

## RESEARCH ARTICLE

# Phylogenetics and phylogeography of *Euphorbia canariensis* reveal an extreme Canarian-Asian disjunction but limited inter-island colonization

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## ABSTRACT

- *Euphorbia canariensis* is an iconic endemic species representative of the lowland xerophytic communities of the Canary Islands. It is widely distributed in the archipelago despite having diaspores unspecialized for long-distance dispersal. Here, we reconstructed the evolutionary history of *E. canariensis* at two levels: a time-calibrated phylogenetic analysis aimed at clarifying interspecific relationships and large-scale biogeographic patterns; and a phylogeographic study focused on the history of colonization across the Canary Islands.
- For the phylogenetic study, we sequenced the ITS region for *E. canariensis* and related species of *Euphorbia* sect. *Euphorbia*. For the phylogeographic study, we sequenced two cpDNA regions for 28 populations representing the distribution range of *E. canariensis*. The number of inter-island colonization events was explored using PAICE, a recently developed method that includes a sample size correction. Additionally, we used species distribution modelling (SDM) to evaluate environmental suitability for *E. canariensis* through time.
- Phylogenetic results supported a close relationship between *E. canariensis* and certain Southeast Asian species (*E. epiphylloides*, *E. lacei*, *E. sessiliflora*). In the Canaries, *E. canariensis* displayed a west-to-east colonization pattern, not conforming to the “progression rule”, i.e. the concordance between phylogeographic patterns and island emergence times. We estimated between 20 and 50 inter-island colonization events, all of them in the Quaternary, and SDM suggested a late Quaternary increase in environmental suitability for *E. canariensis*.
- The extreme biogeographic disjunction between Macaronesia and Southeast Asia (ca. 11,000 km) parallels that found in a few other genera (*Pinus*, *Dracaena*). We hypothesize that these disjunctions are better explained by extinction across north Africa and southwest Asia rather than long-distance dispersal. The relatively low number of inter-island colonization events across the Canaries is congruent with the low dispersal capabilities of *E. canariensis*.

## INTRODUCTION

Oceanic islands have attracted scientific interest for centuries because they provide a clear spatio-temporal framework for evolutionary research (Darwin 1859; Wallace 1880; Whittaker & Fernández-Palacios 2007). Occurrence of terrestrial organisms on oceanic islands can only be explained by long-distance dispersal (LDD) events, as these islands have emerged from the ocean floor and have had no contact with other land masses (Whittaker & Fernández-Palacios 2007; Vargas 2014). In the case of plants, specific morphological structures on diaspores have been traditionally associated with LDD processes (van der Pijl 1982), and these LDD traits are frequently assumed to be responsible for oceanic island colonization (Carlquist 1966). However, recent studies have shown that a large proportion of plant lineages on oceanic archipelagos lack diaspore

specializations for LDD (Vargas *et al.* 2014; Heleno & Vargas 2015). Nonetheless, a study of the xeric flora of the Canary Islands found significant differences in island colonization between species with LDD traits and those without them (Arjona *et al.* 2018). In particular, Arjona *et al.* (2018) observed that species with morphological specializations usually associated with LDD by animals (endozoochorous and epizoochorous diaspores) and by sea currents (thalassochorous diaspores) display wider distribution ranges in the Canarian archipelago, as measured by the number of occupied islands, than plants without such dispersal specialization.

Although general patterns of island colonization have been proposed for entire floras based on floristic studies, distribution ranges and habitat requirements (e.g. Vargas *et al.* 2012; Heleno & Vargas 2015; Arjona *et al.* 2018; Coello *et al.* 2021), accurate inference of colonization patterns for particular

species requires a combination of phylogenetic and phylogeographic analyses (Mairal *et al.* 2015; García-Verdugo *et al.* 2017; Coello *et al.* 2021, 2022; Soto *et al.* 2023). Additionally, as colonization is a two-stage process (i.e. it consists of both dispersal and establishment; van der Pijl 1982; Heleno & Vargas 2015), information about environmental suitability from species distribution models (SDM) helps us to understand patterns of inter-island colonization (Coello *et al.* 2021).

The Canarian archipelago is formed by seven main islands that are the result of a volcanic hotspot in the Atlantic Ocean, active since ca. 60 Ma, although the oldest extant island dates back to ca. 21 Ma (Troll & Carracedo 2016). Because of the proximity of the Canary Islands to the Mediterranean Basin and Africa throughout their geological history, the Canarian flora is mostly related to European and African lineages (Fernández-Palacios *et al.* 2011; del Arco Aguilar & Rodríguez Delgado 2018), which is consistent with a higher probability of island colonization from the closest land masses.

One of the most remarkable species of the Canarian flora is the succulent, cactus-like *Euphorbia canariensis* L. (Fig. 1), a major component of lowland xerophytic communities known, in Spanish, as “cardonal-tabaibal” (Bramwell & Bramwell 1974). Commonly known as “cardón”, *E. canariensis* is endemic to the Canarian archipelago and occurs on at least six of the seven major islands of the archipelago (Acebes *et al.* 2010). Although the LDD process is considered critical to allow oceanic island colonization (Nathan 2006; Traveset *et al.* 2014), *E. canariensis* does not display any LDD traits. For this reason, a low colonization ability would be expected for *E. canariensis*, which makes its wide distribution across the Canarian archipelago intriguing. Similarly, occurrence on multiple islands of the Canarian archipelago is also observed for other *Euphorbia* lineages that also lack LDD traits (e.g. Sun *et al.* 2016; Villaverde *et al.* 2018). In contrast to *E. canariensis*, the other cactus-like species of *Euphorbia* L. in the Canarian flora, *E. handiensis* Burchard, has the same fruit and seed types but is a narrow endemic to a single island (Fuerteventura). Both *E. canariensis* and *E. handiensis* are included in *Euphorbia* subg. *Euphorbia* sect. *Euphorbia* (Bruyns *et al.* 2011; Dorsey *et al.* 2013). However, *E. canariensis* appears to belong to an early-diverging clade together with *E. epiphyllodes* Kurz, sister to the rest of section *Euphorbia* (except for the earlier-diverging *E. abdelkuri* Balf.f.), while *E. handiensis* is distantly related to *E. canariensis* and positioned within one of the major clades of the section (Bruyns *et al.* 2011).

It has been considered that *E. canariensis* displays a Rand Flora pattern (del Arco Aguilar & Rodríguez Delgado 2018). This is a biogeographic pattern in which multiple unrelated lineages display a similar disjunct distribution along the edges of Africa and adjacent areas (such as the southern Arabian Peninsula, Macaronesia, Socotra and Madagascar; Sanmartín *et al.* 2010; Pokorný *et al.* 2015; Rincón-Barrado *et al.* 2021). Another example of this pattern in the genus *Euphorbia* is the clade of *E. balsamifera* Ait. and close relatives, which display a disjunction between Northwest Africa and the Canary Islands on one side, and the southern Arabian Peninsula and the Horn of Africa on the other side (Riina *et al.* 2021). The case of *E. canariensis* appears to be more extreme, as Bruyns *et al.* (2011) inferred that it is sister to the Southeast Asian *E. epiphyllodes*. This is an endangered species with no more than 250 individuals in Saddle Peak, North Andaman Island (Indian Ocean)

(IUCN 1998), which is located ca. 11,000 km away from the Canarian archipelago. Unfortunately, the close relationship between the two species was not well supported (Bruyns *et al.* 2011), and later studies investigating phylogenetic relationships among *Euphorbia* species did not include *E. canariensis* (Dorsey *et al.* 2013; Horn *et al.* 2014). In addition, there are other Asian species that are morphologically similar to *E. epiphyllodes*, i.e. *E. sessiliflora* Roxb. (Nepal, China, Laos, Myanmar, Thailand and Vietnam) and *E. lacei* Craib (Thailand, Myanmar, Vietnam, Indonesia and Philippines). In fact, *E. lacei* is possibly related to the Socotran *E. abdelkuri* (Dorsey *et al.* 2013), and that may be relevant to resolving the phylogenetic relationships of *E. canariensis*. If the close relationship between *E. canariensis* and the Southeast Asian species were corroborated, it would be biogeographically remarkable, given the large geographic distance separating these species (ca. 11,000 km). Extinction in intervening regions between the distribution ranges of *E. canariensis* and its Asian relatives is the most probable explanation for this disjunction. However, knowledge of the colonization abilities of *E. canariensis* would be useful to rule out an alternative hypothesis based on LDD. It would also be relevant to estimate divergence times among species and compare them with the onset of biogeographic barriers between the two territories (e.g. the aridification of Saharan, Arabian and Syrian deserts; Sanmartín 2003; Oberprieler 2005). Bruyns *et al.* (2011) suggested a divergence between *E. canariensis* and *E. epiphyllodes* in the late Miocene (ca. 9 Ma), but the lack of support for this clade, the wide range of uncertainty for the divergence time estimate and the absence of both *E. sessiliflora* and *E. lacei* in that study, make further investigation necessary.

In this study, we analysed the evolutionary history of *E. canariensis* at two levels: (i) a phylogenetic approach to clarify the relationships of *E. canariensis* and to evaluate the potential biogeographic disjunction between the Canary Islands and Southeast Asia; and (ii) a phylogeographic approach to study the pattern of colonization across the islands of the Canarian archipelago in relation to habitat availability for this species through time. We hypothesized that the lack of LDD traits has resulted in a limited number of long-distance colonization events, and therefore the wide disjunction between *E. canariensis* and its Asian relatives is more likely explained by extinction across northern Africa and southwestern Asia. In particular, the following specific objectives were addressed for *E. canariensis*: (i) to investigate its phylogenetic relationships using new ITS sequences for *E. canariensis*, *E. abdelkuri*, *E. epiphyllodes*, *E. handiensis*, *E. officinarum* L. and *E. sessiliflora*; (ii) to estimate divergence times; (iii) to assess the geographic distribution of plastid DNA diversity across the Canarian archipelago; (iv) to estimate the number of inter-island colonization events; and (v) to explore the extent of suitable habitats for the present and past periods in the Canary Islands.

