



**Meise
Botanic Garden**

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1 **TITLE:**

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

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6 Carme Blanco-Gavaldà ^a, Cristina Roquet ^a, Genís Puig-Surroca ^a, Santiago
7 Andrés-Sánchez ^b, Sylvain G. Razafimandimbison ^c, Rokiman Letsara ^d, Nicola
8 Bergh ^{e, f}, Glynis V. Cron ^g, Lucía D. Moreyra ^h, Juan Antonio Calleja ⁱ, Óscar
9 Castillo ^h, Randall J. Bayer ^j, Frederik Leliaert ^k, Alfonso Susanna ^h, Mercè
10 Galbany-Casals ^a.

11
12 **Author affiliations:**

13
14 ^a Systematics and Evolution of Vascular Plants (UAB) – Associated Unit to
15 CSIC by IBB, Departament de Biologia Animal, Biologia Vegetal i Ecologia,
16 Facultat de Biociències, Universitat Autònoma de Barcelona, 08193 Bellaterra,
17 Spain.

18 ^b University of Salamanca, Department of Botany and Plant Physiology,
19 Pharmacy Faculty, C/ Licenciado Méndez Nieto s/n 37007, Salamanca,
20 Spain. santiandres@usal.es

21 ^c Swedish Museum of Natural History, Stockholm, Sweden, BOX 50007, SE-
22 10405 Stockholm, Sweden.

23 ^d Herbarium of the Parc Botanique et Zoologique of Tsimbazaza (PBZT),
24 3G9G+V6C, Antananarivo, Madagascar.

25 ^e South African National Biodiversity Institute, Kirstenbosch NBG, Rhodes Drive,
26 Newlands, Cape Town, South Africa.

27 ^f Gothenburg Botanical Gardens, Carl Skottsbergs Gata 22A, 413 19
28 Gothenburg, Sweden.

29 ^g School of Animal, Plant & Environmental Sciences, University of
30 Witwatersrand, 1 Jan Smuts Avenue, Braamfontein 2000, Johannesburg, South
31 Africa.

32 ^h Botanic Institute of Barcelona (IBB, CSIC- Ajuntament de Barcelona), Pg. del
33 Migdia s.n., 08038 Barcelona, Spain. asusanna@ibb.csic.es

34 ⁱ Autonomous University of Madrid, 28049 Madrid, Spain.

35 ^j University of Memphis, Ellington Hall, 3700 Walker Avenue, Memphis, TN
36 38152-3540, USA.

37 ^k Meise Botanic Garden, Nieuwelaan 38, 1860, Meise, Belgium.

38 Frederik.leliaert@meisebotanicgarden.be

39
40
41 **Abstract**

42
43 Colonization and diversification processes are responsible for the distinctiveness
44 of island biotas, with Madagascar standing out as a biodiversity hotspot
45 exceptionally rich in species and endemism. Regardless of its significance, the
46 evolutionary history and diversification drivers of Madagascar's flora remain
47 understudied. Here we focus on *Helichrysum* (Compositae, Gnaphalieae) to
48 investigate the evolutionary and biogeographic origins of the Malagasy flora. We
49 inferred a highly resolved phylogeny based on target-enrichment data from 327
50 species (including 51% of Malagasy endemics) and conducted ancestral range

51 estimation analyses. Our results revealed at least six trans-oceanic dispersal
52 events from different African regions to Madagascar during the Pliocene. In this
53 process, biome conservatism prevailed, as evidenced by similarities between
54 Malagasy lineages and their African relatives. The southern African grasslands,
55 known to be the center of diversification and the main source of African
56 *Helichrysum* lineages, played a key role in the colonization of Madagascar as the
57 ancestral source area of at least three clades. The Tropical Afrotropical region
58 was revealed as the source of at least two montane Malagasy lineages that
59 substantially radiated *in-situ*. Finally, a dispersal event from southwestern Africa
60 led to a lineage represented by a single species adapted to the island's
61 southwestern arid conditions. The main radiations of *Helichrysum* in
62 Madagascar's mountains occurred within the last 2 My, coinciding with a
63 transition towards cooler and drier conditions and the expansion of open habitats,
64 likely driven by a combination of geographic and ecological speciation. Overall,
65 our findings highlight the affinities between the montane floras of continental
66 Africa and Madagascar.

67

68

69 **Keywords:**

70 Afrotropical flora, Asteraceae, biogeography, dispersal, *Helichrysum*,
71 Madagascar, target-enrichment

72

73 **1. Introduction**

74

75 Unraveling the origins and evolution of island biotas has been of interest to
76 scientists since Darwin's times (Darwin 1859). Due to their isolation,
77 comparatively small size and varying distances from mainlands, islands are
78 considered natural laboratories for the study of ecological and evolutionary
79 processes. Species richness of islands can generally be explained through
80 features such as island area and isolation as well as the age and formation history
81 (Whittaker et al. 2008, Graham et al. 2017). Distinguishing between oceanic
82 islands and continental islands is important as it dictates the mechanisms
83 underlying species colonization. Unlike the relatively younger oceanic islands,
84 which emerge devoid of any resident species and gradually accumulate their
85 diversity through dispersal followed by speciation, older continental islands inherit
86 a baseline biota upon isolation from the mainland (Matthews & Triantis 2021).

87

88 Madagascar, once part of Gondwana, is an ancient continental island. It first
89 broke apart from Africa 155 to 165 million years ago (mya) (Yoder & Nowak 2006
90 and references therein) and later from India 84 to 91 mya (Wells 2003) and has
91 remained separated since then. Madagascar hosts a hyperdiverse biota in a wide
92 range of ecosystems, being home to an estimated total flora of ca. 14900 vascular
93 plant species, of which 87% are endemics (Callmender et al. 2011, Lowry et al.
94 2018, Antonelli et al. 2022), with 310 endemic plant genera and five endemic
95 families (Buerki et al. 2013). Such exceptional species richness and endemism
96 are the result of a complex geological, climatic and evolutionary history. Despite
97 hosting vicariant groups predating Gondwana's breakup (e.g., *Takhtajania*,
98 Winteraceae, Thomas et al. 2014), most of the present-day diversity likely
99 established on Madagascar through long-distance dispersal (Yoder & Novak
100 2006, Buerki et al. 2013), probably assisted by wind and oceanic currents. The

101 existence of land bridges and stepping-stones between Africa and Madagascar
102 has been hypothesized, but not supported by any direct evidence, so their
103 potential role and relevance in facilitating dispersal remains under debate
104 (Warren et al. 2010, Masters et al. 2021, Ali & Hedges 2023, Aslanian et al. 2023).
105 Geographic proximity likely accounts for the Malagasy biota having the strongest
106 taxonomic links to African lineages (Yoder & Novak 2006, Buerki et al. 2013).
107

108 Madagascar's complex topography plays a key role in shaping the main climatic
109 and vegetation zones of the island and has probably had a significant impact on
110 *in-situ* diversification of many groups. The main massifs of the island, reaching
111 1800-2900 m a.s.l., are placed in a north-south axis and are connected through
112 the Central Highlands, a plateau ranging 800 to 1300 m. The orography combined
113 with the prevailing easterly trade winds results in a rainfall gradient from the
114 humid tropical east-northeast to the sub-arid southwest. The Central Highlands
115 have a subhumid climate and are dominated by a grassland-woodland mosaic,
116 heavily modified and degraded by human activity (Yoder et al. 2016; Antonelli et
117 al. 2022). The highest parts of the mountains, above 1800-1900 m, are mainly
118 occupied by sclerophyllous shrublands dominated by Ericaceae and Compositae,
119 in addition to open grasslands and rupicolous plant communities (Burgoyne et al.
120 2005; Yoder et al. 2016; Antonelli et al. 2022, and pers. obs.). Outside the
121 mountains, landscapes are dominated by deciduous forests, sclerophyllous and
122 succulent plants westwards and evergreen rainforests eastwards. This ecological
123 and biological heterogeneity provides the perfect ground for various post-
124 colonization scenarios, such as repeated colonization without subsequent
125 diversification (e.g. the *Inulea-Pulcheinae* group, Nylinder et al. 2016) as well as
126 colonization followed by *in-situ* diversification (e.g. Psychotriaceae alliance,
127 Razafimandimbison et al. 2017; Coffeaceae alliance, Kainulainen et al. 2017; C3
128 grasses, Hackel et al. 2018). Nevertheless, there is a knowledge gap on the
129 relative importance of these scenarios in the evolutionary assembly of the
130 Malagasy biota, given that the biogeographic history of many highly diverse
131 Malagasy plant groups remains largely unexplored (Antonelli et al. 2022).

