

# Reconstruction of the spatio-temporal diversification and ecological niche evolution of *Helianthemum* (Cistaceae) in the Canary Islands using genotyping-by-sequencing data

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- **Background and Aims** Several biogeographical models have been proposed to explain the colonization and diversification patterns of Macaronesian lineages. In this study, we calculated the diversification rates and explored what model best explains the current distribution of the 15 species endemic to the Canary Islands belonging to *Helianthemum* sect. *Helianthemum* (Cistaceae).
- **Methods** We performed robust phylogenetic reconstructions based on genotyping-by-sequencing data and analysed the timing, biogeographical history and ecological niche conservatism of this endemic Canarian clade.
- **Key Results** Our phylogenetic analyses provided strong support for the monophyly of this clade, and retrieved five lineages not currently restricted to a single island. The pristine colonization event took place in the Pleistocene (~1.82 Ma) via dispersal to Tenerife by a Mediterranean ancestor.
- **Conclusions** The rapid and abundant diversification (0.75–1.85 species per million years) undergone by this Canarian clade seems the result of complex inter-island dispersal events followed by allopatric speciation driven mostly by niche conservatism, i.e. inter-island dispersal towards niches featuring similar environmental conditions. Nevertheless, significant instances of ecological niche shifts have also been observed in some lineages, making an important contribution to the overall diversification history of this clade.

**Key words:** Allopatric speciation, Canary Islands, diversification, niche conservatism, genotyping-by-sequencing, *Helianthemum*, phylogenomics.

## INTRODUCTION

The Canary Islands (part of the Macaronesian region) stand out as one of the most attractive and best-studied oceanic island systems in the world from both geological and biogeographical perspectives (Juan *et al.*, 2000; Carracedo and Day, 2002; Ancochea *et al.*, 2006; Sanmartín *et al.*, 2008; Fernández-Palacios *et al.*, 2011; Caujapé-Castells *et al.*, 2017; García-Verdugo *et al.*, 2019a, b). Their complex geological history associated with frequent volcanic activity, the wide geological age-spans of the islands, and their great altitudinal range (El Teide is >3700 m high) give rise to a wide diversity of ecological niches ranging from arid and semiarid coastal scrub, through semiarid sclerophyllous woodlands, humid broad-leaved evergreen laurel forests and xeric pine forests to Canarian high-mountain (subalpine) scrublands (Del Arco and Rodríguez Delgado, 2018). The presence of such a wide-ranging heterogeneity of habitats has been the catalyst for diversification processes in many lineages (for reviews see Juan *et al.*, 2000; Caujapé-Castells *et al.*, 2017), which has yielded an extremely

rich biota characterized by a high degree of endemism (Santos-Guerra, 2001; Fernández-Palacios and Whittaker, 2008; Reyes-Betancort *et al.*, 2008; Carine *et al.*, 2009).

Many of the phylogenetic and biogeographical studies dealing with endemic plant groups in the Canary Islands have revealed the prevalence of strongly supported monophyletic lineages (e.g. Fernández-Palacios and Whittaker, 2008; Kim *et al.*, 2008; Guzmán and Vargas, 2010; Viales *et al.*, 2014; Puppo *et al.*, 2015), which are considered to be the result of a single colonization event from the mainland followed by rapid diversification and spread across the islands (Silvertown *et al.*, 2005). Several models that are not mutually exclusive have been proposed to explain this pattern (Carine, 2005; Herben *et al.*, 2005; Silvertown *et al.*, 2005; Sanmartín *et al.*, 2008). The simplest is the stepping-stone directional model of east–west migration (Sanmartín *et al.*, 2008), in which step-wise colonization from the African continent has led to the presence of just a single species on each island. A variation of this simple model includes subsequent intra-island speciation,

resulting in a species having its closest relatives on the same island (Sanmartín *et al.*, 2008). However, a more complex model including inter-island colonization of similar ecological zones (Francisco-Ortega *et al.*, 2001) considers that independent lineages have radiated throughout the archipelago by colonizing similar habitats on different islands, which results in species having their closest relatives on different islands. Lastly, some authors have argued against the single colonization scenario, stressing that the monophyly of many Canarian endemic lineages is more apparent than real, and that the current patterns of diversification that we observe can also be the result of multiple instances of colonization through hybridization and introgression events (Herben *et al.*, 2005; Saunders and Gibson, 2005; or the ‘surfing syngameon hypothesis’, positing recurrent and relatively swift cycles of introgression and gene flow disruption within and between islands throughout the geological history of the archipelago, alongside a high incidence of multiple colonizations and extinctions; Caujapé-Castells, 2011; Caujapé-Castells *et al.*, 2017).

The genus *Helianthemum* (Cistaceae) provides an excellent opportunity for testing biogeographical hypotheses regarding inter-island colonization patterns in the Canary Islands. The genus consists of ~140 species and subspecies distributed from Cape Verde to Central Asia, and recent phylogenetic studies based on Sanger sequencing (Aparicio *et al.*, 2017) as well as on genotyping-by-sequencing (GBS) using a large amount of data (Martín-Hernanz *et al.*, 2019a) elicited the existence of two independent clades of *Helianthemum* in Macaronesia: a species-poor clade (three species) distributed in Cape Verde and the Canary Islands within sect. *Eriocarpum*, and a species-rich clade with 15 species restricted to the Canary Islands within sect. *Helianthemum*. Although divergence time estimates point to the Late Pliocene or Early Pleistocene for the colonization of the Canary Islands by the ancestor belonging

to sect. *Helianthemum* (Martín-Hernanz *et al.*, 2019a), this clade stands as one of the plant groups with a higher number of species endemic to the Canary Islands (Fernández-Palacios and Whittaker, 2008; Domínguez Lozano *et al.*, 2010), most of which are also remarkably stenochorous and critically endangered (Bañares *et al.*, 2003, 2010) (Table 1).

In the Canary Islands, the clade belonging to sect. *Helianthemum* colonized all the major islands except El Hierro (the youngest and westernmost). With the exception of *H. broussonetii*, which occurs on two islands (Tenerife and La Palma; Fig. 1, Table 1), the remaining 14 species are single-island endemics and they only have one or just a few known populations, a remarkable pattern similar to other oceanic plant lineages [e.g. the Hawaiian silversword alliance (Barrier *et al.*, 2001), Canarian *Sideritis* (Barber *et al.*, 2007) and Canarian *Pericallis* (Jones *et al.*, 2014)]. Biogeographically, based on the apparently low dispersal capability of the diaspores of *Helianthemum* (e.g. Tébar *et al.*, 1997) and the restricted geographical distribution of these Canarian species, a stepping-stone model of dispersal could be a likely hypothesis to interpret the current distribution of sect. *Helianthemum* in the archipelago (Sanmartín *et al.*, 2008). Nevertheless, given the tendency for niche conservatism in *Helianthemum* (S. Martín-Hernanz *et al.*, unpubl. res.), inter-island colonization of similar ecological zones (Francisco-Ortega *et al.*, 2001) could be an equally plausible alternative scenario. Here, we use massive DNA sequencing (GBS data) on a thorough sampling that includes nearly all of the known extant populations of the 15 species of sect. *Helianthemum* in the Canary Islands to infer the colonization sequence that best explains the current distribution of this clade on the archipelago. Specifically, we (1) estimated the timing of arrival and dispersal throughout the archipelago as well as the speed of diversification, (2) reconstructed the dispersal events on islands through biogeographical

TABLE 1. List of species belonging to *Helianthemum* sect. *Helianthemum* in the Canary Islands including three currently undescribed ones. For each one the table shows the distribution, habitat, conservation status and number of extant and sampled populations

