Diversification patterns of *Micromeria* Benth. (Lamiaceae) in the Canary Islands: Genetic and morphological implications of inter-island colonization

Pamela Puppo
Doutoramento em Biodiversidade, Genética e Evolução
Departamento de Biologia
2015

Orientador
Harald Meimberg, Professor, University of Natural Resources and Life Sciences

Nota prévia

Na elaboração desta dissertação, e nos termos do número 2 do Artigo 4º do Regulamento Geral dos Terceiros Ciclos de Estudos da Universidade do Porto e do Artigo 31º do D.L. 74/2006, de 24 de Março, com a nova redação introduzida pelo D.L. 230/2009, de 14 de Setembro, foi efetuado o aproveitamento total de um conjunto coerente de trabalhos de investigação já publicados ou submetidos para publicação em revistas internacionais indexadas e com arbitragem científica, os quais integram alguns dos capítulos da presente tese. Tendo em conta que os referidos trabalhos foram realizados com a colaboração de outros autores, o candidato esclarece que, em todos eles, participou ativamente na sua conceção, na obtenção, análise e discussão de resultados, bem como na elaboração da sua forma publicada.

This research was supported by Programa Operacional Potencial Humano - Quadro de Referência Estratégico National funds from the European Social fund and the Portuguese Ministério de Educação e Ciência through a PhD fellowship (Fundação para a Ciência e a Tecnologia, FCT - SFRH/BD/74747/2010).

This work was developed at:

This thesis should be cited as:

Puppo, P. 2015. Diversification patterns of *Micromeria* Benth. (Lamiaceae) in the Canary Islands: genetic and morphological implications of inter-island colonization. PhD Thesis, University of Porto, Porto, Portugal.

Acknowledgements

First I would like to thank my advisor Harald Meimberg (“Harry”), for his patience during this long learning process. I know it has been an adaptive process for both of us and not always easy, but I want to thank you especially for your support in the difficult moments and for helping me grow as a person and as a researcher. Danke schön!

Secondly, I want to thank Professor Pedro Luis Pérez de Paz from the University of La Laguna (ULL), Tenerife. He provided invaluable help during field work and gave helpful insights throughout the entire project. Le agradezco especialmente su continuo ánimo y consejo, por compartir su conocimiento de *Micromeria* conmigo y, sobre todo, por su amistad. Ha sido una parte esencial de este proyecto, ¡muchas gracias!

FCT made this research possible awarding me a Ph.D. fellowship. Thank you! In this sense, I also want to thank CIBIO and CTM for hosting me these years.

Other persons helped during fieldwork in the islands as well: Francisco Faure, Vicente Lucía, Alfredo Reyes Betancort, and Victor Garzón-Machado. The Department of Botany of ULL made a jeep available for the excursions.

John McAdam from Ervital provided samples from different Lamiaceae genera grown in their greenhouses at El Mezio of Castro Daire, Portugal (http://www.ervital.pt/) that were used for the initial primer screening. Christian Bräuchler (LMU, Germany) provided plant material (dried or DNA) from Baleares and different Canary Islands. Günther Heubl (LMU, Germany) also kindly provided samples from Madeira and Lanzarote. Pedro Luis Pérez de Paz also sent samples from the islands, especially La Palma and El Hierro.

I would like to thank CIBIO and CTM staff, especially Sara João, Bruno Carvalho, Soraia Barbosa, Joana Paupério, Sara Ferreira, and Sandra Rodrigues. Manuel Curto (PhD student) is another keystone of this project. Obrigado Manel pelo teu árduo trabalho, a tua paciência, e amizade. Marcos Nogueiras (PhD student) was also a close-collaborator for this project, Jaqueline Cochofel (M.Sc student) did most of the Gran Canaria samples for the phylogeny. Katya Mamonova and Joana Gusmão Guedes worked as technicians for our group at different stages and helped amplifying some markers for the phylogeny and microsatellites. Guillermo Velo-Antón provided invaluable help with the BEAST analyses and insightful comments on some manuscripts. Cristina García helped me with the SSR analyses. Miguel Faria and Marc Stift were post-docs of the group at different times but I want to acknowledge you both for your support and friendship. Israel Pérez-Vargas (ULL) helped me revise the Spanish Abstract.

Last but (definitively!) not least, I want to thank my husband Francisco, the best field assistant ever! I thank you especially for your constant support, for giving me that hand that I needed to keep walking (not only in the field), for lending me your shoulder in my moments of stress. And to the rest of my family, for being always there cheering me up when I mostly needed it and for being always proud of me even for the most irrelevant things.

Resumo

Fazendo uso de abordagens filogenéticas, da genética populacional e morfométrica, esta tese tem como objetivo contribuir para o conhecimento dos processos de diversificação das espécies do género *Micromeria* Benth. (Lamiaceae) nas Ilhas Canárias. Em primeiro lugar, a história filogenética de *Micromeria* em Tenerife foi investigada para tentar entender o grau de influência que teve a história geológica da ilha na diversificação das suas espécies. Além disso, um conjunto de marcadores microsatélites foram desenvolvidos e analisados com algumas das espécies de Tenerife para avaliar a variabilidade genética destas espécies. A história filogenética de *Micromeria* nas Ilhas Canárias foi, também, inferida para tentar entender o processo de colonização deste arquipélago e para verificar se todas as espécies atualmente reconhecidas estão em consonância com os dados moleculares. Finalmente faz-se uma revisão de várias espécies com o objetivo de reconciliar a sua taxonomia com a evidência molecular gerada no presente estudo.

Os principais resultados obtidos na presente tese revelam que a diversificação de *Micromeria* em Tenerife está de fato relacionada com a história geológica da ilha. *Micromeria* chegou provavelmente pela primeira vez à paleoilha de Anaga onde diversificou. De seguida colonizou a paleoilha de Teno e, desde aqui, colonizou a parte central, mais jovem, de Tenerife, presumivelmente depois da aparição do vulcão Teide. Análises morfométricos revelam que as espécies restringidas às partes antigas de Tenerife são morfologicamente diferentes entre si e das demais espécies, enquanto que as espécies que habitam na parte jovem da ilha são morfologicamente muito parecidas. Dados obtidos com microsatélites sugerem que as espécies restringidas às partes antigas da ilha apresentam uma menor diversidade genética quando comparadas com uma das espécies centrais. Em geral, as espécies de *Micromeria* em Tenerife constituem um exemplo interessante de como a evolução geológica de uma ilha não só afeta a diversificação dos seus *taxa*, como também contribui ao seu elevado endemismo.

Nas Ilhas Canárias, as análises sugerem que *Micromeria* teve dois centros de diversificação: um nas ilhas orientais de Gran Canaria e Lanzarote e outro nas ilhas ocidentais de Tenerife, El Hierro e La Palma. As seguintes direções de colonização entre ilhas são sugeridas: de Gran Canaria para Lanzarote e para La Gomera; de

Tenerife para La Palma (desde Teno), para El Hierro (desde a parte central) e para La Gomera e Madeira (desde as paleoilhas). La Gomera parece ter sido colonizado várias vezes desde Gran Canaria e Tenerife. Em geral, a colonização entre ilhas parece ter desempenhado um papel relevante no processo de diversificação de *Micromeria* no arquipélago Canário.

A revisão taxonómica das espécies teve como resultado a proposta para conservar os nomes *M. varia* e *M. hyssopifolia*, nomes publicados de forma inválida mas amplamente usados, além da descrição de duas novas espécies, três novos híbridos e cinco novas combinações. Com esta revisão, o número de espécies de *Micromeria* nas Ilhas Canárias aumenta para 21, em lugar de 16 como tinha sido reconhecido anteriormente, e está de acordo com a nova evidência molecular disponível. Inclui-se também uma chave para identificar todas as espécies presentes no arquipélago.

Resumindo, esta tese apresenta resultados consistentes e apoia a hipótese de que as espécies de *Micromeria* em cada ilha do arquipélago Canário constituem endemismos insulares. Num sentido mais amplo, esta tese contribui também ao conhecimento dos processos que estão por trás da diversificação de espécies de plantas em arquipélagos oceânicos e em como a geologia das ilhas pode afetar tais processos.

Palavras-chave: *Micromeria*, Ilhas Canárias, Macaronésia, Lamiaceae, biogeografia, diversificação, endemismo, ilhas oceânicas, paleoilhas, filogenética, morfométrica, microssatélites, estrutura genética, taxonomia

Abstract

Using phylogenetics, population genetics and morphometric approaches, this thesis aims to understand the diversification process of the species of *Micromeria* Benth. (Lamiaceae) in the Canary Islands. First, the phylogenetic history of *Micromeria* in Tenerife was investigated in order to comprehend the extent of the influence of the geological history of this island in the diversification of its taxa. A set of microsatellite markers was also developed and analyzed with some of the species from Tenerife to assess the genetic variability of these species. The phylogenetic history of *Micromeria* in the Canary Islands was inferred to understand the colonization process of the archipelago and to verify if all currently recognized species are supported by molecular data. Finally, several species were revised in order to reconcile their taxonomy with the molecular evidence generated in the present study.

The main results obtained in the present thesis reveal that the diversification of *Micromeria* in Tenerife is indeed related to the geological history of the island. *Micromeria* probably first arrived in the palaeoisland of Anaga where it diversified, then colonized the palaeoisland of Teno, and from there colonized the central, younger part of Tenerife, presumably after the formation of the Teide volcano. Morphometric analyses reveal that the species restricted to the older parts of Tenerife are morphologically dissimilar among them and from the rest, while the species inhibiting the younger area of the island are morphologically very similar. Microsatellite data suggests that the species restricted to these older parts present lower levels of genetic diversity when compared to a widespread species. Overall, the species of *Micromeria* in Tenerife constitute an interesting example on how the geological evolution of an island not only affects the diversification of its taxa, but probably contributes to the elevated endemism rate as well.

In the Canary Islands, the analyses suggest that *Micromeria* had two centers of diversification, one in the eastern islands of Gran Canaria and Lanzarote, the other in the western islands of Tenerife, El Hierro and La Palma. The following inter island colonization directions are suggested: Gran Canaria to Lanzarote and La Gomera, Tenerife to La Palma (from Teno), to El Hierro (from the central part), and to La Gomera and Madeira archipelago (from the palaeoislands). La Gomera seems to have been colonized several times from Gran Canaria and Tenerife. Overall, inter island colonization seems to have played a major role in the diversification process of *Micromeria* in the Canary Islands.

The taxonomical revision of the species resulted in the proposal for conserving the names *M. varia* and *M. hyssopifolia,* names not validly published but widely used, and the description of two new species, three new hybrids and five new combinations. With the revision of these taxa, the number of species of *Micromeria* in the Canary Islands increases to 21 instead of 16 as previously stated and is in accordance to the new molecular evidence available. A key for identifying all of the species in the archipelago is also provided.

Overall, this thesis presents consistent results and supports the hypothesis that the species of *Micromeria* in each of the Canary Islands are endemic to each island. In a wider sense, this thesis also contributes to the knowledge of the processes behind the diversification of plant taxa in oceanic archipelagos and how geology might affect these processes.

Keywords: *Micromeria*, Canary Islands, Macaronesia, Lamiaceae, biogeography, diversification, endemism, oceanic islands, palaeoisland, phylogenetics, morphometrics, microsatellites, genetic structure, taxonomy

Resumen

Haciendo uso de enfoques filogenéticos, de genética poblacional y morfometría, esta tesis pretende contribuir al conocimiento de los procesos de diversificación de las especies del género *Micromeria* Benth. (Lamiaceae) en las Islas Canarias. En primer lugar, se investigó la historia filogenética de *Micromeria* en Tenerife para tratar de entender el grado de influencia que tuvo la historia geológica de la isla en la diversificación de sus especies. Además, se desarrollaron un conjunto de marcadores microsatélites que fueron analizados con algunas de las especies de Tenerife para evaluar la variabilidad genética de dichas especies. Asimismo, la historia filogenética de *Micromeria* en las Islas Canarias fue inferida para tratar de entender el proceso de colonización de este archipiélago y para verificar si todas las especies actualmente reconocidas están conforme a los datos moleculares. Finalmente, se revisaron varias especies con el objetivo de reconciliar su taxonomía con la evidencia molecular generada en el presente estudio.

Los principales resultados obtenidos revelan que la diversificación de *Micromeria* en Tenerife está de hecho relacionada con la historia geológica de la isla. *Micromeria* probablemente llegó por la primera vez a la paleoisla de Anaga donde diversificó, luego colonizó la paleoisla de Teno y desde ahí progresó hacia la parte central, más joven, de Tenerife, presumiblemente después de la aparición del volcán Teide. Los análisis morfométricos revelan que las especies restringidas a las partes antiguas de Tenerife son morfológicamente diferentes entre sí y de las demás especies, mientras que las especies que habitan la parte joven de la isla son muy parecidas. Los datos obtenidos con microsatélites sugieren que las especies restringidas a las partes antiguas de la isla presentan niveles más bajos de diversidad genética en comparación con una de las especies centrales. En general, las especies de *Micromeria* en Tenerife constituyen un ejemplo interesante de cómo la evolución geológica de una isla no sólo afecta la diversificación de sus taxones, sino también de cómo contribuye al elevado grado de endemismo de sus especies.

En las Islas Canarias, los análisis sugieren que *Micromeria* tuvo dos centros de diversificación: uno en las islas orientales de Gran Canaria y Lanzarote, y otro en las islas occidentales de Tenerife, El Hierro y La Palma. Se sugieren las siguientes direcciones de colonización entre islas: de Gran Canaria hacia Lanzarote y hacia La

Gomera, de Tenerife hacia La Palma (desde Teno), hacia El Hierro (desde la parte central) y hacia La Gomera y Madeira (desde las paleoislas). La Gomera parece haber sido colonizada varias veces desde Gran Canaria y Tenerife. En general, la colonización entre islas parece haber desempeñado un papel relevante en el proceso de diversificación de *Micromeria* en el archipiélago Canario.

La revisión taxonómica de las especies tuvo como resultado la propuesta para conservar los nombres *M. varia* y *M. hyssopifolia*, nombres publicados de forma inválida pero ampliamente usados, además de la descripción de dos nuevas especies, tres nuevos híbridos y cinco nuevas combinaciones. Con esta revisión, el número de especies de *Micromeria* en las Islas Canarias aumenta a 21 en vez de 16 como había sido reconocido anteriormente y está de acuerdo con la nueva evidencia molecular disponible. Se incluye también una clave para identificar todas las especies en el archipiélago.

En resumen, esta tesis presenta resultados consistentes y apoya la hipótesis de que las especies de *Micromeria* en cada una de las islas del Archipiélago Canario constituyen endemismos insulares. En un sentido más amplio, este trabajo contribuye asimismo al conocimiento de los procesos que están por detrás de la diversificación de especies de plantas en archipiélagos oceánicos y a cómo la geología de las islas puede afectar dichos procesos.

Palabras clave: *Micromeria*, Islas Canarias, Macaronesia, Lamiaceae, biogeografía, diversificación, endemismo, islas oceánicas, paleoislas, filogenética, morfometría, microsatélites, estructura genética, taxonomía

Table of contents

List of Tables

CHAPTER 2

Paper I

Paper II

CHAPTER 3

Paper III

CHAPTER 4

Paper V

List of Figures

CHAPTER 1

CHAPTER 2

Paper I

CHAPTER 3
Paper III

CHAPTER 4

Paper V

CHAPTER 5

Chapter 1:

General Introduction

1.1 Oceanic Islands

Volcanic islands behave more or less like biological individuals in the sense that they are born, grow, attain their highest area and altitude, and are reduced once again below the sea level by erosion and subsidence (Fig. 1) (Fernández-Palacios et al. 2011). Islands are also shaped by catastrophic events such as further volcanism, massive landslides, caldera collapses, etc (Whittaker et al. 2007, 2008; Fernández-Palacios et al. 2011). Six phases have been identified in a volcanic island life cycle (Fernández-Palacios and Whittaker 2010): origin and submarine construction, emergence and subaerial construction, erosion and dismantling, basal plain (or atoll in some cases), disappearance, and flat submarine mount.

The theory of island biogeography (MacArthur and Wilson 1967) states that, the species in an island must be in function of the species that are already there plus those that originated from immigration or speciation minus those that went extinct. This theory is known as the dynamic equilibrium model and proposes that immigration, speciation and extinction vary predictably with time and depend on isolation and island area. Emerson and Kolm (2005) postulated the “diversity begets diversity” model stating that high species richness can also be responsible for generating high speciation rates by genetic drift, competition, and a complex structure in the species communities. Whittaker et al. (2007, 2008) developed the general dynamic model of island biogeography that correlate immigration, speciation and extinction to the life cycle of an island. In young, emerging islands the immigration rate is higher. When the island attains its maximum area and elevation (and thus a higher number of habitats are available), the immigrant species adapt and speciate so the speciation rate is higher. This peak in the speciation rate is interrupted by catastrophic events such as landslides, caldera collapses, volcanic eruptions, etc. Finally, when the area of the island has been significantly reduced by erosion, species richness declines and the extinction rate increases.

Islands constitute perfect natural laboratories in which to study evolutionary processes. Perhaps the most famous study on islands is that on Galapagos’ finches by Darwin (Darwin 1859) which revolutionized the evolutionary thinking during the 19th and 20th centuries. A number of characteristics make islands ideal systems in which to study evolution: they constitute discrete, isolated geographical entities with limited geneflow among them, their flora and fauna are usually well catalogued, they are geologically active, and they possess a high diversity of habitats (Emerson 2002). This combination of factors has produced unique biota resulting in ca. 70 000 vascular plants endemic to islands which roughly represent one fourth of all known species (Kreft et al. 2008).

Biodiversity on oceanic islands is characterized by groups of closely related taxa that may be highly differentiated morphologically due to the diversity of habitats (Crawford et al. 1987). Adaptive radiation is the mechanism most frequently invoked to explain this diversity, especially since the availability of empty ecological niches triggers the formation of new species more rapidly (Crawford et al. 1987; Gillespie 2009). Adaptive radiation assumes that a particular group on an island or archipelago is monophyletic as a consequence of a single colonization event (Crawford et al. 1987). There are several well-known examples of adaptive radiations on islands: Darwin's finches in the Galapagos Islands, silverswords, *Drosophila* and honeycreepers in Hawaii, *Nesotes* beetles in the Canary Islands, *Anolis* lizards in the Greater Antilles, lemurs in Magagascar, among others (reviewed in Emerson 2002, Gillespie 2009). There are also radiations that are not adaptive and might have originated from disruptions or fragmentations of the habitats due to geological events (Gillespie 2009).

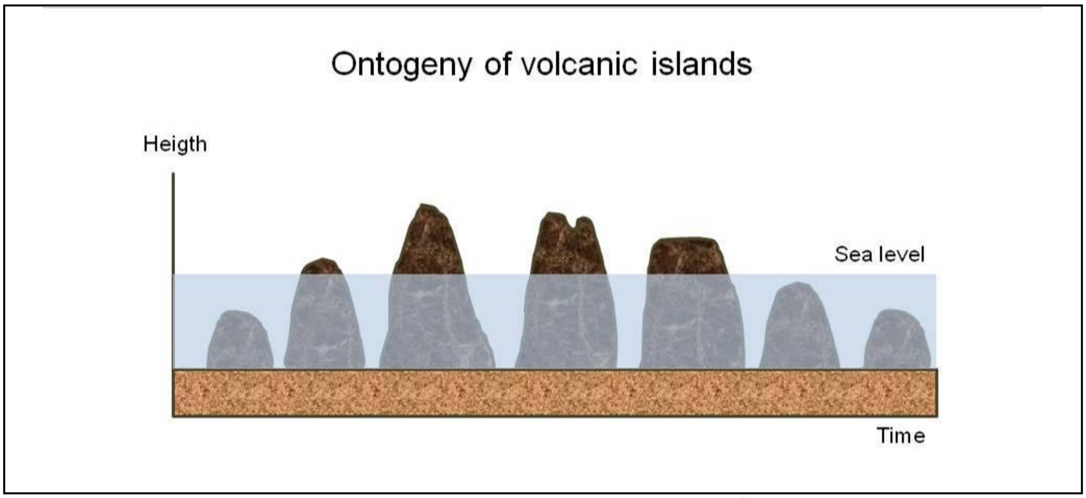


Fig. 1 Different stages in an island life cycle as discussed in the text (sensu Fernández-Palacios et al. 2011).

Diversity on islands can also be the consequence of multiple introductions from the mainland or other nearby islands. Examples of this are yellow warblers in the West Indies, warblers in Madagascar, *Tarentola* geckos in Macaronesia (see Emerson 2002) and numerous plant groups in Macaronesia (reviewed in Silvertown 2004). Examples for multiple introductions have increased in recent years though it seems that single introductions are overall more frequent, especially in isolated islands or archipelagos where colonization events are rare. And at least for Macaronesia flora, it seems that genera originating from a single colonization event are seven times more species-rich than genera introduced multiple times (Silvertown 2004; see Appendix at the end of chapter 5 for a list of genera).

1.2 The Canary Islands

The Canary Islands are one of the five volcanic archipelagos that compose the Macaronesian region together with the Azores, Madeira, Selvagens and Cape Verde (Fig. 2). Unlike most volcanic archipelagos, Macaronesia is located in close proximity to the continent, between 96 and 1500 km off the Iberian Peninsula and North Africa in the Atlantic Ocean (Francisco-Ortega et al. 1996; Fernández-Palacios et al. 2011). The age of the oldest current island (Selvagem Grande) in the Region is 27 Ma, though Macaronesia itself might be 60 Ma (Fernández-Palacios et al. 2011). During the first northern hemisphere glaciations and the desertification of northern Africa in the late Miocene and Pliocene, Macaronesia probably served as refugia for the massive plant and animal migration so its biota is linked to that of the Mediterranean basin and North Africa (Bramwell 1972; Francisco-Ortega et al. 1996). Regarding its flora, Macaronesia possess quite unique elements counting with ca. 700 endemic species (Francisco-Ortega et al. 1996). These endemics consist of palaeoendemics (taxa that survived in a reduced area) and neoendemics (taxa that probably evolved in the islands) (Bramwell 1972; Fernández-Palacios et al. 2011). The palaeoendemics probably colonized younger islands following a stepping-stone model (Kimura and Weiss 1964) thus avoiding extinction, and later gave origin to the neoendemics which probably evolved in situ (Fernández-Palacios et al. 2011). Some of these palaeoendemics are i.e. *Lavatera phoenicea* (Malvaceae), *Plocama pendula* (Rubiaceae), and *Tolpis* (Asteraceae), but overall, it seems that most of the Macaronesian genera are neoendemics derived from continental ancestors (reviewed in Goodson et al. 2006).

The Canary Islands are distinct from other volcanic archipelagos, i.e. they present a complex volcanic evolution, are older, and are in close proximity to the continent (Juan et al. 2000). The Canary Islands are located ca. 95 km off the coast of western Morocco and are composed of seven islands of different ages, older in the east and younger towards the west, that correspond to the different stages in the island life cycle (Fig. 2). Fuerteventura (20 Ma) and Lanzarote (15.5 Ma) are basal plains, Gran Canaria (14-16 Ma) and La Gomera (10 Ma) are already decreasing their surfaces because of erosion, Tenerife (11.6), La Palma (2 Ma), and El Hierro (1 Ma) are still in a building face as evidenced by their active volcanism (Carracedo 1994; Juan et al. 2000; Fernández-Palacios et al. 2011). Among all the islands that compose this archipelago, Tenerife has by far the most complex geological history. In the late Miocene, three islands: Adeje (11.6-3.5 Ma), Teno (6.7-4.5 Ma), and Anaga (6.5-3.5 Ma) occupied the area of today's Tenerife (Ancochea et al. 1990). During the late Miocene-Pliocene, successive volcanic activity secondarily connected these islands until Tenerife attained its current shape ca. 2 Ma (Ancochea et al. 1990). Tenerife is thus composed of three older areas (palaeoislands) and a younger, central part which includes the Teide volcano. In a similar way, Gran Canaria is also composed of older and younger areas though not by secondary connection but by the different stages of its volcanic activity. "Palaeo-canaria" is the SW half of the island which is composed of Miocene substrates while "neo-canaria" in the NE was revitalized by eruptions during the Pliocene (del Arco et al. 2002). Lanzarote and Fuerteventura on the contrary, are one island but its middle part submerged by erosion in the late Miocene currently giving the appearance of two different islands (Fernández-Palacios et al. 2011).

The Canary archipelago has a subtropical climate and present several distinct ecological zones that result from the influence of the northeast humid trade winds and the altitudinal gradient produced by the volcanoes (Francisco-Ortega et al. 1996; Juan et al. 2000). The number of ecological zones varies according to the height of the islands but in general the following zones can be identified: coastal desert, lowland vegetation which can be humid in the NE or arid in the SW, the laurel forest in the NE, the pine forest mostly in the SW, and the dry high mountain (Figs. 3, 4) (Francisco-Ortega et al. 1996; Bramwell 1997; Juan et al. 2000). This variety of habitats has produced an outstanding diversity of ca. 1700 plant species from which around 40% are endemic (Bramwell 1972; Carine et al. 2004).

Most of the plant species inhabiting the Canary Islands are related to the Mediterranean basin flora; so far this pattern has been reported for more than 30 genera from 19 families (reviewed in Juan et al 2000; Carine et al. 2004; see Appendix at the end of Chapter 5 for a list of genera). Although not as frequent as the latter case, relation with the African flora has also been found in several groups, i.e. *Ceropegia* (Asclepiadaceae) (Juan et al. 2000), *Sideroxylon* (Sapotaceae) (Juan et al. 2000), *Helichrysum* (Asteraceae) (Galbany-Casals et al. 2009), *Matthiola* (Brassicaceae) (Jaén-Molina et al. 2009); *Solanum* (Solanaceae) (Olmstead and Palmer 1997). Other plant groups however, are related to more remote regions such as Asia: *Apollonias* (Lauraceae), America: *Bystropogon* (Lamiaceae), or Australia: *Picconia* (Oleaceae) (Juan et al. 2000) (See Appendix).

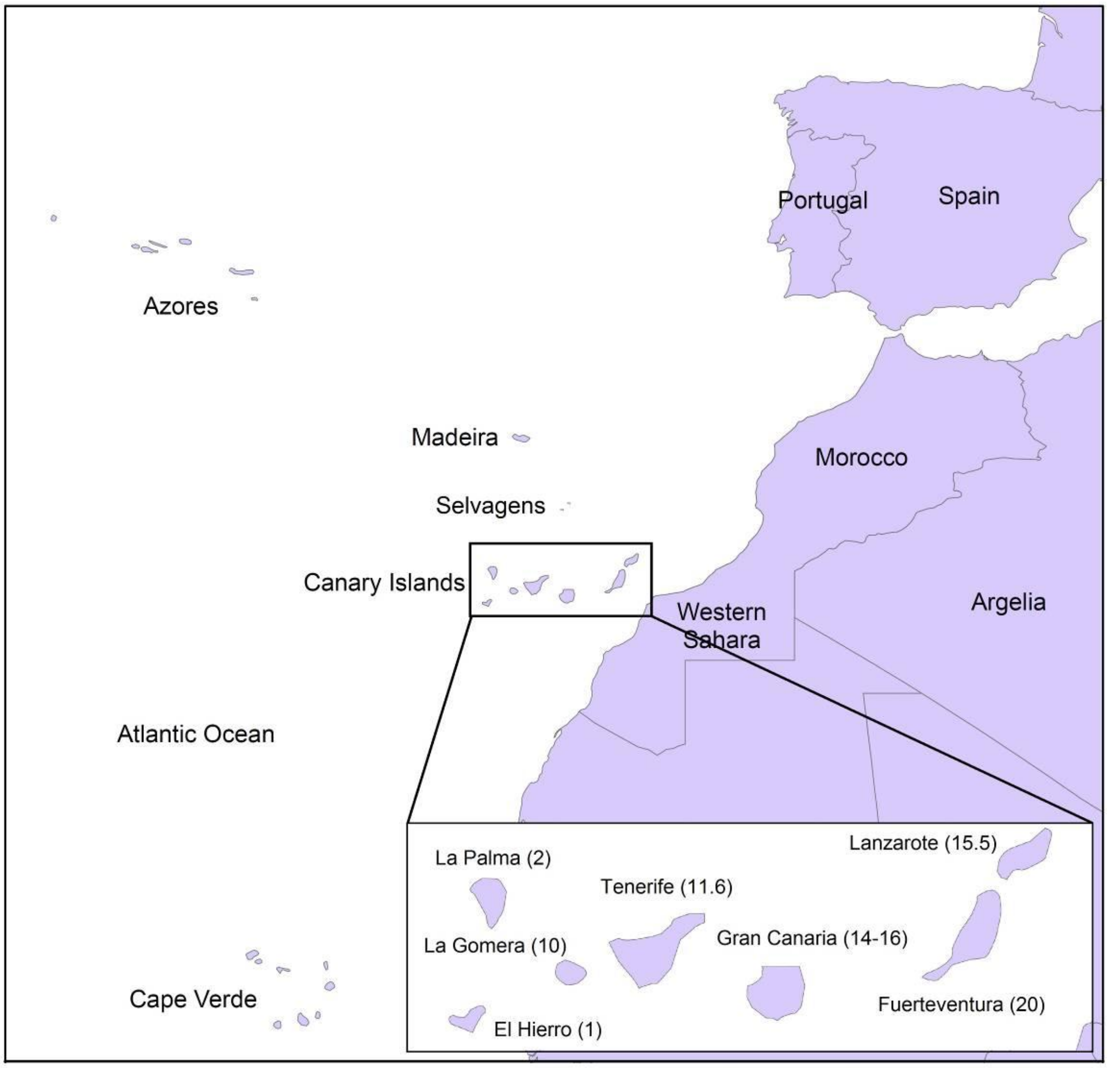


Fig. 2 Map of the five archipelagos that compose the Macaronesian biogeographical region. The Canary Islands (bottom, right) indicating the name and age in Ma of each island (sensu Juan et al. 2000).

Another interesting pattern is that most of the endemic plant genera form a monophyletic group in the Canary Islands suggesting a single colonization event. Examples of this can be found in Fabaceae (Percy and Cronk 2002; Allan et al. 2004), Crassulaceae (Mes et al. 1996; Mort et al. 2002), Lamiaceae (Barber et al. 2000; Trusty et al. 2005), Brassicaceae (Francisco-Ortega et al. 2002), Boraginaceae (Böhle et al. 1996), Gentianaceae (Thiv et al. 1999), and several genera in the Asteraceae (Francisco-Ortega et al. 1996, 2001a, 2001b; Kim et al. 1996; Panero et al. 1999) (See

Appendix). Despite its close proximity to the mainland, multiple colonizations of the Canary Islands have occurred in relatively few genera, i.e. *Helichrysum* (Asteraceae) (Galbany-Casals et al. 2009), *Pulicaria* (Asteraceae) (Francisco-Ortega et al. 2001a), *Matthiola* (Brassicaceae) (Jaén-Molina et al. 2009), *Teline* (Fabaceae) (Percy and Cronk 2002), among others. Probable multiple colonizations in other genera have been discussed as well, i.e. *Artemisia* (Asteraceae), *Campanula* (Campanulaceae), *Daucinae* (Apiaceae), *Dracaena* (Dracaenaceae), *Helianthemum* (Cistaceae), *Hypericum* (Clusiaceae), *Limonium* (Plumbaginaceae), *Salvia* (Lamiaceae), *Senecio* (Asteraceae), *Viola* (Violaceae) (see Carine et al. 2004; Spalik and Downie 2007) (See Appendix).

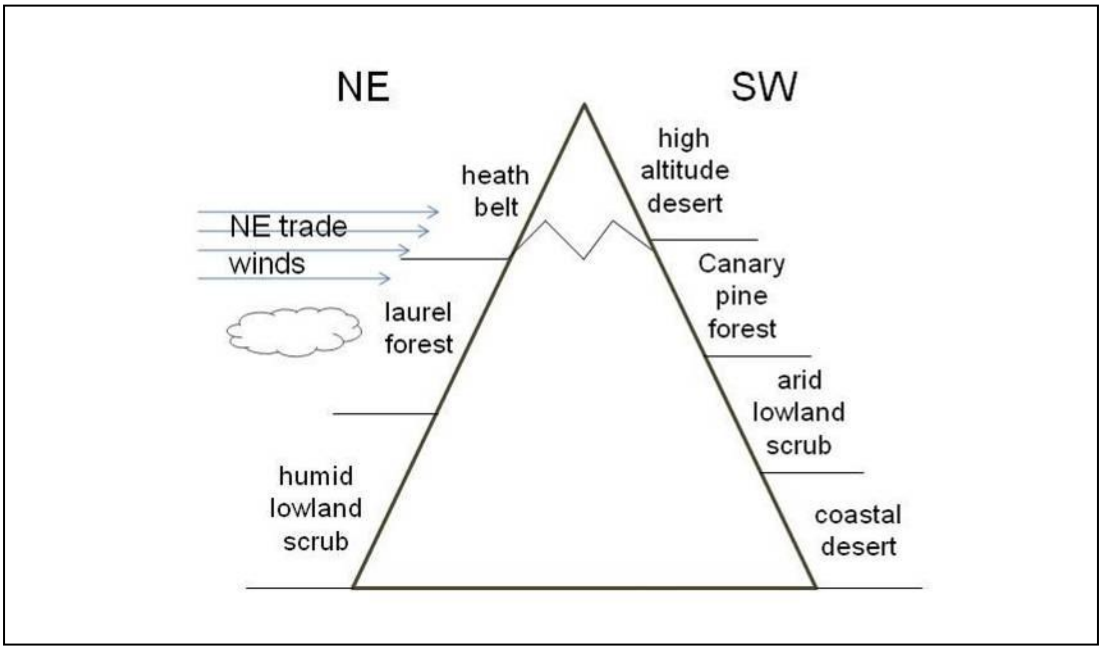


Fig. 3 Vegetational zones in the Canary Islands (sensu Francisco-Ortega et al. 1996) showing the northeast humid trade winds and the cloud layer.

Within the archipelago, diversification patterns predominantly suggest a stepping-stone model of colonization from the older islands towards the younger ones, i.e. from east to west (Juan et al. 2000; Francisco-Ortega et al. 2001a; Allan et al. 2004). Examples of this have been found in *Inulaea* (Asteraceae) (Francisco-Ortega et al. 2001a), *Dendrocrambe* (Brassicaceae) (Francisco-Ortega et al. 2002), and *Lotus* (Fabaceae) (Allan et al. 2004) (See Appendix). This simple pattern however can be tampered by several factors: within island diversification, adaptation, extinction, back colonization, recent colonizations, etc (Juan et al. 2000). For example, radiations from

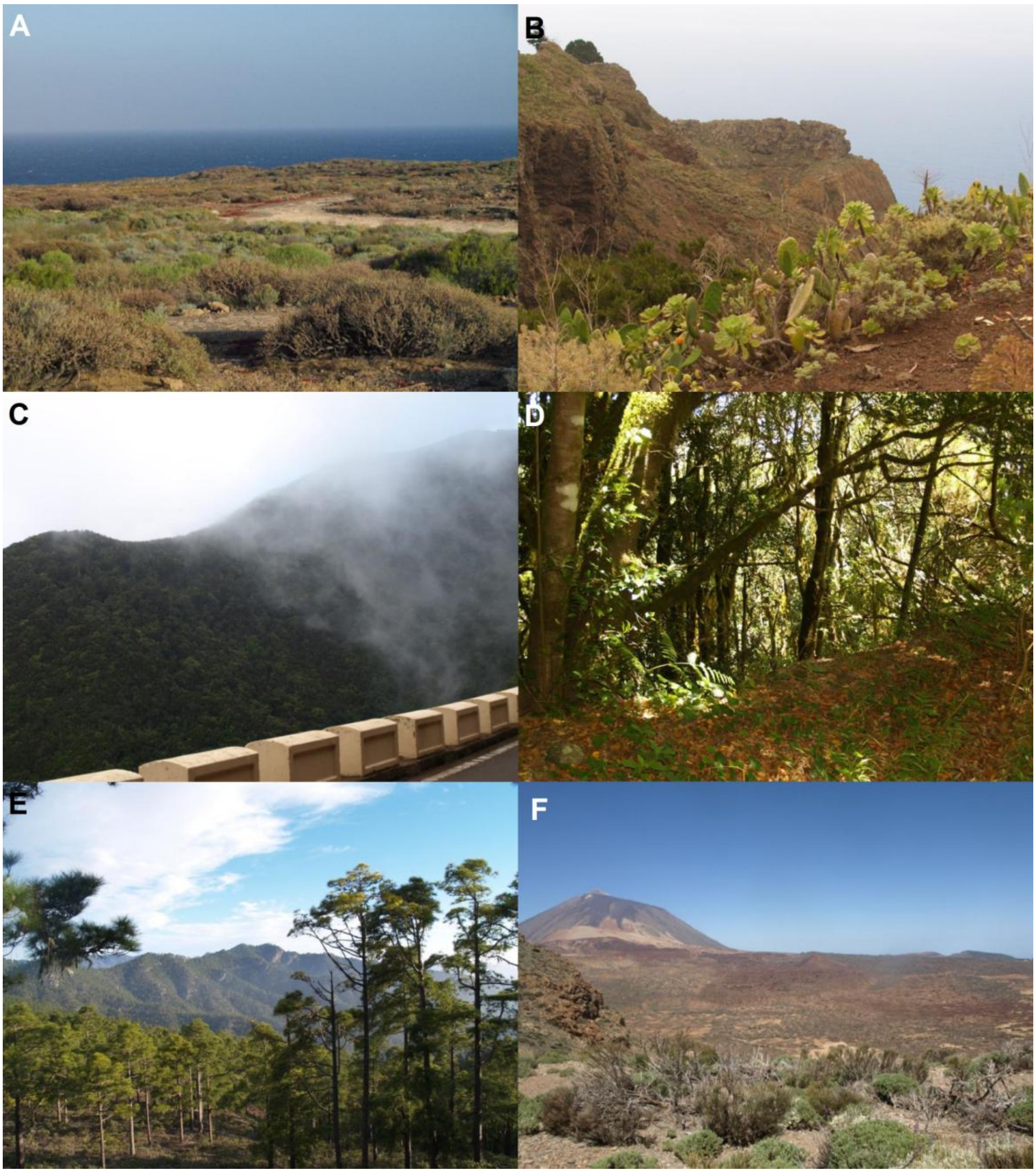


Fig. 4 Different ecological zones: Coastal desert in Los Abades, Tenerife (A); Lowland scrub in Acantilado El Tanque, Teno, Tenerife (B); Laurel forest in Tafur, Anaga, Tenerife (C); Laurel forest in Garajonay National Park, La Gomera (D); Pine forest between Antenara-Tamadaba, Gran Canaria (E); Highland desert in Teide National Park, Tenerife (F). All fotos taken by P. Puppo.

