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ORIGINAL ARTICLE

The influence of geological history on diversification in insular species: genetic and morphological patterns of *Micromeria* Benth. (Lamiaceae) in Tenerife (Canary archipelago)

Pamela Puppo[1], Manuel Curto[1], Guillermo Velo-Antón[1], Pedro Luis Pérez de Paz[2] and Harald Meimberg[1,3,4]*

[1]*Research Center in Biodiversity and Genetic Resources (CIBIO)/InBio Associated Laboratory, University of Porto, Campus Vairão, Vairão 4485-661, Portugal,* [2]*Departamento de Biología Vegetal (Botánica), Universidad de La Laguna, La Laguna (Tenerife) 38271, Spain,* [3]*Restoration Ecology, Technical University Munich, Freising 85350, Germany,* [4]*Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences, Vienna A-1180, Austria*

*Correspondence: Harald Meimberg, Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences, A-1180 Vienna, Austria.
E-mail: meimberg@cibio.up.pt

ABSTRACT

Aim Using phylogenetic and morphometric approaches, our study aims to understand the diversification process of the two groups of *Micromeria* species in Tenerife: the species restricted to the palaeoislands, and the species widely distributed in the younger part of the island.

Location Tenerife, Canary Islands.

Methods We calculated a calibrated phylogeny and a Neighbor-Net network based on eight nuclear loci from 37 samples: 22 of the 8 species currently recognized in Tenerife, and 15 of their closest relatives occurring in neighbouring islands and continental populations. We performed a principal components analysis (PCA) of 27 morphological characters from 54 specimens sampled from Tenerife.

Results Our phylogeny showed that the species from Tenerife can be subdivided into three main clades: one composed of the species inhabiting the palaeoisland of Anaga (*M. teneriffae*, *M. glomerata* and *M. rivas-martinezii*); another composed of the species present in the palaeoisland of Teno (*M. densiflora*); and a third group that includes all the central species (*M. hyssopifolia*, *M. varia*, *M. lachnophylla* and *M. lasiophylla*). Morphometric analyses indicated two main groups corresponding to the palaeoisland species and the central ones.

Main conclusions Our study points to a relationship between the diversification in *Micromeria* and the geological history of Tenerife. We conclude that *Micromeria* first arrived in Anaga where it diversified, subsequently colonized Teno and from there occupied the central part, presumably after the formation of the Teide volcano. The species of *Micromeria* in Tenerife constitute an interesting example of how species diversification on oceanic islands can be shaped by the island's geological history, which probably contributed to the high levels of endemism on Tenerife.

Keywords
Diversification, endemism, island biogeography, island evolution, Lamiaceae, Macaronesia, *Micromeria*, oceanic islands, palaeoislands, Tenerife.

INTRODUCTION

The biodiversity of oceanic islands is characterized by a high rate of endemism and by groups of closely related species that can be highly differentiated morphologically, as an adaptive response to empty ecological niches (e.g. Crawford *et al.*, 1987; Weigelt *et al.*, 2013). Oceanic islands by definition have never had a connection to the mainland, so the diversity of their biota is a result of colonization events, related genetic-drift effects and subsequent evolutionary processes (e.g. Francisco-Ortega *et al.*, 1996; Silvertown, 2004). The rate of diversification after colonization may also depend on the geological history of the islands. The ontogeny of volcanic archipelagos is composed of different phases, beginning

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with a sea mount building above the sea level, its continual growth until it reaches maximum height and area, and its subsequent reduction below sea level by erosion (Fernández-Palacios *et al.*, 2011). The continuous change in area and profile of oceanic islands directly influences the number and type of habitats available and thus limits or broadens speciation opportunities (Whittaker *et al.*, 2008). In consequence, contemporary biodiversity on oceanic islands might be explained by the area of an island, as predicted by the equilibrium theory of island biogeography (MacArthur & Wilson, 1967), but also by the change of profile of the island over time according to the general dynamic model of oceanic island biogeography (Whittaker *et al.*, 2007).

The Canary Islands are located in the Atlantic Ocean, approximately 100 km off the western coast of Morocco. The archipelago is composed of seven islands, each with an independent origin, with the oldest islands in the east and younger islands in the west (Fig. 1; Carracedo, 1994; Juan *et al.*, 2000; Fernández-Palacios *et al.*, 2011). The largest and highest island in the Canaries is Tenerife, which possesses a unique geological history relative to the other islands in the archipelago. In the late Miocene, three islands occupied the area of today's Tenerife: Adeje (11.6–3.5 Ma), Teno (6.7–4.5 Ma) and Anaga (6.5–3.6 Ma; Ancochea *et al.*, 1990). Successive volcanic activity during the late Miocene–Pliocene led to the secondary connection of these formerly separate islands until Tenerife reached its current shape around 2 Ma (Ancochea *et al.*, 1990). Today, Tenerife consists of a younger, central part, including the Teide massif, and three older areas that are the remains of former islands (palaeoislands), which still show distinct geological and geomorphological characteristics (Fernández-Palacios *et al.*, 2011; Fig. 1).

The palaeoislands, in particular Teno and Anaga, harbour unique floral elements, with about 55 plant species endemic to at least one of them (Trusty *et al.*, 2005). We postulate that these endemics may represent early diverging lineages within their respective groups as relicts of formerly isolated areas. The resulting genetic structure between the palaeoislands could therefore constitute a signature of the former isolation of the regions, but might also be a consequence of the secondary split of formerly continuous populations by volcanic activity (i.e. Brochman, 1984). The valleys of Güímar (in the south-east of the island) and La Orotava (in the north-east) were formed after huge landslides between 800 and 600 ka and disconnected the Anaga massif from the rest of the island (Ancochea *et al.*, 1990; Watts & Masson, 1995; Juan *et al.*, 2000). Las Cañadas Caldera (north-central) was formed by another massive landslide less than 200 ka and was successively filled by the Teide volcano (3718 m), forming the highest point of the island today (Ancochea *et al.*, 1990). The La Orotava and Las Cañadas landslides may have functioned to re-isolate, for a time, the Teno and Anaga massifs (see Fig. 1).

Considering the geomorphological history of Tenerife, plant species currently inhabiting the central area of the island could be descendants from species of the older parts. Assuming a stepping-stone model (Kimura & Weiss, 1964), the palaeoislands would have been the nearest colonization source for the central part. Thereby, either adaptation led to diversification in the new areas after colonization of one or more species, or the central species were already present before the formation of the Teide volcano and colonized the central area by range shifts independent of each other. In the latter case, diversification of current species would precede the secondary contact between the palaeoislands, which could be one factor explaining the high species richness of Tenerife.

