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Biome conservatism prevailed in repeated long-distance colonization of Madagascar's mountains by *Helichrysum* (Compositae, Gnaphalieae)

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ABSTRACT

Colonization and diversification processes are responsible for the distinctiveness of island biotas, with Madagascar standing out as a biodiversity hotspot exceptionally rich in species and endemism. Regardless of its significance, the evolutionary history and diversification drivers of Madagascar's flora remain understudied. Here we focus on *Helichrysum* (Compositae, Gnaphalieae) to investigate the evolutionary and biogeographic origins of the Malagasy flora. We inferred a highly resolved phylogeny based on target-enrichment data from 327 species (including 51 % of Malagasy endemics) and conducted ancestral range estimation analyses. Our results revealed at least six *trans*-oceanic dispersal events from different African regions to Madagascar during the Pliocene. In this process, biome conservatism prevailed, as evidenced by similarities between Malagasy lineages and their African relatives. The southern African grasslands, known to be the center of diversification and the main source of African *Helichrysum* lineages, played a key role in the colonization of Madagascar as the ancestral source area of at least three clades. The Tropical Afromontane region was revealed as the source of at least two montane Malagasy lineages that substantially radiated *in-situ*. Finally, a dispersal event from southwestern Africa led to a lineage represented by a single species adapted to the island's southwestern arid conditions. The main radiations of *Helichrysum* in Madagascar's mountains occurred within the last 2 My, coinciding with a transition towards cooler and drier conditions and the expansion of open habitats, likely driven by a combination of geographic and ecological speciation. Overall, our findings highlight the affinities between the montane floras of continental Africa and Madagascar.

1. Introduction

Unraveling the origins and evolution of island biotas has been of interest to scientists since Darwin's times (Darwin 1859). Due to their isolation, comparatively small size and varying distances from mainlands, islands are considered natural laboratories for the study of

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ecological and evolutionary processes. Species richness of islands can generally be explained through features such as island area and isolation as well as the age and formation history (Whittaker et al. 2008, Graham et al. 2017). Distinguishing between oceanic islands and continental islands is important as it dictates the mechanisms underlying species colonization. Unlike the relatively younger oceanic islands, which emerge devoid of any resident species and gradually accumulate their diversity through dispersal followed by speciation, older continental islands inherit a baseline biota upon isolation from the mainland (Matthews & Triantis 2021).

Madagascar, once part of Gondwana, is an ancient continental island. It first broke apart from Africa 155 to 165 million years ago (mya) (Yoder & Nowak 2006 and references therein) and later from India 84 to 91 mya (Wells 2003) and has remained separated since then. Madagascar hosts a hyperdiverse biota in a wide range of ecosystems, being home to an estimated total flora of ca. 14,900 vascular plant species, of which 87 % are endemics (Callmander et al. 2011, Lowry et al. 2018, Antonelli et al. 2022), with 310 endemic plant genera and five endemic families (Buerki et al. 2013). Such exceptional species richness and endemicity are the result of a complex geological, climatic and evolutionary history. Despite hosting vicariant groups predating Gondwana's breakup (e.g., *Takhtajania*, Winteraceae, Thomas et al. 2014), most of the present-day diversity likely established on Madagascar through long-distance dispersal (Yoder & Novak 2006, Buerki et al. 2013), probably assisted by wind and oceanic currents. The existence of land bridges and stepping-stones between Africa and Madagascar has been hypothesized, but not supported by any direct evidence, so their potential role and relevance in facilitating dispersal remains under debate (Warren et al. 2010, Masters et al. 2021, Ali & Hedges 2023, Aslanian et al. 2023). Geographic proximity likely accounts for the Malagasy biota having the strongest taxonomic links to African lineages (Yoder & Novak 2006, Buerki et al. 2013).

Madagascar's complex topography plays a key role in shaping the main climatic and vegetation zones of the island and has probably had a significant impact on *in-situ* diversification of many groups. The main massifs of the island, reaching 1800–2900 m a.s.l., are placed in a north–south axis and are connected through the Central Highlands, a plateau ranging 800–1300 m. The orography combined with the prevailing easterly trade winds results in a rainfall gradient from the humid tropical east-northeast to the sub-arid southwest. The Central Highlands have a subhumid climate and are dominated by a grassland-woodland mosaic, heavily modified and degraded by human activity (Yoder et al. 2016; Antonelli et al. 2022). The highest parts of the mountains, above 1800–1900 m, are mainly occupied by sclerophyllous shrublands dominated by Ericaceae and Compositae, in addition to open grasslands and rupicolous plant communities (Burgoyne et al. 2005; Yoder et al. 2016; Antonelli et al. 2022, and pers. obs.). Outside the mountains, landscapes are dominated by deciduous forests, sclerophyllous and succulent plants westwards and evergreen rainforests eastwards. This ecological and biological heterogeneity provides the perfect ground for various post-colonization scenarios, such as repeated colonization without subsequent diversification (e.g. the Inulea-Pulcheinae group, Nylinder et al. 2016) as well as colonization followed by *in-situ* diversification (e.g. Psychotrieae alliance, Razafimandimbison et al. 2017; Coffeeae alliance, Kainulainen et al. 2017; C3 grasses, Hackel et al. 2018). Nevertheless, there is a knowledge gap on the relative importance of these scenarios in the evolutionary assembly of the Malagasy biota, given that the biogeographic history of many highly diverse Malagasy plant groups remains largely unexplored (Antonelli et al. 2022).

The relative contribution of adaptive evolution to the generation of biodiversity is still under debate. Niche conservatism -i.e., the tendency of species to retain their ancestral niches- has been suggested to be predominant in allopatric (also known as geographic) speciation (Wiens & Graham 2005, Wiens et al. 2010) and has been assumed to prevail in angiosperm evolution (Wiens & Graham 2005, Crisp. et al. 2009, Wiens et al. 2010) since migrating might be easier and quicker to achieve than adapting to new environmental conditions (Donoghue 2008). However, multiple studies support the idea that niche shifts within plant genera are not as rare as previously thought (e.g. *Lonicera* L., Smith & Donoghue 2010; *Coccinia* Wight & Arn., Holstein & Renner 2011; *Ranunculus* L., Hörandl & Emadzade 2011; *Hakea* Schrad & J.C.Wendl., Cardillo et al. 2017) and indeed older and/or more extensive biomes (e.g. sclerophyllous shrubland, tropical rainforest) have often been the source of lineages found in younger and/or less extensive biomes (e.g. grasslands, alpine, Mediterranean shrublands; Crisp et al. 2009, Donoghue & Edwards 2014). To date, few studies have explored the role that niche evolution may have played in the diversification of Malagasy plant groups (but see the case of *Bulbophyllum* Thouars in Gamisch et al. 2016, 2021).

In terms of species richness, Compositae are one of the five dominant plant families in the Malagasy flora (520 spp. and 83 % endemism; Callmander et al. 2011, Catalogue of the Vascular Plants of Madagascar 2024). Within Malagasy Compositae, the most species-rich genus is *Helichrysum* Mill. In the most recent floristic treatment of Malagasy *Helichrysum*, Humbert (1962) recognized 111 species –all but one endemic- and numerous infraspecific taxa at the subspecific and varietal levels. He did not propose a formal infrageneric classification but organized the species into eleven informal taxonomic groups based on morphological affinities. *Helichrysum* occurs in almost all Malagasy biomes, from the extremely arid south-western coastal dunes (e.g., *H. mahafaly* Humbert) to the humid eastern rainforests (e.g. *H. geayi* Humbert), adopting a wide range of growth forms (lianas, herbs, subshrubs, shrubs and small trees). However, most of the Malagasy diversity of *Helichrysum* is found in the Central Highlands and on the highest mountains, distributed across their full elevational and latitudinal gradients. The diversity of *Helichrysum* in Madagascar reflects the extraordinary variation and adaptability of the genus, already evident in the case of continental African lineages (Blanco-Gavaldà et al. 2023).

Previous studies place the origin of *Helichrysum* in southern Africa (Galbany-Casals et al. 2014, Andrés-Sánchez et al. 2019, Blanco-Gavaldà et al. 2023) and suggest that several independent and asynchronous dispersals occurred from the African continent to Madagascar. However, these works treated Madagascar as a single biogeographic area and the sampling of both mainland and Malagasy species was limited. Here, we aim to infer the biogeographic history of *Helichrysum* in Madagascar as a study-case to elucidate the relative contribution of *in-situ* speciation vs. colonization to the generation and maintenance of Malagasy plant diversity. For this purpose, we first generated a highly resolved time-calibrated phylogeny based on target-enrichment sequences, substantially increasing the sampling of African and Malagasy species compared to previous studies. We used the resulting phylogeny to estimate ancestral ranges and infer the source, number and age of colonization events of the genus on Madagascar. Finally, we evaluate whether the dominant mode of diversification within *Helichrysum* has been adaptive speciation associated with biome shifts or non-adaptive speciation associated with allopatry and biome conservatism.

2. Materials and methods

2.1. Taxon sampling

We sampled 327 *Helichrysum* species (ca. 60 % of the genus, see Supplementary Table S1) including 57 (ca. 51 %) of the Malagasy species with representatives of all Humbert's (1962) taxonomic groups, the latest taxonomic treatment of Malagasy *Helichrysum*. For some of these Malagasy species, we included several infraspecific taxa (29 additional samples). While polyploids are known in *Helichrysum* and related genera, previous studies have shown that clades of polyploid origin are confined to specific geographical regions and lineages (the Mediterranean, Macaronesian and Asian members of *Helichrysum*, and the smaller genera *Achyrocline* (Less.) DC., *Anaphalis* DC. and *Pseudognaphalium*

Kirp. in America and Asia; Galbany-Casals et al. 2009, 2014). None of these is closely related to Malagasy lineages (Blanco-Gavaldà et al. 2023). Taking this into account, we excluded well-delimited polyploid clades to optimize read-mapping processes and minimize potential sources of phylogenetic discordance (Tiley et al. 2024a). With this, we also eliminated non-African biogeographic regions, allowing for the recognition of more precise Malagasy areas while keeping the analyses computationally feasible.