Taxon	Island <sup>1</sup>	N <sup>2</sup>	n <sup>3</sup>	Habitat <sup>4</sup>	Altitude	Status <sup>5</sup>
<i>Helianthemum aganae</i>	GO	1	1 (1)	Tabaibal-cardonal (semi-arid shrublands with <i>Euphorbia balsamifera</i> and <i>E. canariensis</i> )	300–350	CR
<i>Helianthemum aguloi</i>	GO	1	1 (1)	Sabinar (sclerophyllous woodlands with <i>Juniperus turbinata</i> subsp. <i>canariensis</i> )	350–400	CR
<i>Helianthemum bramwelliorum</i>	LZ	1	1 (2)	Tabaibal-cardonal (semi-arid shrublands with <i>Euphorbia balsamifera</i> and <i>E. canariensis</i> )	250–300	CR
<i>Helianthemum broussonetii</i>	LP, TF	6	5 (10)	Heathland ( <i>Morella–Erica</i> forests)	400–1500	VU
<i>Helianthemum bystropogophyllum</i>	GC	1	1 (2)	Pine forest ( <i>Pinus canariensis</i> )	1100–1400	CR
<i>Helianthemum cirae</i>	LP	2	2 (4)	Pine forest ( <i>Pinus canariensis</i> )	1350–1430	NE
<i>Helianthemum gonzalezferreri</i>	LZ	2	1 (2)	Sclerophyllous woodlands of <i>Olea cerasiformis</i>	300–580	CR
<i>Helianthemum inaguae</i>	GC	1	1 (3)	Pine forest ( <i>Pinus canariensis</i> )	1200–1400	CR
<i>Helianthemum juliae</i>	TF	3	2 (4)	High mountain scrubland	2000–2300	CR
<i>Helianthemum linii</i>	LP	1	1 (2)	Pine forest ( <i>Pinus canariensis</i> )	1000–1300	NE
<i>Helianthemum teneriffae</i>	TF	1	1 (2)	Heathland ( <i>Morella–Erica</i> forests)	1000–1100	CR
<i>Helianthemum tholiforme</i>	GC	7	1 (2)	Pine forest ( <i>Pinus canariensis</i> )	900–1300	EN
<i>Helianthemum</i> sp. nov. 1	TF	1	1 (2)	Pine forest ( <i>Pinus canariensis</i> )	1140	NE
<i>Helianthemum</i> sp. nov. 2	LP	2	2 (4)	Pine forest ( <i>Pinus canariensis</i> )	1500–1550	NE
<i>Helianthemum</i> sp. nov. 3	FT	1	1 (1)	Sclerophyllous woodlands of <i>Olea cerasiformis</i>	667	NE

<sup>1</sup>GO, La Gomera; LZ, Lanzarote; LP, La Palma; TF, Tenerife; GC, Gran Canaria; FT, Fuerteventura.

<sup>2</sup>Number of known extant populations following Bañares *et al.* (2003, 2010), the original description of species and A. Santos-Guerra, A. Acevedo, and M. Díaz-Bertrana, pers. comm.

<sup>3</sup>Number of sampled populations and number of sampled individuals (in brackets).

<sup>4</sup>Potential habitat type according to <https://visor.grafcan.es/visorweb/> and the original description of species.

<sup>5</sup>Conservation status following Bañares *et al.* (2003, 2010): CR, Critically Endangered; EN, Endangered; NE, Not Evaluated; VU, Vulnerable.

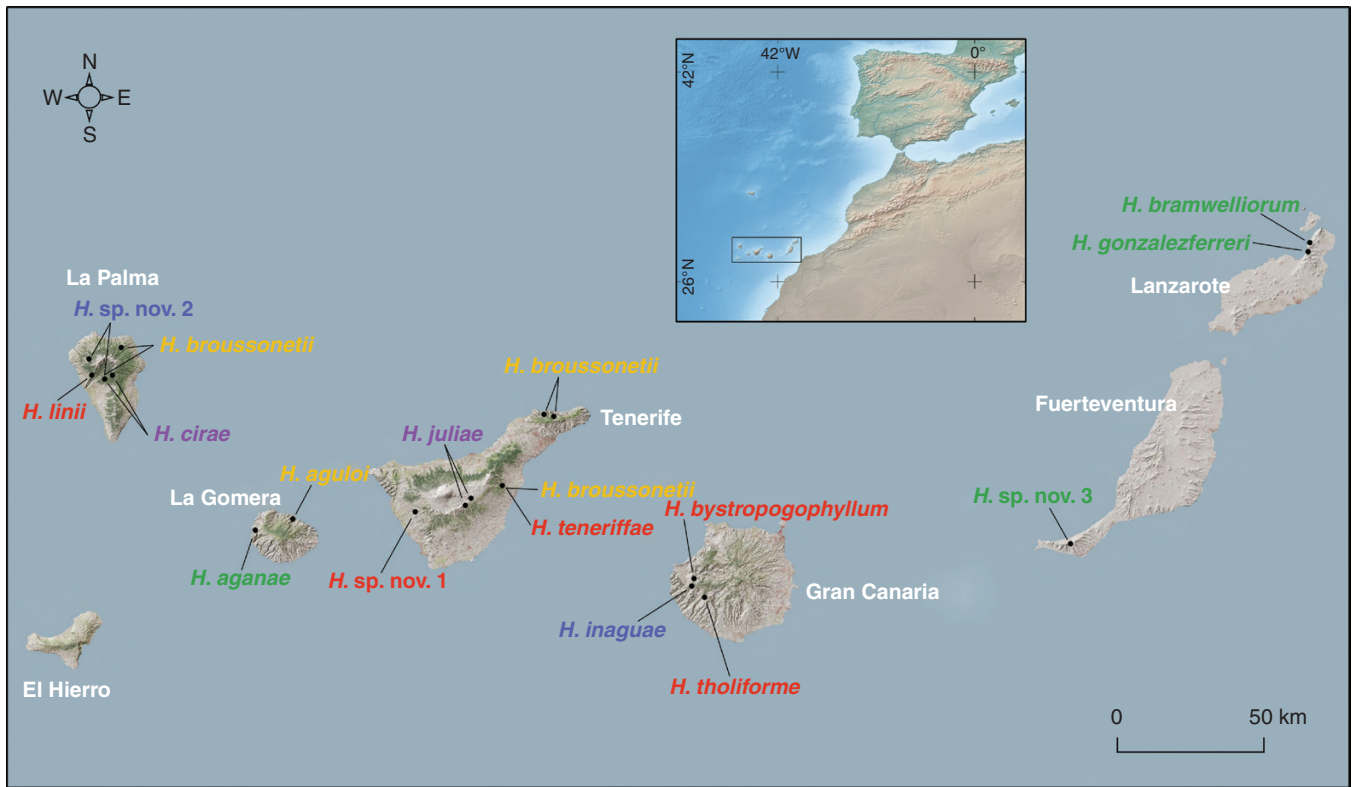


FIG. 1. Geographical location of all 22 populations of the 15 species of *Helianthemum* sect. *Helianthemum* endemic to the Canary Islands included in this study. Note that only *H. broussonetii* is found on two different islands. Species labels are coloured to denote the lineage to which the species was retrieved in the phylogenetic reconstruction (Fig. 2).

dispersal–extinction–cladogenesis (DEC) models including founder-event speciation, explicitly testing whether distance was a limiting factor for colonization, and (3) reconstructed the climatic niche of the species on the phylogenetic tree to reveal the potential role of ecological niche shifts in the evolution of this clade.

## MATERIALS AND METHODS

### *Taxon sampling, DNA extraction and GBS library preparation and sequencing*

*Helianthemum* sect. *Helianthemum* consists of ~43 species: 28 distributed throughout the Mediterranean and the Eurosiberian regions, plus 15 in the Canary Islands, of which three are currently undescribed (Santos-Guerra, 2014; A. Acevedo and M. Díaz-Bertrana, pers. comm.). In this study, we focused on this species-rich Canarian clade, which will be referred to as the ‘Canarian *Helianthemum*’. We performed consequential sampling including all the 15 species and ~71 % of all the extant known populations of the Canarian *Helianthemum* (Fig. 1; Table 1). Whenever possible, we analysed two individual plants per species and population for the ingroup taxa, totalling 42 samples from 22 populations. The outgroup consisted of eight samples of the sister species to the Canarian *Helianthemum* (*H. helianthemoides*, *H. ruficomum*, *H. kotschyannum*, *H. obtusifolium*, *H. grosii*) plus *H. aegyptiacum*, which

represents the early-diverging lineage of sect. *Helianthemum* (Martín-Hernanz *et al.*, 2019a). Other than two samples, all the plant material used for this study was freshly collected in the field from natural populations and stored in silica gel until DNA extraction. In total, 51 samples were examined in this study (Supplementary Data Table S1).

DNA was extracted from the silica-dried leaf material using the Isolate II Plant DNA Kit (Bioline, London, UK) following the manufacturer’s protocol. Paired-end GBS multiplexed libraries were constructed and sequenced by CNAG (Centro Nacional de Análisis Genómicos, Barcelona, Spain) following the protocol used by Elshire *et al.* (2011), with improvements suggested by Poland *et al.* (2012) and Sonah *et al.* (2013) and employing the restriction enzyme ApeKI for genome digestion (see Martín-Hernanz *et al.*, 2019a for details). Two lanes of an Illumina HiSeq 2000 (Illumina, CA, USA) were run to increase the sequencing coverage.