## MATERIAL AND METHODS

### Study species and sampling

*Euphorbia canariensis* (Euphorbiaceae; Fig. 1) is a large cactus-like succulent shrub that produces latex. It usually has four-angled branches and curved spines disposed in pairs along the angles. Mature plants in the field can reach up to 3–4 m in



**Fig. 1.** A large, densely branched individual of *Euphorbia canariensis* in its natural habitat (18 April 2018). The two right-hand insets show a fruiting branch (27 June 2015) and three cyathia bearing highly reduced flowers (27 April 2018). Photos taken in Teno (Tenerife) by Alberto J. Coello and Ricarda Riina.

height (Bramwell & Bramwell 2001). *Euphorbia canariensis* is endemic to the Canary Islands, a volcanic archipelago located between 27.5°–29.5° N and 13°–18.5° W, less than 100 km from the coast of Africa. The archipelago currently comprises seven main islands that emerged from a mantle plume, among which the easternmost islands are the oldest. The age of current emerged islands ranges from >20 Ma (Fuerteventura) to ca. 1 Ma (El Hierro) according to Troll & Carracedo (2016). *Euphorbia canariensis* occurs on every major island of the archipelago except Lanzarote (Acebes *et al.* 2010). Although there are isolated historical references to its presence on Lanzarote, wild populations have not been seen on this island in recent times (Bramwell & Bramwell 2001). The species inhabits rocky slopes, cliffs and lava fields, from sea level to 900 m (Bramwell & Bramwell 1974). Immature capsules have the appearance of a fruit attractive to birds (bright red with a fleshy appearance; Fig. 1), but the immature tissue of the fruit is full of irritant and toxic latex that makes fruits unpalatable to frugivorous animals. Mature capsules are light red-brown, dry and also unsuitable for endozoochory. Seeds are dispersed to the surroundings of the mother plant by explosive dehiscence of capsules (ballochorous dispersal; see Berg 1990). There are reports of the consumption of seeds by a granivorous bird (rock pigeon) in another Canarian *Euphorbia* with similar seeds (*E. balsamifera*; Berg 1990) but, as far as we know, there are no reports of this bird also feeding on *E. canariensis* seeds. Furthermore, mature fruits and seeds of *E. canariensis* lack hairs, adhesive structures (epizoochorous), wings (anemochorous) or the ability to survive in seawater (thalassochorous). Given all these characteristics, the dispersal traits of *E. canariensis* fruits were categorized as not specialized in LDD by Arjona *et al.* (2020). Additionally, vegetative reproduction is limited in *E. canariensis*, and the rooting of stem fragments is uncommon (Kunkel & Kunkel 1978).

We sampled fresh material of *E. canariensis* for 92 individuals from 29 populations (up to five individuals per population) on the six major islands of the Canarian archipelago

where wild populations of the species currently occur (additionally including an old, apparently cultivated individual from Lanzarote; Table S1). Since *E. canariensis* leaves are highly reduced and quickly deciduous, we collected epidermal fragments from branches of each sampled individual. Based on previous phylogenetic results and morphological similarity, we also obtained silica-dried tissue from one cultivated individual of *E. abdelkuri*, one cultivated individual of *E. epiphylloides*, two wild individuals of *E. handiensis*, one cultivated individual of *E. sessiliflora* and two wild individuals of *E. officinarum* (Tables S1 and S2). These species were chosen to obtain a deeper insight into the phylogenetic relationships of *E. canariensis*, in combination with sequences of 24 species available in GenBank for *Euphorbia* sect. *Euphorbia* (considered the ingroup; see below) (Horn *et al.* 2012, 2014). In particular, we sequenced *E. sessiliflora* (Roxburgh & Carey 1832) because it has been described as morphologically similar to *E. epiphylloides*, and therefore could also be related to *E. canariensis*. *Euphorbia handiensis* was sampled to further corroborate the lack of a close phylogenetic relationship with *E. canariensis* and its close relationship to the northwest African *E. officinarum* (= *E. echinus* Hook.f. & Coss.) inferred by Bruyns *et al.* (2011). We extracted total genomic DNA from all samples using the CTAB method (Doyle & Doyle 1987; Cullings 1992).

### DNA sequencing

For the phylogenetic analysis (see below), we sequenced the ITS region from seven individuals of *E. canariensis* and from six individuals of four other *Euphorbia* species mentioned above. Horn *et al.* (2012, 2014) used 10 concatenated regions to build the most recent time-calibrated phylogeny of the genus *Euphorbia*, but we focused on the ITS region because analysis based on this nuclear DNA region alone accurately reflects major phylogenetic patterns within the genus (and particularly in *E. sect. Euphorbia*). This approach also allowed us to take advantage of the wide availability of ITS sequences of

**Table 1.** Primers used for amplification and sequencing of two cpDNA regions (*trnS*–*trnG* and *trnQ*–*rps16*) and the nuclear ITS region for *Euphorbia* species.

region	primer sequences	reference
<i>trnS</i> – <i>trnG</i>	<i>trnS</i> <sup>(GCU)</sup> : GCC GCT TTA GTC CAC TCA GC <i>trnG</i> <sup>(UCC)</sup> : GAA CGA ATC ACA CTT TTA CCA	Hamilton (1999)
<i>trnS</i> – <i>trnG</i>	<i>trnS</i> <sup>(GCU)</sup> : GCC GCT TTA GTC CAC TCA GC <i>trnSG_Euph_int-R</i> : CTA TTT CAA TTG CTA part 1 TAT TTC	This work
<i>trnS</i> – <i>trnG</i>	<i>trnSG_Euph_int-F</i> : CTA TAT AAT TCG AAA part 2 TTG TC <i>trnG</i> <sup>(UCC)</sup> : GAA CGA ATC ACA CTT TTA CCA	This work
<i>trnQ</i> – <i>rps16</i>	<i>trnQ</i> <sup>(UUG)</sup> : GCG TGG CCA AGY GGT AAG GC rpS16x1: GTT GCT TTY TAC CAC ATC GTT T	Shaw et al. (2007)
ITS	ITS5: GGA AGT AAA AGT CGT AAC AAG G ITS4: TCC TCC GCT TAT TGA TAT GC	White et al. (1990)

*Euphorbia* in GenBank. In particular, we retrieved the previously published alignment of 182 ITS sequences from Horn *et al.* (2012) (Table S2). Additionally, we obtained the only ITS sequence of *E. sessiliflora* available in GenBank (Table S2). We also downloaded two sequences of *E. lacei*, given the early-diverging position of this species in *E. sect. Euphorbia* (Dorsey *et al.* 2013) and, therefore, its potential relationship with *E. canariensis* (see Table S2). Finally, we retrieved a sequence of *E. antiquorum* because this is the type species of *E. sect. Euphorbia* (Dorsey *et al.* 2013) (Table S2).

For the phylogeographic analysis, we first conducted a pilot study to select the most variable among 17 cpDNA regions with sequence variation in angiosperms (Shaw *et al.* 2007). As a result, we selected two cpDNA regions: *trnS*–*trnG* and *trnQ*–*rps16* (Table 1). These regions were also sequenced for *E. abdelkuri*, *E. epiphyllodes* and *E. handiensis* as part of the outgroup for the phylogeographic analysis (Table S1).

The studied DNA regions (ITS, *trnS*–*trnG* and *trnQ*–*rps16*) were amplified by conventional PCR in an Eppendorf Mastercycler Eppgradient S with standard primers (Table 1). After 2 min of pretreatment at 94 °C, PCR consisted of 30–35 cycles of 1 min at 94 °C, 1 min at 52 °C (48 °C in some cases) and 1 min at 72 °C, followed by a final elongation period of 10 min at 72 °C. In every 25 µl of reaction, 1 µl bovine serum albumin (BSA) at 1 mg·ml<sup>−1</sup> was included to improve the amplification efficiency. For problematic samples, small modifications were implemented, and internal primers were used (Table 1). PCR products were sequenced by Macrogen (Madrid, Spain), and sequences were assembled in Geneious 11.0.4 (Kearse *et al.* 2012). Ambiguities were coded as “N”, including potential additive polymorphic sites in ITS sequences.

### Phylogenetic analysis

To assess the phylogenetic position of *E. canariensis* and estimate divergence times, new ITS sequences and those retrieved from GenBank were aligned using MAFFT 7.388 (Katoh *et al.* 2002). In order to avoid several uncertain positions detected after visual exploration, we manually deleted a few problematic regions from the alignment because of the

presence of long gaps for a considerable number of samples that resulted in ambiguous alignments (i.e. matrix stretches between positions 65 and 70; 87 and 90; 110 and 162; 197 and 204; 623 and 650; Alignment S1 in Supporting Information). The resulting alignment included 369 (67%) polymorphic sites. We performed a time-calibrated Bayesian phylogenetic analysis in BEAST 1.10.4 (Drummond & Rambaut 2007) using the same calibration points as Horn *et al.* (2014), all of them placed at the crown nodes of the corresponding clades (Figure S1): (i) a fossil of *Hippomanoidea* (exponential prior distribution with an offset of 43 Ma and a mean of 2.5), (ii) a secondary calibration of the root at 69.08 Ma (normal distribution with standard deviation of 6.96 Ma), and (iii) a secondary calibration of Euphorbiaceae excluding Stomatocalyceae at 52.79 Ma (normal distribution with a standard deviation of 5.63 Ma). We applied a GTR + I + G model of nucleotide substitution selected by the AIC criterion in jModelTest 2.1.10 (Darriba *et al.* 2012). An uncorrelated relaxed clock with a log-normal distribution was implemented, with a birth–death speciation process as tree prior. We executed two runs of 100 million generations each, sampled every 10,000 generations, and applied a 10% burn-in. The adequacy of both runs was analysed in Tracer 1.7.1 (Rambaut *et al.* 2018), these were combined using LogCombiner (discarding the burn-in), and trees were summarized in a maximum clade credibility tree using median heights with TreeAnnotator.