Gran Canaria or Tenerife to the rest of the islands have been observed in *Echium* (Boraginaceae) (Böhle et al. 1996) and *Sonchus* (Asteraceae) (Kim et al. 1996); and the colonization of other nearby archipelagos like Madeira has been reported in *Aeonium* (Crassulaceae) (Kim et al. 2008), *Bystropogon* (Lamiaceae) (Trusty et al. 2005), *Sideritis* (Lamiaceae) (Barber et al. 2000), *Crambe* (Brassicaceae) (Kim et al.

2008), *Dendrocrambe* (Brassicaceae) (Francisco-Ortega et al. 2002), *Echium* (Boraginaceae) (Böhle et al. 1996), *Pericallis* (Asteraceae) (Panero et al. 1999), *Teline* (Fabaceae) (Percy and Cronk 2002) (See Appendix). In some groups, inter-island dispersal among similar ecological zones is the mode of diversification, i.e. *Adenocarpus* (Fabaceae) (Percy and Cronk 2002), *Aeonium* (Crassulaceae) (Mes and t'Hart 1996; Mort et al. 2002), *Crambe* (Brassicaceae) (Francisco-Ortega et al. 2002), *Argyranthemum* (Asteraceae) (Francisco-Ortega et al. 1996), *Pericallis* (Asteraceae) (Panero et al. 1999), *Sonchus* (Asteraceae) (Kim et al. 1996). In other groups, hybridization has been implicated in their evolutionary process in the islands, i.e.: *Aeonium* (Crassulaceae) (Jorgensen and Olesen 2001; Mes and t'Hart 1996), *Echium* (Boraginaceae) (Böhle et al. 1996), *Argyranthemum*, *Sonchus* and *Pericallis* (Asteraceae) (Francisco-Ortega et al. 1996; Kim et al. 1996; Jones et al. 2014, respectively) (See Appendix).

1.3 Genus *Micromeria*

Micromeria Benth. is a genus from the mint family Lamiaceae. It is composed of shrubs, subshrubs or herbs with bisexual, entomophilous flowers and the dispersion of the dry fruit is mainly by wind and sometimes by ants or water. This genus, as currently circumscribed, is characterized by a thickened, entire leave margin caused by a sclerenchymatous vein, and the posterior lip of the corolla is emarginated and curved upwards (Bräuchler et al. 2008). *Micromeria* is a monophyletic genus (Bräuchler et al. 2005, 2006, 2010) and is currently composed of ca. 54 species distributed in the Mediterranean basin, Macaronesia, and some parts of Asia and Africa (Bräuchler et al. 2008). Its centers of diversity are in the Mediterranean region and the Canary Islands (Meimberg et al. 2006).

Molecular analyses (Bräuchler et al. 2005; Meimberg et al. 2006) have shown that the species of *Micromeria* present in the Canary Islands are monophyletic with samples from the western Mediterranean (*M. fontanesii* and *M. inodora*) as sister taxa. The species present in Cape Verde form a different clade suggesting that the colonization of this archipelago was independent (Bräuchler et al. 2005). In the Canary Islands, *Micromeria* presents ca. 16 species (Pérez de Paz 1978) most of them single island endemics. The islands with the highest number of species are Tenerife and Gran Canaria with eight and seven species respectively (Figs. 5, 6) (Pérez de Paz 1978).

The remaining islands present lower number of species: one in each Lanzarote and Fuerteventura (Fig. 7), two in each La Gomera (Fig. 8), La Palma and El Hierro (Fig. 9). Only one species, *M. varia*, is present in almost all islands of the Canarian archipelago (except for La Palma) with a different subspecies in each island. *Micromeria varia* is also present in Madeira archipelago. The remaining species are single island endemics except for *M. hyssopifolia* and *M. lasiophylla* that occur in two islands each, the former in Tenerife and El Hierro, the latter in Tenerife and La Palma.

In Tenerife, the composition of the species of *Micromeria* might be linked to the geological evolution of the island. From the eight species that occur in the island, three are narrow endemics and are restricted to the older parts of the island: *M. densiflora* is restricted to the palaeoisland of Teno, *M. glomerata* and *M. rivas-martinezii* to the palaeoisland of Anaga (Fig. 5). These species have been included in the Spanish Red

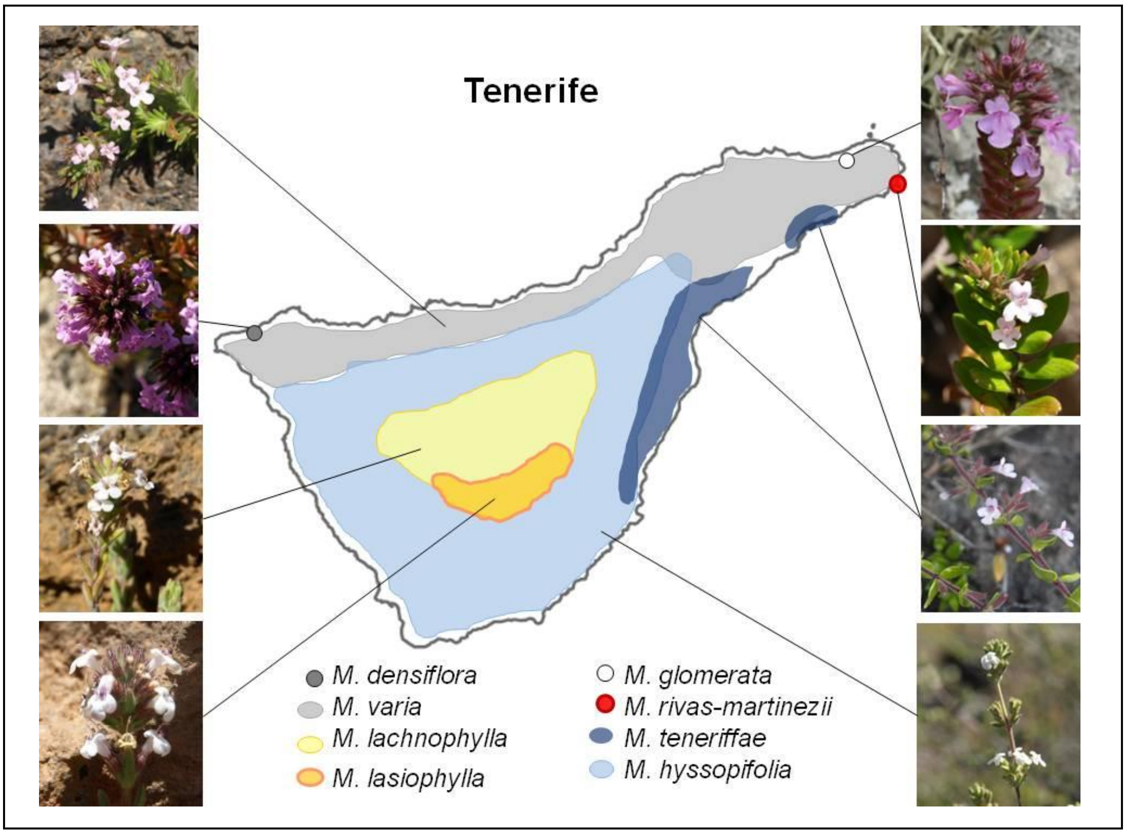


Fig. 5 Distribution of the eight species of *Micromeria* present in Tenerife (sensu Pérez de Paz 1978).

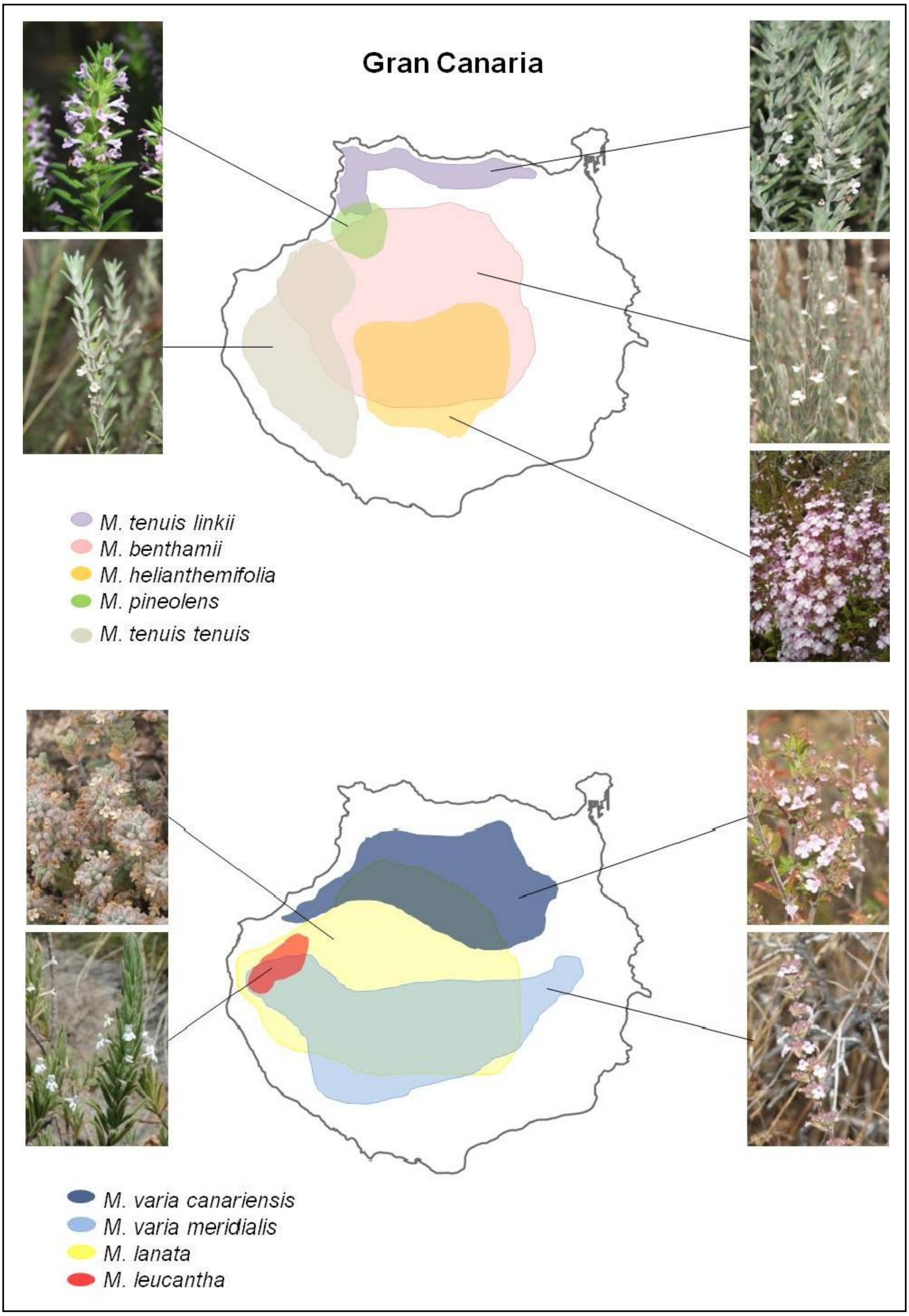


Fig. 6 Distribution of the species and subspecies of *Micromeria* present in Gran Canaria (sensu Pérez de Paz 1978).

List of threatened taxa because of their reduced area (2500-6500 m^2) and number of individuals (ca. 400-900): *M. densiflora* as endangered and *M. glomerata* and *M. rivas-martinezii* as critically endangered (Bañares et al. 2004, 2010). A fourth species, *M. teneriffae*, is also distributed in Anaga but its range extends to Fasnia in SE Tenerife. The four remaining species are distributed in the younger parts of the island: *M. varia* along the northern part, *M. hyssopifolia* throughout the island, *M. lachnophylla* from the pine forest up to the high desert, and *M. lasiophylla* in Las Cañadas cliffs (Fig. 5).

In Gran Canaria, two species are narrow endemics: *M. leucantha* restricted to an area of 7000 m^2 in the W part of the island and a population of ca. 2400 individuals, and *M. pineolens* restricted to an area of ca. 9500 m^2 in the W part and with 4500 individuals (Fig. 6) (Bañares et al. 2004). These species have also been included in the Red List of threatened taxa as endangered (Bañares et al. 2004). The remaining five species are widely distributed (Fig. 6).

The species distribution of *Micromeria* in the Canary Islands makes this genus a good system in which to study the role of interisland colonization in the diversification of plant species in oceanic archipelagos. Furthermore, previous studies using ISSR fingerprinting (Meimberg et al. 2006) suggest that each of the Canary Islands were colonized by one lineage of *Micromeria* except for La Gomera where two lineages were identified. These findings support adaptive radiation as the major mechanism in the evolution of this group in the Canary Islands.

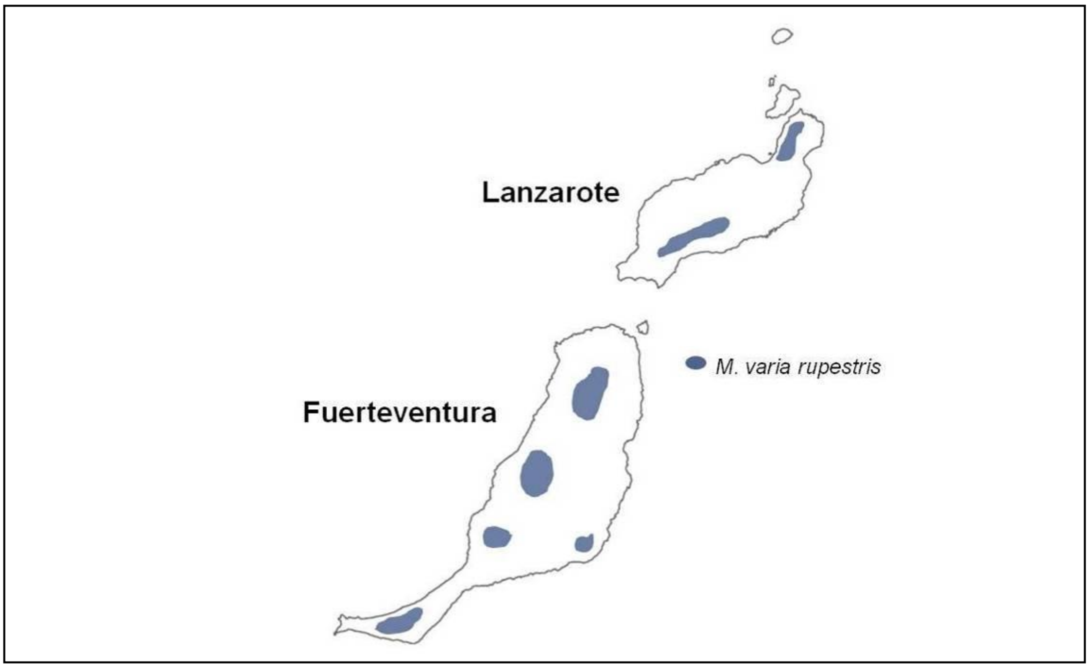


Fig. 7 Distribution of *Micromeria varia* subsp. *rupestris* in Lanzarote and Fuerteventura (sensu Pérez de Paz 1978).

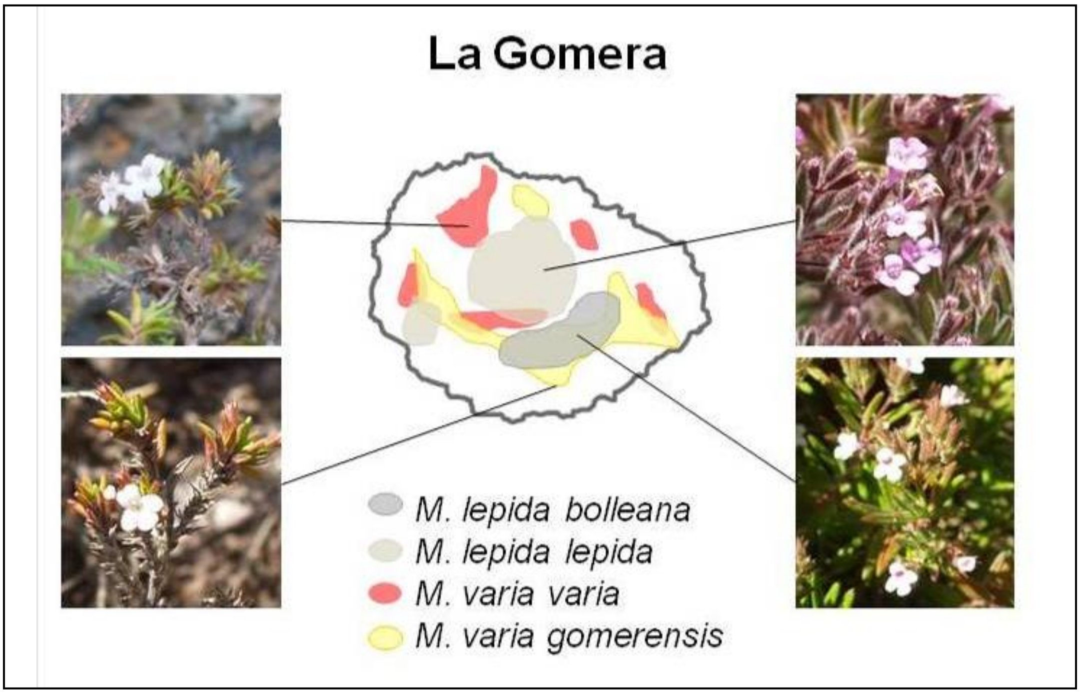


Fig. 8 Distribution of the species and subspecies of *Micromeria* in La Gomera (sensu Pérez de Paz 1978).

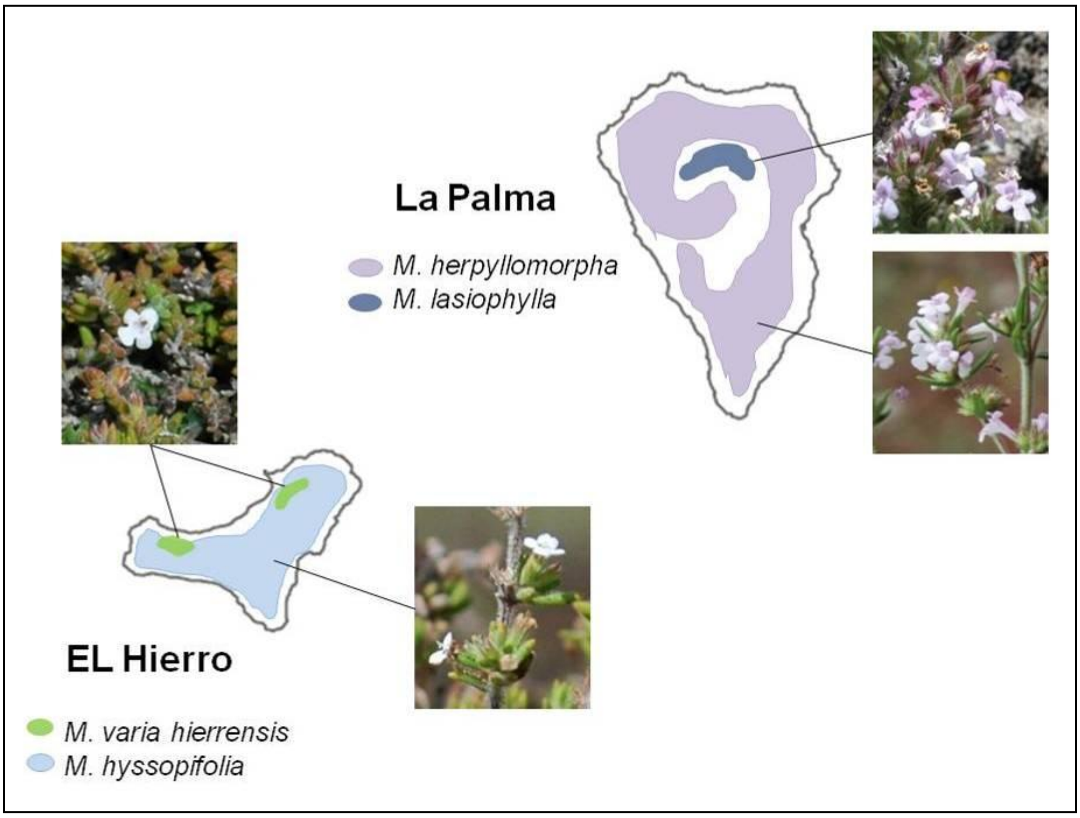


Fig. 9 Distribution of the species of *Micromeria* in El Hierro and La Palma (sensu Pérez de Paz 1978).

1.4 Objectives and thesis structure

The general aim of this thesis is to study the diversification process of *Micromeria* (Lamiaceae) in the Canary Islands using phylogenetics, population genetics, and morphometric approaches. This main goal will be achieved through a series of smaller objectives:

(i) To try to understand the diversification process of the two groups of species of *Micromeria* present in Tenerife: the species restricted to the oldest parts of the island (“palaeoisland species”), and the species inhabiting the central, most recent part of Tenerife (“central species”);

(ii) To develop a set of polymorphic microsatellite markers that can be later used to assess the genetic variability of the palaeoisland species in Tenerife;

(iii) To survey if the species of *Micromeria* present in each of the Canary Islands form a monophyletic group as suggested by previous data and what was the role, if any, of Tenerife in the colonization process of the archipelago;

(iv) To reconcile the taxonomic composition of the species of *Micromeria* in the Canary Islands with the new molecular evidence available.

The research contained in this thesis is presented in five scientific papers which have already been published in international journals or are currently under revision. These papers are organized in chapters and answer the questions proposed above. This thesis is therefore organized in five chapters:

Chapter 1: The present chapter provides a general introduction to the thesis. It provides background information on the research theme, explains the objectives of the thesis, and how it is composed.

Chapter 2: This chapter is composed of two papers that focus on the diversification process of *Micromeria* in the island of Tenerife and how the geological history of the island influenced this process. Paper I (published in Journal of Biogeography) uses phylogenetic and morphometrics approaches to understand the diversification process of the two groups of *Micromeria* species in Tenerife: the palaeoisland species (those restricted to older parts of the island) and the central species (those inhabiting the central, younger part). This paper constitutes an interesting example on how the geological evolution of an oceanic island can shape the diversification process of its taxa. Paper II (published in Conservation Genetics Resources) develops a set of microsatellite markers that will be later used to assess the genetic variability of the palaeoisland species which are also of conservation importance. This paper provides preliminary results that show that the palaeoisland species present lower genetic diversity than the widespread species *M. hyssopifolia*.

Chapter 3: This chapter consists of Paper III (accepted in Molecular Phylogenetics and Evolution) and reconstructs the phylogenetic history of *Micromeria* in the Canary Islands using eight nuclear markers. This paper suggests that *Micromeria* had two centers of diversification in the archipelago, one in the eastern islands of Gran Canaria and Lanzarote, the other in the western islands of Tenerife, La Palma and El Hierro. Multiple colonization of La Gomera is suggested. The taxonomic implications of the results are also discussed.

Chapter 4: This chapter is composed of two papers and deals with reconciling the taxonomic composition of *Micromeria* in the Canary Islands with the molecular evidence provided in previous papers. Paper IV (published in Taxon) aims to untangle the nomenclature of two species: *M. varia* and *M. hyssopifolia*. Paper V (to be submitted to Phytotaxa) revises several taxa describing two new species and hybrids, and proposes several new combinations. This paper also provides an identification key for all the species of *Micromeria* present in the Canary Islands.

Chapter 5: This chapter presents a general discussion of the results obtained from this investigation. The impact of the geological history of an island on the diversification of its taxa is discussed as well as the colonization patters observed in oceanic archipelagos and in the Canary Islands in particular. The implications of the results obtained from the molecular analyses on the taxonomy of the group are also discussed. Finally, main lines of future research are proposed.

1.5 List of papers

Because of the great variation in format and graphical presentation of the articles published in the different journals, the text, tables and figures of the articles that compose this thesis have been formatted in a uniform way without changing their content. The list of papers that compose the present thesis is presented below.

Paper I Puppo, P, M Curto, G Velo-Antón, PL Pérez de Paz and H Meimberg (2014) The influence of geological history on diversification in insular species: genetic and morphological patters of *Micromeria* Benth. (Lamiaceae) in Tenerife (Canary archipelago). Journal of Biogeography 41, 1871-1882. DOI: 10.1111/jbi.12354

Paper II Puppo, P, M Curto, H Meimberg (2015) Development and characterization of 16 microsatellite markers for *Micromeria* (Lamiaceae) from Tenerife (Canary Islands, Spain) using 454 sequencing. Conservation Genetics Resources. DOI: 10.1007/s12686-015-0451-5

Paper III Puppo, P, M Curto, J Gusmão-Guedes, J Cochofel, PL Pérez de Paz, C Bräuchler and H Meimberg (2015) Molecular phylogenetics of *Micromeria* (Lamiaceae) in the Canary Islands and Madeira, diversification and inter-colonization patterns inferred from nuclear genes. Molecular Phylogenetics and Evolution 89, 160-170. DOI: 10.1016/j.ympev.2015.04.017

Paper IV Puppo, P, H Meimberg, PL Pérez de Paz and C Bräuchler (2014) (2326-2327) Proposals to conserve the names *Micromeria varia* with a conserved type and *M. hyssopifolia* against *Thymus ericifolius* (Lamiaceae). Taxon 63, 1137-1138. DOI: 10.12705/635.17

Paper V Puppo, P and H Meimberg (Submitted) New species and new combinations in *Micromeria* (Lamiaceae) from the Canary Islands. Phytotaxa.

1.6 Literature cited

Allan, GJ, J Francisco-Ortega, A Santos-Guerra, E Boerner and EA Zimmer (2004) Molecular phylogenetic evidence for the geographic origin and classification of Canary Island *Lotus* (Fabaceae: Loteae). Molecular Phylogenetics and Evolution 32, 123-138.

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

Bañares, Á, G Blanca, J Güemes, JC Moreno and S Ortiz (2004) Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Dirección General de Conservación de la Naturaleza, Madrid.

Bañares, Á, G Blanca, J Güemes, JC Moreno and S Ortiz (2010) Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Adenda 2010. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino)-Sociedad Española de Biología de la Conservación de Plantas, Madrid.

Barber, JC, J Francisco-Ortega, A Santos-Guerra, A Marrero and RK Jansen (2000) Evolution of endemic *Sideritis* (Lamiaceae) in Macaronesia: Insights from a chloroplast DNA restriction site analysis. Systematic Botany 25, 633-647.

Böhle, U-R, HH Hilger and WF Martin (1996) Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). Proceedings of the Natural Academy of Sciences of Philadelphia 93, 11740-11745.

Bramwell, D (1972) Endemism in the flora of the Canary Islands. Taxonomy, phytogeography, and evolution (ed. by DH Valentine), pp. 141-159. Academic Press, London.

Bramwell, D (1997) Flora de las Islas Canarias, guía de bolsillo. Editorial Rueda, Madrid.

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

Bräuchler, C, H Meimberg and G Heubl (2006) New names in Old world *Clinopodium* - the transfer of the species of *Micromeria* sect. *Pseudomelissa* to *Clinopodium*. Taxon 55, 977-981.

Bräuchler, C, O Ryding and G Heubl (2008) The genus *Micromeria* (Lamiaceae), a synoptical update. Willdenowia 38, 363-410.

Bräuchler, C, H Meimberg and G Heubl (2010) Molecular phylogeny of Menthinae (Lamiaceae, Nepetoideae, Mentheae) -Taxonomy, biogeography and conflicts. Molecular Phylogenetics and Evolution 55, 501-523.

Carine, MA, SJ Russell, A Santos-Guerra and J Francisco-Ortega (2004) Relationships of the Macaronesian and Mediterranean Floras: Molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in Convolvulus (Convolvulaceae). American Journal of Botany 91, 1070-1085.

Carracedo, JC (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, DJ, R Witkus and TF Stuessy (1987) Plant evolution and speciation on oceanic islands. Differentiation patterns in higher plants (ed. by K.M. Urbanska), pp. 183-199. Academic Press, London.

Darwin, C (1859) On the origin of species by means of natural selection (reprint of 1st ed. 1950). Watts, London.

del-Arco, M, M Salas, JR Acebes, M del C Marrero, JA Reyes-Betancort and PL Pérez de Paz (2002) Bioclimatology and climatophilous vegetation of Gran Canaria (Canary Islands). Annales Botanici Fennici 39, 15-41.

Emerson, BC (2002) Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. Molecular Ecology 11, 951-966.

Emerson, BC and N Kolm (2005) Species diversity can drive speciation. Nature 434, 1015-1017.

Fernández-Palacios, JM and RJ Whittaker (2010) El ciclo de la isla. Atlas de la biodiversidad de Canarias (ed. by JL Martín-Esquivel), pp. 18-27. Turquesa Ediciones, Santa Cruz de Tenerife.

Fernández-Palacios, JM, L de Nascimento, R Otto, JD Delgado, E García-del-Rey, JR Arévalo and RJ Whittaker (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, RK Jansen and A Santos-Guerra (1996) Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. Proceedings of the Natural Academy of Sciences of Philadelphia 93, 4085-4090.

Francisco-Ortega, J, SJ Park, A Santos-Guerra, A Benabid and RK Jansen (2001a) Origin and evolution of the endemic Macaronesian Inuleae (Asteraceae): evidence from the internal transcribed spacers of nuclear ribosomal DNA. Biological Journal of the Linnean Society 72, 77-97.

Francisco-Ortega, J, JC Barber, A Santos-Guerra, R Febles-Hernández and RK Jansen (2001b) Origin and evolution of the endemic genera of Gonosperminae (Asteraceae: Anthemideae) from the Canary Islands: evidence from nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA. American Journal of Botany 88, 161-169.

Francisco-Ortega, J, J Fuertes-Aguilar, S-C Kim, A Santos-Guerra, DJ Crawford, and RK Jansen (2002) Phylogeny of the Macaronesian endemic *Crambe* section *Dendrocrambe* (Brassicaceae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. American Journal of Botany 89, 1984-1990.

Galbany-Casals, M, N Garcia-Jacas, L Sáez, C Benedí and A. Susanna (2009) Phylogeny, biogeography, and character evolution in Mediterranean, Asiatic and Macaronesian *Helichrysum* (Asteraceae, Gnaphalieae) inferred from nuclear phylogenetic analyses. International Journal of Plant Sciences 170, 365-380.

Gillespie, RG (2009) Adaptive Radiation. Encyclopedia of Islands (ed. by RG Gillespie and DA Clague), ch. 1. University of California Press, California, USA.

Goodson, BE, A Santos-Guerra and RK Jansen (2006) Molecular systematics of *Descurainia* (Brassicaceae) in the Canary Islands: biogeoraphic and taxonomic implications. Taxon 55, 671-682.

Jaén-Molina, R, J Caujapé-Castells, JA Reyes-Betancort, H Akhani, O Fernández-Palacios, J Pérez de Paz, R Febles-Hernández and A Marrero-Rodríguez (2009) The molecular phylogeny of *Matthiola* R. Br. (Brassicaceae) inferred from ITS sequences, with special emphasis on the Macaronesian endemics. Molecular Phylogenetics and Evolution 53, 972-981.

Jones, KE, JA Reyes-Betancort, SJ Hiscock and MA Carine (2014) Allopatric diversification, multiple habitat shifts, and hybridization in the evolution of *Pericallis* (Asteraceae), a Macaronesian endemic genus. American Journal of Botany 101, 637-651.

Jorgensen, TH and JM Olesen (2001) Adaptive radiation of island plants: evidence from *Aeonium* (Crassulaceae) of the Canary Islands. Perspectives in Plant Ecology, Evolution and Systematics 4, 29-42.

Juan, C, BC Emerson, P Oromí, and GM Hewitt (2000) Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. Trends in Ecology and Evolution 15, 104-109.

Kim, SC, DJ Crawford, J Francisco-Ortega and A Santos-Guerra (1996) A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. Proceedings of the Natural Academy of Sciences of Philadelphia 93, 7743-7748.

Kim, SC, MR McGowen, P Lubinsky, JC Barber, ME Mort and A Santos-Guerra (2008) Timing and tempo of early and successive adaptive radiations in Macaronesia. PLoS ONE 3, e2139. doi:10.1371/journal.pone.0002139

Kimura, M and GH Weiss (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49, 561-576.

Kreft, H, W Jetz, J Mutke, G Kier and W Barthlott (2008) Global diversity of island floras from a macroecological perspective. Ecology Letters 11, 116-127.

MacArthur, RH and EO Wilson (1967) The theory of island biogeography. Princeton University Press, Princeton, New Jersey.

Meimberg, H, T Abele, C Bräuchler, JK McKay, PL Pérez de Paz and G Heubl (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.

Mes, THM and HT Hart (1996) The evolution of growth forms in the Macaronesian genus *Aeonium* (Crassulaceae) inferred from chloroplast DNA RFLPs and morphology. Molecular Ecology 5, 351-363.

Mes, THM, J Van Brederode and H Hart (1996) Origin of the woody Macaronesian Sempervivoideae and the phylogenetic position of the East African species of *Aeonium*. Botanica Acta109, 477-491.

Mort, ME, DE Soltis, PS Soltis, J Francisco-Ortega and A Santos-Guerra (2002) Phylogenetics and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. Systematic Botany 27, 271-288.

Olmstead, RG and JD Palmer (1997) Implications for phylogeny, classification and biogeography of *Solanum* from cpDNA restriction site variation. Systematic Botany 22, 19-29.

Panero, JL, J Francisco-Ortega, RK Jansen and A Santos-Guerra (1999) Molecular evidence for multiple origins of woodiness and a New World biogeographic connection of the Macaronesian Island endemic *Pericallis* (Asteraceae: Senecioneae). Proceedings of the Natural Academy of Sciences of Philadelphia 96, 13886-13891.

Percy, DM and QCB Cronk (2002) Different fates of island brooms: contrasting evolution in *Adenocarpus*, *Genista*, and *Teline* (Genisteae, Fabaceae) in the Canary Islands and Madeira. American Journal of Botany 89, 854-864.

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

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

Spalik, K and SR Downie (2007) Intercontinental disjunctions in *Cryptotaenia* (Apiaceae: Oenantheae): an appraisal using molecular data. Journal of Biogeography 34, 2039-2054.

Thiv, M, L Struwe and JW Kadereit (1999) The phylogenetic relationships and evolution of the Canarian laurel forest endemic *Ixanthus viscosus* (Aiton) Griseb (Gentianaceae): evidence from *mat*K and ITS sequences, and floral morphology and anatomy. Plant Systematics and Evolution 218, 299-317.

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

Whittaker, RJ, RJ Ladle, MB Araújo, JM Fernández-Palacios, JD Delgado and JR Arévalo (2007) The island immaturity – speciation pulse model of island evolution: an alternative to the “diversity begets diversity” model. Ecography 30, 321-327.

Whittaker, RJ, KA Triantis, and RL Ladle (2008) A general dynamic theory of oceanic island biogeography. Journal of Biogeography 35, 977-994.

Chapter 2:

Diversification of *Micromeria* in Tenerife

Paper I Puppo, P, M Curto, G Velo-Antón, PL Pérez de Paz and H Meimberg (2014) The influence of geological history on diversification in insular species: genetic and morphological patters of *Micromeria* Benth. (Lamiaceae) in Tenerife (Canary archipelago). Journal of Biogeography 41, 1871-1882. DOI: 10.1111/jbi.12354

Paper II Puppo, P, M Curto and H Meimberg (2015) Development and characterization of 16 microsatellite markers for *Micromeria* (Lamiaceae) from Tenerife (Canary Islands, Spain) using 454 sequencing. Conservation Genetics Resources. DOI: 10.1007/s12686-015-0451-5

Paper I:

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

Pamela Puppo, Manuel Curto, Guillermo Velo-Antón, Pedro Luis Pérez de Paz, and Harald Meimberg

Journal of Biogeography 41, 1871-1882. DOI: 10.1111/jbi.12354

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]

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

---

[1] Research Center in Biodiversity and Genetic Resources (CIBIO) / InBio Associated Laboratory, University of Porto, Campus Vairão, 4485-661 Vairão, Portugal

[2] Departamento de Biología Vegetal (Botánica), Universidad de La Laguna, 38271 – La Laguna, Tenerife, Spain

[3] Restoration Ecology, Technical University Munich, 85350 Freising, Germany

[4] Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences, A-1180 Vienna, Austria

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 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).