Micromeria Benth. (Lamiaceae) is composed of perennial herbs, subshrubs or shrubs with bisexual, entomophilous

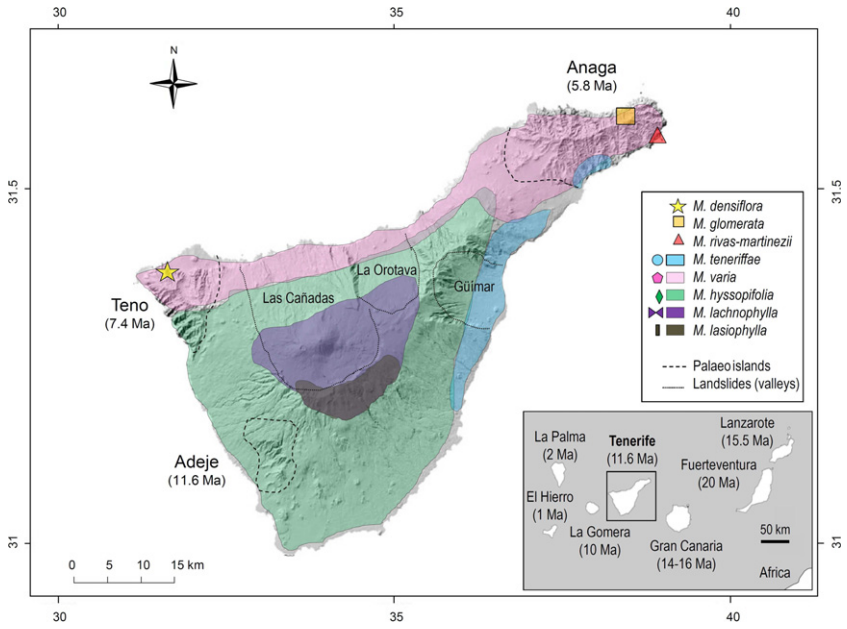

Figure 1 Map of Tenerife showing the distribution of the species of *Micromeria*. Dashed lines indicate remnants of palaeoislands, dotted lines indicate valleys formed after major landslides (*sensu* Ancochea *et al.*, 1990; Juan *et al.*, 2000), and symbol shapes and colours correspond to those used in Figs 3 and 5.

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flowers. The dry calyx bears the nutlets and is dispersed mainly by the wind but also by ants and sometimes water. *Micromeria* is composed of *c.* 54 species distributed in Macaronesia, the Mediterranean basin, Africa and Asia. In the Canary Islands there are *c.* 16 species, most of them single-island endemics. The species from each island are each other's closest relatives, which is consistent with a single colonization event on each island except for La Gomera, which appears to have been colonized twice (Meimberg *et al.*, 2006). Within Tenerife, the *Micromeria* species composition may reflect the geological history of the island. Eight species occur in this island with three species restricted to the palaeoislands. Within the Teno peninsula, *M. densiflora* grows in the walls of a deep cliff in Buenavista (Fig. 1) and to date it is only known from this locality (Santos-Guerra *et al.*, 2011). Within Anaga, *M. glomerata* grows in two small populations on the northern slopes of the massif, while *M. rivas-martinezii* is restricted to a small peninsula on the southern coast (Fig. 1) occupying an area of *c.* 0.1 km^2 (calculated with GE-Path 1.4.6; Sgrillo, 2012). *Micromeria teneriffae* is also distributed in Anaga, although its distribution extends to Fasnia in central Tenerife (Pérez de Paz, 1978; Fig. 1). The remaining four species are found in the central area of the island. Two are widely distributed – *M. varia* along the northern part from Teno to Anaga, and *M. hyssopifolia* throughout the island – while the other two have a narrow range. *Micromeria lachnophylla* is distributed from the high desert in Las Cañadas to the border of the pine forest, and *M. lasiophylla* is restricted to the steep cliffs of Las Cañadas (Fig. 1). Morphologically, the palaeoisland species of *Micromeria* are more easily identified than the central species, which constitute a species complex (Pérez de Paz, 1978; Fig. 2). It had also been hypothesized that the central species descend from *M. varia* (Pérez de Paz, 1978).

In this paper we present a phylogenetic and morphometric study of *Micromeria* on Tenerife, which aims to understand the diversification process of the two groups of species found in this island: the palaeoisland species (i.e. those restricted to the palaeoislands of Anaga and Teno) and the central species (i.e. those widely distributed in the central, younger part of Tenerife). In particular, we sought to determine: (1) whether the palaeoisland species are in fact early diverging lineages that precede the formation of the Teide (central shield); (2) whether *M. teneriffae* originated on a palaeoisland, given that its current distributional range exceeds the area of Anaga; and (3) whether the central species colonized from the palaeoislands. We also investigated the phylogenetic relationships among all species of the genus currently recognized taxonomically in Tenerife.

MATERIALS AND METHODS

Plant material

In order to infer the phylogenetic relationships among the species and populations of *Micromeria*, 80 individual plants were studied; 37 were used in the molecular analyses and 54 in the morphological analyses (Table 1). The samples were collected during 2010–11 and from previous studies (Bräuchler *et al.*, 2005; Meimberg *et al.*, 2006; Curto *et al.*, 2012; see Table 1). Specimens from Tenerife were collected from 24 localities and include all species on the island, including those that are narrowly distributed (see Table 1). At least one individual was collected per population and deposited in the herbarium of the Universidad de la Laguna in Tenerife (TFC), Spain, or at the herbarium of the Ludwig-Maximilians University in Munich, Germany (MSB). For DNA analysis leaves were conserved in silica gel.

Molecular analysis

Silica-preserved leaves were ground and used for DNA analysis using the Macherey-Nagel Plant DNA Extraction Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. For phylogenetic analysis we amplified a total of eight nuclear loci from 37 samples: 35 *Micromeria* (22 from Tenerife and 15 outgroups from other islands and mainland), plus one sample from each of *Mentha* and *Origanum* (see Table 1 and Appendix S1 in Supporting Information). The eight loci used were selected among those published by Curto *et al.* (2012) based on variability (number of polymorphisms) and sequence quality when samples of *Micromeria* from Tenerife were amplified. Amplification was conducted using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA). The final volume reaction was 10 μL and contained: 1 U Taq DNA polymerase, 75 nmol $MgCl_2$, 1 nmol of each dNTP, 4 nmol of each primer, and *c.* 20 ng template DNA. Polymerase chain reaction (PCR) was performed using the following cycle profile: 95 °C for 15 min; 35 cycles of 95 °C for 30 s; specific annealing temperature for 1 min (see Appendix S1); 72 °C for 1 min; and a final extension step of 72 °C for 10 min. Amplification success was confirmed by electrophoresis in 2% agarose gels stained with GelRed (Biotium, Hayward, CA, USA). Lengths of fragments were compared using Lambda – pUC Marker 4 ladder (Fermentas, St. Leon-Rot, Germany). PCR products were cleaned using Exo/Sap digestion in a final volume of 8 μL containing 4 U Exonuclease I (Fermentas) and 1 U Shrimp Alkaline Phosphatase (Fermentas) for 15 min at 37 °C and inactivated for 15 min at 85 °C. The purified DNA was sequenced in both directions for all samples on an ABI 3730 (Applied Biosystems, Carlsbad, CA, USA) at the LMU sequencing facility in Munich, Germany.