### Phylogeographic analysis

Sequences of the *trnS*–*trnG* and *trnQ*–*rps16* regions from *E. canariensis* and the outgroup species (*E. abdelkuri*, *E. epiphyllodes*, *E. handiensis*) (Table S1) were aligned with MAFFT 7.388 (Katoh *et al.* 2002). Minor errors in the resulting alignment were corrected by visual inspection. Furthermore, a 108-bp stretch of the *trnS*–*trnG* region (positions 326–433 in the final alignment; Alignment S2 in Supporting Information) was excluded for downstream analyses because preliminary exploration indicated that this fragment contained several highly homoplastic gaps and variable positions of uncertain alignment. The concatenated alignment of both regions included 45 (4.6%) polymorphic sites and was used to determine the genealogical relationships among haplotypes using the statistical parsimony algorithm (Templeton *et al.* 1992) implemented in TCS 1.21 (Clement *et al.* 2000). Haplotype relationships were calculated using a 95% confidence limit and gaps were treated as missing data. Additionally, to facilitate the connection between the outgroup species and *E. canariensis* haplotypes, we repeated the TCS analysis by reducing the confidence limit to 90%.

### Estimation of inter-island colonization events

The number of inter-island colonization events was estimated based on phylogeographic data using the recently developed method PAICE (Phylogeographic Analysis of Island Colonization Events; Coello *et al.* 2022), which includes a sample size correction. We did not include the individual from Lanzarote because it was apparently cultivated, and this species is currently considered extinct on this island. Nevertheless, Lanzarote and Fuerteventura were considered a single island unit (Mahan) because these islands were connected during the Last

Glacial Maximum (Troll & Carracedo 2016). First, we inferred the minimum number of colonization events required to explain the geographic distribution of *E. canariensis* haplotypes using the *colonization* function of the *PAICE* package (Coello *et al.* 2022). We also calculated asymptotic estimators of colonization events. With this aim, we built rarefaction curves for both sampling variables (genetic and field sampling) using the *rarecol* function of the *PAICE* package. We replicated rarefaction curves a number of times equal to five times the number of levels of each variable, i.e. 140 replicates for field sampling (28 levels, one for each of the 28 populations sampled after the exclusion of the Lanzarote individual, as populations were used as field sampling units, Table S1) and 75 replicates for genetic sampling (15 levels, one for each of the 13 variable positions detected for cpDNA of *E. canariensis*, in addition to one variable position connecting *E. canariensis* populations to the outgroup and the case of 0 variable position, i.e. no genetic information considered) (for details see Coello *et al.* 2022).

These rarefaction curves were later used to calculate the asymptotic estimators of colonization events using the *maxCol* function of the *PAICE* package. We deleted extreme values (i.e. those below the 2.5% quantile and above the 97.5% quantile) using argument *del* = 0.05, and calculated the 95% confidence interval of asymptotic estimators using the argument *level* = 0.95. Finally, we used the argument *method* = 1 to allow the algorithm to fit the accumulation curve of colonization events with fewer parameters if curves could not be fitted initially. As the outgroup was connected to *E. canariensis* at two points in the haplotype network (two missing haplotypes between haplotypes A and B; see Results), we repeated the analysis considering each of the two inferred ancestral haplotypes (see Coello *et al.* 2022 for additional information on PAICE).

For comparison with PAICE results, the colonization history of *E. canariensis* was estimated based on the number of inter-island colonization events as inferred by a Bayesian discrete phylogeographic analysis (DPA; Lemey *et al.* 2009) in BEAST 1.10.4 (Drummond & Rambaut 2007), with *E. abdelkuri*, *E. epiphylloides* and *E. handiensis* as outgroup. The *trnS-trnG* and *trnQ-rps16* regions were used with the HKY + G substitution model following the AIC criterion implemented in jModelTest 2.1.10. We constrained *E. canariensis* as a monophyletic group based on our phylogenetic results and implemented a uniform prior distribution for the crown age between 0.31 and 2.79 Ma, and another calibration point at the node representing the most recent common ancestor of *E. sect. Euphorbia* with a uniform prior distribution for the crown age between 9.32 and 18.25 Ma. All calibration points were obtained from the time-calibrated phylogenetic analysis described above. We used an asymmetric substitution model to reconstruct ancestral areas and a Bayesian stochastic search variable selection (BSSVS) to infer statistically supported migration routes. Six areas were defined: Mahan (palaeoisland comprising Fuerteventura and Lanzarote), Gran Canaria, Tenerife, La Gomera, El Hierro and La Palma. We coded the continental outgroup species (*E. abdelkuri*, *E. epiphylloides*, *E. handiensis*) as missing data to exclude colonization of the Canarian archipelago from the continent and thus focused on inferring inter-island colonization events of *E. canariensis*. An uncorrelated relaxed clock with a log-normal distribution was implemented for the DNA partition, and a strict clock for the area partition, with a simple constant size coalescent tree prior to facilitate convergence. We

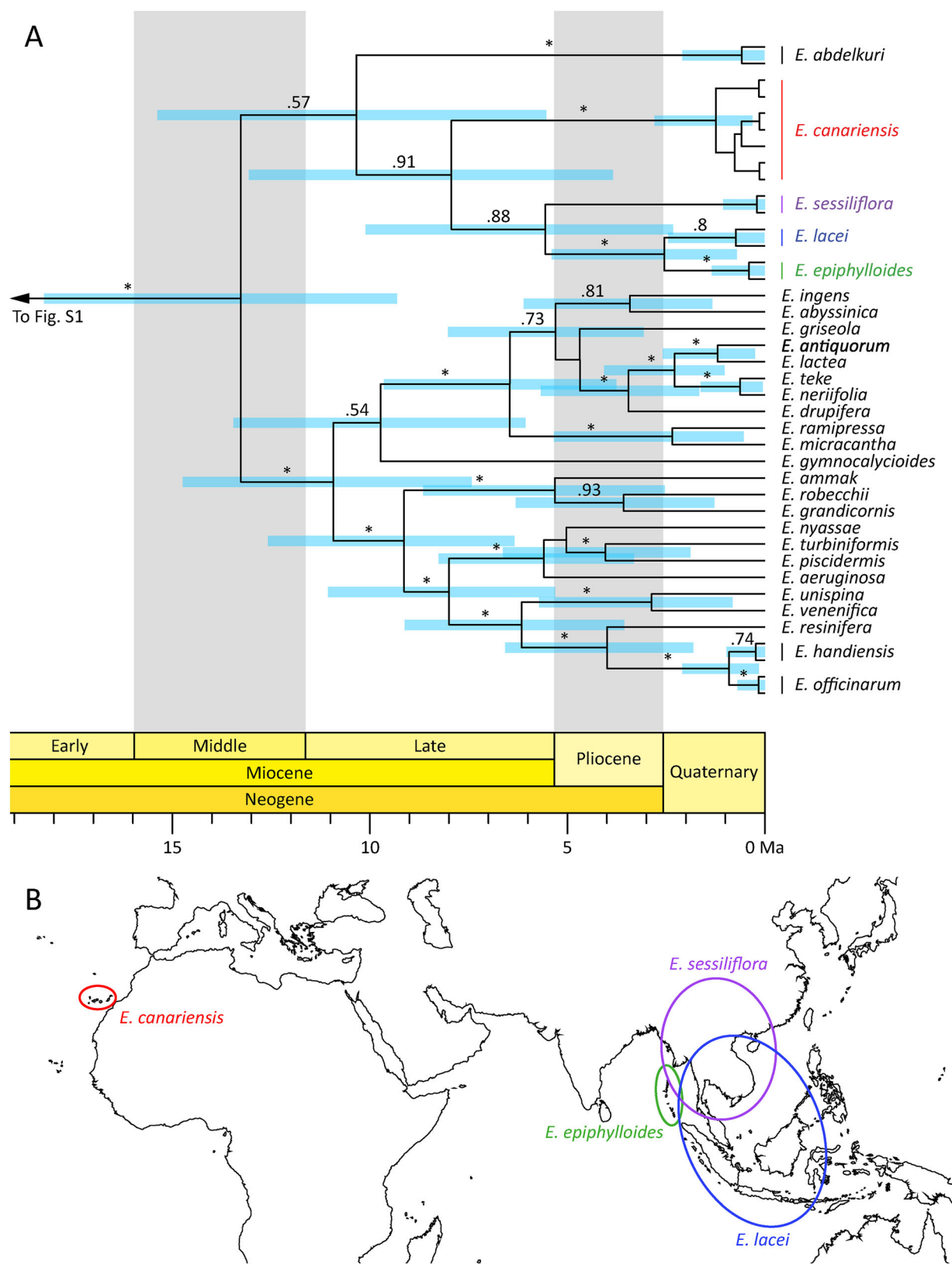
performed three runs of 100 million generations each with a 10% burn-in, and convergence between chains was confirmed with Tracer 1.7.1. All runs were combined using LogCombiner (without burn-in) and trees were summarized in a maximum clade credibility tree using median heights in TreeAnnotator. To calculate Bayes factors (BF) of migration routes we used Spread3 0.9.6 (Bielejec *et al.* 2016), and BF values above 3 were considered as evidence for connection between areas (Kass & Raftery 1995).