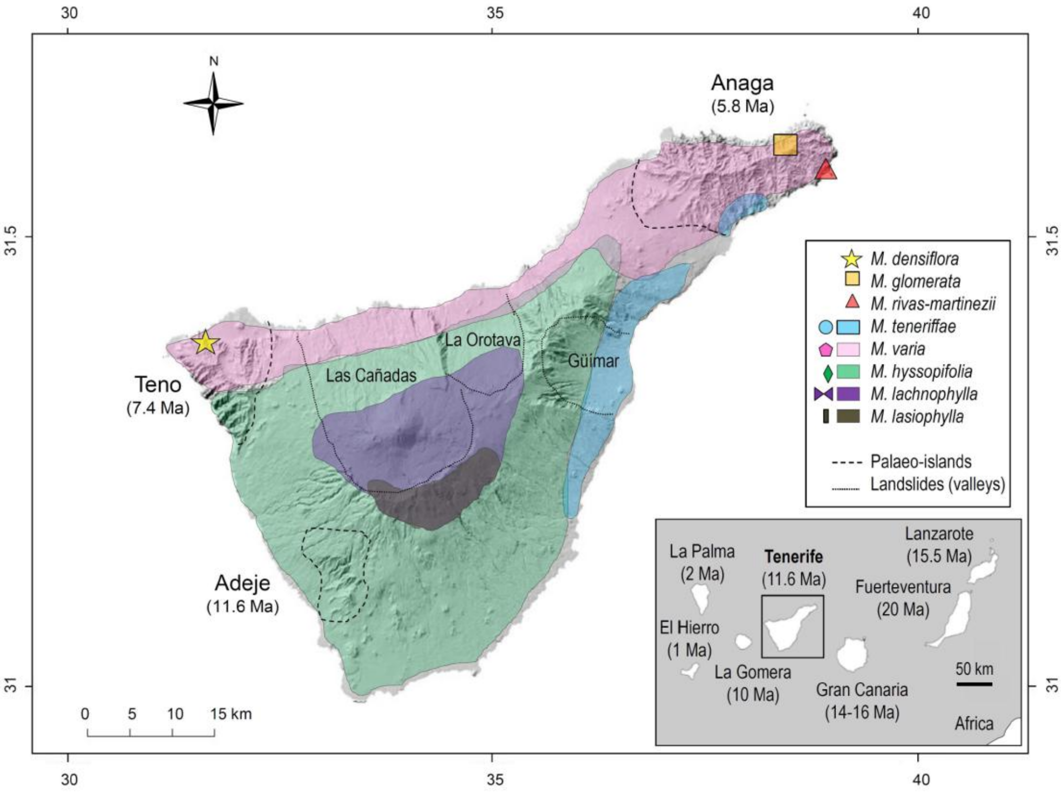


Fig. 1 Map of Tenerife showing distribution of the species of *Micromeria*; dashed lines indicate remnants of palaeoislands, dotted lines indicate valleys formed after major landslides (sensu Ancochea et al., 1990; Juan et al., 2000), symbol shapes and colours correspond to those used in Figs 3 and 5.

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 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 each from *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 ºC and inactivated for 15 min at 85 ºC. 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 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 a 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.

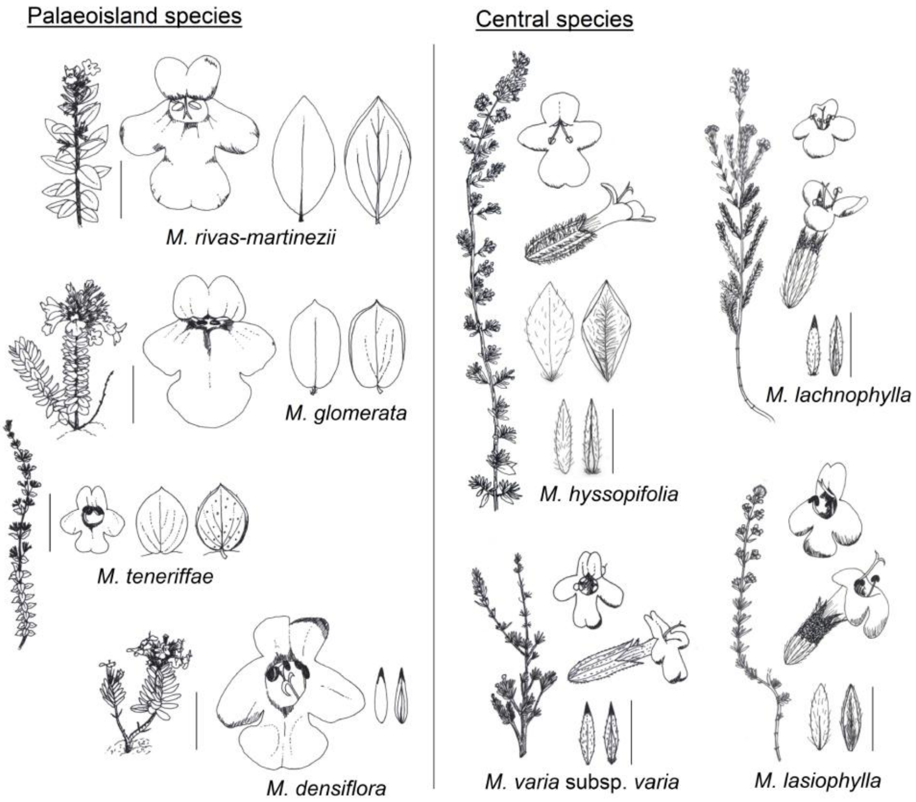


Fig. 2 Drawings of all eight species of *Micromeria* currently recognized in Tenerife showing habit, upper and lower leaf surface, and flower. Scale bars indicate 2.5 cm for habit, 5 mm for leaves and 2.5 mm for flowers.

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. 27º53'23,4"N; 15º33'41,3"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. 31º28'18,1"N; 07º24'05,5"W		Curto MA034, MA035 (MSB)
M. hyssopifolia Webb & Berthel. var. *glabrescens* (Webb & Berthel.) P.Pérez	Tenerife. Lomo Morin, Teno. 28º21'35,3"N; 16º47'20,8"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. 27º48'49,9"N; 17º53'52,8"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. 28º06'33,5"N; 17º12'51"W		Puppo 577.4 (TFC)
M. pineolens Svent.	Gran Canaria. 28º03'14,22"N; 15º41'24,3"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. 28º13'08,2"N; 16º24'55,2"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. *rupestres* (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)	

Table 1 Continued

Species	Locality*	Morphometric analyses	Genetic analyses
M. varia subsp. *varia*	Tenerife. Afur, Anaga. 28°33'09,4"N; 16°14'16,0"W	Puppo 204, 206 (TFC)	
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. Cultivar.		Curto et al. (2012)
Origanum L. sp.	Portugal. Cultivar.		Curto et al. (2012)
TOTAL		54	37

*No geographical coordinates are provided for restricted taxa.

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 the program IBM SPSS STATISTICS 19 (IBM, Armonk, NY, USA) to evaluate the morphological variation among samples. The axes extracted were those corresponding to components with eigen values 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 includes 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.

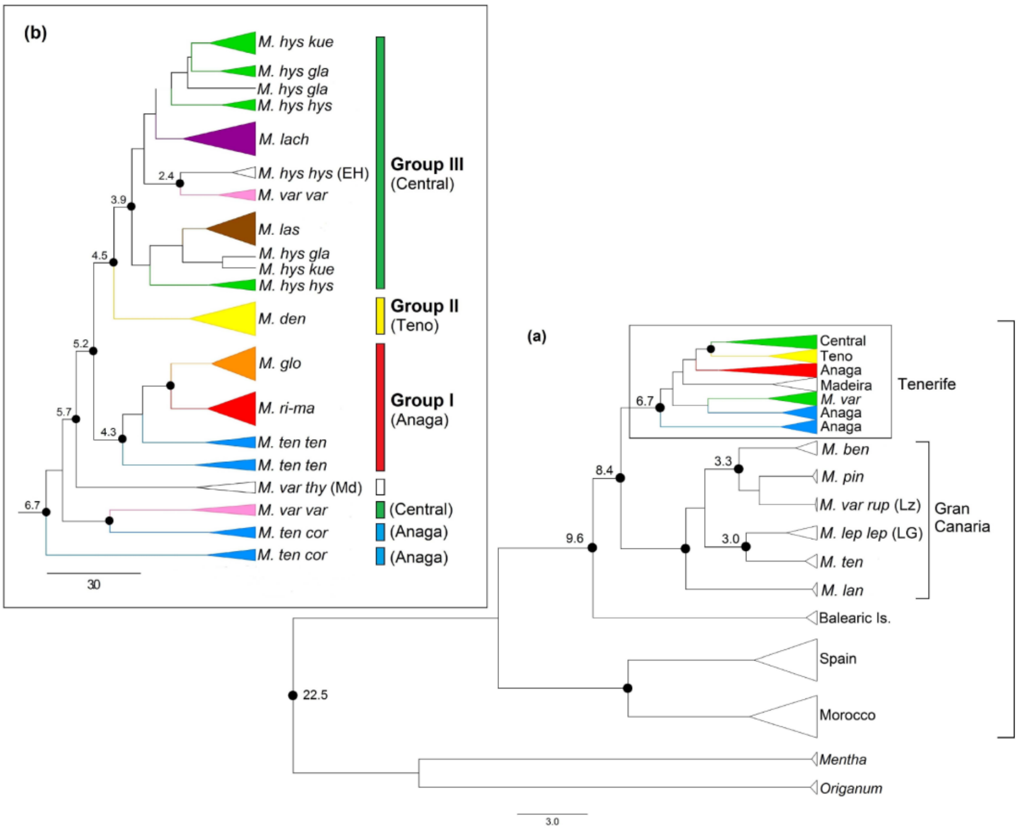


Fig. 3 Bayesian phylogeny calculated in BEAST showing relationships among reconstructed haplotypes for (a) all samples of *Micromeria* included in this study, (b) samples only from Tenerife. See Table 1 for full species names. Filled circles on nodes represent Bayesian posterior probabilities, BPP > 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. Scale bar in Myr.

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 time of the most recent common ancestor (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) while the divergence of the species from Teno and the 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).

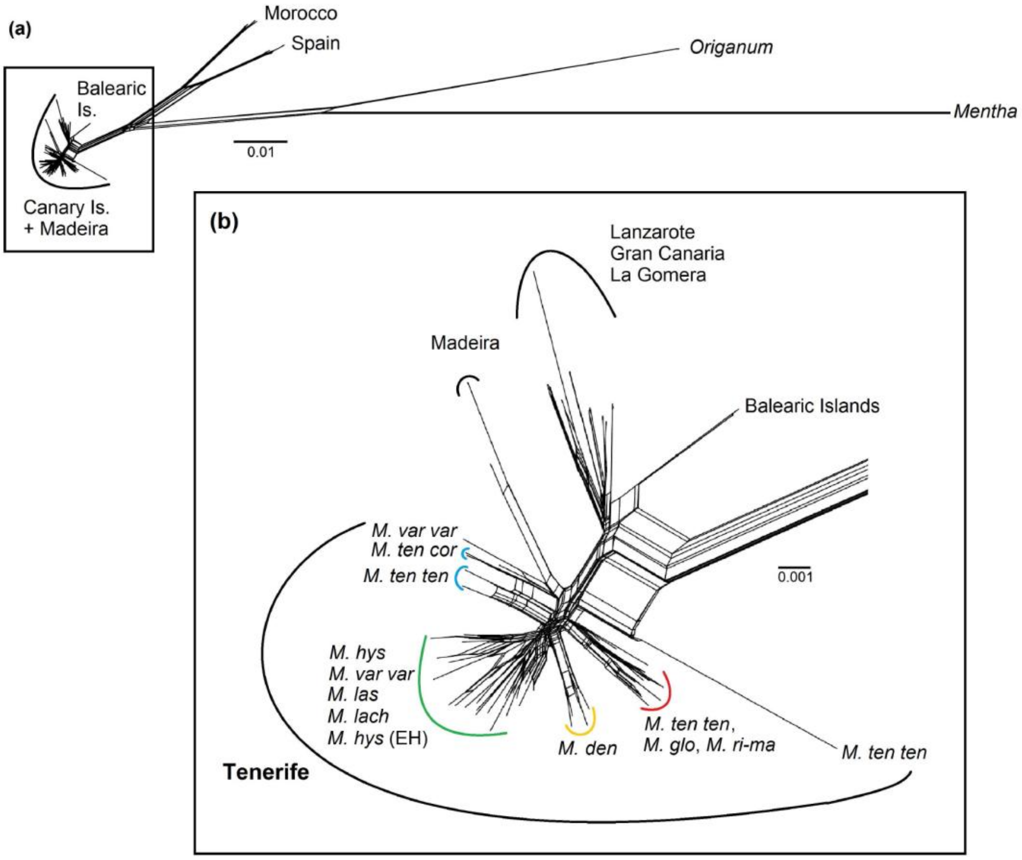


Fig. 4 Neighbor-Net network calculated in SplitsTree showing relationships among reconstructed haplotypes from (a) all samples of *Micromeria* and outgroups and (b) samples from the Canary Islands and Madeira. Colours used for the different Tenerife groups correspond to those in Fig. 3. See Table 1 for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

Morphometric analyses

The results obtained from the PCA including all specimens and all variables indicate that two main clusters are divided by principal component 1 (Fig. 5a). The cluster towards the right of the plot is composed of the three palaeoisland 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 relate mainly to flower size: corolla length, width and calyx length, 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 length and width, and petiole length (Appendix S2).

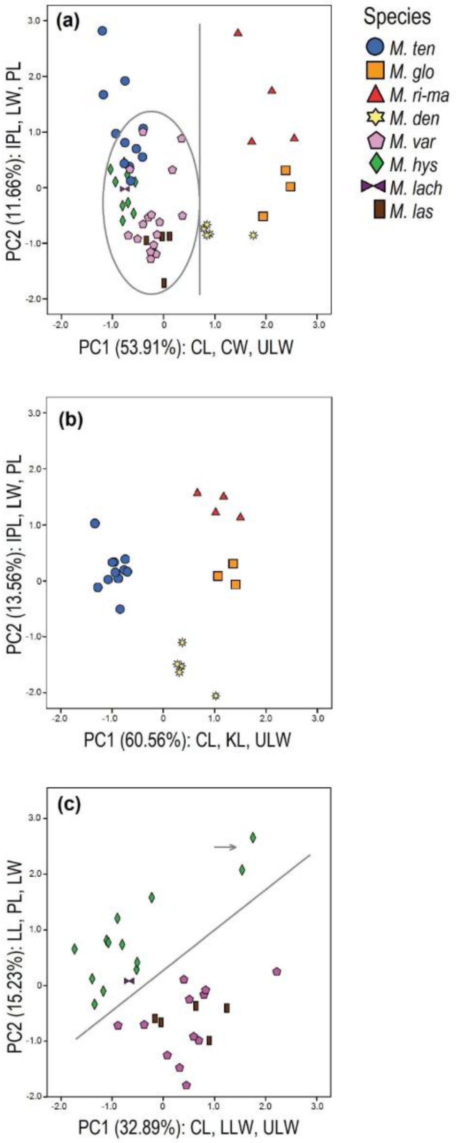


Fig. 5 Bivariate scatter plot formed by the two first components of the principal components analysis (PCA) including: (a) all species of *Micromeria*, (b) species inhabiting only the palaeoislands, and (c) species from the central area only. Each point represents a single specimen; different symbol shapes and colours indicate different species and correspond to those used in Fig. 1. The grey circle in (a) indicates central species, and 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.

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 length and width and the peduncle length on PC2 suggest 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 four 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 internode length suggest that vegetative characters are responsible for the segregation of the first cluster composed of four 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 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. While 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 length vary from *c*. 5-15 and 3.6-14 mm in the palaeoisland species and from 4-11 and 3.6-7.5 mm in the central species, respectively. 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 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.

ACKNOWLEDGEMENTS

The authors thank the University of La Laguna in Tenerife for providing a vehicle during the collection of the plant material in the Canary Islands. Francisco Faure was an invaluable help during fieldwork. Alfredo Reyes Betancort and Vicente Lucía helped as well during the collection of some of the material. We thank Christian Bräuchler for providing some of the DNA samples of the species included as outgroups. Victor Garzón-Machado provided the map for Tenerife. We also thank Robert J. Whittaker, Peter Linder, José Maria Fernández-Palacios and two anonymous referees, who improved this manuscript with their helpful suggestions and comments. Mark P. Simmons helped us improve the English. The research is supported by the Fundação para a Ciência e Tecnología (FCT) with a research grant to H.M. (PTDC/BIA-

BEC/108866/2008), PhD fellowships to P.P. (SFRH/BD/74747/2010) and M.C. (SFRH/BD/79010/2011) and a post-doctoral fellowship to G.V.-A. (SFRH/BPD/74834/2010).

REFERENCES

Ancochea, E, JM Fuster, E Ibarrola, A Cendredo, J Coello, F Hernan, JM Cantagrel and C Jamond (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, H Meimberg, T Abele and G Heubl (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 and V Moulton (2004) Neighbor-Net: An agglomerative method for the construction of phylogenetic networks. Molecular Biology and Evolution 21, 255-265.

Carracedo, JC (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, DJ, R Witkus and TF Stuessy (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, P Puppo, D Ferreira, M Nogueira and H Meimberg (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, GL Taboada, R Doallo and D Posada (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9, 772.

Drew, BT and KJ Sytsma (2012) Phylogenetics, biogeography, and stamina evolution in the tribe Mentheae (Lamiaceae). American Journal of Botany 99, 933-953.

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

Drummond, AJ, MA Suchard, X Dong and A Rambaut (2012) Bayesian phylogenetics with BEAUTi and the BEAST 1.7. Molecular Biology and Evolution 29, 1969-1973.

Fernández-Palacios, JM, L de Nascimento, R Otto, JD Delgado, E García-del-Rey, JR Arévalo and RJ Whittaker (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, RK Jansen and A Santos-Guerra (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, JF Dufayard, V Lefort, M Anisimova, W Hordijk and O Gascuel (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, S Lachmuth, JGB Oostermeijer, H(J)CM den Nijs, PG Meirmans, and PH van Tienderen (2012) Human-induced hybridization among congeneric endemic plants on Tenerife, Canary Islands. Plant Systematics and Evolution 298, 1119-1131.

Huson, DH and D Bryant (2006) Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23, 254-267.

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

Juan, C, BC Emerson, P Oromí and GM Hewitt (2000) Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. Trends in Ecology and Evolution 15, 104-109.

Kimura, M and GH Weiss (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49, 561-576.

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

MacArthur, RH and EO Wilson (1967) *The theory of island biogeography*. Princeton University Press, Princeton, NJ.

Meimberg, H, T Abele, C Bräuchler, JK McKay, PL Pérez de Paz and G Heubl (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, PL (1978) Revisión del género *Micromeria* Bentham (Lamiaceae-Stachyoideae) en la Región Macaronésica. Instituto de Estudios Canarios, Monografías 16, 1-306.

Ronquist, F and JP Huelsenbeck (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572-1574.

Santos-Guerra, A, A Acevedo-Rodríguez and JA Reyes-Betancort (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.

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

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

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

Watts, AB and DG Masson (1995) A giant landslide on the north flank of Tenerife, Canary Islands. Journal of Geophysical Research 100, 24487-24498.

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

Whittaker, RJ, RJ Ladle, MB Araújo, JM Fernández-Palacios, JD Delgado and JR Arévalo (2007) The island immaturity - speciation pulse model of island evolution: an alternative to the "diversity begets diversity" model. Ecography 30, 321-327.

Whittaker, RJ, KA Triantis and RJ Ladle (2008) A general dynamic theory of oceanic island biogeography. Journal of Biogeography 35, 977-994.

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.

SUPPORTING INFORMATION

Appendix S1. Markers used in the present study and GenBank accession numbers.

Table S1 List of markers used in the present study (taken from Curto et al., 2012) including annealing temperature (*Ta*), model as calculated with JMODELTEST, number of polymorphic sites (*S*), number of haplotypes (*h*), and number of informative positions (I.P.).

Marker	Ta (ºC)	Length	Model	*S*	*h*	I.P.
M. pip. 056	55	754	TPM3uf+G	177	24	177
M. pip. 057	55	348	K80	44	19	13
M. pip. 014	55	738	HKY+G	154	33	143
M. pip. 017	55	1498	HKY	398	36	385
M. pip. 047	55	763	TrN+G	236	44	232
M. pip. 027	57	733	HKY	137	25	124
M. pip. 002	55	1180	HKY+G	199	35	187
O. oni. 007	55	524	F81	85	49	61
Combined		6538	GTR+G	1430		

Reference:

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.

Table S2 GenBank accession numbers for all samples included in the phylogenetic analyses. Collection numbers correspond to those in Table 1.

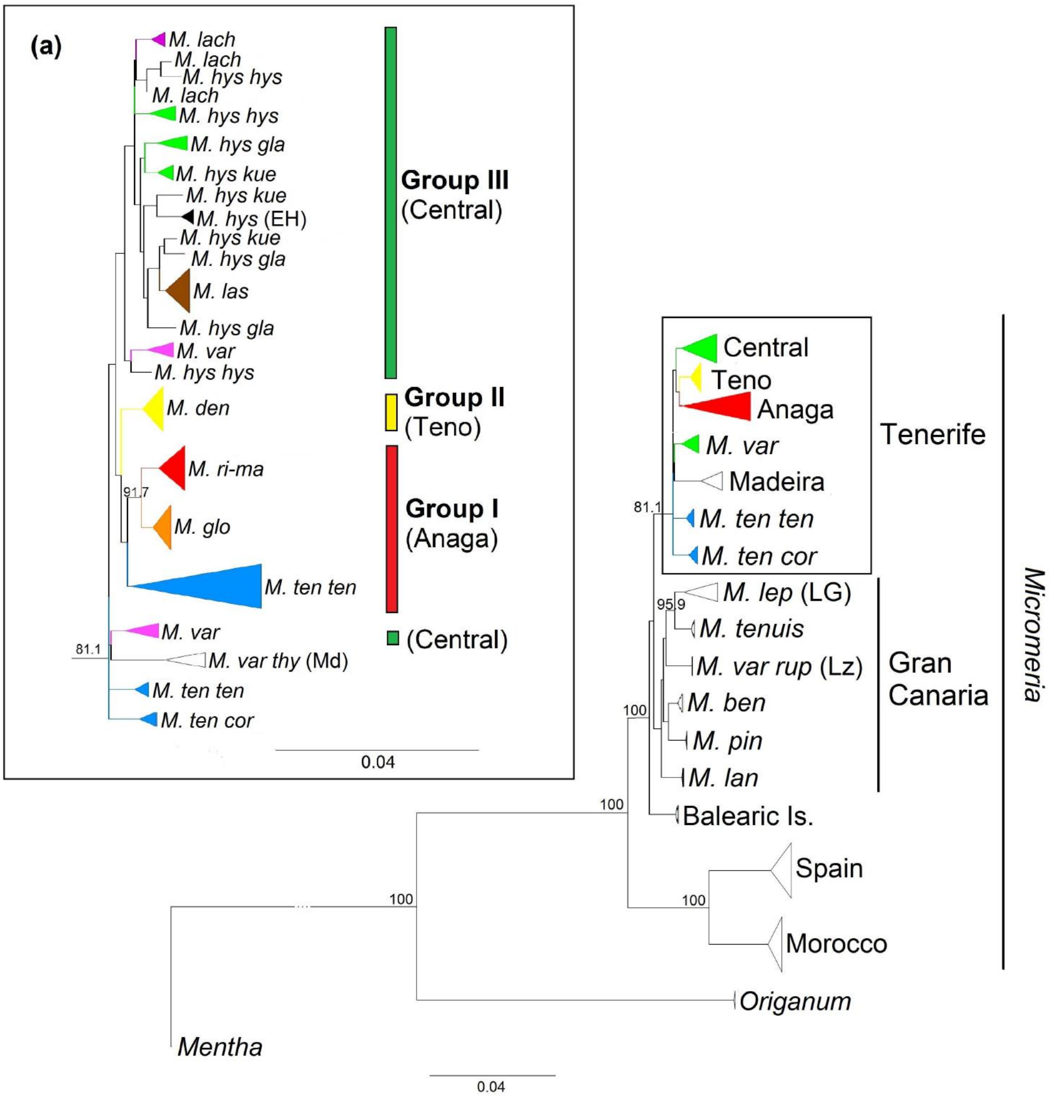
	M.pip.056	M.pip.057	M.pip.014	M.pip.017	M.pip.047	M.pip.027	M.pip.002	O.oni.007
Puppo 562.3	KF667170	KF667162	KF667154	KF667146	KF667138	KF667130	KF667123	KF667115
Puppo 577.4	KF667171	KF667163	KF667155	KF667147	KF667139	KF667131	KF667124	KF667116
Bräuchler *et al.* 2005 (*M. inodora*)	JN587688	JQ934270	JQ934313	JN587598	JQ934352	JQ934370	JQ934393	JQ934414
Puppo 255	JQ934237	JQ934259	JQ934305	JQ934324	JQ934342	JQ934360	JQ934383	JQ934404
Puppo 257	JQ934240	JQ934262	KF727618	JQ934327	JQ934345	JQ934363	JQ934386	JQ934407
Puppo 200	JQ934232	JQ934254	JQ934300	JQ934319	JQ934337	JQ934356	JQ934378	JQ934399
Puppo 202	JQ934231	JQ934253	JQ934299	JQ934318	JQ934336	JQ934355	JQ934377	JQ934398
Bräuchler 170	JQ934249	JQ934273	JQ934314	JQ934334	JQ934353	JQ934373	JQ934394	JQ934417
Puppo 439.1	JQ934250	JQ934274	JQ934315	JQ934335	JQ934354	JQ934374	JQ934395	JQ934418
Puppo 153	JQ934241	JQ934263	JQ934308	JQ934328	JQ934346	JQ934364	JQ934387	JQ934408
Puppo 162	JQ934238	JQ934260	JQ934306	JQ934325	JQ934343	JQ934361	JQ934384	JQ934405
Pérez de Paz H1E	KF667172	KF667164	KF667156	KF667148	KF667140	KF667132	KF667125	KF667117
Puppo 175	KF667173	KF667165	KF667157	KF667149	KF667141	KF667133	KF667126	KF667118
Puppo 181	KF667174	KF667166	KF667158	KF667150	KF667142	KF667134	KF667127	KF667119
Puppo 274	JQ934242	JQ934264	JQ934309	JQ934329	JQ934347	JQ934365	JQ934388	JQ934409
Puppo 276	JQ934234	JQ934256	JQ934302	JQ934321	JQ934339	JQ934357	JQ934380	JQ934401
Puppo 291	JQ934246	JQ934269	JQ934312	JQ934333	JQ934351	JQ934369	JQ934392	JQ934413
Puppo 295	JQ934239	JQ934261	JQ934307	JQ934326	JQ934344	JQ934362	JQ934385	JQ934406
Meimberg *et al.* 2006 (*M. lanata*)	JN587689	JN587700	JN587584	JN587603	JN587670	JN587624	JN587732	JN587751
Bräuchler *et al.* 2005 (*M. varia rupestris*)	JN587690	JQ934268	JN587585	JN587606	JN587671	JN587622	JN587733	JN587752
Curto MA034	JQ934247	JQ934271	KF727616	JQ934294	KF727623	JQ934371	KF727629	JQ934415
Curto MA035	JQ934248	JQ934272	KF727617	KF727621	KF727624	JQ934372	KF727630	JQ934416
Meimberg *et al.* 2006 (*M. tenuis*)	JN587691	JN587702	JN587587	JN587605	JN587675	JN587627	JN587734	JN587754
Meimberg *et al.* 2006 (*M. varia thymoides*)	JN587692	JN587703	JN587588	JN587604	JN587673	JN587625	JN587735	JQ934412
Puppo 212	JQ934235	JQ934257	JQ934303	JQ934322	JQ934340	JQ934358	JQ934381	JQ934402
Puppo 214	JQ934233	JQ934255	JQ934301	JQ934320	JQ934338	KF727627	JQ934379	JQ934400
Meimberg 1.4	JQ934251	JQ934275	JQ934316	JQ934297	KF727625	JQ934375	JQ934396	JQ934419
Meimberg 4.1	JQ934252	JQ934276	JQ934317	JQ934298	KF727626	JQ934376	JQ934397	JQ934420
Puppo 150	JQ934244	JQ934266	JQ934311	JQ934331	JQ934349	JQ934367	JQ934390	JQ934411
Puppo 157	JQ934243	JQ934265	JQ934310	JQ934330	JQ934348	JQ934366	JQ934389	JQ934410
Puppo 188	KF667175	KF667167	KF667159	KF667151	KF667143	KF667135	KF667128	KF667120
Puppo 299	NA	KF667168	KF667160	KF667152	KF667144	KF667136	NA	KF667121
Puppo 184	JQ934245	JQ934267	KF727619	JQ934332	JQ934350	JQ934368	JQ934391	KF727631
Puppo 236	JQ934236	JQ934258	JQ934304	JQ934323	JQ934341	JQ934359	JQ934382	JQ934403
Puppo 239	KF667176	KF667169	KF667161	KF667153	KF667145	KF667137	KF667129	KF667122
Curto *et al.* 2012 (*Mentha*)	JN587699	JN587707	JN587591	JN587599	KF727622	JN587630	KF727628	NA
Curto *et al.* 2012 (*Origanum*)	JN587697	JN587709	NA	KF727620	JN587677	NA	JN587739	JN587757

NA, failed amplification

Appendix S2 Three principal components (PC1, PC2, PC3) of the principal components analyses (PCA) when including all species of *Micromeria*, palaeoisland species only and central species only.

	All			Palaeoisland species			Central species		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalue	14.555	3.147	1.876	16.350	3.662	2.305	8.879	4.112	3.008
Percentage of total variance explained	53.908	11.655	6.949	60.555	13.563	8.538	32.884	15.231	11.140
Cumulative percentage of total variance	53.908	65.563	72.511	60.555	74.118	82.656	32.884	48.115	59.255
Component loading:									
Largest leaf length (mm)	0.639	0.556	0.200	0.605	0.642	−0.300	0.210	0.751	0.263
Largest leaf width (mm)	0.475	0.696	−0.129	0.219	0.826	0.146	0.297	0.687	0.093
Largest leaf petiole length (mm)	0.461	0.646	0.224	0.477	0.675	−0.266	0.061	0.706	−0.249
Largest leaf margin revoluteness (mm)	0.086	−0.448	0.544	0.771	−0.040	−0.279	−0.406	−0.092	0.334
Space between leaf nodes/branches (mm)	−0.393	0.168	0.675	−0.480	0.585	0.074	0.001	0.679	−0.050
Inflorescence peduncle length (mm)	0.015	0.717	0.303	−0.163	0.893	−0.140	−0.153	0.487	0.110
Calyx length (mm)	0.938	0.133	−0.092	0.964	0.144	−0.041	0.634	−0.501	−0.323
Calyx width at the beginning of the lobes (mm)	0.923	−0.002	−0.077	0.934	0.021	0.038	0.607	−0.303	0.031
Deepest calyx lobes length (mm)	0.892	0.084	−0.184	0.905	−0.108	−0.124	0.619	−0.275	−0.231
Deepest calyx lobe width (mm)	0.688	−0.134	0.201	0.839	0.220	0.055	0.601	−0.299	−0.496
Corolla length (mm)	0.968	0.042	0.025	0.976	0.054	0.016	0.849	−0.039	−0.256
Opened corolla width (mm)	0.945	0.048	0.037	0.946	−0.024	−0.089	0.791	0.363	−0.001
Upper lip length (mm)	0.826	0.206	0.213	0.854	0.194	−0.303	0.720	0.457	−0.160
Upper lip width (mm)	0.941	−0.018	0.171	0.953	0.127	0.058	0.808	0.092	0.160
Lower lip length (mm)	0.913	0.012	0.058	0.942	−0.063	−0.160	0.683	0.284	−0.197
Lower lip width (mm)	0.934	0.037	0.081	0.938	0.133	−0.135	0.834	−0.087	−0.031
Lateral lobe length (mm)	0.723	−0.116	−0.079	0.752	−0.357	−0.380	0.741	0.189	−0.253
Lateral lobe width (mm)	0.875	0.006	0.047	0.901	−0.140	−0.156	0.774	0.492	−0.031
Larger stamens theca length (mm)	0.576	−0.492	0.347	0.833	0.064	0.273	0.504	−0.529	0.028
Larger stamens anther width (mm)	0.662	−0.475	0.009	0.692	−0.271	0.503	0.626	−0.237	0.226
Larger stamens filaments length (mm)	0.745	0.059	0.068	0.709	0.226	0.531	0.308	0.167	0.540
Shorter stamens theca length (mm)	0.524	−0.612	0.295	0.765	−0.091	0.509	0.557	−0.388	0.342
Shorter stamens anther width (mm)	0.666	−0.473	−0.051	0.710	−0.246	0.570	0.490	−0.322	0.306
Shorter stamens filaments length (mm)	0.685	0.083	0.027	0.702	0.231	0.527	−0.244	0.116	0.478
Style length (mm)	0.915	0.008	−0.044	0.924	−0.091	−0.235	0.739	0.012	0.133
Upper stigma length (mm)	0.683	−0.026	−0.451	0.695	−0.328	−0.279	0.401	−0.045	0.812
Lower stigma length (mm)	0.578	−0.102	−0.566	0.552	−0.545	−0.280	0.394	−0.105	0.810

Appendix S3 Maximum likelihood phylogeny showing relationships among reconstructed haplotypes for all samples of *Micromeria* included in this study. Numbers above branches represent bootstrap values greater than 80%, samples from Tenerife and Gran Canaria are indicated in vertical lines, other islands are indicated in parenthesis: EH, El Hierro; Md, Madeira; LG, La Gomera; Lz, Lanzarote. See Table 1 in the main paper for full species names. Scale bar indicates average number of nucleotide substitutions per site.



Paper II:

Development and characterization of 16 microsatellite markers for *Micromeria* (Lamiaceae) from Tenerife (Canary Islands, Spain) using 454 sequencing

Pamela Puppo, Manuel Curto, and Harald Meimberg

Conservation Genetics Resources (In Press) DOI: 10.1007/s12686-015-0451-5

Development and characterization of 16 microsatellite markers for *Micromeria* (Lamiaceae) from Tenerife (Canary Islands, Spain) using 454 sequencing

Pamela Puppo[1], Manuel Curto[1], and Harald Meimberg[1,2]

ABSTRACT

Micromeria presents eight species in Tenerife, and from these, three are endangered or critically endangered. In this paper we use a 454 run from *M. hyssopifolia* to develop and characterize 16 new microsatellite markers and test them using 19 individuals from two populations of this species. The number of alleles per locus varied from 2 to 10 and observed heterozygozity ranged from 0.125 to 0.900. Most loci successfully amplified in the threatened species *M. densiflora*, *M. glomerata* and *M. rivas-martinezii* and were also polymorphic. These loci will be valuable tools for assessing the genetic diversity of these endangered species.

Keywords: Lamiaceae; *Micromeria*; microsatellites; 454 GS-FLX

---

[1] CIBIO, Research Center in Biodiversity and Genetic Resources, InBio Associate Laboratory, University of Porto, Campus Vairão, Vairão 4485-661, Portugal

[2] Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences, 1180 Vienna, Austria

Micromeria presents ca. 16 species endemic to the Canary Islands (Spain) and half of these grow in Tenerife. Within Tenerife, one species is endangered (*M. densiflora*) and two are critically endangered (*M. glomerata* and *M. rivas-martinezii*) following the IUCN criteria (Bañares et al. 2004; 2010). Unfortunately, these endangered species are reduced to single small populations located in almost inaccessible areas so material for isolation and characterization of microsatellite markers are scarce and difficult to obtain. In this sense, we used the common *M. hyssopifolia* to develop 16 microsatellite markers to study the genetic diversity and population structure of the species of *Micromeria* present in Tenerife.

DNA was extracted from grounded silica-preserved leaves from one sample of *M. hyssopifolia* from central Tenerife using the Macherey-Nagel Plant DNA Extraction Kit (Macherey-Nagel). DNA was sent to ecogenics GmbH (Zurich, Switzerland) for pyrosequencing on a Roche 454 GS-FLX Titanium platform. Marker screening and primer design was also conducted by this company resulting in 46 candidate loci. Each primer was tagged (5'-end) with one of four different florescent universal primers using the M13-tailed primer method (Oetting et al. 1995). The respective primer sequence was added to the 5'-end of the forward microsatellite primer as described in Curto et al. (2013).

Initial primer screening was conducted using five samples of *M. hyssopifolia* including the sample used for pyrosequencing. The 46 primers were multiplexed in single polymerase chain reactions (PCR) of four or five markers with different fragment lengths and dyes. Amplification was performed using HotStarTaq Plus Master Mix Kit (Qiagen). The multiplex primer combination consisted of 4 nmol of each forward primer, 40 nmol of each reverse primer and the florescent universal primer. The final volume reaction was 10 µL and contained: 5 µL of QIAGEN Multiplex PCR Master Mix (Qiagen), 1 µL of primer mix and 0.5 µL of template DNA (about 40 ng/µL). PCR was performed as follows: 95 ºC for 15 min; 7 cycles of 95 ºC for 30 s; touchdown from 58 ºC to 55 ºC, decreasing 0,5 ºC per cycle for 45 s; 72 ºC for 30 s; 25 cycles of 95 ºC for 30 s; 55 ºC for 45 s; 72 ºC for 30 s; 8 cycles of 95 ºC for 30 s; 54 ºC for 45 s; 72 ºC for 30 s; and 60 ºC for 30 min. Amplification success was confirmed using 2% agarose gels stained with GelRed (Biotium). Sixteen markers amplified consistently, were multiplexed in different PCR (Table 1) as described above, and were used to genotype 19 individuals from two populations of *M. hyssopifolia* from Tenerife. Test for cross amplification was conducted using 5 individuals from *M. glomerata,* 9 individuals from

Table 1 Characteristics of 16 polymorphic microsatellite markers developed for *M. hyssopifolia*.