We also included 34 specimens of other genera representing the main lineages of the tribe (Nie et al. 2016, Smissen et al. 2020) so that we could implement secondary age calibrations. The complete dataset comprises a total of 386 samples, 289 from previous studies (273 from Blanco-Gavaldà et al. 2023 BioProject PRJNA936872; six from Mandel et al. 2019, BioProject PRJNAS40287; and ten from Schmidt-Lebuhn & Bovill 2021, BioProject PRJNA665592). Here we sequenced for the first time 97 samples using the same baits set, representing mainly *Helichrysum* species from continental Africa and Madagascar.

2.2. Next-generation sequencing

DNA was extracted from 10–30 mg of dried leaf material obtained from herbarium specimens and from our field expeditions (see Supplementary Table S1) using the E.N.Z.A® SP Plant DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) following manufacturer instructions. We measured DNA concentration using Qubit^{TM} Flex Fluorometer (Thermo Scientific, Waltham, MA, USA). Then, we used a Qsonica Q800R3 Sonicator (Qsonica LLC, Newton, CT, USA) at 20 % amplitude for 45 sec to 8 min to shear 0.2–1 ug in 50 uL of DNA into 300–400 bp fragments. We performed gel electrophoresis (1.2 % agarose) to check fragment length.

We prepared target-enrichment libraries from 25ul of the sonicated DNA using the NEBNext Ultra II DNA Library Prep Kit for Illumina ® (New England Biolabs, Ipswich, MA, USA) employing half of the volumes specified by the manufacturer and 15 cycles of PCR amplification. We barcoded the libraries using NEBNext Multiplex Oligos sets with unique single or dual index combinations. Afterward, we pooled the indexed libraries with more than 17 ng of DNA in groups of up to 10 samples and around 250 ng of DNA per library. We evaporated or filled with water the arranged pools to 7 ul of total volume to perform target-enrichment (protocol from Mandel et al. 2014) using the Microarray MyBaits COS kit (Daicel Arbor Biosciences, Ann Arbor, MI, USA), specifically developed for the Compositae family. The final sequencing pools were prepared by pooling enriched libraries with unenriched libraries at a 60:40 ratio and the samples were sequenced (PE 150 bp) on Illumina HiSeq2500 and HiSeqX platforms.

We deposited newly generated raw sequence reads in the NCBI Short Read Archive database (SRA; access: https://www.ncbi.nlm.nih.gov/sra) under the BioProject accession number PRJNA1121119.

2.3. Molecular data processing

We used HybPhyloMaker, a bioinformatic workflow developed to process Hyb-Seq data (Fér & Schmickl 2018, available at https://github.com/tomas-fer/HybPhyloMaker, indicated hereafter as HPM, followed by the number of the corresponding script) in combination with ParalogWizard (Ufimov et al. 2022, available at https://github.com/rufimov/ParalogWizard), which detects and separates paralogs of a given locus based on sequence similarity to generate orthologous alignments.

Specifically, we used Trimmomatic v.0.39 (Bolger et al. 2014) to remove adaptors and low-quality reads, and BBMap v.38.42 (Bushnell 2014) to remove duplicates, both implemented in HPM1 (read statistics in Supplementary Table S2). We generated a reference file for initial read mapping with BWA (Li & Durbin 2009) and SPAdes (Bankevich et al. 2012) based on sunflower genome sequences from the Compositae1061 probe set (Mandel et al. 2014). Then, we generated a customized reference based on our ingroup samples to increase mapping specificity. Pairwise exonic sequence divergence was calculated to identify paralogs. The first resulting peak represents putative allelic variation, while the second peak represents highly divergent sequences corresponding to putative paralogs. We used the value of the latter as the threshold to retrieve putative paralogous sequences. We then aligned orthologous matrices using MAFFT v.7.475 (Katoh & Toh 2008) to finally concatenate exons into putative loci. To reduce missing data, we excluded sequences missing more than 70 % of the data and removed loci for which less than 75 % of all samples were represented (HPM5).

2.4. Phylogenetic analyses

We applied concatenation as well as coalescence summary approaches to infer phylogenetic relationships. We concatenated all nuclear loci into a single supermatrix and conducted maximum likelihood (ML) partitioned analyses using RAxML-NG v.1.1.0 (modified HPM8f; Kozlov et al. 2019). Specifically, we first estimated the best nucleotide substitution model for each locus with ModelTest-NG (Darriba et al. 2020) and then performed 20 independent ML tree searches. We assessed branch support with 1000 bootstrap replicates and annotated the best-scoring ML tree with Felsenstein's Bootstrap (BS, Felsenstein 1985) and Transfer Bootstrap Expectation (TBE, Lemoine et al. 2018) proportions considering branches with BS $\geq$ 70 % and TBE $\geq$ 0.7 to be statistically supported (Hillis & Bull 1993, Lemoine et al. 2018).

We performed summary-coalescence inference with ASTRAL-III v.5.7.8 (Zhang et al. 2018). To do so, we first inferred individual gene trees for each retrieved locus generated with RAxML v.8.2.12 (Stamatakis 2014) and performed 100 bootstrap replicates (HPM6a, HPM7 and HPM8a). For the ASTRAL tree, we calculated branch support values as local posterior probabilities (LPP), considering well-supported branches those with LPP $\geq$ 0.95 (Sayyari & Mirarab 2016). In addition, we conducted a second summary-coalescence analysis with the same parameters but using only the 25 most informative loci, which we selected with SortaDate (Smith et al. 2018) based on three criteria: bipartition support, clock-likeness, and tree length (which is proportional to information content).

2.5. Divergence time estimation

We applied the RelTime method (Tamura et al. 2012, 2018) implemented in MEGA 11 (Tamura et al. 2021) to time-calibrate the most likely ML tree, and computed confidence intervals using the method of Tao et al. (2020). This methodology is especially suited for large empirical genomic datasets and allows the use of calibration densities (Costa et al. 2022). The tribe Gnaphalieae lacks old enough fossils to be useful as primary calibration points. Therefore, we used four secondary calibration points (CP, Supplementary Figure S1) from previously inferred divergence time estimates (Nie et al. 2016) as age constraints. We applied a normal density to each calibration point providing appropriate mean and standard deviation values to reflect the 95 % confidence interval values reported by Nie et al. (2016): the tribe crown node (mean age 25 mya, std ± 2.55, CP1), the "crown radiation" node (mean age 20.7 mya, std ± 2.55, CP2), the "HAP clade" crown node (mean age 15.39 mya, std ± 1.95, CP3) and the "FLAG clade" crown node (mean age 12.78 mya, std ± 1.85, CP4).

2.6. Ancestral geographic range reconstruction

To infer the most probable ancestral geographic ranges of *Helichrysum* lineages, we defined 17 areas. Regarding continental Africa and Arabia, we applied the same criteria as in Blanco-Gavaldà et al. (2023) resulting in the following areas: (A) the tropical Afroalpine area, (C) the Indian Ocean Coastal Forest Belt, (D) the high Drakensberg area, (F) the Fynbos Biome, (G) the southern African grasslands, (L) the tropical African lowlands, (N) the arid to semi-arid southern African area, (P) the Arabian Peninsula in Asia, (S) the southern African savannah, and (T) the tropical Afromontane area.

For our delimitation of Malagasy regions (Fig. 1) we consulted many bibliographic sources: the definitions of the main vegetation types described in Jenkins (1987), Burgess et al. (2004), Goodman & Benstead (2003) –which in turn integrates previous classifications by Faramalala (1995), Du Puy & Moat (1996) and Lowry et al. (1997)- and Gautier et al. (2018); the delimitation of Madagascar's six principal biomes by Yoder & Nowak (2006), based on Goodman & Benstead (2003); the map of predominant vegetation types shown in Antonelli et al. (2022) simplified from Moat & Smith (2007); and the bioclimatic zones by Rakotoarivelo et al. (2019) modified in Razafimandimbison et al. (2022). However, our final proposal is not completely coincident with any of the cited sources, as it also integrates distribution patterns of the genus and our field experience. We used the following seven distribution areas: (B) southern and southwestern arid lowlands, an area that ranges from 0 to 800 m and mainly includes Madagascar's arid spiny thicket, with drought-resistant species adapted to extreme aridity and secondary grasslands; (W) dry western lowlands, an area that ranges approximately from 0 to 800 m and mainly includes dry and highly seasonal forests, succulent woodlands and secondary grasslands; (O) humid eastern lowlands, an area that ranges approximately from 0 to 800 m and mainly includes very humid lowland rainforests with some herbaceous clearings; (H) subhumid central highlands, an area that ranges from 800 to 1300 m and is mainly constituted by grassland formations and small patches of Tapia forests; (M) subhumid central montane belt, an area that ranges from 1300 to 1800 m and includes a mosaic of Tapia forest, woodlands, grasslands and rocky habitats; (R) humid eastern and northern montane belt, an area that ranges from 800 to 1800 m and mainly constituted by moist, broadleaf forests that, because they grow at certain altitude, are not as tall as lowland rainforests; and (E) highest areas, which are disjoint small patches comprising all areas above 1800 m and up to the summit of Maromokotro (2876 m) and include a mosaic of ericoid thickets, mountain grasslands and rocky habitats (these correspond, broadly speaking, to the massifs of Tsaratanana [and its surrounding mountain chains], Marojejy, Anjanaharibe, Ankaratra, Vavavato, Ibity [and its neighbor mountains], Itremo, Andringitra [and its satellite peaks] and Beampingaratra). We excluded the Lowland Sambirano Rainforest biome (grey area in Fig. 1) represented in some maps at the north-western tip of the island, and coastal mangroves (not shown in Fig. 1), because none of the included *Helichrysum* species inhabits these regions.

Based on all these criteria, our proposed biogeographical areas can be viewed as macroecological units, which despite not being geographically continuous in some cases, share bioclimatic conditions determined by factors such as temperature and precipitation that likely filtered out lineages according to their general bioclimatic preferences.

