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journal homepage: www.elsevier.com/locate/ympevBiogeographic history and environmental niche evolution in the palearctic genus *Helianthemum* (Cistaceae)Sara Martín-Hernanz^{a,*}, Rafael G. Albaladejo^a, Sébastien Lavergne^b, Encarnación Rubio^a, Aurélie Grall^c, Abelardo Aparicio^a^a Departamento de Biología Vegetal y Ecología, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain^b Laboratoire d'Ecologie Alpine (LECA), CNRS – Université Grenoble Alpes – Université Savoie Mont Blanc, FR-38000 Grenoble, France^c Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom

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ABSTRACT

The biogeographic history and the degree of environmental niche conservatism provide essential clues to decipher the underlying macroevolutionary processes of species diversification and to understand contemporary patterns of biodiversity. The genus *Helianthemum* constitutes an excellent case study to investigate the impact of the geo-climatic changes and the environmental niche shifts on the origins of plant species diversity in the Mediterranean hotspot. It is a palearctic species-rich lineage with c. 140 species and subspecies mostly belonging to three distinct evolutionary radiations, almost confined to the Mediterranean region and occurring across varied environmental conditions. In this work, we studied the ample and rapid diversification of the genus *Helianthemum* across its whole distribution range by performing phylogenetic reconstructions of ancestral ranges and environmental niche evolution. We observed a striking synchrony of biogeographic movements with niche shifts between the three major clades of the genus *Helianthemum*, likely related to the geo-climatic events occurred in the Mediterranean Basin since the Upper Miocene. In particular, Late Miocene and Early Pliocene were dominated by episodes of range expansions, the Late Pliocene by range contraction and vicariance events, and Pleistocene by most intense environmental niche shifts and *in-situ* diversification. Our study also provides evidence for four main environmental niches in *Helianthemum* (i.e., Mediterranean, subdesert, humid-montane and subtropical-insular) and a tendency toward environmental niche conservatism within different subclades, with few niche shifts mostly occurring from Mediterranean ancestors. The relative longer time spent in Mediterranean areas by the ancestors of *Helianthemum* suggests that the larger species diversity observed in the Mediterranean (i.e. Northern Africa and Southern Europe) may have been generated by a time-for-speciation effect reinforced by environmental niche conservatism. Overall, our work highlights the role of the Mediterranean Basin as a 'cradle of diversity' and an 'evolutionary hub', facilitating the environmental transitions and determining the building up of a global plant biodiversity hotspot.

1. Introduction

The study of how geo-climatic events have driven the biogeographic history of different lineages and their degree of niche conservatism over time, provide essential clues to interpret the underlying macroevolutionary processes of species diversification and to understand contemporary patterns of biodiversity (Condamine et al., 2018; Šmíd et al., 2019). A geographical area where geo-climatic changes strongly imprinted the biogeographic history of many plant lineages is the Mediterranean Basin, a global biodiversity hotspot harbouring c. 25,000

plant species (Myers et al., 2000; Thompson, 2020). In particular, the formation of land bridges during the Oligocene and Miocene periods led to biotic expansions across the Mediterranean Basin (Oosterbroek and Arntzen, 1992; Lavergne et al., 2013), and the climatic oscillations that occurred from the early Pliocene onwards (i.e. onset of the Mediterranean Climate and the subsequent Quaternary glacial episodes) opened up a massive heterogeneity of habitats and glacial refugia (Médail and Diadema, 2009), which promoted a rapid diversification of several groups of plants. The complex geo-climatic history of the Mediterranean Basin thus generated a high level of endemism mainly circumscribed

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within a few unrelated species-rich lineages (i. e. families, genera, tribes or sections; Euro + Med PlantBase, 2006; Vargas et al., 2018).

During the last two decades, much effort has been devoted to reconstruct the phylogenetic and biogeographic histories of many species-rich lineages in the Mediterranean [e.g. *Anthemis* (Lo Presti and Oberprieler, 2009), *Antirrhinum* (Vargas et al., 2009), *Aquilegia* (Bastida et al., 2010), the *Cistus-Halimium* complex (Guzmán et al., 2009), *Dianthus* (Valente et al., 2010a), *Erodium* (Fiz-Palacios et al., 2010), *Erysimum* (Moazzeni et al., 2014), *Limonium* (Koutroumpa et al., 2021), *Linaria* sect. *Supinae* (Blanco-Pastor et al., 2012), *Linaria* sect. *Versicolores* (Fernández-Mazuecos and Vargas, 2011), *Narcissus* (Santos-Gally et al., 2012), *Nigella* (Bittkau and Comes, 2009), *Ophrys* (Breitkopf et al.,

2015), *Reseda* sect. *Glaucoreseda* (Martín-Bravo et al., 2010) or *Tragopogon* (Bell et al., 2012)]. In spite of this considerable effort, the phylogenetic trees reconstructed in these studies sometimes remain poorly resolved due to limited informative genetic polymorphisms, so that divergence time estimations and biogeographic conclusions should therefore be taken with caution. On the other hand, many of these species-rich Mediterranean lineages thrive in a wide-range heterogeneity of habitats and also present an asymmetric geographic distribution, i. e. by having a small number of species in adjacent areas outside the Mediterranean Basin (a pattern also documented for the other Mediterranean-climate regions; Cowling et al., 1996; Beard et al., 2000; Hopper and Gioia, 2004; Skeels and Cardillo, 2017). Some possible

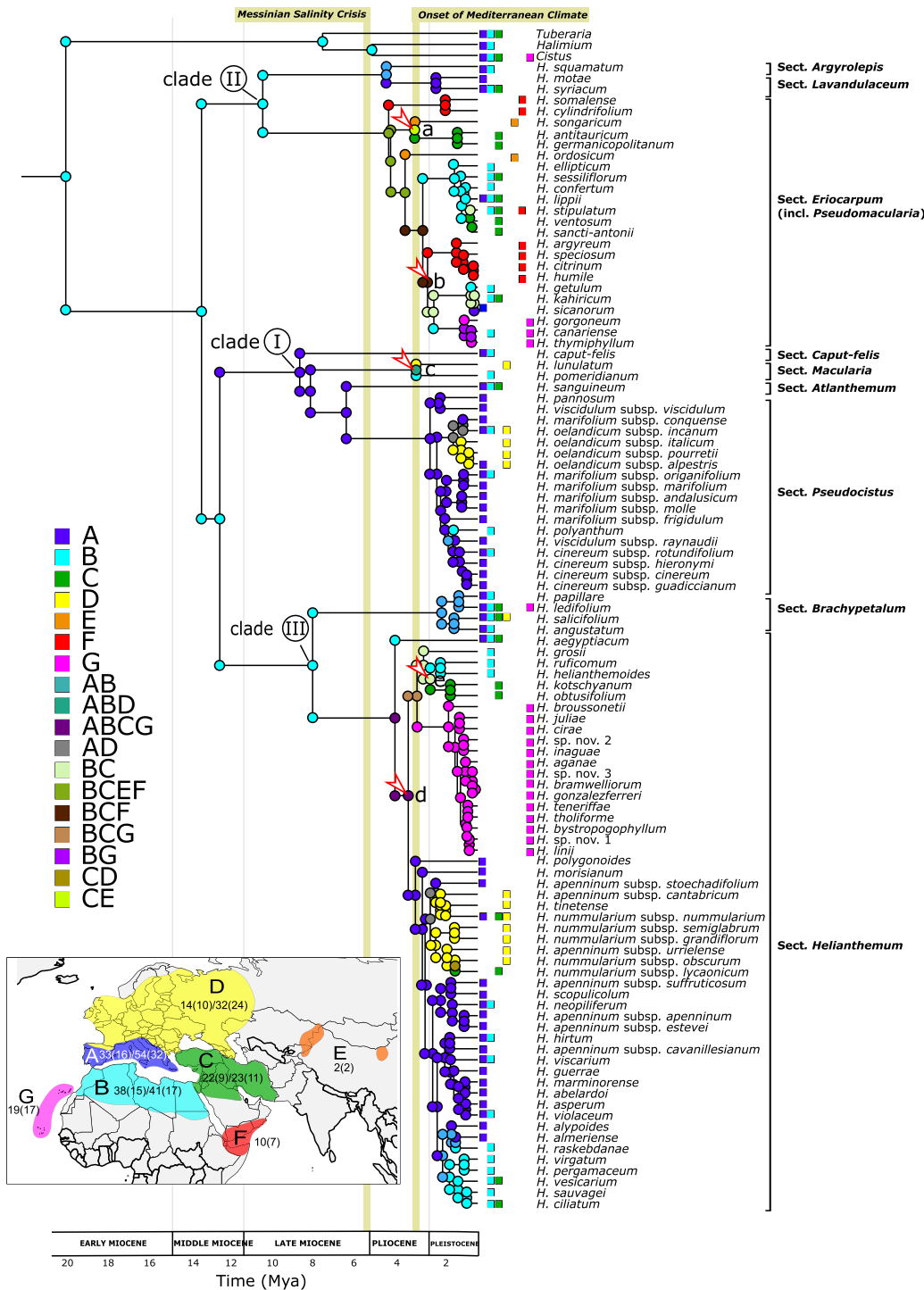


Fig. 1. Operational areas (A: Southern Europe, B: Northern Africa, C: The Middle East, D: Central and Northern Europe, E: Central Asia, F: Horn of Africa, and G: Macaronesia) and ancestral area reconstruction in *Helianthemum* under the dispersal-extinction-cladogenesis (DEC) analysis applying the model with highest log-likelihood value on the 'RD updated GBS-trees' (see text for explanation). Coloured circles in the nodes of the phylogenetic tree represent the ancestral area receiving the maximum relative posterior probability and circles above and below the nodes the most likely ranges inherited from each ancestor (colour pattern according to the retrieved ancestral areas). Current distribution of taxa is indicated by coloured squares before taxa names. Red arrowheads represent vicariance events, which are denoted by lowercase letters a-e. The number of species and taxa (species + subspecies) in each operational area is indicated in the inset as follows: number of total species in the area (number of endemic species in the area) / number of total taxa in the area (number of endemic taxa in the area). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

drivers of higher Mediterranean diversity in these lineages include (i) higher diversification rates in the Mediterranean Basin than elsewhere, (ii) higher immigration rates into the Mediterranean Basin from adjacent areas, or (iii) more time to accumulate diversity in the Mediterranean Basin than elsewhere (time-for-speciation effect) (Sauquet et al., 2009; Valente et al., 2010b; Buerki et al., 2012, 2013; Lancaster and Kay, 2013; Onstein et al., 2014). Regarding the last hypothesis, recent studies have documented that the greater permanence of lineages in biodiversity hotspots was reinforced by strong niche conservatism, stressing the need to jointly reconstruct biogeographic and niche evolution (Crisp et al., 2009; Skeels and Cardillo, 2017; Segovia et al., 2020), which has rarely been performed for Mediterranean lineages (Lo Presti and Oberprieler, 2009).