					Repeat motif	Size range (bp)	Cross Ampl (%)			hys1 (N=10)		
Mix	Locus	Primer sequence (5'-3')	Genebank	Dye			den	glo	riv	Na	Ho	He
M1	2438	F: TGATGGATGAGATGAGAATAATCCG R: ATCTGTCCCAACCGCTACAG	KP090417	VIC	$(GT)_{13}$	178-209	80	**20**	**23**	8	0.778	0.809
M1	3172	F: GCAGCGAGAGAGATTGCTTG R: TGCACATACAAAACACAATCAGC	KP090418	NED	$(TG)_{14}$	199-217	100	100	100	7	0.900	0.820
M1	4418	F: TGCTGAAACTTGAGAGAGAGTG R: GGTCCTACGCGTTTACACAG	KP090421	PET	$(GA)_{21}$	115-151	**40**	80	67	5	0.222	0.457
M1	5232	F: ACCTCACATGCCCCATTCAC R: CGACTGGAATCAACACCACC	KP090422	FAM	$(AG)_{16}$	182-243	100	**0**	100	6	0.400	0.650
M1	5978	F: ACAAAACAAAGGAAATTAGTACACAC R: GAGTCCTCGGCTTCATCACC	KP143519	NED	$(AC)_{11}$	138-146	**0**	**0**	**23**	2	0.400	0.320
M2	234	F: GGTCGGCAAGAGAGAGAGAG R: TCGCATACGAGTCTTCCCC	KP090411	VIC	$(GA)_{14}$	176-222	100	100	100	8	0.600	0.825
M2	753	F: CGCTCAACTTACAGCACCTAC R: GCAGCATGAGCCTTGTGAG	KP090412	NED	$(CAT)_{10}$	131-140	100	100	100	4	0.500	0.655
M2	1763	F: AACCACGCAACCTCTTCTTC R: GGAGAAGGGAAAGGGAAATGG	KP090415	VIC	$(AC)_{12}$	132-148	100	100	100	7	0.600	0.810
M2	3963	F: AGCAAGTCAGTTTTTCTGGC R: AGGTCGAAAATGACGCACAC	KP090420	NED	$(CT)_{13}$	176-211	100	100	100	8	0.600	0.850
M2	5960	F: AAACAAAACAAAGGAAATTATACACAC R: TGTGCCATGTGAGTCGAAAG	KP090424	PET	$(AC)_{11}$	240-260	**20**	100	100	4	0.600	0.580
M3	1018	F: AAGGGGAAGGATCAAAGGATAAG R: GTGTGCCTTGGCAGTCTTG	KP090413	PET	$(AGA)_{8}$	89-120	100	100	100	3	0.200	0.185
M3	1333	F: GGGACTCGATTGCAAAGAAGG R: TCCAGCTCAAGATCAAGACCC	KP090414	VIC	$(TATG)_{9}$	135-166	100	100	100	5	0.500	0.585
M3	2073	F: AGTCATCCACCCCATTCTCC R: CACGATGCTCCGTTCTCATC	KP090416	VIC	$(GA)_{14}$	216-262	100	100	67	9	0.556	0.796
M3	3289	F: GCCACGAAAGAAGGAACGAG R: GTTGCATCGGGCTCCTTTAC	KP090419	FAM	$(TG)_{14}$	205-222	100	100	100	6	0.600	0.790
M3	5419	F: CCACTTCTCCTCATTCTCCCC R: TCTGGGTCAGGTAGCTGTTG	KP090423	PET	$(ATAC)_{10}$	232-259	100	100	100	7	0.600	0.690
M3	6493	F: CAATGGCACCTGGAGATGAG R: CTTACTTGCAGCCGAAGCAC	KP090425	FAM	$(CA)_{12}$	236-279	**20**	80	**45**	8	0.889	0.821

Number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), test for deviation from Hardy-Weinberg Equilibrium (HWE; *P* value), Null allele frequency (Nulls). In bold: loci with significant deviations from HWE ($P < 0.05$), Null allele frequency > 0.1, and cross amplification success of <50%. Cross amplification was conducted using 5 individuals from *M. densiflora* (den), 5 individuals from *M. glomerata* (glo), and 9 individuals from *M. rivas-martinezii* (riv).

Table 1 (Continued)

hys1 (N=10)		hys 2 (N=9)					den (N=5)				glo (N=5)				riv (N=9)			
HWE	Nulls	Na	Ho	He	HWE	Nulls	Na	Ho	He	HWE	Na	Ho	He	HWE	Na	Ho	He	HWE
0.373	0.000	6	0.375	0.773	0.114	**0.233**	5	1.000	0.750	0.285	-	-	-	-	-	-	-	-
0.669	0.000	5	0.667	0.698	0.482	0.000	4	0.600	0.580	0.580	3	1.000	0.580	0.172	6	0.556	0.722	0.263
0.003	**0.163**	6	0.125	0.773	**0.005**	**0.367**	-	-	-	-	2	0.000	0.500	**0.046**	4	0.000	0.722	**0.006**
0.029	**0.117**	7	0.375	0.813	**0.029**	**0.241**	1	0.000	0.000	NA	-	-	-	-	3	0.222	0.494	**0.019**
0.576	0.000	4	0.750	0.656	0.677	0.000	-	-	-	-	-	-	-	-	-	-	-	-
0.012	**0.122**	8	0.889	0.821	0.832	0.000	4	0.600	0.640	0.847	3	0.400	0.540	0.644	4	0.444	0.519	0.125
0.471	0.106	3	0.222	0.549	0.086	**0.212**	1	0.000	0.000	NA	2	0.200	0.180	0.804	3	0.222	0.370	**0.027**
0.452	0.104	4	0.556	0.735	0.108	**0.116**	3	0.400	0.340	0.958	2	0.800	0.480	0.136	4	0.667	0.611	0.719
0.506	**0.130**	10	0.889	0.870	0.525	0.000	3	0.600	0.460	0.821	3	0.200	0.460	0.164	3	0.333	0.549	0.241
0.367	0.000	6	0.667	0.716	0.820	0.026	-	-	-	-	2	0.400	0.320	0.576	1	0.000	0.000	NA
0.989	0.000	2	0.444	0.346	0.391	0.000	2	0.200	0.180	0.804	2	0.000	0.320	**0.025**	2	0.111	0.105	0.860
0.137	0.081	5	0.778	0.568	0.962	0.000	2	0.600	0.420	0.338	1	0.000	0.000	NA	2	0.111	0.278	0.072
0.034	**0.110**	8	0.667	0.772	0.877	0.014	3	0.400	0.460	0.223	1	0.000	0.000	NA	1	0.000	0.000	NA
0.128	**0.118**	6	0.778	0.765	0.507	0.000	1	0.000	0.000	NA	1	0.000	0.000	NA	2	0.111	0.105	0.860
0.818	0.020	4	0.500	0.617	0.148	0.094	2	0.600	0.420	0.338	2	0.400	0.320	0.576	2	0.111	0.278	0.072
0.637	0.000	6	0.333	0.531	**0.027**	**0.130**	-	-	-	-	2	0.000	0.375	**0.046**	-	-	-	-

M. rivas-martinezii, and 5 individuals from *M. densiflora*. Genotyping was performed with an internal size standard (Genescan-500 LIZ; Applied Biosystems) in an ABI3130xl automatic sequencer (Applied Biosystems). Alleles were called using GeneMapper ver. 4.0 (Applied Biosystems). Number of alleles (Na), expected and observed heterozygosity (He and Ho), and deviations of Hardy-Weinberg equilibrium (HWE) were calculated using GenAlEx 6 (http://biology-assets.anu.edu.au/GenAlEx/). Tests for Linkage disequilibrium (LD) were calculated with Genepop v.4.2 (http://genepop.curtin.edu.au/) and null allele frequencies were estimated using FreeNA (http://www1.montpellier.inra.fr/CBGP/software/FreeNA/).

All 16 loci were shown to be polymorphic when amplified with *M. hyssopifolia* (Table 1). Na varied from 2 to 10, Ho and He varied from 0.125 to 0.900 and 0.185 to 0.870 respectively. Four and three loci significantly deviated from HWE ($P < 0.05$) in population one and two respectively (Table 1). All locus pairs across populations were in linkage equilibrium. Null alleles (frequency > 0.1) were detected in six loci in each population (Table 1). Most loci had an amplification success higher than 50% in the congener species tested: 12 loci in *M. densiflora*, 13 in *M. glomerata*, and 13 in *M. rivas-martinezii*. From these, most were polymorphic (Table 1). Even though the use of some of these markers will require caution due to the presence of null alleles, these 16 microsatellite markers will be useful to study the genetic variability of the endangered *Micromeria* species.

ACKNOWLEDGEMENTS

The University of La Laguna in Tenerife provided a vehicle for the field trips. P.L. Pérez de Paz (ULL) was an invaluable help during collection of the plant material. This research was supported by the Fundação para a Ciência e Tecnologia (FCT) with a research grant to H.M. (PTDC/BIA-BEC/108866/2008) and PhD fellowships to P.P. (SFRH/BD/74747/2010) and M.C. (SFRH/BD/79010/2011).

REFERENCES

Bañares, Á, G Blanca, J Güemes, JC Moreno and S Ortiz, eds. (2004) Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Dirección General de Conservación de la Naturaleza, Madrid.

Bañares, Á, G Blanca, J Güemes, JC Moreno and S Ortiz, eds. (2010) Atlas y Libro Rojo de la Flora Vascular Amenazada de España, Adenda 2010. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino)-Sociedad Española de Biología de la Conservación de Plantas, Madrid.

Curto, MA, LR Tembrock, P Puppo, M Nogueira, MP Simmons, H Meimberg (2013) Evaluation of microsatellites of *Catha edulis* (qat; Celastraceae) identified using pyrosequencing. Biochemical Systematics and Ecology 49, 1-9. doi: 10.1016/j.bse.2013.02.002

Oetting, WS, HK Lee, DJ Flanders, GL Wiesner, TA Sellers and RA King (1995) Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. Genomics 30, 450-458. doi: 10.1006/geno.1995.1264

ELECTRONIC SUPPLEMENTARY MATERIAL

Sequences of the 16 loci developed in the present study:

```
>234
ATACGGTCGGCAAGAGAGAGAGAGAAACTGAAGAAGAGGAATTTGATTCAAGAGT
GAGAGAGTGGAGGGGGAGATTTAAAAGATGAGAGAGAGAGAGAGAGAGAGAGAG
AGAAGCAGTGTAGTTGGGGAAGAAGAGTGAGAGAGCTATTAAATTAAAGAATTTAA
AGGGAAGGGGAAGACTCGTATGCGAGGGGAGTGGTGCGCACACGTATACGTATA
CTCAGCTGCGTTA
>753
ACAACCTATGTCACCCATATCTAAGTTCAACTCCAAGTCACGATTGTGATATACGC
TCAACTTACAGCACCTACATTAGGAACAAACATATTCTTGGGAGTGGGATAGCATC
ATCGGCATCATCATCATCATCATCATCATCATCATCCTCGAGCTCACAAGGCTCAT
GCTGCGCGTGGTAG
>1018
ACCTTACGGCTGTTGTCAACACCCTTGTTGATGATGTTAAAAAGGGGAAGGATCAA
AGGATAAGTCAGAGAAGAAGAAGAAGAAGAAGAAGAGGAAAGAGCAC
>1333
AGTGACCGTTGGGTTAGAATGGAAGATCGGCATTGCTACTAAAGATTGGGACTCG
ATTGCAAAGAAGGAAAAGATCGAGGTTAGTCCTCTCGATCCATCGATATGTATGTA
TGTATGTATGTATGTATGTATGTATGATGGTAGTAATGATATATGGTGTGTAGGGT
CTTGATCTTGAGCTGGAAAAGCTTCAGAGTTATGTGAATGCAATCAGTGAAAAAAT
GTTGGATTTGATTGGAAGGTAATT
>1763
ATCATTTGAAAATCCAACTAAATTCAACCACGCAACCTCTTCTTCCTATAAACACCA
AATCATCGCATTGGAAACACACACACACACACACACACACTCATCTACACACACAA
TGATGAGTTCCCTCAAATCCATTTCCCTTTCCCTTCTCCTTTTCCTCTTCCTCCTCC
ACGCCGACGCCATCCGCT
>2073
TACAAAGTCATCCACCCCATTCTCCGCCAGCACCTTCGCCGCCGTTATTCCTATAC
ATACATACACATTCATAACCTAACAATTTATAACCATCATGATGGAATTATATCATAT
ACATGTACTATTTTATGGAGAGAGAGAGAGAGAGAGAGAGAGAGATAGAATGAGT
TGTGTACCGAGAAATACCGGCTACCAACGATGAGAACGGAGCATCGTGGT
>2438
```

```
ACATGGTTTTCTTTTTGATGGATGAGATGAGAATAATCCGATCCATAAAAAGCTTGT
TGAAGGTTAGAATATTTACAGTAGTTATAGAATGGAATAAATTTACTCTCTTTCTCT
CTCGTGTGTGTGTGTGTGTGTGTGTGTTTAATCTACTTCAATTCACCTGTAGCG
GTTGGGACAGATAGGAAATACAAACCCACCATGTAATCAGAGCTGCTGCTGAGTC
TCTTCTACTTCCTTTGACCTAAATTTACACACACTACGAACAGAGCTCTTA
>3172
TATTAGCAACGAGAAGCAGCGAGAGAGATTGCTTGAATGCATCCAGCTTCATCTT
GTCGCTTTGAGAAATATACATATGGAAAACACATAGTTGCTCGCTTTGAACAATTG
TCCGGCGAAGAAGGTCTCCCCCTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTGCA
TTCTGTTCTTTTAGCTGATTGTGTTTTGTATGTGCATGCAGATAATGGATCTTCTGA
GTCGTAGACTAGTTTCTCTACACAGCC
>3289
AGGGCCACGAAAGAAGGAACGAGCCTATGTGTGTGTGTGTGTGTGTGTGTGTGT
GCTATTTGCCAGTAACTCAAAGCATGGTAAGTTGATAAAGATGAATCTGAGTGATC
CATGAAAATCATACTGGCTGTAGAGAATCTATACCAACATCTGTGCAGAAAACTAA
TTCAATATAAAATGTAGGTAAAGGAGCCCGATGCAACAAAATCACTTACATCACTC
ATACAAGTCTTGGGGCCTTCCTTGGATGTGCCATAGGAATCCAGTATAGGCCTCA
CCACAGCTGGAAGGAGGAGTCCTGCAATGCCACATCTCTTAGAAATGGATGGATA
TATTGTTTAACCCACGAAACATGAGGACACAAATTGTTACAGAGAAAGG
>3963
ATAGCAAGTCAGTTTTTCTGGCATTCGACTCTCTCTCTCTCTCTCTCTCTCTCTGCA
TGCTTATGGCGAGTATCGTGAAAACTGAAGCGACGGATGCGGCGTCGCTTTACGA
CCTAGAGTACAAAGCTAGCGAGGACGACGCCTGGTACAGTGTGTGCGTCATTTTC
GACCTTGAAGCCGAGGCGCTCACCGTCAAGTACTTGT
>4418
AAAGTGTCTCTCTGCTGAAACTTGAGAGAGAGTGAGAGAGAGAGAGAGAGAGAG
AGAGAGAGAGAGAGAGAGAGATGATTATCTATGTACTTAAAATGGAATGCACAAA
GAAACAGTAGCACATGACTGTGTAAACGCGTAGGACCTAAATAGAGGCACAAGAA
CATAACTACCAACATAGAAAATGT
>5232
ATAACCGGCTCTACCTCACATGCCCCATTCACTTTACTTTATACTTATACGCATCAA
GTTTTTTCTAATTATGCCCCATTCACTTTACTTTATACTTATACGCATCAAGTTTTTT
CTAATTAAAGTTCCAAAAATCATACAATTATTAATTTCCATTGTCGACCTATCAGAT
CTGTTCATCCACTCAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAAGTGGAG
GTGGTGTTGATTCCAGTCGGCCAGTGAGTGGGGCACGTGCAGATCGTGGAGTTT
```

GCGAAGCTCATCATCCATCATCGTAACCTTCTCCCTCCTGGTATATTAATTAATTTC
TATATATCTCCTTCTTCCTTCTCTAATCTTCT
>5419
ATAGACCACCACCTCTCCTTTTCCTTTTCCTTCTCAACAAACCAACTCCTCCAATTA
AGGACAAAGAACCTTTCTCCTACAGAACACCTAAACTCAAGCACTCCCTTCTCATA
CAAATACCCACTTCTCCTCATTCTCCCCTTCATCACTTCAACTTCAACAACATTCCT
CTCCACAATATACCAATACATACATACATACATACATACATACATACATACATACAT
GATGAAGATGTGTTCCCCCCTNTCTCTCCTCTTCTCTCTCTTCCTCCTAATCCACA
CCACCAATGCCTTAGACATCACCCAAGTCCTCAATCAATACCCTGACTTCACCAAC
TTCAACAGCTACCTGACCCAGACCGGGCTCGCCGTCATCATCAACGGC
>5960
AGGCCAAAAACAAAGAAAAAACAAAACAAAGGAAATTATACACACATACACACACA
CACACACACACACTCATCCGCCACCTTCATTCTAAAGATGTCATGAAATGTCACGA
CCCGACTTTCATAAGGGTGATGAAGCCGAGGACTCGTGATTTGGAATAAGATTAT
CGGAACGGAAATACAATAAAAGAACTTGAAACCTGGTTAACTTGAAGATAAACATT
AGATAGAGAATTCTTTCGACTCACATGGCACAAGGCCTTAAGGATACAAACGGAAT
>6493
AGAAACTTCCACCCCCCTCCAACTTACTAGTACTCTGTTAAATAATTAACTATTAAA
AAAGGTAAAAGTACAATAAAGTTACTATTAAAGTACTAACCCCTTTACGTTTCTTAA
TATTCGGGTCGAAATTACCCTTTTTCCTTGAAATAGGAGTTCTGAATTCTACAATGG
CACCTGGAGATGAGAAATCTGTCAAGACTCTAGTTTTCGCTACTCAGGTCTCTCTC
TCTCTCTCTCACACACACACACACACACACACATTAATCTTGGAAGNGGGATCTTG
AAAAGTAGAATTTGTATGGATGTTTCGAGTACCCTTTGAATCTGAGAGATGGGATA
AACTGAATTTACGCATTTCATATTTGTGCTTCGGCTGCAAGTAAGCATAC
>5978
AGGCCAAAAACAAAGAAAAAACAAAACAAAGGAAATTAGTACACACATACACACAC
ACACACACACACACTCATCCGCCACCTTCATTCTAAAGATGTCATGAAATGTCACG
ACCCGACTTTCATAAGGGTGATGAAGCCGAGGACTCGTGATTTGGAATAAGATTA
TCGGAACGGAAATACAATAAAGAACTTGAAACCTGGTTAACTGAAGATAAACATTA
GATAGAGAATTCTTCGACTCACATGGCACAAGGCCTTAAGGATACAAACGGAAT

Chapter 3:

Diversification of *Micromeria* in the Canary Islands

Paper III Puppo, P, M Curto, J Gusmão-Guedes, J Cochofel, PL Pérez de Paz, C Bräuchler and H Meimberg. (2015) Molecular phylogenetics of *Micromeria* (Lamiaceae) in the Canary Islands, diversification and inter-colonization patterns inferred from nuclear genes. Molecular Phylogenetics and Evolution 89, 160-170. DOI: 10.1016/j.ympev.2015.04.017

Paper III:

Molecular phylogenetics of *Micromeria* (Lamiaceae) in the Canary Islands, diversification and inter-island colonization patterns inferred from nuclear genes

Pamela Puppo, Manuel Curto, Joana Gusmão-Guedes, Jaqueline Cochofel, Pedro Luis Pérez de Paz, Christian Bräuchler, and Harald Meimberg

Molecular Phylogenetics and Evolution 89, 160-170.DOI: 10.1016/j.ympev.2015.04.017

Molecular phylogenetics of *Micromeria* (Lamiaceae) in the Canary Islands, diversification and inter-island colonization patterns inferred from nuclear genes

Pamela Puppo[1], Manuel Curto[1,2], Joana Gusmão-Guedes[1], Jaqueline Cochofel[1], Pedro Luis Pérez de Paz[3], Christian Bräuchler[4,5], and Harald Meimberg[2]

ABSTRACT

Here we reconstruct the evolutionary history of *Micromeria* in the Canary Islands using eight nuclear markers. Our results show two centers of diversification for *Micromeria*, one in the eastern islands Gran Canaria and Lanzarote, the other in the western islands, Tenerife, La Palma and El Hierro. Suggested directions of inter-island colonization are the following: Gran Canaria to Lanzarote and La Gomera; Tenerife to La Palma (from the paleoisland of Teno), to El Hierro (from the younger, central part), and to La Gomera and Madeira (from the paleoislands). Colonization of La Gomera probably occurred several times from Gran Canaria and Tenerife. The taxonomic implications of these results are discussed. Incongruence among the different markers was evaluated and, using next generation sequencing, we investigated if this incongruence is due to gene duplication.

Keywords: Canary Islands; diversification; gene duplication; island biogeography; *Micromeria*; oceanic islands

---

[1] Research Center in Biodiversity and Genetic Resources (CIBIO) / InBio Associated Laboratory, University of Porto, Campus Vairão, R. Monte-Crasto, 4485-661 Vairão, Portugal.

[2] Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences, A-1180 Vienna; Austria.

[3] Departamento de Biología Vegetal (Botánica), Universidad de La Laguna, 38271 - La Laguna, Tenerife, Spain.

[4] Department of Biology I, Section: Biodiversity Research, Ludwig-Maximilians-University Munich, Menzinger Str. 67, 80638 Munich, Germany

[5] Restoration Ecology, Technical University Munich, Emil Raman Str. 6, 85350 Freising, Germany.

INTRODUCTION

Oceanic islands by definition have never been in contact to the mainland so their unique biodiversity evolved after colonization events, isolated from continental biota (Emerson, 2002). The intense volcanic activity that characterizes most of these islands provides a variety of altitudinal gradients that, in addition to pronounced differences in rainfall regimes and a high heterogeneity in edaphic conditions, creates a wide range of habitats. This mosaic of habitats produces high levels of endemic taxa. In fact, about one fourth of all known plant species are endemic to islands (Kreft et al., 2008). Volcanic islands have a life cycle in which they are born, grow and emerge, gain their maximum area and height, and finally are reduced by erosion until below the sea level (Fernández-Palacios et al., 2011). According to the general dynamic model of oceanic islands (Whittaker et al., 2007, 2008), the speciation opportunities in these islands would be directly related to the island's life cycle, while catastrophic events such as landslides, caldera collapses, etc, can further shape the island's area also impacting its diversity levels.

The Canary Archipelago is composed of seven islands located in the Atlantic Ocean, ca. 100 km off the coast of Northwestern Africa. The archipelago is of volcanic origin, being older in the east (20 Ma, Fuerteventura) and younger in the west (1 Ma, El Hierro), the islands being at different stages in this cycle. Fuerteventura and Lanzarote are already much eroded, being little more than low-altitude plains, the erosion process in Gran Canaria and La Gomera has already begun, while Tenerife, La Palma, and El Hierro are still growing as evidenced by their recent volcanic activity (Juan et al., 2000; Fernández-Palacios et al., 2011). Of the latter, Tenerife has the most complex geological history. It started as three islands (palaeoislands), Adeje (11.6-3.5 Ma), Teno (6.7-4.5 Ma) and Anaga (6.5-3.6 Ma), in the late Miocene that became connected during the late Miocece-Pliocene forming the current island ca. 2 Ma ago (Ancochea et al., 1990). Gran Canaria is also heterogeneous, although not due to the secondary connection of once separate parts, but rather by different areas being at different phases of volcanic activity. The SW half of the island is known as "paleo-canaria" and is composed of Miocene substrates, the NE is called "neocanaria" and it was revitalized by eruptions during the Pliocene (del Arco et al., 2002). On the contrary, Lanzarote and Fuerteventura formed one island (Mahan) until the late Pliocene when, due to erosion and changes in the sea level, its middle part submerged making the two current islands (Fernández-Palacios et al., 2011).

The Canary Islands have been the focus of evolutionary studies not only because of its well-known geological history but also because of its high proportion of endemic taxa (ca. 40% of its flora; Carine et al., 2004). The Canarian archipelago served as refugia in the late Miocene and Pliocene when the first glaciation of the northern hemisphere and the desertification of northern Africa led to massive migrations of plants and animals, so its fauna and flora are mainly related to that of the Mediterranean basin (Francisco-Ortega et al. 1996). Other elements of the archipelago’s biodiversity however, are related to more distant regions such as East Africa, Asia, America and even Australia as indicated by several molecular studies (see Juan et al. 2000). According to Francisco-Ortega et al. (1996), most plant taxa in the Canary archipelago have undergone inter-island diversification while remaining in similar ecological zones. This is supported by phylogenies where taxa inhabiting similar habitats form a single clade suggesting that adaptation to ecological zones happened only once. Inter-island diversification has been observed in several genera: *Adenocarpus* DC. (Fabaceae) (Percy and Cronk, 2002), *Lotus* L. (Fabaceae) (Allan et al., 2004), *Aeonium* Webb & Berthel. (Crassulaceae) (Mes and Hart, 1996; Mort et al., 2002), *Crambe* L. (Brassicaceae) (Francisco-Ortega et al., 2002), *Argyranthemum* Webb (Asteraceae) (Francisco-Ortega et al., 1996), *Pericallis* D. Don (Asteraceae) (Panero et al., 1999), and *Sonchus* L. (Asteraceae) (Kim et al., 1996). However, in some cases, all taxa on one island form a clade, and adaptation to similar habitats on different islands occurs independently. This can be interpreted as signature of adaptive radiation, the rapid adaptive diversification of a clade, and this has been observed for example in *Bystropogon* L’Hér. (Lamiaceae) (Trusty et al., 2005), *Sideritis* L. (Lamiaceae) (Barber et al., 2000), *Gonospermum* Less. and *Pericallis* D.Don (Asteraceae) (Francisco-Ortega et al., 2001 and Jones et al., 2014 respectively), *Teline* Medik. (Fabaceae) (Percy and Cronk, 2002) and the Madeiran species of *Argyranthemum* and *Sonchus* (Asteraceae) (Francisco-Ortega et al., 1996 and Kim et al., 1996 respectively).

The genus *Micromeria* Benth. (Nepetoideae, Lamiaceae) is composed of ca. 54 species in Macaronesia, the Mediterranean region, and some parts of Africa and Asia (Bräuchler et al., 2008). In the Macaronesian region, it is present in the Canary Islands, Madeira and Cape Verde archipelagos. Previous molecular studies using cpDNA (Bräuchler et al. 2005, 2010) show that *Micromeria* forms a monophyletic group in the Canary Islands (it includes the Madeiran *M. varia* subsp. *thymoides)*, and is mostly related to taxa present in Morocco (*M. fontanesii*) and the Balearic Islands (*M. inodora*).

The species from Cape Verde form a separate group suggesting an independent colonization. In the Canary Islands, *Micromeria* is represented by ca. 16 species distributed in all islands, Tenerife and Gran Canaria being centers of diversity with 8 and 7 species respectively (Pérez de Paz, 1978; see Table 1). The other five islands have one (Fuerteventura and Lanzarote share the same taxon) or two species (La Gomera, La Palma, El Hierro). Most species are endemic to one island and only one species, *M. varia*, is present in all islands of the archipelago (Table 1). Previous work using ISSR fingerprint patterns indicate that the species present on more than one island might in fact be different lineages since in most other cases species from the same island form one group (Meimberg et al., 2006). These findings suggest that *Micromeria* might be another example where adaptation to different habitats within an island might be the prevailing mechanism explaining species richness. On the other hand, multi-locus phylogenetic analyses showed that *Micromeria* species from Tenerife seemed to have undergone at least three phases of diversification, two on the palaeoislands and a more recent one in the young, central part of Tenerife (Puppo et al., 2014). This might indicate that diversification of *Micromeria* in Tenerife is influenced by ongoing adaptation to relatively recently originated habitats but also by the secondary contact of the palaeoislands caused by the elevation of the central Teide massif (Puppo et al., 2014). *Micromeria* thus is an interesting example where the relative importance of inter-island dispersal and adaptation as explanations for a genus' diversity on an archipelago can be compared.

Here, we present a phylogenetic analysis of the species of *Micromeria* from the Canary Islands based on eight nuclear markers, including a complete sampling of all species and subspecies present on all islands. With this approach we aim to determine: (1) if the species in each island form a monophyletic group as suggested by previous data; (2) whether species diversity on Gran Canaria can be explained by one or several diversification events, and if there is any relation to the geology of the island; and (3) if there is a connection between the diversifications on Tenerife and those of the other islands. We also aim to verify if all currently recognized species are supported by molecular data. And finally, we investigate locus congruence and duplication using a low coverage Illumina run to determine copy numbers of the markers in the genome.

Table 1. Taxa recognized in the Canary Islands and Madeira indicating abbreviations used in the different figures (Abbrev.), the island they inhabit, and their distribution (following Pérez de Paz 1978). Altitude in parenthesis indicates rare occurrences.

Species	Abbrev.	Island	Distribution
M. benthamii Webb & Berthel.	M. ben	Gran Canaria	highland and pine forest, above 700 m
M. helianthemifolia Webb & Berthel. var. helianthemifolia	M. hel	Gran Canaria	rocky cliffs in the W, 200-1400 m
var. mary-annae P.Pérez & Kunkel		Gran Canaria	scarps in central part, above 1000 m
M. lanata Benth.	M. lan	Gran Canaria	dry montain forest, 700-1900 m
M. leucantha Svent. ex P.Pérez	M. leu	Gran Canaria	cliffs near San Nicolás de Tolentino (SW), 500-1000 m
M. pineolens Svent.	M. pin	Gran Canaria	pine forest and cliffs in the NW, 700-1400 m
M. tenuis Benth. subsp. tenuis*	M. tenuis	Gran Canaria	stony soils and cliffs in the SW, 200-800 m
subsp. linkii (Webb & Berthel.) P.Pérez	M. ten lin	Gran Canaria	rocky walls in the N, NW, below 200(400) m
M. densiflora Benth.	M. den	Tenerife	old rocky cliffs in Buenavista (Teno), 450-600 m
M. glomerata P.Pérez	M. glo	Tenerife	old rocky cliffs in N Anaga, 300-400 m
M. hyssopifolia Webb & Berthel. var. hyssopifolia	M. hys hys	Tenerife, El Hierro	Tenerife: pine forest, 400-2000 m; El Hierro: around all the island, 0-1500 m
var. glabrescens P.Pérez	M. hys gla	Tenerife	degraded areas in the N, 300-600 m
var. kuegleri (Bornm.) P.Pérez	M. hys kue	Tenerife	coastal desert in the SW, S, SE, 0-400 m
M. lachnophylla Webb & Berthel.	M. lach	Tenerife	highland in central part, above 1500 m
M. lasiophylla Webb & Berthel. subsp. lasiophylla	M. las las	Tenerife	highland cliffs in the SE, above 2000 m
subsp. palmensis (Bolle) P.Pérez	M. las pal	La Palma	highland cliffs in the N, 2000-2400 m
M. rivas-martinezii Wildpret	M. riv-mar	Tenerife	old rocks in Juan Bay (Anaga), 0-150 m
M. teneriffae (Poir) Benth. var. teneriffae*	M. ten ten	Tenerife	rocky walls in the SE from Anaga to Fasnia, 100-500 m
var. cordifolia P.Pérez*	M. ten cor	Tenerife	coastal desert in the SW (Fasnia and Güímar), 20-150 m
M. herpyllomorpha Webb & Berthel.	M. herp	La Palma	the whole island, (5)50-1500 (1800) m
M. lepida Webb & Berthel. subsp. lepida*	M. lep lep	La Gomera	forest clearings throughout the island, above 800 m
subsp. bolleana P.Pérez*	M. lep bol	La Gomera	humid areas in the SE, (100)300-700 m
M. varia Benth. subsp. varia	M. var var	Tenerife, La Gomera	Tenerife: in the N from Teno to Anaga, (5)50-1000 m; La Gomera: N half of the island, 0-1400 m
subsp. canariensis P.Pérez	M. var can	Gran Canaria	humid cliffs and rocky soils in the NE half of the island, 400-1000 m
subsp. gomerensis P.Pérez	M. var gom	La Gomera	around all the island, 200-800 m
subsp. hierrensis P.Pérez	M. var hie	El Hierro	in the two N extremes of the island (Sabinosa and Mirador de la Peña), 400-800 m
subsp. meridialis P.Pérez	M. var mer	Gran Canaria	dry areas in the SE half of the island, 100-1500 m
subsp. rupestris (Webb & Berthel.) P.Pérez	M. var rup	Lanzarote, Fuerteventura	scattered in both islands, above 500 m
subsp. thymoides (Sol. ex Lowe) P.Pérez*	M. var thy	**Madeira archipelago**	scattered in the islands, 0-1800 m

*Pérez de Paz (1978) described two varieties / forms for each of this subspecies / varieties. These are not included here.

MATERIAL AND METHODS

Plant Material

Seventy six individuals were studied: 74 from all currently recognized species and subspecies of *Micromeria* in the Canary Islands (sensu Pérez de Paz, 1978), one from Madeira, and one (*M. inodora*) from the Balearic Islands (Table 2). The latter species, shown to be sister to *Micromeria* in the Canary Islands in previous phylogenetic analyses (Bräuchler et al. 2005, 2010; Puppo et al., 2014), was used as outgroup. Only one sample from the taxon inhabiting Lanzarote and Fuerteventura (*M. varia* subsp. *rupestris*) was available since fieldwork on this island was not possible in the course of this study. Most samples were collected during 2010-2012, a few samples from earlier studies also being included (Bräuchler et al., 2005; Meimberg et al., 2006; see Table 2). At least one individual was collected per population sampled 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 (MSB), Germany. For DNA analysis, leaves were conserved in silica gel.

Amplification and sequencing

Silica-preserved leaves were ground and used for DNA purification with the Macherey-Nagel Plant DNA Extraction Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. For phylogenetic analyses we amplified all 76 samples of *Micromeria* using eight nuclear loci. These loci were selected from those published by Curto et al. (2012) based on sequence quality and variability (number of polymorphisms). Curto et al. (2012) identified the loci by comparing EST sequences from several Lamiaceae species to the *Arabidopsis thaliana* genome and designing primers targeting potential intron regions. These markers were also used in Puppo et al. (2014) and details concerning sequence structure can be found in Table 3. Amplification was performed using HotStarTaq Plus Master Mix Kit (QIAGEN, Valencia, California, USA) with a final volume of 10ul and containing 1 U Taq DNA polymerase, 75 nmol MgCl2, 1 nmol of each dNTP, 4 nmol of each primer, and ca. 20 ng template DNA. Polymerase Chain Reaction (PCR) was conducted with the following cycle program: 95°C for 15 min; 40 cycles of 95°C for 30 s, 55ºC for 1 min, 72°C for 1 min; and a final extension step of 60°C for 30 min. Successful amplifications were confirmed by electrophoresis in 2% agarose gels stained with GelRed (Biotium, Hayward, California, USA) and fragment lengths were compared with Lambda - pUC Marker 4 ladder (Fermentas, St. Leon-Rot, Germany). Cleaning of PCR products was performed using Exo/Sap digestion in a final volume of 8ul containing 4 U Exonuclease I (Fermentas) and 1 U Shrimp Alkaline Phosphatase (Fermentas) for 15 min at 37ºC and inactivated for 15 min at 85ºC. The purified DNA was sequenced for all samples in both directions using an ABI 3730 (Applied Biosystems, Carlsbad, California, USA) at the

LMU sequencing facility in Munich, Germany. All sequences used in the present study can be accessed in GenBank (see Appendix A).

Table 2. *Micromeria* samples used in the present study indicating voucher/source and locality. MSB: Herbarium of the Ludwig-Maximilians University in Munich; TFC: Herbarium of the Universidad de la Laguna in Tenerife. *No geographical coordinates are provided for restricted taxa; **sample only used for genome sequencing (see text).