Species occurrence in the geographical areas was assigned considering information on distribution and elevational range (based on Humbert 1962, Catalogue of the Vascular Plants of Madagascar 2024, herbarium records and own field observations). Some species are only occasionally or marginally present in other areas outside of their main distribution range. In these cases, we only considered the core area/s or

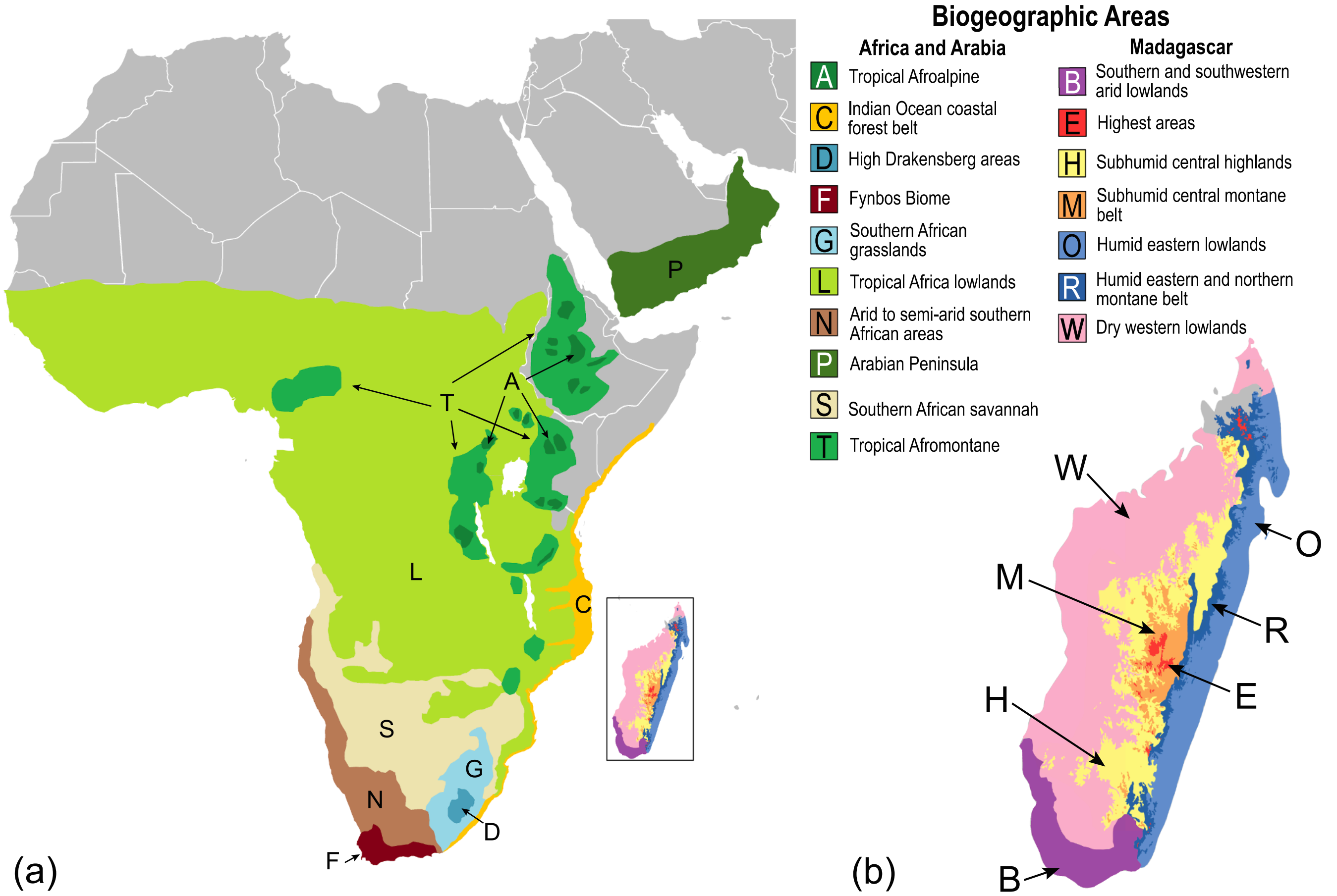

Fig. 1. Maps illustrating the 17 geographic areas defined in this study. The colors and labels correspond to those in the biogeographical reconstruction analyses. (a) General map at scale, including continental Africa, Arabia and Madagascar. The dark green spots within the tropical Afromontane area are an overrepresentation of the tropical Afroalpine area. (b) Closeup of Madagascar's map to facilitate area distinction.

elevational belts in which there are a significant number of occurrences of the species, or that can be interpreted as the central distribution of the species.

We carried out biogeographic range evolution analyses in R with the package BioGeoBEARS (Matzke 2013), using the ML time-calibrated tree as input. The maximum number of areas for any node was set to three, which is the highest number of areas occupied by the most widespread extant taxon in our study. We tested the fit of three biogeographical models: Dispersal-Extinction-Cladogenesis (DEC; Ree et al. 2005; Ree & Smith 2008), a likelihood implementation of the

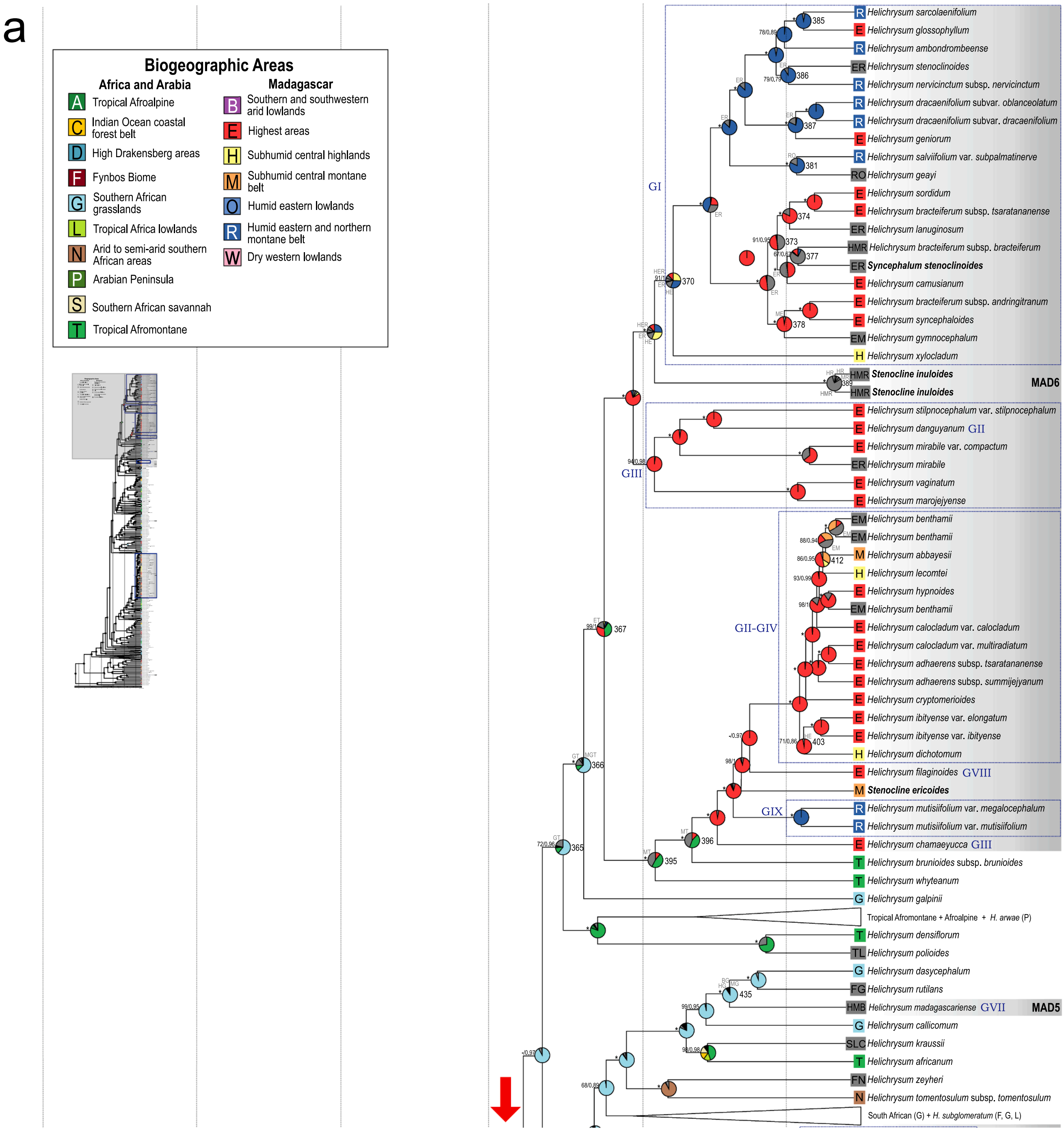

Fig. 2. Ancestral range estimation of Helichrysum using the best-fitting model DEC + j. It is based on a time-calibrated phylogeny generated under the concatenation approach using target-enrichment data (Compositae1061 probe set). Pie charts at nodes show the relative probability of the possible states (areas in primary colors, combinations of areas in grey). Relevant node numbers are to the right of the node. Support values are to the left of the node. The first numerical value corresponds to the BS metric and the second to the TBE metric. Asterisks indicate nodes supported with a BS and TBE of 100 and 1, respectively. Names of species corresponding to genera other than Helichrysum are in bold. Malagasy clades highlighted in grey and labeled MAD1 to MAD6 are shown in pairs across panels: (a) includes clades MAD5 and MAD6; (b) includes clades MAD3 and MAD4; (c) includes clades MAD1 and MAD2. Humbert's (1962) taxonomic groups are indicated in blue. Unrelated African clades are collapsed and their geographical distribution is indicated to the right. The complete tree obtained from the ancestral range estimation analyses is shown in Supplementary Figure S6.