The genus *Helianthemum* (Cistaceae) constitutes an excellent biological model to investigate the impact of the geo-climatic changes and the environmental niche shifts on the origins of species diversity in Mediterranean plant lineages. *Helianthemum* is a palearctic species-rich monophyletic lineage with c. 140 species and subspecies, mostly distributed across the Mediterranean Basin, but with several species distributed in adjacent non-Mediterranean areas such as Macaronesia, Central and Northern Europe, the Horn of Africa and Central Asia (see Fig. 1, and the Fig. 5 in Aparicio et al., 2017). Remarkably, the species of *Helianthemum* thrive in quite disparate environmental conditions from arid and semi-arid scrubs to subalpine vegetation on a variety of bedrocks such as limestone, dolomite, schist, gypsum, saline, volcanic and sandy soils (Aparicio et al., 2017; Martín-Hernanz et al., 2019a, 2019b). Phylogenetically, every species of *Helianthemum* belongs to one of the three major clades constituting the genus, which mostly fit its three largest taxonomical sections *Eriocarpum*, *Pseudocistus* and *Helianthemum*. Furthermore, each of these sections represent an evolutionary radiation, which started to diversify from the Late Miocene onwards (Martín-Hernanz et al., 2019a). The almost synchronous onset of the three evolutionary radiations in *Helianthemum* (i.e., temporal congruence) may thus be a consequence of the dramatic geo-climatic changes that have occurred in the Mediterranean Basin since that period. If so, a pattern of biogeographical congruence during the rapid diversification of the three major clades of the genus would be expected (Vargas et al., 2014). So far, the events of biogeographic movements and environmental niche shifts that have occurred during the diversification of the genus *Helianthemum* remains unexplored. Resolving the past evolutionary scenario of this species-rich lineage could serve as an important example to better understand the historical and biogeographic relationships between the Mediterranean Basin and other adjacent regions.

Here, we study the broad and rapid diversification of the genus *Helianthemum* across its whole distribution range (Mediterranean Basin and adjacent areas) by performing phylogenetic comparative analyses of historical biogeography and environmental niche evolution. Firstly, we inferred a large and robust time-calibrated phylogenetic tree primarily based on genotyping-by-sequencing (GBS) data (Martín-Hernanz et al., 2019a), and used to analyse species distribution (chorology) and abiotic niches (climatic and soil requirements). Specifically, we (i) estimated the ancestral ranges for the genus *Helianthemum* and its three major clades, (ii) inferred the main colonization routes and predominant biogeographic events involved in its diversification (i.e. range expansion, range contraction, vicariance, and *in-situ* diversification), (iii) identified main environmental niches inhabited by *Helianthemum* and reconstructed its evolution along the phylogeny, and (iv) compared species diversification rates among its different environmental niches. Specifically, we address the following questions: (i) Have the three major lineages of the genus *Helianthemum* followed congruent biogeographic histories? (ii) Was the onset of the three evolutionary radiations in *Helianthemum* linked to environmental niche shifts or rather to pervasive niche conservatism? and (iii) which biogeographic hypothesis (higher diversification rates, higher immigration rates, or the time-for-speciation effect) better explains the higher diversity of *Helianthemum*

within the Mediterranean Basin?

2. Materials and methods

2.1. Phylogenetic framework

The input tree for the biogeographic and environmental niche reconstruction performed in this study is a modification of the robust time-calibrated tree generated by Martín-Hernanz et al. (2019a) based on an extensive GBS dataset of *Helianthemum* (98 taxa: 73 species and 25 subspecies) under the assembly configuration which minimized error rates, which would be referred hereafter in this paper as the ‘updated GBS-tree’. The update consisted in the inclusion of six species from Somalia and the southern of the Arabian Peninsula (i.e. the Horn of Africa area), a very relevant biogeographic region where seven endemic exclusive species exist (Gillett, 1954). Nevertheless, given the high DNA quality required to obtain readable GBS libraries and the unavailability of freshly collected material for these additional species from the Horn of Africa area, we used plastid (*ndhF*, *psbA-trnH*, *trnL-trnF*) and nuclear (ITS) DNA Sanger sequences obtained from herbarium-preserved specimens (Table S1) and proceeded as follows.

To infer the phylogenetic emplacement of the species from the Horn of Africa area as accurate as possible, as well as to increase the reliability in the divergence time estimations for the whole genus, we reanalysed the concatenated cpDNA (*ndhF*, *psbA-trnH*, *trnL-trnF*) ± nrDNA (ITS) dataset in Aparicio et al. (2017) to which 57 new samples were included: five populations from five species from the Horn of Africa area, 18 populations from ten species belonging to sect. *Eriocarpum* coming from different geographic areas, and 34 populations from 19 species belonging to sect. *Helianthemum* mostly from the Canary Islands (Table S1; Fig S1). Details about DNA extraction, PCR amplification and sequencing, phylogenetic analyses and lineage divergence times are in Aparicio et al. (2017). However, for the divergence time estimation in this reanalysis, the position of the fossil calibration point derived from pollen found in the Middle Pleistocene from the Elsterian Glaciation in Poland (Hrynowiecka and Winter, 2016) was moved to a more external position instead of the crown of sect. *Helianthemum* (where it was placed by Aparicio et al., 2017), according to the information retrieved by the robust GBS-tree (see Fig. S1; Martín-Hernanz et al., 2019a). In addition, two topological constraints were enforced in those nodes where a particular resolution was needed based on prior phylogenetic knowledge: the monophyly of the family Cistaceae and the relationship between clades I and III (Martín-Hernanz et al., 2019a). Since the precise emplacement of the clades containing the species from the Horn of Africa was not well-supported, we assessed all possible phylogenetic hypotheses through constraining tree topologies in BEAST v. 1.8.4 (Drummond et al., 2012), computing marginal likelihoods from each alternative topology and by statistical tests using Bayes factors (see Fig. S2). None of the alternative topologies was favoured by the Bayes factor so we selected the configuration recovered in BEAST without constraints for the following steps.

Once the phylogenetic emplacement and the divergence times of the species from the Horn of Africa were estimated in the reanalysis of the cpDNA + nrDNA dataset, we firstly extracted the resulting subclades over 900 random BEAST chronograms and added to them a root edge with branch lengths corresponding to the mean values estimated over all BEAST chronograms using the phytools R package (Revell, 2012). Secondly, we inserted the subclades into the corresponding nodes of the 900 TreePL trees used to compute the dated GBS-tree (Martín-Hernanz et al., 2019a) at different positions of the corresponding branch respecting the 95% HPD estimated in BEAST using the ape R package (Paradis et al., 2004). Finally, we summarized the modified 900 TreePL trees (now representing c. 75% of the genus *Helianthemum* with 104 taxa: 80 species and 24 subspecies) in a single chronogram with their corresponding 95% confidence intervals (‘updated GBS-tree’) using TreeAnnotator v.1.8.4 (Fig. S3). In order to integrate phylogenetic uncertainty in some of the

following biogeographic and phylogenetic comparative analyses (see below), a subset of 100 dated trees (hereafter ‘RD updated GBS-trees’) was also randomly sampled.

2.2. Biogeographic reconstruction

2.2.1. Distributional data and operational areas

The geographic distribution of the species and subspecies included in the ‘updated GBS-tree’ was determined using different literature sources (see Appendix S1 in Aparicio et al., 2017) and the Euro + Med Plant-Base. Operational areas for biogeographic analyses were defined as geographic ranges that reflected the main centres of diversity or area of endemism in *Helianthemum*, considered as a congruent distributional range shared by, at least, two endemic species (Sanmartín, 2003). As shown in Fig. 1, the areas under consideration were: (A) Southern Europe (Mediterranean areas of Spain, France, Italy, Western Mediterranean Islands and Greece), (B) Northern Africa (from Morocco to Egypt), (C) Middle East (Sinai, northern Arabian Peninsula, Israel, Jordan, Iraq, Iran, Syria, Turkey and Eastern Mediterranean Islands), (D) Central and Northern Europe (from North of Spain to the Caucasus), (E) Central Asia (China and Mongolia), (F) Horn of Africa (Somalia, Oman and Yemen) and (G) Macaronesia (Canary Islands and Cape Verde). The outgroup taxa were assigned to a whole Mediterranean distribution (i.e., occurring in A, B and C), which matches the real distribution of the genus *Tuberaria* and the *Cistus-Halimium* complex (in this case, also including Macaronesia; Guzmán and Vargas, 2009).