### *GBS sequence data assembly*

Demultiplexed Illumina FASTQ reads were run via ipyrad v. 0.7.24 (Eaton and Overcast, 2020) to generate *de novo* assemblies. The following stringent parameters were used in the assembly process to remove low-quality sequences and potential paralogous regions: (1) reads with more than five bases of low quality ( $Q < 20$ ) were excluded; (2) a minimum sequence depth of ten per individual locus was required for statistical



base-calling; (3) a clustering threshold of similarity between sequences of 0.90 was set for recovering loci; and (4) the maximum number of alleles allowed in consensus sequences was set at two since all species in the study are diploid  $2n = 20$  (Aparicio *et al.*, 2019). Minimum sequence depth and clustering threshold values correspond to those minimizing allele and SNP error rates, which were established after a previous study of the whole genus using replicate samples (Martín-Hernanz *et al.*, 2019a). To account for the effect of missing data, we constructed different datasets with three values of minimum sample loci, namely 15 % (all loci shared by at least eight samples; dataset min15%), 25 % (all loci shared by at least 15 samples; dataset min25%) and 50 % (all loci shared by at least 25 samples; dataset min50%), and performed phylogenetic analyses using all three datasets.

#### Phylogenetic reconstruction

For the phylogenetic reconstructions, we used both a concatenation approach entailing the concatenation of all recovered loci, and a coalescence-based approach, which is less prone to suffering from the incomplete lineage sorting of loci, a frequent phenomenon in rapidly diversified taxa (e.g. Fernández-Mazuecos *et al.*, 2018). For the concatenation approach, we reconstructed phylogenetic relationships under maximum likelihood (ML) and Bayesian inference (BI). For ML we used RAxML v.7.2.8 (Stamatakis, 2006) with a GTR+G nucleotide substitution model for all concatenated loci and branch support with rapid bootstrap analyses (BS), and the extended majority-rule consensus tree with automatic bootstrap stopping criterion, following a search for the best-scoring ML tree. For BI we used Exabayes v.1.4.1 (Aberer *et al.*, 2014) with the GTR+G substitution model and two runs of four Metropolis-coupled Monte Carlo Markov chains (MCMCs) with trees sampled every 500 generations for a total of  $3 \times 10^5$  generations. Convergence was assessed with Tracer 1.7 (Rambaut *et al.*, 2018) using summary statistics from the parameter files. Fifty-percent majority-rule consensus phylograms and posterior probabilities (PPs) were obtained using the consensus command with a burn-in fraction of 10 %.

For the coalescent-based approach, we used the software tetrad (built-in ipyrad v.0.7.24) (Eaton and Overcast, 2020), a quartet-based method that applies the algorithm of Chifman and Kubatko (2014) implemented in SVDQuartet. We inferred all possible quartet trees based on a matrix of one randomly selected SNP per locus. One thousand non-parametric bootstrap replicates were conducted and the results were summarized into a 50 % majority-rule consensus tree.

Since traditional branch-support metrics (BS, PP) can be unreliable and suffer from interpretation problems when applied to phylogenomic datasets (Pease *et al.*, 2018), we implemented the quartet sampling method (Pease *et al.*, 2018) to estimate the robustness of the inferred relationships. This method represents a generalized framework for quantifying phylogenetic uncertainty (specifically, branch support) that distinguishes branches with low information from those with multiple highly supported – but mutually exclusive – phylogenetic histories by calculating three different metrics: quartet concordance (QC),

quartet differential (QD) and quartet informativeness (QI) scores (Pease *et al.*, 2018). We performed the quartet sampling method with the Exabayes-inferred tree topologies for the three datasets with 500 replicates per branch and a minimum assembly overlap of 100 000 bp.

Bioinformatic processing in ipyrad as well as phylogenetic analyses were performed using the computer cluster located at the Centro Informático Científico de Andalucía (CICA, Seville, Spain) and the CIPRES Science Gateway (Miller *et al.*, 2010).

#### Estimates of divergence time and diversification rate

To reconstruct the temporal scale of colonization by the Canarian *Helianthemum* in the archipelago and date the inter-island dispersal events, we used the relaxed-clock Bayesian MCMC approach (Drummond *et al.*, 2006) implemented with the software BEAST v.2.4.5 (Lemey *et al.*, 2009; Bouckaert *et al.*, 2014). Since BEAST is computationally very intensive, we set several constraints to help run convergence.

Given that the inferred phylogenetic relationship between species was identical in all datasets (see Results section), we chose the smallest dataset (min50%) to include a single individual per species and island of distribution (the one with the highest number of recovered loci). We selected GTR+G as the model for nucleotide substitution and the uncorrelated log-normal model for rate variation within branches. For the tree prior we selected a birth–death process that we provided with informative log-normal prior distributions for the birth–death differentiation and relative death rate based on values for speciation and extinction rates of  $\lambda = 0.76$  and  $\mu = 0.14$ , respectively, estimated for sect. *Helianthemum* (Martín-Hernanz *et al.*, 2019a).

Unfortunately, no fossils of *Helianthemum* are known from the Canary Islands; hence, we used a secondary calibration point in the root node that we obtained from the most recent dated phylogenetic reconstruction of the whole family Cistaceae modified from Aparicio *et al.* (2017) (S. Martín-Hernanz *et al.*, unpubl. data). We used a normal distribution prior with a mean of 2.22 Ma; the uncertainty around this calibration point was set to include the 95 % highest posterior density (95 % HPD) of this estimate from 0.84 to 4.03 Ma (roughly corresponding to a standard deviation of 0.70). We fed BEAST with an initial tree displaying a topology concordant with that of the phylogenetic analyses (see Results section), which was not fixed during runs. This tree was an ultrametric tree constructed with the function chronos in the R package ape v.5.3 (Paradis and Schliep, 2018) by assigning the same ample minimum and maximum values to the age of the root node. We ran two MCMC analyses for 20 million generations each, sampling every 2000 generations. Convergence of chains and adequate effective sample size (ESS) of parameters  $>200$  was checked with Tracer. The maximum clade credibility (MCC) tree was obtained with TreeAnnotator after combining the chains with LogCombiner and discarding the first 10 % of sampled generations as burn-in.

We also estimated the absolute net diversification rate for the Canarian *Helianthemum* and compared it with other notable examples of plant radiations (Table 2) in the Canary Islands using the standardized whole-clade method of Magallón and

TABLE 2. Diversification rates of notable plant radiations in the Canary Islands (some of them also distributed in other Macaronesian archipelagos), including the *Helianthemum* sect. *Helianthemum* clade. Diversification rates were calculated using Magallón and Sanderson's (2001) method based on the number of species and mean (and 95 % HPD intervals) of the crown ages at two extremes of the relative extinction rate ( $r = 0$ , no extinction;  $r = 0.9$ , high extinction rate). Numbers in bold represent fast diversification rates following Vargas et al.'s criterion (2018)

Lineage	No. of species (genera)	Stem age	Crown age	Diversification rate at $r = 0$	Diversification rate at $r = 0.9$	Family	Reference
<i>Helianthemum</i> (sect. <i>Helianthemum</i> )	15	1.82 (0.61–3.04)	1.09 (0.32–1.90)	<b>1.85 (1.06–6.30)</b>	0.75 (0.43–2.57)	Cistaceae	Reyes-Betancort et al., 2008; Martín-Hernanz et al., 2019a; S. Martín-Hernanz et al., unpubl. res.; this paper
<i>Aeonium</i> alliance	61 (4)	9.7 (5.6–13.7)	6.8 (3.8–9.7)	0.50 (0.35–0.90)	0.28 (0.20–0.50)	Crassulaceae	Mort et al., 2002; Kim et al., 2008; Kondraskov et al., 2015
<i>Argyranthemum</i>	24	2.2 (1.5–3.0)	?	<b>1.13* (0.83–1.67)*</b>	0.52* (0.38–0.76)*	Asteraceae	Francisco-Ortega et al., 1996, 1997; Susanna et al., 1999; Vitales et al., 2014
<i>Cheirolophus</i>	20	8.5 (4.7–12.5)	1.7 (0.8–2.9)	<b>1.35 (1.15–2.89)</b>	0.60 (0.51–1.27)	Asteraceae	Francisco-Ortega et al., 2002; Kim et al., 2008
<i>Crambe</i> (sect. <i>Dendrocrambe</i> )	14	14.9 (9.3–20.3)	8.2 (3.9–12.5)	0.24 (0.16–0.50)	0.10 (0.06–0.20)	Brassicaceae	Böhle et al., 1996; Kim et al., 2008; García-Maroto et al., 2009
<i>Echium</i>	27	7.9 (4.3–11.1)	3.7 (1.5–5.9)	0.70 (0.44–1.74)	0.33 (0.21–0.82)	Boraginaceae	Meimberg et al., 2006; Puppo et al., 2015
<i>Micromeria</i>	16	9.6 (4.2–12.6)	8.4 (4.2–12.6)	0.25 (0.50–0.17)	0.10 (0.07–0.21)	Lamiaceae	Jones et al., 2014
<i>Pericallis</i>	14	>5.0	4.8 (2.5–7.4)	0.41 (0.26–0.78)	?	Asteraceae	Barber et al., 2007; Kim et al., 2008
<i>Sideritis</i> (subgenus <i>Marrubiastrum</i> )	23	11.9 (6.0–17.8)	3.3 (1.2–5.4)	0.74 (0.45–2.04)	0.34 (0.21–0.93)	Lamiaceae	Kim et al., 1996; Kim et al., 2008
<i>Sonchus</i> alliance	31 (6)	13.2 (7.7–18.7)	8.5 (3.0–13.9)	0.32 (0.20–0.92)	0.16 (0.10–0.44)	Asteraceae	