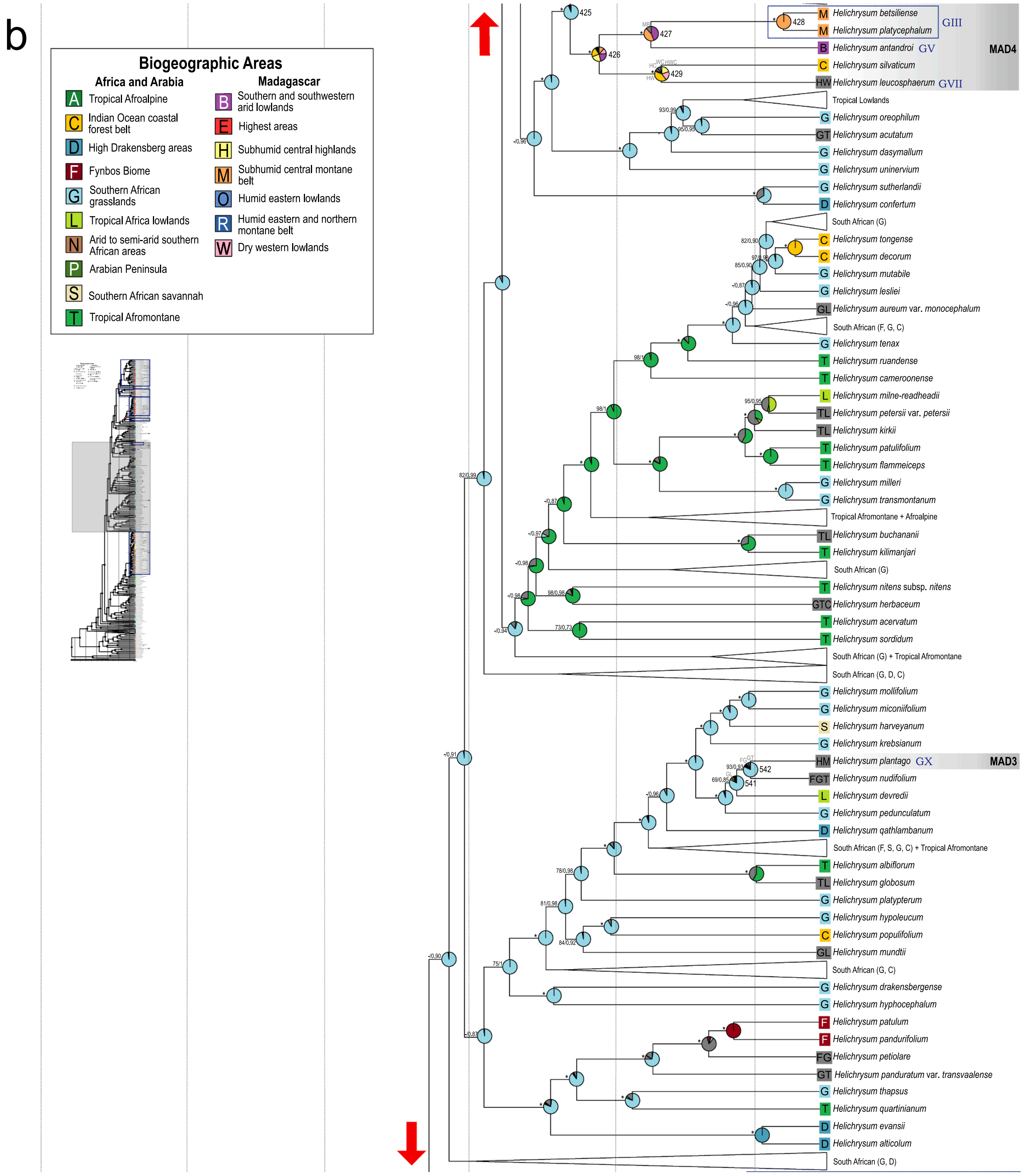

Fig. 2. (*continued*).

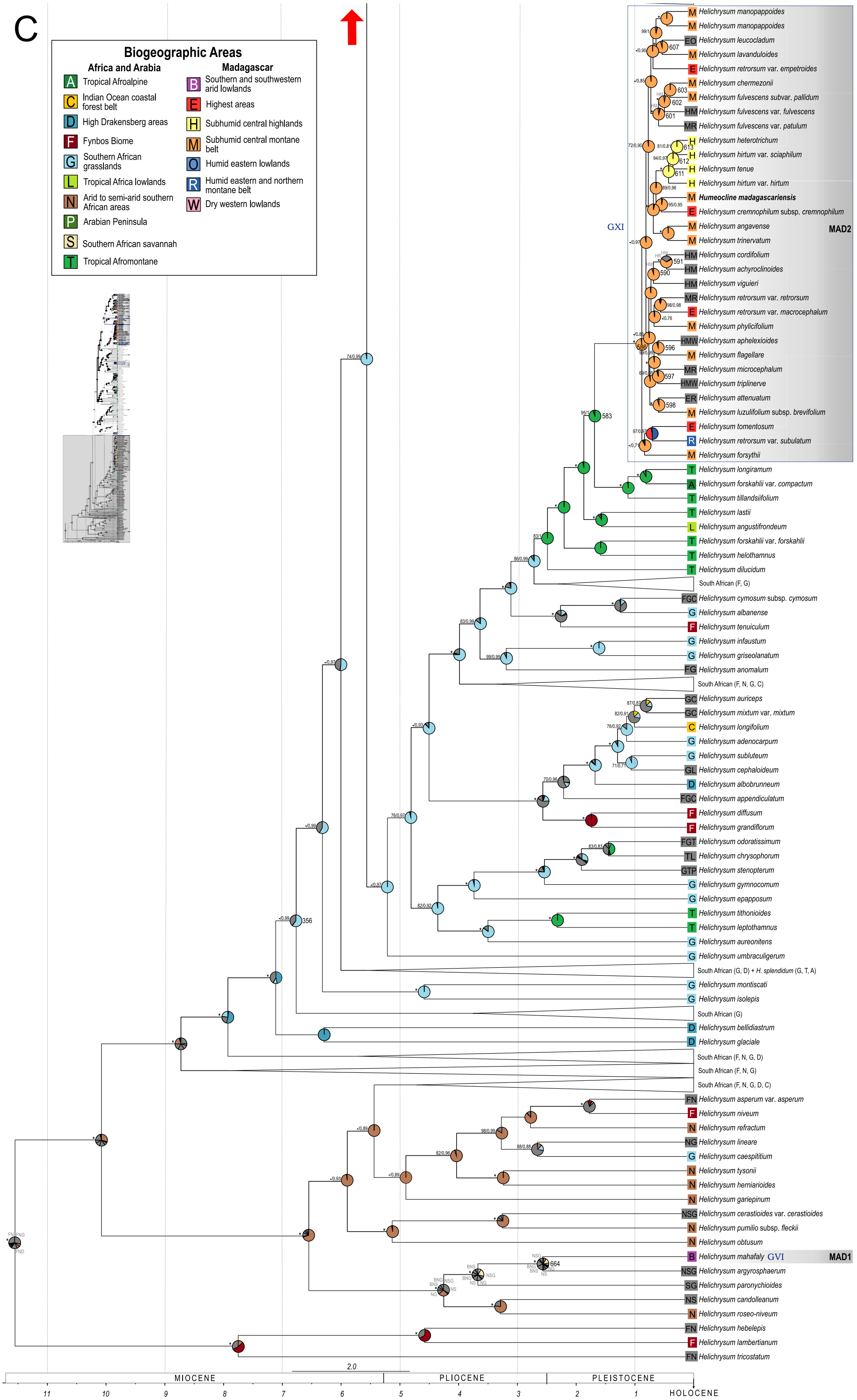

Fig. 2. (*continued*).

Dispersal-Vicariance model (DIVAlike; Ronquist 1997), and the BayArea model (BAYAREAlike; Landis et al. 2013). We also tested a more complex version of each model that accounts for founder-event speciation by adding the jump-dispersal parameter (+j). We compared and chose the best fitting model based on the Akaike Information Criterion (AIC) and AIC weight (AICw), considering that the comparison of DEC and DEC + j models is statistically valid based on Matzke's (2022) reply to Ree & Sanmartín (2018). We also performed Biogeographic Stochastic Mapping (BSM; Dupin et al. 2017) on the best fitting model (DEC + j) to estimate the frequency and types of biogeographical events, taking the mean and standard deviation of event counts from 100 BSM replicates.

3. Results

3.1. Molecular data processing

Out of the 1061 loci targeted, we initially recovered 929 loci. Based on pairwise sequence divergence histograms (Supplementary Figure S2), we estimated that values of 7.0 to 19.0 % of divergence indicated paralogy. Using the *Helichrysum*-customized reference tailored to our dataset, 228 (± 58) paralogous loci were detected on average (Supplementary Table S3). After filtering for missing data and splitting alignments containing paralogs, which resulted in 322 new alignments, we performed phylogenetic inference with a total of 971 loci. The aligned length of each locus averaged 278 bp (ranging from 37 to 735 bp, see Supplementary Table S4). Each locus had on average 77 (ranging from 4 to 264) parsimony informative sites and 113 (ranging from 5 to 383) variable sites. The average proportion of missing data was 2.8 % (ranging from 0 to 65 %). Concatenation of all loci resulted in a supermatrix with 295610 bp and 384 taxa.

3.2. Phylogenetic analyses

The topologies of the phylogenetic trees inferred using the concatenation approach (hereafter, ML tree, collapsed version Fig. 2 and full version Supplementary Figure S3) and the summary-coalescence approach (hereafter, ASTRAL tree, Supplementary Figure S4) based on 971 loci were congruent except for a few intermediate nodes, which are supported in both trees but show different relationships between species. In particular, two montane Malagasy clades are recovered as close relatives in the ML tree, but distant in the ASTRAL tree (ML tree node 367). In both trees, there are six lineages constituted by Malagasy species (MAD1-MAD6, as shown in Fig. 2). Three of them are represented by a single species –*Helichrysum mahalafy* (MAD1), *H. plantago* DC. (MAD3) and *H. madagascariense* DC.(MAD5)-, each of them sister to an African species; a fourth one is constituted by four Malagasy species and a South African species; and the rest of Malagasy species constitute two speciose lineages, MAD2 and MAD6, the latter including some African species.