2.2.2. Ancestral area reconstruction analyses

To reconstruct the biogeographic history of *Helianthemum*, we employed a model-based maximum-likelihood approach for ancestral area optimization: the dispersal-extinction-cladogenesis (DEC) model implemented in BioGeoBEARS R package (Matzke, 2013). This method applies a continuous-time stochastic model for geographical range evolution in discrete areas, with maximum likelihood (ML) parameters estimated from dispersal rates between areas (range expansion) and local extinction within areas (range contraction), according to

exponential rate parameters. The probability of dispersal and extinction events along any branch of the phylogenetic tree depends on the evolutionary time (Ree and Smith, 2008). Furthermore, the model allows dispersal rates between areas to be constrained based on paleogeographical information and assumptions on the ability for long-distance dispersal of species.

To evaluate the long-distance dispersal capability in *Helianthemum* and whether the spatial arrangement of the operational areas defined has effect on biogeographic patterns of evolution, we compared six models (M0–M5; Table 1) differing in the number of areas allowed for the ancestral ranges and constancy of dispersal rates through time. In M0–M2 all ancestral area combinations were possible (unconstrained) whilst in M3–M5 non-adjacent ranges of more than two areas between them were not allowed (constrained). In M0 and M3, the dispersal rate was kept constant through time and between areas. M1 and M4 incorporated a dispersal probability matrix constant through time to determine the effect of geographic distance on dispersal ability (e.g. Santos-Gally et al., 2012). We used a dispersal rate of 1 between adjacent areas, 0.5 to areas separated by only one intermediate area or by a sea-strait, 0.1 to areas separated by two areas between them, one area and a sea-strait or a long sea-barrier and 0.01 to areas separated by more than two areas or by one area and a long sea-barrier. In M2 and M5, dispersal rate was set to vary according to historical connections among areas: being maximum between the three Mediterranean areas during the Messinian Salinity Crisis (MSC) in the Late Miocene (5.96–5.33 Mya; Krijgsman et al., 1999), when the contact between the Eurasian and African plates and the desiccation of the Mediterranean Sea decreased marine barriers among areas (dispersal probabilities between areas A, B and C equal to 1), and as was described above during the time intervals before and after the Messinian event, when marine barriers were active. We assumed that the dispersal ability of the ancestors was not higher than that of extant species (Sanmartín, 2003) and limited the maximum number of areas in ancestral distributions to four in all the models. Reconstructions described above rely on both single ‘updated GBS-tree’ and ‘RD updated GBS-trees’ to account for uncertainty in tree topology and node ages.

Table 1

Results for the biogeographic models tested under the dispersal-extinction-cladogenesis analyses, including values of log-likelihood (lnL), Akaike Information Criterion (AIC), dispersal rate (d), extinction rate (e) and ancestral areas inferred with the highest relative probabilities for selected clades. Values or areas before and after the slashes were estimated using the ‘updated GBS-tree’ and the ‘RD updated GBS-trees’, respectively. The row marked in bold represents the best model.

Range constrains	Dispersal matrix	Model name	lnL	AIC	d	e	anc Genus	anc Clade II	anc <i>Eriocarp.</i>	anc Clade I	anc <i>Pseudoc.</i>	anc Clade III	anc <i>Helianthem.</i>
UNCONSTRAIN DISJUNCT RANGES	No dispersal matrix	M0	−245.08 / −245.20	494.16 / /	0.0303 / /	0.0025 / /	B / B	B / B	BCEF / BCEF	B / B	A / A	B / B	ABCG / ABCG
	Dispersal matrix constant through time	M1	−230.56 / −230.72	494.40 / 465.43	0.0303 / 0.0725	0.0025 / 0.0105	A / A	B / B	BCEF / BCEF	A / A	A / A	A / A	ABCG / ABCG
	Dispersal matrix variable through time	M2	−230.20 / −230.49	464.40 / 464.98	0.0718 / 0.0718	0.01 / 0.0104	ABCG / /	B / B	BCEF / BCEF	A / A	A / A	ABCG / /	ABCG / ABCG
	Dispersal matrix variable through time	M3	−230.90 / −231.32	465.80 / /	0.0478 / /	0.0144 / /	B / B	B / B	BCEF / BCEF	A / A	A / A	B / B	ABCG / ABCG
CONSTRAIN DISJUNCT RANGES	Dispersal matrix constant through time	M4	−219.81 / −221.46	443.62 / 446.92	0.0948 / 0.0948	0.0144 / 0.0125	B / B	B / B	BCEF / BCEF	A / A	A / A	B / B	ABCG / ABCG
	Dispersal matrix variable through time	M5	−228.17 / −228.17	460.35 / 460.35	0.0716 / / 0.716	0.0665 / /	B / B	B / B	BCEF / BCFE	A / A	A / A	B / B	ABCG / ABCG

2.3. Environmental niche reconstruction

2.3.1. Environmental data collection and characterisation of the environmental niches

In order to reconstruct the environmental niche evolution along the phylogenetic tree of *Helianthemum*, we first characterised the environmental niches for the 104 study taxa based on the geological substrate and climatic data. Geological substrate and climatic conditions may be involved in the diversification of a large number of Mediterranean plant lineages and have long been considered as important triggers for the emergence of local endemism in the region (Nieto-Feliner, 2014; Molina-Venegas et al., 2015; Rundel et al., 2016; Thompson, 2020).

First, we extracted the geological substrate inhabited by each taxon from numerous literature sources (see Appendix S1 in Aparicio et al., 2017) and coded it as a discrete variable (see Table S2). Second, we compiled a dataset containing 1058 geo-referenced occurrence points across the whole distribution range of *Helianthemum* for which we extracted eight continuous climatic variables from the WorldClim2 database (Fick and Hijmans, 2017) by QGIS 3.8 (<http://www.qgis.org>; see Table S2). The geographical coordinates for the 1058 occurrence points were obtained from: (i) the original descriptions of some species (9 occurrence points), (ii) our own field surveys (596 occurrence points) or (iii) downloaded from the Global Biodiversity Information Facility (GBIF) (453 occurrence points). We queried the GBIF database applying the following filters: ‘*Helianthemum* Mill.’, ‘Preserved specimen’, ‘has coordinate’, ‘WGS84’, a query that produced more than 18,000 records (GBIF.org, 2019). Then we just selected the most recent and reliable records, since taxa identification in *Helianthemum* is often challenging and requires analysis by expert taxonomists (Aparicio et al., 2017). Finally, we plotted the records in the geographical space to ensure that no outliers were processed (Fig. S4). On average, we were able to retrieve ten highly reliable geographical occurrences for each taxon, since most species in the genus have restricted ranges or are endemic to very small regions (Aparicio et al., 2017).

We performed a multivariate analysis from the geological substrate type and the mean values of all climatic variables for each taxon to characterise the environmental niche of the 104 study taxa. As we used both discrete (geological) and continuous (climatic) variables, we conducted PCA analyses using the Hill and Smith (1976) principal component method with the ade4 R package (Chessel et al., 2004) to obtain an interpretable two-dimensional ordination according to the niche similarities among taxa. Then, we determined the optimal number of clusters (i.e. distinct environmental niches) conducting two approaches: (i) the “elbow method”, which maps the within-cluster sum of squares onto the number of possible clusters, so the optimum number of clusters corresponds with the value displaying the steepest decrease in the within-cluster dissimilarity (called elbow); and (ii) the “hierarchical clustering”, which represent graphically the structure and relationships in the data through a dendrogram and allow indirectly to decide the appropriate number of clusters based on the branch lengths. Once established the optimal number of clusters, we assigned the corresponding environmental niche to each taxon using the *k*-means clustering approach (Hartigan and Wong, 1979) using the stats R package. These analyses allowed clustering taxa among environmental niches, which we subsequently treated as new discrete characters.

2.3.2. Comparative analyses of niche evolution

To study the evolution of the environmental niche of *Helianthemum* we used two different phylogenetic datasets, (i) the ‘whole taxa’ phylogenetic dataset including all the 104 study taxa (species and subspecies), and (ii) the ‘species-level taxa’ phylogenetic dataset, obtained by pruning the previous tree so to keep all subspecies represented by one tip at species level (80 tips). In this ‘species-level’ tree we followed the nomenclature and taxonomic circumscriptions of taxa of López-González (1993) but also took in consideration the supported systematic implications of the phylogenetic reconstructions by Aparicio et al.

(2017) and Martín-Hernanz et al. (2019a). We compared the results obtained from both datasets to assess possible biases derived from the lack of consensus in the consideration of taxonomic ranks (see Section 4), particularly within species complexes [some authors consider some subspecies in that complexes at species level such as, for example, *H. marifolium* subsp. *origanifolium* sub *H. origanifolium* (Crespo et al., 2016) or *H. nummularium* subsp. *grandiflorum* sub *H. grandiflorum* (Le Floch et al., 2010)], and considering that most species complexes are non-monophyletic (Martín-Hernanz et al., 2019a).

2.3.2.1. Phylogenetic signal. To assess the mode and tempo of evolution of the environmental niches in *Helianthemum*, we measured their respective amount of phylogenetic signal by estimating λ and κ Pagel’s statistics (Pagel, 1999). Both statistics were estimated on the ‘updated GBS-tree’ and averaged through the ‘RD updated GBS-trees’ from the two axes extracted in the PCA Hill-Smith analysis using phytools (Revell, 2012).