Sequence analyses

Electropherograms were checked and edited by eye and sequences were aligned using Geneious 6.1.4 (Biomatters, Auckland, New Zealand), with default parameters for gap opening and extension (Geneious alignment). Heterozygous point mutations were included using IUPAC ambiguity codes (W, R, Y, S, K and M). Haplotype reconstructions for each

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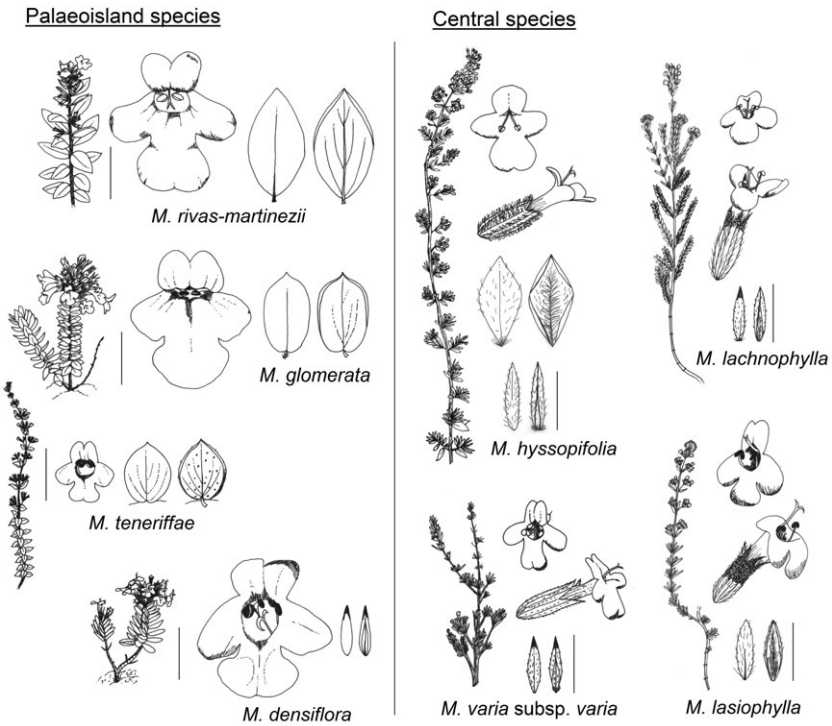

Figure 2 Drawings of all eight species of *Micromeria* currently recognized in Tenerife showing habit, upper and lower leaf surface, and flower. Scale bars indicate 2.5 cm for habit, 5 mm for leaves and 2.5 mm for flowers.

gene were conducted using default parameters in PHASE 2.1 (Stephens *et al.*, 2001) as implemented in DnaSP 5.10 (Librado & Rozas, 2009). Summary statistics for each locus were calculated using DnaSP. jModelTest 2.1.3 (Darriba *et al.*, 2012) was used to determine the most likely substitution model for each locus independently and for the combined alignment and tested for all 88 models implemented in this program. The most suitable model was chosen using the Akaike information criterion (AIC). All loci were combined and used for phylogenetic analysis. Phylogenetic trees were inferred using maximum likelihood (ML) and Bayesian inference (BI). ML was performed using PhyML 3.0 (Guindon *et al.*, 2010) using the model calculated for the combined alignment and 1000 bootstrap values. BI analyses were performed with unlinked substitution models for each gene. MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) was run using a strict clock and a uniform tree model; BEAST 1.7.5 (Drummond *et al.*, 2012) was used with a relaxed uncorrelated lognormal clock and a Yule process tree. Analyses were started with random trees and were run for 10 million generations (MrBayes) or 200 million generations (BEAST) sampling every 1000 generations, and a burn-in of 25%. Two independent runs were conducted using each program and results were analysed using Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/). Trees were combined using LogCombiner 1.7 (Drummond & Rambaut, 2007) and the resulting trees were edited using FigTree 1.7 (http://tree.bio.ed.ac.uk/software/figtree/).

Time to the most recent common ancestor (TMRCA) of main clades was estimated using BEAST calibrated by the age of the subtribe Menthinae (*c.* 23 Ma; as calculated in Drew & Sytsma, 2012) defined as following a normal distribution with an SD of 2 Myr. A haplotype Neighbor-Net network (Bryant & Moulton, 2004) was constructed using the combined alignment of the eight markers used in this study to show nuclear genetic relationships. The analysis was performed using SplitsTree 4.13.1 (Huson & Bryant, 2006) based on uncorrected patristic distances and a bootstrap analysis with 1000 replicates.

Morphometric analysis

For morphometric analysis, 54 flowering specimens from Tenerife were measured (Table 1). Specimens were assigned to the eight species recognized by Pérez de Paz (1978). The number of samples per species varied from one (*M. lachnophylla*) to 15 (*M. varia*) and was limited by the presence of flowers in the specimens. In the case of *M. lachnophylla*, all of the specimens but one lacked flowers and thus had to be excluded from the morphometric analyses. Twenty-seven measurements were taken from each sample including five vegetative and 22 floral characters (see Appendix S2). The characters were selected to reflect the morphological variation observed throughout the specimens. Fruit characters were not taken into consideration because specimens were collected during flowering season and thus lacked fruits. Vegetative characters and measurements from the calyx were taken from dried individuals; the remaining floral characters were taken from rehydrated flowers. Each character was measured once per sample using a ruler and a dissecting microscope (Nikon SMZ-10) and entered in a data matrix in mm.

Distance analyses were conducted using principal components analysis (PCA) as implemented in the program IBM

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Table 1 *Micromeria* samples used in the present study including locality and voucher/source for the different analyses. MSB, Herbarium of the Ludwig-Maximilians University in Munich; TFC, Herbarium of the Universidad de la Laguna in Tenerife.

