, White et al. 2020; *Helianthemum*, Albaladejo et al. 2021; *Scalesia*, Fernández-Mazuecos et al. 2020). Besides these advances, a greater sampling effort for mainland species and populations potentially related to thermophilous plant species may help resolve sister-group relationships. On the one hand, an extensive sampling may reduce type I error (due to a mistaken rejection of an actually true null hypothesis), i.e. mistakenly inferring a thermophilous lineage as pre-mediterranean when it is actually more recent. On the other hand, a greater sampling effort across the archipelago, including populations from all the islands (especially from the oldest, Fuerteventura and Lanzarote), will capture more insular genetic diversity.



This will potentially result in older crown ages, thus reducing type II error (mistaken acceptance of an actually false null hypothesis), i.e. mistakenly accepting a thermophilous lineage to be mediterranean. In addition, new fossil records yet to be discovered could provide palaeoecological evidence for the onset of mediterranean-like conditions in the Canary Islands and paleobotanical evidence of past occurrence of currently extinct plant lineages on the mainland. In addition, more reliable fossil calibration points would help reduce temporal uncertainty at the stem and crown nodes, as well as shorten the time gap between these two nodes.

To sum up, many lineages already present on the Canary Islands prior to the development of the current mediterranean-like climate, while others became important components of the thermophilous vegetation since the original establishment of that climate type (mostly during the Pleistocene). Different climatic and temporal 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 current endangered status of its few surviving 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. Fig. S1. Time-calibrated phylogeny of *Asparagus* by BEAST using a previously published phylogenetic dataset (Norup et al. 2015) and newly generated sequences in this study. Fig. S2. Time-calibrated phylogeny of Amaranthaceae by BEAST using a previously published phylogenetic dataset (Di Vincenzo et al. 2018) and newly generated sequences in this study. Fig. S3. Time-calibrated phylogeny of *Bryonia*

by BEAST using a previously published phylogenetic dataset (Volz and Renner 2008).

Fig. S4. Time-calibrated phylogeny of *Chrysojasminum* by BEAST using a previously published phylogenetic dataset (Jeyarani et al. 2018) and newly generated sequences in this study. Fig. S5. Time-calibrated phylogeny of *Arum* (and other genera of Araceae including *Dracunculus*) by BEAST using a previously published phylogenetic dataset (Mansion et al. 2008) and newly generated sequences in this study. Fig. S6. Time-calibrated phylogeny of *Ephedra* by BEAST using a previously published phylogenetic dataset (Ickert-Bond et al. 2009) and newly generated sequences in this study. Fig. S7. Time-calibrated phylogeny of *Gymnosporia* by BEAST using a previously published phylogenetic dataset (Oberprieler et al. 2017) and newly generated sequences in this study. Fig. S8. Time-calibrated phylogeny of *Juniperus* by BEAST using a previously published phylogenetic dataset (Mao et al. 2010) and newly generated sequences in this study. Fig. S9. Time-calibrated phylogeny of *Myrsine* by BEAST using a previously published phylogenetic dataset (Appelhans et al. 2020) and newly generated sequences in this study. Fig. S10. Time-calibrated phylogeny of *Pistacia* by BEAST using a previously published phylogenetic dataset (Xie et al. 2014) and newly generated sequences in this study. Fig. S11. Time-calibrated phylogeny of *Rhamnus* by BEAST using a previously published phylogenetic dataset (Bolmgren and Oxelman 2004) and newly generated sequences in this study. Fig. S12. Time-calibrated phylogeny of *Sideroxylon* by BEAST using a previously published phylogenetic dataset (Stride et al. 2014) and newly generated sequences in this study. Fig. S13. Time-calibrated phylogeny of *Thesium* by BEAST using a previously published phylogenetic dataset (Zhigila et al. 2020) and newly generated sequences in this study. 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 (archipelago 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. 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,  
2    DNA regions and bibliographic references of the DNA sequence datasets used are detailed, as well as calibration approach and bibliographic  
3    reference on which the strategy for calculating divergence times is based. The last column indicates the reasons why the original datasets had to be  
4    improved, 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. \* Macaronesian endemics; \*\* Canarian endemics. Species with no  
7    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> *	Genus <i>Asparagus</i> (Asparagaceae)	PHYC, <i>trnH-psbA</i> , <i>trnD-T</i> , <i>ndhF</i>	Norup et al. 2015	Secondary calibration (Fig. S1)	Chen et al. 2013	1, 3, 4
<i>Asparagus umbellatus</i> *						
<i>Bosea yervamora</i> **	Family Amaranthaceae	<i>matK</i>	Di Vincenzo et al. 2017	Fossil and secondary calibration (Fig. S2)	Di Vincenzo et al. 2017	2, 3
<i>Bryonia verrucosa</i> **	Genus <i>Bryonia</i> (Cucurbitaceae)	ITS, LFY, <i>trnL</i> , <i>trnL-trnF</i> , <i>psbA-trnH</i> , <i>trnH2</i> , <i>trnR-atpA</i>	Volz and Renner 2008	Secondary calibration (Fig. S3)	Schaefer et al. 2009	5
<i>Chrysojasminum odoratissimum</i> *	Genus <i>Chrysojasminum</i> (Oleaceae)	ITS, <i>matK</i> , <i>trnL-F</i> and <i>trnH-psbA</i>	Jeyarani et al. 2018	Secondary calibration (Fig. S4)	Vargas et al. 2014	2, 3, 4
<i>Dracunculus canariensis</i> *	Genus <i>Arum</i> (Araceae)	<i>trnL</i> , <i>trnF</i> , <i>matK</i> , <i>trnK</i> , <i>rbcL</i> , <i>rps16</i>	Mansion et al. 2008	Fossil and secondary calibration (Fig. S5)	Mansion et al. 2008, Nauheimer et al. 2012	3, 5
<i>Ephedra fragilis</i>	Genus <i>Ephedra</i> (Ephedraceae)	26S, 18S, ITS, <i>atpB</i> , <i>rbcL</i> , <i>matK</i> , <i>rps4</i> , <i>psbA-trnH</i> , <i>trnL</i> , <i>trnL-trnF</i>	Ickert-Bond et al. 2009	Fossil calibration (Fig. S6)	Ickert-Bond et al. 2009	2, 3
<i>Gymnosporia cryptopetala</i> **	Genus <i>Gymnosporia</i> (Celastraceae)	ITS	Oberprieler et al. 2017	Secondary calibration (Fig. S7)	Davis et al. 2005	3, 5
<i>Gymnosporia cassinoides</i> **						
<i>Juniperus turbinata</i> subsp. <i>canariensis</i>	Genus <i>Juniperus</i> (Cupressaceae)	<i>matK</i> , <i>rbcL</i> and <i>trnL-F</i> , <i>rps4</i> , <i>trnS-G</i> , <i>trnD-T</i> , <i>trnV</i> , <i>petB-D</i> and <i>psbB1-B2</i>	Mao et al. 2010	Fossil calibration (Fig. S8)	Mao et al. 2010	2, 3
<i>Myrsine excelsa</i> *	Genus <i>Myrsine</i> (Primulaceae)	ITS, ETS	Appelhans et al. 2020	Secondary calibration (Fig. S9)	Rose et al. 2018	3, 4
<i>Pistacia atlantica</i>	Genus <i>Pistacia</i> (Anacardiaceae)	ITS, ETS	Xie et al. 2014	Secondary calibration (Fig. S10)	Xie et al. 2014	2, 3
<i>Pistacia lentiscus</i>						
<i>Rhamnus crenulata</i> **	Genus <i>Rhamnus</i> (Rhamnaceae)	ITS, <i>trnL-trnF</i>	Bolmgren and Oxelman 2004	Secondary calibration (Fig. S11)	Onstein et al. 2015	2, 3
<i>Sideroxylon canariense</i> **	Genus <i>Sideroxylon</i> (Sapotaceae)	ITS, <i>trnH-psbA</i>	Stride et al. 2014	Fossil calibration (Fig. S12)	Stride et al. 2014	3
<i>Thesium retamoides</i> **	Genus <i>Thesium</i> (Santalaceae)	ITS, <i>matK</i> , <i>rpl32-trnL</i> , <i>trnL-F</i>	Zhigila et al. 2020	Secondary calibration (Fig. S13)	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

<b>Mediterranean</b>	<b>Pre-mediterranean</b>	<b>Undetermined</b>
<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. 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.

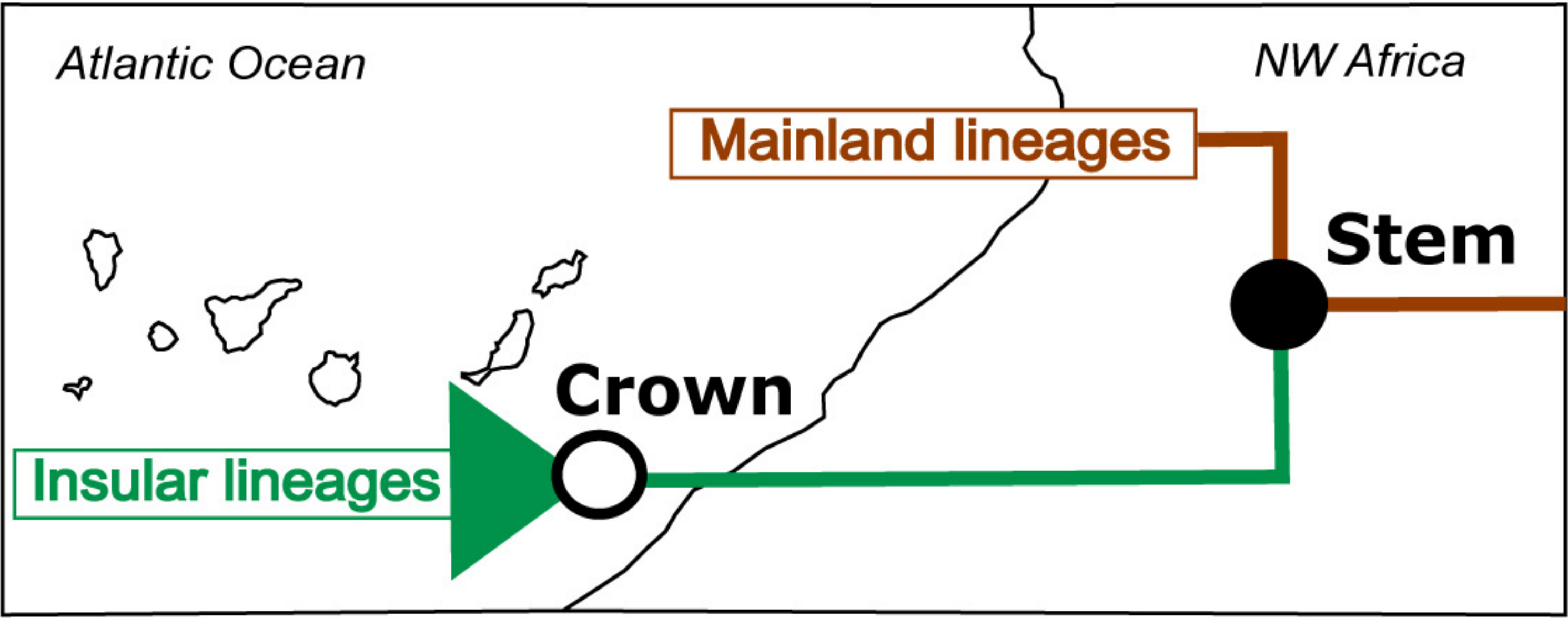
**Fig. 5.** The three pre-mediterranean lineages (i.e. crown and thus stem ages predate 2.8 Ma) and related Canarian and mainland species from the 13 time-calibrated phylogenies inferred in this study using BEAST. Mean stem and crown ages and 95% posterior credibility intervals (blue bars and values 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.