\*Diversification rates calculated from the stem ages, when the crown age was not available. ? Lack of information for that particular estimate.

Sanderson (2001) as implemented in the R package *geiger* v.2.0.6.1 (Harmon *et al.*, 2008). In all cases we used the mean and 95 % HPD crown ages, at two extremes of the relative extinction rate ( $r = 0$ , no extinction;  $r = 0.9$ , high extinction rate), to calculate net diversification rates. For the genus *Argyranthemum* (Francisco-Ortega *et al.*, 1996, 1997), whose crown age is unknown, the stem age was used instead.

#### Biogeographical reconstruction

The historical biogeography of the Canarian *Helianthemum* was reconstructed using the DEC model implemented in the R package BioGeoBEARS v.1.1.2 (Matzke, 2013). Specifically, we tested for models including ‘founder-event speciation’ processes that assume speciation events at the nodes of the phylogeny, leaving one daughter lineage in a new range and the other lineage retaining the ancestral range. This model is accounted for in BioGeoBEARS through the addition of a founder-event jump dispersal parameter (DEC+ $j$ ; Matzke, 2014) and seems especially suited for oceanic islands (or island-like systems; e.g. Van Dam and Matzke, 2016). In these contexts, a small number of individuals may take part in a rare long-distance colonization event and found a population that is instantly isolated from the ancestral population (Gillespie *et al.*, 2012). We refrained from making comparisons between the DEC and DEC+ $j$  models as suggested by Ree and Sanmartín (2018).

To account for uncertainty in node age estimates, BioGeoBEARS analyses were performed on a set of 100 random trees from the BEAST results using consensus results showing average values of ancestral area probabilities. We set eight operational areas: the six islands on which the Canarian *Helianthemum* species occur (La Palma, La Gomera, Tenerife, Gran Canaria, Fuerteventura and Lanzarote) plus Eastern and Western Mediterranean, where the outgroup taxa currently occur. As only one species (*H. broussonetii*) of the 15 Canarian *Helianthemum* species thrives on two islands (Fig. 1), we allowed for a maximum of two islands at each node, assuming that the dispersal ability of the ancestors was probably not higher than that of the extant species (e.g. Sanmartín, 2003; Van Dam and Matzke, 2016). Given that the emergence of the Canary Islands took place prior to the diversification processes of Canarian *Helianthemum* in the archipelago (Martín-Hernanz *et al.*, 2019a, and see Results section), we did not place any time constraint on the dispersal process. To evaluate the long-distance dispersal capacity of the Canarian *Helianthemum* throughout the archipelago, we compared two models by including a constraint matrix indicating relative distances between discrete areas (islands and mainland Africa): M0 was an unconstrained model where dispersal rates between island pairs and the continent were equally likely, while M1 was a model in which dispersal rates between island pairs were constrained by the nearest coast-to-coast geographical distance, allowing for a higher dispersal likelihood between neighbouring operational areas (i.e. islands; Supplementary Data Table S2). In this latter scenario, the distances from the Eastern and Western Mediterranean were set at the minimum distance in the distance matrix. All distances were rescaled by dividing by the minimum distance

to avoid very large values (Van Dam and Matzke, 2016). To assess the statistical significance of likelihood differences between M0 and M1 models, we employed the Akaike information criterion corrected for small sample sizes (AICc) (Burnham and Anderson, 2002).

#### Ancestral niche reconstruction

We mapped ecological information for the Canarian *Helianthemum* onto the MCC species tree obtained with BEAST, with *H. grosii* (restricted to the Mediterranean coast of north-eastern Morocco) as the outgroup. To characterize the climatic niche of each species, we extracted values for eight temperature and precipitation variables from the WorldClim 2 database (Fick and Hijmans, 2017) using QGIS 3.8 ([www.qgis.org](http://www.qgis.org)): annual temperature, maximum temperature in the warmest month, minimum temperature in the coldest month, annual precipitation, precipitation in the wettest month, precipitation in the driest month, precipitation in the wettest quarter, and precipitation in the driest quarter. These data were obtained for 31 geo-referenced occurrence points according with our own field surveys, published references and personal communications, which included all currently known localities for the 15 species (one to seven points per species; Table 1), and three locations for *H. grosii*. This dataset was subjected to a principal component analysis (PCA) with the R package *ade4* v.1.17 (Dray and Dufour, 2007). Since the first principal component extracted in the PCA yielded a clear gradient of temperature and precipitation and explained a high percentage of the total variance of the climatic data (65 %; see Results section), we used the values of each species along this axis as a proxy for its climatic niche. The phylogenetic signal of this climatic niche value across the phylogeny was assessed using Pagel’s  $\lambda$ , while the ML ancestral state inference was conducted under the following three models of continuous trait evolution: Brownian motion (BM; random drift), Ornstein–Uhlenbeck (OU; selective-adaptive model) and white noise (WN; lack of phylogenetic signal). Models were compared with AICc as implemented in the R package *geiger* v.2.0.6.1 (Harmon *et al.*, 2008). The best model of trait evolution was selected for ML ancestral reconstruction of climatic values onto the phylogeny of species using the function *anc.ML* in *phytools* v.0.6 (Revell, 2012).

## RESULTS

#### GBS sequence data assembly

The two lanes of the HiSeq Illumina run generated an average of 2 053 664 paired reads per sample (from 231 625 to 6 920 137), of which an average of 2 049 258 per sample (from 231 425 to 6 903 833) passed the quality filters (99.8 %). Average heterozygosity per sample was 0.013 and the average estimated error rate was 0.0055. The number of recovered loci per sample ranged from 900 (*Helianthemum* sp. nov. 2\_432\_3B) to 104 074 (*H. ruficomum*\_111). After filtering data according to the minimum number of samples per locus, we obtained a matrix with 4265 loci for the dataset min50%, 15 780 loci for

the dataset min25% and 24 505 loci for the dataset min15% (Supplementary Data Table S3).

Phylogenetic reconstruction

Under both the concatenation and coalescent approaches, the three datasets provided mostly congruent topologies and generally strongly supported phylogenetic relationships (Fig. 2, Supplementary Data Figs S1–S5), albeit with lower

values of branch support at some nodes in the coalescent trees (Supplementary Data Figs S3–S5). The Canarian *Helianthemum* formed a strongly supported monophyletic clade composed of five lineages (Fig. 2, Supplementary Data Figs S1–S5): (A) an early-diverging sister lineage incorporating *H. broussonetii* (Tenerife and La Palma) and *H. aguloi* (La Gomera); (B) a lineage incorporating *H. juliae* (Tenerife) and *H. cirae* (La Palma); (C) a lineage incorporating *H. inaguae* (Gran Canaria) and *Helianthemum* sp. nov. 2 (La Palma); (D) a lineage incorporating *H. bramwelliorum* (Lanzarote), *H. gonzalezferreri* (Lanzarote),