Species	Locality*	Morphometric analyses	Genetic analyses
Micromeria benthamii Webb & Berthel.	Gran Canaria, 27°53′23.4″ N; 15°33′41.3″ W		Puppo 439.1 (TFC)
M. densiflora Benth.	Tenerife, Buenavista, Teno	Puppo 255–259 (TFC)	Puppo 255, 257 (TFC)
M. glomerata P. Pérez	Tenerife, Taganana, Anaga	Puppo 201–203 (TFC)	Puppo 200, 202 (TFC)
M. graeca (L.) Benth.	Spain		Meimberg 1.4, 4.1 (MSB)
M. hochreutineri R.Maire	Morocco, 31°28′18.1″ N; 07°24′05.5″ W		Curto MA034, MA035 (MSB)
M. hyssopifolia Webb & Berthel. var. *glabrescens* (Webb & Berthel.) P. Pérez	Tenerife, Lomo Morin, Teno, 28°21′35.3″ N; 16°47′20.8″ W		Puppo 562.3 (TFC)
M. hyssopifolia var. *glabrescens*	Tenerife, Rambla de Castro, 28°23′45.3″ N; 16°35′23.3″ W	Puppo 241–244 (TFC)	Puppo 239 (TFC)
M. hyssopifolia var. *hyssopifolia*	El Hierro, Costa de Valverde, 27°48′49.9″ N; 17°53′52.8″ W		Pérez de Paz H1E (TFC)
M. hyssopifolia var. *hyssopifolia*	Tenerife, Güímar, 28°17′40.1″ N; 16°24′10.8″ W		Puppo 153 (TFC)
M. hyssopifolia var. *hyssopifolia*	Tenerife, Bco. Herques, Fasnia-Güímar, 28°14′59.2″ N; 16°26′17.5″ W	Puppo 162, 165 (TFC)	Puppo 162 (TFC)
M. hyssopifolia var. *hyssopifolia*	Tenerife, Arico, 28°10′59″ N; 16°27′11.4″ W	Puppo 169 (TFC)	
M. hyssopifolia var. *hyssopifolia*	Tenerife, Arico, 28°09′08.3″ N; 16°29′29.7″ W	Puppo 172 (TFC)	
M. hyssopifolia var. *hyssopifolia*	Tenerife, Arafo, 28°22′21.7″ N; 16°25′39.5″ W	Puppo 269, 271 (TFC)	
M. hyssopifolia var. *kuegleri* (Bornm.) P. Pérez	Tenerife, Los Abades, 28°08′28.1″ N; 16°27′16.4″ W	Puppo 175–176 (TFC)	Puppo 175 (TFC)
M. hyssopifolia var. *kuegleri*	Tenerife, Acantilado La Hondura, 28°12′03.1″ N; 16°25′29.5″ W	Puppo 180 (TFC)	Puppo 181 (TFC)
M. inodora (Desf.) Benth.	Formentera, Cult. Botanical Garden Munich		Bräuchler *et al.* (2005)
M. lachnophylla Webb & Berthel.	Tenerife, Las Cañadas	Puppo 297 (TFC)	Puppo 291, 295 (TFC)
M. lanata Benth.	Gran Canaria		Meimberg *et al.* (2006)
M. lasiophylla Webb & Berthel. subsp. *lasiophylla*	Tenerife, Las Cañadas	Puppo 276–278, 280, 285 (TFC)	Puppo 274, 276 (TFC)
M. lepida Webb & Berthel. subsp. *lepida*	La Gomera, Mirador de los Roques, 28°06′33.5″ N; 17°12′51″ W		Puppo 577.4 (TFC)
M. pineolens Svent.	Gran Canaria, 28°03′14.22″ N; 15°41′24.3″ W		Bräuchler 170 (MSB)
M. rivas-martinezii Wildpret	Tenerife, Roque de Juan Bay, Anaga	Puppo 209–211, 213 (TFC)	Puppo 212, 214 (TFC)
M. teneriffae (Poir) Benth. var. *cordifolia* P. Pérez	Tenerife, Roques de Fasnia, 28°13′08.2′′ N; 16°24′55.2′′ W	Puppo 299–300 (TFC)	Puppo 299 (TFC)
M. teneriffae var. *teneriffae*	Tenerife, Güímar, 28°17′40.1″ N; 16°24′10.8″ W	Puppo 151, 156 (TFC)	Puppo 150 (TFC)
M. teneriffae var. *teneriffae*	Tenerife, Bco. Herques, Fasnia-Güímar, 28°14′59.2″ N; 16°26′17.5″ W	Puppo 157, 159 (TFC)	Puppo 157 (TFC)
M. teneriffae var. *teneriffae*	Tenerife, Bco. Eras, Fasnia. 28°14′59.2″ N; 16°27′11.4″ W	Puppo 166, 167 (TFC)	
M. teneriffae var. *teneriffae*	Tenerife, Bco. del Tahodio, Anaga, 28°29′53.5″ N; 16°15′32.1″ W	Puppo 185, 186, 192 (TFC)	Puppo 188 (TFC)
M. tenuis Benth.	Gran Canaria		Meimberg *et al.* (2006)
M. varia Benth. subsp. *rupestris* (Webb & Berthel.) P. Pérez	Lanzarote		Bräuchler *et al.* (2005)
M. varia subsp. *thymoides* (Sol ex Lowe) P. Pérez	Madeira		Meimberg *et al.* (2006)
M. varia subsp. *varia*	Tenerife, San Andrés, Anaga, 28°30′58.3″ N; 16°10′29.1″ W	Puppo 183 (TFC)	Puppo 184 (TFC)
M. varia subsp. *varia*	Tenerife, Bco. del Tahodio, Anaga, 28°30′14.3″ N; 16°15′51.8″ W	Puppo 195 (TFC)	
M. varia subsp. *varia*	Tenerife, Parque rural de Anaga, 28°31′46.6″ N; 16°11′38.8″ W	Puppo 197 (TFC)	
M. varia subsp. *varia*	Tenerife, Afur, Anaga, 28°33′09.4″ N; 16°14′16.0″ W	Puppo 204, 206 (TFC)	

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Table 1 Continued

Species	Locality*	Morphometric analyses	Genetic analyses
M. varia subsp. *varia*	Tenerife, Entre Roque de Juan Bay y Roque de Antequera, Anaga	Puppo 219 (TFC)	
M. varia subsp. *varia*	Tenerife, Entre Bco. de Antequera y Bco. de Hijuana, Anaga	Puppo 225 (TFC)	
M. varia subsp. *varia*	Tenerife, Bco. de Antequera, Anaga	Puppo 231 (TFC)	Puppo 236 (TFC)
M. varia subsp. *varia*	Tenerife, Degollado de Teno alto, Teno, 28°20′31.4″ N; 16°51′41.4″ W	Puppo 246, 248 (TFC)	
M. varia subsp. *varia*	Tenerife, Buenavista, Teno	Puppo 262, 263 (TFC)	
Mentha L. sp.	Portugal. DNA extracted from cultivated material		Curto *et al.* (2012)
Origanum L. sp.	Portugal. DNA extracted from cultivated material		Curto *et al.* (2012)
Total		54	37

*No geographical coordinates are provided for restricted taxa.

SPSS Statistics 19 (IBM, Armonk, NY, USA) to evaluate the morphological variation among samples. The axes extracted were those corresponding to components with eigenvalues over 1, which means that only components presenting a variation of at least one of the original variables are retained (Kaiser criterion).

RESULTS

Phylogenetic analysis

The combined alignment of the eight loci used in this study consisted of 6538 bp and included 1430 polymorphic positions (Appendix S1). The number of polymorphic positions among loci varied from 44 (M.pip.057) to 398 (M.pip.017) and the number of haplotypes per locus varied from 19 (M.pip.057) to 49 (O.oni.007; Appendix S1). Three samples failed to amplify with one or more markers: *M. teneriffae* var. *cordifolia* with markers M.pip.056 and M.pip.002; *Origanum* sp. with M.pip.014 and M.pip.027; and *Mentha* sp. with O.oni.007.

Our phylogenetic analyses show that *Micromeria* is monophyletic and can be subdivided into two major clades: Spain + Morocco; and Balearic Islands + Gran Canaria + Tenerife (Fig. 3a). The position of the samples from the Balearic Islands varied as sister to Gran Canaria in MrBayes or as outgroup to the Gran Canaria + Tenerife group in beast and ML. In all analyses, the samples from Tenerife form a well-supported group (Bayesian posterior probabilities, BPP = 1), which includes the samples from Madeira and El Hierro. The samples from Madeira appear between the earliest diverging lineages from Tenerife and the Anaga group (Group I in Fig. 3b), while the samples from El Hierro are included within the samples from central Tenerife (Group III in Fig. 3b). Likewise, the species from Gran Canaria form a well-supported group (BPP = 1) and include the samples from Lanzarote and La Gomera.