**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. 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.

**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.

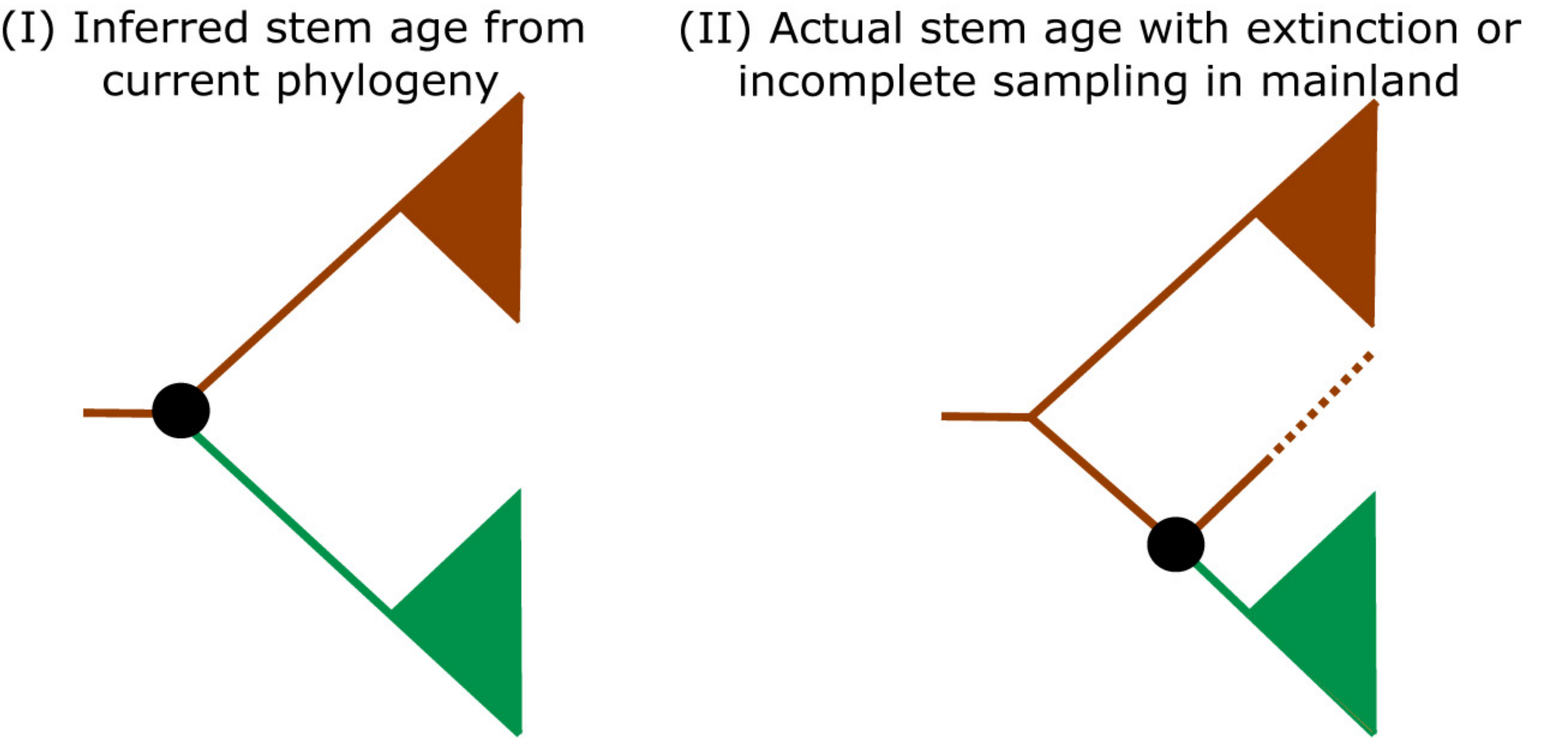
**Fig. 8.** Twenty-eight crown ages of Canarian thermophilous plant lineages (i.e., including more than one sample from the Canaries) of the 43 shown in Fig. 7. Results from the new phylogenies obtained in this study are indicated with plant names in bold, while results from previously published time-calibrated 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.