2.3.2.2. Ancestral niche reconstruction with discrete characters. To reconstruct past evolution of clusters of those environmental niches inferred earlier, we first fitted three ML evolution models in geiger R package: the “ER” (with equal state transition rates), “SYM” (symmetrical), and “ARD” (with all rates unequal) models. We identified the best-fitting model using comparisons performed on the corrected Akaike Information Criterion (AICc), and likelihood ratio test. When likelihood difference between models was not significant, we selected the simplest model to avoid over-parameterization. We then estimated their respective absolute number of shifts between niche clusters, the dates of these shifts and the time spent in each state by running stochastic character mappings (Huelsenbeck et al., 2003), in phytools. We launched 1000 simulations using an estimated prior distribution on the root node, the best-fitting ML model recovered in the previous analyses and applied first to the ‘updated GBS-tree’, and then to the ‘RD updated GBS-trees’ to obtain most likely envelopes of past shift numbers and ages.

2.3.2.3. Environmental niche shifts analysis with continuous characters. Due to the high uncertainty of the ancestral niches estimated for the root and deepest nodes of the tree under discrete characters, as well as the slight overlapping among environmental niches (especially between Mediterranean and subdesert environments, see Section 3), we estimated the placement, magnitude and number of environmental niche shifts using the first two axes retrieved in the Hill-Smith PCA analysis. To that end, we conducted the reversible-jump Bayesian method of fitting multi-optima OU models in the R package bayou (Uyeda and Harmon, 2014). We allowed only one shift per branch and assigned an equal probability of each branch having a shift. We placed a corrected Poisson distribution as prior on adaptive optima and a probability density for a half-Cauchy distribution on the number of shifts between adaptive regimes. We run MCMC for 10^6 cycles, verified that MCMC analyses converged and discarded the first 30% of samples. We obtained and plotted the shift locations with posterior probabilities above 0.50 and the environmental regimes (i.e. set of lineages sharing an OU optimum).

2.3.2.4. Multi-state trait-based diversification analyses. To investigate whether a particular niche state have affected clade diversification rates of *Helianthemum*, we conducted multi-state trait-based analyses using the diversitree R package (FitzJohn et al., 2009; FitzJohn, 2012), applied to the ‘updated GBS-tree’. We fitted 46 distinct models make a range of assumptions, going from a model with no difference in speciation, extinction and transition rates between niche classes to the most complex model with different speciation, extinction and transition rates for all niche states. We selected the best-fitting model using the AICc. We performed subsequent Bayesian analyses for the best-fitting model and for the most complex model to estimate the parameters and plot them respectively through a MCMC, run for 10,000 generations on the 100

'RD updated GBS-trees' (100 steps per tree) with exponential prior distributions, and discarding the first 10% of the generations as burn-in. Sampling was calculated and fixed to each character state through the allocation of a state according to the PCA and clustering analyses to the included taxa (see above) and according to the taxonomic knowledge of the missing taxa (see Appendix S1 from Aparicio et al., 2017). Posterior probability (PP) distributions of all parameters were summarized and plotted.

3. Results

3.1. Phylogenetic framework

The reanalysis of the concatenated cpDNA + nrDNA dataset performed in this study to assess the phylogenetic position of the species from the Horn of Africa provided a congruent topology and slightly higher Bayesian support values than previous studies, although unresolved polytomies still remain towards more recent nodes of the tree. However, the phylogenetic tree retrieved the species from the Horn of Africa in two distinct well-supported lineages (PP \geq 0.99) within clade II (Fig. S1). In particular, *H. somalense* and *H. cylindrifolium* were emplaced in an early-diverging position within sect. *Eriocarpum*, whereas *H. argyreum*, *H. speciosum*, *H. citrinum* and *H. humile* were nested in a more external position related to other North African (*H. kahiricum*, *H. getulum* and *H. sicanorum*) and Macaronesian (*H. canariense*, *H. gorgoneum* and *H. thymiphyllum*) species (see Figs. S1 and S2) also belonging to sect. *Eriocarpum*. The stem and crown ages inferred in this new analysis were 2.8 Mya (95% highest posterior density – HPD – intervals: 1.1–4.8) and 1.4 Mya (95% HPD: 0.3–2.8) for the clade integrated by *H. somalense* and *H. cylindrifolium* and 1.7 Mya (95% HPD: 0.6–2.9) and 1.0 (95% HPD: 0.3–1.9) for *H. argyreum*, *H. speciosum*, *H. citrinum* and *H. humile* (Fig. S1). The 'updated GBS-tree' including the phylogenetic emplacement and diverging times of the species from the Horn of Africa is presented in Fig. S3.

3.2. Biogeographic reconstruction

The six biogeographic models tested for the input 'updated GBS-tree' in BioGeoBEARS produced mostly congruent biogeographic reconstructions, albeit with different optimal ancestral areas inferred for the deepest nodes (Table 1). Those models constraining disjunct ranges (M3–M5) or including dispersal probability matrices (M1, M2, M4, M5) provided better fit to the data than those unconstrained or with the same dispersal rates between areas respectively, suggesting that long-distance dispersal capability of *Helianthemum* has been low throughout its evolution. Overall, the model in which disjunct areas were constrained and a dispersal matrix constant through time was implemented (M4) is the model that received the highest log-likelihood value (-221.46) and lowest AIC value (446.92). Hence, we used this model to plot the most likely ancestral area reconstructions (Fig. 1) and to describe and discuss its underlying biogeographic processes.

The ancestral area reconstruction analysis in model M4 provided relative probabilities (rp) above 0.50 as optimal reconstruction in most of the shallow nodes, while some uncertainty remained in the deepest nodes (Fig. S5). First, the optimal reconstruction for the crown node of *Helianthemum* supports a common ancestor distributed in an area corresponding to the current Northern Africa (B; $rp = 0.19$; Fig. S5) during the Middle Miocene (13.00–13.07 Mya; Fig. S3). Nevertheless, two alternative scenarios in which the ancestor was widely distributed in the Mediterranean Basin (ABC) plus another adjacent area in Macaronesia (G) or the Horn of Africa (F) were also retrieved with about half probability ($rp = 0.11$ and $rp = 0.10$ respectively). Second, the highest probability for the origin of clade II (9.93–10.95 Mya) also corresponded to Northern Africa (B; $rp = 0.21$), although a widespread ancestor distributed in Northern Africa, the Horn of Africa, the Middle East and Central Asia was also retrieved with about half probability (BCEF; $rp =$

0.12). Third, an optimal ancestral area for the divergence between clades I and III (12.10–12.85 Mya) was inferred in Northern Africa (B; $rp = 0.38$) but a Southern European distribution cannot be dismissed (A; $rp = 0.30$). The highest uncertainty was retrieved in the ancestral area of clade III (7.69–8.29 Mya), in which four possible scenarios are possible: Northern Africa (B; $rp = 0.27$), Mediterranean Basin and Macaronesia (ABCG; $rp = 0.17$), Southern Europa (A; $rp = 0.16$) and southern Europa and North Africa (AB; $rp = 0.14$).

Despite this uncertainty in the deepest nodes of the phylogenetic tree, a pattern of biogeographical congruence of the three major clades is detected. In particular, episodes of range expansions via colonization of adjacent areas may have occurred during the Late Miocene and the Early Pliocene (clade II spreading eastward from Northern Africa to Central Asia, ancestors of clade I colonizing Southern Europe, and ancestors of clade III expanding in both sides of the Mediterranean; Figs. 1, 2) followed by range contraction and vicariance events during the Late Pliocene, and high *in-situ* diversification (i.e. speciation within each area) during the Pleistocene. High *in-situ* diversification during the last 3 Mya is reflected in the overall geographic structure along the different sections and clades. It is remarkable that there were multiple independent colonization events followed by *in-situ* diversification out of the Mediterranean Basin in the last 3 Mya: at least two instances towards Macaronesia (clades II and III), two towards the Horn of Africa (clade II) and three towards Central and Northern Europe (clades I and III).

3.3. Environmental niche reconstruction

3.3.1. Characterisation of the environmental niches

The PCA–Hill Smith analyses allowed the separation of four distinct environmental niches from the geological substrate types and the climatic variables (Fig. 3). The first two axes explain together 55.41% of total variance. The first principal component of the PCA (PC1, explaining 42.25% of total variance) represents an aridity and bedrock gradient with positive values indicating higher annual precipitation and a predominance of schist, dolomites and limestone substrates, and negative values showing warmer annual temperatures as well as gypsum, sandy and saline substrates (Fig. 3). The second principal component (PC2, accounting for 13.16% of total variation) is related to climate seasonality or continentality, allowing the separation between mainland and insular environments defined primarily by the minimum temperature of coldest month (higher in insular taxa), the maximum temperature of warmest month (lower in insular taxa), and the presence of volcanic substrates (only in insular taxa) (Fig. 3).

Both the hierarchical clustering and the "elbow" method suggested four clusters as the optimal number of groups (Fig. S6A–B). Based upon these results, the following four major environmental niches were recognized for *Helianthemum*: (a) Mediterranean environment on limestone and dolomitic (rarely schist) bedrocks (hereafter, we will refer to the pre-Mediterranean environment for those ancestral states corresponding to Mediterranean environmental conditions prior to the establishment of the Mediterranean Climate 3.4–2.8 Mya ago, and to the Mediterranean environment for those states corresponding to Mediterranean conditions after the onset of the Mediterranean Climate, see Section 4 for details), (b) humid-montane environment on limestone (rarely schist) bedrocks (hereafter, humid-montane environment), (c) subdesert environment on gypsum, sandy and saline soils (hereafter, subdesert environment) and (d) subtropical-insular environment on volcanic (rarely limestone) bedrocks (hereafter, subtropical-insular environment) (Fig. 3).