Within the Tenerife group, our phylogenetic analysis shows three early divergent lineages: one *M. teneriffae* var. *teneriffae* sample; a sample of *M. teneriffae* var. *cordifolia* plus *M. varia*; and the sample from Madeira. These lineages are followed by two highly supported clades. The first is composed of the species endemic to Anaga: *M. glomerata*, *M. rivas-martinezii* and the remaining samples of *M. teneriffae* var. *teneriffae* (BPP > 0.97, Group I in Fig. 3b). The second clade is formed of the species that is endemic to Teno – *M. densiflora* (Group II in Fig. 3b), which is sister to a subclade composed of all the central species: *M. hyssopifolia, M. lachnophylla, M. lasiophylla* and *M. varia* (BPP = 1, Group III in Fig. 3b). Even though the species in this subclade are well supported, relationships among them are not and *M. hyssopifolia* and *M. varia* appear polyphyletic in all analyses. The ML tree also shows the two main clades of *Micromeria* in the Canary Islands (Gran Canaria + Lanzarote + La Gomera and Tenerife + Madeira + El Hierro), although relationships within the Tenerife group are not well resolved and most nodes are poorly supported (bootstrap < 50%, Appendix S3). The Neighbor-Net network (Fig. 4) recovered the same relationships depicted in the BI analyses.

Divergence-time estimates

Overall, times of divergence are largely congruent with the geological events in the Canary Islands, especially in Tenerife, although the estimated dates represent the coalescence time of the different haplotypes within each clade (i.e. they indicate the upper limit for the most divergent populations; Fig. 3). Thus, the species could have diverged at a much more recent time and our results must be interpreted carefully. Our analysis suggests that the TMRCA for Gran Canaria and Tenerife would be around 8.4 Ma (± 4.2 Myr). Within Tenerife, the divergence time of the species restricted to Anaga would have occurred around 4.3 Ma (± 2.5 Myr), whereas the divergence of the species from Teno and the

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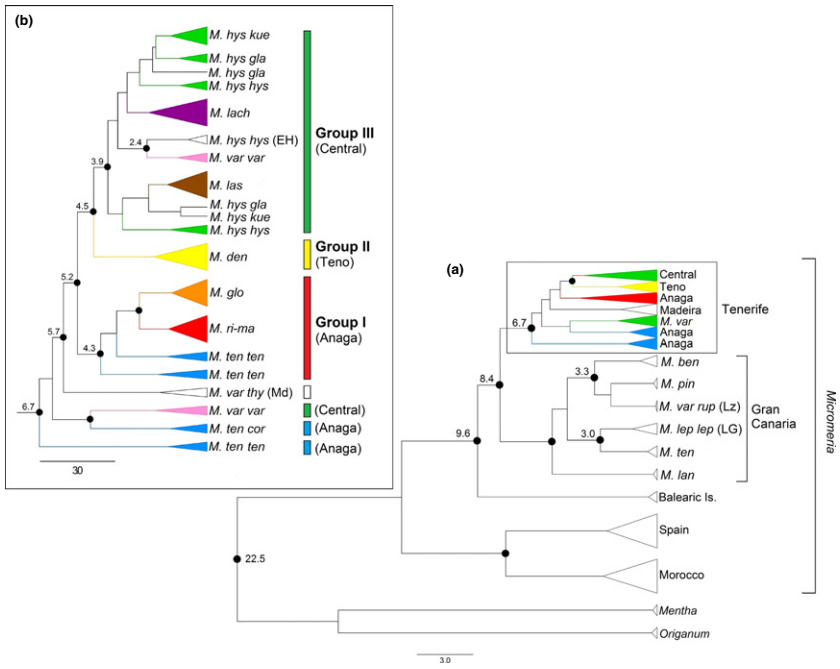

Figure 3 Bayesian phylogeny calculated in BEAST showing relationships among reconstructed haplotypes for (a) all samples of *Micromeria* included in this study, (b) samples only from Tenerife. See Table 1 for full species names. Filled circles on nodes represent Bayesian posterior probabilities, BPP > 0.95 in (a) and BPP > 0.9 in (b), numbers above branches indicate divergence time average in Ma calculated using BEAST, colours used correspond to those in Figs 1, 4 and 5. EH, El Hierro; LG, La Gomera; Lz, Lanzarote; Md, Madeira. The scale bar represents Myr.

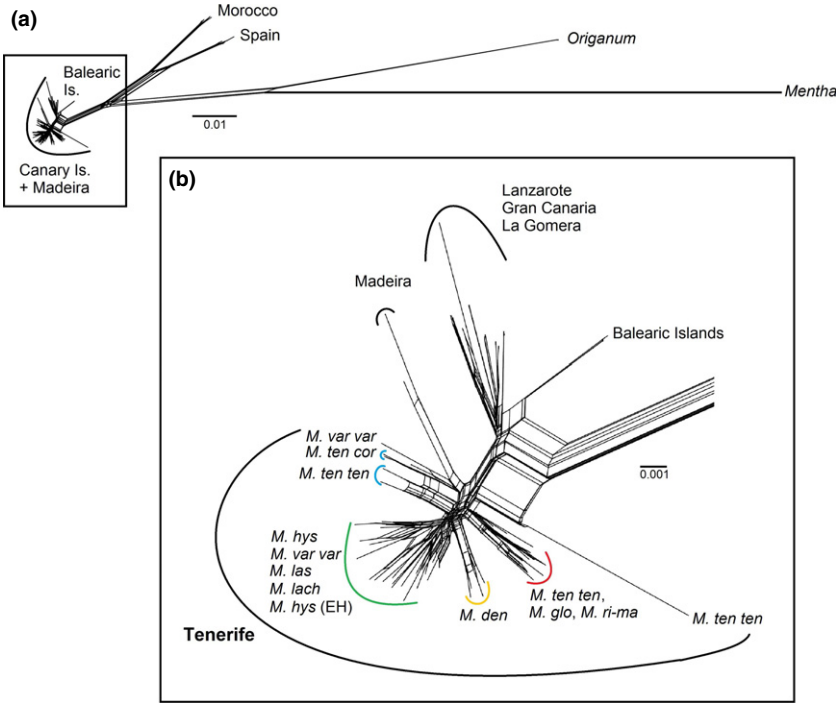

Figure 4 Neighbor-Net network calculated in SPLITSTREE showing relationships among reconstructed haplotypes from (a) all samples of *Micromeria* and outgroups and (b) samples from the Canary Islands and Madeira. Colours used for the different Tenerife groups correspond to those in Fig. 3. See Table 1 for full species names. The bar represents the network scale based on uncorrected patristic distances among haplotypes.

central subclade would have happened *c.* 4.5 Ma (± 2.3 Myr). Finally, diversification of the central subclade would have started around 3.9 Ma (± 2.1 Myr) (Fig. 3b).