**A. Estimates of colonization times on oceanic islands:  
Stem vs. crown ages**

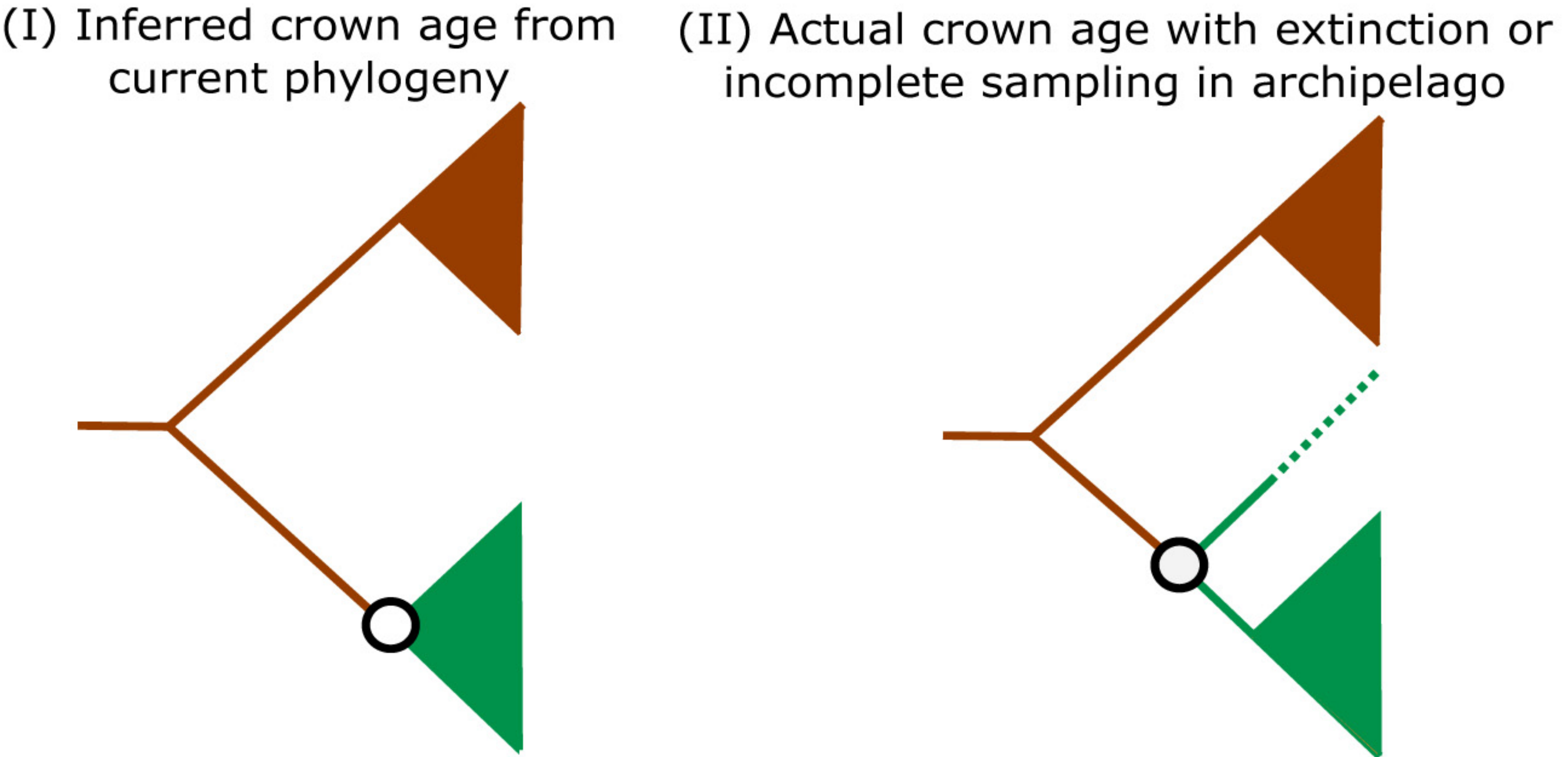


Modified from García-Verdugo et al. 2019a

**B. Stem age overestimation**



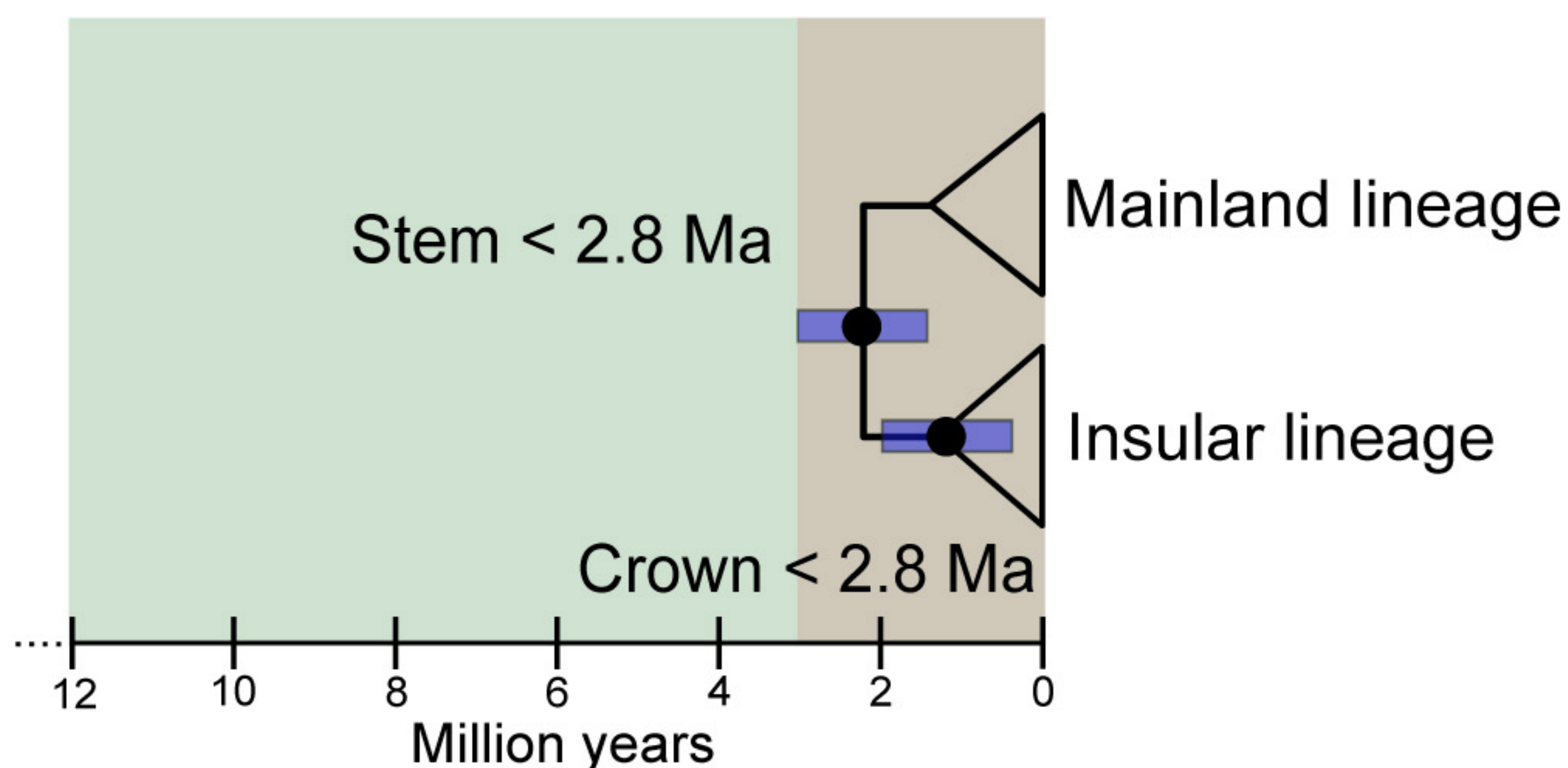
**C. Crown age underestimation**





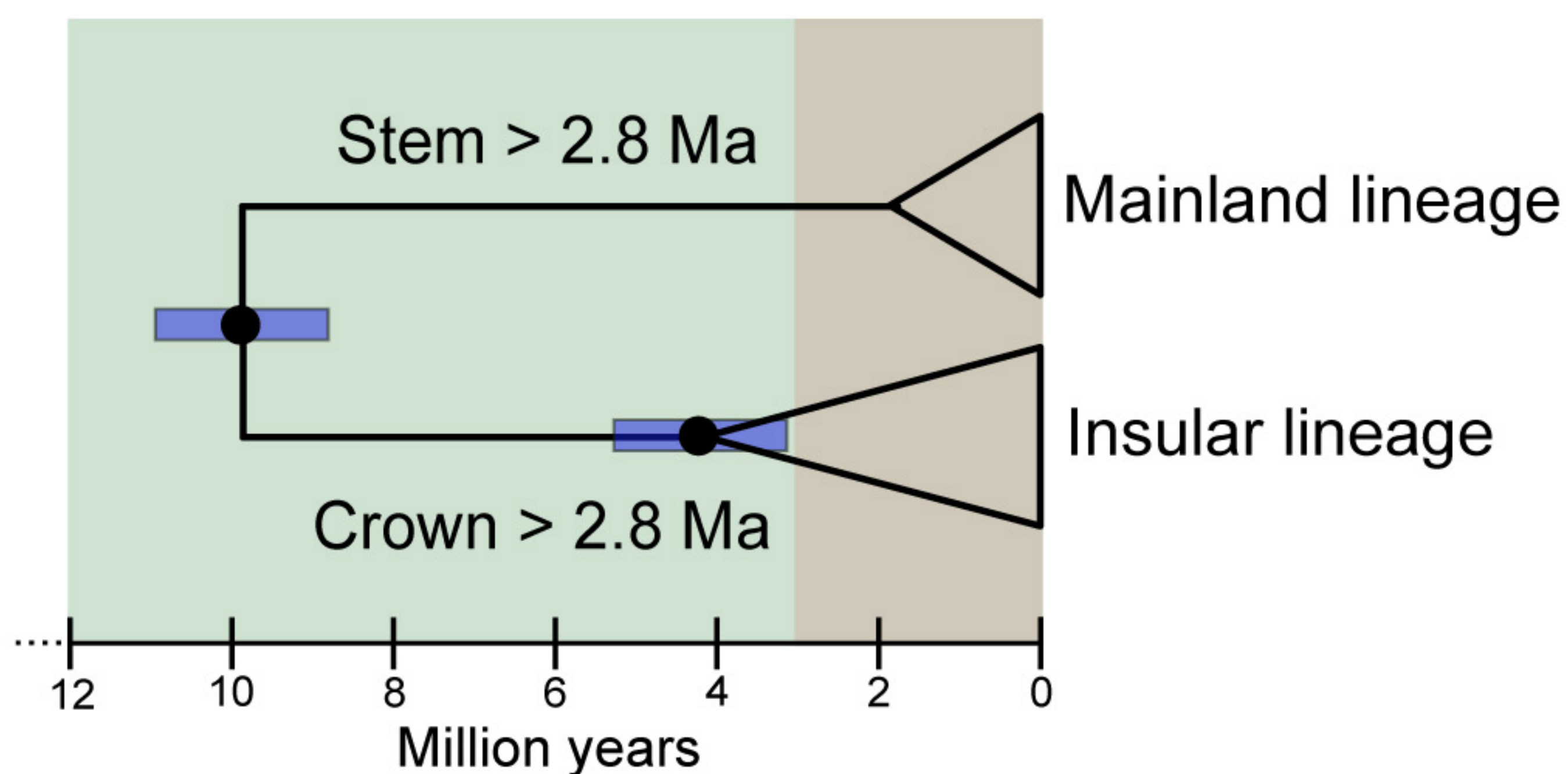
### (1) Mediterranean lineages

(Stem < 2.8 Ma)



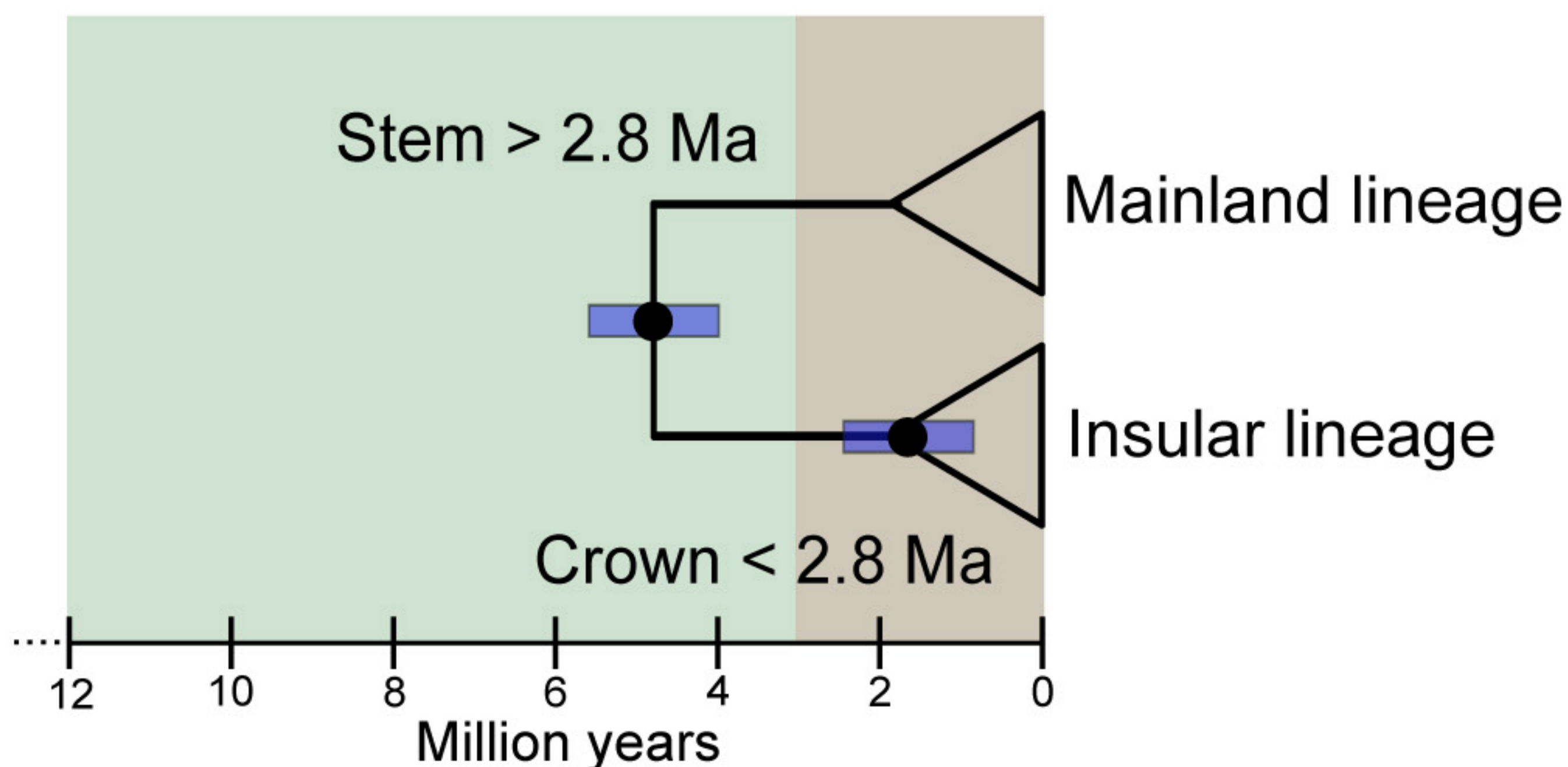
### (2) Pre-mediterranean lineages

(Crown > 2.8 Ma)