### Species distribution modelling

For SDM, occurrences of *E. canariensis* were obtained from two sources: locations of populations sampled for the phylogeographic analysis (Table S1); and occurrences obtained from GBIF (GBIF.org 2021) after applying a filter to exclude those with error >1 km in their coordinates (occurrences without coordinates were also excluded). We only considered GBIF occurrences from well-established databases (i.e. Anthos, MAGRAMA, iNaturalist and Observation.org). In the case of citizen science databases (i.e. iNaturalist and Observation.org), we carefully checked every occurrence that met the above requirements to validate its accuracy. Specifically, based on the unequivocal plant characteristics (large size, cactus-like features), we only selected occurrences with attached photos in order to confirm the identification of every specimen. We also checked if the environment shown in the photos corresponded to the coordinates (e.g. we discarded observations in which the sea was close in the photos but coordinates did not correspond to coastal localities, indicating that coordinates were wrong) and whether the environment of the coordinates corresponded to the habitat of the species (e.g. we discarded occurrences at high elevations on Mount Teide in Tenerife). To reduce spatial bias due to oversampling, we followed suggestions of Boria *et al.* (2014) and applied a buffer of 0.05° (i.e. ca. 3 arc-min). With this procedure, we obtained a final set of 53 occurrences that were used in SDM (Table S3).

We downloaded 19 bioclimatic variables for current conditions from CHELSA (Karger *et al.* 2017) at 30 arc-second resolution, and 10 edaphic layers from SoilGrids (Hengl *et al.* 2017) at 250 m resolution (Table S4). We cropped all layers to the extent of the study area, spanning from 19° to 13° W and from 27° to 30° N. The study area comprised the entire Canarian archipelago, including the six main islands where *E. canariensis* currently occurs (Acebes *et al.* 2010) and Lanzarote (see above). For edaphic layers, we reduced resolution to match that of bioclimatic layers using the *resample* function of the R package *raster* with the bilinear method (i.e. argument *method* = "bilinear"). We followed recommendations of Dormann *et al.* (2013) and discarded variables with  $|r| > 0.85$  and a variance inflation factor  $VIF > 10$ . VIFs were calculated using the R package *HH* (Heiberger 2017). This procedure resulted in a selection of eight variables: temperature seasonality (bio 4), minimum temperature of the coldest month (bio 6), precipitation seasonality (bio 15), precipitation of the driest quarter (bio 17), volumetric percentage of coarse fragments with size >2 mm (eda 2), weight percentage of clay particles with size <0.0002 mm (eda 3), cation exchange capacity of soil (eda 6) and pH index measured in water solution (eda 9). Climatic variables were also downloaded from PaleoClim (Brown *et al.* 2018) for the following time slices of the late Quaternary: Meghalayan (late-Holocene: 0.3–4.2 ka),

Northgrippian (mid-Holocene: 4.2–8.326 ka), Greenlandian (early-Holocene: 8.326–11.7 ka), Younger Dryas Stadial (11.7–12.9 ka), Bølling-Allerød (12.9–14.7 ka), Heinrich Stadial 1 (14.7–17.0 ka), Last Glacial Maximum (ca. 22 ka) and Last Interglacial (ca. 130 ka). Three other time slices (MIS19, ca. 787 ka; mid-Pliocene warm period, 3.205 Ma; and M2, ca.



**Fig. 2.** Phylogenetic relationships, divergence times and distribution ranges of *Euphorbia canariensis* and its closest living relatives (*E. epiphyllodes*, *E. lacei*, *E. sessiliflora*). A: Time-calibrated Bayesian phylogenetic analysis of representative lineages of *E. sect. Euphorbia* (the complete phylogeny is provided in Figure S1). Numbers above branches indicate posterior probabilities (PP) when  $PP \geq 0.5$  and asterisks (\*) indicate  $PP \geq 0.95$ . Bars at nodes with  $PP \geq 0.5$  indicate 95% highest posterior density intervals for ages. *Euphorbia canariensis* and its closest extant relatives are highlighted in colour. B: Approximate distribution ranges of *E. canariensis* and its closest living relatives according to POWO (2023).

3.3 Ma) were not used because not all bioclimatic variables were available for them. We assumed that edaphic variables were constant throughout all periods and therefore used the same edaphic layers for every period (they were resampled to match the extension and resolution of climatic layers).

We estimated the current distribution model of *E. canariensis* by employing the maximum entropy algorithm implemented in Maxent 3.4.1 (Phillips *et al.* 2006), as it performs well when using presence-only data (Elith *et al.* 2006). We conducted 100 sub-sample replicates. For each replicate, we randomly selected 60% of occurrences for training the algorithm and the other 40% of occurrences for testing. The model based on current conditions was projected to late Quaternary time slices (see above) to reconstruct the potential distribution of this species through time.

## RESULTS

### Phylogenetic origin of *Euphorbia canariensis*

The ITS phylogeny (Fig. 2A; Figure S1, Alignment S1) confirmed that both cactus-like species of *Euphorbia* (*E. canariensis* and *E. handiensis*) occurring in the Canary Islands are only distantly related (Fig. 2A). All sequenced individuals of *E. canariensis* (at least one per island) formed a monophyletic group ( $PP = 1$ , 1.39 Ma, 95% HPD: 0.31–2.79 Ma) that was sister ( $PP = 0.91$ , 8.18 Ma; 95% HPD: 3.84–13.06 Ma) to the clade of Southeast Asian *E. sessiliflora*, *E. lacei* and *E. epiphyllodes* ( $PP = 0.88$ , 5.85 Ma, 95% HPD: 2.33–10.11 Ma). *Euphorbia abdelkuri*, from the Socotra Archipelago (Yemen), was recovered as sister to this clade, but with low support ( $PP = 0.57$ ). This small clade formed by Canarian, Southeast Asian, and Yemeni species was sister to a much larger clade representing the core of *E. sect. Euphorbia*, including also *E. handiensis* as sister to *E. officinarum* ( $PP = 1$ , 1.02 Ma, 95% HPD: 0.15–2.09 Ma).

### Phylogeographic relationships

We obtained DNA sequences of the *trnS–trnG* and *trnQ–rps16* plastid DNA regions for 92 individuals from 29 populations of *E. canariensis* (Table S1). The individual from Los Canarios (La Palma) could not be sequenced for one of the two regions and this population was only used for SDM. Concatenation of *trnS–trnG* and *trnQ–rps16* sequences of *E. canariensis* and the outgroup samples resulted in an alignment of 1107 bp (564 bp for *trnS–trnG* and 543 bp for *trnQ–rps16*; Alignment S2) before excluding the problematic 108 bp region (see above). We observed 13 variable positions in the cpDNA sequences of *E. canariensis*, and the TCS analysis resulted in 10 substitution-based haplotypes for *E. canariensis* (Fig. 3). The most frequent haplotype was haplotype I, observed in 47 of 92 individuals (51%). The haplotype network showed no loops among *E. canariensis* haplotypes, few (four) missing haplotypes, and

three main groups of haplotypes: one group endemic to La Gomera (haplotype A); a second group found on the westernmost islands of La Palma and El Hierro (haplotypes B and C); and a third group containing seven haplotypes (D–J) occurring on central and eastern islands (Tenerife, Gran Canaria and Fuerteventura). The haplotype I was also observed in the apparently cultivated individual from Lanzarote. Tenerife had the highest number of haplotypes (five), while La Gomera had the lowest (one haplotype). The outgroup species (*E. abdelkuri*, *E. epiphyllodes*, *E. handiensis*) were not connected to *E. canariensis* haplotypes at a 95% confidence interval, but they were connected to the two missing haplotypes between A and B at a 90% confidence interval.