Morphometric analyses

The results obtained from the PCA including all specimens and all variables indicate that two main clusters are divided by principal component (PC) 1 (Fig. 5a). The cluster towards the right of the plot is composed of the three palaeo-island species: *M. rivas-martinezii*, *M. glomerata* and *M. densiflora*. The cluster towards the left is composed of the central species and *M. teneriffae*, which slightly segregates from the other two species. These two clusters were also observed when only flower or vegetative characters were analysed separately (not shown). PC1 and PC2 explained 53.91% and 11.66% of the total variation, respectively (Appendix S2). The characters that loaded heavily on PC1

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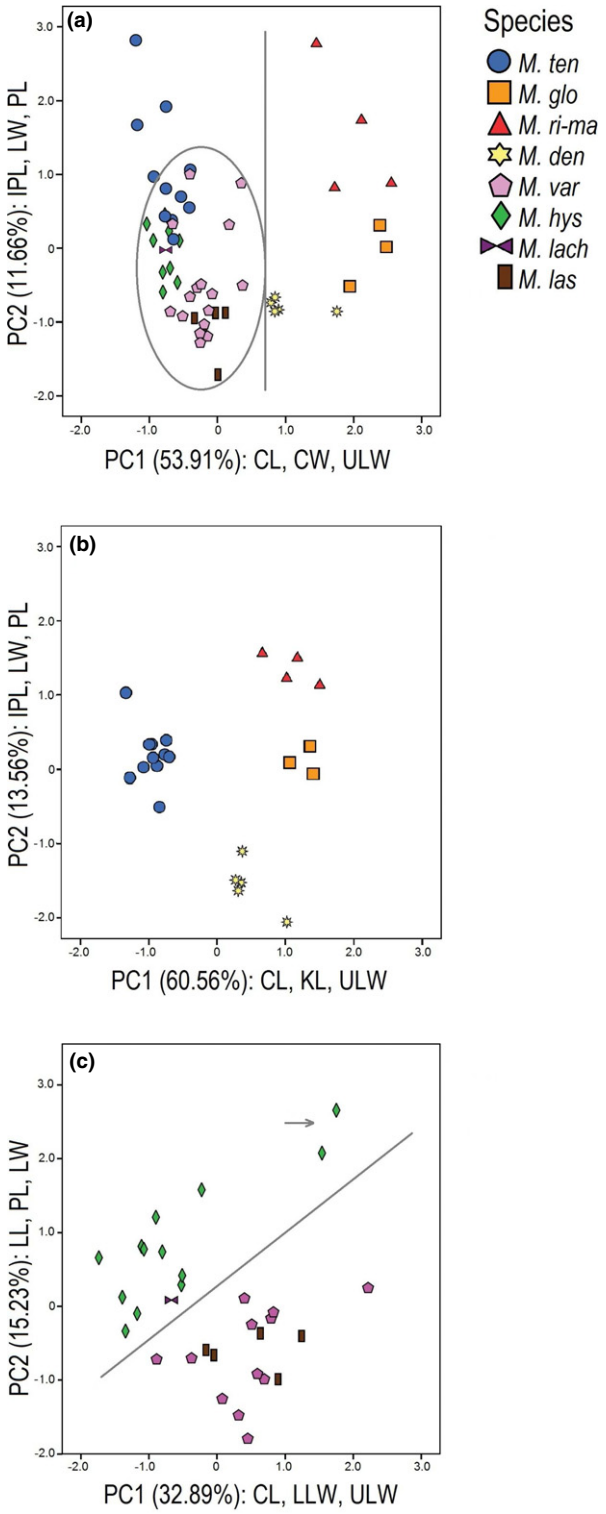

Figure 5 Bivariate scatter plot formed by the two-first components of the principal components analysis (PCA) including: (a) all species of *Micromeria*, (b) species inhabiting only the palaeoislands, and (c) species from the central area only. Each point represents a single specimen; different symbol shapes and colours indicate different species and correspond to those used in Fig. 1. The grey circle in (a) indicates central species, the grey arrow in (c) and the line in (a) and (c) indicate the segregation of samples as discussed in the text. The abbreviations used in the axis labels indicate the characters that loaded heavily on each PC: CL, corolla length; CW, corolla width; IPL, inflorescence peduncle length; KL, calyx length; LL, leaf lamina length; LW, leaf lamina width; LLW, lower lip of the corolla width; PL, leaf petiole length; ULW, upper lip of the corolla width.

relate mainly to flower size: corolla length, width and upper lip of the corolla width, suggesting that flower size was the main character separating the two clusters (Fig. 5a). The characters that mainly contributed to the variation on PC2 were peduncle length, leaf width, and petiole length (Appendix S2).

When only the palaeoisland species (*M. glomerata*, *M. rivas-martinezii*, *M. densiflora* and *M. teneriffae*) were analysed, the samples segregated into four distinct clusters (Fig. 5b) corresponding to each of the species. The first and second PC accounted for 60.56% and 13.56% of the total variation, respectively (Appendix S2). Characters related to flower size loaded heavily on PC1. This suggests that the main difference between *M. teneriffae* and the other species is in flower size (Fig. 5b). The strong influence of leaf width, petiole length, and the peduncle length on PC2 suggests that the three other species are mainly distinguished by vegetative characters (Fig. 5b, Appendix S2).

The analysis of the central species (*M. hyssopifolia*, *M. lachnophylla*, *M. lasiophylla* and *M. varia*) resulted in the segregation of three main clusters (Fig. 5c). One of the clusters is composed of two of the samples identified as *M. hyssopifolia*, the second cluster includes the remaining *M. hyssopifolia* specimens and *M. lachnophylla*. The third cluster is composed of the *M. varia* samples and those identified as *M. lasiophylla*. Principal component 1 accounts for 32.89% and PC2 for 15.23% of the total variation (Appendix S2). Character loadings on PC1 suggest that the principal difference among the second and third cluster is in the corolla. On PC2 the loadings of leaf length, width and petiole length suggest that vegetative characters are responsible for the segregation of the first cluster composed of two of the *M. hyssopifolia* samples as shown in Fig. 5c.

DISCUSSION

Signature of Tenerife's geological events on *Micromeria* diversification

Recent theoretical development in island biogeography postulate that the geological evolution of an island has a high impact on speciation rate and diversity (Whittaker *et al.*, 2007, 2008). According to the general dynamic model of oceanic island biogeography (Whittaker *et al.*, 2007, 2008), speciation rates and species richness should have a direct relationship to the island's life cycle. In young, emerging islands, the immigration rate should be higher. When the

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island starts reaching its maturity – this is, its maximum area and highest elevation – a higher number of habitats are present, allowing the immigrant species to speciate and adapt to the empty niches forming new, endemic species. It is in this phase of the island life cycle where the single-island endemics appear. These species could afterwards be the source of migrants to other younger, nearby islands. This peak in the speciation curve is interrupted, however, by catastrophic events that lead to extinction: volcanic eruptions, landslides, etc. Finally, when the island's area is significantly reduced after a long period of erosion, the speciation rate diminishes and species richness declines, with species persisting within progressively contracting ranges until they go extinct (Whittaker *et al.*, 2007, 2008).

The different islands that compose the Canary archipelago are representatives of the different stages of this island life cycle (Fernández-Palacios *et al.*, 2011). Among these islands, Tenerife has a complex history including the fusion of three islands – Adeje, Anaga and Teno – and secondary isolation between island parts caused by massive landslides and volcanic eruptions. This island constitutes a special case with a complex island life cycle. Whereas the remnants of the palaeoislands are in the last stage of their life cycle, the central part of the island is presumably in its growth stage. This means that, in the older parts of Tenerife the speciation rate might be significantly low and many species could have gone extinct as a consequence of the habitat reduction due to erosion.