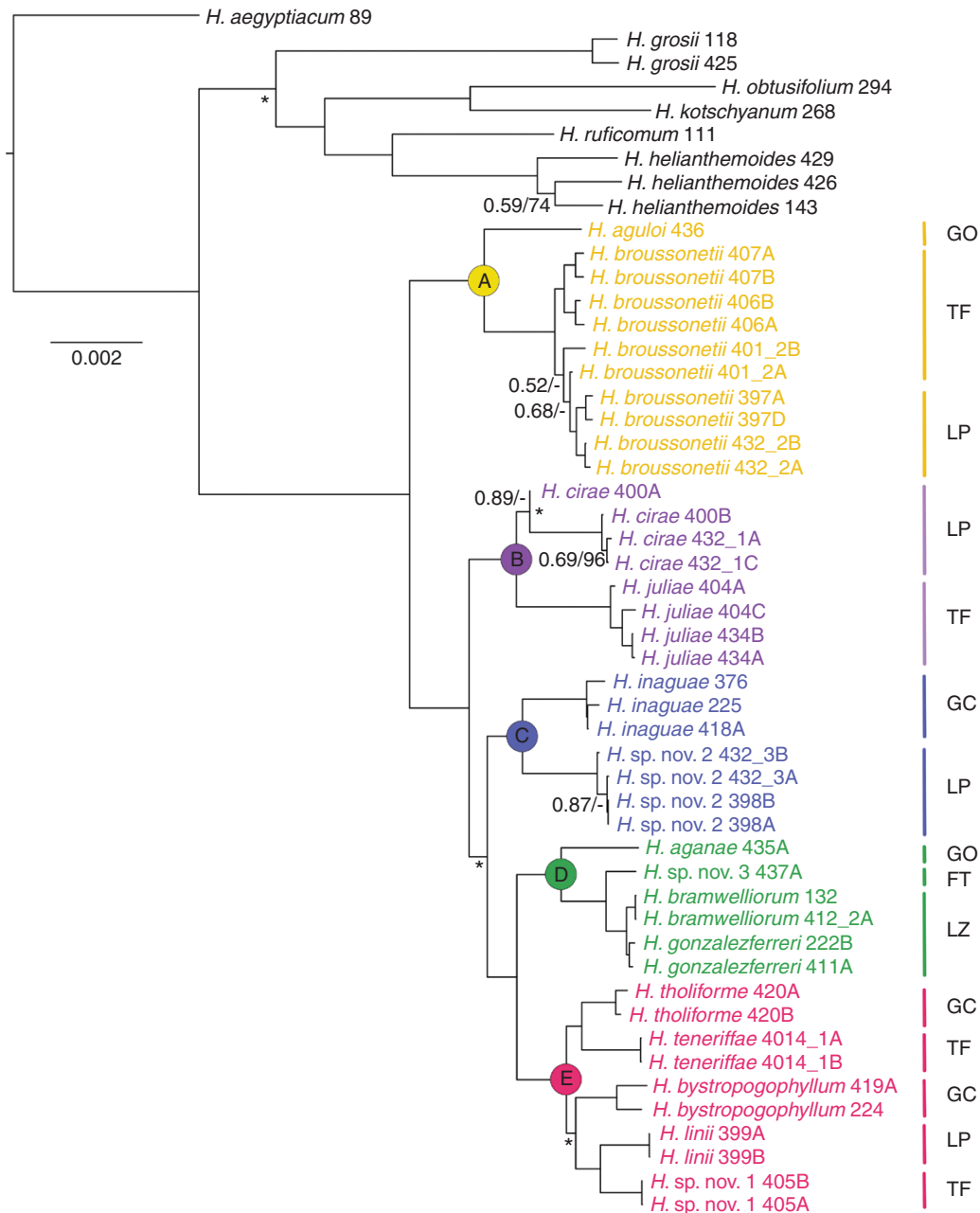


Fig. 2. Majority-rule consensus tree estimated under concatenation analyses (ML conducted in RAxML and BI in Exabayes) from the dataset min50% including the species of *Helianthemum* sect. *Helianthemum* in the Canary Islands and the outgroup species. PP/BS are shown above or below branches only when PP < 0.95 and BS < 75 %. Dashes indicate nodes not recovered by RAxML. The five lineages retrieved are identified with capital letters from A to E and coloured accordingly. Nodes with low QC (<0.5) and QD (<0.75) scores after the quartet sampling analyses are indicated by asterisks. The island of origin of the studied populations is also shown: GO, La Gomera; TF, Tenerife; LP, La Palma; GC, Gran Canaria; FT, Fuerteventura; LZ, Lanzarote.



*H. sp. nov.* 3 (Fuerteventura) and *H. aganae* (La Gomera); and, finally, (E) a lineage incorporating *H. teneriffae* (Tenerife), *H. tholiforme* (Gran Canaria), *H. bystropogophyllum* (Gran Canaria), *Helianthemum sp. nov.* 1 (Tenerife) and *H. linii* (La Palma). All species with at least two samples were recovered as monophyletic groups (Fig. 2, Supplementary Data Figs S1–S5), except for *H. gonzalezferreri*, which appeared as paraphyletic in the coalescent analyses (Supplementary Data Figs S3–S5).

Quartet sampling analyses showed all nodes with very high QI values (>0.89) throughout the datasets, although some shallow nodes depicting intraspecific relationships could not be analysed due to the low number of available sites, below the imposed threshold of 100 000 bp (Supplementary Data Figs S6–S8). Overall, QC values of the resolved branches were also very high throughout the datasets (QC mean values of 0.892, 0.878 and 0.877 for min50, min25 and min15 datasets, respectively), indicating that most relationships inferred by concatenating all loci (under ML and BI approaches) were concordant and strongly supported by the quartet sampling method. However, three clades within the ingroup displayed consistently low QC and QD values throughout the phylogenetic trees (Fig. 2, Supplementary Data Figs S6–S8), indicating weak support and a bias in discordance compatible with the presence of a secondary evolutionary history: (1) the clade including lineages C, D and E; (2) the clade including *H. bystropogophyllum* and *H. linii* plus *Helianthemum sp. nov.* 1, both strongly supported across datasets using the concatenation and coalescence methods; and (3) the clade supporting the monophyly of *H. cirae*, which had weak support in the coalescence and ML inference methods (Supplementary Data Figs S1–S8).

The concatenated min50% dataset provided strongly supported phylogenetic relationships and was the most suitable dataset for estimating divergence times because of its smaller size; as such it is the only dataset that will be illustrated and discussed in this paper (Fig. 2). The results of the phylogenetic analyses for the different datasets are depicted in Supplementary Data Figs S1–S8.

#### Divergence time and diversification rate estimates

The topology recovered by BEAST depicted the same phylogenetic relationships between species as in previous analyses, with all nodes displaying a Bayesian posterior probability of 1.0 (Supplementary Data Fig. S9). However, uncertainty is large in some instances, as reflected in the large confidence interval linked to the divergence estimates. The divergence time of the Canarian *Helianthemum* from its Mediterranean ancestor was dated at 1.82 Ma (95 % HPD confidence interval 0.61–3.04 Ma), while the starting date of the diversification process through the archipelago was established at 1.09 Ma (0.32–1.90 Ma) in the Middle Pleistocene (Supplementary Data Fig. S9).

The Canarian *Helianthemum* clade displayed a net diversification rate of 1.85 (1.06–6.30) species per million years assuming no extinction, and 0.75 (0.43–2.57) species per million years assuming a high extinction rate. This figure exceeds the rates estimated for other radiations in the Canary Islands (Table 2), being slightly higher than *Cheirolophus* (1.15–2.89

species per million years at  $r = 0$  and 0.51–1.27 at  $r = 0.9$ ) and *Argyranthemum* (0.83–1.67 species per million years at  $r = 0$  and 0.38–0.76 at  $r = 0.9$ ), and considerably higher than the rest of the lineages here revised (<0.74 species per million years at  $r = 0$  and <0.34 at  $r = 0.9$ ). However, it is important to note that the diversification rates obtained for *Argyranthemum* are based on stem age, and that higher diversification rate values may be retrieved if the crown age were available.