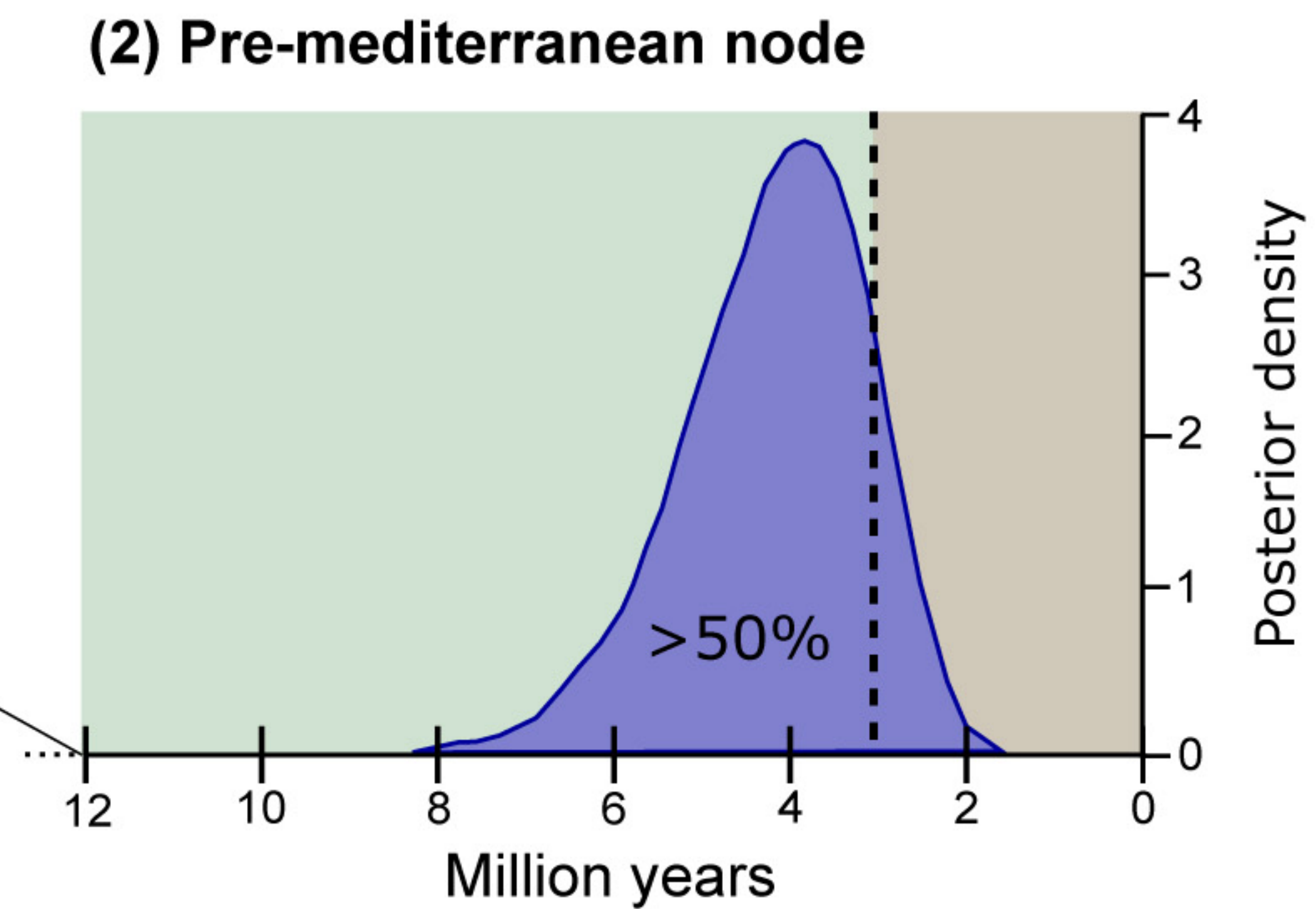
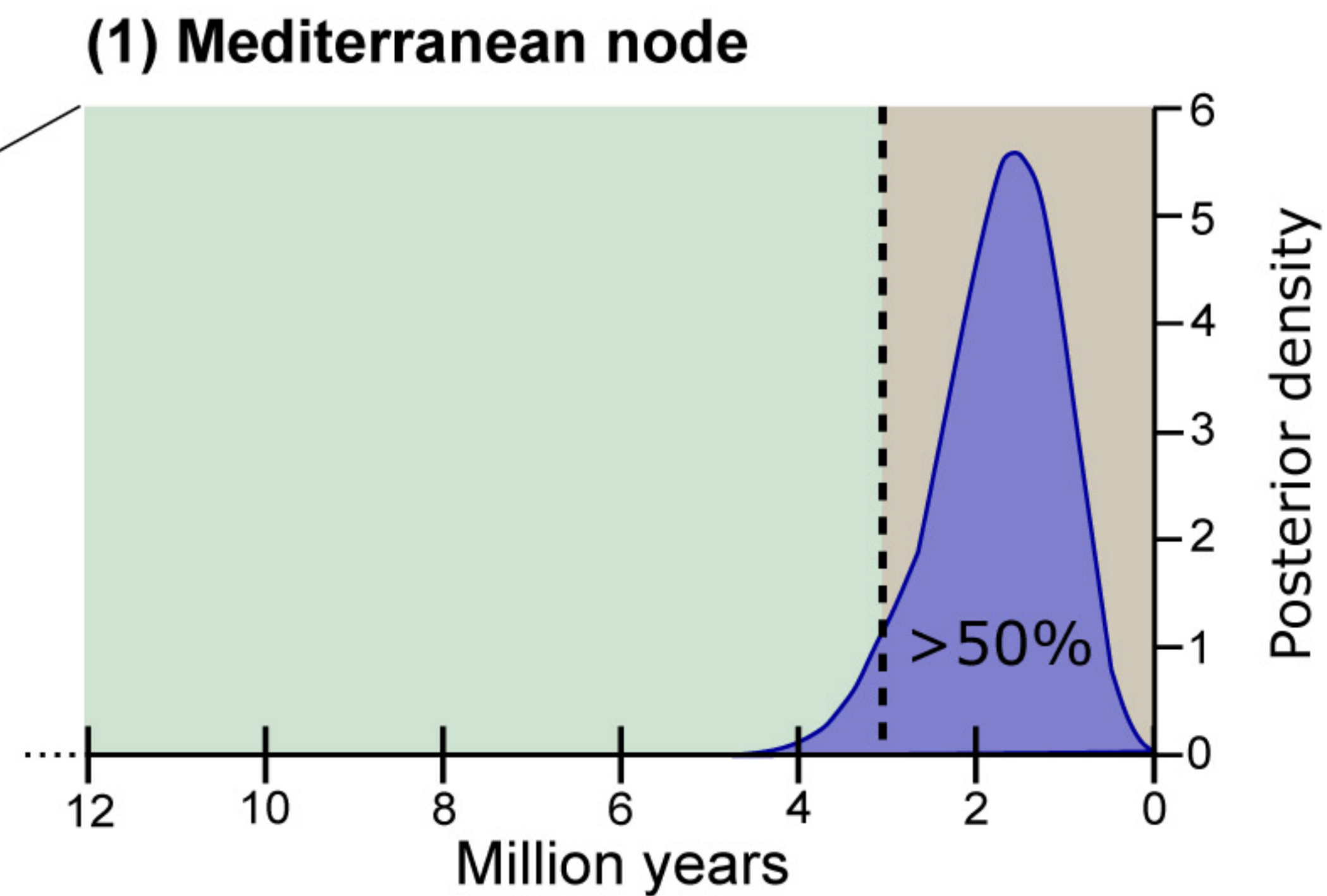
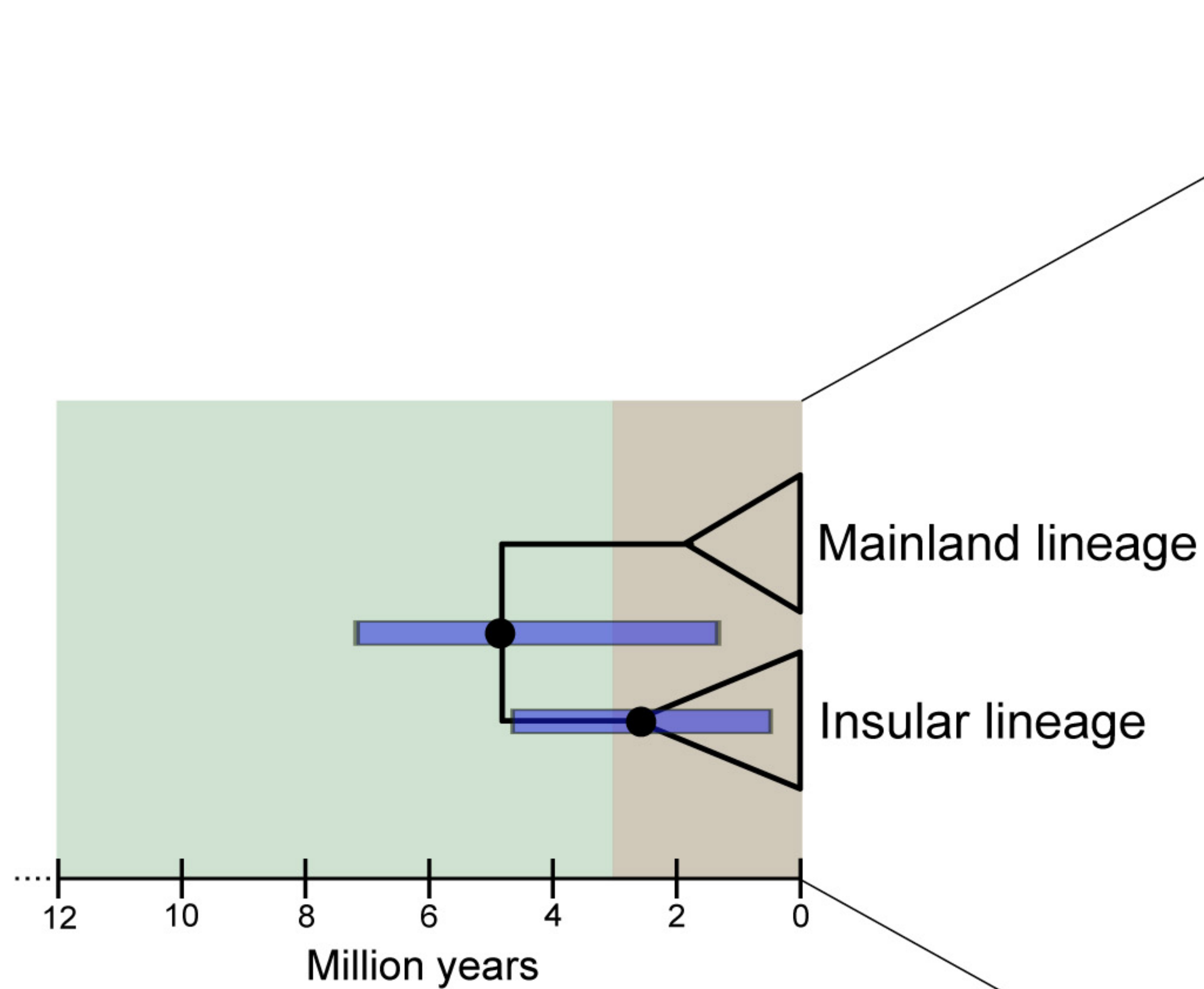


### (3) Undetermined lineages

(Stem > 2.8, Crown < 2.8 Ma)