132 The relative contribution of adaptive evolution to the generation of biodiversity is
133 still under debate. Niche conservatism –i.e., the tendency of species to retain
134 their ancestral niches– has been suggested to be predominant in allopatric (also
135 known as geographic) speciation (Wiens & Graham 2005, Wiens et al. 2010) and
136 has been assumed to prevail in angiosperm evolution (Wiens & Graham 2005,
137 Crisp. et al. 2009, Wiens et al. 2010) since migrating might be easier and quicker
138 to achieve than adapting to new environmental conditions (Donoghue 2008).
139 However, multiple studies support the idea that niche shifts within plant genera
140 are not as rare as previously thought (e.g. *Lonicera* L., Smith & Donoghue 2010;
141 *Coccinia* Wight & Arn., Holstein & Renner 2011; *Ranunculus* L., Hörandl &
142 Emadzade 2011; *Hakea* Schrad & J.C.Wendl., Cardillo et al. 2017) and indeed
143 older and/or more extensive biomes (e.g. sclerophyllous shrubland, tropical
144 rainforest) have often been the source of lineages found in younger and/or less
145 extensive biomes (e.g. grasslands, alpine, Mediterranean shrublands; Crisp et al.
146 2009, Donoghue & Edwards 2014). To date, few studies have explored the role
147 that niche evolution may have played in the diversification of Malagasy plant
148 groups (but see the case of *Bulbophyllum* Thouars in Gamisch et al. 2016, 2021).

149

150 In terms of species richness, Compositae are one of the five dominant plant
151 families in the Malagasy flora (520 spp. and 83% endemism; Callmänder et al.
152 2011, Catalogue of the Vascular Plants of Madagascar 2024). Within Malagasy
153 Compositae, the most species-rich genus is *Helichrysum* Mill. In the most recent
154 floristic treatment of Malagasy *Helichrysum*, Humbert (1962) recognized 111
155 species –all but one endemic– and numerous infraspecific taxa at the subspecific
156 and varietal levels. He did not propose a formal infrageneric classification but
157 organized the species into eleven informal taxonomic groups based on
158 morphological affinities. *Helichrysum* occurs in almost all Malagasy biomes, from
159 the extremely arid south-western coastal dunes (e.g., *H. mahafaly* Humbert) to
160 the humid eastern rainforests (e.g. *H. geayi* Humbert), adopting a wide range of
161 growth forms (lianas, herbs, subshrubs, shrubs and small trees). However, most
162 of the Malagasy diversity of *Helichrysum* is found in the Central Highlands and
163 on the highest mountains, distributed across their full elevational and latitudinal
164 gradients. The diversity of *Helichrysum* in Madagascar reflects the extraordinary
165 variation and adaptability of the genus, already evident in the case of continental
166 African lineages (Blanco-Gavaldà et al. 2023).

167

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

184

185

186 **2. Materials and Methods**

187

188 *2.1 Taxon Sampling*

189

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

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

206
207 We also included 34 specimens of other genera representing the main lineages
208 of the tribe (Nie et al. 2016, Smissen et al. 2020) so that we could implement
209 secondary age calibrations. The complete dataset comprises a total of 386
210 samples, 289 from previous studies (273 from Blanco-Gavaldà et al. 2023
211 BioProject PRJNA936872; six from Mandel et al. 2019, BioProject
212 PRJNAS40287; and ten from Schmidt-Lebuhn & Bovill 2021, BioProject
213 PRJNA665592). Here we sequenced for the first time 97 samples using the same
214 baits set, representing mainly *Helichrysum* species from continental Africa and
215 Madagascar.

216 217 218 *2.2 Next-generation sequencing*

219
220 DNA was extracted from 10–30 mg of dried leaf material obtained from herbarium
221 specimens and from our field expeditions (see Supplementary Table S1) using
222 the E.N.Z.A.[®] SP Plant DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA)
223 following manufacturer instructions. We measured DNA concentration using
224 Qubit[™] Flex Fluorometer (Thermo Scientific, Waltham, MA, USA). Then, we
225 used a Qsonica Q800R3 Sonicator (Qsonica LLC, Newton, CT, USA) at 20%
226 amplitude for 45 sec to 8 min to shear 0.2-1 ug in 50 uL of DNA into 300-400 bp
227 fragments. We performed gel electrophoresis (1.2% agarose) to check fragment
228 length.

229
230 We prepared target-enrichment libraries from 25ul of the sonicated DNA using
231 the NEBNext Ultra II DNA Library Prep Kit for Illumina[®] (New England Biolabs,
232 Ipswich, MA, USA) employing half of the volumes specified by the manufacturer
233 and 15 cycles of PCR amplification. We barcoded the libraries using NEBNext
234 Multiplex Oligos sets with unique single or dual index combinations. Afterward,
235 we pooled the indexed libraries with more than 17 ng of DNA in groups of up to
236 10 samples and around 250 ng of DNA per library. We evaporated or filled with
237 water the arranged pools to 7 ul of total volume to perform target-enrichment
238 (protocol from Mandel et al. 2014) using the Microarray MyBaits COS kit (Daicel
239 Arbor Biosciences, Ann Arbor, MI, USA), specifically developed for the
240 Compositae family. The final sequencing pools were prepared by pooling
241 enriched libraries with unenriched libraries at a 60:40 ratio and the samples were
242 sequenced (PE 150 bp) on Illumina HiSeq2500 and HiSeqX platforms.

243
244 We deposited newly generated raw sequence reads in the NCBI Short Read
245 Archive database (SRA; access: <https://www.ncbi.nlm.nih.gov/sra>) under the
246 BioProject accession number PRJNA1121119.

247
248

249 *2.3 Molecular data processing*

250

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

258

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

275

276

277 *2.4 Phylogenetic Analyses*

278

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

290

291 We performed summary-coalescence inference with ASTRAL-III v.5.7.8 (Zhang
292 et al. 2018). To do so, we first inferred individual gene trees for each retrieved
293 locus generated with RAxML v.8.2.12 (Stamatakis 2014) and performed 100
294 bootstrap replicates (HPM6a, HPM7 and HPM8a). For the ASTRAL tree, we
295 calculated branch support values as local posterior probabilities (LPP),
296 considering well-supported branches those with $LPP \geq 0.95$ (Sayyari & Mirarab
297 2016). In addition, we conducted a second summary-coalescence analysis with
298 the same parameters but using only the 25 most informative loci, which we

299 selected with SortaDate (Smith et al. 2018) based on three criteria: bipartition
300 support, clock-likeness, and tree length (which is proportional to information
301 content).

302

303

304 *2.5 Divergence Time Estimation*

305

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

320

321

322 *2.6 Ancestral geographic range reconstruction*

323

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

332

333 For our delimitation of Malagasy regions (**Figure 1**) we consulted many
334 bibliographic sources: the definitions of the main vegetation types described in
335 Jenkins (1987), Burgess et al. (2004), Goodman & Benstead (2003) –which in
336 turn integrates previous classifications by Faramalala (1995), Du Puy & Moat
337 (1996) and Lowry et al. (1997)– and Gautier et al. (2018); the delimitation of
338 Madagascar’s six principal biomes by Yoder & Nowak (2006), based on
339 Goodman & Benstead (2003); the map of predominant vegetation types shown
340 in Antonelli et al. (2022) simplified from Moat & Smith (2007); and the bioclimatic
341 zones by Rakotoarivelo et al. (2019) modified in Razafimandimbison et al. (2022).
342 However, our final proposal is not completely coincident with any of the cited
343 sources, as it also integrates distribution patterns of the genus and our field
344 experience. We used the following seven distribution areas: (B) southern and
345 southwestern arid lowlands, an area that ranges from 0 to 800 m and mainly
346 includes Madagascar’s arid spiny thicket, with drought-resistant species adapted
347 to extreme aridity and secondary grasslands; (W) dry western lowlands, an area
348 that ranges approximately from 0 to 800 m and mainly includes dry and highly

349 seasonal forests, succulent woodlands and secondary grasslands; (O) humid
350 eastern lowlands, an area that ranges approximately from 0 to 800 m and mainly
351 includes very humid lowland rainforests with some herbaceous clearings; (H)
352 subhumid central highlands, an area that ranges from 800 to 1300 m and is
353 mainly constituted by grassland formations and small patches of *Tapia* forests;
354 (M) subhumid central montane belt, an area that ranges from 1300 to 1800 m
355 and includes a mosaic of *Tapia* forest, woodlands, grasslands and rocky habitats;
356 (R) humid eastern and northern montane belt, an area that ranges from 800 to
357 1800 m and mainly constituted by moist, broadleaf forests that, because they
358 grow at certain altitude, are not as tall as lowland rainforests; and (E) highest
359 areas, which are disjoint small patches comprising all areas above 1800 m and
360 up to the summit of Maromokotro (2876 m) and include a mosaic of ericoid
361 thickets, mountain grasslands and rocky habitats (these correspond, broadly
362 speaking, to the massifs of Tsaratanana [and its surrounding mountain chains],
363 Marojejy, Anjanaharibe, Ankaratra, Vavavato, Ibity [and its neighbor mountains],
364 Itremo, Andringitra [and its satellite peaks] and Beampingaratra). We excluded
365 the Lowland Sambirano Rainforest biome (grey area in **Figure 1**) represented in
366 some maps at the north-western tip of the island, and coastal mangroves (not
367 shown in **Figure 1**), because none of the included *Helichrysum* species inhabits
368 these regions.