These environmental niches are partly coincident with the operational areas defined for the biogeographic reconstruction (Figs. 1, 3), particularly with regard to the humid-montane taxa, most of them distributed in Central and Northern Europe (a few humid-montane taxa also widespread in Southern Europe; operational area D) and the subtropical-insular species, all of them distributed in Macaronesia (G). The Mediterranean environment is mainly represented by taxa

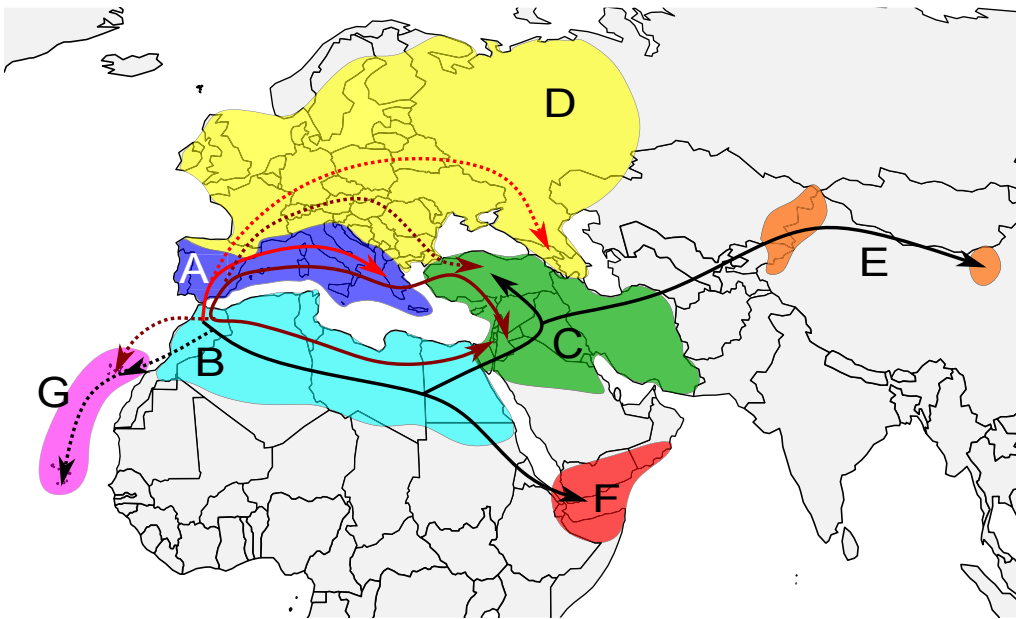


Fig. 2. Biogeographic scenario of colonization of the three main clades in the genus *Helianthemum* interpreted from the dispersal–extinction–cladogenesis (DEC) analysis. Operational areas coded for ancestral area reconstructions appear in different colours: A, Southern Europe, in dark blue; B, Northern Africa, in light blue; C, The Middle East, in green; D, Central and Northern Europe, in yellow; E, Central Asia, in orange; F, Horn of Africa, in red; G, Macaronesia, in pink. Black lines represent major colonization routes of clade II, red lines represent major colonization routes within clade I, and brown lines correspond to the colonization routes of clade III. Solid lines represent the main colonization events during the Miocene-Pliocene, while dotted lines represent the main dispersal events during the Pleistocene. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

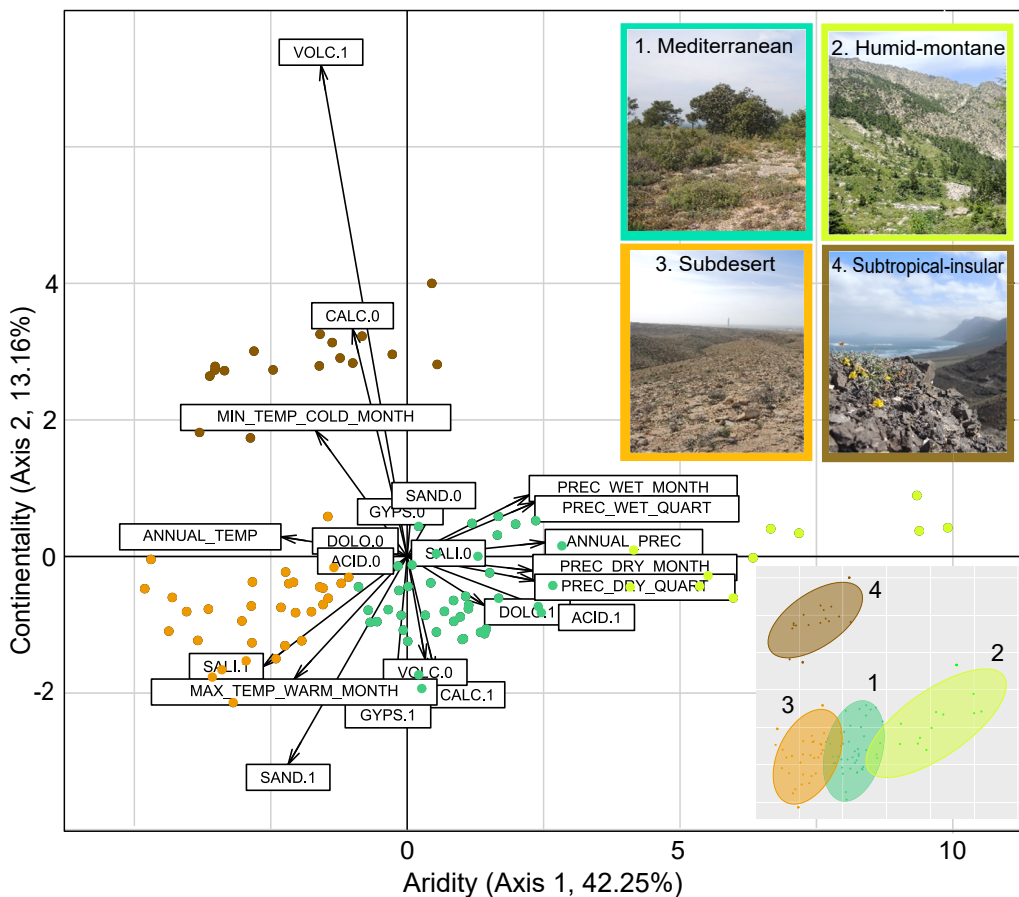


Fig. 3. Scatterplot of the first two components of the PCA-Hill-Smith of environmental variables (geological substrate and climatic data) and representation of the four environmental niches identified. Abbreviations: DOLO = dolomite, GYPS = gypsum, LIME = limestone, SALI = saline soils, SAND = sandy, SCHI = schist, VOLC = volcanic, ANNUAL_TEMP = annual temperature, MAX_TEMP_WARM_MONTH = maximum temperature of warmest month, MIN_TEMP_COLD_MONTH = minimum temperature of coldest month, ANNUAL_PREC = annual precipitation, PREC_WET_MONTH = precipitation of wettest month, PREC_DRY_MONTH = precipitation of driest month, PREC_WET_QUART = precipitation of wettest quarter, PREC_DRY_QUART = precipitation of driest quarter). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

distributed in Southern Europe (A), Northern Africa (B) and Middle East (C) while the subdesert environment are represented in the same areas than Mediterranean but also in the Horn of Africa (F). Subdesert and subtropical-insular environments represent the most arid habitats while the humid-montane environment is the wettest, being the Mediterranean environment located in the middle of the aridity gradient.

3.3.2. Comparative analyses of niche evolution

3.3.2.1. Phylogenetic signal. Our estimates of λ Pagel's statistic showed high phylogenetic signal with values close to 1 in the analyses resulting from both phylogenetic datasets (i.e. the 'whole taxa' and the 'species-level' datasets; Table S4), suggesting that environmental niches have

diversified quite early and remained under similar conditions in the genus *Helianthemum* (i.e. tendency towards environmental niche conservatism). Estimates of the κ statistic were lower than 0.5 (Table S4) indicating a tendency towards punctual niche evolution.