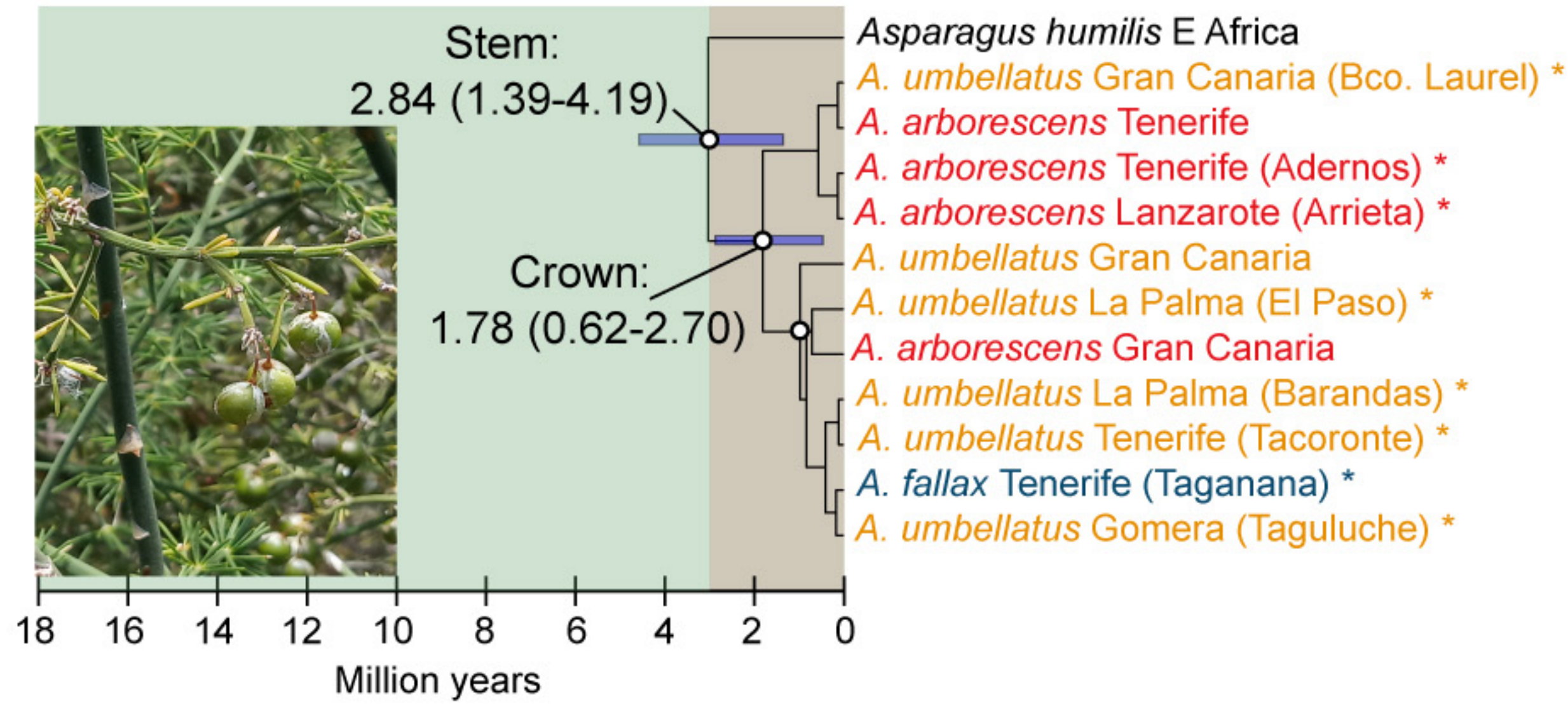




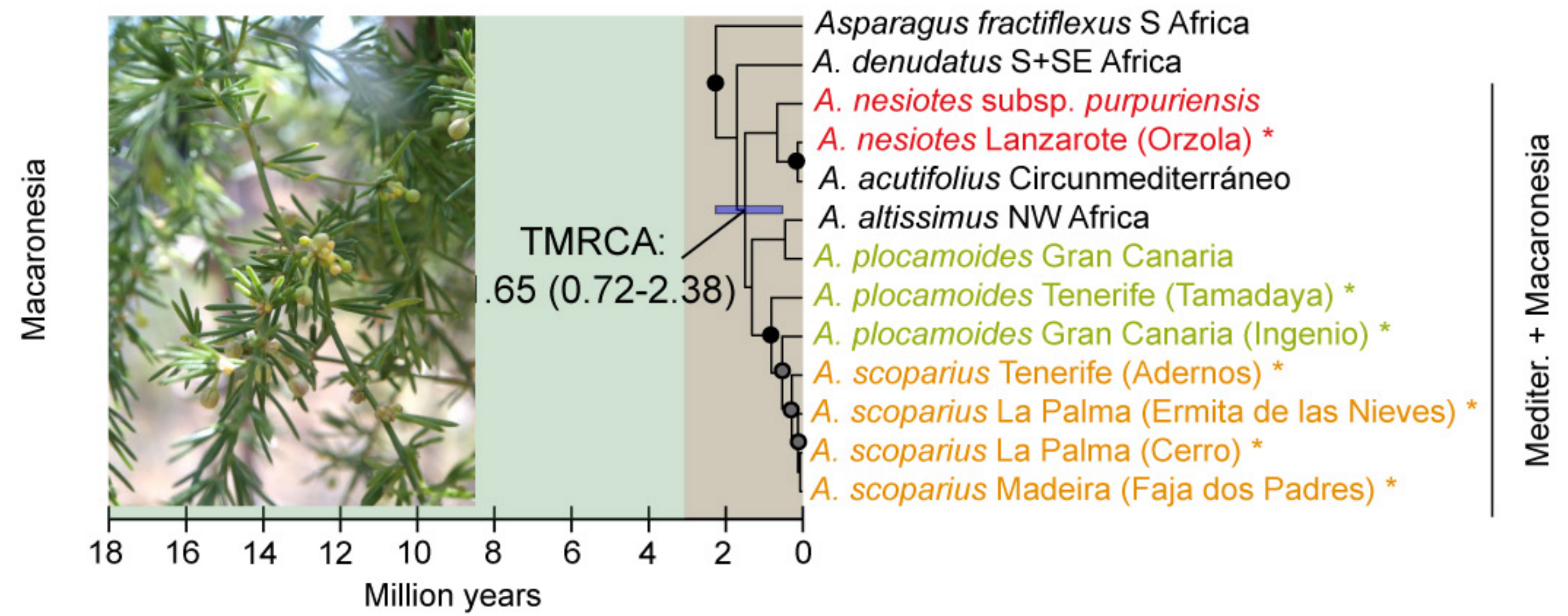




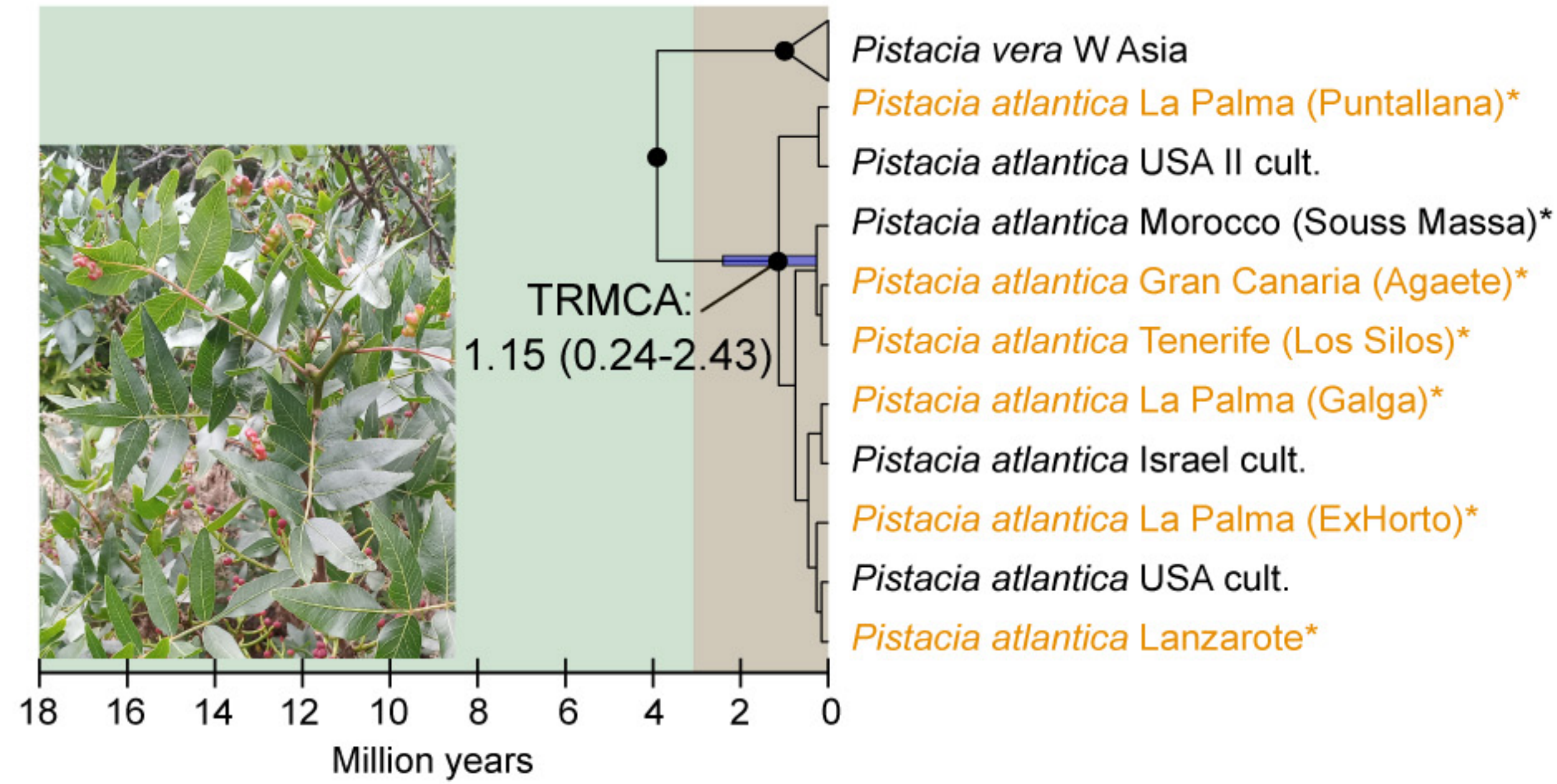
## A. *Asparagus* lineage I



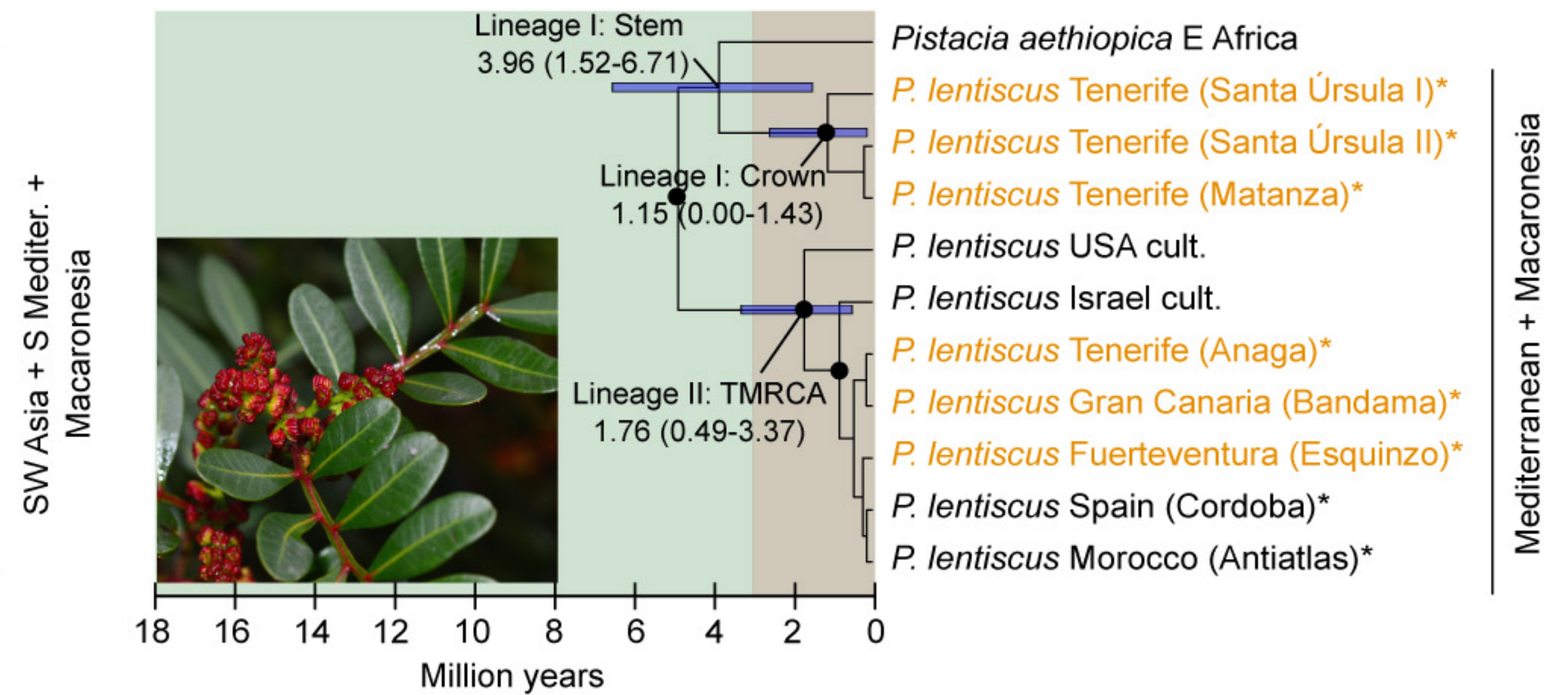
## B. *Asparagus* lineage II



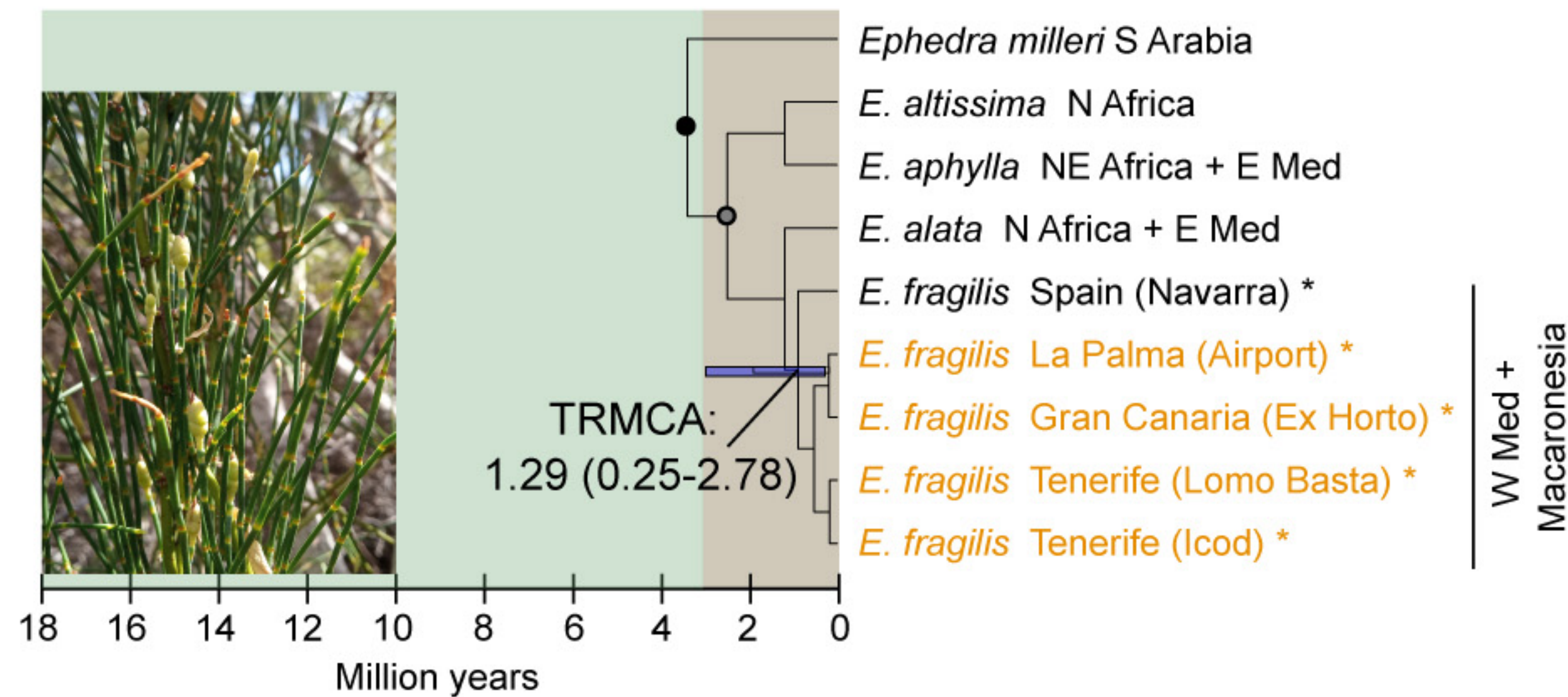
## C. *Pistacia atlantica*



## D. *Pistacia lentiscus*



## E. *Ephedra fragilis*



W Med +  
Macaronesia

### Statistical support values

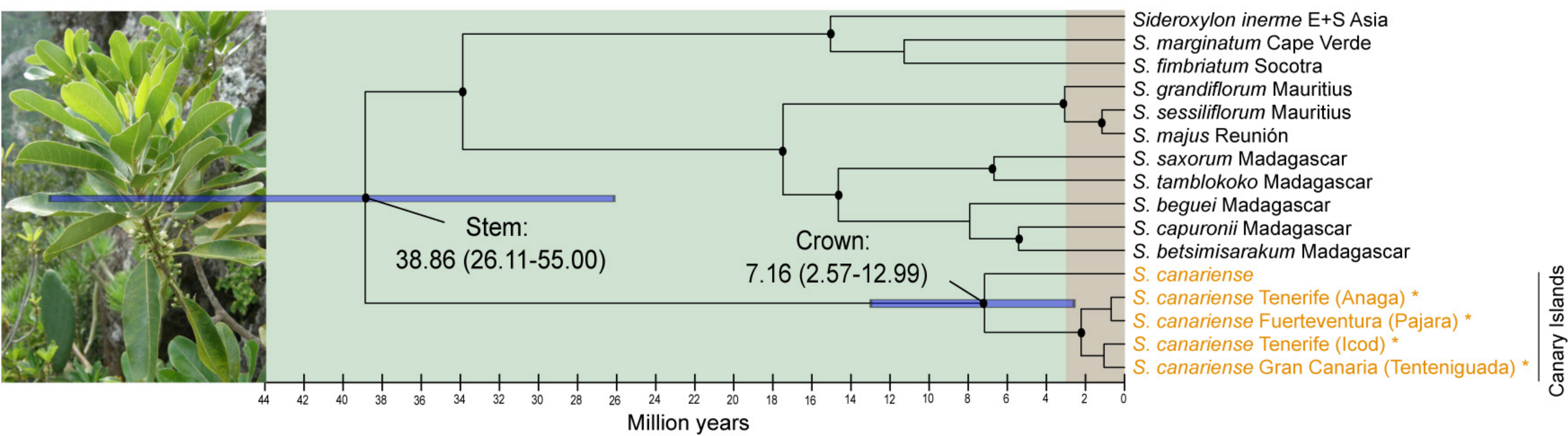