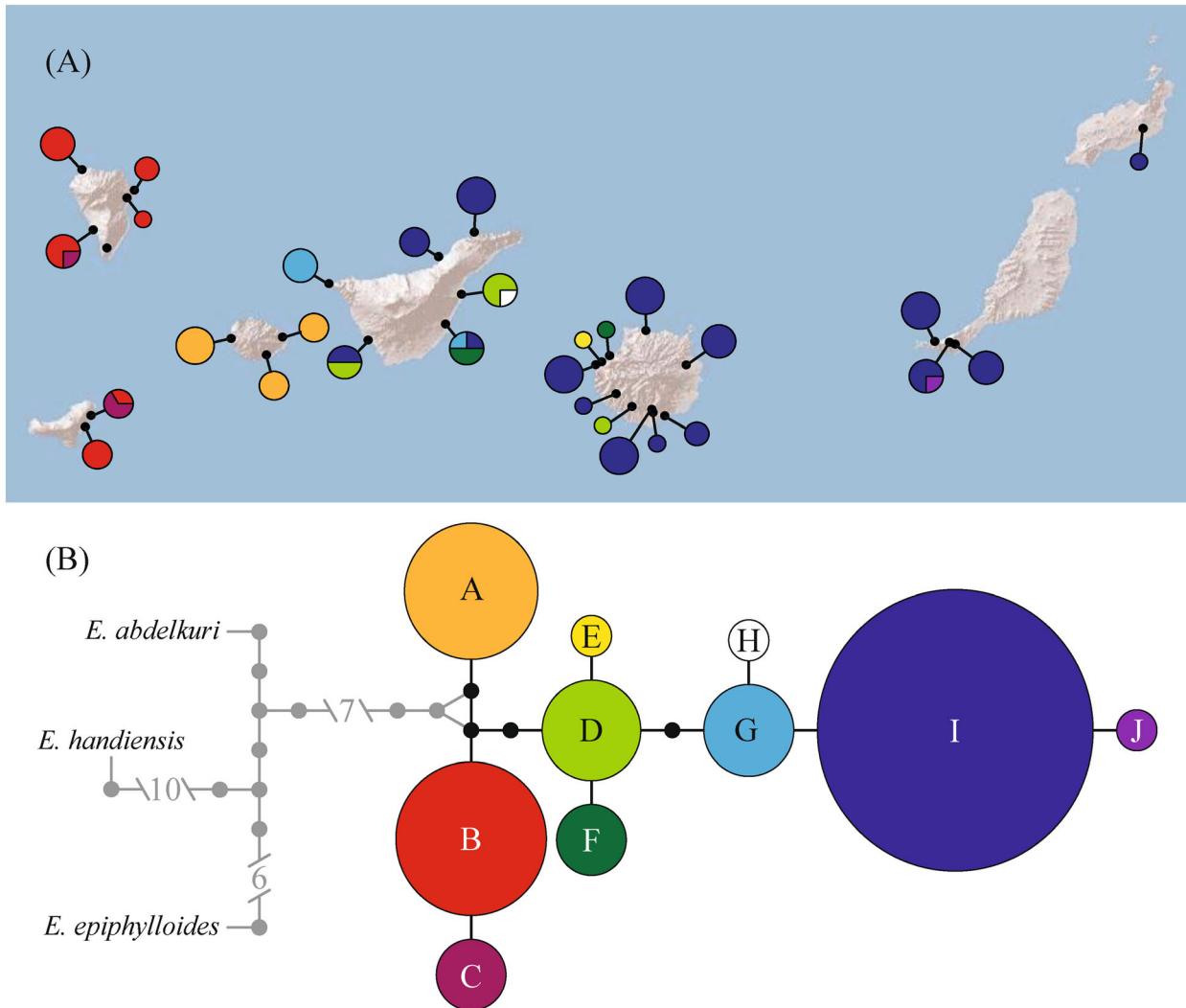
### Estimation of inter-island colonization events

According to PAICE results, haplotypes of *E. canariensis* required a minimum of eight inter-island colonization events across the Canarian archipelago to explain their geographic distribution and genealogical relationships. When sampling bias was considered, rarefaction curves fitted well to theoretical curves (Fig. 4) and the asymptotic estimators for the number of colonization events were as follows: (i) when using the missing haplotype closest to haplotype A as ancestral, values of 22.5 (95% CI: 20.6–25.1) colonization events for the genetic estimator and 28.1 (95% CI: 23.9–34.5) colonization events for the field estimator; and (ii) when using the missing haplotype closest to haplotype B as ancestral, values of 26.4 (95% CI: 22.8–32.7) colonization events for the genetic estimator and 38.3 (95% CI: 31.4–49.7) colonization events for the field estimator.

Bayesian discrete phylogeographic analysis (DPA) estimated 13.1 (95% CI: 8–18) colonization events. La Gomera was suggested as the most probable ancestral area of *E. canariensis* in the Canarian archipelago (46%), followed by Tenerife (28%). From this common ancestor, three well supported clades were differentiated, including two western clades (haplotype A, with La Gomera as the most probable ancestral area, 100%; and haplotypes B–C, with La Palma as the most probable ancestral area, 69%, and subsequent colonization of El Hierro); and a central-eastern clade (haplotypes D–J), with Tenerife as the most probable ancestral area (90%) and subsequent colonization of Gran Canaria and Mahan. The Bayesian stochastic search variable selection (BSSVS) showed a general pattern of west-to-east colonization of the Canarian archipelago. The most probable routes of colonization of *E. canariensis* are shown in Table 2. All these colonization events seem to have taken place between 1.62 and 2.79 Ma ( $PP = 1$ ) (Fig. 5).

### Species distribution modelling

The inferred potential distribution of *E. canariensis* for the present and past periods is shown in Fig. 6. Current potential

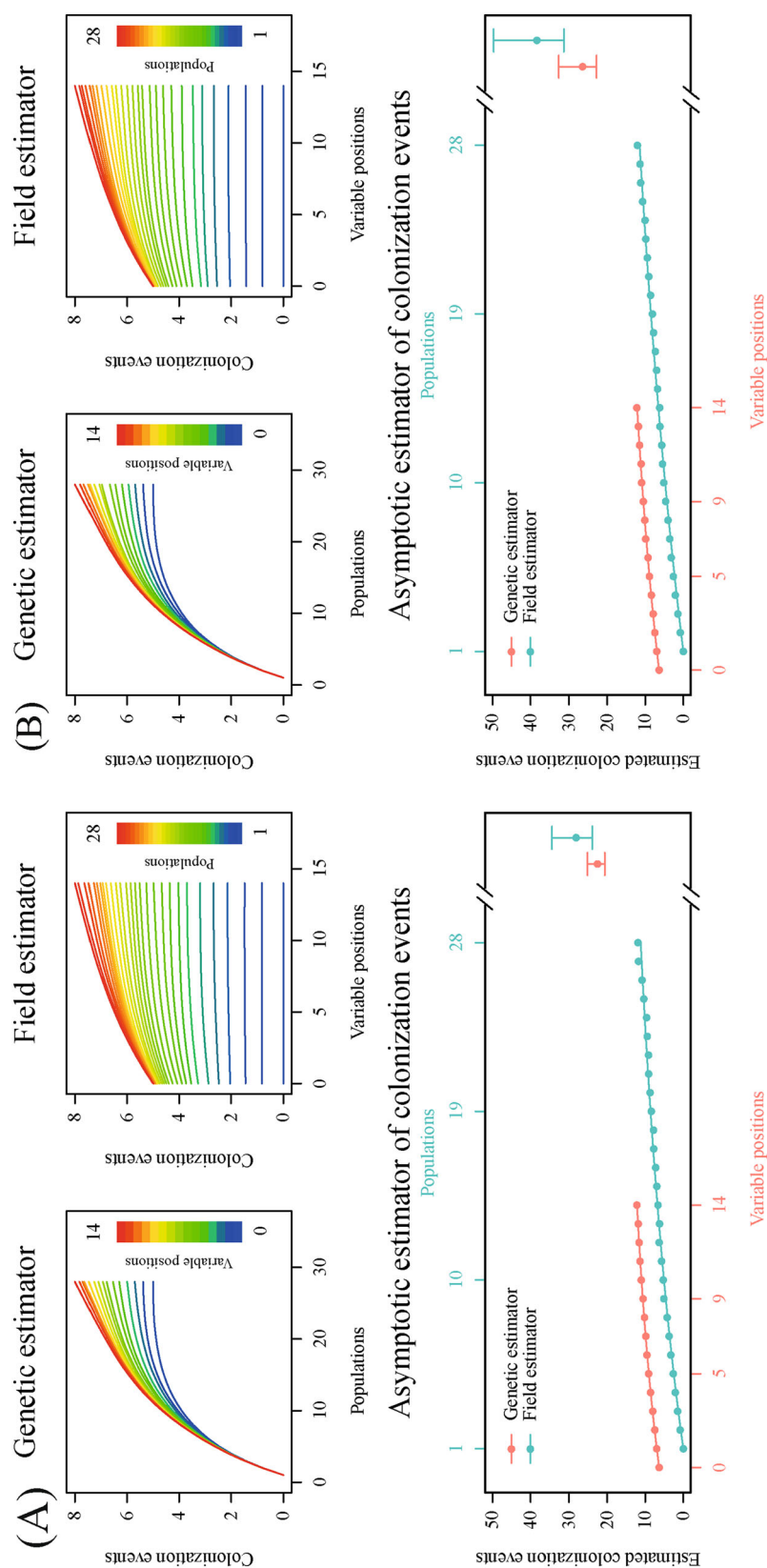


**Fig. 3.** Phylogeographic relationships in *Euphorbia canariensis*. A: Geographic distribution of haplotypes of *E. canariensis* in the Canary Islands based on two cpDNA regions (*trnS-trnG* and *trnQ-rps16*). Pie charts indicate the proportions of different haplotypes with colours, and their sizes are proportional to the numbers of sampled individuals (note that the southernmost population of La Palma could not be sequenced). B: Statistical parsimony network of *E. canariensis* haplotypes; lines correspond to single nucleotide substitutions, dots indicate missing haplotypes (either extinct or not found), and circle sizes are proportional to haplotype frequencies (each haplotype is named with a unique letter); the outgroup, in grey, is only connected to *E. canariensis* haplotypes at lower confidence intervals (see text). Major island names, from west to east: El Hierro, La Palma, La Gomera, Tenerife, Gran Canaria, Fuerteventura, Lanzarote.

distribution of this species is located mainly in the lowlands of every island of the Canarian archipelago. The narrowest potential habitats available (relative to island size) were found on the easternmost islands (Lanzarote and Fuerteventura), while the other islands showed wider habitat availability (Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro). The predictive power of the model was supported by an AUC = 0.871, and the two variables that contributed the most to the model were the minimum temperature of the coldest month (bio 6) and the pH index measured in water solution (eda 9) (Table 3). Projections displayed a tendency to narrower habitat availability in the past, with the narrowest areas in the Hainrich Stadial 1 (14.7–17.0 ka) and Last Glacial Maximum (ca. 22 ka). The oldest period studied (Last Interglacial, ca. 130 ka) did not follow this pattern, and showed a large potential distribution for this species across the Canarian archipelago.