3.3.2.2. Ancestral niche reconstruction with discrete characters. The best-fitting evolutionary model identified in the ancestral niche reconstruction analyses of the ‘whole taxa’ dataset was the “SYM” model. The most likely ancestral environmental niches of *Helianthemum* were subdesert ($rp = 0.56$) or pre-Mediterranean ($rp = 0.43$) environments (Fig. 4A). This ambiguity was also retrieved in the ancestral environmental niche

recovered for the divergence between clades I and III (subdesert: $rp = 0.53$; pre-Mediterranean: $rp = 0.47$) and the crown node of clade III (subdesert: $rp = 0.50$; pre-Mediterranean: $rp = 0.48$). Most of the diversification within clade II occurred in the subdesert environment while in clade I it took place mainly in the pre-Mediterranean or Mediterranean environment, occurring equally in both subdesert and Mediterranean environments in clade III. The analysis of stochastic character mapping estimated that the number of environmental shifts during the evolution of *Helianthemum* mainly occurred between Mediterranean and subdesert environments followed by shifts between Mediterranean and humid-montane, and more rarely between Mediterranean or subdesert

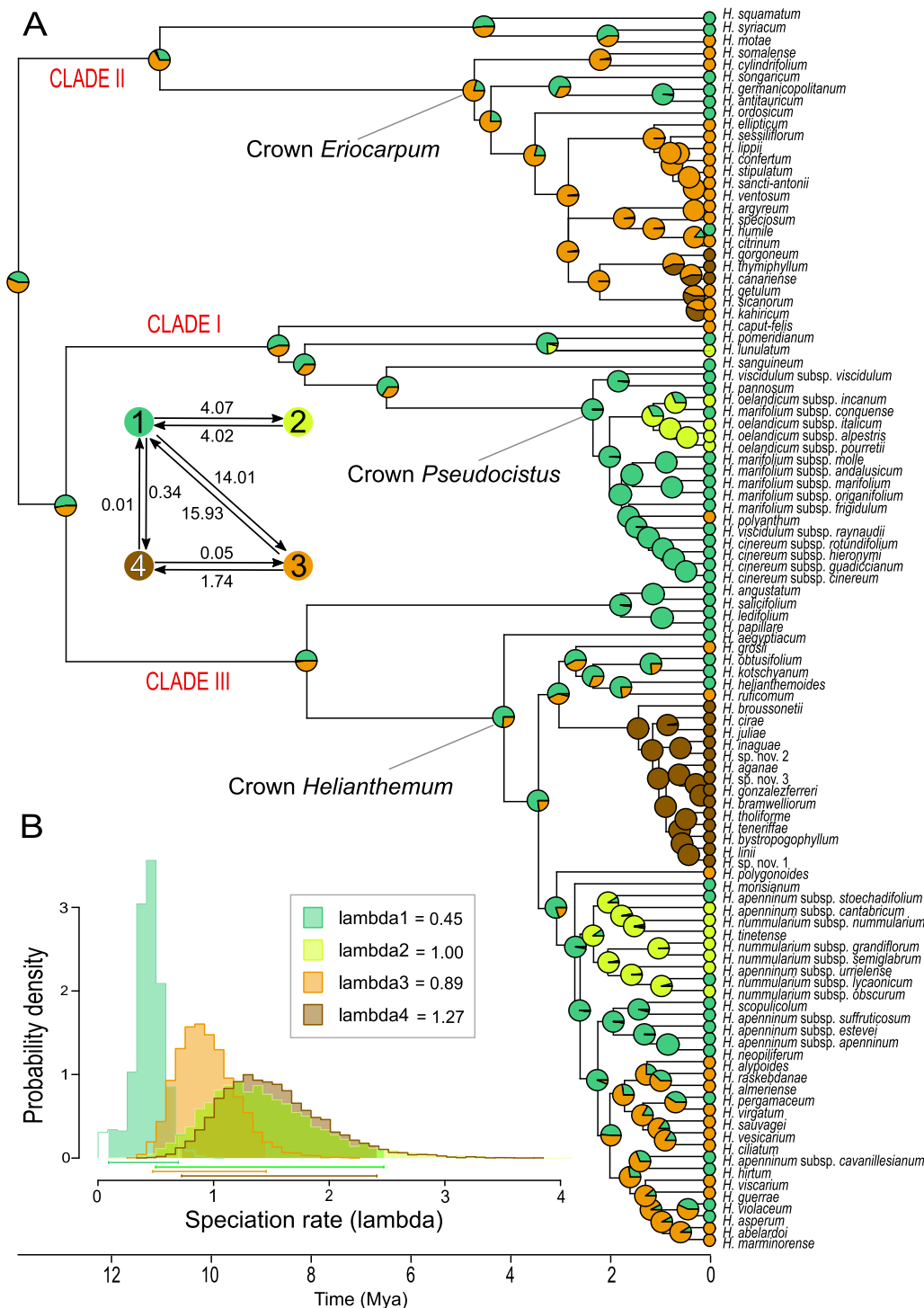


Fig. 4. Environmental niche reconstruction analyses of the genus *Helianthemum*. A) Reconstruction of ancestral states of the four environmental niches in *Helianthemum* by applying stochastic character mapping analysis on the ‘RD updated GBS-trees’ for the ‘whole taxa’ phylogenetic dataset, and the transition diagram inferred under the SYM model (see text for details). Pie charts in each node report the relative probabilities for each environmental niche. B) Marginal distribution of speciation rates determined from multi-state trait-based analysis under the best-fitting MuSSE model for the ‘whole taxa’ phylogenetic dataset, and the mean speciation rates value calculated for each environmental niche. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

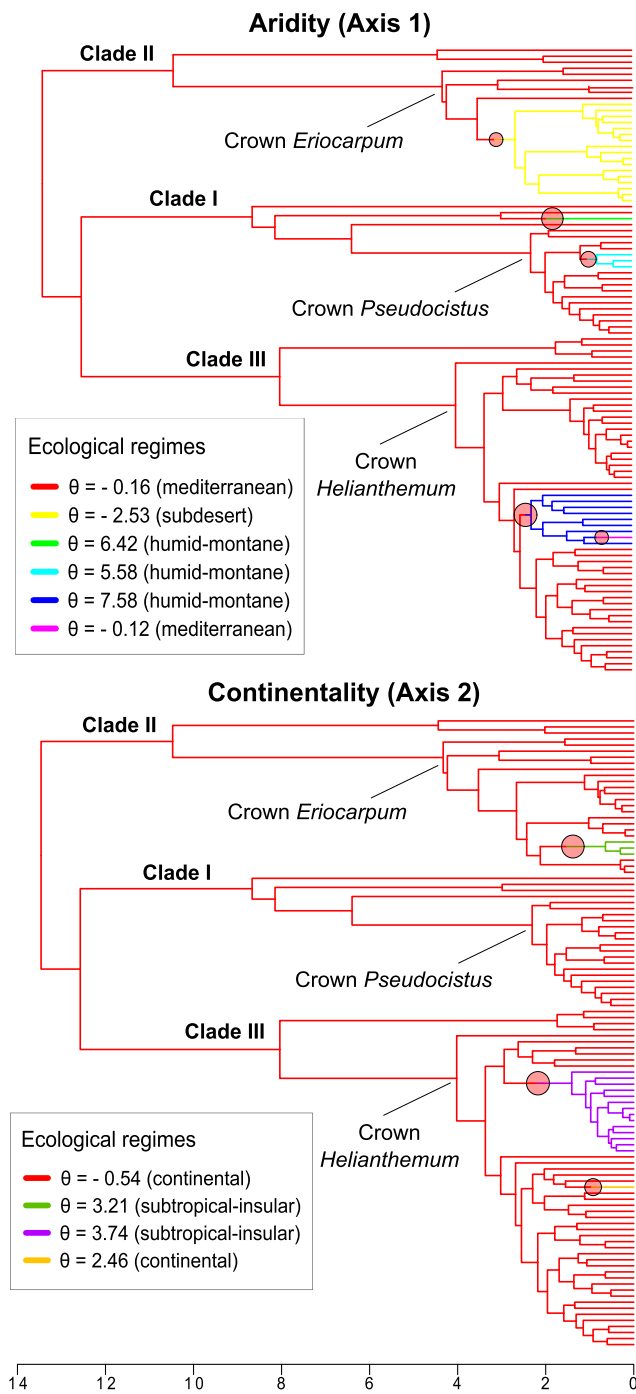


Fig. 5. Environmental niche shifts with posterior probabilities above 0.5 identified by applying the multi-optima OU model in bayou R package from the first two axes of the Hill-Smith principal component method for the ‘whole taxa’ phylogenetic dataset. The red circles on the branches denote the place where shifts have taken place. Circle diameter is proportional to the posterior probability of the shift. Branches are coloured according to the environmental regimes inferred. The insets indicate the mean optimum value associated to each environmental regime and the corresponding environmental conditions according to Fig. 3. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

and subtropical-insular environments (Fig. 4A). Transitions between humid-montane and subdesert environments and between the humid-montane and the subtropical-insular environments were never recovered. Lastly, the relative time spent in the pre-Mediterranean or Mediterranean environment was the highest (proportion of total time =

0.48), followed by the subdesert (0.35), humid-montane (0.11) and subtropical-insular (0.06) environments.

The results derived from the ‘species-level’ phylogenetic dataset also identified SYM as the best-fitting evolutionary model and recovered high uncertainty in the deepest nodes between Mediterranean and subdesert environments as ancestral niches (Fig. S7). However, the transition matrix was not biologically meaningful, recovering transitions between extreme environmental niches. This is probably due to the fact that a significant part of the environmental variability, provided by the subspecies, is not being considered in this dataset.

3.3.2.3. Environmental niche shifts analysis. Bayesian fitting of multi-optima OU models to species position into the PCA-based niche space estimated the placement and magnitude of five shifts in axis 1 (aridity) and three shifts in axis 2 (continentality) with posterior probabilities above 0.5 using the ‘whole taxa’ phylogenetic dataset (Fig. 5). The same environmental niche shifts were inferred under the ‘species-level’ phylogenetic dataset, except for two undetected shifts occurring at subspecies level (Fig. S9). All environmental niche shifts were estimated to have occurred from the Pliocene onwards and from intermediate environmental conditions along the first two axes of the PCA (Figs. 3, 5). In particular, the ancestral regime inferred from axis 1 displays an optimum value corresponding to Mediterranean environment and the ancestral regime from axis 2 shows an optimum value represented by a continental environment (see Figs. 3, 5). Six of the eight total shifts were followed by diversification: an environmental niche shift towards greater aridity (optimum value of the new regime corresponding to subdesert conditions in Fig. 3) during the evolution of clade II, two shifts into colder and wetter conditions (optimum value corresponding to humid-montane conditions in Fig. 3) in clades I and III, and two shifts towards insular conditions (optimum value corresponding to subtropical-insular conditions in Fig. 3) in clades II and III.