369

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

375

376 Species occurrence in the geographical areas was assigned considering
377 information on distribution and elevational range (based on Humbert 1962,
378 Catalogue of the Vascular Plants of Madagascar 2024, herbarium records and
379 own field observations). Some species are only occasionally or marginally
380 present in other areas outside of their main distribution range. In these cases, we
381 only considered the core area/s or elevational belts in which there are a significant
382 number of occurrences of the species, or that can be interpreted as the central
383 distribution of the species.

384

385 We carried out biogeographic range evolution analyses in R with the package
386 BioGeoBEARS (Matzke 2013), using the ML time-calibrated tree as input. The
387 maximum number of areas for any node was set to three, which is the highest
388 number of areas occupied by the most widespread extant taxon in our study. We
389 tested the fit of three biogeographical models: Dispersal-Extinction-Cladogenesis
390 (DEC; Ree et al. 2005; Ree & Smith 2008), a likelihood implementation of the
391 Dispersal-Vicariance model (DIVAlize; Ronquist 1997), and the BayArea model
392 (BAYAREAlize; Landis et al. 2013). We also tested a more complex version of
393 each model that accounts for founder-event speciation by adding the jump-
394 dispersal parameter (+j). We compared and chose the best fitting model based
395 on the Akaike Information Criterion (AIC) and AIC weight (AICw), considering that
396 the comparison of DEC and DEC+j models is statistically valid based on Matzke's
397 (2022) reply to Ree & Sanmartín (2018). We also performed Biogeographic
398 Stochastic Mapping (BSM; Dupin et al. 2017) on the best fitting model (DEC+j) to

399 estimate the frequency and types of biogeographical events, taking the mean and
400 standard deviation of event counts from 100 BSM replicates.

401 **3. Results**

402

403 *3.1 Molecular Data Processing*

404

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

417

418

419 *3.2 Phylogenetic analyses*

420

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

435

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

445

446 *3.3 Biogeographic Reconstruction*

447

448 The best-fitting biogeographical model was DEC+j according to AIC values
 449 (**Table 1**). Regarding dispersal types, cladogenetic founder-event processes (j =
 450 0.0085) had a slightly larger contribution than anagenetic range expansions (d =
 451 0.0076).
 452

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

453 **Table 1.** Summary statistics of the biogeographic models tested in BioGeoBEARS. The best-fitting model
 454 used to infer the most likely area occupied by the ancestors of *Helichrysum* is highlighted in bold (DEC+j).
 455 Values of parameters for dispersal (d), extinction (e), founder effect (j), likelihood scores (lnL) and Akaike
 456 Information Criterion (AIC) are provided.
 457

458
 459 Ancestral range reconstruction analyses (probabilities in Supplementary Table
 460 Table S5, full tree in Supplementary Figure S6) suggest that the genus most
 461 probably originated and initially diversified in the winter rainfall regions of western
 462 South Africa (Fynbos Biome, F and arid to semi-arid southern Africa, N) around
 463 11.6 mya (11.4 – 15.0 95% CI), although the ancestral range may have also
 464 included some of the surrounding areas (the southern African grasslands, G;
 465 and/or the high Drakensberg area, D). The largest lineage in our tree notably
 466 diversified in the southern African grasslands and dispersals northwards and
 467 eastwards occurred from the late Miocene onwards (c. 8 mya, 6.5 – 11.7 95% CI,
 468 node 356). Repeated dispersals gave rise to the current global distribution of
 469 *Helichrysum*.
 470

471 Within Madagascar, Biogeographic Stochastic Mapping (BSM) analyses reveal
 472 at least six founder dispersal events from continental Africa to Madagascar
 473 (**Figure 3**; BSM summaries in Supplementary Table S6), all of them giving rise
 474 to endemic lineages. The main geographic source of Madagascar's colonization
 475 events was the southern African grasslands (G, with a mean of 3 colonization
 476 events) followed by the Tropical Afromontane area (T, with a mean of 2
 477 colonization events).
 478

479 According to our results, the oldest Malagasy clade (**Figure 2**, clade MAD6) is
 480 sister to *Helichrysum galpinii* N.E.Br., likely descending from an ancestor
 481 occurring in the southern African grasslands that dispersed at an inferred age of
 482 3.7 mya (2.2 – 6.2 95% CI, node 366, BS = 100, TBE = 0.99). This clade
 483 comprises mainly Malagasy montane species found in the highest areas (area
 484 E), followed by species found in the subhumid northern and eastern montane belt
 485 (area R), but also includes two Tropical Afromontane species from mainland
 486 Africa. The probabilities of the ancestral range of the whole clade involve both
 487 Afromontane areas (tropical continental Africa and the highest areas of
 488 Madagascar). This clade is made up of two large and highly diversified Malagasy
 489 montane lineages (one inferred to have originated around 3.5 mya, 2.0 – 5.9 95%
 490 CI, node 367, BS = 99, TBE = 0.99 and the other at about 2.3 mya, 1.2 – 4.5 95%

491 CI, node 396, BS = 100, TBE = 1). However, these two lineages are not closely
492 related to each other in the ASTRAL phylogeny. Therefore, these results should
493 be taken with caution since concatenation and coalescence-based phylogenetic
494 inferences provide conflicting relationships around these nodes. The two
495 Malagasy montane clades notably diversified during the Pleistocene, with some
496 species colonizing or extending their area into the subhumid central montane belt
497 (area M, e.g. *H. abbayesii* Humbert), the grasslands of Madagascar's central
498 highlands (area H, e.g. *H. lecomtei* R. Vig. & Humbert and *H. xylocladum* Baker)
499 or the humid eastern lowlands (area O) in the case of *H. geayi* (node 381).

500

501 The second oldest colonization of Madagascar has an inferred age of 3.6 mya
502 (2.2 – 5.9 95% CI, node 425, BS = 100, TBE = 1, MAD4), also from a southern
503 African grassland ancestor. The ancestral range of the Malagasy descendant is
504 highly uncertain due to the wide range of the extant species belonging to this
505 clade, which include four areas in Madagascar (southern and southwestern arid
506 lowlands, area B; subhumid central montane belt, area M; subhumid central
507 highlands, area H and dry western lowlands, area W) and one area from
508 continental Africa (Indian Ocean Coastal Forest Belt, area C). These results
509 suggest a back-colonization to the southern African coast, inferred to have
510 occurred 2.3 mya (1.2 – 4.5 95% CI, node 429, BS = 100, TBE = 1) giving rise to
511 *H. silvaticum* Hilliard.

512

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

525

526 Three other independent dispersal events to Madagascar took place, each one
527 giving rise to a single Malagasy species. *Helichrysum mahafaly* (MAD1) from the
528 southern and south-western arid lowlands (area B) of Madagascar is nested
529 within a clade of species from the geographically distant arid to semi-arid
530 southwestern African area (area N). This dispersal event is inferred to have taken
531 place 2.6 mya (1.4 – 4.7 95% CI, node 664, BS = 100, TBE = 1). The Malagasy
532 widespread *H. madagascariense* (MAD5) diverged from its sister from the
533 southern African grasslands around 1.8 mya (0.8 – 3.7 95% CI, node 435, BS =
534 100, TBE = 1). Lastly, the ancestor of *H. plantago* (MAD3) is also inferred to have
535 dispersed from the southern African grasslands at about 1.1 mya (0.5 – 2.6 95%
536 CI, node 542, BS = 93, TBE = 0.93).