## DISCUSSION

In this study, we have contributed to clarify the biogeographic patterns of the cactus-like *E. canariensis* at different evolutionary scales. The phylogenetic position of *E. canariensis* as sister to Southeast Asian species indicates a disjunction much wider than that of the Rand Flora pattern, which is observed in several Macaronesian lineages with sister lineages in opposite margins of Africa and the Eritreo-Arabian region (Sanmartín *et al.* 2010; Pokorný *et al.* 2015). The coincidence of the stem age of *E. canariensis* (Figs 2 and 5) and the origin of the Saharan, Arabian and Syrian deserts (Sanmartín 2003; Oberprieler 2005) is consistent with the possibility of extinction of intermediate African lineages between *E. canariensis* and its Asian relatives. Therefore, this wide disjunction could be the result of relatively recent events of climatic aridification in



**Fig. 4.** Rarefaction curves of colonization events of *Euphorbia canariensis* in the Canary Islands according to Phylogeographic Analysis of Island Colonization Events (PAICE). A: Results when considering the missing haplotype closest to A as ancestral. B: Results when considering the missing haplotype closest to B as ancestral. Top plots represent raw rarefaction curves of both genetic (left) and field (right) estimators, and bottom plots show rarefaction curves of the final estimation for both genetic (red) and field (blue) estimators, in which asymptotic estimators are plotted with their 95% confidence intervals.

**Table 2.** Most probable routes of colonization of *Euphorbia canariensis* in the Canary Islands according to Bayesian stochastic search variable selection (BSSVS). Only routes of colonization with a Bayes Factors (BF) >3 are shown.

from	to	Bayes Factor (BF)
Tenerife	Gran Canaria	74.46
La Palma	El Hierro	15.88
Gran Canaria	Mahan	6.11
Tenerife	Mahan	5.43
La Gomera	Tenerife	4.65
El Hierro	La Palma	3.72
La Gomera	La Palma	3.62

northern Africa and southwestern Asia rather than long-distance dispersal between distant regions. In fact, the lack of LDD traits in *E. canariensis* is mirrored by a relatively low number of inter-island colonization events (considering the old time of arrival in the archipelago) in comparison with other Canarian species, including examples with and without LDD traits (*Olea europaea* subsp. *guanchica* and *Cistus monspeliensis*, see below; Coello *et al.* 2021). As a whole, this study supports the hypothesis of low dispersal and colonization abilities of *E. canariensis*.

**An extreme disjunction between the Canary Islands and Southeast Asia**

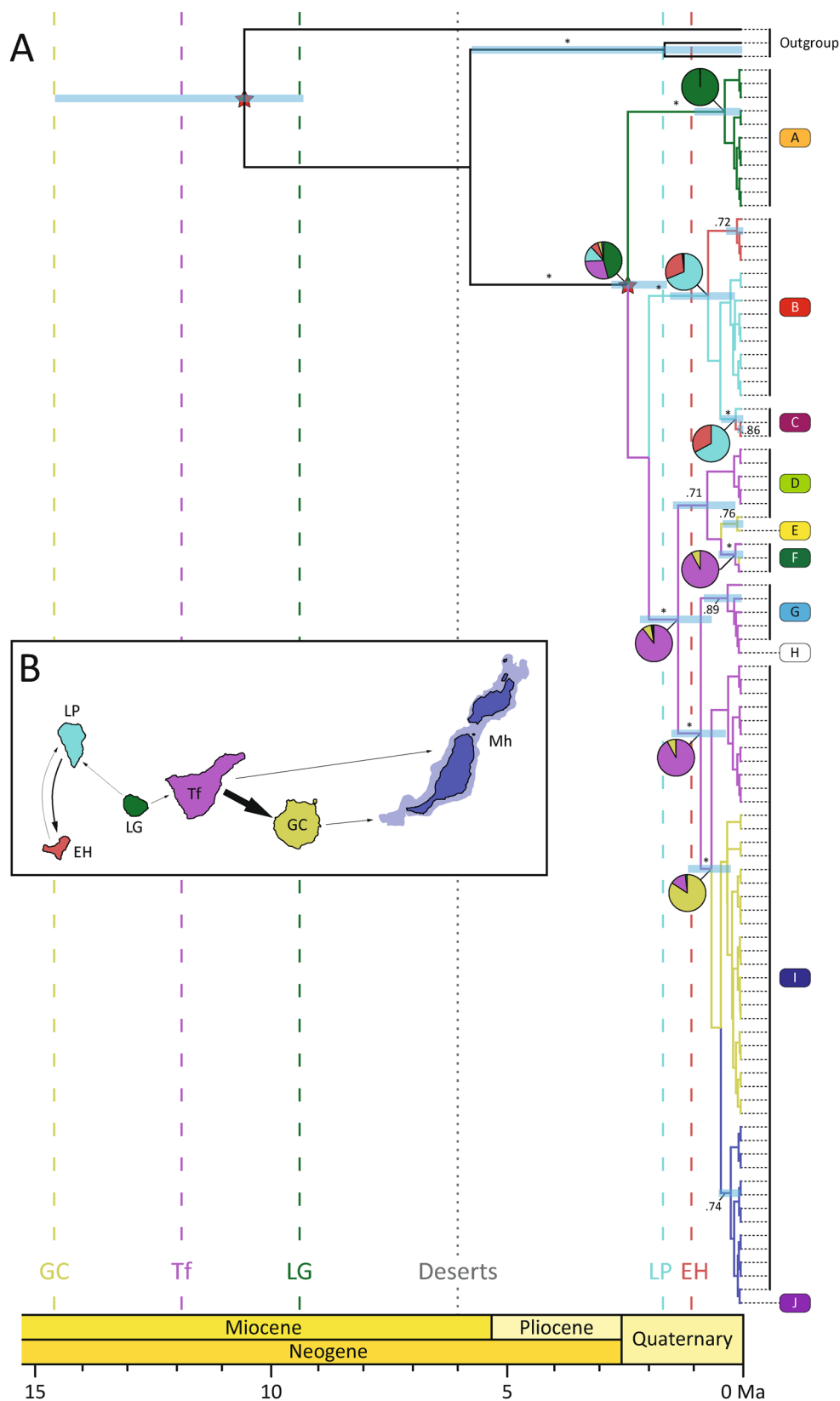
Our phylogenetic results confirm the pattern suggested by Bruyns *et al.* (2011). *Euphorbia canariensis* is located in an early-diverging clade in *Euphorbia* sect. *Euphorbia*, sister to a clade containing most species of the section (Fig. 2A). We recovered a well-supported clade including *E. canariensis* and three Southeast Asian species (*E. sessiliflora*, *E. lacei* and *E. epiphyllodes*) (Fig. 2A,B), separated by an extremely large area including the northern African continent, the Arabian Peninsula and the Indian subcontinent.

It has been suggested that *E. canariensis* conforms to a Rand Flora biogeographic pattern (Sanmartín *et al.* 2010; Pokorný *et al.* 2015; del Arco Aguilar & Rodríguez Delgado 2018). However, the apparent lack of any close relatives to *E. canariensis* in continental Africa (Fig. 2; Bruyns *et al.* 2011) suggests that the disjunction pattern of this species goes beyond the Rand Flora. Likewise, some other Canarian species show a similar extreme disjunction between Macaronesia and Southeast Asia. For example, the Macaronesian dragon trees (*Dracaena draco* (L.)

L. from the Canary Islands, Cape Verde and southwestern Morocco; and *D. tamaranae* Marrero Rodr., R.S.Almeira & M.González-Martin from Gran Canaria in the Canary Islands) diverged ca. 15 Ma from their closest relatives *D. cambodiana* Pierre ex Gagnep. and *D. cochinchinensis* (Lour.) S.C.Chen, which are distributed in Southeast Asia (Edwards *et al.* 2018; Celiński *et al.* 2020; Durán *et al.* 2020). Similarly, the Canary Island pine (*Pinus canariensis* C.Sm. ex DC.) is sister to the Himalayan *P. roxburghii* Sarg., and they diverged ca. 13.5–25 Ma (Jin *et al.* 2021).