- PP > 0.90, BS > 70
- PP > 0.90, BS < 70
- PP < 0.90, BS > 70

### Vegetation types

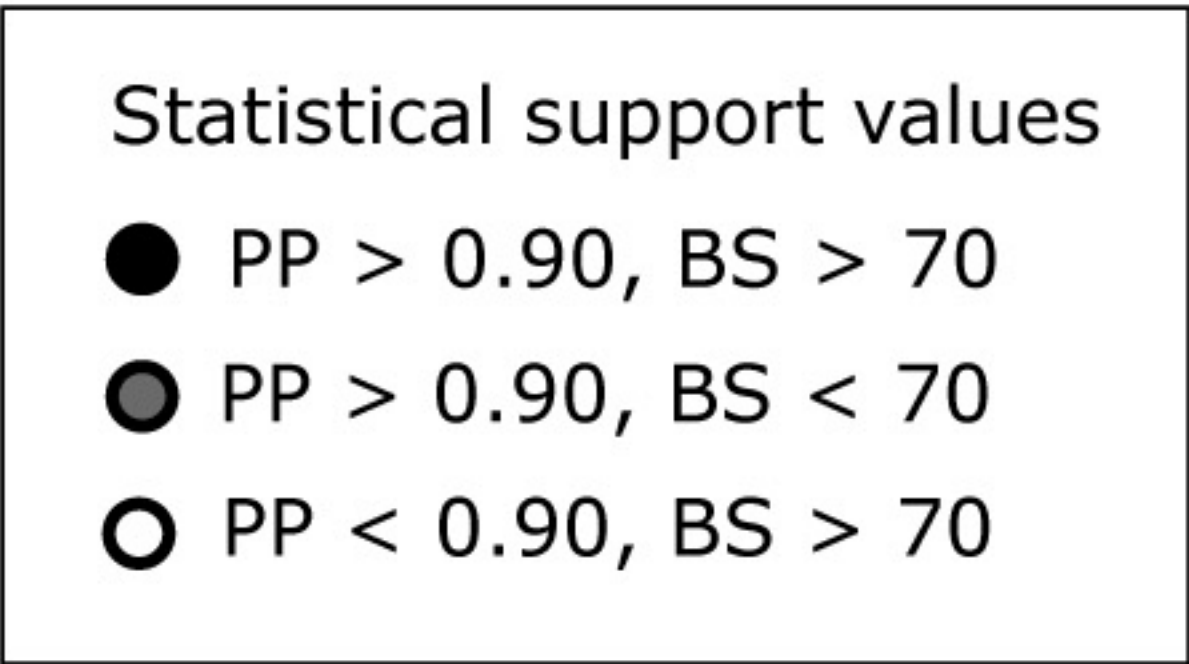
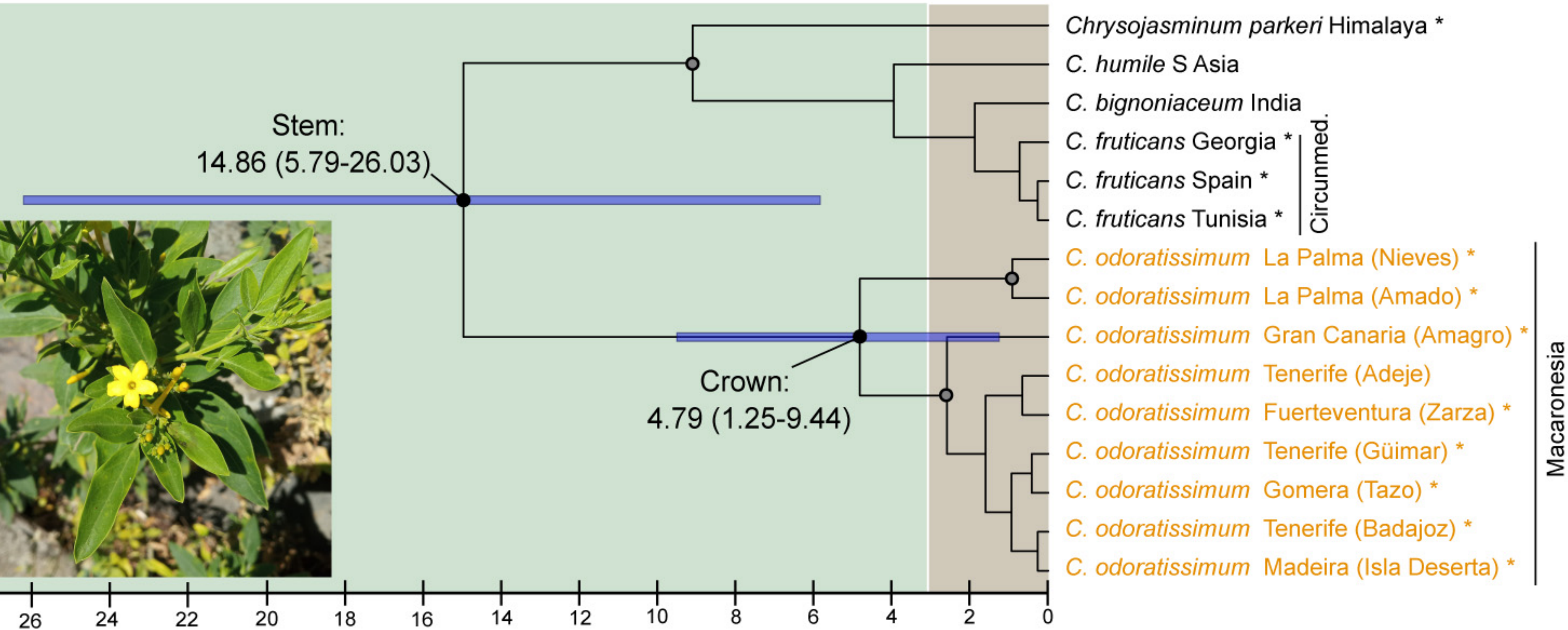
- Mainland
- Canarian thermophilous
- Canarian xerophytic shrubland
- Canarian laurel forest
- Canarian pine forest



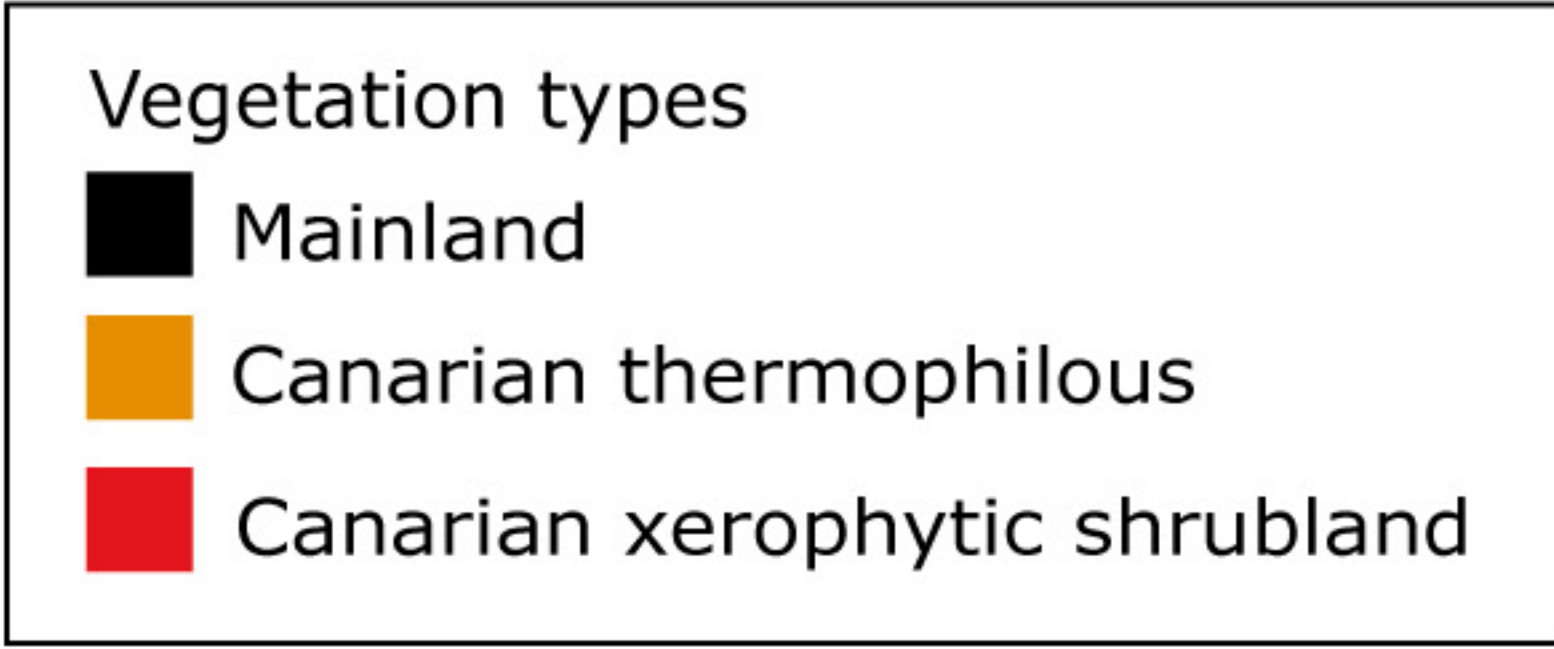
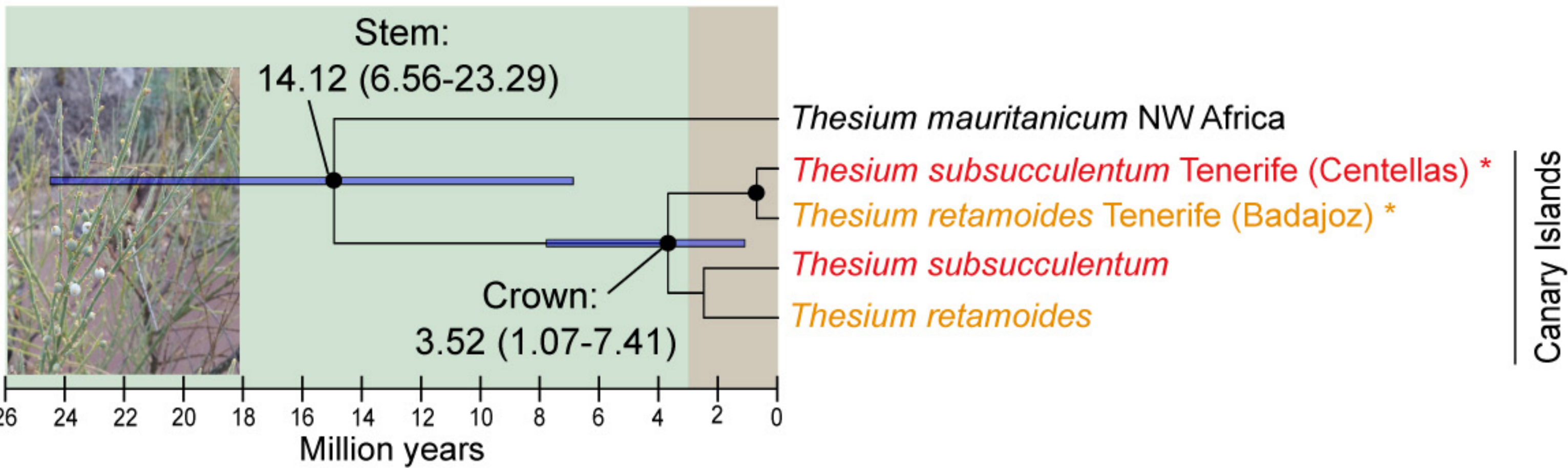
A. *Sideroxylon canariense*