The composition of the species of *Micromeria* present in Tenerife reflects the geological evolution of the island. A first diversification event is indicated in the palaeoisland of Anaga, giving rise to early diverging lineages: *M. teneriffae* (*c.* 6.7 Ma) and afterwards to *M. glomerata* and *M. rivas martinezii* (*c.* 2.7 Ma). A second diversification event probably took place in the palaeoisland of Teno and gave rise to *M. densiflora* (*c.* 4.5 Ma). As observed for the Canary Islands in general, it is likely that the diversification of *Micromeria* in Tenerife also followed an east (Anaga) to west (Teno) direction, at least before the central area arose. Interestingly, the diversification of *Micromeria* in the palaeoisland of Anaga appears older than in Teno, as depicted by their position in the phylogeny. Times of emergence of Teno and Anaga have been calculated to be around 7 and 6 Ma, respectively (Ancochea *et al.*, 1990), and the fusion of the three palaeoislands presumably started around 3.5 Ma (see Fernández-Palacios *et al.*, 2011), which coincides roughly with the TMRCA of the central subclade. In this context, it could also be possible that some *Micromeria* species inhabited the third palaeoisland, Adeje, but went extinct during the erosion processes that reduced this palaeoisland to its current extent. A disjointed distribution of species in the three palaeoislands has also been observed in other plant groups such as *Convolvulus* L. (Convolvulaceae; Trusty *et al.*, 2005), *Pericallis* D. Don (Asteraceae; van Hengstum *et al.*, 2012) and in the beetle genus *Pimelia* (Juan *et al.*, 1996), making this inference plausible. Finally, a third diversification event would have given origin to the group of species inhabiting the central area of the island: *M. hyssopifolia*, *M. lachnophylla*, *M. lasiophylla* and *M. varia* (*c.* 3.9 Ma). Our phylogeny suggests that central Tenerife might have been colonized from Teno rather than from another island, given that *M. densiflora* is the sister taxon to the central species.

According to the phylogeny, the palaeoisland species consist of the three narrowly distributed species – *M. glomerata*, *M. rivas-martinezii* and *M. densiflora* – and one widely distributed species – *M. teneriffae*. The samples assigned to *M. teneriffae* are indicated as early diverging lineages within the Tenerife group but do not form a monophyletic group. Instead, some of the samples of var. *teneriffae* group with *M. glomerata* and *M. rivas-martinezii* (Group I; Fig. 3b). This resolution supports the hypothesis that *M. teneriffae* originated on Anaga and subsequently extended its range. The narrow-endemics, on the other hand, are presumably persisting today within contracted ranges in the area of the palaeoislands as remnant populations of a formerly wider range. Morphologically, all four species from the palaeoislands seem more diverse than the central species group. Not only are the species to a higher extent morphologically differentiated from each other, they also show a broader range of variation within the morphometric space. For example, leaf and corolla lengths were in the range *c.* 5–15 and 3.6–14 mm, respectively, for the palaeoisland species, and 4–11 and 3.6–7.5 mm, respectively, for the central species. These morphological characteristics seem to be correlated with the age and geological nature of their habitat (basaltic or salic rocks), which is much older than those habitats found in the central part of the island.

The central-species group consists of *M. hyssopifolia, M. lachnophylla, M. lasiophylla* and *M. varia*. *Micromeria varia* is found on the humid northern slopes of Tenerife, almost continually from Teno to Anaga. It is replaced in the centre and south by the remaining three species, *M. hyssopifolia*, *M. lachnophylla* and *M. lasiophylla*. The species with comparably small ranges, *M. lasiophylla* and *M. lachnophylla*, occur at high elevations and are likely to be derived from the low- and middle-elevation representatives. Morphometric analysis differentiated specimens assigned to *M. hyssopifolia* from those assigned to *M. varia*. The specimens from *M. lasiophylla* appeared nested within the *M. varia* group and the individual included from *M. lachnophylla* fell within the *M. hyssopifolia* cluster. These results corroborate what has already been observed in the field, that *M. lachnophylla* is more similar to *M. hyssopifolia* (they both have white corollas and are almost impossible to differentiate when growing in sympatry) and that *M. lasiophylla* is more similar to *M. varia* (e.g. they both have pink corollas). Also, the PCA showed that two samples of *M. hyssopifolia* segregate from the rest (indicated by an arrow in Fig. 5c). These samples have been described as var. *glabrescens* by Pérez de Paz (1978). The phylogenetic analysis on the other hand is not conclusive and the relations among the different taxa within the central subclade are poorly supported possibly because of

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introgression or incomplete lineage sorting. It could also be that these four species are in fact one species presenting a high degree of phenotypic plasticity, although a more detailed molecular analysis, possibly at the population level, would be needed to further elucidate relationships within this species group.

Even though the general dynamic model of oceanic island biogeography aims to explain global biodiversity patterns of oceanic islands, the species of *Micromeria* in Tenerife provide an interesting example of how an island life cycle and the secondary connection of old and new areas might play major roles in species diversification on oceanic islands in individual evolutionary lineages. *Micromeria varia*, *M. hyssopifolia* and the high-elevation endemics *M. lachnophylla* and *M. lasiophylla* might be examples of the formation of new species by adaptation to recently formed ecological niches in the central part of Tenerife and an increase in the levels of diversity would be expected with time. In the palaeoislands, where erosion already resulted in a decrease of number and area of ecological niches, *M. glomerata, M. rivas-martinezii* and *M. densiflora* can be regarded as persisting within contracted ranges, which are likely to become extinct in time with proceeding erosion. On the other hand, the secondary contact of the old and young areas of Tenerife might have provided new opportunities for the palaeoisland species for shifting their original range, allowing them to maintain extended range sizes despite the decrease of their original area by erosion. *Micromeria teneriffae* might be one such case.

Polyphyly of some species and taxonomic implications

In our phylogeny, three species appear as polyphyletic: *M. varia*, *M. teneriffae* and *M. hyssopifolia*. *Micromeria varia* is present in all of the Canary Islands (except La Palma) and Madeira. A different subspecies has been described on each island (Pérez de Paz, 1978), indicating the morphological differentiation present among islands. In their analysis for *Micromeria* in the Canary Islands, Meimberg *et al.* (2006) showed that *M. varia* might be polyphyletic because the species from each island are each other's closest relatives, suggesting a single colonization event on each island (except for La Gomera, which seems to have been colonized twice). This same pattern is observed in our phylogeny where the samples of *M. varia* from different islands do not cluster together. This might suggest that *M. varia* is composed of a group of morphologically similar forms or species that have adapted to similar ecological conditions in different islands. In Tenerife, the lack of monophyly of *M. varia* could be explained by introgression events because (1) the *M. varia* samples were collected in Anaga, and (2) there is morphological evidence that *M. varia* hybridizes with *M. teneriffae* and the other species inhabiting the palaeoislands (see Pérez de Paz, 1978).

The second species that seems to lack monophyly in our phylogeny is *M. teneriffae*. Two of the samples of this species appear as early divergent lineages in the Tenerife group (one from var. *teneriffae* and the other from var. *cordifolia*), while the other samples of var. *teneriffae* are members of Group I. Samples of *M. teneriffae* form a cohesive cluster in our morphometric study and subdivision of this species is not suggested. Introgression with *M. varia* from Anaga as well as the inclusion of the sample from Madeira might be causing the lack of monophyly of this species in our analyses.

In the central subclade, samples of *M. hyssopifolia* are not clustered together but appeared mixed among the other species. The segregation of these samples seems stochastic because they are not grouping according to variety or collection locality. However, the lack of monophyly in this group should be taken cautiously because of the low support among the samples within the central subclade (BPP < 0.9). As with *M. varia*, a population-level molecular analysis will allow a better understanding of *M. hyssopifolia* species boundaries.