Lower overall clade support was obtained with the summary-coalescence approach: 61 % of the nodes received significant support (LPP ≥ 0.95), whereas the percentage of significantly supported nodes in the ML tree was 85 % and 96 % according to BS and TBE metrics (≥70 %), respectively. The summary-coalescence analysis based on the 25 most informative loci resulted in a tree (Supplementary Figure S5) that is congruent with the former ASTRAL tree, but even overall lower supports were obtained (only 25 % of the nodes received significant support). Because of that, we will not further discuss the results of this tree.

3.3. Biogeographic reconstruction

The best-fitting biogeographical model was DEC + j according to AIC values (Table 1). Regarding dispersal types, cladogenetic founder-event processes (j = 0.0085) had a slightly larger contribution than anagenetic range expansions (d = 0.0076).

Ancestral range reconstruction analyses (probabilities in Supplementary Table S5, full tree in Supplementary Figure S6) suggest that the genus most probably originated and initially diversified in the winter rainfall regions of western South Africa (Fynbos Biome, F and arid to semi-arid southern Africa, N) around 11.6 mya (11.4 – 15.0 95 % CI), although the ancestral range may have also included some of the surrounding areas (the southern African grasslands, G; and/or the high Drakensberg area, D). The largest lineage in our tree notably diversified in the southern African grasslands and dispersals northwards and eastwards occurred from the late Miocene onwards (c. 8 mya, 6.5 – 11.7 95 % CI, node 356). Repeated dispersals gave rise to the current global distribution of *Helichrysum.*

Within Madagascar, Biogeographic Stochastic Mapping (BSM) analyses reveal at least six founder dispersal events from continental Africa to Madagascar (Fig. 3**;** BSM summaries in Supplementary Table S6), all of them giving rise to endemic lineages. The main geographic source of Madagascar's colonization events was the southern African grasslands (G, with a mean of 3 colonization events) followed by the Tropical Afromontane area (T, with a mean of 2 colonization events).

According to our results, the oldest Malagasy clade (Fig. 2, clade MAD6) is sister to *Helichrysum galpinii* N.E.Br., likely descending from an ancestor occurring in the southern African grasslands that dispersed at an inferred age of 3.7 mya (2.2 – 6.2 95 % CI, node 366, BS = 100, TBE = 0.99). This clade comprises mainly Malagasy montane species found in the highest areas (area E), followed by species found in the subhumid northern and eastern montane belt (area R), but also includes two Tropical Afromontane species from mainland Africa. The probabilities of the ancestral range of the whole clade involve both Afromontane areas (tropical continental Africa and the highest areas of Madagascar). This clade is made up of two large and highly diversified Malagasy montane lineages (one inferred to have originated around 3.5 mya, 2.0 – 5.9 95 % CI, node 367, BS = 99, TBE = 0.99 and the other at about 2.3 mya, 1.2 – 4.5 95 % CI, node 396, BS = 100, TBE = 1). However, these two lineages are not closely related to each other in the ASTRAL phylogeny. Therefore, these results should be taken with caution since concatenation and coalescence-based phylogenetic inferences provide conflicting relationships around these nodes. The two Malagasy montane clades notably diversified during the Pleistocene, with some species colonizing or extending their area into the subhumid central montane belt (area M, e.g. *H. abbayesii* Humbert), the grasslands of Madagascar's central highlands (area H, e.g. *H. lecomtei* R. Vig. & Humbert and *H. xylocladum* Baker) or the humid eastern lowlands (area O) in the case of *H. geayi* (node 381).

The second oldest colonization of Madagascar has an inferred age of 3.6 mya (2.2 – 5.9 95 % CI, node 425, BS = 100, TBE = 1, MAD4), also from a southern African grassland ancestor. The ancestral range of the Malagasy descendant is highly uncertain due to the wide range of the extant species belonging to this clade, which include four areas in

Table 1
Summary statistics of the biogeographic models tested in BioGeoBEARS. The best-fitting model used to infer the most likely area occupied by the ancestors of *Helichrysum* is highlighted in bold (DEC + j). Values of parameters for dispersal (d), extinction (e), founder effect (j), likelihood scores (lnL) and Akaike Information Criterion (AIC) are provided.

Biogeographic models	lnL	No. free parameters	d	e	j	AIC	AIC weight
DEC	−1077	2	0.012	0.011	0	2157	2.6E-33
DEC + J	**−1001**	**3**	**0.0082**	**1.00E-12**	**0.0084**	**2007**	**1**
DIVALIKE	−1099	2	0.014	3.4E-09	0	2201	7.4E-43
DIVALIKE + J	−1028	3	0.0091	1.00E-12	0.0087	2062	1.3E-12
BAYAREALIKE	−1088	2	0.010	0.23	0	2181	2.10E-38
BAYAREALIKE + J	−1012	3	0.0068	0.0077	0.011	2031	7.6E-06

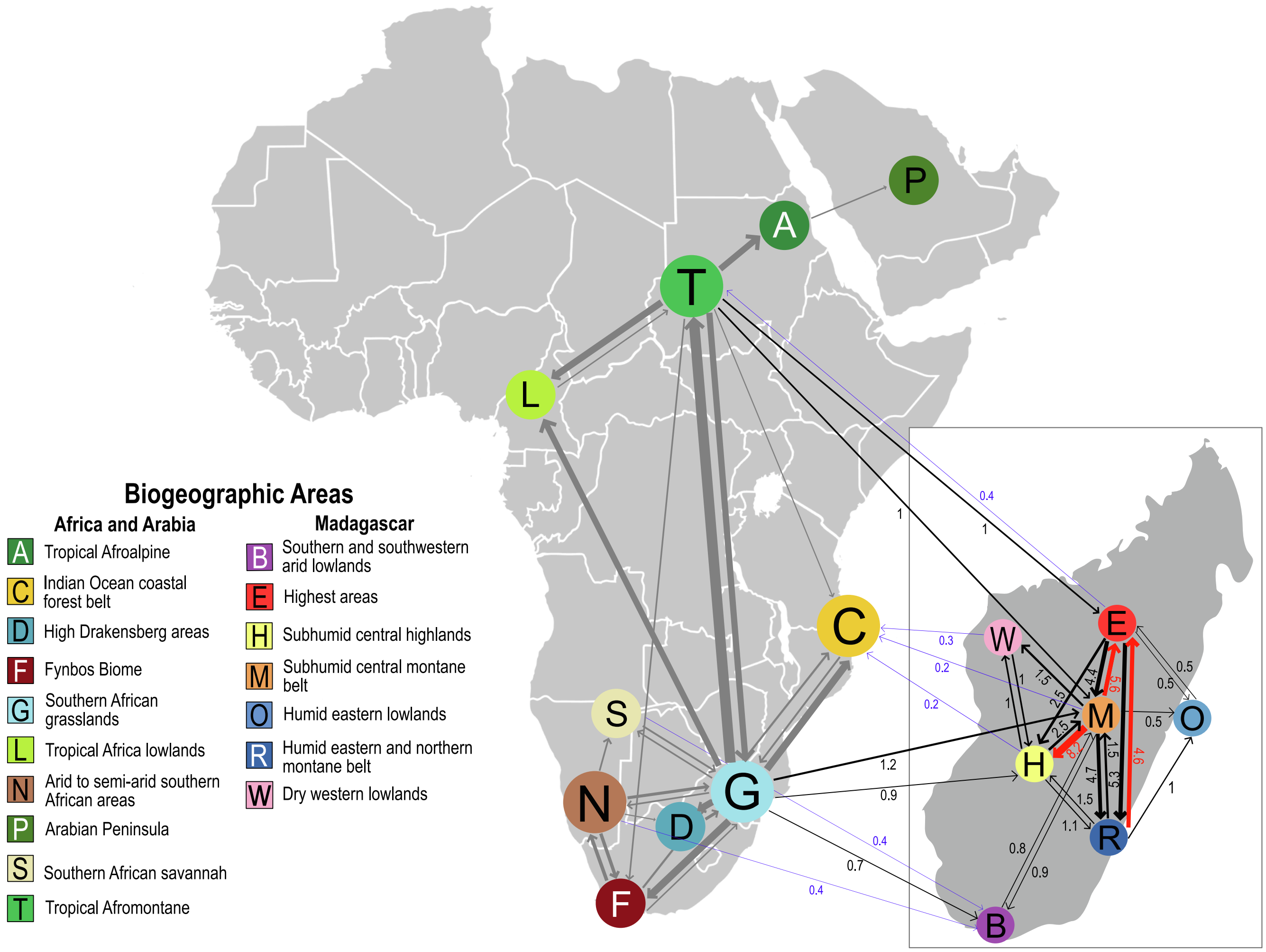

Fig. 3. Summary of mean dispersal events estimated from 100 Biogeographic Stochastic Mappings (BSM) in *Helichrysum* (see all event counts in Supplementary Table S6). Arrow tips indicate the directionality of the dispersal. Numbers on the arrows are the mean of dispersal event counts (only given for dispersals related to Madagascar). Arrow thickness is proportional to the mean number of dispersals. For events involving Madagascar, either within Madagascar or between continental Africa and Madagascar: blue arrows represent dispersals with mean counts below 0.5; black arrows represent dispersals with mean counts between 0.5 and 4.9; red arrows represent dispersals with mean counts ≥ 5. Gray arrows represent dispersal events within continental Africa and the Arabian Peninsula. For readability, dispersals below 0.7 involving only these regions have not been represented.