Island	Code	Species	Source	Locality
El Hierro	H1E	M. hyssopifolia var. hyssopifolia	Pérez de Paz H1E (TFC)	Costa de Valverde. 27º48'49,9"N; 17º53'52,8"W
	H7D	M. hyssopifolia var. hyssopifolia	Pérez de Paz H7D (TFC)	Cumbre de Malpaso. 27º43'34"N; 18º02'09"W
	H6C	M. hyssopifolia var. hyssopifolia	Pérez de Paz H6C (TFC)	El Julán. “Arenales”. 27º43'02"N; 18º04'28"W
	H5A	M. varia subsp. hierrensis	Pérez de Paz H5A (TFC)	Mirador de Risco Bascos. 27º45'16"N; 18º07'06"W
	H5B	M. varia subsp. hierrensis	Pérez de Paz H5B (TFC)	Mirador de Risco Bascos. 27º45'16"N; 18º07'06"W
Gran Canaria	H4_1	M. benthamii	Meimberg 4_1 (MSB)	27°53'46,8"N; 15°41'3,5"W
	GC439_1	M. benthamii	Puppo 439.1 (TFC)	27º53'23,4"N; 15º33'41,3"W
	C154	M. benthamii	Bräuchler 154 (MSB)	27º56'29,4"N; 15º38'57,6"W
	P343	M. benthamii	Puppo 343 (TFC)	Sobre pueblo Fontanales. 28º03'35,5"N; 15º36'32,8"W
	H25	M. helianthemifolia	Meimberg 25 (MSB)	27°49'11,6"N; 15°34'40,9"W
	C179	M. helianthemifolia	Bräuchler 179 (MSB)	27º49'09,3"N; 15º34'42"W
	P428	M. helianthemifolia	Puppo 428 (TFC)	Mirador del Bco de Fataga. 27º49'14,6"N; 15º34'39,4"W
	lanGC	M. lanata	Meimberg et al. 2006	
	H8_1	M. lanata	Meimberg 8_1 (MSB)	27°54'34,2"N; 15°41'14,1"W
	C150	M. lanata	Bräuchler 150 (MSB)	27º54'33,9"N; 15º41'57,8"W
	P547	M. lanata	Puppo 547 (TFC)	Carretera Ayacata-Tejera (GC-60) km 12. 27º57'32,4"N; 15º37'34"W
	C175	M. leucantha	Bräuchler 175 (MSB)	27º59'14,3"N; 15º44'23,58"W
	P500	M. leucanta	Puppo 500 (TFC)	Sobre pueblo de San Nicolás. 27º58'42,5"N; 15º45'48,4'"W
	GC170	M. pineolens	Bräuchler 170 (MSB)	28º03'14,22"N; 15º41'24,3"W
	C169	M. pineolens	Bräuchler 169 (MSB)	28º03'27,6"N; 15º41'18,8"W
	P387	M. pineolens	Puppo 387 (TFC)	Pinar de Tamadaba, zona recreativa. 28º03'27"N; 15º41'18,7"W
	H20_2	M. tenuis	Meimberg 20_2 (MSB)	27°59'22,8"N; 15°41'26,6"W
	Mten	M. tenuis	Meimberg et al. 2006	
	C146	M. tenuis	Bräuchler 146 (MSB)	27º52'08,4"N; 15º44'9,8"W
	P477	M. tenuis subsp. linkii	Puppo 477 (TFC)	Agaete. 28º05'33,4"N; 15º42'3,7"W
	P487	M. tenuis subsp. linkii	Puppo 487 (TFC)	Andén verde. 28º01'51,1"N; 15º45'56,2"W
	P320	M. tenuis subsp. linkii	Puppo 320 (TFC)	Cuesta de Silva, sobre El Roque. 28º08'32,1"N; 15º35'28"W
	P458	M. tenuis subsp. tenuis	Puppo 458 (TFC)	Barranco de Arguineguin. 27º51'46,8"N; 15º39'54,9"W
	P496	M. tenuis subsp. tenuis	Puppo 496 (TFC)	Sobre pueblo de San Nicolás. 27º58'45"N; 15º45'48,1"W
	LF1	M. varia subsp. canariensis	Meimberg LF1 (MSB)	28º55'48,4"N; 15º35'39,8"W
	LF2	M. varia subsp. canariensis	Meimberg LF2 (MSB)	28º55'48,4"N; 15º35'39,8"W
	LF3	M. varia subsp. canariensis	Meimberg LF3 (MSB)	28º55'48,4"N; 15º35'39,8"W
	LF4	M. varia subsp. canariensis	Meimberg LF4 (MSB)	28º55'48,4"N; 15º35'39,8"W
	P330	M. varia subsp. canariensis	Puppo 330 (TFC)	Bco de los Tiles de Moya. 28º05'33,8"N; 15º35'39,2"W
	P352	M. varia subsp. canariensis	Puppo 352 (TFC)	Fontanales. 28º03'29,2"N; 15º36'43,1"W
	P416	M. varia subsp. meridialis	Puppo 416 (TFC)	Bco de Fataga. 27º48'33,4"N; 15º34'59,5"W
	P508	M. varia subsp. meridialis	Puppo 508 (TFC)	Sobre pueblo de Tasarte. 27º55'39,2"N; 15º44'59,6"W
	H21_3**	M. varia	Meimberg 21_3 (MSB)	27º49'10,6"N; 15º34'38,2"W

Table 2 (Continued)

Island	Code	Species	Source	Locality
La Gomera	574_4	M. lepida subsp. bolleana	Puppo 574.4 (TFC)	Entrada del Bco Benchijigua. 28º04'48,3"N; 17º12'33"W
	573_2	M. lepida subsp. bolleana	Puppo 573.2 (TFC)	Mirador de la Laja. 28º05'58,5"N; 17º11'5,6"W
	577_4	M. lepida subsp. lepida	Puppo 577.4 (TFC)	Mirador de los Roques. 28º06'33,5"N; 17º12'51"W
	579_6	M. varia subsp. gomerensis	Puppo 579.6 (TFC)	Carretera las Hayas-Cercado. 28º07'33,6"N; 17º17'01,9"W
	581_2	M. varia subsp. gomerensis	Puppo 581.2 (TFC)	Pueblo de Arure. 28º07'55,5"N; 17º19'14,7"W
	565_10	M. varia subsp. varia	Puppo 565.10 (TFC)	Carretera Agulo-Las Rosas. 28º11'13,2"N; 17º12'38,1"W
	582_3	M. varia subsp. varia	Puppo 582.3 (TFC)	Mirador de El Rejo, PN Garajonay. 28º07'30,5"N; 17º12'31,5"W
La Palma	34A	M. herpyllomorpha	Pérez de Paz 34A (TFC)	Barranco de Las Palmas, Jedey, El Paso. 28º34'29"N; 17º52'24"W
	35A	M. herpyllomorpha	Pérez de Paz 35A (TFC)	Camino de Las Angustias, Llanos de Aridane. 28º39'26"N; 17º56'04"W
	32A	M. herpyllomorpha	Pérez de Paz 32A (TFC)	Casa Camineros, Tiguerorte, Mazo. 28º33'36"N; 17º47'57"W
	28C	M. lasiophylla subsp. palmensis	Pérez de Paz 28C (TFC)	Entre Morro Negro y Pico de La Cruz. 28º44'57"N; 17º50'54"W
	30A	M. lasiophylla subsp. palmensis	Pérez de Paz 30A (TFC)	Inmediaciones Pico de La Nieve. 28º43'48"N; 17º50'02"W
	29A	M. lasiophylla subsp. palmensis	Pérez de Paz 29A (TFC)	Mirador Degollada Bco. de Franceses. 28º45'39"N; 17º51'59"W
Lanzarote	Lanz	M. varia subsp. rupestris	Bräuchler et al. 2005	
Tenerife	D255	M. densiflora*	Puppo 255 (TFC)	Buenavista, Teno.
	D257	M. densiflora*	Puppo 257 (TFC)	Buenavista, Teno.
	G200	M. glomerata*	Puppo 200 (TFC)	Taganana, Anaga.
	G202	M. glomerata*	Puppo 202 (TFC)	Taganana, Anaga.
	562_3	M. hyssopifolia var. glabrescens	Puppo 562.3 (TFC)	Lomo Morin, Teno. 28º21'35,3"N; 16º47'20,8"W
	V239	M. hyssopifolia var. glabrescens	Puppo 239 (TFC)	Rambla de Castro. 28°23'45,3"N; 16°35'23,3"W.
	560	M. hyssopifolia var. glabrescens	Puppo 560.1 (TFC)	Rambla, desembocadura del bco de Ruiz. 28º23'42,9"N; 16º37'29"W
	H162	M. hyssopifolia var. hyssopifolia	Puppo 162 (TFC)	Bco. Herques, Fasnia-Güímar. 28°14'59,2"N; 16°26'17,5"W
	H153	M. hyssopifolia var. hyssopifolia	Puppo 153 (TFC)	Güímar. 28°17'40,1"N; 16°24'10,8"W
	HK181	M. hyssopifolia var. kuegleri	Puppo 181 (TFC)	Acantilado La Hondura. 28°12'03,1"N; 16°25'29,5"W
	HK175	M. hyssopifolia var. kuegleri	Puppo 175 (TFC)	Los Abades. 28°08'28,1"N; 16°27'16,4"W
	L291	M. lachnophylla*	Puppo 291 (TFC)	Las Cañadas.
	L295	M. lachnophylla*	Puppo 295 (TFC)	Las Cañadas.
	L274	M. lasiophylla subsp. lasiophylla*	Puppo 274 (TFC)	Las Cañadas.
	L276	M. lasiophylla subsp. lasiophylla*	Puppo 276 (TFC)	Las Cañadas.
	R212	M. rivas-martinezii*	Puppo 212 (TFC)	Roque de Juan Bay, Anaga.
	R214	M. rivas-martinezii*	Puppo 214 (TFC)	Roque de Juan Bay, Anaga.
	TC188	M. teneriffae var. teneriffae	Puppo 188 (TFC)	Bco. del Tahodio, Anaga. 28°29'53,5"N; 16°15'32,1"W
	TC299	M. teneriffae var. cordifólia	Puppo 299 (TFC)	Roques de Fasnia. 28º13'08,2"N; 16º24'55,2"W
	T157	M. teneriffae var. teneriffae	Puppo 157 (TFC)	Bco. Herques, Fasnia-Güímar. 28°14'59,2"N; 16°26'17,5"W
	T150	M. teneriffae var. teneriffae	Puppo 150 (TFC)	Güímar. 28°17'40,1"N; 16°24'10,8"W
	V236	M. varia subsp. varia	Puppo 236 (TFC)	Bco. de Antequera, Anaga.28º32'53,6"N; 16º08'54,8"W
	V184	M. varia subsp. varia	Puppo 184 (TFC)	San Andrés, Anaga. 28°30'58,3"N; 16°10'29,1"W
Madeira	Mvthy	M. varia subsp. thymoides	Meimberg et al. 2006	
Outgroup				
Balearic Is.	BG65	M. inodora (Desf.) Benth.	Bräuchler et al. 2005	Formentera. Cult. Botanical Garden Munich.

Sequence analyses

Geneious v.6.1.4 (Biomatters, Auckland, New Zealand) was used to check and edit electropherograms and to align sequences using default parameters for gap opening and extension (Geneious alignment); heterozygous point mutations were included using IUPAC codes (W, R, Y, S, K, and M). DnaSP v.5.10 (Librado and Rozas, 2009) was used for calculating summary statistics for each locus. The most likely substitution model for the combined alignment as well as for each locus was determined with jModelTest 0.1.1 (Posada, 2008) using all the 88 models implemented in this program and the most suitable model was chosen using the Akaike Information Criterion (AIC; Akaike, 1974). Phylogenetic analyses were conducted on the combined alignment of all 8 loci and phylogenetic trees were inferred using maximum likelihood (ML) and Bayesian inference (BI). ML was performed with 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 with MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) and BEAST 1.7.5 (Drummond et al., 2012) using the CIPRES Science Gateway (Miller et al., 2010). MrBayes was conducted in two independent runs with a total of 4 chains; BEAST was used with a relaxed uncorrelated lognormal clock and a Yule process tree. Analyses started with random trees and ran for 30 (MrBayes) or 100 (BEAST) million generations sampling every 1000 generations, and with a burn-in of 30%. Trees were combined using MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) (MrBayes) or TreeAnnotator 1.7.4 (Drummond et al., 2012) (BEAST) and the resulting trees were edited using FigTree v.1.7 (http://tree.bio.ed.ac.uk/software/figtree). Loci were concatenated for ML and BI analyses to increase support of the phylogenetic inference (Gatesy and Baker, 2005).

Haplotype reconstructions were conducted using PHASE (Stephens et al., 2001) as implemented in DnaSP using default parameters. In order to visualize the relationships among the different haplotypes, Network v.4.6.1.2 (http://www.fluxus-engineering.com) was used for reconstructing median-joining (MJ) networks (Bandelt et al., 1999) of each of the eight loci used in this study. Indices of nucleotide (Pi) and haplotype (Hd) diversity for each locus were calculated with DnaSP at three different levels: total (all samples), per island (only for islands with more than one sample), and per group of islands using the groups observed in the phylogeny. The partition of genetic variability was calculated in Arlequin v.3.11 (Excoffier et al., 2005) using a hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992) among groups, among islands within groups, and within islands, using the groups depicted in the phylogeny. A Neighbor-Net Network of the reconstructed haplotypes was constructed in SplitsTree v.4.13.1 (Huson and Bryant, 2006) using the combined alignment of the 8 nuclear markers. The analysis is based on uncorrelated patristic distances with a bootstrap analysis of 1000 replicates.

Gene duplication

To test for gene duplication, we compared one sequence per marker against a de novo assembly draft of a low coverage run using one lane of an Illumina HiSeq, on genomic sequences of one *Micromeria varia* individual from Gran Canaria (H21_3; see Table 2). The sequences used were *M. varia* sequences taken from Curto et al. (2012). To create a whole genome reference, a library with an average insert size of 500 bp was prepared and paired end sequenced on an Illumina HiSeq 2000 (Illumina, San Diego, California, USA) for 100 cycles each end. Library preparation and sequencing was performed by the University of Chicago Genomics Core facility. Illumina sequencing read quality was assessed using the program FastQC v.0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). De novo assembly was performed by the software Velvet v.1.2.10 (Zerbino and Birney, 2008) with a K-mer size of 61 bp. Sequences were compared to the reference using Blastn v.2.2.25 (Altschul et al., 1997) using the default settings with no DUST filtering. Only hits with an overlap of at least 50%, a minimum identity of 95% and an e-value of at least 0.001 were considered.

Table 3. List of the eight markers used in the present study (taken from Curto et al., 2012) indicating gene, length, number of introns (NI), percentage of intron coverage (IC), number of exons (NE), percentage of exon coverage (EC), intron (I) and exon (E) positions, model and nst as calculated with jMODELTEST, number of polymorphic sites (S), number of haplotypes (h), and number of informative positions (IP).

Marker	Gene	Length	NI	IC (%)	NE	EC (%)	I Positions	E Positions	Model	nst	S	h	IP
M. pip. 056	gapdH	694	4	53	4	47	3-99; 198-299; 443-535; 620-694	1-2; 100-197; 300-442; 536-619	HKY+G	6	55	57	32
M. pip. 057	SINAC1	338	0	0	1	100		1-338	K80	2	10	20	4
M. pip. 014	LeMa-1	727	2	81	3	19	54-249; 338-675	1-53; 250-337; 676-727	TPM2uf+G	2	49	57	27
M. pip. 017	HRGP1	1400	5	72	4	28	1-85; 256-634; 712-1126; 1193-1295; 1371-1400	86-255; 635-711;1127-1192; 1296-1370	HKY+G	2	84	99	49
M. pip. 047	adk	708	3	62	3	38	1-90; 149-225; 408-689	80-148; 226-409; 690-708	TrN+G	6	48	91	26
M. pip. 027	CamKK1	684	2	65	2	35	1-366; 483-563	367-482; 564-684	HKY	2	30	44	24
M. pip. 002	DXR	901	5	63	5	37	128-218; 444-506	1-127; 219-443	HKY+I	2	69	94	31
O. oni. 007	MAPKK1	506	2	30	2	70	1-81; 204-283; 391-473; 586-690; 760-869	82-203; 284-390; 474-585; 691-758; 870-901	HKY+I	1	28	97	15

RESULTS

The combined alignment of the eight loci used in this study consisted of 5958 positions, 373 of which were polymorphic between 10 (M.pip.057) to 84 (M.pip.017) per locus (Table 3). Some samples failed to amplify with single markers: sample TC299 failed to amplify with markers M.pip.056 and M.pip.002, and samples 34A and P428 failed to amplify with marker M.pip.017. Two other samples (P428 and P458) were excluded from the M.pip.014 alignment because their sequences were too different from the others and amplification of unspecific products or pseudo genes was suspected.

Samples from the Canary Islands are divided in two highly supported clades (Fig. 1). One includes the samples from the eastern islands: Gran Canaria and Lanzarote (Bayesian posterior probabilities, BPP=0.98), the other includes the samples from the western islands: Tenerife, La Palma and El Hierro (BPP=1). The ML tree also shows these two major clades but a sample of *M. lanata* from Gran Canaria is sister to all other Canarian samples (Appendix B). Samples from La Gomera occur in both clades (Fig. 1). This grouping is observed also in the analyses of all single markers, shown as haplotype networks in Fig. 2.

The eastern island clade consists of two subgroups (subgroup I and II) (Fig. 1). Subgroup I has medium support (BPP=0.80) and includes all *M. varia* from Gran Canaria, Lanzarote and *M. varia* subsp. *gomerensis* from La Gomera. Samples of *M. pineolens* and *M. helianthemifolia* are also in this group, together with one sample of *M. tenuis*, *M. leucantha* and *M. benthamii*. Subgroup II is highly supported (BPP=0.95) and is composed of *M. lepida* from La Gomera, the other sample of *M. leucantha*, and most samples of *M. lanata*, *M. tenuis* and *M. benthamii*. BI and ML analyses are congruent, but with one small difference. In the ML tree samples of *M. helianthemifolia* and *M. pineolens* are placed at the base of subgroup II instead of the base of subgroup I., but support is weak in both analyses (Appendix B).

The western island clade is more complex and is divided into three subgroups: a, b, and c (Fig. 1). The general pattern of the samples from Tenerife is in agreement to the previous study of Puppo et al. (2014). One sample from both *M. teneriffae* var. *teneriffae* and *M. varia* are early-divergent branches and then there are the three subgroups. Subgroup a (BPP=0.89) is formed by the species narrowly restricted to the palaeoisland of Anaga, *M. glomerata* and *M. rivas-martinezii*, plus the other samples of *M. teneriffae*. Subgroup b (BPP=0.80) is composed of *M. densiflora*, the species restricted to the palaeo-island of Teno. Subgroup c (BPP=0.93) is composed of the species from central Tenerife: *M. varia*, *M. hyssopifolia*, *M. lasiophylla*, and *M. lachnophylla*. Samples from the remaining western islands and Madeira occur within this group. *Micromeria varia* subsp. *thymoides* from Madeira and the samples of *M. varia* subsp. *varia* from La Gomera form a polytomy with the subgroups. Samples from La Palma (*M. herpyllomorpha* and *M. lasiophylla* subsp. *palmensis*) form a clade sister to *M. densiflora* in subgroup b. Samples from El Hierro (*M. varia* subsp. *hierrensis* and *M. hyssopifolia*) are placed within subgroup c, together with the species from Central Tenerife. ML and BEAST analyses show some slight differences, but the three subgroups are recovered (Anaga, *M. densiflora* + La Palma, and central Tenerife + El Hierro) (see Appendices B and C).

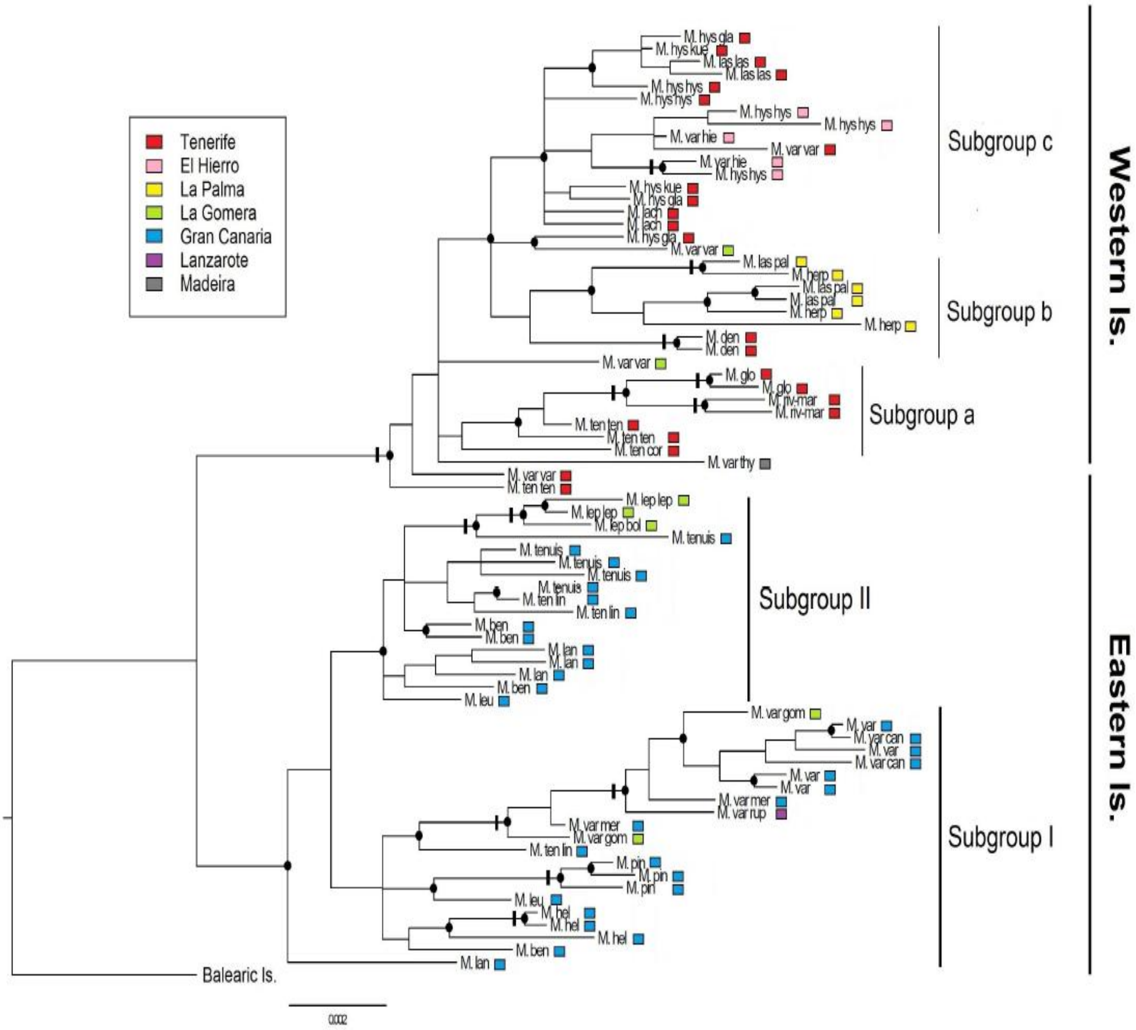


Fig. 1. Bayesian phylogeny calculated in MrBayes showing relationships of all taxa of *Micromeria* present in the Canary Islands and Madeira. Filled circles indicate BPP ≥ 0.90; vertical bars indicate bootstrap ≥ 80%; different colors correspond to the different islands. See Table 1 for full species names.

The haplotype reconstructions showed variable levels of haplotype diversity among the loci, most of them high (Hd>0.9) except for M.pip.057 (Hd=0.6704), while nucleotide diversity varied from Pi=0.00477 (M.pip.057) to Pi=0.01457 (O.oni.007; Table 4). Most MJ networks show the split between the western islands (Tenerife, La Palma, El Hierro) and the eastern islands (Gran Canaria and Lanzarote), while samples from La Gomera are found in both groups (Fig. 2). Levels of diversity within islands vary between the different markers but on average, Tenerife, La Gomera, and Gran Canaria show the highest levels of Hd (>0.85) and Pi (>0.006) (Table 4). Haplotype and nucleotide diversity and AMOVA were calculated at a group level for the western and eastern groups as observed in the phylogeny. Levels of Hd are relatively high for most markers (Hd>0.9) while Pi values are moderate (Pi>0.004), AMOVA results show that most of the genetic variation (55%) is found within islands. Polyphyly of La Gomera was also investigated at the marker level by including all samples of both species from this island in the western or eastern group only and then comparing them with the original groupings. Our results show that even though Hd and Pi values do not vary significantly from the original groupings, the genetic differentiation in the AMOVA among groups diminishes for each locus when samples from La Gomera were included only in the western or eastern groups suggesting that polyphyly of La Gomera is supported at the individual marker level. The Neighbor-Net network (Fig. 3) also showed the distinction between the western and eastern islands providing a graphical representation of the haplotype relationships.

Illumina sequencing resulted in 53 519 272 sequences corresponding to an expected coverage of 14.6 x considering an estimated *Micromeria varia* C value of 367Mbp (Suda et al., 2003). Because of generally good sequence quality no trimming was necessary. A total of 39 525 269 reads were used for the genome assembly creating 168 488 contigs with an average coverage of 6.2 x and a N50 of 8817 bp. The resulting contigs summed up to a total of 224 450 543 bp (~57 % of estimated genome size) being the largest one 137 351 bp. The blast search was able to find all loci on the reference with high similarity (Table 5). Two loci, M.pip.002 and O.oni.007, were not completely mapped because the overlap length was shorter than the sequence length. This was a consequence of either the assembly regions not being covered enough and coded with Ns during scaffolding, or parts of the sequence being out of the contig range (see S start and S end on Table 5). Of the eight loci analyzed, only O.oni.007 had more than one hit and thus has signs of duplication corresponding to four positive matches of an overlap superior to 50%.

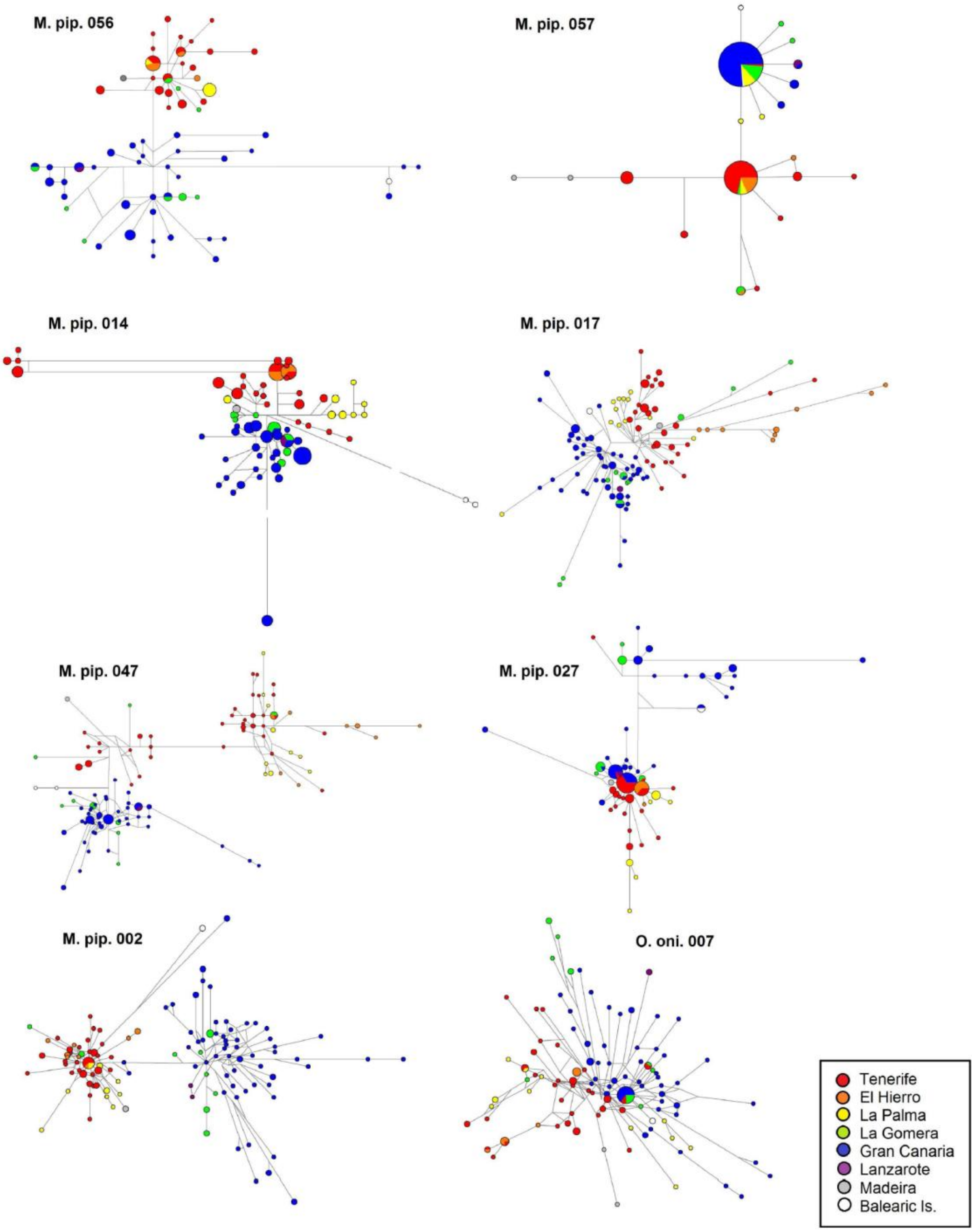


Fig. 2. Median Joining haplotypes for each locus used in this study. The area of each circle is proportional to the frequency of the haplotype represented. Different colors correspond to the different islands. See Table 1 for full species names.

Table 4. Measures of haplotype (Hd) and nucleotide (Pi) diversity for each marker at different scales: all samples (total), per island (only for islands with more than one sample), per group as observed in the phylogeny, and per group when all samples from La Gomera are included in the western (W) or eastern (E) island group. LP: La Palma, EH: EL Hierro, T: Tenerife, LG: La Gomera, GC: Gran Canaria; N: number of samples included in each group; *significant at p<0.01; ** significant at p<0.05.

			M. pip. 056	**M. pip. 057**	**M. pip. 014**	**M. pip. 017**	**M. pip. 047**	**M. pip. 027**	**M. pip. 002**	**O. oni. 007**	**Avg.**
Total		Hd	0.979	0.670	0.979	0.995	0.990	0.945	0.995	0.984	**0.942**
		Pi	0.011	0.005	0.009	0.007	0.012	0.008	0.009	0.015	**0.009**
Per island	LP (N=6)	Hd	0.303	0.636	0.909	1	0.97	0.727	0.894	0.985	**0.803**
		Pi	0.001	0.003	0.006	0.005	0.008	0.005	0.003	0.012	**0.005**
	EH (N=5)	Hd	0.622	0.378	0.556	0.978	0.911	0.2	0.956	0.8	**0.675**
		Pi	0.003	0.002	0.001	0.009	0.011	0.000	0.004	0.013	**0.005**
	T (N=22.23)	Hd	0.921	0.532	0.948	0.971	0.961	0.789	0.961	0.984	**0.883**
		Pi	0.004	0.003	0.009	0.005	0.009	0.003	0.003	0.012	**0.006**
	LG (N=7)	Hd	0.934	0.593	0.89	0.967	0.912	0.769	0.934	0.912	**0.864**
		Pi	0.011	0.005	0.007	0.007	0.010	0.009	0.007	0.014	**0.009**
	GC (N=30.32)	Hd	0.971	0.178	0.935	0.983	0.947	0.915	0.988	0.955	**0.859**
		Pi	0.007	0.001	0.005	0.005	0.006	0.009	0.007	0.009	**0.006**
Groups as in phylogeny	W (N=36.37)	Hd	0.908	0.647	0.951	0.988	0.974	0.85	0.963	0.986	**0.908**
		Pi	0.004	0.004	0.009	0.007	0.009	0.004	0.004	0.013	**0.007**
	E (N=36.38)	Hd	0.973	0.222	0.943	0.982	0.954	0.924	0.987	0.953	**0.867**
		Pi	0.007	0.001	0.005	0.005	0.006	0.009	0.006	0.011	**0.006**
	% AMOVA	a. groups	48.4	48.92	19.02	19.26	54.15	19.35	46.75	10.73	**33.32**
		a. islands	7.14	25.09	13.81	22.63	9.96	11.66	7.14	14.72	**14.02**
		w. islands	44.45	25.99	67.17	58.11	35.89	68.99	46.11	74.55	**52.66**
	F. indices	Fsc	0.138*	0.491*	0.171*	0.280*	0.217*	0.145*	0.134*	0.165*	**0.218**
		Fst	0.555*	0.740*	0.328**	0.419*	0.641*	0.310*	0.539*	0.254*	**0.473**
		Fct	0.484**	0.489	0.190	0.193	0.541	0.194	0.468	0.107	**0.333**
W group + all LG samples	W (N=41.42)	Hd	0.927	0.696	0.96	0.99	0.978	0.878	0.97	0.985	**0.923**
		Pi	0.007	0.005	0.009	0.007	0.011	0.005	0.006	0.014	**0.008**
	E (N=31.33)	Hd	0.97	0.226	0.937	0.983	0.944	0.908	0.986	0.958	**0.864**
		Pi	0.007	0.001	0.005	0.005	0.006	0.009	0.006	0.009	**0.006**
	% AMOVA	a. groups	26.26	21.29	10.43	1.03	30.92	1.82	26.54	2.17	**15.06**
		a. islands	26.02	50.32	20.68	37.57	30.41	25.44	24.28	21.36	**29.51**
		w. islands	47.72	28.39	68.89	61.4	38.67	72.74	49.18	76.47	**55.43**
	F. indices	Fsc	0.353*	0.639*	0.231*	0.379*	0.440*	0.259*	0.330*	0.218*	**0.356**
		Fst	0.523*	0.716*	0.311*	0.386*	0.613*	0.273*	0.508*	0.235*	**0.446**
		Fct	0.263	0.213	0.104	0.010	0.309	0.182	0.265	0.022	**0.171**

Table 4 (Continued)

			M. pip. 056	**M. pip. 057**	**M. pip. 014**	**M. pip. 017**	**M. pip. 047**	**M. pip. 027**	**M. pip. 002**	**O. oni. 007**	**Mean**
E group + all LG samples	W (N=34.35)	Hd	0.907	0.624	0.946	0.987	0.977	0.834	0.96	0.986	**0.903**
		Pi	0.004	0.004	0.009	0.007	0.009	0.004	0.004	0.013	**0.007**
	E (N=38.40)	Hd	0.975	0.298	0.949	0.984	0.958	0.929	0.988	0.952	**0.879**
		Pi	0.008	0.001	0.006	0.005	0.007	0.009	0.007	0.011	**0.007**
	% AMOVA	a. groups	41.26	42.41	18.06	14.8	48.8	16.91	42.59	10.48	**29.41**
		a. islands	13.29	31.06	14.57	26.31	14.7	13.62	10.74	14.94	**17.40**
		w. islands	45.45	26.53	67.36	58.89	36.5	69.48	46.68	74.58	**53.18**
	F. indices	Fsc	0.226*	0.539*	0.178*	0.309*	0.287*	0.164*	0.187*	0.167*	**0.257**
		Fst	0.545*	0.735*	0.326*	0.411*	0.635*	0.305*	0.533*	0.254*	**0.468**
		Fct	0.413	0.424	0.181	0.148	0.488	0.169	0.426	0.105**	**0.294**

DISCUSSION

Colonization patterns of Micromeria in the Canary and Madeira archipelagos

A number of studies have showed that many species groups in the Canary Islands follow an east to west colonization direction, from Lanzarote and Fuerteventura, the older islands, towards the younger western islands according to a stepping stone model (Kimura and Weiss, 1964; see e.g. Juan et al., 2000; Silvertown et al., 2005; Dlugosch and Parker, 2007). In the present study, the split among *Micromeria* samples between eastern and western islands is also evident (Fig. 4). Furthermore, our data shows that *Micromeria* in the Canary Islands probably radiated from two centers: one on Gran Canaria, the other on Tenerife. Radiations from these two islands have also been observed in other groups of plants (Böhle et al., 1996; Kim et al., 1996) and animals (Thorpe et al., 1994; Ribera et al., 2003).

Within the eastern islands, samples from Gran Canaria form two main groups that are apparently not correlated with geological age but may be a consequence of massive extinctions or vicariance due to volcanic activity (Juan et al., 2000), incomplete lineage sorting, or ongoing gene flow among some of the species. According to the relationships within the eastern group, *Micromeria* from Gran Canaria could have colonized Lanzarote at least once and La Gomera twice. Lanzarote may have been the site of the initial colonization of the Canary Islands, but because of its extensive erosion, some of its original flora may have become extinct and it has since been recolonized from Gran Canaria. In La Gomera, at least one colonization event gave origin to *M. varia* subsp. *gomerensis* and at least one other to *M. lepida*.

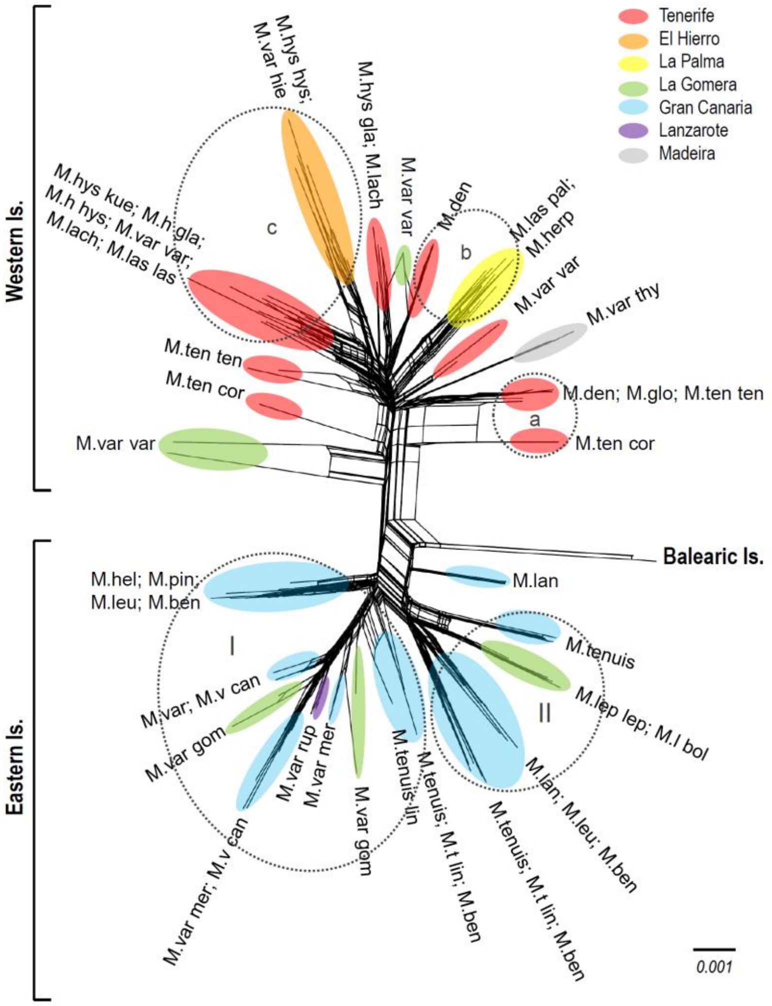


Fig. 3. Neighbor-Net network calculated in SplitsTree showing relationships among reconstructed haplotypes of all species of *Micromeria* present in the Canary Islands and Madeira. Different colors correspond to the different islands. Dotted circles indicate subgroups corresponding to Fig. 1. See Table 1 for full species names.

Unlike the eastern group, the clades in the western group seem related to the geological evolution of Tenerife and generally agree with the results obtained by Puppo et al. (2014). Our study suggests that El Hierro was colonized from the central part of Tenerife. La Palma was probably colonized from Teno as indicated by the phylogeny, where it groups with the samples of *M. densiflora*. This is also most likely in a stepping stone model since Teno is geographically closer to La Palma. La Gomera might have been colonized once from Anaga and once from Teno or central Tenerife, hence the position of *M. varia* subsp. *varia* on the tree (Fig.1). Studies in other groups of plants and animals have also shown Tenerife to be the source for the colonization of other western islands such as La Gomera, El Hierro, and La Palma (Juan et al., 2000; Ribera et al., 2003; Dlugosch and Parker, 2007). Colonizations from Tenerife's palaeoislands have also been described for some groups (Juan et al., 2000; Trusty et al., 2005), e.g. *Hypericum* (Hypericaceae) from Anaga colonized El Hierro (Dlugosch and Parker, 2007), and *Gallotia* (Lacertidae) from Adeje colonized La Gomera and from Anaga colonized La Palma (Thorpe et al., 1994). The initial colonization of Madeira may have been from Anaga or central Tenerife as suggested by the position of *M. varia* subsp. *thymoides* in the phylogeny. Our previous study suggested colonization from Anaga (Puppo et al., 2014) so we consider this as the most plausible scenario. Fernández-Palacios et al. (2011) found that the Canary Islands were linked to Madeira via the Savage Islands making a stepping stone connection among these archipelagos possible. The colonization of Madeira via Tenerife has also been observed in numerous other plant groups (Böhle et al., 1996; Barber et al., 2000; Lee et al., 2005; Trusty et al., 2005; Dlugosch and Parker, 2007).