One of the hypotheses proposed to explain the Rand Flora pattern is that related extant lineages occurring on the margins of Africa are remnants of more widely distributed African lineages affected by extinction (i.e. the vicariance hypothesis; Sanmartín *et al.* 2010). The estimated divergence time between *E. canariensis* and the most closely related species (3.84–13.06 Ma; Fig. 2A) reflects a time window similar to that of the origin of the Saharan, Arabian and Syrian deserts around 6 Ma (Sanmartín 2003; Oberprieler 2005). It is reasonable to hypothesize that extinction of putative ancestors of *E. canariensis* and other intermediate lineages in continental Africa and the Arabian Peninsula resulted in this extreme disjunction pattern, rather than being the result of a long-distance dispersal event. This is similar to large-scale extinctions that occurred in southern Africa because of the expansion of deserts (Linder 2003). In fact, the tropical xerophytic shrubland that prevailed in northern Africa during the late Miocene (Pound *et al.* 2011) seems to be more similar to the dry habitats in which *E. canariensis* currently occurs than the tropical-subtropical habitats of its Asian relatives, which supports the idea of extinction of closer relatives of *E. canariensis* in Africa. For *Pinus* L. (Kvaček *et al.* 2014) and *Dracaena* Vand. ex L. (Denk *et al.* 2014), the presence of fossils related to Macaronesian lineages in the Mediterranean Region supports wider distribution ranges in the past, subsequently fragmented by extinction, resulting in wide Macaronesian–Asian disjunctions. However, fossils related to the *E. canariensis* lineage have not yet been found in Africa, Asia or elsewhere to the best of our knowledge. Therefore, deeper paleobotanical research might provide additional support for the hypothesis of extinction across Africa. Alternatively, some unsampled or even unknown *Euphorbia* species from eastern Africa or the southern Arabian Peninsula could be part of the *E. canariensis* clade. In fact, available phylogenetic studies (Zimmermann *et al.* 2010; Bruyns *et al.* 2011; Horn *et al.* 2012; Dorsey *et al.* 2013) have only analysed 20 of the 92 species of section *Euphorbia* distributed in the eastern side of the Sahara (Ethiopia, Yemen, Socotra, Somalia and Oman;

**Fig. 5.** Discrete phylogeographic analysis (DPA) and haplotype divergence times of *Euphorbia canariensis* based on two cpDNA regions (*trnS–trnG* and *trnQ–rps16*). A: Phylogeographic relationships according to the maximum clade credibility tree. Letters (A–J) and colours of terminal labels in represent haplotypes in *E. canariensis* as in Fig. 3. Branch colours correspond to the most probable ancestral area as shown in the map: El Hierro (EH) in red, La Palma (LP) in light blue, La Gomera (LG) in dark green, Tenerife (Tf) in purple, Gran Canaria (GC) in yellow and Mahan (Mh, palaeoisland comprising Lanzarote and Fuerteventura) in dark blue (outgroup branches are not coloured). Numbers above branches are posterior probabilities (PP), only shown when PP ≥ 0.5; asterisks (\*) indicate PP ≥ 0.95. Node bars indicate the 95% highest posterior density intervals for ages when PP ≥ 0.5. Pie charts indicate the posterior probability distributions of ancestral areas for well-supported clades (PP ≥ 0.95) in *E. canariensis*. Red stars indicate secondary calibration points according to ages shown in Fig. 2A. Ages of emergence of five islands of the Canarian archipelago are indicated (GC: 14.6 Ma, Tf: 11.9 Ma, LG: 9.4 Ma, LP: 1.7 Ma, EH: 1.1 Ma; Mh is older than 20 Ma and therefore it is not shown along the time scale; Troll & Carracedo 2016). The grey dotted line at 6 Ma indicates the origin of the Saharan, Arabian and Syrian deserts (Sanmartín 2003; Oberprieler 2005). B: Direction of the most probable migration routes inferred from ancestral reconstruction and supported by Bayes factors (BF). Thickness of arrows is proportional to BF values (routes with BF <3 were omitted).

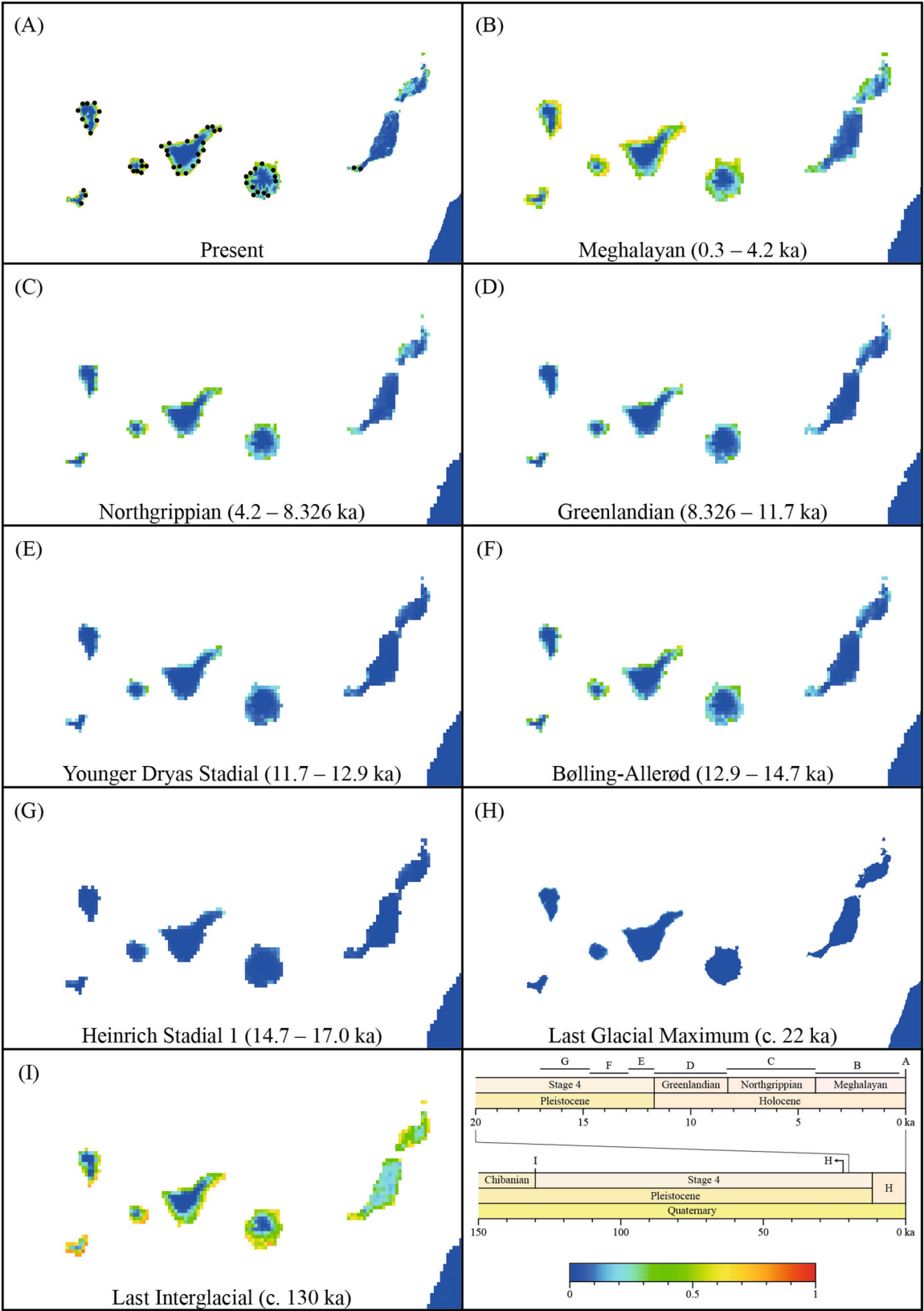


Riina & Berry 2023). After studying the morphology of the 72 unsampled species from this region — using herbarium specimens (including types), descriptions in regional floras and

protologues — we identified at least six species with some similarities to members of the *E. canariensis* clade. They are *E. borenensis* M.G.Gilbert (Ethiopia), *E. colubrina* P.R.O.Bally

& S.Carter (Somalia), *E. cryptospinosa* P.R.O.Bally (Ethiopia, Somalia), *E. fascicaulis* S.Carter (Somalia), *E. mosaica* P.R.O.Bally & S.Carter (Somalia) and *E. tenuirama* Schweinf. ex

A.Berger (Ethiopia). Since we could not sample these candidate species, further studies including them are needed to test the hypothesis of a larger *E. canariensis* clade and consequently a



**Fig. 6.** Potential past and current environmental suitability for *Euphorbia canariensis* in the Canary Islands based on species distribution modelling (SDM). A: Inferred suitability for the present; dots indicate occurrences used in the SDM analysis (see text); B: projected distribution for the Meghalayan (0.3–4.2 ka); C: projected distribution for the Northgrippian (4.2–8.326 ka); D: projected distribution for the Greenlandian (8.326–11.7 ka); E: projected distribution for the Younger Dryas Stadial (11.7–12.9 ka); F: projected distribution for the Bølling-Allerød (12.9–14.7 ka); G: projected distribution for the Heinrich Stadial 1 (14.7–17.0 ka); H: projected distribution for the Last Glacial Maximum (ca. 22 ka); I: projected distribution for the Last Interglacial (ca. 130 ka). In all maps, suitability for *E. canariensis* is indicated as colours according to the scale shown at the bottom right (blue for no suitability; green for low suitability, yellow and red for high suitability). A time scale for the past periods used in projections is also shown.

**Table 3.** Variable contributions to the SDM analysis of *Euphorbia canariensis*. Variables listed in order of importance.

abbreviation	variable	contribution (%)
Bio 6	Minimum temperature of the coldest month	46.6
Eda 9	pH index measured in water solution	31.8
Bio 17	Precipitation of the driest quarter	13.6
Eda 2	Volumetric percentage of coarse fragments with size >2 mm	3.3
Bio 15	Precipitation seasonality	2.6
Eda 3	Weight percentage of clay particles with size <0.0002 mm	1.4
Eda 6	Cation exchange capacity of soil	0.3
Bio 4	Temperature seasonality	0.3

narrower geographic gap between *E. canariensis* and its closest relatives.

### The colonization history of cactus-like *Euphorbia* species across the Canary Islands

In the Canarian archipelago, *E. canariensis* (occurring on six islands) and *E. handiensis* (occurring only on the Jandía Peninsula of Fuerteventura) are the only cactus-like species of *Euphorbia*. Phylogenetic results indicate that both species belong to different lineages of *E. sect. Euphorbia* (Fig. 2A). While *E. canariensis* is related to Southeast Asian species, *E. handiensis* is sister to Northwest African *E. officinarum* (Fig. 2; Bruyns *et al.* 2011). This pattern of multiple colonizations has also been found in other subgenera and sections of *Euphorbia* (e.g., Riina *et al.* 2013; Sun *et al.* 2016; Barres *et al.* 2017; Riina *et al.* 2021) and several other genera, such as *Cistus* (Guzmán & Vargas 2010) and *Lotus* (Jaén-Molina *et al.* 2021). The difference between the origins of the two cactus-like species of *Euphorbia* in the Canary Islands is that the ancestor of *E. canariensis* appears to have arrived in the Canarian archipelago earlier than that of *E. handiensis* (Fig. 2). This has resulted in the narrower geographic range of *E. handiensis* at present (a single island) compared to *E. canariensis*, which has had a wider time window to colonize six of the major islands.