537

538

539 **4. Discussion**

540

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

559
560

561 *4.1 Helichrysum colonized Madagascar multiple times from Africa since the Late* 562 *Pliocene and likely returned once to the continent*

563

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

591

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

603

604 The already mentioned prevailing westward currents and winds, which have
605 dominated since the Miocene, should have increased the likelihood of dispersal
606 from Madagascar to Africa, thus the potential of back-colonizations to the
607 mainland. However, documented examples of such events are scarcely found in
608 the literature. Some notable exceptions include Dombeyoideae (Malvaceae s.l.)
609 with at least five migrations back to the continent (Skema et al. 2023), the
610 Coffeae and Psychotrieae alliances (Rubiaceae, Kainulainen et al. 2017;
611 Razafimandimbison et al. 2017), *Croton* (Euphorbiaceae, Haber et al. 2017) and
612 grammitid ferns (Bauret et al. 2017). Here, we inferred at least one potential back-
613 colonization from Madagascar to continental Africa in *Helichrysum*, involving *H.*
614 *silvaticum*, which occurs in the Indian Ocean Coastal Forest Belt (area C, in
615 Mozambique and South Africa). This biome, separated from Madagascar by the
616 415 km wide Mozambique Channel (Masters et al. 2021), is the geographically
617 closest continental biome to the island. This species is sister to *H. leucosphaerum*
618 Baker which inhabits Madagascar's subhumid central highlands (area H) and the
619 dry western lowlands (area W). Both species belong to the MAD4 clade (**Figure**
620 **2**), which originated from a dispersal from the southern African grassland around
621 3.6 mya (2.2 – 5.9 95% CI), giving rise to five species, four of which are endemic
622 to Madagascar. The most likely scenario in our reconstruction is a single dispersal
623 to Madagascar, followed by diversification and a back-colonization of the
624 ancestor of *H. silvaticum*. However, uncertainty at nodes 426 and 429 suggests
625 an alternative possibility in which the ancestor of both species remained in the
626 continent, in which case *H. leucosphaerum* would be the result of a second,
627 independent colonization of Madagascar within the MAD4 clade. Given the
628 clade's high morphological diversity, increased taxonomic sampling –particularly
629 of continental African species, since Madagascar morphotypes and taxonomic
630 groups are all represented– could reduce uncertainty regarding the
631 biogeographic and evolutionary history of this lineage.

632

633

634 *4.2 Biome conservatism prevails in the colonization of Madagascar by*
635 *Helichrysum: the continental Afrotropical region reveals as the main source of*
636 *Malagasy lineages.*

637

638 Our results for *Helichrysum* are consistent with previous findings of biome
639 conservatism following transoceanic dispersal (Crisp et al. 2009, Vences et al.
640 2009) as well as the maintenance of biome-related adaptations (Wiens & Graham

641 2005, Wiens et al. 2010). Specifically, we infer that five out of the six hypothesized
642 independent colonization events of Madagascar by *Helichrysum* happened in the
643 island's highlands and mountains by ancestors from the continental Afromontane
644 region *s.l.*, which apart from the tropical Afromontane area also includes the high
645 southern African grasslands in the Drakensberg mountains. This suggests that
646 *Helichrysum* dispersed from areas with similar broad-scale climatic conditions in
647 continental Africa, reinforcing the idea that long-distance dispersal to
648 Madagascar by *Helichrysum* was associated with biome conservatism. In greater
649 detail, we identify two colonization events from the southern African montane
650 grasslands to the Malagasy montane grasslands. In these cases, the colonizers
651 preserved not only broad-scale bioclimatic preferences but also morphological
652 traits. For example, *H. plantago* (MAD3) shares traits with its sister species *H.*
653 *nudifolium* (L.) Less (**Figure 4**, more details in Table S7) and other closely related
654 species. Likewise, *H. madagascariense* (MAD5) is strikingly similar to the species
655 comprising its sister group, *H. dasycephalum* O.Hoffm. and *H. rutilans* D.Don,
656 and to *H. callicomum* Harv., the sister to all three (**Figure 4**, more details in Table
657 S7).

658
659 Malagasy grasslands, now extensive, have a complex history, and whereas some
660 grassland formations are ancient, some others are much more recent and
661 anthropogenic (Vorontsova et al. 2016, Hackel et al. 2018, Joseph & Seymour
662 2020, Solofondranohatra et al. 2020, Joseph et al. 2021, Lehmann et al. 2022,
663 Bond et al. 2023, Tiley et al. 2024b). Thus, some form of grassland environment
664 was undoubtedly already available for *Helichrysum* with relevant adaptations to
665 “dispersify” (Donoghue 2008) from similar African environments. Dispersals from
666 southern Africa to Madagascar have been reported for multiple grasses and
667 sedge clades (e.g. Linder et al. 2014, Larridon et al. 2021). However, little is
668 known about non-graminoid grassland species, although *Alchemilla* L. (Gehrke
669 et al. 2016) and *Stoebe* (Bergh & Linder 2009), currently found in the highest
670 areas of Malagasy mountains, probably dispersed from the montane southern
671 African grasslands.

672
673 Two ancestors from the tropical Afromontane area successfully established and
674 later radiated in the mountains of Madagascar, one radiation resulting in most of
675 the current species growing in the subhumid central montane belt (area M, **Figure**
676 **2**, clade MAD2) and the other resulting in most of the species occurring in the
677 highest areas (area E, **Figure 2**, clade MAD6). The Tapia woodland mosaics in
678 the subhumid central montane belt are structurally equivalent to the miombo
679 woodland savannas of continental Africa (Alvarado et al. 2014) and the iconic
680 montane ericoid shrublands found in Africa display similar physiognomy to those
681 growing on the summits of the highest Malagasy mountains (Silander et al. 2024).
682 Examples of Afromontane migrations from tropical Africa to Madagascar involve
683 several grass clades (Vorontsova et al. 2016, Hackel et al. 2018), sedge lineages
684 (Larridon et al. 2021), *Kniphofia* Moench (Ramdhani et al. 2009), and the diverse
685 *Erica* L. (Pirie et al. 2019). In the case of clade MAD2, morphological traits have
686 been conserved in this jump between montane biomes in Africa and Madagascar
687 by *Helichrysum* (**Figure 4**, detailed information in Table S7).

688
689 Lastly, we propose an alternative interpretation of the ancestral range
690 reconstruction for the clade MAD6 (**Figure 2**). According to our ML phylogeny,

691 *Helichrysum galpinii* (inhabiting the southern African grasslands, **Figure 2**, node
692 366) is sister to two Malagasy lineages, suggesting that the entire clade likely
693 originated from an ancestor in the southern African grasslands. This clade also
694 includes a couple of tropical Afrotropical species, introducing uncertainty about
695 the biogeographic range of the ancestor of these two Malagasy montane
696 lineages. However, our ASTRAL phylogeny (Supplementary Figure S4) presents
697 a different topology, where the two Malagasy montane clades are recovered as
698 unrelated: one is sister to *H. galpinii* and the other is sister to *H. whyteanum*
699 Britten and *H. brunioides* Moeser. This topology thus suggests two independent
700 colonizations of Madagascar's montane area, one from the southern African
701 grasslands and the other from the tropical Afrotropical region. Indeed, the
702 morphological distinctness of these two Malagasy montane lineages is consistent
703 with the hypothesis of two independent colonizations.
704

705 Finally, we would like to note a striking case of conservatism in bioclimatic
706 preferences in the colonization of Madagascar by the ancestor of *Helichrysum*
707 *mahafaly* (**Figure 2**, clade MAD1). This species, which is endemic to the
708 extremely hot and arid southwestern part of the island, is nested within a southern
709 African clade from the western arid to semi-arid regions. In other words, *H.*
710 *mahafaly* occupies a habitat characterized by environmental stressors strikingly
711 similar to those experienced by its sister species in southern Africa (*H.*
712 *argyrosphaerum*, **Figure 4**). Other examples of Malagasy plants adapted to arid
713 environments and likely originating from African ancestors include baobabs
714 (*Adansonia* L., Leong Pock Tsy et al. 2009), Portulacaceae members (Eggli 1997,
715 Hershkovitz & Zimmer 2000) and *Neopaloxylon* Rauschert (Leguminosae,
716 Choo et al. 2020).
717