Overall, inter-island colonization seems to have played a major role in the diversification of *Micromeria* in the Canary Islands and Madeira archipelagos. For most of the islands, adaptation to different environments after a single colonization event seems to have been the primary mode of speciation. In the case of La Gomera, multiple colonizations from Gran Canaria and Tenerife gave origin to at least three different lineages: *M. varia* subsp. *varia*, *M. varia* subsp. *gomerensis* and *M. lepida*. These taxa inhabit different ecological zones than their respective sister taxa so diversification in allopatry of taxa with similar ecological requirements does not seem able to explain these multiple lineages. Further studies are needed, perhaps at a population level, to help elucidate the diversification of *Micromeria* on La Gomera.

Lack of monophyly in some species and taxonomic implications

In our phylogeny, the status of several species is confirmed while other species or subspecies appear to be polyphyletic, so being at odds with the taxonomy of Pérez de Paz (1978). An explanation of incongruence between taxonomy and phylogenetic analyses may be hybridization or incomplete lineage sorting. Furthermore, it seems that hybridization is common in plant groups with a single colonization event (Carine et al., 2004) and at least for Canarian taxa, it has been well-documented in *Sideritis* (Barber et al., 2000), *Argyranthemum* (Brochman, 1984; Francisco-Ortega et al., 1996), and *Pericallis* (Jones et al., 2014).

Among the eastern islands, *M. pineolens* and *M. helianthemifolia* from Gran Canaria as well as *M. lepida* from La Gomera are supported as species forming monophyletic groups in the phylogeny. One sample from *M. leucantha* and most samples from *M. tenuis*, *M. benthamii,* and *M. lanata* from Gran Canaria group together in subgroup II except from one sample each that are scattered within subgroup I. Hybridization might explain this lack of monophyly, perhaps especially in *M. benthamii* and *M. lanata*, since hybrids have been frequently observed in the field with most other species from Gran Canaria.

In Tenerife, the species narrowly restricted to the palaeoislands of Anaga (*M. glomerata* and *M. rivas-martinezii*) and Teno (*M. densiflora*) are all supported. *Micromeria teneriffae* from Anaga, and the species from central Tenerife are not monophyletic; similar results were obtained and discussed in detail by Puppo et al. (2014). The two species on La Palma are not supported in the present analysis since *M. herpyllomorpha* and *M. lasiophylla* subsp. *palmensis* are intermixed on the tree. Also, *M. lasiophylla* from La Palma and *M. lasiophylla* from Tenerife are well apart on the tree. The relationship between the samples from El Hierro (*M. varia* subsp. *hierrensis* and *M. hyssopifolia*) and samples from central Tenerife (*M. varia* and *M. hyssopifolia*) seems to be somewhat supported but the lack of resolution within subgroup c makes it difficult to conclude that these taxa are directly linked.

Table 5. Blast analysis of the markers used against a reference genome of *Micromeria varia* including: Genbank reference number of the sequences used (Genbank Nº), markers name (Query), matching contig identity number attributed by the assembler (Subject), query (Q) and subject (S) length, starting and ending positions of the matching regions, overlap length, and percentage of identity.

Genbank Nº	Query	Subject	Q length	S length	Q start	Q end	S start	S end	Overlap length	% overlap	% identity
JN587737	M. pip. 002	561652	992	13078	15	600	12287	11702	586	59.07	99.66
JN587590	M. pip. 014	377346	738	30442	21	754	19015	19748	734	99.46	100
JN587606	M. pip. 017	434788	1425	24487	16	1439	11084	9661	1424	99.93	99.86
JN587624	M. pip. 027	446118	751	11018	17	764	7448	6701	748	99.60	98.93
JN587671	M. pip. 047	55108	746	3826	16	760	1096	354	745	99.87	99.6
JN587692	M. pip. 056	362346	761	9513	28	784	2210	2964	757	99.47	98.15
JN587704	M. pip. 057	17379	428	2012	13	437	225	649	425	99.30	98.35
JN587752	O. oni. 007	252359	736	1234	22	592	1132	560	573	77.85	97.91
JN587752	O. oni. 007	257829	736	6374	22	570	554	4	551	74.86	96.01
JN587752	O. oni. 007	510473	736	13740	22	608	589	1	589	80.03	97.28
JN587752	O. oni. 007	533712	736	808	152	608	459	1	459	62.36	95.42

Samples from *M. varia* appear scattered among the phylogeny making the species polyphyletic. This same pattern has been observed in previous studies (Meimberg et al., 2006; Puppo et al., 2014). In the eastern islands, samples from *M. varia* from Gran Canaria, Lanzarote and *M. varia* subsp. *gomerensis* from La Gomera form a single group indicating they are probably related. In the western islands however, *M. varia* might be more than one taxon since samples of *M. varia* subsp. *varia* from Tenerife and La Gomera do not group together. From our phylogeny, the two subspecies of *M. varia* from La Gomera are widely separate and would need to be separated as species: subsp. *gomerensis* probably originated from a source on Gran Canaria and subsp. *varia* most likely from one on Tenerife. A taxonomical revision based on the present results is currently being prepared and will be published elsewhere.

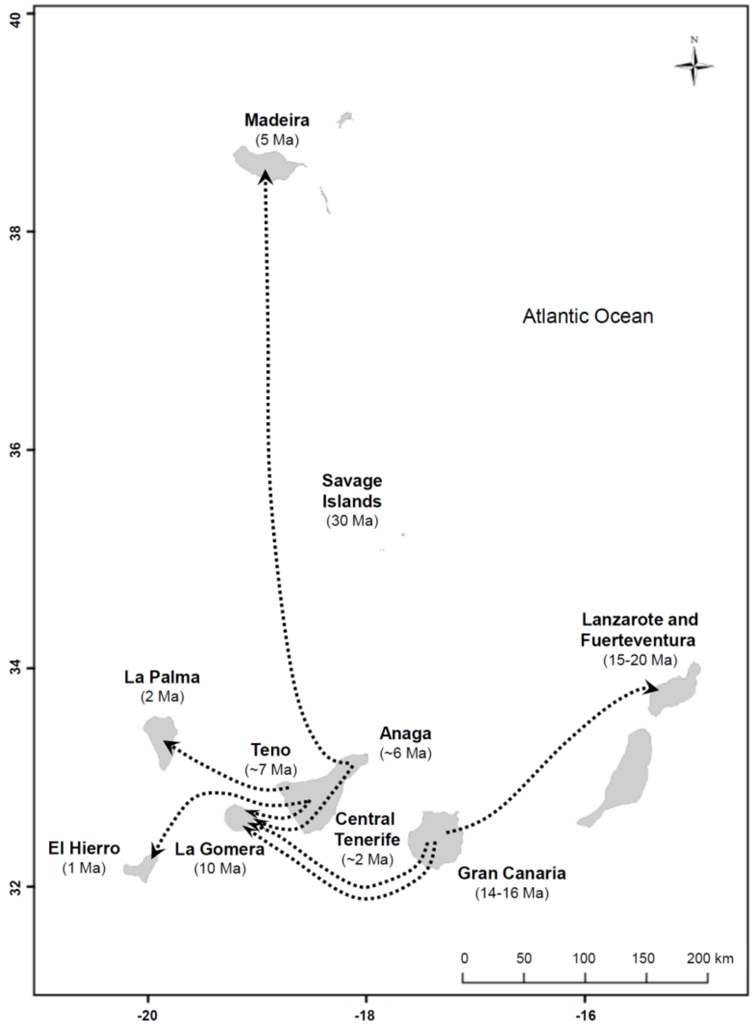


Fig. 4 Map of the Canary Islands and Madeira showing the islands' ages. Black dotted lines represent colonization routes of *Micromeria* as inferred from the Bayesian phylogeny on Fig. 1.

Gene duplication

We tried to assess the number of copies of the loci used in this study by comparing their sequences to a reference genome. This way, if multiple significant hits are found it is very likely for that locus to be duplicated in the genome. Although indirect approaches, such as looking for excess of heterozygosity, may be good indications of gene duplication they are still sensitive to sequencing errors and contamination. By direct comparison to the genome it is possible to confirm the duplication status of a gene though this still depends on the quality of the reference used. In our case, the assembly of the reference is still in a preliminary stage so there is a risk of not detecting the duplication of some genes because the whole genome is not represented. For that reason we can only confirm duplication status but not exclude it. Among the markers used in the present study, we detected that the locus O.oni.007 has at least four copies in the *Micromeria* genome. This is concordant with the observations of excess of heterozygosity reported by Curto et al. (2012) and it may explain the lack of resolution of this marker in the individual MJ networks. On the other hand, this lack of resolution may rather be an artifact of haplotype reconstruction that infers that the locus has two paralog sequences rather than the two orthologs. Nonetheless, when unphased data is analyzed, duplicated markers can still contribute some true phylogenetic signal if there is some degree of convergent evolution among paralogs. Examples of duplicated loci frequently used in phylogenetics are the ribosomal genomic regions ITS and ETS (Nieto-Feliner and Rosselló, 2007). In our case, when O.oni.007 is removed from the concatenated analyses (Appendix D) the position of the sample from the Balearic Islands is not resolved and forms a polytomy with the eastern and western groups. Relationships within the eastern group are maintained, but relationships within the western group are less resolved (Appendix D). This indicates that O.oni.007 contains phylogenetic signal especially for the western group.

The genome of *M. varia* is being sequenced as part of another study, and data are still being collected and analyzed. We expect in the future to have a good quality reference genome that can be used as an important resource for the study of *Micromeria* species from the Canary Islands.

ACKNOWLEDGEMENTS

The authors thank the University of La Laguna in Tenerife for facilitating a car for the collection of the plant material in Gran Canaria, Tenerife, and La Gomera during 2010-2012. Francisco Faure was an important help during field work. Victor Garzón-Machado provided the map that served as base for Fig. 4. Saurabh Dilip helped with the de novo genome assembly and Blast analyses. Guillermo Velo-Antón helped with the BEAST run. Peter Stevens helped improve the English. The authors also thank the two anonymous reviewers who helped improve this manuscript with their helpful comments and suggestions. The research is supported by the Fundação para a Ciência e Tecnología (FCT) with a research grant to H.M. (PTDC/BIA-BEC/108866/2008), and Ph.D. fellowships to P.P. (SFRH/BD/74747/2010) and M. C. (SFRH/BD/79010/2011).

Appendices A, B, C, D. Supplementary data

REFERENCES

Akaike, H (1974) A new look at the statistical model identification. IEET Transactions on Automatic Control 19, 716-723.

Allan, GJ, J Francisco-Ortega, A Santos-Guerra, E Boerner and EA Zimmer (2004) Molecular phylogenetic evidence for the geographic origin and classification of Canary Island *Lotus* (Fabaceae: Loteae). Molecular Phylogenetics and Evolution 32, 123-138.

Altschul, SF, TL Madden, AA Schaffer, JH Zhang, Z Zhang, W Miller and DJ Lipman (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25, 3389-3402.

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

Bandelt, HJ, P Forster and A Rohl (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16, 37-48.

Barber, JC, J Francisco-Ortega, A Santos-Guerra, A Marrero and RK Jansen (2000) Evolution of endemic *Sideritis* (Lamiaceae) in Macaronesia: Insights from a chloroplast DNA restriction site analysis. Systematic Botany 25, 633-647.

Böhle, U-R, HH Hilger and WF Martin (1996) Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). Proceedings of the Natural Academy of Sciences of Philadelphia 93, 11740-11745.

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

Bräuchler, C, O Ryding and G Heubl (2008) The genus *Micromeria* (Lamiaceae), a synoptical update. Willdenowia 38, 363-410.

Bräuchler, C, H Meimberg and G Heubl (2010) Molecular phylogeny of Menthinae (Lamiaceae, Nepetoideae, Mentheae) -Taxonomy, biogeography and conflicts. Molecular Phylogenetics and Evolution 55, 501-523.

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

Carine, MA, SJ Russell, A Santos-Guerra and J Francisco-Ortega (2004) Relationships of the Macaronesian and Mediterranean Floras: Molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Convolvulus* (Convolvulaceae). American Journal of Botany 91, 1070-1085.

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

del-Arco, M, M Salas, JR Acebes, M del C Marrero, JA Reyes-Betancort and PL Pérez de Paz (2002) Bioclimatology and climatophilous vegetation of Gran Canaria (Canary Islands). Annales Botanici Fennici 39, 15-41.

Dlugosch, KM and IM Parker (2007) Molecular and quantitative trait variation across the native range of invasive species *Hypericum canariense*: evidence of ancient patterns of colonization via pre-adaptation? Molecular Ecology 16, 4269-4283.

Drummond, AJ, MA Suchard, X Dong and A Rambaut (2012) Bayesian phylogenetics with BEAUTi and the BEAST 1.7. Molecular Biology and Evolution 29, 1969-1973.

Emerson, BC (2002) Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. Molecular Ecology 11, 951-966.

Excoffier, L, PE Smouse, and JM Quattro (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes -Application to human mitochondrial DNA restriction data. Genetics 131, 479-491.

Excoffier, L, G Laval and S Schneider (2005) Arlequin v.3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1, 47-50.

Fernández-Palacios, JM, L de Nascimento, R Otto, JD Delgado, E García-del-Rey, JR Arévalo and RJ Whittaker (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, RK Jansen and A Santos-Guerra (1996) Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. Proceedings of the Natural Academy of Sciences of Philadelphia 93, 4085-4090.

Francisco-Ortega, J, JC Barber, A Santos-Guerra, R Febles-Hernández and RK Jansen (2001) Origin and evolution of the endemic genera of Gonosperminae (Asteraceae: Anthemideae) from the Canary Islands: evidence from nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA. American Journal of Botany 88, 161-169.

Francisco-Ortega, J, J Fuertes-Aguilar, S-C Kim, A Santos-Guerra, DJ Crawford and RK Jansen (2002) Phylogeny of the Macaronesian endemic *Crambe* section *Dendrocrambe* (Brassicaceae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. American Journal of Botany 89, 1984-1990.

Gatesy, J and RH Baker (2005) Hidden likelihood support in genomic data: can forty-five wrongs make a right? Systematic Biology 54, 483-492.

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

Huson, DH and D Bryant (2006) Application of Phylogenetic Networks in Evolutionary Studies. Molecular Biology and Evolution 23, 254-267.

Jones, KE, JA Reyes-Betancort, SJ Hiscock and MA Carine (2014) Allopatric diversification, multiple habitat shifts and ancient hybridization in the evolution of

Pericallis (Asteraceae), a Macaronesian endemic genus. American Journal of Botany 101, 637-651.

Juan, C, BC Emerson, P Oromí and GM Hewitt (2000) Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. Trends in Ecology and Evolution 15, 104-109.

Kim, SC, DJ Crawford, J Francisco-Ortega and A Santos-Guerra (1996) A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. Proceedings of the Natural Academy of Sciences of Philadelphia 93, 7743-7748.

Kimura, M and GH Weiss (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49, 561-576.

Kreft, H, W Jetz, J Mutke, G Kier and W Barthlott (2008) Global diversity floras from a macroecological perspective. Ecology Letters 11, 116-127.

Lee, C, SC Kim, K Lundy and A Santos-Guerra (2005) Chloroplast DNA phylogeny of the woody Sonchus alliance (Asteraceae: Sonchinae) in the Macaronesian Islands. American Journal of Botany 92, 2072-2085.

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

Meimberg, H, T Abele, C Bräuchler, JK McKay, PL Pérez de Paz and G Heubl (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.

Mes, THM and HT Hart (1996) The evolution of growth forms in the Macaronesian genus *Aeonium* (Crassulaceae) inferred from chloroplast DNA RFLPs and morphology. Molecular Ecology 5, 351-363.

Miller, MA, W Pfeiffer and T Schwartz (2010) Creating the CIPRES Science Gateway for inference of large phylogenetics trees, in: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp. 1-8.

Mort, ME, DE Soltis, PS Soltis, J Francisco-Ortega and A Santos-Guerra (2002) Phylogenetics and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. Systematic Botany 27, 271-288.

Nieto-Feliner, G and JA Rosselló (2007) Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. Molecular Phylogenetics and Evolution 44, 911-919.

Panero, JL, J Francisco-Ortega, RK Jansen and A Santos-Guerra (1999) Molecular evidence for multiple origins of woodiness and a New World biogeographic connection of the Macaronesian Island endemic *Pericallis* (Asteraceae: Senecioneae). Proceedings of the Natural Academy of Sciences of Philadelphia 96, 13886-13891.

Percy, DM and QCB Cronk (2002) Different fates of island brooms: contrasting evolution in *Adenocarpus, Genista,* and *Teline* (Genisteae, Fabaceae) in the Canary Islands and Madeira. American Journal of Botany 89, 854-864.

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

Posada, D (2008) jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution 25, 1253-1256.

Puppo, P, M Curto, G Velo-Antón, PL Pérez de Paz and H Meimberg (2014) The influence of geological history on diversification of insular species: genetic and morphological patterns of *Micromeria* (Lamiaceae) in Tenerife (Canary archipelago). Journal of Biogeography 41, 1871-1882.

Ribera, I, DT Bilton and AP Vogler (2003) Mitochondrial DNA phylogeography and population history of *Meladema* diving beteles on the Atlantic Islands and in the Mediterranean basin (Coleoptera, Dytiscidae). Molecular Ecology 12, 153-167.

Ronquist, F and JP Huelsenbeck (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572-1574.

Silvertown, J, J Francisco-Ortega and M Carine (2005) The monophyly of island radiations: an evaluation of niche pre-emption and some alternative explanations. Journal of Ecology 93, 653-657.

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

Suda, J, T Kyncl and R Freiova (2003) Nuclear DNA amounts in Macaronesian angiosperms. Annals of Botany 92, 153-164.

Thorpe, RS, DP McGregor, AM Cumming and WC Jordan (1994) DNA evolution and colonization sequence of island lizards in relation to geological history: mtDNA RFLP, cytochrome B, cytochrome oxidase, 12S ribosomal RNA sequence, and nuclear RAPD analysis. Evolution 48, 230-240.

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

Whittaker, RJ, RJ Ladle, MB Araújo, JM Fernández-Palacios, JD Delgado and JR Arévalo (2007) The island immaturity -speciation pulse model of island evolution: an alternative to the “diversity begets diversity” model. Ecography 30, 321-327.

Whittaker, RJ, KA Triantis and RJ Ladle (2008) A general dynamic theory of oceanic island biogeography. Journal of Biogeography 35, 977-994.

Zerbino, DR and E Birney (2008) Velver: algorithms for de novo short read assembly using de Brujin graphs. Genome Research 18, 821-829.

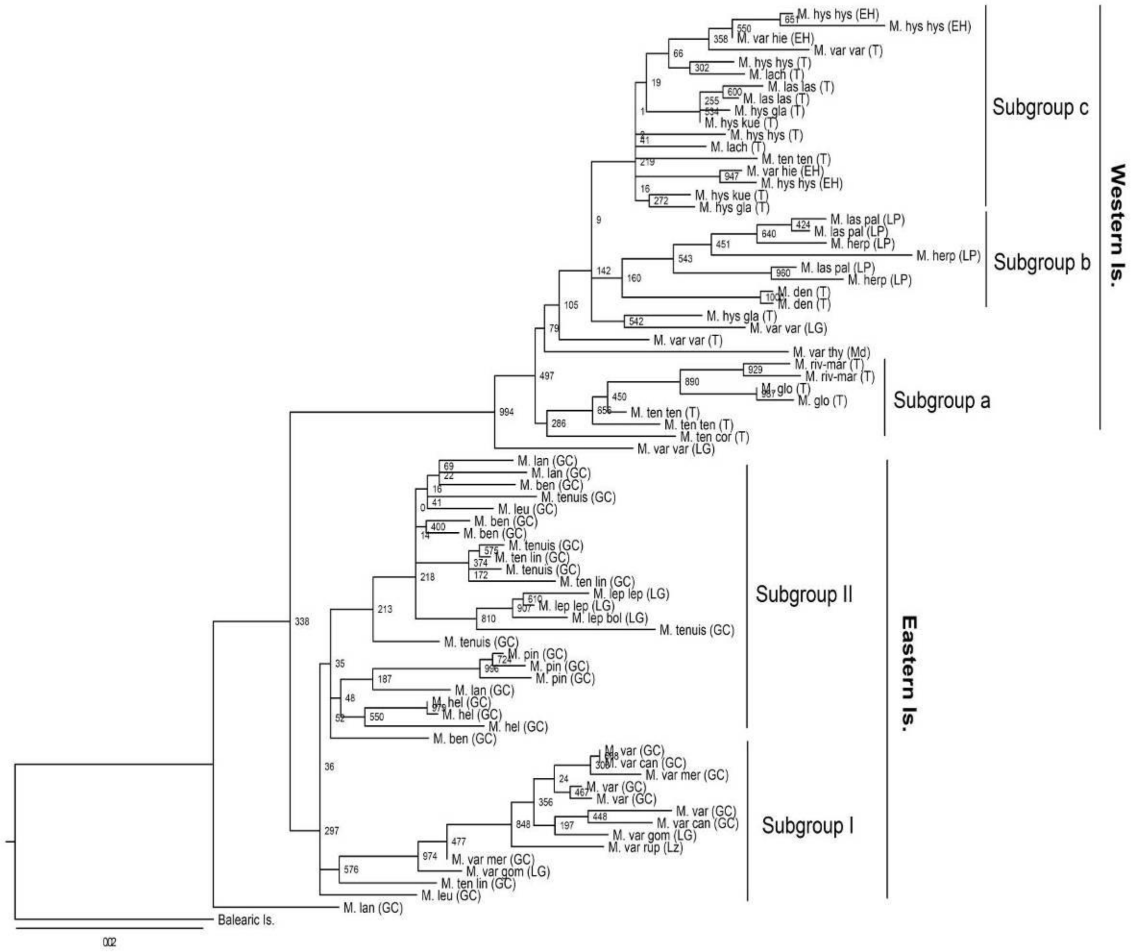
SUPPLEMENTARY DATA

Appendix A. GenBank accession numbers for all samples included in the phylogenetic analyses. Sample codes correspond to those in Table 2; NA= failed amplification.

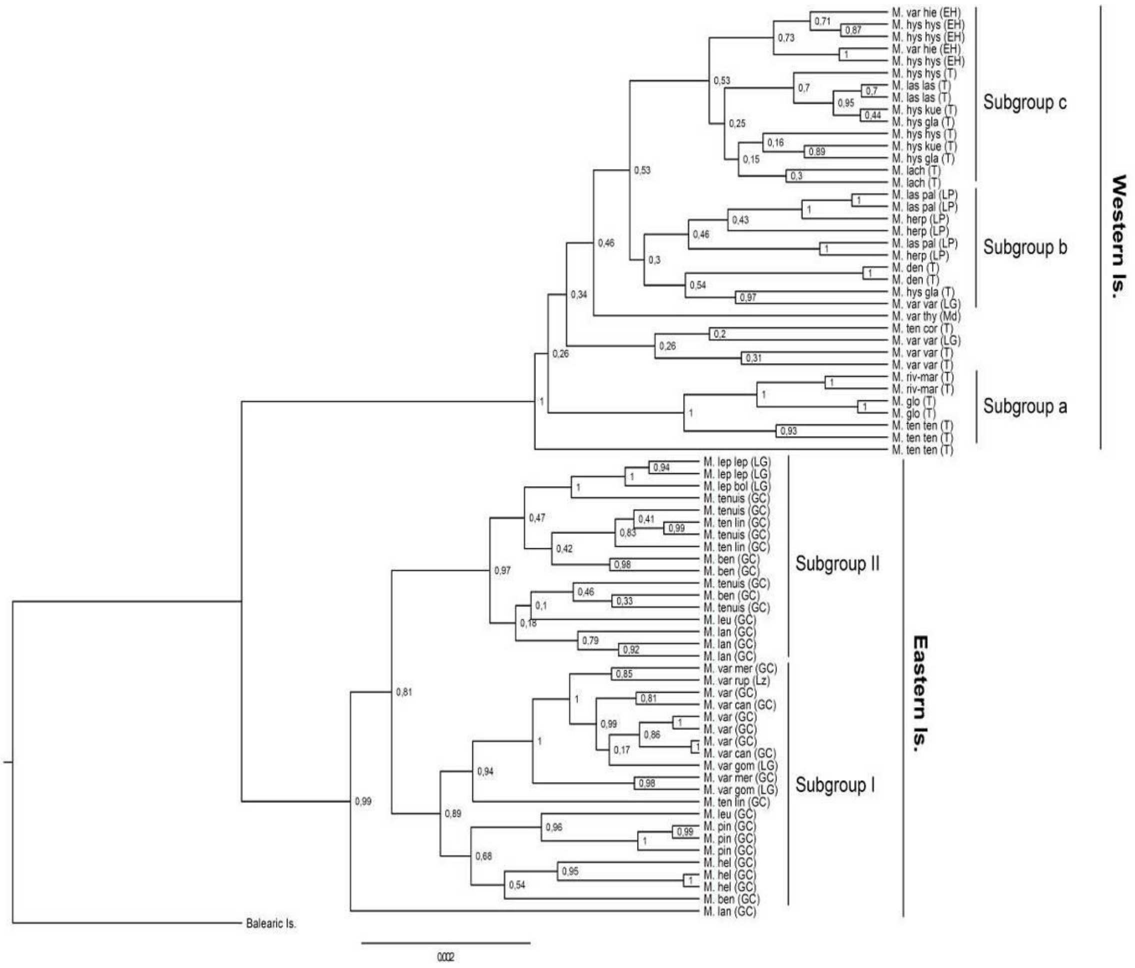
Sample Code	M.pip.056	M.pip.057	M.pip.014	M.pip.017	M.pip.047	M.pip.027	M.pip.002	O.oni.007
560	KF877342	KF877387	KF877432	KF877474	KF877518	KF877563	KF877608	KF877650
28C	KF877336	KF877381	KF877426	KF877469	KF877512	KF877557	KF877602	KF925329
29A	KF877337	KF877382	KF877427	KF877470	KF877513	KF877558	KF877603	KF925330
30A	KF877338	KF877383	KF877428	KF877471	KF877514	KF877559	KF877604	KF925331
32A	KF877339	KF877384	KF877429	KF877472	KF877515	KF877560	KF877605	KF877647
34A	KF877340	KF877385	KF877430	NA	KF877516	KF877561	KF877606	KF877648
35A	KF877341	KF877386	KF877431	KF877473	KF877517	KF877562	KF877607	KF877649
562_3	KF667170	KF667162	KF667154	KF667146	KF667138	KF667130	KF667123	KF667115
565_10	KF877343	KF877388	KF877433	KF877475	KF877519	KF877564	KF877609	KF877651
573_2	KF877344	KF877389	KF877434	KF877476	KF877520	KF877565	KF877610	KF877652
574_4	KF877345	KF877390	KF877435	KF877477	KF877521	KF877566	KF877611	KF877653
579_6	KF667171	KF667163	KF667155	KF667147	KF667139	KF667131	KF667124	KF667116
579_6	KF877346	KF877391	KF877436	KF877478	KF877522	KF877567	KF877612	KF877654
581_2	KF877347	KF877392	KF877437	KF877479	KF877523	KF877568	KF877613	KF877655
582_3	KF877348	KF877393	KF877438	KF877480	KF877524	KF877569	KF877614	KF877656
BG65	JN587688	JQ934270	JQ934313	JN587598	JQ934352	JQ934370	JQ934393	JQ934414
C146	KF877349	KF877394	KF877439	KF877481	KF877525	KF877570	KF877615	KF877657
C150	KF877350	KF877395	KF877440	KF877482	KF877526	KF877571	KF877616	KF877658
C154	KF877351	KF877396	KF877441	KF877483	KF877527	KF877572	KF877617	KF877659
C169	KF877352	KF877397	KF877442	KF877484	KF877528	KF877573	KF877618	KF877660
C175	KF877353	KF877398	KF877443	KF877485	KF877529	KF877574	KF877619	KF877661
C179	KF877354	KF877399	KF877444	KF877486	KF877530	KF877575	KF877620	KF877662
D255	JQ934237	JQ934259	JQ934305	JQ934324	JQ934342	JQ934360	JQ934383	JQ934404
D257	JQ934240	JQ934262	KF727618	JQ934327	JQ934345	JQ934363	JQ934386	JQ934407
G200	JQ934232	JQ934254	JQ934300	JQ934319	JQ934337	JQ934356	JQ934378	JQ934399
G202	JQ934231	JQ934253	JQ934299	JQ934318	JQ934336	JQ934355	JQ934377	JQ934398
GC170	JQ934249	JQ934273	JQ934314	JQ934334	JQ934353	JQ934373	JQ934394	JQ934417
GC439_1	JQ934250	JQ934274	JQ934315	JQ934335	JQ934354	JQ934374	JQ934395	JQ934418
H153	JQ934241	JQ934263	JQ934308	JQ934328	JQ934346	JQ934364	JQ934387	JQ934408
H162	JQ934238	JQ934260	JQ934306	JQ934325	JQ934343	JQ934361	JQ934384	JQ934405
H1E	KF667172	KF667164	KF667156	KF667148	KF667140	KF667132	KF667125	KF667117
H20_2	KF877355	KF877400	KF877445	KF877487	KF877531	KF877576	KF877621	KF877663
H25	KF877356	KF877401	KF877446	KF877488	KF877532	KF877577	KF877622	KF877664
H4_1	KF877357	KF877402	KF877447	KF877489	KF877533	KF877578	KF877623	KF877665
H5A	KF877358	KF877403	KF877448	KF877490	KF877534	KF877579	KF877624	KF877666
H5B	KF877359	KF877404	KF877449	KF877491	KF877535	KF877580	KF877625	KF877667

H6C	KF877360	KF877405	KF877450	KF877492	KF877536	KF877581	KF877626	KF877668
H7D	KF877361	KF877406	KF877451	KF877493	KF877537	KF877582	KF877627	KF877669
H8_1	KF877362	KF877407	KF877452	KF877494	KF877538	KF877583	KF877628	KF877670
HK175	KF667173	KF667165	KF667157	KF667149	KF667141	KF667133	KF667126	KF667118
HK181	KF667174	KF667166	KF667158	KF667150	KF667142	KF667134	KF667127	KF667119
L274	JQ934242	JQ934264	JQ934309	JQ934329	JQ934347	JQ934365	JQ934388	JQ934409
L276	JQ934234	JQ934256	JQ934302	JQ934321	JQ934339	JQ934357	JQ934380	JQ934401
L291	JQ934246	JQ934269	JQ934312	JQ934333	JQ934351	JQ934369	JQ934392	JQ934413
L295	JQ934239	JQ934261	JQ934307	JQ934326	JQ934344	JQ934362	JQ934385	JQ934406
lanGC	JN587689	JN587700	JN587584	JN587603	JN587670	JN587624	JN587732	JN587751
Lanz	JN587690	JQ934268	JN587585	JN587606	JN587671	JN587622	JN587733	JN587752
LF1	KF877363	KF877408	KF877453	KF877495	KF877539	KF877584	KF877629	KF877671
LF2	KF877364	KF877409	KF877454	KF877496	KF877540	KF877585	KF877630	KF877672
LF3	KF877365	KF877410	KF877455	KF877497	KF877541	KF877586	KF877631	KF877673
LF4	KF877366	KF877411	KF877456	KF877498	KF877542	KF877587	KF877632	KF877674
MA034	JQ934247	JQ934271	KF727616	JQ934294	KF727623	JQ934371	KF727629	JQ934415
MA035	JQ934248	JQ934272	KF727617	KF727621	KF727624	JQ934372	KF727630	JQ934416
Mten	JN587691	JN587702	JN587587	JN587605	JN587675	JN587627	JN587734	JN587754
Mvthy	JN587692	JN587703	JN587588	JN587604	JN587673	JN587625	JN587735	JQ934412
P320	KF877367	KF877412	KF877457	KF877499	KF877543	KF877588	KF877633	KF877675
P330	KF877368	KF877413	KF877458	KF877500	KF877544	KF877589	KF877634	KF877676
P343	KF877369	KF877414	KF877459	KF877501	KF877545	KF877590	KF877635	KF877677
P352	KF877370	KF877415	KF877460	KF877502	KF877546	KF877591	KF877636	KF877678
P387	KF877371	KF877416	KF877461	KF877503	KF877547	KF877592	KF877637	KF877679
P416	KF877372	KF877417	KF877462	KF877504	KF877548	KF877593	KF877638	KF877680
P428	KF877373	KF877418	NA	NA	KF877549	KF877594	KF877639	KF877681
P458	KF877374	KF877419	NA	KF877505	KF877550	KF877595	KF877640	KF877682
P477	KF877375	KF877420	KF877463	KF877506	KF877551	KF877596	KF877641	KF877683
P487	KF877376	KF877421	KF877464	KF877507	KF877552	KF877597	KF877642	KF877684
P496	KF877377	KF877422	KF877465	KF877508	KF877553	KF877598	KF877643	KF877685
P500	KF877378	KF877423	KF877466	KF877509	KF877554	KF877599	KF877644	KF877686
P508	KF877379	KF877424	KF877467	KF877510	KF877555	KF877600	KF877645	KF877687
P547	KF877380	KF877425	KF877468	KF877511	KF877556	KF877601	KF877646	KF877688
R212	JQ934235	JQ934257	JQ934303	JQ934322	JQ934340	JQ934358	JQ934381	JQ934402
R214	JQ934233	JQ934255	JQ934301	JQ934320	JQ934338	KF727627	JQ934379	JQ934400
Sp1_4	JQ934251	JQ934275	JQ934316	JQ934297	KF727625	JQ934375	JQ934396	JQ934419
Sp4_1	JQ934252	JQ934276	JQ934317	JQ934298	KF727626	JQ934376	JQ934397	JQ934420
T150	JQ934244	JQ934266	JQ934311	JQ934331	JQ934349	JQ934367	JQ934390	JQ934411
T157	JQ934243	JQ934265	JQ934310	JQ934330	JQ934348	JQ934366	JQ934389	JQ934410
TC188	KF667175	KF667167	KF667159	KF667151	KF667143	KF667135	KF667128	KF667120
TC299	NA	KF667168	KF667160	KF667152	KF667144	KF667136	NA	KF667121
V184	JQ934245	JQ934267	KF727619	JQ934332	JQ934350	JQ934368	JQ934391	KF727631
V236	JQ934236	JQ934258	JQ934304	JQ934323	JQ934341	JQ934359	JQ934382	JQ934403
V239	KF667176	KF667169	KF667161	KF667153	KF667145	KF667137	KF667129	KF667122

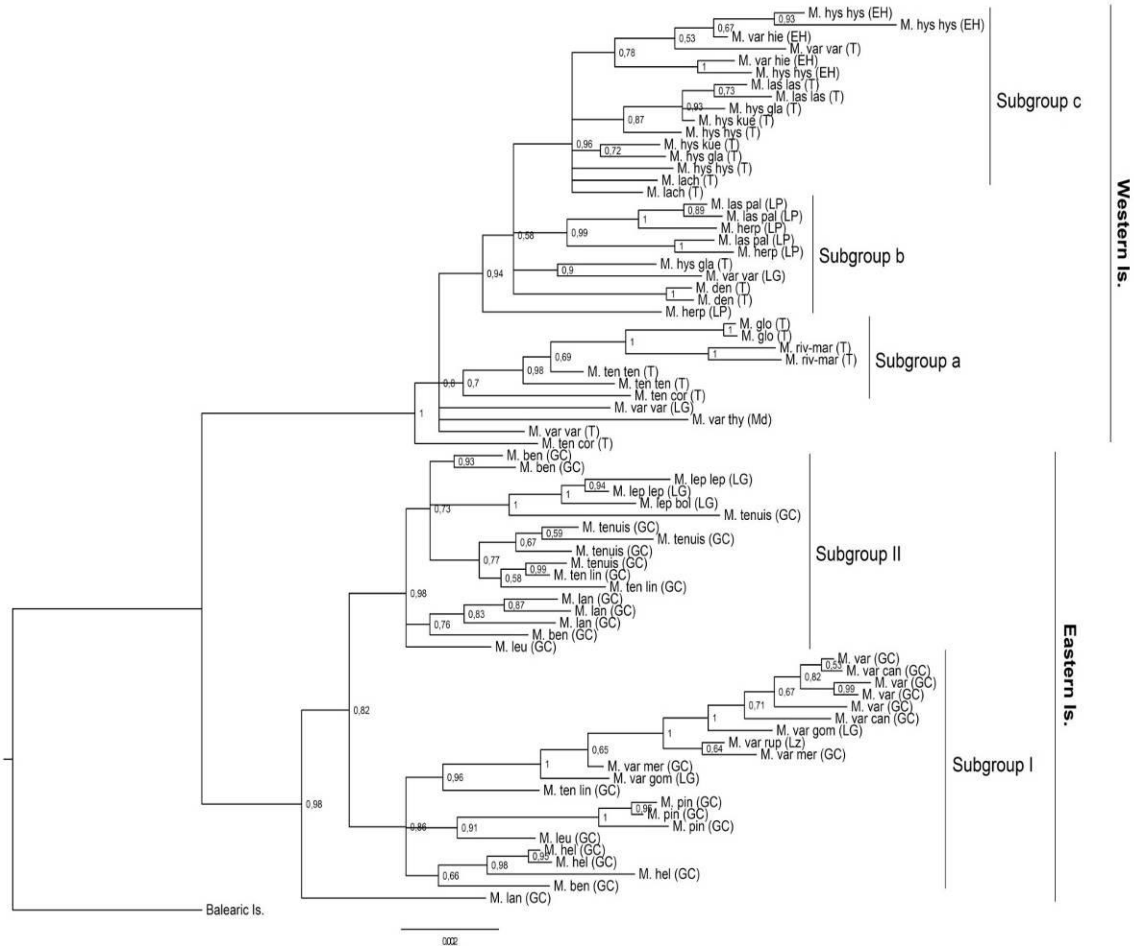
Appendix B. Maximum likelihood phylogeny calculated in PhyML showing relationships of all taxa of *Micromeria* present in the Canary Islands and Madeira. Numbers in nodes indicate bootstrap values. Vertical lines indicate groups and subgroups and correspond to those in Fig. 1. Islands are indicated in parenthesis: GC, Gran Canaria; LG, La Gomera; Lz, Lanzarote; T, Tenerife; Md, Madeira; LP, La Palma; EH, El Hierro. See Table 1 for full species names.