The relatively recent divergence among *E. canariensis* haplotypes in the Canary Islands (crown age, 1.62–2.79 Ma; Fig. 5) contrasts with the older divergence between this species and its Asian sister group (stem age, 3.84–13.06 Ma; Fig. 2A). This implies a high uncertainty on the time of colonization of the Canary Islands from the continent, which may have happened at any point between the stem age and the crown age (Martín-

Hernanz *et al.* 2023). It is possible that the colonization of the Canarian archipelago from the continent occurred long before the estimated crown age if the *E. canariensis* lineage experienced significant extinction in the Canary Islands in the past (García-Verdugo *et al.* 2019b). However, a relatively recent colonization is also plausible if there was substantial extinction among related lineages on the continent.

In oceanic archipelagos, it is common to observe lineages of organisms whose phylogeographic relationships are concordant with the geological sequence of island emergence. This pattern is known as the “progression rule”, according to which a lineage of organisms colonizes islands of an oceanic archipelago as they emerge (Shaw & Gillespie 2016). In the case of the Canary Islands, the hotspot responsible for the emergence of islands has historically followed an east-to-west trajectory, as shown by the fact that eastern islands are older (Troll & Carracedo 2016). As a result, some species show an east-to-west pattern of lineage divergence (e.g. Villa-Machío *et al.* 2020; Rincón Barrado *et al.* 2024), although the Canarian biota as a whole provides mixed evidence for and against this “progression rule” (Shaw & Gillespie 2016). In the case of *E. canariensis*, we observe a deviation from the “progression rule” (Figs 3 and 5). Our data suggest that central-western islands were colonized earlier in the evolutionary history of the species, in contrast to the older eastern islands, when considering extant lineages.

The overall west-to-east colonization pattern of *E. canariensis*, starting from La Gomera and Tenerife (Fig. 5), is congruent with the set of islands of the Canarian archipelago that were emerged at the time of the most recent common ancestor of extant lineages (1.62–2.79 Ma; Fig. 5). This set included all major islands except for the two westernmost ones, El Hierro and La Palma. According to the reconstruction of ancestral ranges obtained by DPA, the most recent common ancestor was probably found in La Gomera or Tenerife with 74% probability (Fig. 5). A recent colonization of the easternmost islands, Fuerteventura and Lanzarote, is clearly supported, as already documented for other organisms (García-Verdugo *et al.* 2019a). This pattern has been related to the aridity of these islands (Troll & Carracedo 2016). In the case of *E. canariensis*, SDM partly supports this hypothesis, as suitable habitats appear to have been less common in eastern islands than in the rest of the archipelago throughout the late Quaternary, including a limited habitat availability during the Holocene (Fig. 6). The west-to-east colonization pattern of *E. canariensis* (Figs 3 and 5) is also congruent with the apparent lack of wild populations of this species in Lanzarote (only one individual observed in 1990; Bramwell & Bramwell 2001), together with its scarcity in Fuerteventura. In fact, despite the assumed drought tolerance of succulent species (Griffiths & Males 2017), *E. canariensis* struggles to survive on the driest islands of the archipelago,

as shown by the lack of natural populations in Lanzarote and the small populations in Fuerteventura (personal observation). Furthermore, the limited connectivity of the Jandía populations (southern Fuerteventura; Figs 3 and 5) could be the result of a lack of suitable habitats in the easternmost islands (Fig. 6) and the wide separation between them and the rest of the archipelago.

Additionally, the relatively recent colonization of the two westernmost islands, La Palma and El Hierro, is congruent with their emergence time. As shown in Fig. 5, the clade composed of haplotypes B and C (occurring on La Palma and El Hierro) diverged 0.18–1.55 Ma (PP = 1), and thus post-dates the time of emergence of La Palma (1.7 Ma; Troll & Carracedo 2016), which was inferred as ancestral island for this clade (69%). Similarly, divergence times of individuals from El Hierro in both clades B and C were estimated to be more recent than the emergence of this island (1.1 Ma; Troll & Carracedo 2016).

### Colonization ability of *Euphorbia canariensis*

Given the occurrence of *E. canariensis* on the six major islands of the Canarian archipelago (considering Lanzarote and Fuerteventura as the single palaeoisland of Mahan; Troll & Carracedo 2016), *E. canariensis* has experienced at least five inter-island colonization events based on species distributions alone (Vargas *et al.* 2015; Arjona *et al.* 2018). The geographic distribution of plastid DNA diversity (Fig. 3) indicates a minimum of eight colonization events required to explain the geographic distribution of haplotypes in the archipelago. It should be noted that this value does not necessarily match the number of most probable colonization routes (seven; Fig. 5, Table 2), as each colonization route may involve multiple colonization events, and the route of some colonization events may be uncertain (see Coello *et al.* 2022 for further details). The estimates obtained in PAICE considering sample size (Coello *et al.* 2022) suggested ca. 20–50 colonization events (see Results, Fig. 4). Results of this study corroborate those of Coello *et al.* (2022) regarding the fact that DPA analysis (ca. 13 colonization events for *E. canariensis* with a 95% confidence interval of 8–18) estimates fewer inter-island colonization events than PAICE.

The estimate of inter-island colonization events for *E. canariensis*, although apparently high, is comparatively lower than those previously obtained for other species. In particular, the comparison between *E. canariensis* (unspecialized for seed dispersal) and *Olea europaea* subsp. *guanchica* P.Vargas, J.Hess, Muñoz Garm. & Kadereit (zoochorous) agrees with the higher colonization ability of species with LDD traits reported by Arjona *et al.* (2018). The estimates for the number of inter-island colonization events for these species are similar, but *O. europaea* subsp. *guanchica* achieved those values (Coello *et al.* 2022) in a shorter time (Besnard *et al.* 2009) and with a smaller number of occupied islands (Acebes *et al.* 2010). The same happens when *E. canariensis* is compared to *Cistus monspeliensis* L., another species without LDD traits, which seems to have experienced ca. 25–33 inter-island colonization events (Coello *et al.* 2022) in a shorter time (Fernández-Mazuecos & Vargas 2011; Coello *et al.* 2021) and occurs on five islands (as *C. monspeliensis* does not occur on Mahan; Acebes *et al.* 2010). Considering that *E. canariensis* occurs on six islands, probably colonized the archipelago earlier (Fig. 5), and

its environmentally suitable areas are more widespread (Fig. 6), it seems reasonable to hypothesize that *E. canariensis* has lower colonization abilities than the other two species. A more robust comparison will require additional analyses of colonization patterns accounting for sample size of other Canarian species, including species of the genus *Euphorbia*. In any case, the apparently low colonization capacity of *E. canariensis* supports the low likelihood of a LDD event from Southeast Asia to the Canarian archipelago in the clade including *E. canariensis*, *E. lacei*, *E. epiphyllloides* and *E. sessiliflora* (Fig. 2), and is in agreement with a vicariance scenario (see above).

## CONCLUSIONS

This study supports that the Canarian *E. canariensis* is closely related to Southeast Asian species, representing one of the widest biogeographic disjunctions for Macaronesian plant species. This fact, together with the apparently low colonization capabilities of *E. canariensis* across the Canary Islands, supports the idea of extinction of both the mainland ancestor and closely related species in Africa and other areas further east in Asia. This extreme Canarian–Asian disjunction shared with other plant families, such as Pinaceae (*Pinus canariensis*–*P. roxburghii*), Asparagusaceae (*Dracaena*) and Lauraceae (*Apollonias* Nees), deserves further exploration (Li *et al.* 2011; Martín-Hernanz *et al.* 2023). Indeed, the high number of unsampled species (in phylogenies) of *E.* section *Euphorbia* occurring in eastern Africa and the southern Arabian Peninsula should be further investigated. Finally, the scarce environmental suitability in the two easternmost islands of the Canarian archipelago highlights the importance for conservation of *E. canariensis* populations in the Jandía Natural Park of southern Fuerteventura, and the interest in searching for any extant populations in Lanzarote.

## AUTHOR CONTRIBUTIONS

A.J.C., M.F.-M. and P.V. conceived and designed the study; A.J.C., M.F.-M., P.V. and R.R. collected the samples; A.J.C. and E.C. performed lab work; A.J.C. analysed data; A.J.C., M.F.-M., R.R. and P.V. discussed results; A.J.C. and M.F.-M. wrote the paper, and all authors critically revised it.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Complete Bayesian chronogram of *Euphorbia*.

**Table S1.** *Euphorbia* species used in the phylogeographic analysis and accession numbers of DNA sequences.

**Table S2.** Accession number of ITS sequences used in phylogenetic analysis.

**Table S3.** Final set of *E. canariensis* occurrences used for SDM analysis, obtained from GBIF occurrences and our own sampling points.

**Table S4.** Climate variables considered for SDM analysis.

**Tree S1.** Tree file for the Bayesian chronogram of *Euphorbia*.

**Alignment S1.** Alignment of ITS sequences of *Euphorbia* used in the phylogenetic analysis.

**Alignment S2.** Alignment of *trnS-trnG* and *trnQ-rps16* sequences of *Euphorbia* used in the phylogeographic analyses.

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