718 4.3 Diversification within Madagascar

719

720 Over time, lineages that colonize new territories, such as islands, can lead to
721 various evolutionary outcomes. These may include limited or extensive
722 speciation, remaining relatively unchanged or adapting to new conditions. In
723 *Helichrysum*, we observed instances of different diversification patterns. Some
724 lineages have speciated considerably. The timing of colonization and speciation
725 on Madagascar by the ancestors of these lineages coincided with the transition
726 towards the so-called "Icehouse Climate State" (Westerhold et al. 2020). The
727 cooling trend resulted in increased aridity, pronounced seasonality and lower
728 atmospheric CO₂ concentrations, which have been linked to several plant
729 radiations (especially in the two most species-rich grassland families Poaceae
730 and Compositae, Palazzesi et al. 2022) in the open grassland biomes that had
731 proliferated worldwide during the Miocene (Spriggs et al. 2014). In parallel, ocean
732 circulation patterns changed around 3 – 4 mya, resulting in a decrease in rainfall
733 and increased aridity in both East Africa and Madagascar (De Wit 2003). Most
734 speciation events leading to extant *Helichrysum* species are inferred to have
735 occurred during the Pleistocene, as reported for other Malagasy groups such as
736 orchid genera (*Aeranthes* Lindl., *Angraecum* Bory and *Jumellea* Schltr.,
737 Andriananjamanantsoa et al. 2016) and scaly tree ferns (Cyntheaeaceae, Janssen
738 et al. 2008).
739

740 As is common for many groups that have diversified in archipelagos (Rundell &
741 Price 2009), *Helichrysum* likely experienced a combination of adaptive and non-
742 adaptive radiations (summarized in Table S7). Some clades such as the clade
743 MAD4 (Figure S7) and the youngest subclade in MAD6 (**Figure 5**), comprise
744 ecologically and morphologically well-differentiated species. This heterogeneity
745 suggests ecological speciation (i.e. reproductive isolation arises through
746 divergent natural selection in populations adapted to different ecological
747 environments; Rundle & Nosil 2005) as the main evolutionary force. Similar
748 evolutionary patterns have been reported for other groups, such as the radiation
749 of the small Afromontane genus *Arrowsmithia* DC. (formerly *Macowania* Oliv.,
750 also belonging to the tribe Gnaphalieae) in the Drakensberg mountains (Bentley
751 et al. 2014). Nonetheless, members of the more species-rich clades within
752 *Helichrysum* tend to be morphologically uniform, making them easily identifiable
753 as a cohesive group. One of such examples is a subclade in MAD6, which
754 comprises all sampled species from Humbert's taxonomic group I (GI, Humbert
755 1962). Despite their morphological similarities (**Figure 5**), species in this group
756 show notable variation in geographic distribution and ecological preferences. This
757 suggests that diversification in GI likely resulted from a combination of geographic
758 (or allopatric) and ecological speciation events. However, despite the occurrence
759 of several shifts in broad-scale bioclimatic preferences, the overall pattern within
760 this clade points towards biome conservatism. Each of the two major subclades
761 within GI has a distinct primary distribution area (see **Figure 2**): one diversified in
762 the northern and eastern montane belt, while the other diversified on mountain
763 summits. A similar pattern is observed in MAD2, which includes all sampled
764 species of taxonomic group XI (GX1, Humbert 1962). Although the group is
765 morphologically well-defined (more details in Table S7), it exhibits considerable
766 variation in growth form, capitula size, bract color, and ecological preferences.

767
768 Our findings indicate that biome shifts likely occurred in Madagascar, following
769 speciation within the colonized area and/or expansion of the distribution range
770 across Madagascar's biomes. Most shifts in bioclimatic preferences in Malagasy
771 *Helichrysum* are associated with independent dispersals from higher to lower
772 elevation environments. The colder glacial periods of the Pleistocene may have
773 promoted this transition to mid-low elevations. For instance, the elevational range
774 of many species in group XI (GX1, clade MAD2) appears to have expanded
775 downward to the highland grasslands (e.g. *H. fulvescens* DC. and *H. viguieri*
776 Humbert), while multiple species that are currently restricted to the highlands'
777 grasslands have montane ancestors. An example is the clade comprising *H.*
778 *hirtum* Humbert, *H. tenue* Humbert and *H. heterotrichum* Humbert (GX1), in which
779 biome conservatism prevailed after an initial shift to lower elevations.
780 Exceptionally, a few montane species have colonized the dry western lowlands
781 (e.g. *H. triplinerve* DC) or the humid eastern lowlands (e.g. *H. geayi*), two biomes
782 with few *Helichrysum* species. Such repeated downward migrations are
783 infrequently reported in the literature (e.g. *Dendrosenecio* (Hauman ex Hedberg)
784 B.Nord., Knox & Palmer 1995), the general evolutionary trend being migration
785 from lower to higher elevations (see Gamisch et al. 2016 and references therein).

786
787 Lastly, two of the three Malagasy lineages represented by a single species, and
788 descending from African ancestors, are morphologically distinct from all other
789 Malagasy *Helichrysum* species. This was recognized in the most recent

790 taxonomic treatment (Humbert 1962), which placed these species into
791 monospecific taxonomic groups: *H. mahafaly* in group VI (**Figure 4**, GVI, clade
792 MAD1) and *H. plantago* in group X (**Figure 4**, GX, clade MAD3). This is not
793 surprising as these species closely resemble their relatives in continental Africa,
794 both morphologically and ecologically. Even if our sampling is not complete, we
795 hypothesize that these two species are the only extant descendants of their
796 respective colonizing ancestors. The lack of diversification in the lineage
797 represented by *H. mahafaly* could be attributed to its highly specialized niche,
798 which results in geographical isolation to the southwestern coast. Conversely, *H.*
799 *plantago* is a widespread and opportunistic species that thrives in the heavily
800 degraded highlands. Its recent origin and its ability to exploit this transformed
801 landscape, which lacks significant geographical barriers, may have allowed it to
802 spread widely, and all these factors may explain lack of further diversification in
803 this clade. The sole member of the third lineage, *H. madagascariense* (**Figure 2**,
804 GVII, clade MAD5, see **Figure 4**) was classified by Humbert (1962) in group VII
805 together with three other species. However, our tree shows that group VII is not
806 monophyletic, as *H. leucosphaerum* (node 429) is placed in a separate Malagasy
807 clade containing species from different taxonomic groups (clade MAD4 in **Figure**
808 **2**, see Figure S7). Without sampling the other two members of group VII, we
809 cannot confirm whether *H. madagascariense* truly constitutes a monospecific
810 lineage.

811

812

813 **5. Conclusions**

814

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

839 coast of continental Africa, supporting the idea of sporadic biogeographical
840 dispersals from Madagascar to mainland Africa.

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842

843 **CRedit authorship contribution statement:**

844 Carme Blanco-Gavaldà – conceptualization, methodology, investigation, formal
845 analyses, data curation, writing—original draft preparation.

846 Mercè Galbany-Casals – conceptualization, methodology, resources,
847 investigation, formal analyses, data curation, funding acquisition, project
848 administration, writing – review and editing.

849 Cristina Roquet – conceptualization, methodology, investigation, formal
850 analyses, data curation, writing – review and editing.

851 Lucía D. Moreyra – software, validation.

852 Santiago Andrés-Sánchez – methodology, resources, investigation, writing –
853 review and editing.

854 Genís Puig-Surroca – resources, investigation, formal analysis, data curation,
855 writing – review and editing.

856 Óscar Castillo – investigation, data curation, writing – review and editing.

857 Alfonso Susanna – conceptualization, methodology, resources, funding
858 acquisition, project administration, writing – review and editing.