Appendix C. Bayesian phylogeny calculated in BEAST showing relationships of all taxa of *Micromeria* present in the Canary Islands and Madeira. Numbers in nodes indicate Bayesian posterior probabilities. Vertical lines indicate groups and subgroups and correspond to those in Fig. 1. Islands are indicated in parenthesis: GC, Gran Canaria; LG, La Gomera; Lz, Lanzarote; T, Tenerife; Md, Madeira; LP, La Palma; EH, El Hierro. See Table 1 for full species names.



Appendix D. Bayesian phylogeny calculated in MrBayes excluding duplicated locus O.oni.007 showing relationships of all taxa of *Micromeria* present in the Canary Islands and Madeira. Numbers in nodes indicate Bayesian posterior probabilities. Vertical lines indicate groups and subgroups and roughly correspond to those in Fig. 1. Islands are indicated in parenthesis: GC, Gran Canaria; LG, La Gomera; Lz, Lanzarote; T, Tenerife; Md, Madeira; LP, La Palma; EH, El Hierro. See Table 1 for full species names.



Chapter 4:

Morphological consequences of insularity: taxonomical revision of *Micromeria*

Paper IV Puppo, P, H Meimberg, PL Pérez de Paz and C Bräuchler (2014) (2326–2327) Proposals to conserve the names *Micromeria varia* with a conserved type and *M*. *hyssopifolia* against *Thymus ericifolius* (*Lamiaceae*). Taxon 63, 1137-1138. DOI: 10.12705/635.17

Paper V Puppo, P and H Meimberg (Submitted) New species and new combinations in *Micromeria* (Lamiaceae) from the Canary Islands. Phytotaxa

Paper IV:

(2326–2327) Proposals to conserve the names *Micromeria varia* with a conserved type and *M*. *hyssopifolia* against *Thymus ericifolius* (Lamiaceae)

Pamela Puppo, Harald Meimberg, Pedro Luis Pérez de Paz, and Christian Bräuchler

Taxon 63, 1137-1138. DOI: 10.12705/635.17

(2326-2327) Proposals to conserve the names *Micromeria varia* with a conserved type and *M*. *hyssopifolia* against *Thymus ericifolius* (Lamiaceae)

Pamela Puppo[1], Harald Meimberg[1,2], Pedro Luis Pérez de Paz[3], and Christian Bräuchler[4,5,6]

(2326) **Micromeria varia** Benth., Labiat. Gen. Spec.: 374. Mai 1834 [*Angiosp*.: *Lab*.], nom. cons. prop.

Typus: [Spain, Canary Islands] Ins. Teneriffa (K barcode K000193466), typ. cons. prop.

(2327) **Micromeria hyssopifolia** Webb & Berthel., Hist. Nat. Iles Canaries 3(2,3): 72. Dec 1844 [*Angiosp*.: *Lab*.], nom. cons. prop.

Typus: [Spain, Canary Islands] “*Thymus polymorpha* sp. n.?; in rupestribus siccis Ins. Canar. vulgatissima”, *Barker-Webb* (FI, upper left-hand specimen of 5 on sheet).

(=) *Thymus ericifolius* Roth, Catal. Bot. 2: 50. 1800 *(‘ericaefolius’)*, nom. rej. prop.

Neotypus (hic designatus per Bräuchler): [Spain, Canary Islands] “Habitat in Hispania” [on folder B-W 11017-000], “Hort. bot. Berol. W.” (B-W barcode 11017-010).

---

[1] Research Center in Biodiversity and Genetic Resources (CIBIO) / InBio Associated Laboratory, University of Porto, Campus Vairão, R. Monte-Crasto, 4485-661 Vairão, Portugal.

[2] Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences, A-1180 Vienna; Austria.

[3] Departamento de Biología Vegetal (Botánica), Universidad de La Laguna, 38271 - La Laguna, Tenerife, Spain.

[4] Department Biology I, Systematic Botany & Mycology, Ludwig-Maximilians-University Munich, Menzinger Str. 67, 80638 Munich, Germany.

[5] Botanische Staatssammlung München, Menzinger Str. 67, 80638 Munich, Germany.

[6] Technische Universität München, Restoration Ecology, Emil-Ramann-Str. 6, 85350 Freising, Germany.

The name *Micromeria varia* Benth. has been widely used for the most common species of the genus in the Canary Islands and Madeira (e.g. Webb & Berthelot, Hist. Nat. Iles Canaries 3(2,3):71. 1845; Bentham in Candolle, Prodr. 12: 212. 1848; Pérez de Paz, Rev. Gen. Micromeria Reg. Macaronesica: 173. 1978; Hohenester & Wells, Exkursionsfl. Kanar. Ins.: 219. 1993; Morales in Bot. Complut. 18: 165. 1993; Bramwell, Fl. Isl. Canar.: 120. 1997; IUCN-Red List of threatened plants: 311. 1997; Bräuchler & al. in Taxon 54: 639–650. 2005, in Willdenowia 38: 363-410. 2008, in Molec. Phylogen. Evol. 55: 501–523. 2010; Meimberg & al. in Molec. Phylogen. Evol. 41: 566–578. 2006; Govaerts, World Checkl. Lamiaceae: http://apps.kew.org/wcsp/, retrieved 2014-03-12). *Micromeria varia* is a perennial subshrub characterized by leaves condensed on short side branches that are ericoid with margins revolute and laminas green and frequently tinged with red as well as flowers less than 1 cm long, with corollas pink to purple.

Initially the species was listed as *Thymus inodorus* Desf. by Bentham (in Linnaea 6: 77. 1831), without description based on a specimen collected by Chamisso (K000193457, identified as a possible hybrid between *M*. *varia* and *M*. *hyssopifolia* Webb & Berthel. by Pérez de Paz and mounted on the same sheet as the proposed conserved type at K). Recognizing it as distinct from *T. inodorus* Desf., Bentham (Labiat. Gen. Spec.: 374. 1834) chose the name *M*. *varia* to account for the considerable variation among the material cited (according to Pérez de Paz, l.c.: 175, including also specimens of *M*. *hyssopifolia*, and *M*. *herpyllomorpha*). Even in a more narrow circumscription excluding these latter collections the species today remains the most variable of all taxa on the Canary Islands.

Inclusion of *Thymus ericifolius* Roth (Catal. Bot. 2: 50. 1800) in the original synonymy of *M*. *varia*, however, renders the name illegitimate, for the epithet of this older name ought to have been adopted under *Micromeria*. Formally the type of *M*. *varia* thus is that of *T*. *ericifolius*, the designation of a “lectotype” by Pérez de Paz (l.c.: 174) is ineffective (see Art. 7.5). Pérez de Paz was unable to trace type material of *T*. *ericifolius*, but kept it in the synonymy of *M*. *varia*. He suggested *M*. *varia* should be conserved against *T*. *ericifolius*, but did not submit a formal proposal to Taxon as required by the ICN. According to the original description and the neotype designated by us, application of the name *T*. *ericifolius* contrasts with the definition of *M*. *varia* in the literature cited above (see below for further details).

To avoid disruption of current usage and guarantee stability, the well-established and well-descriptive name *Micromeria varia* is here formally proposed for conservation with a conserved type (Pérez de Paz’s suggested “lectotype”).

As already noted, *Thymus ericifolius* Roth was considered a synonym of *Micromeria varia* by Bentham (l.c.), as well as by later authors (e.g. Pérez de Paz, l.c.: 173; Hohenester & Wells, l.c.; Govaerts, l.c.). After its original description, *T. ericifolius* was only accepted by Willdenow (Enum. Pl.: 624. 1809) and not listed anymore except in the synonymy cited above. Bornmüller (in Repert. Spec. Nov. Regni Veg. 19: 198. 1924) rediscovered the name and published the legitimate combination *M. ericifolia* (Roth) Bornm. ("M. varia Bth. (richtiger M. ericifolia [Roth sub Thymo] Bornm.)"). Subsequently the name was not used again except in the publication of *Satureja ericifolia* (Roth) R.H. Willemse (in Willdenowia 21: 83. 1991) necessitated by the suggested inclusion of *Micromeria* in *Satureja*.

Many of Roth's own collections according to Stafleu & Cowan (TL-II, IV: 914. 1983) had been incorporated in the general herbarium at B and were mostly destroyed during WW-II in 1943. The type of *T. ericifolius* most likely was among them. The specimen present at the Willdenow herbarium at B may or may not be part of the original material. Due to citation of Roth's name and description by Willdenow (l.c.), that material, however, represents the best available for typification. A specimen labelled "Thymus ericaefolius Roth" and most likely stemming from the same source is at GOET (via Herbarium C.A. Fischer). After carefully examining the available material and description the conspecifity of *T. ericifolius* with *M. hyssopifolia* Webb & Berthel. (1844) could be confirmed due to indumentum, shape of leaves and colour of corollas. The neglected *M. ericifolia* thus would become the oldest available name for the latter taxon.

However, *Micromeria hyssopifolia* is another very common species of the genus on Tenerife and El Hierro islands (e.g. Christ in Bot. Jahrb. Syst. 9: 131–132. 1888; Engler in Engler & Drude, Veg. Erde 9(I,2-3): 840. 1910; Bannerman, Canary Isl.: 82, 359. 1922; Pérez de Paz, l.c.: 173; Rodríguez & al. in Revista Acad. Canar. Ci. 2: 138. 1990; Hohenester & Wells, l.c.; Morales, l.c.; Izquerdo & al. (eds.), Lista de Espec. Silvestr. Canar.: 128. 2004; Bräuchler & al., l.c. 2005, l.c. 2008, l.c. 2010; Meimberg & al., l.c.; Govaerts, l.c.). The species comprises perennial subshrubs in contrast to *M. varia* characterized by strigose indumentum, giving the plant a greyish aspect, leaves lanceolate or oblong-lanceolate and flowers less than 1 cm long, with white, barely exerted corollas. Being the long accepted and most widely used name, *Micromeria hyssopifolia*, lectotypified by Pérez de Paz (l.c.: 209) on the specimen cited above, is here formally proposed for conservation against *T. ericifolius*.

ACKNOWLEDGEMENTS

The authors express their gratitude to John McNeill and John Wiersema for improvements to the manuscript.

Paper V:

New species and new combinations in *Micromeria* (Lamiaceae) from the Canary Islands

Pamela Puppo and Harald Meimberg

Submitted to Phytotaxa

New species and new combinations in *Micromeria* (Lamiaceae) from the Canary Islands and Madeira

Pamela Puppo[1] and Harald Meimberg[2]

ABSTRACT

Based on recent molecular evidence, two new species of *Micromeria* are described for the Canary Islands: *M. ferrensis* and *M. pedro-luisii.* Five new combinations are proposed: *M. canariensis*, *M. canariensis* subsp. *meridialis*, *M. gomerensis*, *M. rupestris,* and *M. herpyllomorpha* subsp. *palmensis*. Three new hybrids are described for La Gomera: *M. lepida* subsp. *bolleana* × *M. canariensis* subsp. *gomerensis*, *M. lepida* subsp. *bolleana* × *M. pedro-luisii*, and *M. lepida subsp. lepida* × *M. pedro-luisii*. And, a new name is given to the taxon from Madeira: *M. madeirensis.* A table including all the species of *Micromeria* found in the Canary Islands and Madeira is provided, as well as an identification key for all the species present in the Canary archipelago.

Keywords: Distribution, endemism, Lamiaceae, Macaronesia, *Micromeria*.

RESUMEN

Con base a nueva evidencia molecular, se describen dos nuevas especies de *Micromeria* para las Islas Canarias: *M. ferrensis* y *M. pedro-luisii.* Se proponen cinco nuevas combinaciones: *M. canariensis*, *M. canariensis* subsp. *meridialis*, *M. gomerensis*, *M. rupestris* y *M. herpyllomorpha* subsp. *palmensis*. Se describen tres nuevos híbridos para La Gomera: *M. lepida* subsp. *bolleana* × *M. canariensis* subsp. *gomerensis*, *M. lepida* subsp. *bolleana* × *M. pedro-luisii*, and *M. lepida* subsp*. lepida* × *M. pedro-luisii*. Y un nuevo nombre es dado para el taxon de Madeira: *M. madeirensis.* Se incluye una tabla con todas las especies de *Micromeria* que se encuentran en las Islas Canarias y Madeira, así como una clave de identificación para todas las especies presentes en el archipiélago Canario.

Palabras clave: Distribución, endemismo, Lamiaceae, Macaronesia, *Micromeria.*

---

[1] Research Center in Biodiversity and Genetic Resources (CIBIO) / InBio Associated Laboratory, University of Porto, Campus Vairão, R. Monte-Crasto, 4485–661 Vairão, Portugal; p.puppo@cibio.up.pt; p_puppo@hotmail.com

[2] Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences, 1180 Vienna, Austria.

INTRODUCTION

Micromeria Bentham (1829: 1282) belongs to the mint family Lamiaceae, subfamily Nepetoideae, tribe Mentheae, subtribe Menthinae. It is mostly distributed along the Macaronesian and Mediterranean regions, eastern Africa, India, and south China, and is composed of ca. 54 species (Bräuchler *et al.* 2008). *Micromeria* is characterized by the thickened margin in the leaves caused by a continuous sclerenchymatous vein, bracteoles always present, calyx lobes usually straight or spreading, posterior lip of the corolla emarginated and curved upwards (Bräuchler *et al.* 2008).

In Macaronesia, *Micromeria* is present in three of the five archipelagos that compose this biogeographic region: the Canary Islands, Madeira, and Cape Verde. Molecular analyses showed that the species of *Micromeria* from the Canary Islands and Madeira constitute a monophyletic group while the species present in Cape Verde cluster with other species from the West Mediterranean Region (Bräuchler *et al.* 2005). These studies show that the species of *Micromeria* form monophyletic groups in each of the Canary Islands, except in La Gomera where several colonization events from Tenerife and Gran Canaria are suggested (Meimberg *et al.* 2006, Puppo *et al.* 2015).

According to the last revision for Macaronesian *Micromeria* (Pérez de Paz 1978), there are 16 species present in the Canary Islands and Madeira, most of them single island endemics. Two species are present in two islands: *M. hyssopifolia* Webb & Berthelot (1844: 72) in Tenerife and El Hierro, and *M. lasiophylla* Webb & Berthelot (1844: 74) in Tenerife and La Palma. Only one species, *M. varia* Bentham (1834: 374), is distributed among all islands though different subspecies have been described for each highlighting the morphological variation present in this species group. Phylogenetic evidence (Puppo *et al.* 2014, 2015) suggests that there is no relationship between *M. hyssopifolia* from Tenerife and the specimens identified as *M. hyssopifolia* from El Hierro. Likewise, it seems that *M. lasiophylla* from La Palma is not related with *M. lasiophylla* from Tenerife and rather constitute two different taxa. In this sense, both *M. hyssopifolia* and *M. lasiophylla* would be endemic to Tenerife. Similarly, *M. varia* constitutes an endemic taxon from Tenerife and different from the ones present in other islands of the Canary archipelago and Madeira (Meimberg *et al.* 2006, Puppo *et al.* 2014, 2015; Fig. 1–A). In La Gomera, two species were described: *M. lepida* Webb & Berthelot (1844: 74) and *M. varia* with two subspecies: subsp. *varia* and subsp. *gomerensis* P. Pérez (1978: 179) (Pérez de Paz 1978). Molecular analyses revealed

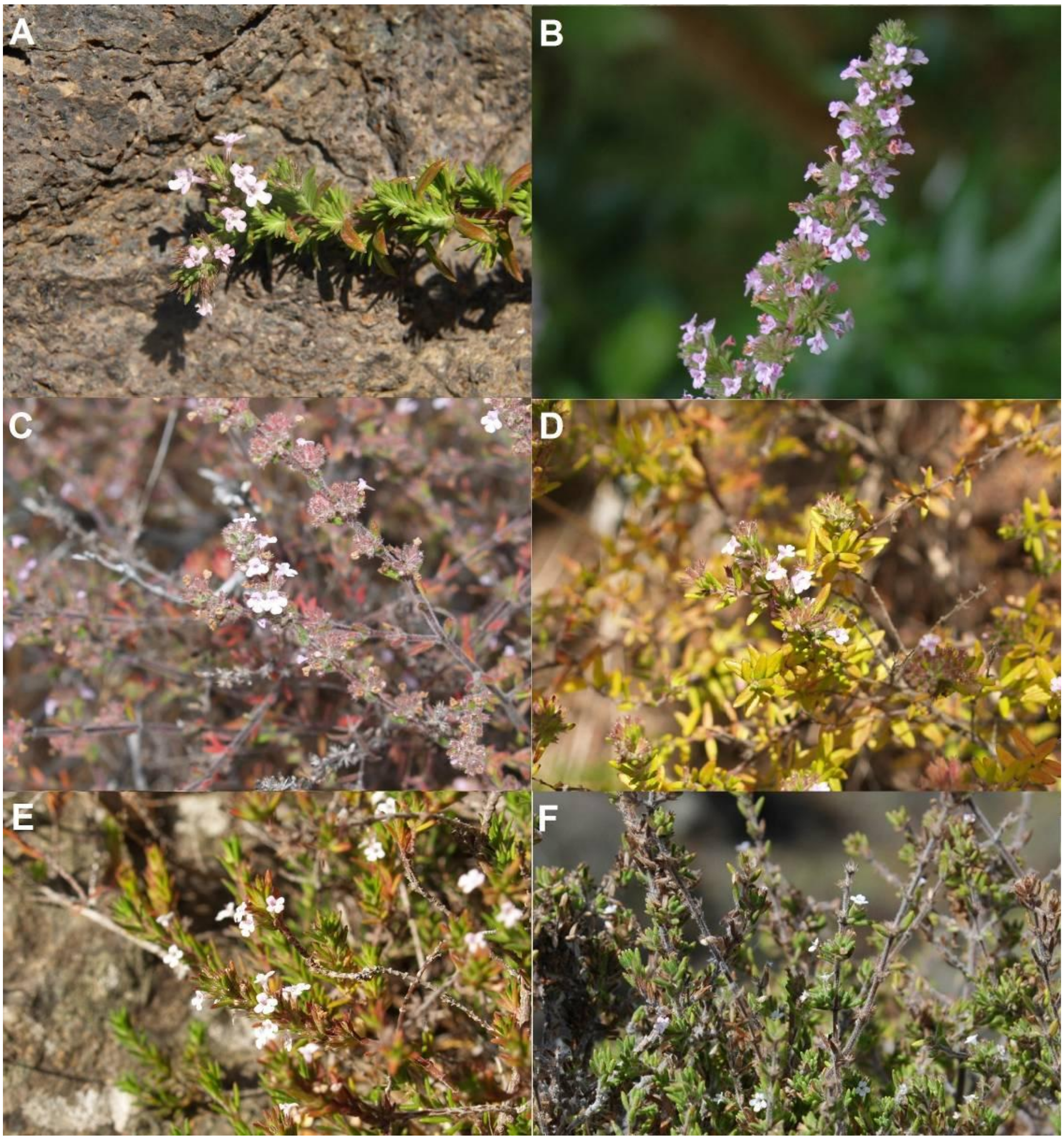


Fig. 1 A. *Micromeria varia* (Tenerife). B. *Micromeria canariensis* subsp. *canariensis* (Gran Canaria). C. *Micromeria canariensis* subsp. *meridialis* (Gran Canaria). D. *Micromeria gomerensis* (La Gomera). E. *Micromeria ferrensis* (El Hierro). F. *Micromeria pedro-luisii* (La Gomera). Photos A, C, D, F from P. Puppo; B and E from P.L. Pérez de Paz.

that these three taxa constitute in fact different lineages (Puppo *et al.* 2015) deriving from different colonization events. *Micromeria varia* subsp. *gomerensis* and *M. lepida* most likely resulted from colonization from Gran Canaria while *M. varia* subsp. *varia* probably originated from Tenerife (Puppo *et al.* 2015).

In order to reconcile the taxonomic composition of *Micromeria* in the Canary Islands and Madeira with the new molecular evidence available, the present study revises several of these species proposing the recognition of two new species, one new name, and five new combinations. Three new hybrids are described as well for the island of La Gomera. With the present revision, the number of total species of *Micromeria* present in these islands increases to 22 instead of 16 as previously stated (Pérez de Paz 1978; Table 1; Fig. 2). A key to the species of *Micromeria* present in the Canary Islands is included as well.

Table 1. Species of *Micromeria* in the Canary Islands sensu Pérez de Paz (1978) and the present study showing new species and new combinations in bold.

Island	Species sensu Pérez de Paz (1978)	This study
Tenerife	*M. densiflora*	*M. densiflora*
	M. glomerata	*M. glomerata*
	M. hyssopifolia	*M. hyssopifolia*
	M. lachnophylla	*M. lachnophylla*
	M. lasiophylla subsp. *lasiophylla*	*M. lasiophylla*
	M. rivas-martinezii	*M. rivas-martinezii*
	M. teneriffae	*M. teneriffae*
	M. varia	*M. varia*
La Palma	*M. herpyllomorpha*	*M. herpyllomorpha*
	M. lasiophylla subsp. *palmensis*	***M. herpyllomorpha* subsp. *palmensis***
La Gomera	*M. lepida*	*M. lepida*
	M. varia subsp. *varia*	***M. pedro-luisii***
	M. varia subsp. *gomerensis*	***M. gomerensis***
Gran Canaria	*M. benthamii*	*M. benthamii*
	M. helianthemifolia	*M. helianthemifolia*
	M. lanata	*M. lanata*
	M. leucantha	*M. leucantha*
	M. pineolens	*M. pineolens*
	M. tenuis	*M. tenuis*
	M. varia subsp. *canariensis*	***M. canariensis***
	M. varia subsp. *meridialis*	***M. canariensis* subsp. *meridialis***
Lanzarote | Fuerteventura	*M. varia* subsp. *rupestris*	***M. rupestris***
El Hierro	*M. hyssopifolia* subsp. *hyssopifolia*	***M. ferrensis***
	M. varia subsp. *hierrensis*	***M. ferrensis***
Madeira	*M. varia* subsp. *thymoides*	***M. madeirensis***
Total species	**16**	**22**

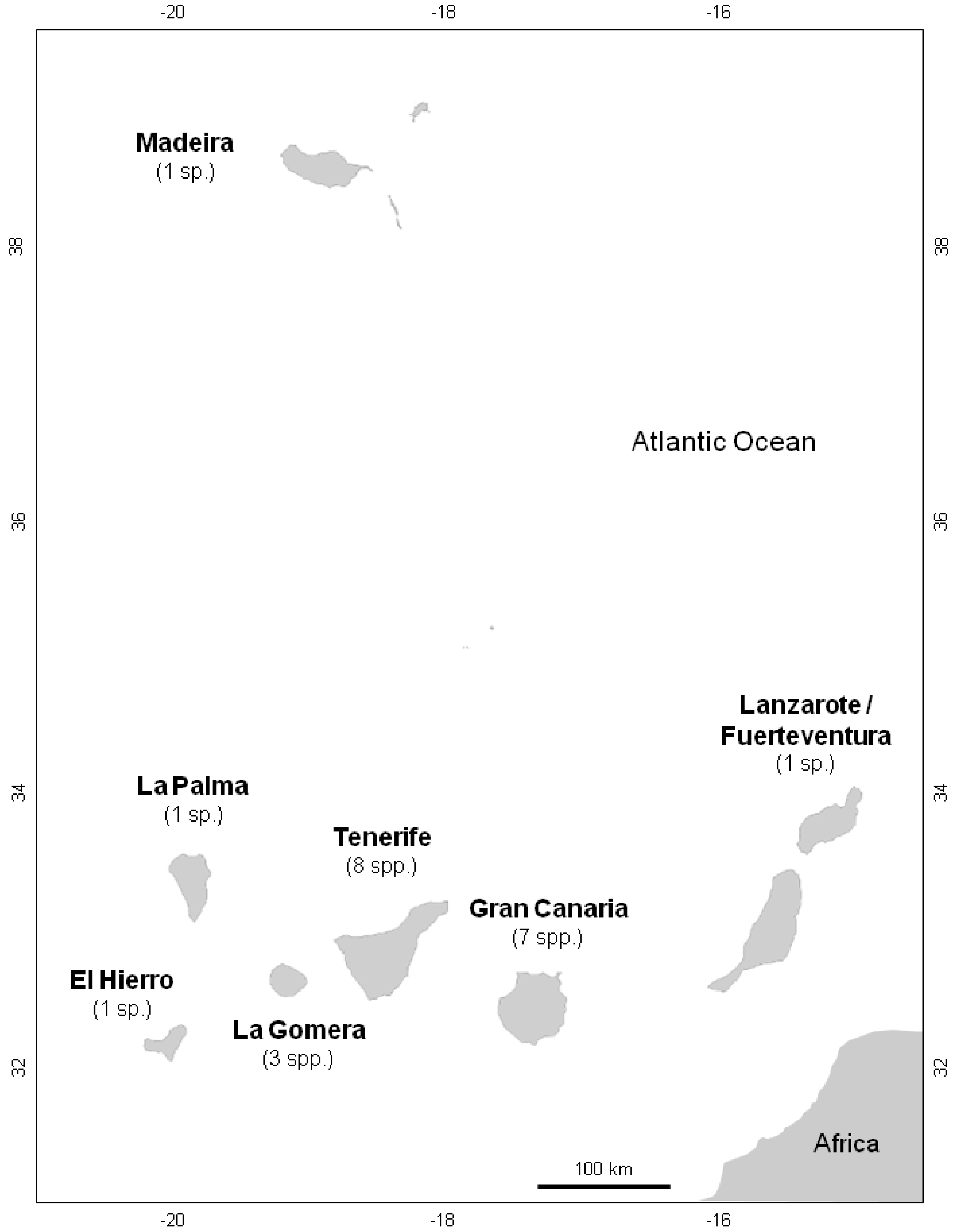


Fig. 2 Map of the Canary Islands and Madeira showing number of species of *Micromeria* present in each island.

TAXONOMIC TREATMENT

Micromeria canariensis (P. Pérez) Puppo, *comb. nov*. *Micromeria varia* subsp. *canariensis* P. Pérez, Rev. Gen. Micromeria Reg. Macaronesica 180. 1978. *Satureja ericifolia* (Roth) R.H. Willemse subsp. *canariensis* (P. Pérez) R.H. Willemse, Willdenowia 21(1–2): 84. 1991. *Satureja varia* subsp. *canariensis* (P. Pérez) A. Hansen & Sunding, Sommerfeltia 17: 7. 1993.

Type:—SPAIN. Canary Islands: "Ex Insula Canaria Magna (G. Canaria dicta) in magno anfractu "Bco. Oscuro", dictum, prope Tamadaba, versus 900 m. supra Mare", 10 July 1974, *P. Pérez 9* (holotype: herb. Pérez, TFC!).

Micromeria varia Benth. subsp. *angustissima* Bolle ex Christ, Bot. Jahrb. Syst. 9: 131. 1888. Type:—SPAIN. Canary Islands: "In Canaria magna supra Tafira", March 1884, *Christ* (BAS?).

a. subsp. *canariensis.* Fig. 1–B, Fig. 3.

Erect subshrub, up to 80 cm high, highly branched; branches persisting and entangled, basal parts of branches glabrate shedding bark, younger parts puberulent. Leaves sessile or inconspicuously petiolated; blades herbaceous 5–10 × 0.5–3 mm, green, basal blades lanceolate, flat, glabrate on both sides, abaxially puberulent on the midrib, upper blades linear, revolute, puberulent throughout. Cymes arranged on the tip of young branches; peduncles 2–5 mm long. Calyx green tinged with purple, puberulent, 2.5–3.5 mm long, calyx apices subulate, acute, ciliate. Corolla light purple, 3.5–5 mm long, exerted, lower lip projected upwards almost forming a 90º angle with the upper lip. Anthers purple. Style slightly exerted.

Representative Specimens Examined:—SPAIN. Gran Canaria: Bco. de los Tiles de Moya, 24 May 2011, *P. L. Pérez de Paz 674/C-2* (TFC); *P. Puppo et al. 330* (TFC); *P. Puppo et al. 334* (TFC); Brezal de el Palmital, 24 May 2011, *P. L. Pérez de Paz 674/C-3* (TFC); *P. Puppo et al. 337* (TFC); *P. Puppo et al. 338* (TFC); Fontanales, 24 May 2011, *P. L. Pérez de Paz 674/C-5* (TFC); *P. Puppo et al. 358* (TFC); *P. Puppo et al. 360* (TFC); *P. Puppo et al. 361* (TFC); *P. Puppo et al. 362* (TFC).

Habitat and Geographical Distribution:—This subspecies is restricted to the island of Gran Canaria where it grows in several localities in the NE side of the island between 400–1000 m. It is especially abundant in rocky, humid areas, in the border of the Laurisilva forest and humid gorges.

Notes:—This taxon was described as one of the *M. varia* subspecies present in Gran Canaria by Pérez de Paz (1978). Molecular studies (Meimberg *et al.* 2006, Puppo *et al.* 2015) showed that this is a different taxon than the *M. varia* present in Tenerife so a new combination is proposed.

b. subsp. *meridialis* (P. Pérez) Puppo, *comb. nov*. Fig. 1–C, Fig. 4. *Micromeria varia* subsp. *meridialis* P. Pérez, Rev. Gen. Micromeria Reg. Macaronesica 181. 1978. *Satureja ericifolia* (Roth) R.H. Willemse subsp. *meridialis* (P. Pérez) R.H. Willemse, Willdenowia 21(1–2): 84. 1991. *Satureja varia* subsp. *meridialis* (P. Pérez) A. Hansen & Sunding, Sommerfeltia 17: 7. 1993.

Type:—SPAIN. Canary Islands, “In regione austral insulae Canarie Magnae (Gran Canaria dicta), in rupibus circumstantibus loco Fataga dicto, ubi est frequens”, 20 July 1974, *P. Pérez 10* (holotype: herb. Pérez, TFC!).

Micromeria varia Benth. f. *microphylla* Christ, Bot. Jahrb. Syst. 9: 134. 1888. Type:—SPAIN. Canary Islands: “In convallibus reg. marit. insular. fere omnium”, *Barker-Webb* (FI?).

Erect subshrub, up to 30 cm high, highly branched; branches persisting and entangled, basal parts of branches densely puberulent shedding bark, younger parts canescent. Leaves sessile or inconspicuously petiolated; blades herbaceous 3–6 × 0.5–0.9 mm, reddish or green tinged with red, all blades linear, revolute, densely puberulent to hirsute. Cymes sessile or shortly pedunculated arranged on the tip of young branches; peduncles less than 2 mm long when present. Calyx reddish or lilac, tomentose, less than 3 mm long, calyx apices lanceolate, acute. Corolla light purple, less than 3 mm long, barely exerted. Anthers purple. Style slightly exerted.

Representative Specimens Examined:—SPAIN. Gran Canaria: Bco. de Fataga, 25 May 2011, *P. L. Pérez de Paz 674/C-9* (TFC); *P. Puppo et al. 417* (TFC); *P. Puppo et al. 419* (TFC); *P. Puppo et al. 423* (TFC); sobre pueblo de Fataga, 25 May 2011, *P. L. Pérez de Paz 674/C-11* (TFC); Bco. de Arguineguin, cerca a Soria, 25 May 2011, *P. L. Pérez de Paz 674/C-13* (TFC); *P. Puppo et al. 467* (TFC); *P. Puppo et al. 469* (TFC); *P. Puppo et al. 470* (TFC); Carretera Tasarte-Mogán, sobre pueblo de Tasarte, 26 May 2011, *P. L. Pérez de Paz 674/C-18* (TFC); *P. Puppo et al. 508* (TFC); *P. Puppo et al.*

509 (TFC); Lomo Maguyo (Telde), sobre ciudad de Maguyo, 27 May 2011, *P. L. Pérez de Paz 674/C-20* (TFC); *P. Puppo et al. 528* (TFC).

Geographical Distribution:—This subspecies is widely distributed in the S side of Gran Canaria island from 100 m up to the highest areas in the island. This subspecies grows in dry areas and is especially common colonizing abandoned fields.

Notes:—This taxon was the other *M. varia* subspecies described by Pérez de Paz (1978) for Gran Canaria. Molecular studies (Puppo *et al.* 2015) do not support subsp. *meridialis* as a different taxon so it has been left as a subspecies of *M. canariensis* until a more detailed molecular analysis is conducted to determine its degree of differentiation.

Micromeria gomerensis (P. Pérez) Puppo, *comb. nov*. Fig. 1–D, Fig. 5–A, B. *Micromeria varia* subsp. *gomerensis* P. Pérez, Rev. Gen. Micromeria Reg. Macaronesica 179. 1978. *Satureja ericifolia* (Roth) R.H. Willemse subsp. *gomerensis* (P. Pérez) R.H. Willemse, Willdenowia 21(1–2): 83. 1991. *Satureja varia* subsp. *gomerensis* (P. Pérez) A. Hansen & Sunding, Sommerfeltia 17: 7. 1993.

Type:—SPAIN. Canary Islands: "Ex Insula Junonia Minor (Insula Gomera dicta) in loco vulgo dicto "Ladera de Pilas" in anfractu "Bco. de la Villa", juxta S. Sebastián"; May 1976, *P. Pérez 8* (holotype: herb. Pérez; isotypes: TFC!, MA).

Micromeria teneriffae (Poir.) Benth. ex G. Don var. *brevidens* Bornmüller in Repert. Spec. Nov. Regni Veg. 6: 2. 1909. Type:—SPAIN. Canary Islands: sine loco, *Bornmüller, Pl. exicc. Canar. 2719* (B?).

Erect subshrub 15–40 cm height, highly branched, branches ascending, glabrate to slightly strigose. Leaves shortly pedicelated; blades herbaceous less than 6 × 1 mm, bright green sometimes tinged with purple or yellow, lanceolate or linear, margins revolute, sparsely strigose throughout. Cymes shortly pedunculated arranged in the upper parts of the branches. Calyx green tinged with purple, strigose, ca. 2.5 mm long, calyx apices lanceolate, acute, ciliate. Corolla lilac to purple, 3–4 mm long, exerted, lower lip slightly projected upwards. Anthers purple, barely exerted, almost included. Style included.

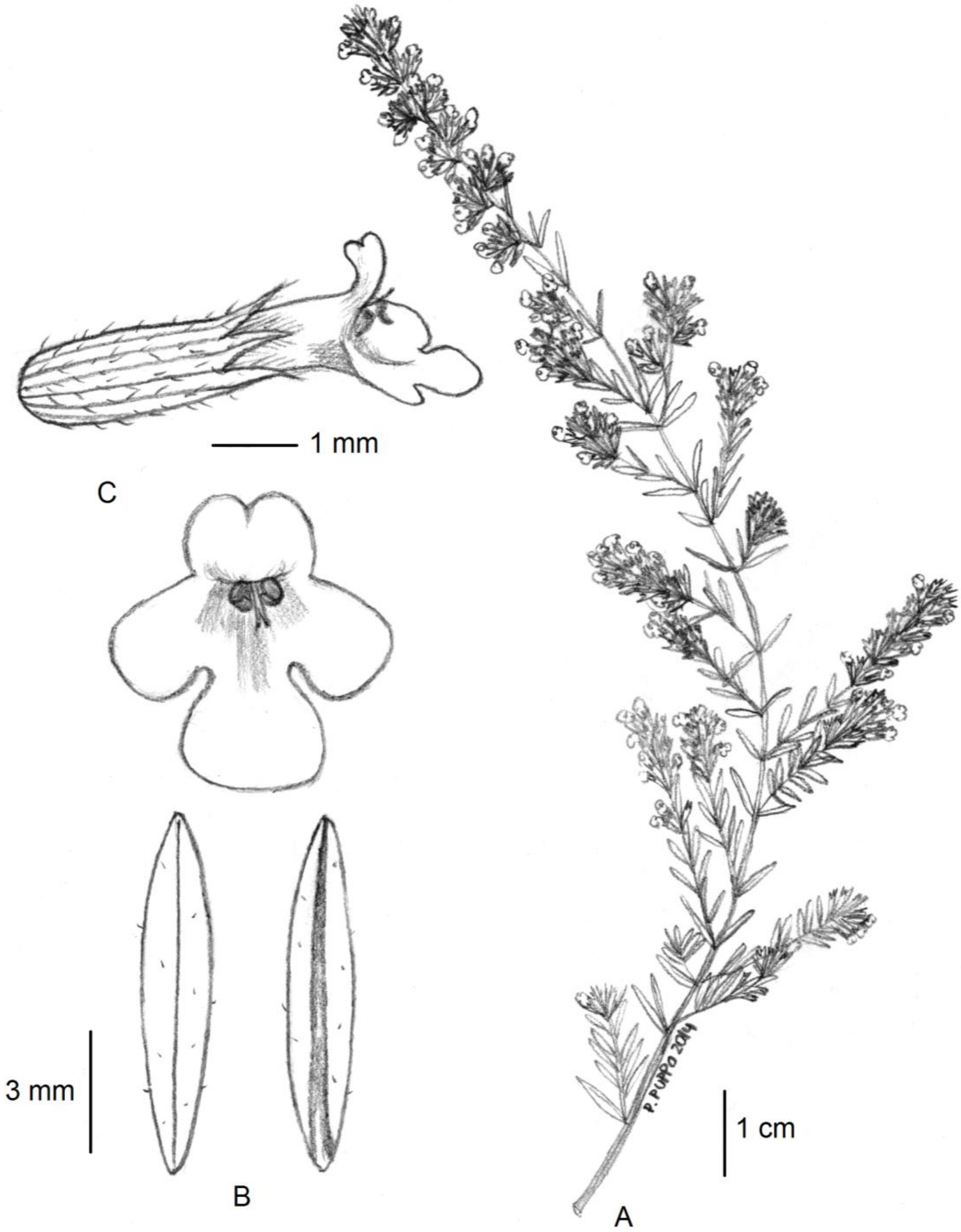


Fig. 3 *Micromeria canariensis* subsp*. canariensis* flowering branch (A), leaf axial and abaxial (B) and flower, frontal and side view (C). Drawn by P. Puppo from *Puppo et al. 330* (TFC).