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REFERENCES

Ancochea, E., Fuster, J.M., Ibarrola, E., Cendredo, A., Coello, J., Hernan, F., Cantagrel, J.M. & Jamond, C. (1990) Volcanic evolution of the island of Tenerife (Canary Islands) in the light of new K-Ar data. *Journal of Volcanology and Geothermal Research*, **44**, 231–249.

Bräuchler, C., Meimberg, H., Abele, T. & Heubl, G. (2005) Polyphyly of the genus *Micromeria* (Lamiaceae) – evidence from cpDNA sequence data. *Taxon*, **54**, 639–650.

Brochman, C. (1984) Hybridization and distribution of *Argyranthemum coronopifolium* (Asteraceae–Arthemideae) in the Canary Islands. *Nordic Journal of Botany*, **4**, 729–736.

Bryant, D. & Moulton, V. (2004) Neighbor-Net: an agglomerative method for the construction of phylogenetic networks. *Molecular Biology and Evolution*, **21**, 255–265.

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Carracedo, J.C. (1994) The Canary Islands: an example of structural control on the growth of large oceanic-island volcanoes. *Journal of Volcanology and Geothermal Research*, **60**, 225–241.

Crawford, D.J., Witkus, R. & Stuessy, T.F. (1987) Plant evolution and speciation on oceanic islands. *Differentiation patterns in higher plants* (ed. by K.M. Urbanska), pp. 183–199. Academic Press, London.

Curto, M., Puppo, P., Ferreira, D., Nogueira, M. & Meimberg, H. (2012) Development of phylogenetic markers from single-copy nuclear genes for multi-locus, species level analyses in the mint family (Lamiaceae). *Molecular Phylogenetics and Evolution*, **63**, 758–767.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**, 772.

Drew, B.T. & Sytsma, K.J. (2012) Phylogenetics, biogeography, and stamina evolution in the tribe Mentheae (Lamiaceae). *American Journal of Botany*, **99**, 933–953.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Drummond, A.J., Suchard, M.A., Dong, X. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUTi and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Fernández-Palacios, J.M., de Nascimento, L., Otto, R., Delgado, J.D., García-del-Rey, E., Arévalo, J.R. & Whittaker, R.J. (2011) A reconstruction of Palaeo-Macaronesia, with particular reference to the long-term biogeography of the Atlantic island laurel forests. *Journal of Biogeography*, **38**, 226–246.

Francisco-Ortega, J., Jansen, R.K. & Santos-Guerra, A. (1996) Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. *Proceedings of the National Academy of Sciences USA*, **93**, 4085–4090.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, **59**, 307–321.

van Hengstum, T., Lachmuth, S., Oostermeijer, J.G.B., den Nijs, H. & (J.) C.M., Meirmans, P.G. & van Tienderen, P.H., (2012) Human-induced hybridization among congeneric endemic plants on Tenerife, Canary Islands. *Plant Systematics and Evolution*, **298**, 1119–1131.

Huson, D.H. & Bryant, D. (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, **23**, 254–267.

Juan, C., Ibrahim, K.M., Oromí, P. & Hewitt, G.M. (1996) Mitochondrial DNA sequence variation and phylogeography of *Pimelia* darkling beetles on the island of Tenerife (Canary Islands). *Heredity*, **77**, 589–598.

Juan, C., Emerson, B.C., Oromí, P. & Hewitt, G.M. (2000) Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology and Evolution*, **15**, 104–109.

Kimura, M. & Weiss, G.H. (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561–576.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

MacArthur, R.H. & Wilson, E.O. (1967) *The theory of island biogeography*. Princeton University Press, Princeton, NJ.

Meimberg, H., Abele, T., Bräuchler, C., McKay, J.K., Pérez de Paz, P.L. & Heubl, G. (2006) Molecular evidence for adaptive radiation of *Micromeria* Benth. (Lamiaceae) on the Canary Islands as inferred from chloroplast and nuclear DNA sequences and ISSR fingerprint data. *Molecular Phylogenetics and Evolution*, **41**, 566–578.

Pérez de Paz, P.L. (1978) Revisión del género *Micromeria* Bentham (Lamiaceae–Stachyoideae) en la Región Macaronésica. *Instituto de Estudios Canarios, Monografías*, **16**, 1–306.

Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Santos-Guerra, A., Acevedo-Rodríguez, A. & Reyes-Betancort, J.A. (2011) Redescubrimiento del endemismo tinerfeño *Micromeria densiflora* Benth. (Labiatae). *Anales del Jardín Botánico de Madrid*, **68**, 155–159.

Sgrillo, R. (2012) *GE-Path v.1.4.6*. Available at: http://www.sgrillo.net/googleearth/gepath.htm (accessed 15 March 2012).

Silvertown, J. (2004) The ghost of competition past in the phylogeny of island endemic plants. *Journal of Ecology*, **92**, 168–173.

Stephens, M., Smith, N. & Donnelly, P. (2001) A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, **68**, 978–989.

Trusty, J., Olmstead, R.G., Santos-Guerra, A., Sá-Fontinha, S. & Francisco-Ortega, J. (2005) Molecular phylogenetics of the Macaronesian-endemic genus *Bystropogon* (Lamiaceae): palaeoislands, ecological shifts and interisland colonizations. *Molecular Ecology*, **14**, 1177–1189.

Watts, A.B. & Masson, D.G. (1995) A giant landslide on the north flank of Tenerife, Canary Islands. *Journal of Geophysical Research*, **100**, 24487–24498.

Weigelt, P., Jetz, W. & Kreft, H. (2013) Bioclimatic and physical characterization of the world's islands. *Proceedings of the Natural Academy of Sciences of Philadelphia*, **110**, 15307–15312.

Whittaker, R.J., Ladle, R.J., Araújo, M.B., Fernández-Palacios, J.M., Delgado, J.D. & Arévalo, J.R. (2007) The island immaturity – speciation pulse model of island evolution: an alternative to the "diversity begets diversity" model. *Ecography*, **30**, 321–327.

Whittaker, R.J., Triantis, K.A. & Ladle, R.J. (2008) A general dynamic theory of oceanic island biogeography. *Journal of Biogeography*, **35**, 977–994.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Markers used in the present study and GenBank accession numbers.
Appendix S2 Principal components analyses.
Appendix S3 Maximum likelihood phylogeny.

BIOSKETCH

Pamela Puppo is mainly interested in plant speciation patterns. After obtaining her MSc at the University of St. Louis Missouri and the Missouri Botanical Garden, she is currently working on her PhD at CIBIO, University of Porto, investigating the influence of ecology and geology as drivers of the diversification in *Micromeria* within the Canary Islands. She also studies the taxonomy and systematics of the Andean genus *Calceolaria* (Calceolariaceae).

Author contributions: H.M. and P.P. developed the research questions; H.M. obtained funding for fieldwork and laboratory data collection; P.P. and P.L.P.P. collected most samples; P.P. and M.C. performed the molecular analyses in the laboratory under H.M.'s supervision; P.P. carried out the morphometric analyses; P.P., H.M., M.C. and G.V.-A. analysed the data; and P.P. led the writing of the paper with important contributions from all authors.

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