859 Sylvain G. Razafimandimbison – methodology, resources, investigation, writing
860 – review and editing.

861 Nicola Bergh – resources, investigation, writing – review and editing

862 Glynis V. Cron – resources, writing – review and editing.

863 Frederik Leliaert – resources, writing – review and editing.

864 Juan Antonio Calleja - resources, investigation, writing – review and editing

865 Rokiman Letsara – resources, writing – review and editing.

866 Randall J. Bayer – resources.

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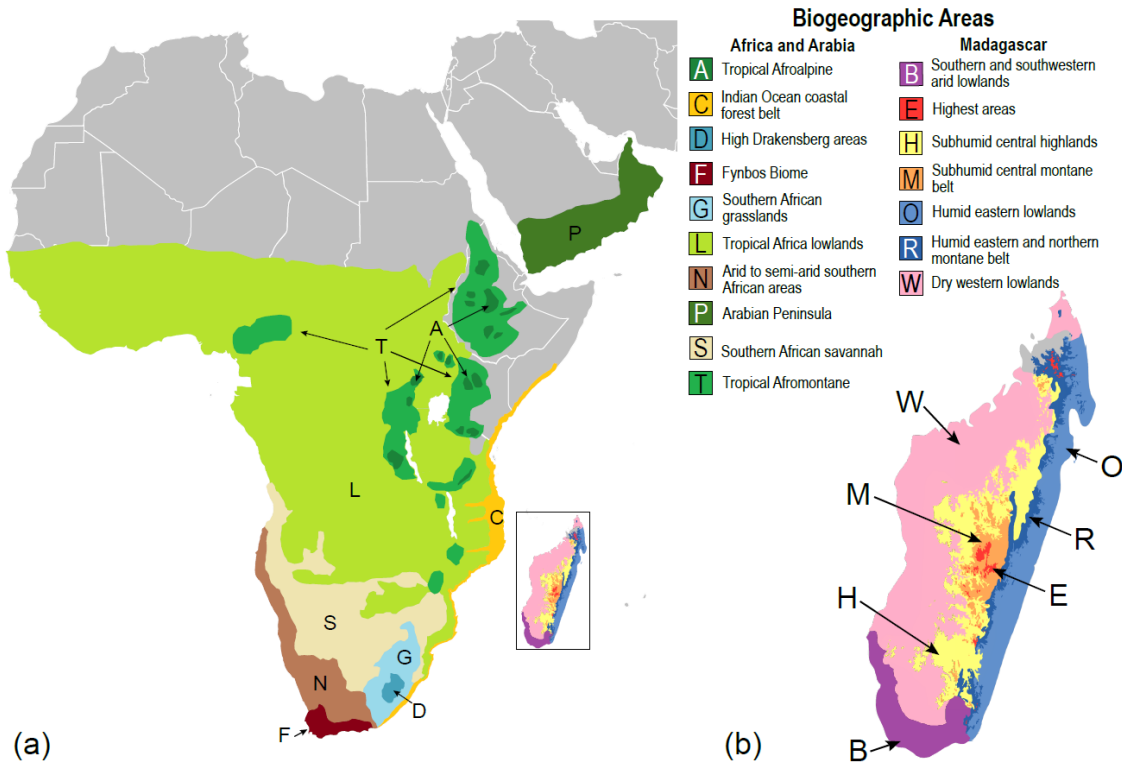


Figure 1. Maps illustrating the 17 geographic areas defined in this study. The colors and labels correspond to those in the biogeographical reconstruction analyses. (a) General map at scale, including continental Africa, Arabia and Madagascar. The dark green spots within the tropical Afromontane area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

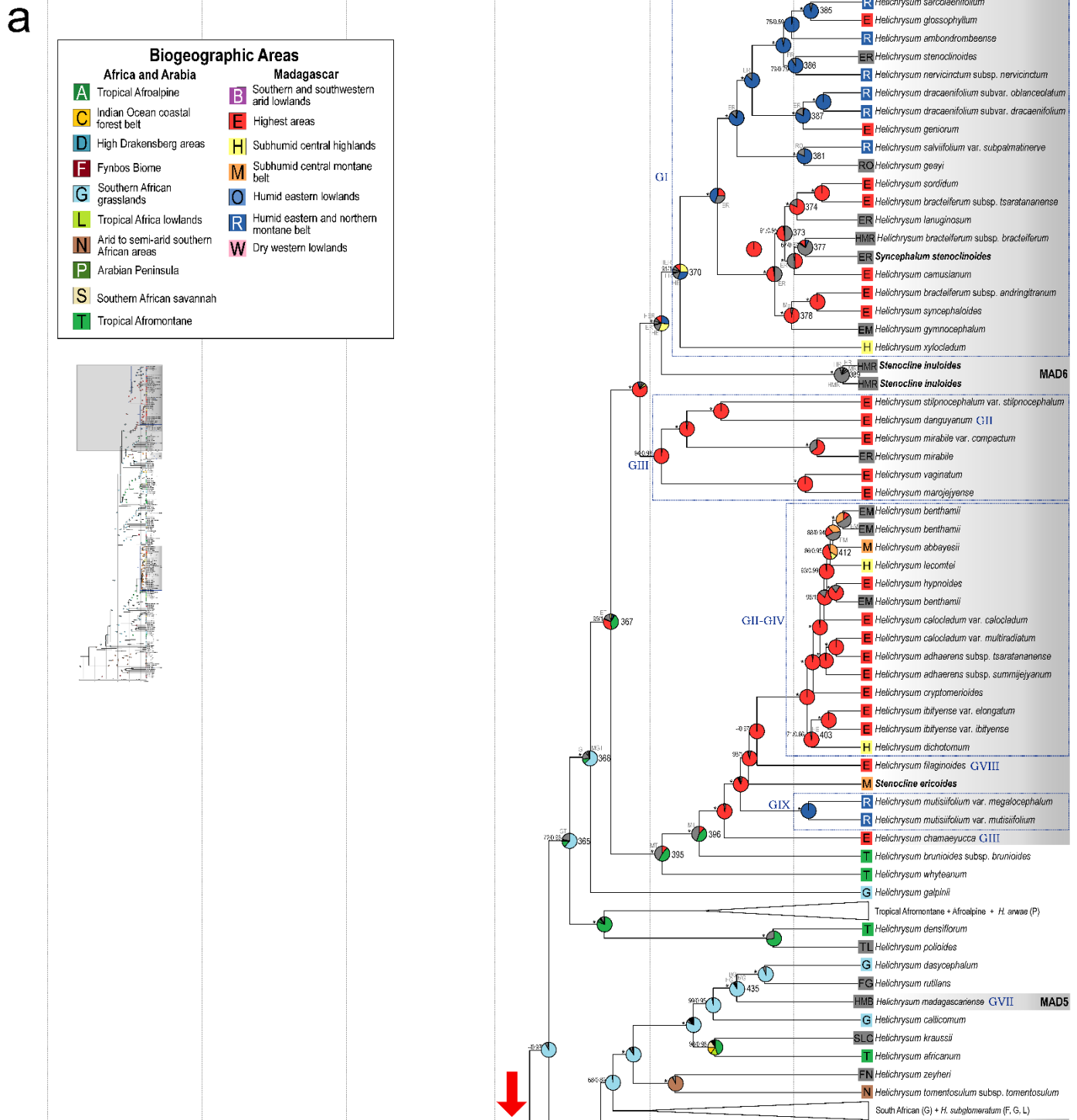


Figure 2. Ancestral range estimation of *Helichrysum* using the best-fitting model DEC+j. It is based on a time-calibrated phylogeny generated under the concatenation approach using target-enrichment data (Compositae1061 probe set). Pie charts at nodes show the relative probability of the possible states (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.