B. *Chrysojasminum odoratissimum*

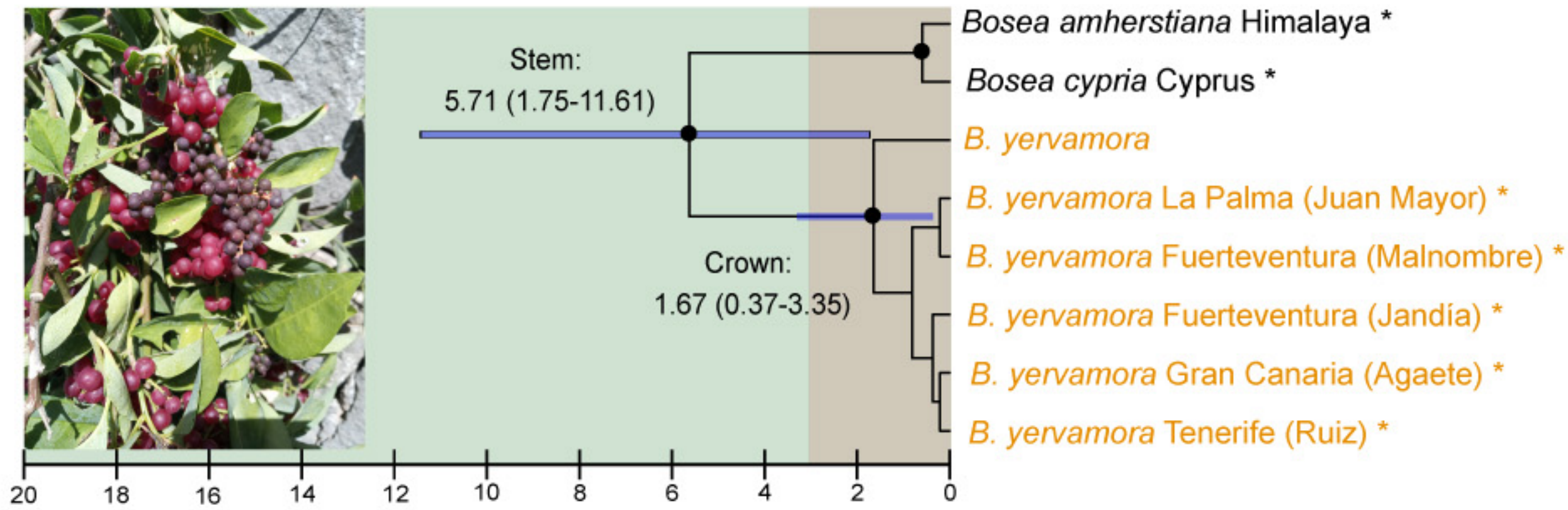


C. *Thesium* lineage

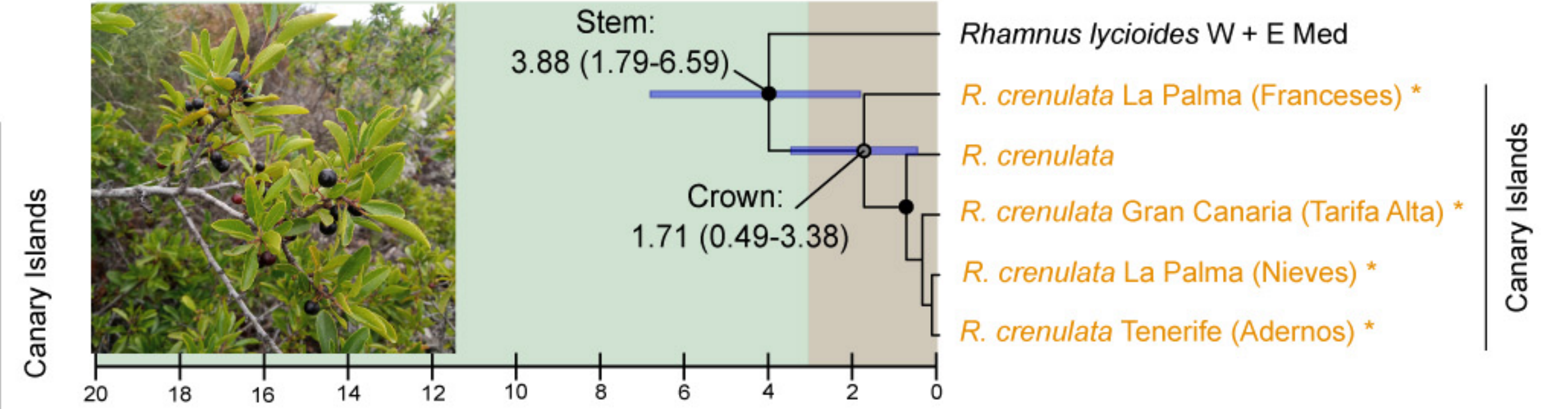




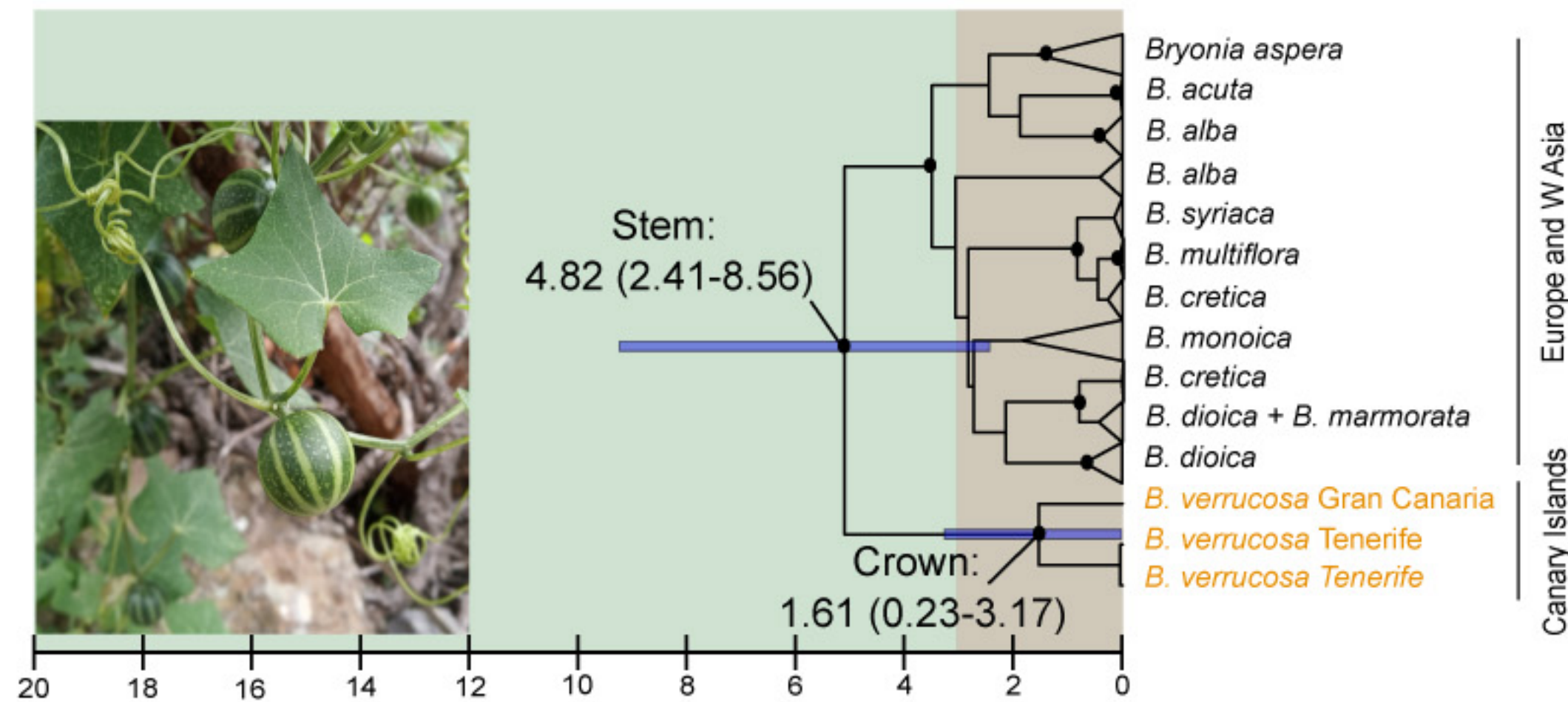
## A. *Bosea yervamora*



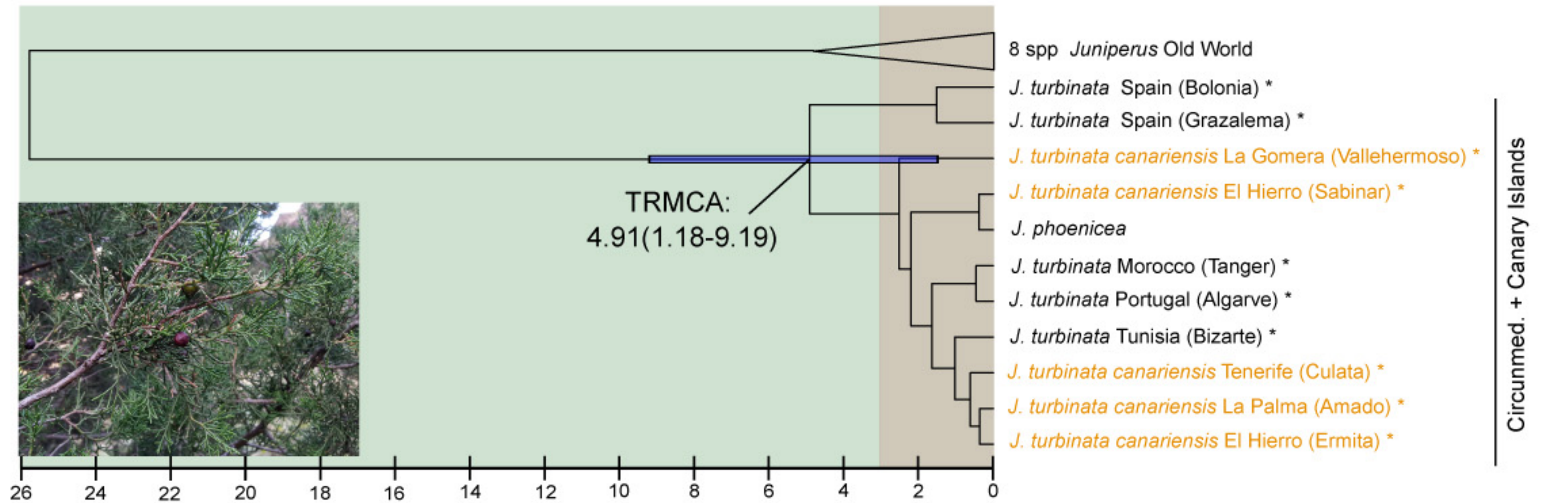
## B. *Rhamnus crenulata*



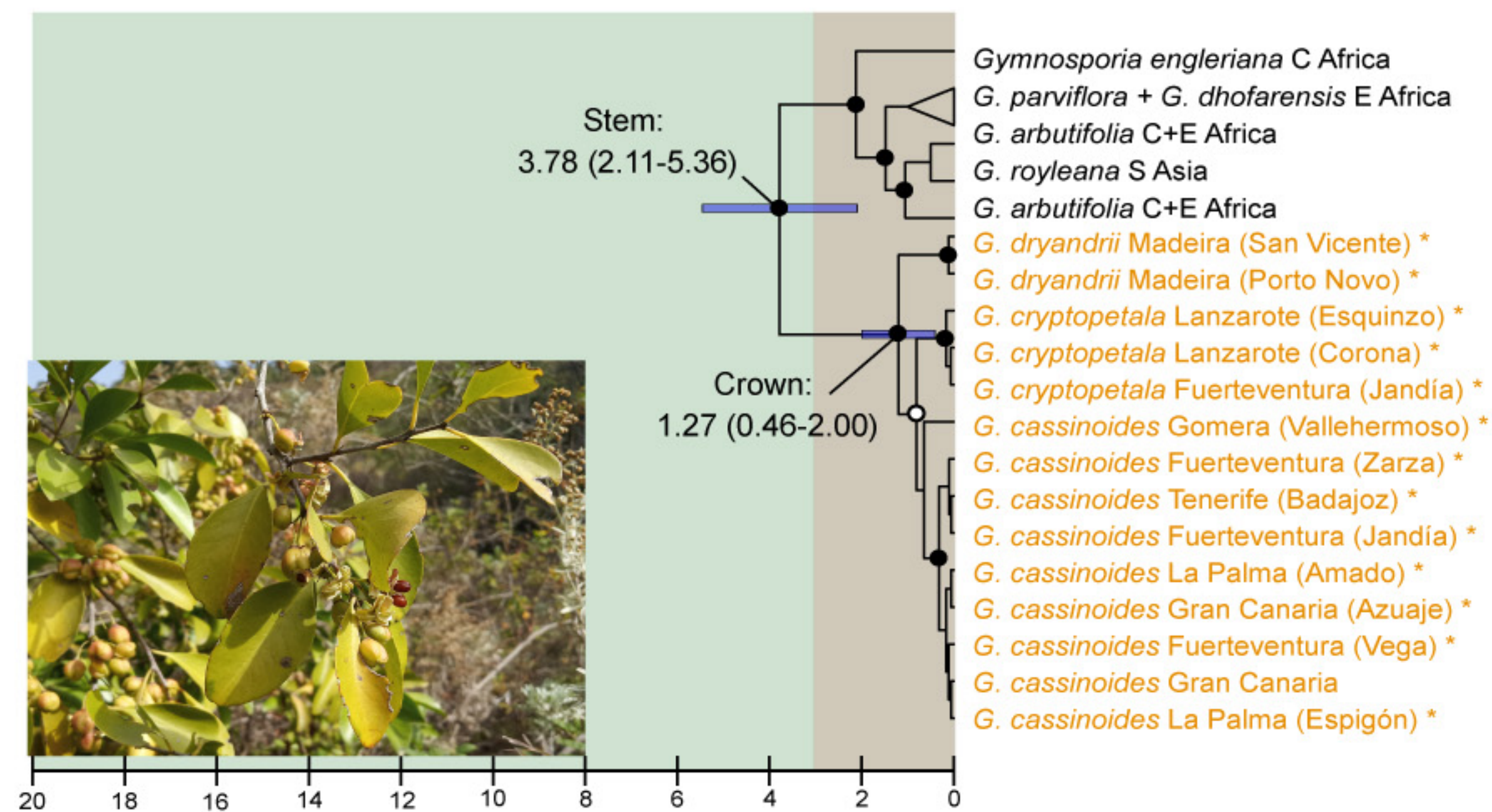
## C. *Bryonia verrucosa*



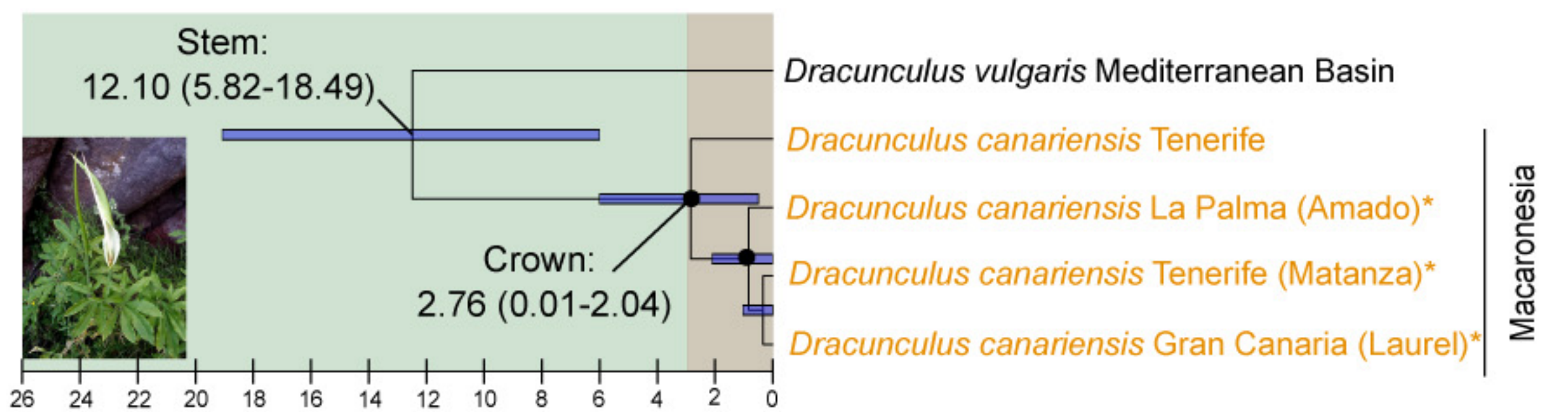