#### Biogeographical reconstruction

The results of the biogeographical analysis for models M0 and M1 were identical, with the same values of (log)likelihood (–44.91) and AICc (84.56), indicating that geographical distance was not a limiting factor that has constrained the historical biogeography of Canarian *Helianthemum* in the archipelago. The positive value for the parameter  $j$  (0.161) and the virtually zero value for the dispersal ( $d$ ) and extinction ( $e$ ) parameters indicate that founder-event speciation after dispersal is the only process needed to explain the current distribution pattern. The most likely biogeographical result points to north-west Africa as the origin of the Canarian colonization and to the island of Tenerife as the place of arrival and source of initial diversification (Fig. 3, Supplementary Data Fig. S10). The highest uncertainty when inferring the ancestral range occurred in the node comprising lineages B–E, for which BioGeoBEARS gave virtually the same probability of ancestry for Tenerife and for La Palma (38 %) (Supplementary Data Fig. S10). We believe, however, that Tenerife is a more parsimonious and plausible scenario than La Palma as the origin of this clade for two reasons: because the estimated mean age for the stem of this clade (1.82 Ma) is slightly greater than the oldest parts of La Palma (1.77 Ma); and because it implies at least two fewer inter-island dispersal events. With Tenerife as the geographical origin of this clade, the reconstructed history of colonization and diversification consists of 11 inter-island dispersal events including multiple colonization events to islands such as La Palma (four times), La Gomera (twice) and Tenerife (twice) (Fig. 3).

#### Ancestral niche reconstruction

The PCA of the climatic variables depicted a clear trend in temperature and precipitation along the first axis extracted, which explained 65 % of the total variance. The five variables related to precipitation were positively correlated with this axis, while the three variables related to temperature were negatively correlated (Fig. 4A, Supplementary Data Table S4). The species occupying the extremes of variation were *H. gonzalezferreri* (value = –3.72), which occurs in a single population in the semi-arid shrublands of northern Lanzarote at 300–580 m a.s.l. in the *tabaibal-cardonal*, and *H. juliae* (value = 3.85), which occurs in a few subpopulations in the *retamar-codesar* zone in the high mountain area of Tenerife at 2050–2300 m a.s.l.

The values of the first axis of the PCA used as a proxy for the climatic niche for the species displayed a high value for Pagel's  $\lambda$  (0.98), suggesting a strong signal over the phylogenetic tree. The best evolutionary model for this trait throughout the phylogeny



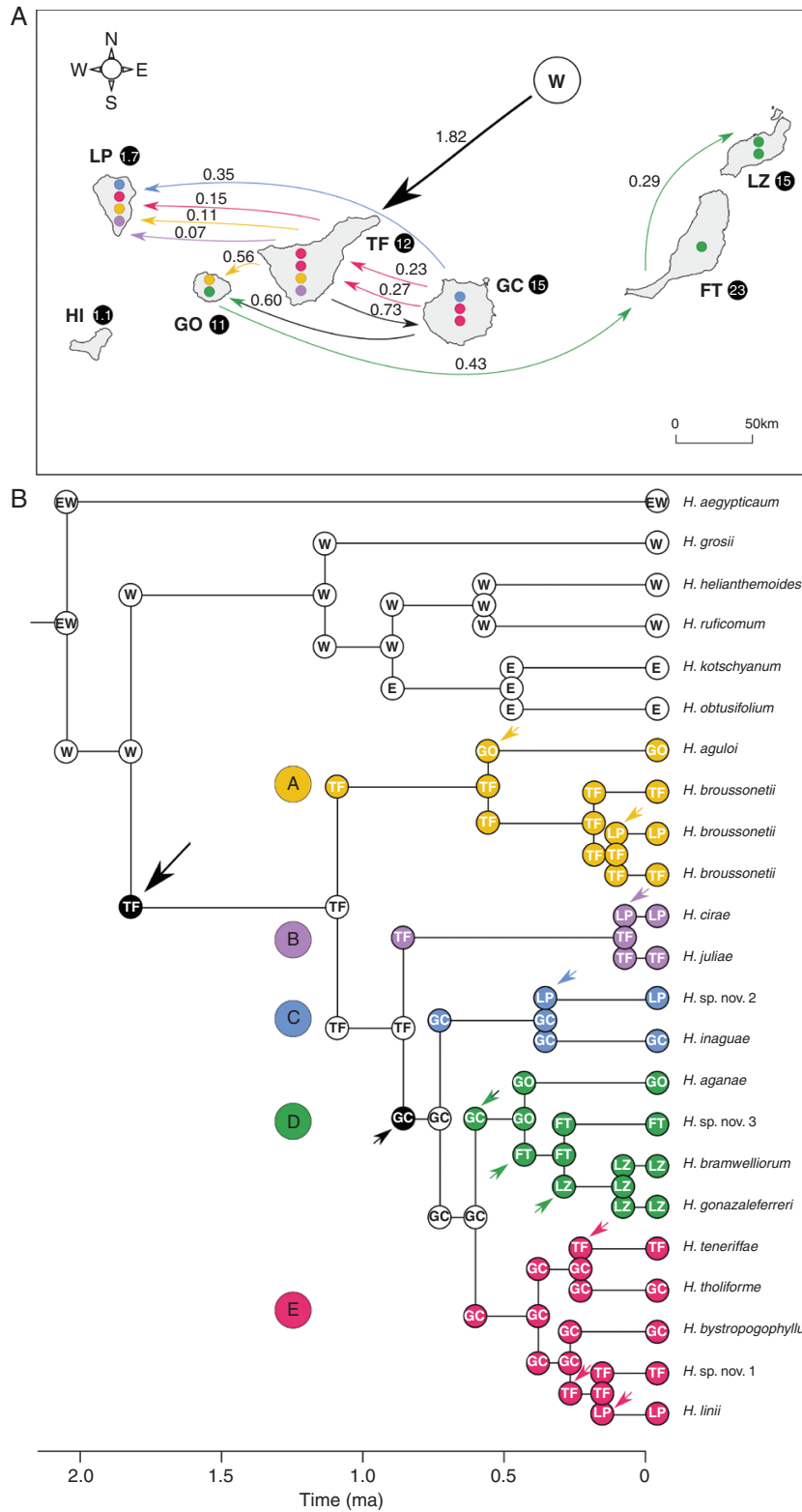


FIG. 3. Spatio-temporal reconstruction of the diversification of *Helianthemum* sect. *Helianthemum* in the Canary Islands. (A) Arrows represent the most parsimonious dispersal events that best explain the current distribution of these species in the archipelago, and are coloured according to the lineage involved in each event. Numbers above or below arrows are the mean estimated ages (in Ma) for each dispersal event. The number of species and the lineage to which they belong are indicated by dots inside the outlines of the island. (B) Ancestral area reconstruction under the DEC analysis with founder-event speciation parameters (DEC+j) conducted in BioGeoBEARS using the MCC tree obtained with BEAST from the dataset min50%. Arrows show inter-island dispersal events and indicate either an east–west or west–east direction of dispersal. Black lines, arrowheads and nodes correspond to ancestral (not extant) species or lineages: W, Western Mediterranean; E, Eastern Mediterranean; GO, La Gomera; TF, Tenerife; LP, La Palma; GC, Gran Canaria; FT, Fuerteventura; LZ, Lanzarote. Oldest island ages (van den Bogaard, 2013) are shown near the island’s abbreviated name.