Madagascar (southern and southwestern arid lowlands, area B; subhumid central montane belt, area M; subhumid central highlands, area H and dry western lowlands, area W) and one area from continental Africa (Indian Ocean Coastal Forest Belt, area C). These results suggest a back-colonization to the southern African coast, inferred to have occurred 2.3 mya (1.2 – 4.5 95 % CI, node 429, BS = 100, TBE = 1) giving rise to *H. silvaticum* Hilliard.

Our results suggest that a recent dispersal from the tropical Afromontane area took place at an inferred age of 1.7 mya (0.9 – 3.3 95 % CI, node 583, BS = 100, TBE = 1, MAD2) giving rise to a recent radiation in the subhumid central montane belt of Madagascar (area M; ca. 0.9 mya, 0.4 – 2.0 95 % CI, node 586, BS = 95, TBE = 1). Again, this was followed by several independent dispersals within Madagascar, mainly consisting of populations establishing in lower-elevation habitats. The distribution range of some species extended beyond the Central Highlands (e.g. *Helichrysum leucocladum* Humbert into the humid eastern lowlands and *H. aphelexioides* DC. into the western arid lowlands). Nevertheless, there were also a few instances of upward dispersal to higher elevations, such as the case of *H. cremnophilum* Humbert, whose distribution range reached the highest areas.

Three other independent dispersal events to Madagascar took place, each one giving rise to a single Malagasy species. *Helichrysum mahafaly* (MAD1) from the southern and south-western arid lowlands (area B) of Madagascar is nested within a clade of species from the geographically distant arid to semi-arid southwestern African area (area N). This dispersal event is inferred to have taken place 2.6 mya (1.4 – 4.7 95 % CI, node 664, BS = 100, TBE = 1). The Malagasy widespread *H. madagascariense* (MAD5) diverged from its sister from the southern African grasslands around 1.8 mya (0.8 – 3.7 95 % CI, node 435, BS = 100, TBE = 1). Lastly, the ancestor of *H. plantago* (MAD3) is also inferred to have dispersed from the southern African grasslands at about 1.1 mya (0.5 – 2.6 95 % CI, node 542, BS = 93, TBE = 0.93).

4. Discussion

Here, we explore the evolutionary dynamics of colonization and diversification on Madagascar using the species-rich genus *Helichrysum*. This is the first time that the origin and geographic diversification of a Malagasy plant group have been explored using a large phylogeny based on NGS data from over 300 taxa, including more than half of the species endemic to the island. We report at least six independent colonizations from continental Africa from the Pliocene onwards, most of these from the southern African grasslands and the tropical Afromontane areas. We also recover a single back-colonization to the southeastern African coastal region. Our results suggest a clear tendency to retain bioclimatic preferences and morphological characters in the colonization of Madagascar by *Helichrysum*. However, our findings indicate that once lineages established on the island, their diversification was partly associated with biome shifts, especially shifts from high-elevation to lower-elevation habitats. We also identify several simultaneous and recent *in-situ* radiations in the montane areas of the island, including the subhumid central montane belt and the highest areas, which enriched the diversity of their flora. Although some lineages are morphologically and functionally very uniform, others include a great variety of life forms and morphological characters.

4.1. Helichrysum colonized Madagascar multiple times from Africa since the late Pliocene and likely returned once to the continent

Given the geological history of Madagascar, the two possible mechanisms by which Madagascar's biota was established are Gondwanan vicariance and long-distance dispersal. We infer a recent colonization of Madagascar by *Helichrysum*, with the earliest dispersal event estimated at around 3.7 mya (2.2 – 6.2 95 % CI), followed by multiple independent colonizations until at least 1 mya (0.5 – 2.6 95 % CI). Considering that Madagascar separated from Africa more than 150 mya, *Helichrysum* must therefore have colonized the island via long-distance dispersal across the Mozambique Channel. Short-lived land bridges across this ancient biogeographical barrier at different geological times have been proposed (Masters et al. 2021), but their existence has not been demonstrated, keeping their true biogeographical impact under debate (Ali & Hedges 2023, Aslanian et al. 2023). In any case, the last potentially available land bridges would have probably disappeared before the first colonization of Madagascar by *Helichrysum*. Most Compositae species produce anemochorous fruits, and *Helichrysum* cypselae are extremely small (< 1 mm long), which enables wind dispersal (Nathan et al. 2009). Since the Miocene, dominant sea currents and winds in the southwestern Indian Ocean region have predominantly flowed westward, reducing the likelihood of dispersal from Africa to Madagascar, except through cyclones crossing the Mozambique Channel in the opposite direction. At the same time, this shift in ocean currents and wind directionality increased the probability of dispersal from Asia to Madagascar, as reflected in the strong floristic affinities between southeastern Asia, India and Madagascar, especially in the humid northern and eastern regions (Schatz 1996, Ali & Huber 2010, Warren et al. 2010, Buerki et al. 2013). Another plausible mechanism that could have acted in parallel is avian dispersal, as there is evidence of direct seed dispersal by birds, either stuck in the plumage or contained in the digestive tract of the consumed prey (Padilla et al. 2012).

According to Buerki et al. (2013), during the Miocene there was an increase in colonizations of Madagascar by non-endemic genera, coinciding also with the emergence of most Malagasy endemic genera. Our dating indicates a much more recent colonization of Madagascar by *Helichrysum,* occurring during the Pliocene and Pleistocene. Buerki et al. (2013) identified a few genera with similarly recent inferred colonization dates, such as *Hibiscus* L. (Malvaceae), *Paracorynanthe* Capuron (Rubiaceae), *Colvillea* Bojer and *Lemuropisum* H.Perrier (Fabaceae). Another notable example within the tribe Gnaphalieae with a similar age of colonization is the genus *Stoebe* L. (Bergh & Linder 2009). Nevertheless, unlike the diversification patterns observed in *Helichrysum*, all these genera are represented in Madagascar by few species.

The already mentioned prevailing westward currents and winds, which have dominated since the Miocene, should have increased the likelihood of dispersal from Madagascar to Africa, thus the potential of back-colonizations to the mainland. However, documented examples of such events are scarcely found in the literature. Some notable exceptions include Dombeyoideae (Malvaceae s.l.) with at least five migrations back to the continent (Skema et al. 2023), the Coffeeae and Psychotrieae alliances (Rubiaceae, Kainulainen et al. 2017; Razafimandimbison et al. 2017), *Croton* (Euphorbiacee, Haber et al. 2017) and grammitid ferns (Bauret et al. 2017). Here, we inferred at least one potential back-colonization from Madagascar to continental Africa in *Helichrysum*, involving *H. silvaticum*, which occurs in the Indian Ocean Coastal Forest Belt (area C, in Mozambique and South Africa). This biome, separated from Madagascar by the 415 km wide Mozambique Channel (Masters et al. 2021), is the geographically closest continental biome to the island. This species is sister to *H. leucosphaerum* Baker which inhabits Madagascar's subhumid central highlands (area H) and the dry western lowlands (area W). Both species belong to the MAD4 clade (Fig. 2), which originated from a dispersal from the southern African grassland around 3.6 mya (2.2 – 5.9 95 % CI), giving rise to five species, four of which are endemic to Madagascar. The most likely scenario in our reconstruction is a single dispersal to Madagascar, followed by diversification and a back-colonization of the ancestor of *H. silvaticum*. However, uncertainty at nodes 426 and 429 suggests an alternative possibility in which the ancestor of both species remained in the continent, in which case *H. leucosphaerum* would be the result of a second, independent colonization of Madagascar within the MAD4 clade. Given the clade's high morphological diversity, increased taxonomic sampling –particularly of continental African species, since Madagascar morphotypes and taxonomic groups are all represented– could reduce uncertainty regarding the biogeographic and evolutionary history of this lineage.

4.2. Biome conservatism prevails in the colonization of Madagascar by Helichrysum: The continental Afromontane region reveals as the main source of Malagasy lineages

Our results for *Helichrysum* are consistent with previous findings of biome conservatism following transoceanic dispersal (Crisp et al. 2009, Vences et al. 2009) as well as the maintenance of biome-related adaptations (Wiens & Graham 2005, Wiens et al. 2010). Specifically, we infer that five out of the six hypothesized independent colonization events of Madagascar by *Helichrysum* happened in the island's highlands and mountains by ancestors from the continental Afromontane region *s.l.,* which apart from the tropical Afromontane area also includes the high southern African grasslands in the Drakensberg mountains. This suggests that *Helichrysum* dispersed from areas with similar broad-scale climatic conditions in continental Africa, reinforcing the idea that long-distance dispersal to Madagascar by *Helichrysum* was associated with biome conservatism. In greater detail, we identify two colonization events from the southern African montane grasslands to the Malagasy montane grasslands. In these cases, the colonizers preserved not only broad-scale bioclimatic preferences but also morphological traits. For example, *H. plantago* (MAD3) shares traits with its sister species *H. nudifolium* (L.) Less (Fig. 4**,** more details in Table S7) and other closely related species. Likewise, *H. madagascariense* (MAD5) is strikingly similar to the species comprising its sister group, *H. dasycephalum* O. Hoffm. and *H. rutilans* D.Don, and to *H. callicomum* Harv., the sister to all three (Fig. 4**,** more details in Table S7).

Malagasy grasslands, now extensive, have a complex history, and whereas some grassland formations are ancient, some others are much more recent and anthropogenic (Vorontsova et al. 2016, Hackel et al. 2018, Joseph & Seymour 2020, Solofondranohatra et al. 2020, Joseph et al. 2021, Lehmann et al. 2022, Bond et al. 2023, Tiley et al. 2024b). Thus, some form of grassland environment was undoubtedly already available for *Helichrysum* with relevant adaptations to "dispersify" (Donoghue 2008) from similar African environments. Dispersals from southern Africa to Madagascar have been reported for multiple grasses and sedge clades (e.g. Linder et al. 2014, Larridon et al. 2021). However, little is known about non-graminoid grassland species, although *Alchemilla* L. (Gehrke et al. 2016) and *Stoebe* (Bergh & Linder 2009), currently found in the highest areas of Malagasy mountains, probably dispersed from the montane southern African grasslands.