## D. *Juniperus turbinata* subsp. *canariensis*



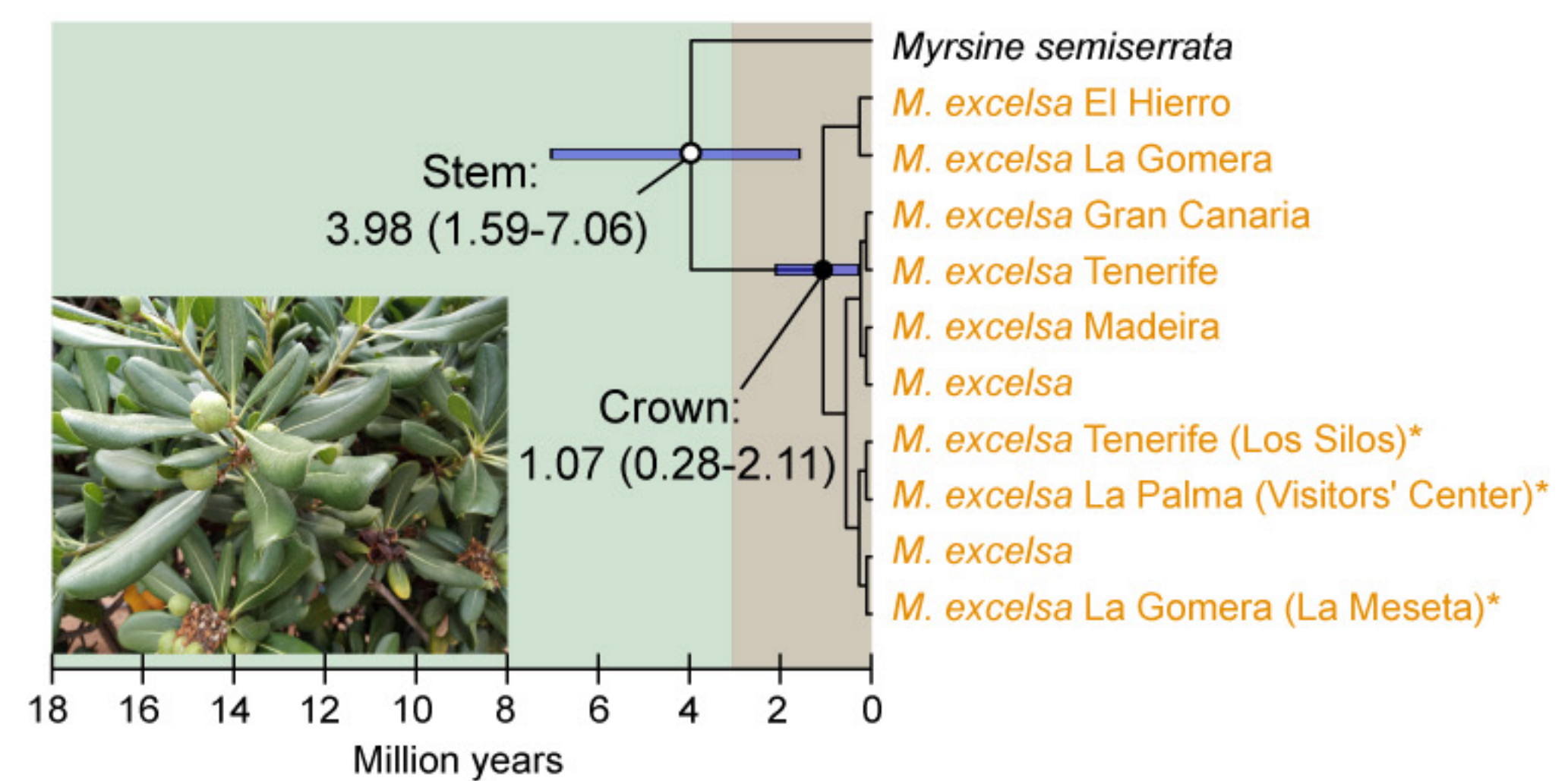
## E. *Gymnosporia* lineage



## F. *Dracunculus canariensis*



## G. *Myrsine excelsa*



### Statistical support values

- PP > 0.90, BS > 70
- PP > 0.90, BS < 70
- PP < 0.90, BS > 70

### Vegetation types

- Mainland
- Canarian thermophilous