3.3.2.4. Multi-state trait-based diversification analyses. The best-fitting model identified when the ‘whole taxa’ phylogenetic dataset was applied (AIC = 505.4) showed different speciation rates for each state. In particular, speciation rates were higher in the subtropical-insular environment ($\lambda = 1.27$) and the lowest speciation rates were retrieved in the pre-Mediterranean or Mediterranean environment ($\lambda = 0.45$). This model also recovered no significant differences in the extinction rates between states ($\mu = 0.11$), except for the subdesert ($\mu = 0.54$), which was higher (Fig. 4B, Table S3, Fig. S8). The second best-fitting model using the ‘whole taxa’ phylogenetic dataset (AIC = 505.4, Table S3) provided a similar pattern since the lowest speciation rates were retrieved in the pre-Mediterranean or Mediterranean environment (results not shown). The analysis for the ‘species-level’ phylogenetic dataset recovered a similar best-fitting model than the previous ones (AIC = 397.7), just differing in the speciation rates from humid-montane environment ($\lambda = 0.30$) that were as low as the Mediterranean, since most of the humid-montane representatives are here considered as infra-specific taxa (Fig. S9; see Section 4). The second best-fitting model using the ‘species level’ phylogenetic dataset (AIC = 398.5; Table S3) equally retrieved different speciation rates for each state, being the highest in the subtropical-insular environments and the lowest in the pre-Mediterranean or Mediterranean environment (results not shown). Thus, the results were mostly consistent between the two data sets.

4. Discussion

4.1. Biogeographical congruence in the three major clades of the genus *Helianthemum*

The macroevolutionary history of the genus *Helianthemum* reconstructed in this study revealed a strong imprint of major geo-climatic

events that have impacted the Mediterranean Basin during the last 6 Mya (i.e. Messinian Salinity Crisis in the Late Miocene, the onset of the Mediterranean Climate in the Pliocene, and the glacial-interglacial oscillations in the Pleistocene; Fiz-Palacios and Valcárcel, 2013). This is illustrated by the synchrony in the major events of biogeographic movements and environmental niche shifts during the diversification of the three main clades that integrate the genus *Helianthemum*, and which reflect a clear pattern of biogeographical congruence (Vargas et al., 2014). In particular, our results indicate that the Late Miocene and Early Pliocene was dominated by range expansions across the Mediterranean Basin and adjacent areas, the Late Pliocene by range contraction and vicariance events of the previously achieved widespread distribution, and the Pleistocene define the period of most intense environmental niche shifts and *in-situ* diversification. These results make the genus *Helianthemum* a unique case in which the imprint of paleoenvironmental events is clearly visible, and the improved robust phylogenetic tree inferred here provides an insightful window into the past biogeography of the Mediterranean Basin.

4.1.1. Origin and early diversification in the Middle Miocene

According to our best ancestral area and niche reconstructions, the ancestor of the genus *Helianthemum* was originally present in Northern Africa under pre-Mediterranean environmental conditions during the Middle Miocene (Fig. 1; Fig. 5; Table 1), albeit a subdesert ancestral conditions cannot be ruled out given the observed uncertainty of the environmental niche reconstruction when discrete variables were applied (see Fig. 4). Nevertheless, the facts that there is evidence of the existence of a pre-Mediterranean climate by the Middle to Late Miocene (Rundel et al., 2016), prior to the onset of the Mediterranean climate type (3.2–2.8 Mya; Suc, 1984), and that the time of differentiation of the three main clades of *Helianthemum* nearly coincides with that period, lead us to tentatively consider the pre-Mediterranean environmental condition as the more likely starting point for the evolution of *Helianthemum* (Fig. 5). Currently, the Iberian Peninsula has the highest number of taxa of *Helianthemum* including subspecies (Parejo-Farnés et al., 2013; Aparicio et al., 2017), but Northern Africa in general and Morocco in particular harbour the highest number of species and the most heterogeneous environmental conditions. This result converges with previous studies (*Cistus-Halimium*, Guzmán and Vargas, 2009; *Fumana*, Carrió et al., 2020) and provides additional evidence for an early differentiation of the family Cistaceae as a whole in the Mediterranean Basin, where all major lineages, 5 genera and about 100 species can be found (Guzmán and Vargas, 2009).

4.1.2. Range expansion across the Mediterranean Basin during the Late Miocene and early Pliocene

The Late Miocene and Early Pliocene were characterized by low phylogenetic divergence in *Helianthemum* and considerable range expansion along the European and African fringe via colonization of adjacent areas, probably associated with the increase of land surfaces, land bridges and the progressive aridification of this region (De Jong, 1998; cf. Hellwig, 2004), considering the xeric preferences of most *Helianthemum* species (e.g. López-González, 1993). Interestingly, the colonization routes followed by the ancestors of the three main lineages in *Helianthemum* were different (Figs. 1, 2): ancestors of clade II spread eastward from Northern Africa to Central Asia throughout subdesert environments (Fig. 5), probably coinciding with the beginning of the aridification of the Sahara (Gillett, 1954; Caujapé-Castells et al., 2001; Barres et al., 2013) and the appearance of temperate steppe vegetation in Central Asia (Sanmartín, 2003; Su et al., 2011); ancestors of clade I mostly remained in Southern Europe under pre-Mediterranean conditions, and ancestors of clade III expanded along both sides of the Mediterranean Basin achieving a Circum-Mediterranean distribution and reaching Macaronesia through long distance dispersal (Sanmartín, 2003; Oberprieler, 2005; Lo Presti and Oberprieler, 2009).

4.1.3. Range contraction and vicariance during the Late Pliocene

The Late Pliocene was characterized by the fragmentation of the previous widespread distributions achieved by different lineages of *Helianthemum* because of recurrent range contractions and vicariance events (Fig. 1), which in extreme cases gave rise to pronounced disjunct distributions. The major uplifting of the Qinghai-Tibetan Plateau during the Plio-/Pleistocene (3.6–1.8 Mya; Zhang and Fritsch, 2010) may have favoured the disjunction between lineages in Central Asia (currently represented by *H. songaricum* in Central China) and Middle East (now composed by *H. germanicopolitanum* and *H. antitauricum* in Anatolia) in clade II (2.82–3.23 Mya; letter a in Fig. 1). Similarly, the opening of the Red Sea during the Pliocene-Pleistocene transition (Sanmartín, 2003) might have promoted the disjunction between Northern Africa (currently represented by six-species from Northern Africa plus Macaronesia) and the Horn of Africa (now composed by *H. argyreum*, *H. citrinum*, *H. humile* and *H. speciosum*) in clade II (2.06–2.85 Mya; letter b in Fig. 1). It is also worth noticing that, coinciding with the onset of the Mediterranean climate (2.8–3.4 Mya; Suc, 1984), an example of European/Northern African disjunction appeared in clade I (2.7–3.3 Mya; letter c in Fig. 1), with *H. lunulatum* restricted to the Maritime Alps and *H. pomeridianum* in northern Algeria and the High Atlas in Morocco.

Finally, two relevant vicariance events are noticeable in clade III, which resulted in an African-European disjunction (3.24–3.44 Mya; letter d in Fig. 1) and an East-West Mediterranean disjunction (2.1–2.4 Mya; letter e in Fig. 1). The first vicariance split the widespread ancestral Circum-Mediterranean range in two areas located at both sides of the Mediterranean Basin (also including Macaronesia) after the refilling of the Mediterranean Sea (e.g. Yesson et al., 2009; Fernández-Mazuecos and Vargas, 2011; Santos-Gally et al., 2012). The second vicariance probably occurred as a consequence of the Saharan desert expansion from the Late Pliocene (e.g. Sanmartín, 2003; Oberprieler, 2005) which now comprises *H. ruficomum* and *H. helianthemoides* in the Maghreb and *H. kotschyannum* and *H. obtusifolium* in the Middle East.

4.1.4. Inter-continental colonizations, environmental niche shifts and *in-situ* diversification during the Pleistocene

The fragmentation of the ancestral widespread distributions of *Helianthemum* in the Late Pliocene was followed by four dispersal events outside the Mediterranean Basin and by several post-Messinian colonizations between Northern Africa and Southern Europe, as well as an intensive subsequent *in-situ* diversification in all biogeographic areas during the Pleistocene.

The dispersal events outside the Mediterranean Basin correspond to two colonizations of Macaronesia (in clades II and III) and two colonizations of Central and Northern Europe (in clades I and III), which strikingly coincided with punctual environmental niche shifts from Mediterranean to humid-montane and to subtropical-insular environmental conditions, respectively (Figs. 1, 2, 4, 5). These environmental niche shifts are likely the result of greater availability of empty niches in higher latitudes (i.e. Northern and Central Europe) after the Pleistocene glacial periods (Fuertes-Aguilar et al., 2011) and a greater climatic stability in lower latitudes (i.e. Macaronesia) during that period (Rodríguez-Sánchez and Arroyo, 2008). Indeed, the differential effects of glacial-interglacial cycles during the Pleistocene in both geographic areas appear to have resulted in different diversification patterns.