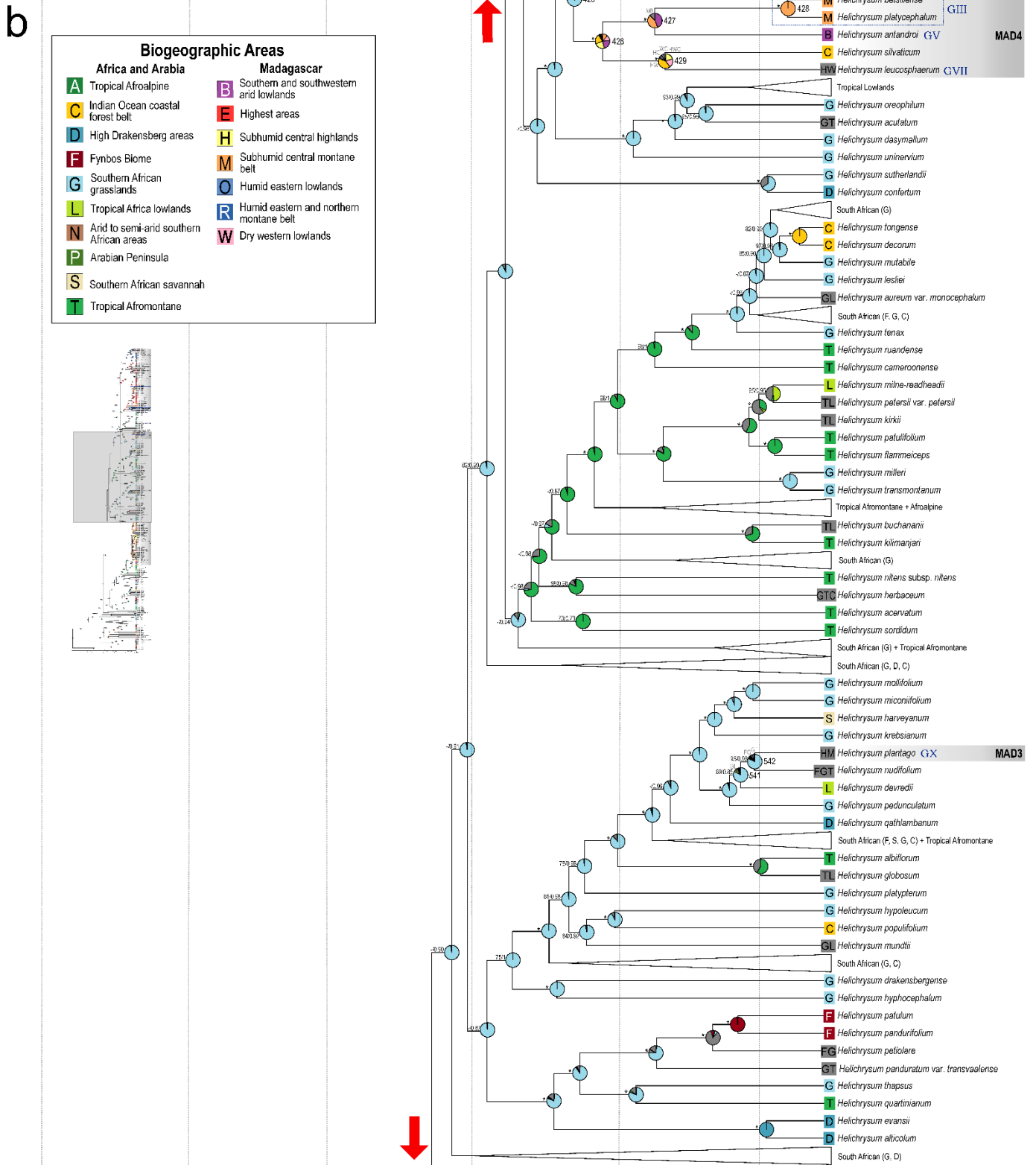


Figure 2. continued

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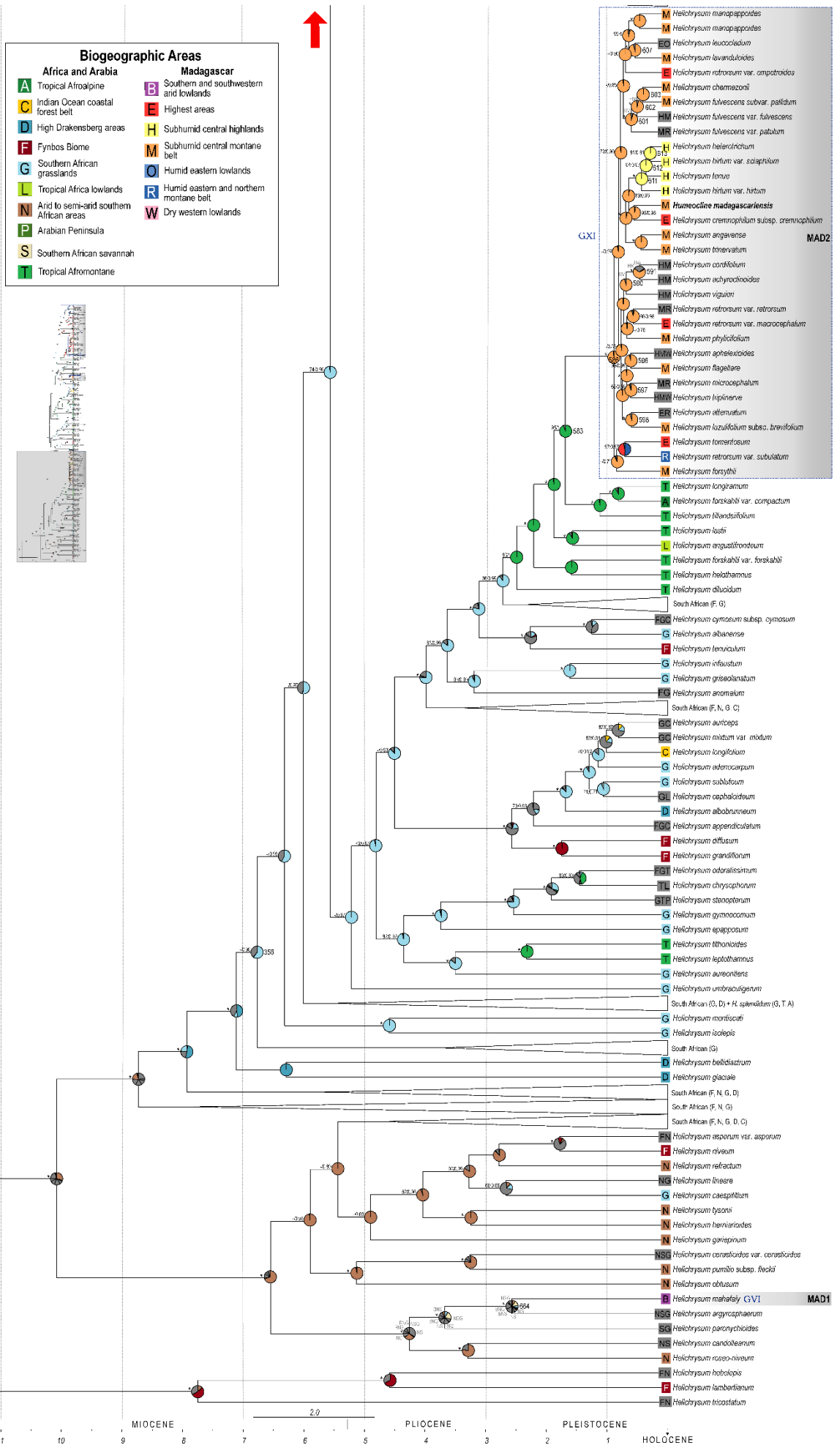


Figure 2. continued

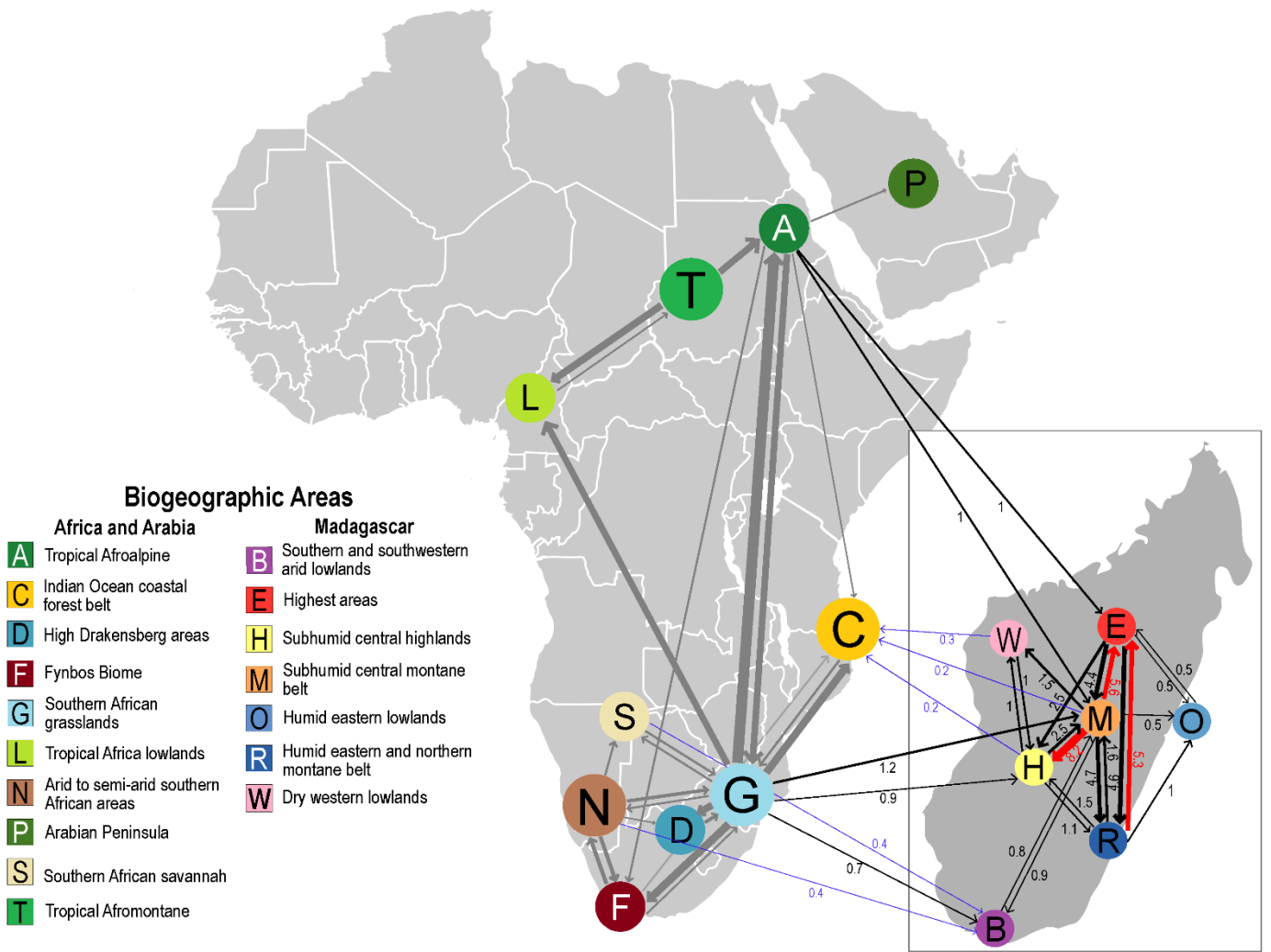


Figure 3. Summary of mean dispersal events estimated from 100 Biogeographic Stochastic Mappings (BSM) in *Helichrysum* (see all event counts in Supplementary Table S6). Arrow tips indicate the directionality of the dispersal. Numbers on the arrows are the mean of dispersal event counts (only given for 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.