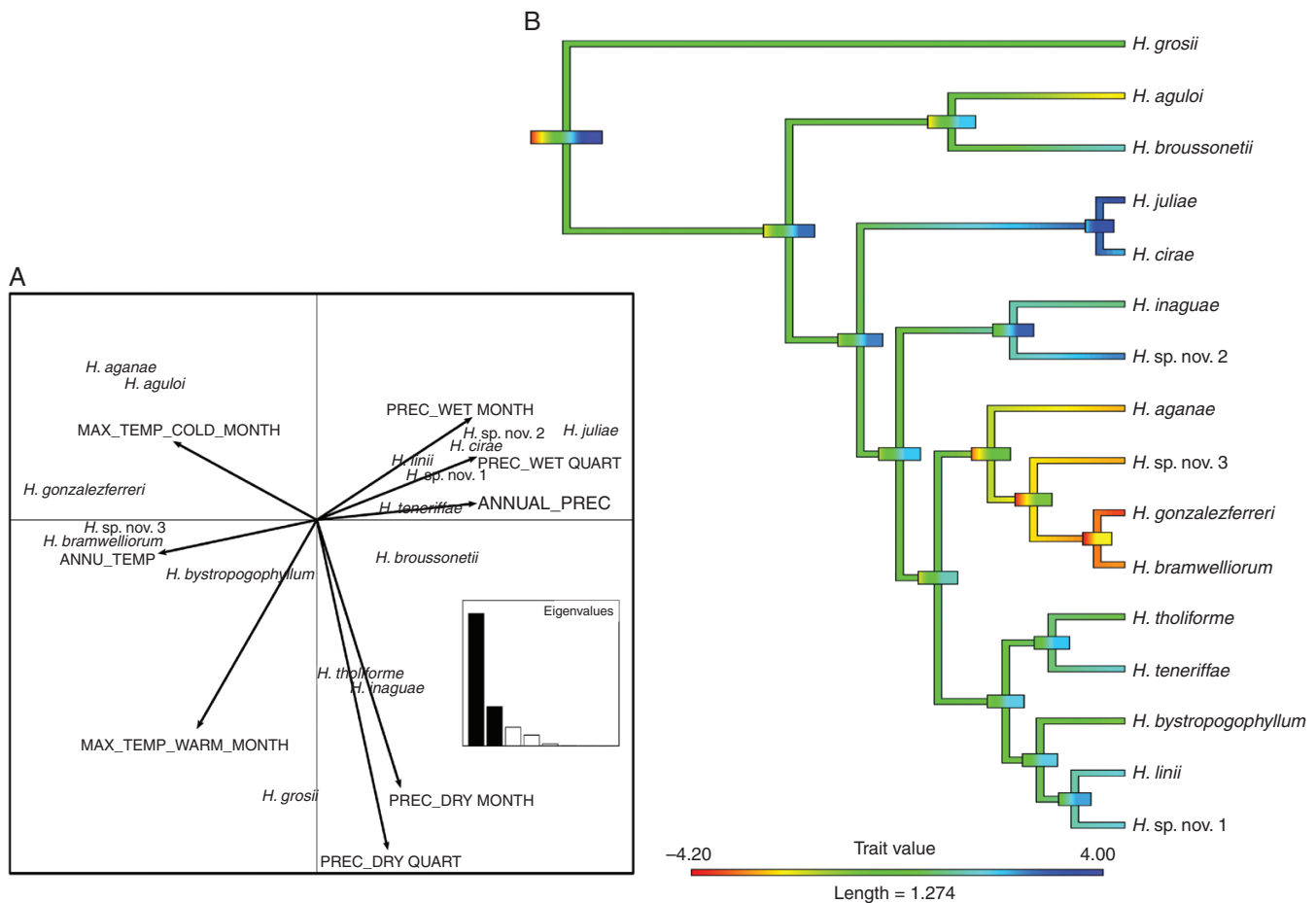


FIG. 4. Climatic niche reconstruction analysis of *Helianthemum* sect. *Helianthemum* in the Canary Islands. (A) Representation of the PCA obtained using climatic data from the occurrence points of the study species. (B) Character state reconstruction of species' climatic niche (according to the values on the first axis of the PCA) on the MCC tree obtained with BEAST from the dataset min50%. The ML reconstruction is represented as gradational colours along the branches (warmer and drier climatic conditions in red, colder and wetter in blue and intermediate values in green). The lengths of the horizontal bars at the nodes represent the 95 % confidence intervals of the ancestral niche estimates (error range).

according to the AICc value was the Brownian motion model (BM = 71.35, OU = 72.87 and WN = 76.70). The inferred history of evolution of the climatic niche of the Canarian *Helianthemum* is shown in Fig. 4B. The climatic niche reconstruction shows that the most likely ancestral habitat for this group of species was a mild climate, similar to the climate of the habitats currently occupied by *H. broussonetii* in open areas of *Morella*–*Erica* forests in Tenerife and La Palma. Despite the strong conservation of the climatic niche across the phylogenetic tree, two strong shifts in climatic niche occupancy followed by diversification were detected: (1) a change towards higher precipitation and colder conditions in lineage B (*H. juliae* and *H. cirae*) and (2) a shift towards warmer and drier climatic conditions at the base of lineage D in La Gomera (*H. aganae*), which subsequently dispersed to Fuerteventura (*Helianthemum* sp. nov. 3) and Lanzarote (*H. bramwelliorum* and *H. gonzalezferreri*).

## DISCUSSION

### Phylogenetic reconstruction

Our phylogenetic reconstructions provided strong support for the monophyly of the Canarian *Helianthemum*, as is the case for

many other Canarian plant taxa (e.g. Francisco-Ortega *et al.*, 1996; Mort *et al.*, 2002; Viales *et al.*, 2014). Moreover, the two phylogenetic approaches, concatenation and coalescence, provided congruent topologies and good support for the retrieved relationships. Both approaches recovered five lineages (Fig. 2, Supplementary Data Figs S1–S5), with concatenation displaying greater values of branch support. The consistency between these methods was also supported by the overall high consistency values (QC) of the nodes raised by the quartet sampling approach. This concordance suggests that incomplete lineage sorting had little influence on our phylogenetic reconstruction, thereby showing that, in this specific case, concatenation may explain more accurately the retrieved phylogenetic relationships between species (Chou *et al.*, 2015). However, it is important to notice that the quartet sampling analyses consistently revealed three clades with low QD values, evidence for a non-tree-like mode of evolution (Fig. 2, Supplementary Data Figs S6–S8). Specifically, our biogeographical analyses place the origin of the common ancestor of lineages C, D and E, and the sublineage including *H. bystropogophyllum*, *H. linii* and *Helianthemum* sp. nov. 1, in Gran Canaria in a time frame of 0.86–0.27 Ma (Fig. 3). The island of Gran Canaria emerged ~15 Ma (van den Bogaard, 2013) and reached its highest degree

of topographical complexity in the Late Pliocene–Pleistocene due to several intense episodes of volcanism and erosion that dismantled its last big volcanoes (Pérez-Torrado, 2000; Caujapé-Castells et al., 2017). These intense geological episodes could have created the opportunity for the genetic admixture of ancient lineages via secondary contact on the island (Caujapé-Castells et al., 2017) and the low QD values recovered for these clades might reflect the imprint of introgression events occurring at that time. Similarly, the low QC and QD values accounting for the monophyly of *H. cirae* (Fig. 2, Supplementary Data Figs S6–S7) might also reflect past instances of introgression by an ancient lineage in Tenerife previous to dispersal to La Palma. With the data to hand we cannot confirm that ancient hybridization and introgression events fostered the successful colonization of certain insular regions, as predicted by the surfing syngameon hypothesis (Caujapé-Castells, 2011, 2017). Thus, future population-level studies (e.g. Curto et al., 2017) are still required to assess the real impact of natural hybridization and introgression in the evolutionary history of the Canarian *Helianthemum*.

Finally, the fact that none of the retrieved five lineages was restricted to a single island suggests a complex evolutionary history including frequent instances of inter-island dispersal events (Fig. 3). Even within *H. broussonetii*, the only species occurring on two islands, the clustering of samples from La Palma with samples from eastern Tenerife is evidence of subsequent dispersal between islands.

#### Colonization history of the Canarian *Helianthemum*

As in other Canarian clades (e.g. Carine, 2005; Guzmán and Vargas, 2010), and according to previous estimates (Aparicio et al., 2017; Martín-Hernanz et al., 2019a), our analyses show that Canarian *Helianthemum* colonized the Canary Islands in the early Pleistocene (around 1.82 Ma; 0.61–3.04 Ma) from a Mediterranean ancestor probably originating in north-west Africa (Fig. 3), which was followed by quick diversification from the Middle Pleistocene onwards (around 1.09 Ma; 0.32–1.90 Ma). This period was a time of profound climatic change and active volcanism that significantly reduced the humid habitats available for hygrophilous taxa in the Canary Islands (Fernández-Palacios et al., 2016), thereby generating ‘windows of opportunity’ for diversification in more xeric-adapted lineages (Carine, 2005). In this regard, Canarian *Helianthemum* seems to be one of the most recent and rapidly diversified clades in the Canary Islands yet reported, whose diversification was comparable, or even faster, than the *Cheirolophus* (Vitales et al., 2014) or *Argyranthemum* (Francisco-Ortega et al., 1996, 1997) explosive radiations (Table 2).