On the one hand, the effects of the glacial-interglacial cycles were much more marked in Central and Northern Europe than in Macaronesia (Rodríguez-Sánchez and Arroyo, 2008), which exposed the humid-montane widespread European species *H. nummularium* (sect. *Helianthemum*) and *H. oelandicum* (sect. *Pseudocistus*) to recurrent episodes of range expansions and contractions. Specifically, these processes might have provided new possibilities for isolation during contractions, and subsequently for hybridization and introgression in secondary contact zones during northward expansion from the Southern European refugia (i.e. Iberian, Italian and Greek peninsulas), the last process hindering the differentiation of taxa (Soubani et al., 2014a, b; Volkova

et al., 2016; Widén, 2018; Martín-Hernanz et al., 2019a). Indeed, both species are young species-complexes integrated by an array of morphological variation, in some cases described by taxonomists at subspecies level (e.g. Proctor and Heywood, 1968; Greuter et al., 1984; Volkova et al., 2016), while at species level in others (e.g. Crespo et al., 2016; Yuzepchuk, 1974; Tzvelev, 2006; Le Floc'h et al., 2010).

In Macaronesia, conversely, the extreme glacial periods might have facilitated colonization from mainland through emerged seamounts facilitating stepping-stone dispersal (e.g., Fernández-Palacios et al., 2016; Menezes et al., 2018), whereas the increase of the sea level during the interglacial periods coupled with the complex topographic relief of the islands may have promoted genetic divergence (Caujapé-Castells et al., 2017; Albaladejo et al., 2021). The genus *Helianthemum* colonized Macaronesia in two independent events at different time intervals (Figs. 1, 2) and with disparate consequences. A first colonization happened in the Late Miocene or Pliocene from a Mediterranean ancestor belonging to sect. *Helianthemum* from Northern Africa, then radiated during the middle Pleistocene (1.3–1.5 Mya) onwards to give rise to 15 narrow-endemic species (Santos-Guerra, 2014). The second dispersal event to Macaronesia occurred during the second half of the Pleistocene (0.6–0.7 Mya) from a subdesert ancestor belonging to sect. *Eriocarpum* also from Northern Africa, just originating three more or less widely distributed species in the Canary Islands (*H. canariense* and *H. thymiphyllum*) and Cape Verde (*H. gorgoneum*). So, the Canary Islands is one of the adjacent areas to the Mediterranean where *Helianthemum* has undergone the highest rates of diversification (Albaladejo et al., 2021), and, by far, the Palearctic island system with the highest number of species of *Helianthemum*.

Lastly, our biogeographic reconstructions support at least two post-Messinian dispersal events from Europe to Northern Africa across the Strait of Gibraltar in clades I and III involving sections *Pseudocistus* (1.35–1.49 Mya) and *Helianthemum* (1.59–1.77 Mya; Fig. 1). Similar post-Messinian colonizations between the Iberian Peninsula and Northern Morocco seem to have been frequent in different angiosperms lineages, and examples can be found in *Linaria* sect. *Versicolores* (Fernández-Mazuecos and Vargas, 2011), *Linaria* sect. *Supinae* (Blanco-Pastor et al., 2013) and *Cistus* (Guzmán and Vargas, 2009; Fernández-Mazuecos and Vargas, 2010).

4.2. Prevalence of environmental niche conservatism during the rapid diversification of *Helianthemum*

Our study provides evidence for four environmental niches in *Helianthemum* (Mediterranean, subdesert, humid-montane and subtropical-insular) and a tendency toward an environmental niche conservatism. In particular, the scarcity of significant environmental niche shifts retrieved by our analyses (only 12.4% out of the approximately 97 evolutionary divergences recovered; Fig. 5) suggests that species and lineages tend to diversify primarily within the same niche (Crisp et al., 2009, 2011; Cruz et al., 2017). Furthermore, the few niche shifts mostly occurred between close environmental conditions within an aridity gradient, particularly from Mediterranean to subdesert, subtropical-insular and the humid-montane environments (Fig. 3). Accordingly, we did not detect any transition between humid-montane and subdesert environments or between humid-montane and subtropical-insular environments (Fig. 4A, 5). Thus, our results are consistent with the idea that environmental niche shifts are more likely to occur between areas with overlapping or rather close environmental conditions (Harvey and Pagel, 1991; Holt and Gaines, 1992; Crisp et al., 2009).

4.3. The time-for-speciation effect as the best hypothesis to explain the higher species diversity in *Helianthemum* within the Mediterranean Basin

The relatively longer time period spent into the Mediterranean Basin (i. e. Northern Africa and Southern Europe) by *Helianthemum* lineages (Fig. 1) suggests that the larger species diversity observed in the

Mediterranean compared to surrounding areas (i. e. Central and Northern Europe, Horn of Africa, Macaronesia and Central Asia) may have been generated by a time-for-speciation effect. This hypothesis is also supported by the lowest speciation rates undergone by Mediterranean lineages compared to subdesert, subtropical-insular or humid-montane lineages (Fig. 4B), despite most taxa in *Helianthemum* thrive in these environmental conditions (Fig. 4A-B). The striking disparity between diversification rates and current species diversity may have been reinforced by the above-mentioned environmental niche conservatism limiting the capacity to break out from Mediterranean conditions to adapt to different environments (Crisp et al., 2009; Skeels and Cardillo, 2017). This is congruent with the point of view of Wiens et al. (2010) who suggest that geographical patterns of species diversity can be explained by the degree of niche conservatism, so that in a group with high levels of conservatism, the greatest diversity of species will be found near the medium part of a given environmental gradient.

Its geographic location at the crossroads of three continents (Europe, Africa, and Asia) and its intermediate environmental conditions within an aridity gradient thus makes the Mediterranean Basin a 'cradle of diversity' for plant lineages (Sanmartín, 2003; Torrecilla et al., 2004; Oberprieler, 2005; Inda et al., 2008), facilitating their persistence within the region; and an 'evolutionary hub' (Willis et al., 2014), driving environmental niche transitions and colonization of adjacent areas. Remarkably, shifts from Mediterranean to subtropical-insular conditions, which coincided with the colonization of Macaronesia (the Canary Islands in particular), were followed by greater diversification rates (Fig. 4B). This oceanic region has been subjected to frequent environmental and geological upheavals since the Miocene (Weigelt et al., 2016). Despite the Canary Islands were not substantially affected by the Pleistocene glaciations (Rodríguez-Sánchez and Arroyo, 2008), recent phylogenetic literature strongly suggests that species turnover and diversification on these islands may have been much faster than in the Mediterranean Basin (Carine et al., 2004; Vitales et al., 2014; García-Verdugo et al., 2019; see also Albaladejo et al., 2021 for the Canarian *Helianthemum* lineage). These results reflect that equilibrium and non-equilibrium dynamics can promote high species diversity in continental and insular hotspots respectively (Skeels and Cardillo, 2019).

4.4. Concluding remarks and future directions

Given the congruence between geo-climatic changes and biogeographical processes among the three main clades of *Helianthemum* found in this paper, combined with the prevalence of niche conservatism and the presumed low dispersal capacity of the species (see also Martín-Hernanz et al., 2019b), it is tempting to recall allopatric speciation (more than ecological differentiation) as the preeminent driving force for the rapid diversification observed in this group (Lo Presti and Oberprieler, 2009; Smid et al., 2019; see also Albaladejo et al., 2021). However, additional criteria need to be strictly tested for pinpointing the nature of the radiations within *Helianthemum* as adaptive or non-adaptive (e. g. phenotype-environment correlations; Schluter, 2000), and precise microecological environmental data required to assess the effects of the local-scale conditions not considered here. Until more information is available, the high niche conservatism here hypothesized in the evolution of *Helianthemum* should be taken with caution (Wiens and Graham, 2005; Benítez-Benítez et al., 2018; Albaladejo et al., 2021; Thompson, 2020).

It is also necessary to keep in mind that one of the most important challenge in the study of recently diversified groups is the delimitation of taxa, particularly complex species, in many instances consisting of several cryptic species (Bickford et al., 2007). It is implicitly assumed that one individual sample is representative of the whole geographic distribution or environmental niche of the species (e.g. Roquet et al., 2009; Buerki et al., 2011; Valente et al., 2011), which may have relevant implications for downstream analyses when complex species are involved (e.g. ancestral areas reconstructions, diversification rate

analyses; see Etienne and Rosindell, 2012; Fernández-Mazuecos et al., 2019; Jiménez-Lobato et al., 2019). In *Helianthemum*, the array of subspecies that characterize the most complex and most widely distributed species confers a more than notable contribution to the morphological and ecological diversity of the whole genus (e. g. the stenochorous edaphic specialists *H. apenninum* subsp. *estevei* or *H. marifolium* subsp. *conquense*; see also Vasconcelos et al., 2019). Therefore, in this study, we put our effort to analyze and discuss the results including all the sub-specific taxa, also considering that some of them have diverged even earlier than more recent sister species (see Fig. S3), and that most of the complex species are non-monophyletic (see Martín-Hernanz et al., 2019a). Future population genomic approaches of complex species such as *H. oelandicum* or *H. nummularium* are necessary to evaluate in detail the taxonomic ranks of the entities currently considered, and to test if the high speciation rate that we have retrieved in these species (see Fig. 4B) is not merely a case of range expansion.

Beyond the above methodological considerations, the biogeographic history of *Helianthemum* here inferred not only recovers the traces of the past geological and climatic events that impacted the Mediterranean Basin since the Late Miocene and the tendency towards niche conservatism, but also its unique contribution to the extraordinary floristic richness and diversity of the Mediterranean hotspot. Our study highlights the importance of reconstructing both ancestral areas and environmental niche evolution to achieve an in-depth understanding of evolution in species-rich lineages within hotspots of biodiversity.

CRedit authorship contribution statement

Sara Martín-Hernanz: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Rafael G. Albaladejo:** Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Sébastien Lavergne:** Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Encarnación Rubio:** Data curation, Project administration, Resources. **Aurélié Grall:** Data curation, Resources. **Abelardo Aparicio:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmpev.2021.107238>.

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