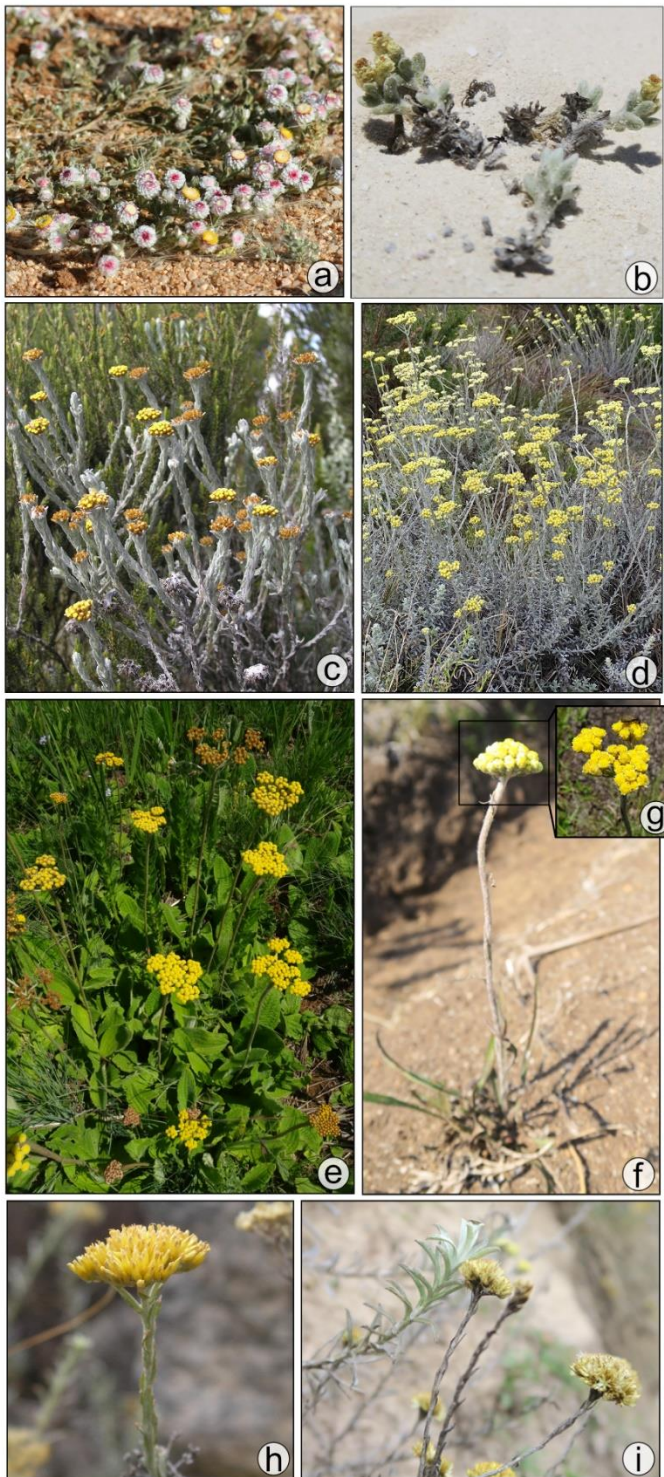


Figure 4. Species of *Helichrysum* illustrating the similarity between closely related continental Africa and Malagasy taxa. First row: (a) *Helichrysum argyrosphaerum* from South Africa, which is the inferred sister to (b) *Helichrysum mahafaly* (MAD1) from Madagascar. Both are prostrate herbs with 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 fimbriiferous 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).

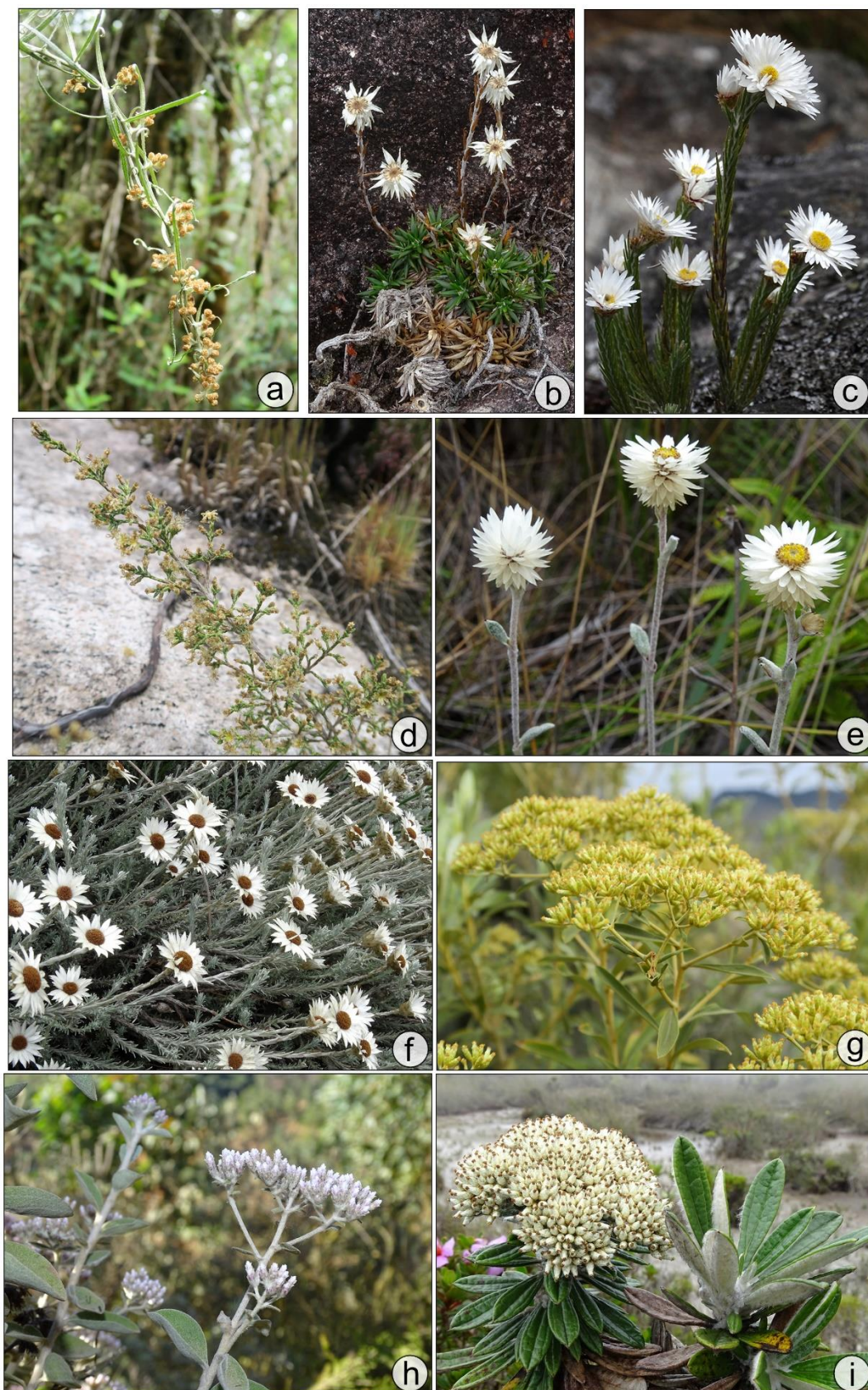


Figure 5. Morphological diversity of clade MAD6. (a) *Helichrysum mutisiifolium* (GIX), a lianioid species; (b) *Helichrysum chamaeyucca*, (e) *Helichrysum marojejense*, and (f) *Helichrysum danguyanum*, the three from GIII, all with long white radiant involucre bracts; (c) *Helichrysum ibityense* var. *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).