The ancestral area reconstructions suggest that Tenerife was the first island to be colonized by Canarian *Helianthemum* from the mainland. Because of its large size, diversity of habitats and central position within the archipelago, Tenerife has also been discovered to be the source of diversification of many other Canarian clades (Sanmartín et al., 2008), including *Cheirolophus* (Vitales et al., 2014), *Cistus* (Guzmán and Vargas, 2010), *Crambe* (Francisco-Ortega et al., 2002), *Descurainia* (Goodson et al., 2006) and *Euphorbia* sect. *Aphyllis* subsect. *Macaronesicae* (Sun

et al., 2016). After an apparent initial period of stasis, early intra-island diversification occurred in Tenerife, which gave rise to two out of the five retrieved lineages in the Canarian *Helianthemum* (lineages A and B; Fig. 3). This period was followed by a complex inter-island pattern of colonizations that mostly took place in an east–west direction (Fig. 3). Firstly, a single dispersal event (one of the few west–east dispersal events retrieved in our study) to Gran Canaria occurring around 0.86 Ma (0.24–1.53 Ma) leading to an intra-island diversification that originated the ancestors of the remaining extant lineages (C, D and E), thereby making Gran Canaria a remarkable secondary source of diversification. The first dispersal event from Gran Canaria involved the ancestor of lineage D, which was dispersed to La Gomera and then to Fuerteventura and Lanzarote (the only other two west–east dispersals retrieved in our study). Three additional dispersal events from Gran Canaria took place: two independent back-dispersals to Tenerife in lineage E and one to La Palma in lineage C. Lastly, three more recent dispersal events from Tenerife to La Palma (lineages A, B and E) and one to La Gomera (lineage A) have given rise to the current distribution of these species in the archipelago. Remarkably, the two westernmost islands in the archipelago represent the two opposite extremes of diversification: El Hierro has no Canarian *Helianthemum* species, while La Palma, with four species belonging to four different lineages (A, B, C and E; Fig. 3), harbours the greatest phylogenetic diversity in the archipelago. The absence of species in El Hierro could be explained by the highly volcanic and geomorphologically active conditions prevailing on the island since its formation, demoting permanent settlement and promoting extirpation of colonizing species (Carracedo et al., 2001).

The complex colonization patterns in the Canary archipelago obtained from the biogeographical analyses performed in this study are better explained by recurrent inter-island dispersal events. This is a remarkable result given that the diaspores of *Helianthemum* species are supposed to disperse only over short distances (e.g. Tébar et al., 1997). Indeed, some *Helianthemum* species forming large and continuous populations exhibit fine-scale positive spatial genetic structures demonstrating that seed dispersal is mostly constrained by distance (Martín-Hernanz et al., 2019b). On the other hand, the fact that most inter-island dispersal events detected by our biogeographical analyses occurred in an east–west direction may be suggestive of the direct or indirect influence of the trade winds, which convert inter-island dispersal into a passive mechanism in sect. *Helianthemum*. Thus, inter-island dispersal in our case study could be viewed not just as the consequence of stochastic events but as deterministic non-standard dispersal processes (Nogales et al., 2012), as underlined by many other studies (e.g. Mort et al., 2001; Guzmán and Vargas, 2005; Fernández-Mazuecos and Vargas, 2011). In fact, some lineages may be unaffected or even display greater dispersal potential under insular conditions (García-Verdugo et al., 2019b).

#### Niche conservatism throughout the history of the Canarian *Helianthemum*

Our results suggest that both inter- and intra-island allopatric speciation are the main evolutionary forces affecting

*Helianthemum* in the Canary Islands. The predominance of climatic niche conservatism revealed by this genus's high phylogenetic signal (Fig. 4), the fact that only two closely related species inhabit the same island (Figs 2 and 3), and the mixed mating system suspected to exist in most species of Canarian *Helianthemum* (S. Martín-Hernanz, unpubl. res.) point to this speciation mechanism. In fact, the few population-level studies carried out in the Canarian *H. gonzalezferreri* and *H. juliae* have consistently shown strong spatial genetic structure and a very low level of among-population gene flow (González-Pérez et al., 2013, 2015).

However, our ancestral climatic reconstruction showed that diversification in some of the lineages was also assisted by pre-adaptation to new ecological conditions, i.e. it occurred before the inter-island dispersal (Fig. 4). In this regard, we detected two more or less concomitant significant ecological niche shifts followed by diversification. On the one hand, a shift in adaptation towards colder and wetter conditions occurred on Tenerife that facilitated adaptation to the higher altitudes currently inhabited by *H. juliae* and the dispersal of this lineage towards a similar climatic niche on La Palma (*H. cirae*) [similar patterns of diversification at higher elevation in the Canaries have also been observed in *Descourainia* (Goodson et al., 2006)]. On the other hand, a contrasting climatic niche shift occurred on La Gomera that allowed the colonization of hotter and more xeric environments, leading to the origin of *H. aganae*. This adaptation to arid conditions further stimulated the successful west-east dispersal towards the more xeric landscapes of Fuerteventura and Lanzarote, probably assisted by the prevalence of westerly winds at some time during the late Pleistocene (Rognon and Coude-Gaussen, 1996), which promoted the subsequent diversification of this lineage consisting of *H. gonzalezferreri*, *H. bramwelliorum* and *Helianthemum* sp. nov. 3 (Fig. 4). The relative floristic poverty of Fuerteventura and Lanzarote, which harbour only three species, all belonging to the phylogenetic lineage D, is not exclusive to *Helianthemum*. Despite emerging ~15–23 Ma, massive extinctions due to intense volcanic activity on these islands in the past 6 Ma (Juan et al., 1998) and/or to the impact of Pleistocene climatic oscillations (García-Verdugo et al., 2019a) may have wiped out the local biota, thereby creating new empty niches for colonization from the mainland or from other islands, in our case from La Gomera, as also suggested for *Sideritis* (Barber et al., 2000) and *Micromeria* (Meimberg et al., 2006; Curto et al., 2017). Indeed, our results show that these species diversified very recently, within the last 0.43 Ma (0.07–0.88 Ma) (Supplementary Data Fig. S9).

### Conclusions

Our results provide support for the tenet that complex patterns of inter-island dispersal to similar environmental conditions is a primary mode of species diversification in endemic Canarian plant clades, a biogeographical pattern that has seemingly only occurred in Macaronesia (e.g. Francisco-Ortega et al., 1996; Sanmartín et al., 2008; Curto et al., 2017). Among them, the Canarian *Helianthemum* stands out as one of the clades that has diversified not only most intensely but also rapidly in the Canary Islands (Fernández-Palacios and Whittaker, 2008; Domínguez Lozano et al., 2010) and, likely, both intrinsic (breeding systems, seed

dispersal syndrome) and extrinsic (fragmented landscapes, isolated habitats, climatic and geological changes) factors have contributed to the formation of these neo-endemic species (Mansion et al., 2009). Moreover, ancestral hybridization and adaptation towards new climatic niches may have also played a relevant role in the current distribution of this clade of endemic species. Future research should delve into answering why some lineages have profusely radiated in Macaronesia while others have failed to do so (e.g. García-Verdugo et al., 2015), and also to explore why this ample array of neo-endemic species in the Canarian archipelago are currently so critically restricted and endangered.

### SUPPLEMENTARY DATA

Supplementary data are available at <https://academic.oup.com/aob> and consist of the following. Figure S1: majority-rule consensus tree estimated under concatenation analyses from the dataset min15% including the species of *Helianthemum* sect. *Helianthemum* in the Canary Islands. Figure S2: majority-rule consensus tree estimated under concatenation analyses from the dataset min25% including the species of *Helianthemum* sect. *Helianthemum* in the Canary Islands. Figure S3: phylogenetic tree of *Helianthemum* sect. *Helianthemum* in the Canary Islands from applying the coalescence-based analysis of tetrads from the dataset min15%. Figure S4: phylogenetic tree of *Helianthemum* sect. *Helianthemum* in the Canary Islands from applying the coalescence-based analysis of tetrads from the dataset min25%. Figure S5: phylogenetic tree of *Helianthemum* sect. *Helianthemum* in the Canary Islands from applying the coalescence-based analysis of tetrads from the dataset min50%. Figure S6: quartet sampling score for branches of the majority-rule consensus tree estimated under Exabayes from the dataset min15%. Figure S7: quartet sampling score for branches of the majority-rule consensus tree estimated under Exabayes from the dataset min25%. Figure S8: quartet sampling score for branches of the majority-rule consensus tree estimated under Exabayes from the dataset min50%. Figure S9: maximum clade credibility tree produced by relaxed molecular clock analysis conducted in BEAST from the dataset min50%. Figure S10: ancestral area reconstruction under the DEC+j model conducted in BioGeoBEARS using the maximum clade credibility tree obtained with BEAST from the dataset min50%. Table S1: sampling information of the studied taxa and samples of *Helianthemum* sect. *Helianthemum* included in this study. Table S2: nearest coast-to-coast geographical distance between neighbouring operational areas calculated to evaluate the long-distance dispersal capacity of the Canarian *Helianthemum* throughout the archipelago through ancestral area reconstruction using BioGeoBEARS. Table S3: characteristics of the three assembled datasets generated with ipyrad. Table S4. Summary of the principal component analysis of the eight climatic variables extracted from *Helianthemum* sect. *Helianthemum* species in the Canary Islands.

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