Two ancestors from the tropical Afromontane area successfully established and later radiated in the mountains of Madagascar, one

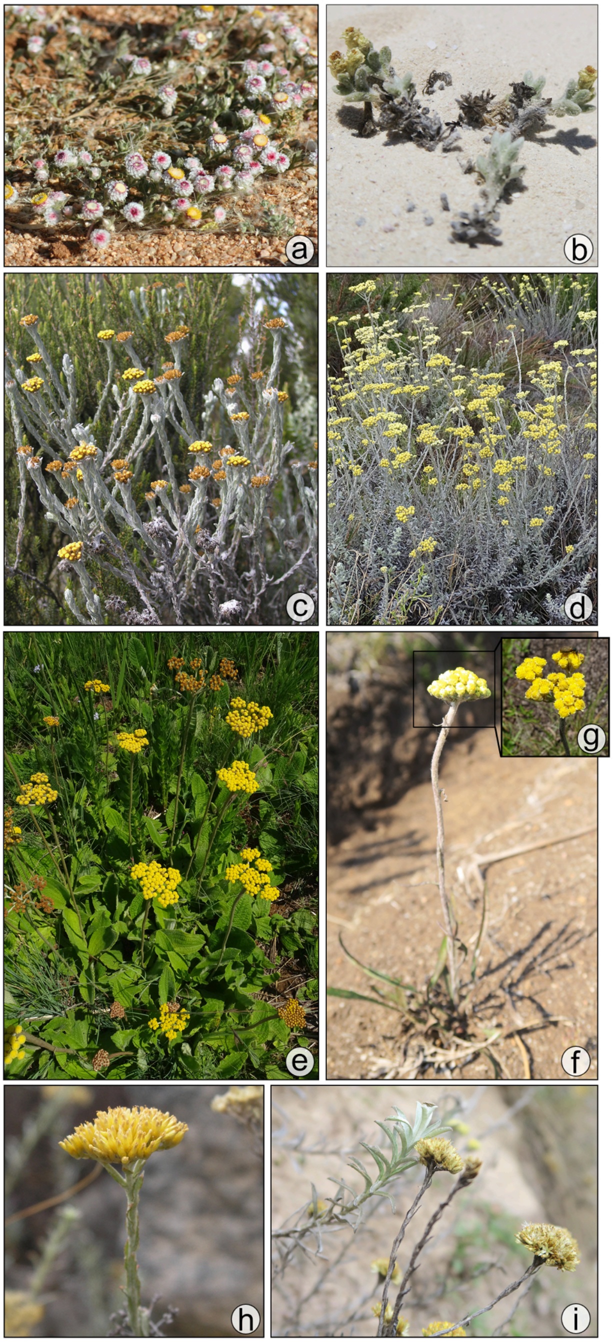

Fig. 4. Species of *Helichrysum* illustrating the similarity between closely related continental Africa and Malagasy taxa. First row: (a) *Helichrysum argyrosphaerum* from South Africa, which is the inferred sister to (b) *Helichrysum mahafaly* (MAD1) from Madagascar. Both are prostrate herbs with solitary, subglobose capitula, inhabiting arid sandy habitats. Second row: (c) *Helichrysum forskahlii* var. *compactum* a tropical Afromontane species closely related to taxa from the Malagasy clade MAD2; (d) *Helichrysum fulvescens,* a Malagasy species from clade MAD2, mostly subshrubs with numerous small cylindrical capitula arranged in corymbs and fimbrilliferous receptacles. Third row: (e) *Helichrysum nudifolium* var. *pilosellum* from South Africa, inferred sister species of *Helichrysum plantago* (f) and (g) from Madagascar (MAD3). Both are perennial herbs with basal leaf rosette and dense corymbs of capitula with yellow involucral bracts. Fourth row: (h) *Helichrysum rutilans*, a southern African species closely related to (i) *Helichrysum madagascariense* (MAD5) from Madagascar, both subshrubs with dense corymbs of narrow cylindrical capitula. (Photos: Mercè Galbany-Casals, except (a) and (e): Marinda Koekemoer).

radiation resulting in most of the current species growing in the subhumid central montane belt (area M, Fig. 2, clade MAD2) and the other resulting in most of the species occurring in the highest areas (area E, Fig. 2, clade MAD6). The Tapia woodland mosaics in the subhumid central montane belt are structurally equivalent to the miombo woodland savannas of continental Africa (Alvarado et al. 2014) and the iconic montane ericoid shrublands found in Africa display similar physiognomy to those growing on the summits of the highest Malagasy mountains (Silander et al. 2024). Examples of Afromontane migrations from tropical Africa to Madagascar involve several grass clades (Vorontsova et al. 2016, Hackel et al. 2018), sedge lineages (Larridon et al. 2021), *Kniphofia* Moench (Ramdhani et al. 2009), and the diverse *Erica* L. (Pirie et al. 2019). In the case of clade MAD2, morphological traits have been conserved in this jump between montane biomes in Africa and Madagascar by *Helichrysum* (Fig. 4**,** detailed information in Table S7).

Lastly, we propose an alternative interpretation of the ancestral range reconstruction for the clade MAD6 (Fig. 2). According to our ML phylogeny, *Helichrysum galpinii* (inhabiting the southern African grasslands, Fig. 2, node 366) is sister to two Malagasy lineages, suggesting that the entire clade likely originated from an ancestor in the southern African grasslands. This clade also includes a couple of tropical Afromontane species, introducing uncertainty about the biogeographic range of the ancestor of these two Malagasy montane lineages. However, our ASTRAL phylogeny (Supplementary Figure S4) presents a different topology, where the two Malagasy montane clades are recovered as unrelated: one is sister to *H. galpinii* and the other is sister to *H. whyteanum* Britten and *H. brunioides* Moeser. This topology thus suggests two independent colonizations of Madagascar's montane area, one from the southern African grasslands and the other from the tropical Afromontane region. Indeed, the morphological distinctness of these two Malagasy montane lineages is consistent with the hypothesis of two independent colonizations.

Finally, we would like to note a striking case of conservatism in bioclimatic preferences in the colonization of Madagascar by the ancestor of *Helichrysum mahafaly* (Fig. 2, clade MAD1). This species, which is endemic to the extremely hot and arid southwestern part of the island, is nested within a southern African clade from the western arid to semi-arid regions. In other words, *H. mahafaly* occupies a habitat characterized by environmental stressors strikingly similar to those experienced by its sister species in southern Africa (*H. argyrosphaerum,* Fig. 4). Other examples of Malagasy plants adapted to arid environments and likely originating from African ancestors include baobabs (*Adansonia* L., Leong Pock Tsy et al. 2009), Portulacaceae members (Eggli 1997, Hershkovitz & Zimmer 2000) and *Neoapaloxylon* Rauschert (Leguminoseae, Choo et al. 2020).

4.3. Diversification within Madagascar

Over time, lineages that colonize new territories, such as islands, can lead to various evolutionary outcomes. These may include limited or extensive speciation, remaining relatively unchanged or adapting to new conditions. In *Helichrysum*, we observed instances of different diversification patterns. Some lineages have speciated considerably. The timing of colonization and speciation on Madagascar by the ancestors of these lineages coincided with the transition towards the so-called "Icehouse Climate State" (Westerhold et al. 2020). The cooling trend resulted in increased aridity, pronounced seasonality and lower atmospheric CO_2 concentrations, which have been linked to several plant radiations (especially in the two most species-rich grassland families Poaceae and Compositae, Palazzesi et al. 2022) in the open grassland biomes that had proliferated worldwide during the Miocene (Spriggs et al. 2014). In parallel, ocean circulation patterns changed around 3 – 4 mya, resulting in a decrease in rainfall and increased aridity in both East Africa and Madagascar (De Wit 2003). Most speciation events leading to extant *Helichrysum* species are inferred to have occurred during the Pleistocene, as reported for other Malagasy groups such as orchid genera

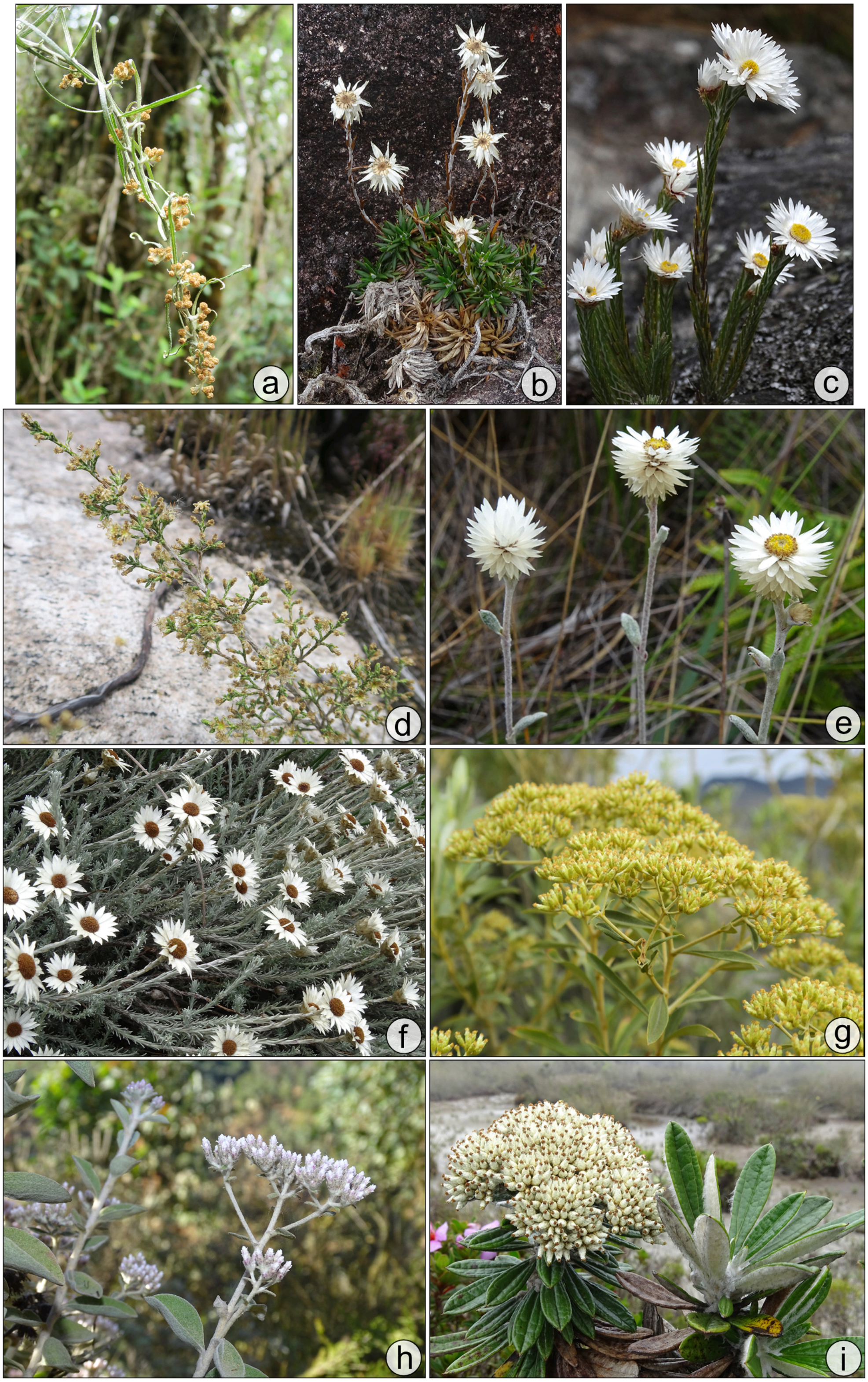

Fig. 5. Morphological diversity of clade MAD6. (a) *Helichrysum mutisiifolium* (GIX), a lianioid species; (b) *Helichrysum chamaeyucca*, (e) *Helichrysum marojejyense*, and (f) *Helichrysum danguyanum*, the three from GIII, all with long white radiant involucral bracts; (c) *Helichrysum ibityense* var. *ibityense* (GII) and (d) *Helichrysum hypnoides* (GIV), both with ericoid leaves; (g) *Helichrysum gymnocephalum*, (h) *Helichrysum xylocladum* and (i) *Helichrysum geniorum*, the three from GI, shrubs or treelets with tiny capitula grouped in glomerules surrounded by leaves and gathered in big corymbose synflorescences. (Photos: Mercè Galbany-Casals).

(*Aeranthes* Lindl.*,* *Angraecum* Bory and *Jumellea* Schltr., Andriananjamanantsoa et al. 2016) and scaly tree ferns (Cynthaeaceae, Janssen et al. 2008).

As is common for many groups that have diversified in archipelagos (Rundell & Price 2009), *Helichrysum* likely experienced a combination of adaptive and non-adaptive radiations (summarized in Table S7). Some clades such as the clade MAD4 (Figure S7) and the youngest subclade in MAD6 (Fig. 5**),** comprise ecologically and morphologically well-differentiated species. This heterogeneity suggests ecological speciation (i.e. reproductive isolation arises through divergent natural selection in populations adapted to different ecological environments; Rundle & Nosil 2005) as the main evolutionary force. Similar evolutionary patterns have been reported for other groups, such as the radiation of the small Afromontane genus *Arrowsmithia* DC. (formerly *Macowania* Oliv., also belonging to the tribe Gnaphalieae) in the Drakensberg mountains (Bentley et al. 2014). Nonetheless, members of the more species-rich clades within *Helichrysum* tend to be morphologically uniform, making them easily identifiable as a cohesive group. One of such examples is a subclade in MAD6, which comprises all sampled species from Humbert's taxonomic group I (GI, Humbert 1962). Despite their morphological similarities (Fig. 5), species in this group show notable variation in geographic distribution and ecological preferences. This suggests that diversification in GI likely resulted from a combination of geographic (or allopatric) and ecological speciation events. However, despite the occurrence of several shifts in broad-scale bioclimatic preferences, the overall pattern within this clade points towards biome conservatism. Each of the two major subclades within GI has a distinct primary distribution area (see Fig. 2): one diversified in the northern and eastern montane belt, while the other diversified on mountain summits. A similar pattern is observed in MAD2, which includes all sampled species of taxonomic group XI (GXI, Humbert 1962). Although the group is morphologically well-defined (more details in Table S7), it exhibits considerable variation in growth form, capitula size, bract color, and ecological preferences.

Our findings indicate that biome shifts likely occurred in Madagascar*,* following speciation within the colonized area and/or expansion of the distribution range across Madagascar's biomes. Most shifts in bioclimatic preferences in Malagasy *Helichrysum* are associated with independent dispersals from higher to lower elevation environments. The colder glacial periods of the Pleistocene may have promoted this transition to mid-low elevations. For instance, the elevational range of many species in group XI (GXI, clade MAD2) appears to have expanded downward to the highland grasslands (e.g. *H. fulvescens* DC. and *H. viguieri* Humbert), while multiple species that are currently restricted to the highlands' grasslands have montane ancestors. An example is the clade comprising *H. hirtum* Humbert*, H. tenue* Humbert and *H. heterotrichum* Humbert (GXI), in which biome conservatism prevailed after an initial shift to lower elevations. Exceptionally, a few montane species have colonized the dry western lowlands (e.g. *H. triplinerve* DC) or the humid eastern lowlands (e.g. *H. geayi*), two biomes with few *Helichrysum* species. Such repeated downward migrations are infrequently reported in the literature (e.g. *Dendrosenecio* (Hauman ex Hedberg) B.Nord., Knox & Palmer 1995), the general evolutionary trend being migration from lower to higher elevations (see Gamisch et al. 2016 and references therein).

Lastly, two of the three Malagasy lineages represented by a single species, and descending from African ancestors, are morphologically distinct from all other Malagasy *Helichrysum* species. This was recognized in the most recent taxonomic treatment (Humbert 1962), which placed these species into monospecific taxonomic groups: *H. mahafaly* in group VI (Fig. 4, GVI, clade MAD1) and *H. plantago* in group X (Fig. 4, GX, clade MAD3). This is not surprising as these species closely resemble their relatives in continental Africa, both morphologically and ecologically. Even if our sampling is not complete, we hypothesize that these two species are the only extant descendants of their respective colonizing ancestors. The lack of diversification in the lineage represented by *H. mahafaly* could be attributed to its highly specialized niche, which results in geographical isolation to the southwestern coast. Conversely, *H. plantago* is a widespread and opportunistic species that thrives in the heavily degraded highlands. Its recent origin and its ability to exploit this transformed landscape, which lacks significant geographical barriers, may have allowed it to spread widely, and all these factors may explain lack of further diversification in this clade. The sole member of the third lineage, *H. madagascariense* (Fig. 2, GVII, clade MAD5, see Fig. 4) was classified by Humbert (1962) in group VII together with three other species. However, our tree shows that group VII is not monophyletic, as *H. leucosphaerum* (node 429) is placed in a separate Malagasy clade containing species from different taxonomic groups (clade MAD4 in Fig. 2**,** see Figure S7). Without sampling the other two members of group VII, we cannot confirm whether *H. madagascariense* truly constitutes a monospecific lineage.

5. Conclusions

This study has focused on the Malagasy radiations of the highly diverse plant genus *Helichrysum* to unravel its biogeographic and evolutionary history within this long-isolated fragment of Gondwana. Our findings reveal a scenario of repeated recent transoceanic dispersal events, in which biome conservatism played a critical role in shaping speciation and diversification of Malagasy lineages. Some colonization events were followed by *in-situ* diversification, likely driven by a combination of allopatric and ecological speciation processes. The primary source of Malagasy lineages can be traced to the Afromontane region *s.l.*, including the highest mountains of tropical Africa and southern Africa. The ancestors were probably preadapted to high-elevation environments, which facilitated the colonization of the island's montane areas, harboring now most of the genus' diversity. Once established, the descendants of these African ancestors appear to have radiated *in-situ,* possibly through geographic speciation, giving rise to several high-elevation Malagasy endemics distributed across different massifs. In addition, our inferences suggest that some montane species shifted their ranges to lower-elevation environments, such as the highlands' grasslands, western dry deciduous forests or eastern evergreen forests. In some cases, these range shifts resulted in expanded distribution ranges, while in others, they promoted further speciation. In contrast, Malagasy clades coming from southern African grasslands ancestors consist of one or few widespread species. Notably, we identified a rare example of conservatism in bioclimatic preferences after long-distance dispersal from the arid southwestern Africa to Madagascar's similarly arid southwestern coastal dunes. Our findings also reveal a potential case of back-dispersal by *Helichrysum* to the southeastern coast of continental Africa, supporting the idea of sporadic biogeographical dispersals from Madagascar to mainland Africa.

CRediT authorship contribution statement

Carme Blanco-Gavaldà: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cristina Roquet:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Genís Puig-Surroca:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Santiago Andrés-Sánchez:** Writing – review & editing, Resources, Methodology, Investigation. **Sylvain G. Razafimandimbison:** Writing – review & editing, Resources, Methodology, Investigation. **Rokiman Letsara:** Writing – review & editing, Resources. **Nicola Bergh:** Writing – review & editing, Resources, Investigation. **Glynis V. Cron:** Writing – review & editing, Resources. **Lucía D. Moreyra:** Validation, Software. **Juan Antonio Calleja:** Writing – review & editing, Resources, Investigation. **Òscar Castillo:** Writing – review & editing, Investigation, Data curation. **Randall J. Bayer:** Resources. **Frederik Leliaert:** Writing – review & editing, Resources. **Alfonso Susanna:** Writing – review & editing, Resources, Methodology, Investigation,

Conceptualization. **Mercè Galbany-Casals:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2024.108283.

Data availability

The Sequence Read Archive (SRA) identifier for each sample can be found in the Supplementary Table S1 as well as the link to the repository.

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