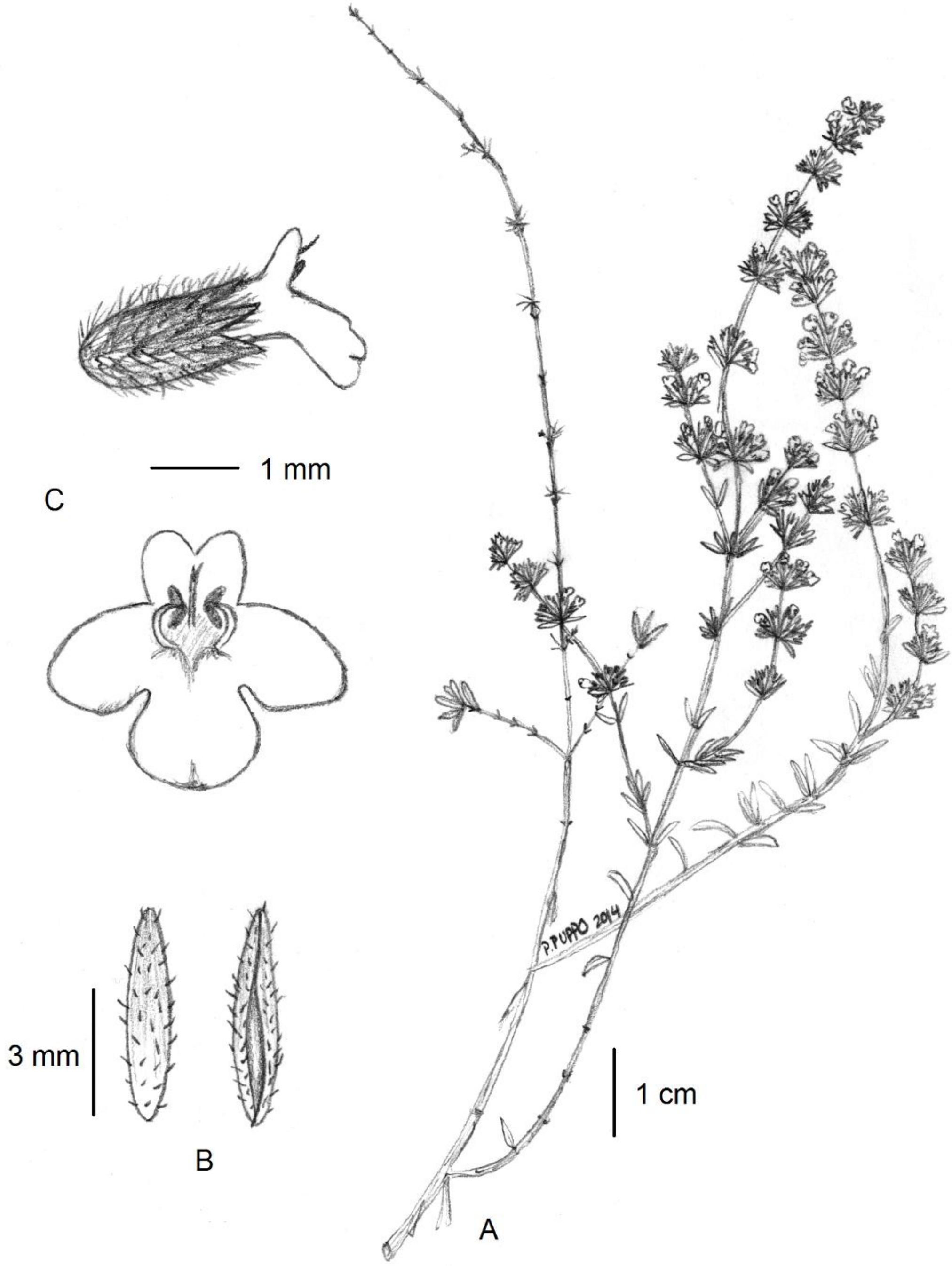


Fig. 4 *Micromeria canariensis* subsp*. meridialis* flowering branch (A), leaf axial and abaxial (B) and flower, frontal and side view (C). Drawn by P. Puppo from *Puppo et al. 419* (TFC).

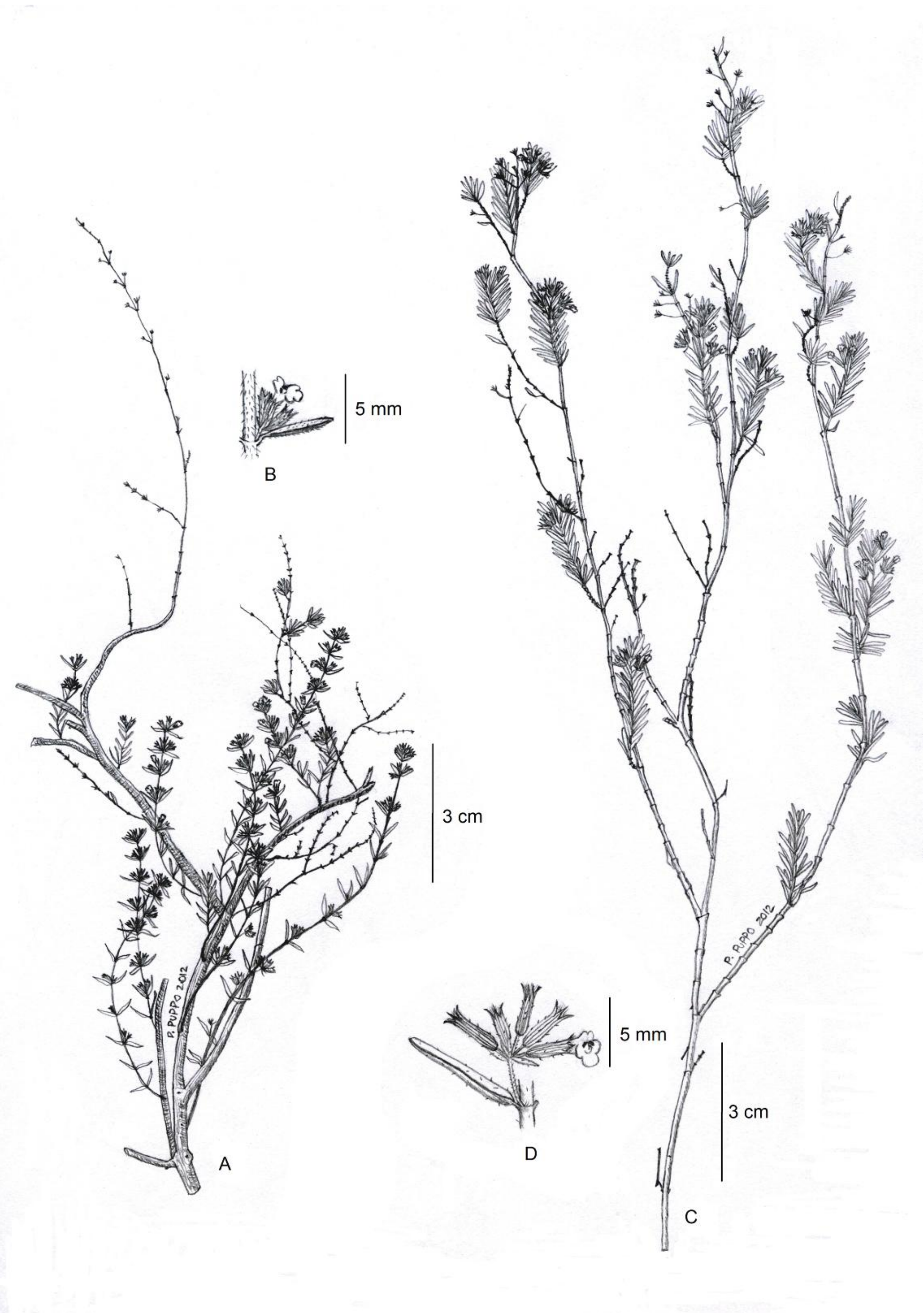


Fig. 5 *Micromeria gomerensis* flowering branch (A) and inflorescence (B). Drawn by P. Puppo from *Puppo et al. 572.2* (TFC). *Micromeria* × *tolomensis* flowering branch (C) and inflorescence (D). Drawn by P. Puppo from *Puppo et al. 573.2* (TFC).

Representative Specimens Examined:—SPAIN. La Gomera: Sobre Ayamosna, camino entre Ayamosna y Tagamiche, 16 June 2012, *P. L. Pérez 705-G5* (TFC); Mirador de La Laja, 16 June 2012, *P. L. Pérez 705-G6* (TFC); *P. Puppo 572.7* (TFC); Carretera Las Hayas-Cercado, 16 June 2012, *P. L. Pérez 705-G10* (TFC); *Puppo et al.* 579.2 (TFC); *Puppo et al.* 579.3 (TFC); *Puppo et al.* 579.6 (TFC); *Puppo et al.* 579.7 (TFC); Pueblo de Arure, 16 June 2012, *P. L. Pérez 705-G11* (TFC); Mirador de El Rejo, PN Garajonay, 17 June 2012, *P. L. Pérez 705-G12* (TFC).

Geographical Distribution:—Restricted to the island of La Gomera where it grows abundantly throughout the island. It is especially frequent between 200–800 m.

Notes:—Pérez de Paz (1978) distinguished two subspecies of *M. varia* in La Gomera: subsp. *varia* and subsp. *gomerensis.* Molecular studies showed that these two taxa are not only different but have a different origin, subsp. *varia* probably colonized from Tenerife while subsp. *gomerensis* from Gran Canaria (Puppo *et al.* 2015). Here, we propose a new combination placing subsp. *gomerensis* as a different species endemic to the island of La Gomera.

Hybrids between this subspecies and another species present in La Gomera (*M. lepida* subsp. *bolleana*) have been found in the field. This hybrid is formally described below.

Micromeria* × *tolomensis Puppo & P. Pérez, *nothosp. nov*. Fig 5–C, D.

Type:—SPAIN. La Gomera: Mirador de La Laja, 28º05’58,5’’N, 17º11’05,6’’W, Alt. 955 m, 16 July 2012, *P. Puppo, P. Pérez & F. Faure 573.2* (holotype: TFC).

Hybrid between *Micromeria lepida* subsp. *bolleana* and *M. gomerensis* presenting intermediate characteristics from both parents: leaves densely arranged in younger branches and calyx apices slightly oriented outwards from the former, habit ascendant, erect branches and lilac corolla from the latter.

Subshrub, 20–40 cm high; branches ascendant and erect, puberulous or glabrous, basal part of branches shedding bark. Leaves subsessile, ericoids, arranged in the upper parts of young branches; blades 5–8 × 0.8–1 mm, sublinear, revolute, puberulous adaxially, abaxially densely strigose. Cymes pedunculated, densely arranged on younger branches; peduncles 3–5 mm long; bracts 1–2 mm long, subulate, puberulous or velutinous; flowers shortly pedicelated, pedicels less than 1 mm long; bracteoles less than 1 mm long, subulate, puberulous or velutinous. Calyx tubular, green tinged with violet, puberulous with glandular trichomes, 3–4 mm long, calyx apices slightly oriented outwards. Corolla lilac to violet, slightly exerted, lower lip projected downwards. Anthers white, included. Style included.

Etymology:—The epithet of this new hybrid refers to the old name of La Gomera island, “Tolomeo”.

Paratypes:—SPAIN. La Gomera: Mirador de La Laja, 28º05’58,5’’N, 17º11’05,6’’W, 955 m, 16 July 2012, *P. Puppo, P. Pérez & F. Faure 573.3* (TFC 50472); *P. Puppo et al. 573.4* (TFC 50473).

Geographical Distribution:—This hybrid has been observed only in the type locality, Mirador de La Laja, where both parents occur. It grows in rocky slopes with little soil and shrubberies near 950 m elevation.

Notes:—*M.* × *tolomensis* presents an intermediate habit from both parents. The younger branches resemble those in *M. lepida* subsp *bolleana* (see Fig. 10–C, D) with larger, densely arranged leaves, though the general size of the hybrid plants is larger like in *M. gomerensis.* The cymes are conspicuously pedunculated and the calyx apices are subulate as in *M. lepida* subsp *bolleana* though the flowers are of an intermediate size between the parents. Likewise, the corolla of *M.* × *tolomensis* is lilac or violet while in *M. lepida* subsp. *bolleana* is white and in *M. gomerensis* is purple. All the hybrid specimens examined had stamens and styles normally developed.

Micromeria × *tolomensis* was found growing in a large population composed by both parents in Mirador de La Laja. In this locality, flies (probably Syrphidae) were found visiting the flowers of the different individuals. These bee-like flies are known to feed on flower nectar and are probably contributing to the occurrence of these hybrids.

Micromeria rupestris (Webb & Berthel.) Puppo, *comb. nov*. Fig. 6. *Micromeria varia* subsp. *rupestris* (Webb & Berthel.) P. Pérez, Rev. Gen. Micromeria Reg. Macaronesica 182. 1978. *Micromeria varia* f. *rupestris* (Webb & Berthel.) Christ, Bot. Jahrb. Syst. 9: 133. 1888. *Micromeria thymoides* Webb & Berthel. var. *rupestris* Webb & Berthel., Hist. Nat. Iles Canaries, 3: 71, Tab. 153, 1844. *Satureja ericifolia* (Roth) R.H. Willemse subsp. *rupestris* (P. Pérez) R.H.

Willemse, Willdenowia 21(1–2): 84. 1991. *Satureja varia* subsp. *rupestris* (P. Pérez) A. Hansen & Sunding, Sommerfeltia 17: 7. 1993.

Type:—SPAIN. Canary Islands, “in rupibus Lancerotta septentriorum” (lectotype: FI! [labeled “*Micromeria thymoides*”, left hand individual in lower part of sheet], designated by P. Pérez 1978: 183).

Small subshrub 5–20 cm height, highly branched; branches short, curved inwards, semi prostrated, basal parts of branches glabrate, shedding bark, younger parts densely strigose to tomentose. Leaves sessile or inconspicuously petiolated, petiole less than 1 mm long.; blades herbaceous, 3–7 × 1–3 mm, green sometimes tinged with purple, minutely pubescent on both surfaces, shortly strigose on midrib abaxially, basal blades ovate, slightly revolute, upper blades linear, revolute. Cymes densely covering the tip of the branches, almost sessile to pedunculated, peduncule up to 2 mm long. Calyx green, puberulent to shortly strigose, 2.5–3.5 mm long, calyx apices shortly lanceolate, densely white-ciliate. Corolla dark purple, 3–4 mm long, barely exerted. Anthers purple, barely exerted. Style barely exerted.

Specimens Examined:—SPAIN. Lanzarote: Alrededores de las Peñitas del Chache, 25 March 1976, *P. Pérez & J. R. Acebes s.n.* (TFC); Altos del Bco. de la Madre del Agua, 23 February 1994, *Wolfredo Wildpret de la Torre et al. s.n.* (TFC); San Bartolomé, Tomaren, 24 February 1995, *Reyes-Betancort, J. Alfredo s.n.* (TFC); Tinajo, Caldera Blanca, 24 April 1996, *J. A. Reyes-Betancort s.n.* (TFC). Fuerteventura: Mª de Tindaya, La Oliva, 29 March 1975, *P. Pérez & J. R. Acebes s.n.* (TFC); Risco del Carnicero, 30 March 1975, *P. Pérez & J. R. Acebes s.n.* (TFC); Mª de los Cardones, Gran Tarajal, 31 March 1975, *P. Pérez & J. R. Acebes s.n.* (TFC); Entre Picos de la Zarza y de la Palma, Jandía, 31 May 2003, *Stephan Scholz s.n.* (TFC); Castillejo Alto, Jandía, 17 April 2003, *Stephan Scholz s.n.* (TFC).

Geographical Distribution:—This species is only present in the islands of Lanzarote and Fuerteventura and its distribution is scattered in small populations throughout the islands.

Notes:—Pérez de Paz (1978) designated subsp. *rupestris* as the subspecies of *M. varia* present in Lanzarote and Fuerteventura. Molecular evidence (Meimberg *et al.* 2006, Puppo *et al.* 2015) has shown that *M. varia* (sensu Pérez de Paz 1978) is polyphyletic so this new combination is proposed to elevate the former subsp. *rupestris* to species status.

Micromeria ferrensis Puppo, *sp. nov*. Fig. 1–E, Fig. 7.

Type:—SPAIN. El Hierro: Los Cangrejos, Costa de Valverde, UTM 214503-3079961, Alt. 130 m, 14 September 2011, *P. L. Pérez de Paz H-1* (holotype: TFC).

Micromeria varia subsp. *hierrensis* P. Pérez, Rev. Gen. Micromeria Reg. Macaronesica 184. 1978, *syn. nov*. *Satureja ericifolia* (Roth) R.H. Willemse subsp. *hierrensis* (P. Pérez) R.H. Willemse, Willdenowia 21(1–2): 84. 1991. *Satureja varia* subsp. *hierrensis* (P. Pérez) A. Hansen & Sunding, Sommerfeltia 17: 7. 1993.

Type:—SPAIN. Canary Islands: “Ex Insula Hierro dicta, in rupibus abruptis super pagum Sabinosa dictum proclivibus ad nebulas diurnas exposistis”, July 1973, *P. Pérez 11* (holotype: herb. Pérez; isotypes: MA, TFC!, TFMC).

Subshrub up to 60 cm high, upper branches incanous to lanuginose; leaves sessile, blades ovate to linear, revolute, 2–5 mm long, green sometimes tinged with red, strigose on the adaxial side, abaxial side lanate; calyx green tinged with red, 2–3 mm long, apices lanceolate to subulate, ciliate; corolla white or slightly pink 2.5–3.5 mm long, barely exerted; anthers lilac, included; style included or exerted.

Subshrub, up to 60 cm high, highly branched; branches persisting and entangled, basal part of branches tomentose to villous, younger parts incanous to lanuginose. Leaves sessile or inconspicuously petiolated; blades herbaceous 2–5 × 0.5–2 mm, green sometimes tinged with red, sparsely strigose adaxially, abaxially lanate, basal blades ovate, slightly revolute, upper blades linear, revolute. Cymes sessile or shortly pedunculated arranged on the tip of young branches; peduncles less than 2 mm long when present. Calyx tubular to slightly campanulated, green tinged with purple, pubescent to sparsely pilose, 2–3 mm long, calyx apices lanceolate to subulate, acute, ciliate. Corolla white or slightly pink, 2.5–3.5 mm long, barely exerted. Anthers lilac, included or barely exerted. Style included or exerted.

Etymology:—The specific epithet alludes the island of El Hierro, where the new species grows.

Conservation Status:—This new species is abundant and has a wide area of distribution in El Hierro so it is considered as LC (Least Concern) according to the categories of the IUCN (2012).

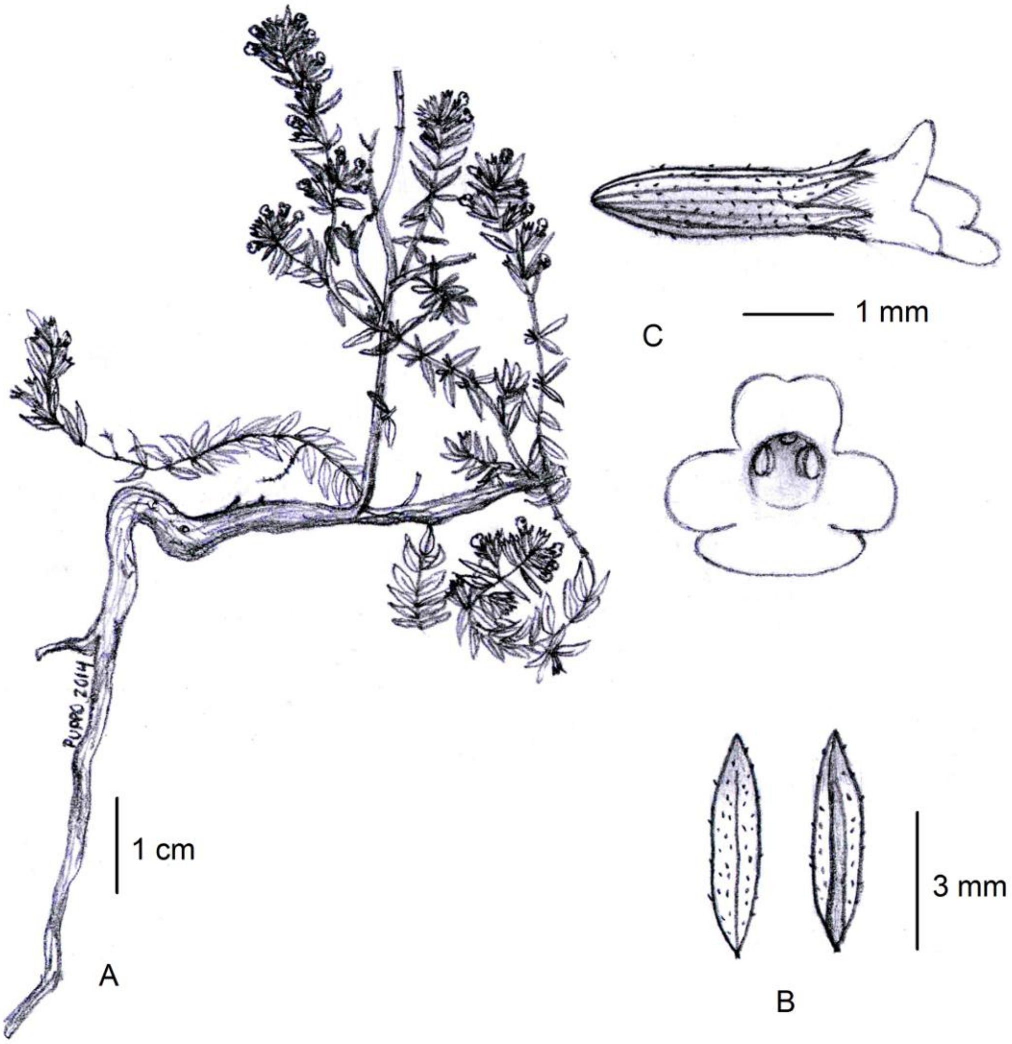


Fig. 6 *Micromeria rupestris* flowering branch (A), leaf axial and abaxial (B) and flower, frontal and side view (C). Drawn by P. Puppo from *P. Pérez & J.R Acebes s.n.* (TFC).

Specimens Examined:—SPAIN. El Hierro: La Restinga, 15 September 2011, *P. L. Pérez de Paz H-2* (TFC); El Pinar, 15 September 2011, *P. L. Pérez de Paz H-3* (TFC); Brezal de La Llanía, 15 September 2011, *P. L. Pérez de Paz H-4* (TFC); Risco de Bascos, Mirador, 15 September 2011, *P. L. Pérez de Paz H-5* (TFC); Arenales de El Julan, 15 September 2011, *P. L. Pérez de Paz H-6* (TFC); Cumbre de Malpaso, 17 September 2011, *P. L. Pérez de Paz H-7* (TFC).

Geographical Distribution:—This species is distributed all over the island of El Hierro, from the sea-level up to the highest points. Large populations are found in some areas where the individuals cover vast areas forming "tomillares" (patches of thymes).

Notes:—Pérez de Paz (1978) recognized one highly variable species in this island which he identified as *M. hyssopifolia*. *Micromeria ferrensis* is the new name we are giving to this taxon since it has been shown in molecular analyses (Meimberg *et al.* 2006; Puppo *et al.* 2015) that the lineage present in El Hierro is different from the *M. hyssopifolia* found in Tenerife. Also, these studies support the occurrence of only one distinct lineage in this island though morphologically *M. ferrensis* is very variable. In some parts this species has a grayish appearance due to the pubescence in branches and leaves, imbricate branches, and white flowers (i.e. Costa de Valverde, La Restinga, Malpaso). In other localities, this taxon presents green leaves, reduced pubescence, and slightly pink flowers (e.g. Sabinosa, Mirador de la Peña). Pérez de Paz (1978) described these specimens as *M. varia* subsp. *hierrensis* alluding to the morphological resemblance between these individuals and the *M. varia* from Tenerife. Since these specimens constitute an extreme morphological form, *M. varia* subsp. *hierrensis* is placed here as a synonym of *M. ferrensis*.

Micromeria herpyllomorpha Webb & Berthel. Hist. Nat. Iles Canaries (Phytogr.). iii. 72. t. 155. 1844. *Micromeria varia* Benth. f. *herpyllomorpha* (Webb & Berthel.) Christ in Bot. Jahr. Syst. 9: 133. 1888. *Satureja herpyllomorpha* (Webb & Berthel.) Briq. in H.G.A. Engler & K.A.E. Prantl, Nat. Pflanzenfam. 4(3a): 299. 1896.

Type (lectotype designated by P. Pérez 1978: 238):—SPAIN. Canary Islands, “In ins. Palma”, *Barker-Webb* (FI!, labeled “*Thymus herpylloides* Nob.”).

Micromeria serpyllomorpha Webb, Benth. in Candolle, Prodr.12: 217. 1848. *Micromeria perezii* Bolle, Bonplandia (Hannover) 8: 282. 1860. *Satureja perezii* (Bolle) Briq. in H.G.A. Engler & K.A.E. Prantl, Nat. Pflanzenfam. 4(3a): 299. 1896.

Type:—SPAIN. Canary Islands, “In Caldera ins. Palmae”, Sep, *Bolle* (B).

Micromeria varia Benth. var. *citriodora* (Webb & Berthel.) Burchard in Biblioth. Bot. (Stuttgard) 98: 182. 1929. *Micromeria varia* f. *citriodora* (Webb & Berthel.) Christ in Bot. Jahrb. Syst. 9: 133. 1888. *Micromeria thymoides* (Sol. ex Lowe) Webb & Berthel. var. *citriodora* Webb & Berthel. , Hist. Nat. Iles Canaries 3: 71. 1844, nom. illeg.?

Type:—SPAIN. Canary Islands, “In sylvis Teneriffae”, *Barker-Webb* (not traced, FI?).

a. subsp. *herpyllomorpha*

Subshrub up to 70 cm high, highly branched; branches persisting, basal parts of branches strigose or velutinous shedding bark, younger parts villose. Leaves inconspicuously petiolated; blades herbaceous 5–11 × 1–4 mm, green, basal blades ovate-lanceolate, flat, sparsely pilose or strigose on both sides, abaxially densely villose on the midrib, upper blades linear, revolute, villose adaxially, lanuginose abaxially. Cymes arranged throughout the upper part of the branches; sessile or with peduncles ca. 1 mm long. Calyx green tinged with purple, somewhat strigose, 2.5–3.5 mm long, calyx apices subulate or lanceolate. Corolla white to light purple, 3–6 mm long, exerted, slightly curved downwards. Anthers light purple, slightly exerted. Style exerted.

Specimens Examined:—SPAIN. La Palma: Casa Camineros, Tiguerorte, Mazo, 18 June 2010, *P. L. Pérez 32* (TFC); Entre Montaña del Viento y Montaña Lagi, Fuentecaliente, 18 June 2010, *P. L. Pérez 33* (TFC); Barranco de Las Palmas, Jedey, El Paso, 18 June 2010, *P. L. Pérez 34* (TFC); Camino de Las Angustias, Los Llanos de Aridane, 18 June 2010, *P. L. Pérez 35* (TFC); Barranco de La Galga, Los Sauces, 19 June 2010, *P. L. Pérez 36* (TFC); Barranco Seco, Puntallana, 19 June 2010, *P. L. Pérez 37* (TFC).

Geographical Distribution:—This species is abundantly distributed throughout the island of La Palma, especially between 50–1500 m.

Notes:—This species presents ample morphological variation, presumably associated with the variation in humidity and elevation present in the different localities where it grows. The typical *M. herpyllomorpha* grow in open areas, especially in the laurisilva and fayal-brezal ecological zones. At lower elevations, the plants present sparsely leaves and larger internodes while specimens at the highest elevations are smaller in size and densely pubescent.

b. subsp. *palmensis* (Bolle) Puppo, *comb. nov*. *Micromeria lasiophylla* Webb & Berthel. subsp. *palmensis* (Bolle) P. Pérez, Rev. Gen. Micromeria Reg. Macaronesica 265. 1978. *Micromeria palmensis* (Bolle) Lid in Skr. Norske Vidensk.-Akad. Oslo, Mat.-Naturvidensk. Kl., ser. 2, 23: 152. 1968. *Micromeria julianoides* Webb & Berthel. var. *palmensis* Bolle, Bonplandia, 8: 283. 1860.

Type (lectotype designated by P. Pérez 1978: 266):—SPAIN. Canary Islands, "Cumbre von Inseln Palma, über von Caldera. 1852, August", *Bolle* (Z!, labeled "*Micromeria lasiophylla* Webb").

Small subshrub up to 20 cm high, highly branched; branches short, persisting, basal parts of branches puberulous shedding bark, younger parts densely villose. Leaves sessile; blades herbaceous up to 5 × 2 mm, green, densely villose to lanuginose throughout, basal blades ovate-lanceolate, slightly revolute, upper blades linear, revolute. Cymes arranged in the upper part of the branches, sessile. Calyx purple, somewhat strigose, 3–3.5 mm long, calyx apices lanceolate. Corolla light pink to purple, 4–7 mm long, slightly exerted, straight. Anthers purple, slightly exerted. Style exerted.

Specimens Examined:—SPAIN. La Palma: Entre Morro Negro y Pico de La Cruz, 15 June 2010, *P. L. Pérez 28* (TFC); Mirador Degollada, Bco. de Franceses, 15 June 2010, *P. L. Pérez 29* (TFC); Inmediaciones Pico de La Nieve, 16 June 2010, *P. L. Pérez 30* (TFC); Espigón de El Roque, 17 June 2010, *P. L. Pérez 31* (TFC).

Geographical Distribution:—This subspecies is abundant in the higher parts of the island between 2000–2400 m.

Notes:—This subspecies constitutes the highest elevation extreme form of *M. herpyllomorpha* being shorter in size and more pubescent than the typical form. Pérez de Paz (1978) described this taxon as a subspecies of *M. lasiophylla* alluding to the similarities between the high elevation habitats from Tenerife and La Palma. Molecular studies (Meimberg *et al.* 2006, Puppo *et al.* 2015) suggest that there is only one lineage present in La Palma and thus we place subsp. *palmensis* under *M. herpyllomorpha*.

Micromeria pedro-luisii Puppo, *sp. nov.* Fig. 1–F, Fig. 8.

Type:—SPAIN. La Gomera: Sobre Ayamosna, camino entre Ayamosna y Tagamiche, UTM 286523-3110078, Alt. 780–800 m, 16 June 2012, *P. Puppo 568.2* (holotype: TFC).

Subshrub up to 50 cm high, upper branches glabrate to velutinous; leaves sessile, arranged in fascicles, blades linear, revolute, less than 6 × 1.5 mm long, green sometimes tinged with red, minutely pubescent adaxially, lanuginose abaxially; calyx green tinged with red, 3–5 mm long, apices lanceolate to subulate, ciliate; corolla white 4–6 mm long, exerted; anthers lilac, included; style included.

Subshrub, up to 50 cm high, highly branched; branches persisting and entangled, basal part of branches glabrous shedding bark, younger parts glabrate to velutinous, buds densely lanuginose. Leaves sessile arranged in fascicles in the upper parts of the branches; blades herbaceous less than 6 × 1.5 mm, linear, revolute, green sometimes tinged with red, minutely pubescent adaxially, lanuginose abaxially. Cymes sessile arranged on the tip of young branches, with few flowers. Calyx slightly campanulated, green tinged with purple, minutely pubescent, 3–5 mm long, calyx apices lanceolate to subulate, densely white-ciliate. Corolla white, 4–6 mm long, exerted. Anthers lilac, included or barely exerted. Style included.

Etymology:—This new species is dedicated to Professor Pedro Luis Pérez de Paz as a recognition for his contribution to the knowledge of genus *Micromeria* throughout the years.

Conservation Status:—This new species is abundant and has a wide area of distribution in La Gomera so it is considered as LC according to the categories of the IUCN (2012).

Paratypes:— SPAIN. La Gomera: Entre Ayamosna y Tagamiche, 16 June 2012, *P. L. Pérez 705-G5* (TFC); *Puppo et al.* 568.3 (TFC).

Other Specimens Examined:—SPAIN. La Gomera: Túneles de Aguajilva, Bco. de Aguajilva, 15 June 2012, *P. L. Pérez 705-G1* (TFC); Carretera Agulo-Las Rosas, 15 June 2012, *P. L. Pérez 705-G2* (TFC); Presa El Garabato, El Tion, 15 June 2012, *P. L. Pérez 705-G3* (TFC); Epina, 15 June 2012, *P. L. Pérez 705-G4* (TFC); Entrada Bco. de Benchijigua, desde Las Torres, 16 June 2012, *P. L. Pérez 705-G7* (TFC); Arure, 16 June 2012, *P. L. Pérez 705-G11* (TFC); *Puppo et al.* 580.2 (TFC); *Puppo et al.* 580.5 (TFC); El Rejo, 17 June 2012, *P. L. Pérez 705-G12* (TFC); *Puppo et al.* 582.3 (TFC).

Geographical Distribution:—This species grows throughout La Gomera island being especially frequent in the northern part where it grows from the sea level up to the higher parts of the Garajonay National Park.

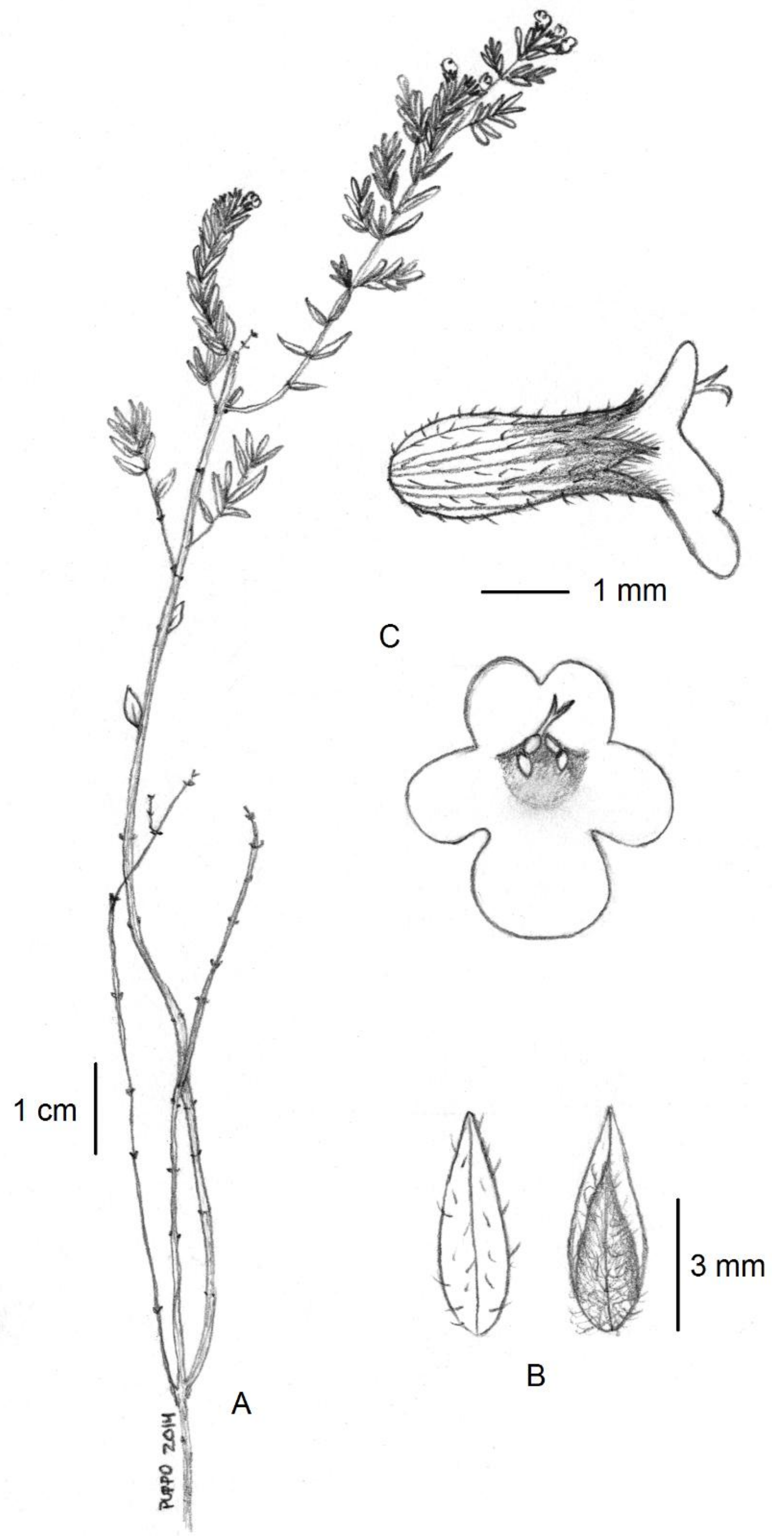


Fig. 7 *Micromeria ferrensis* flowering branch (A), leaf axial and abaxial (B) and flower, frontal and side view (C). Drawn by P. Puppo from the holotype.

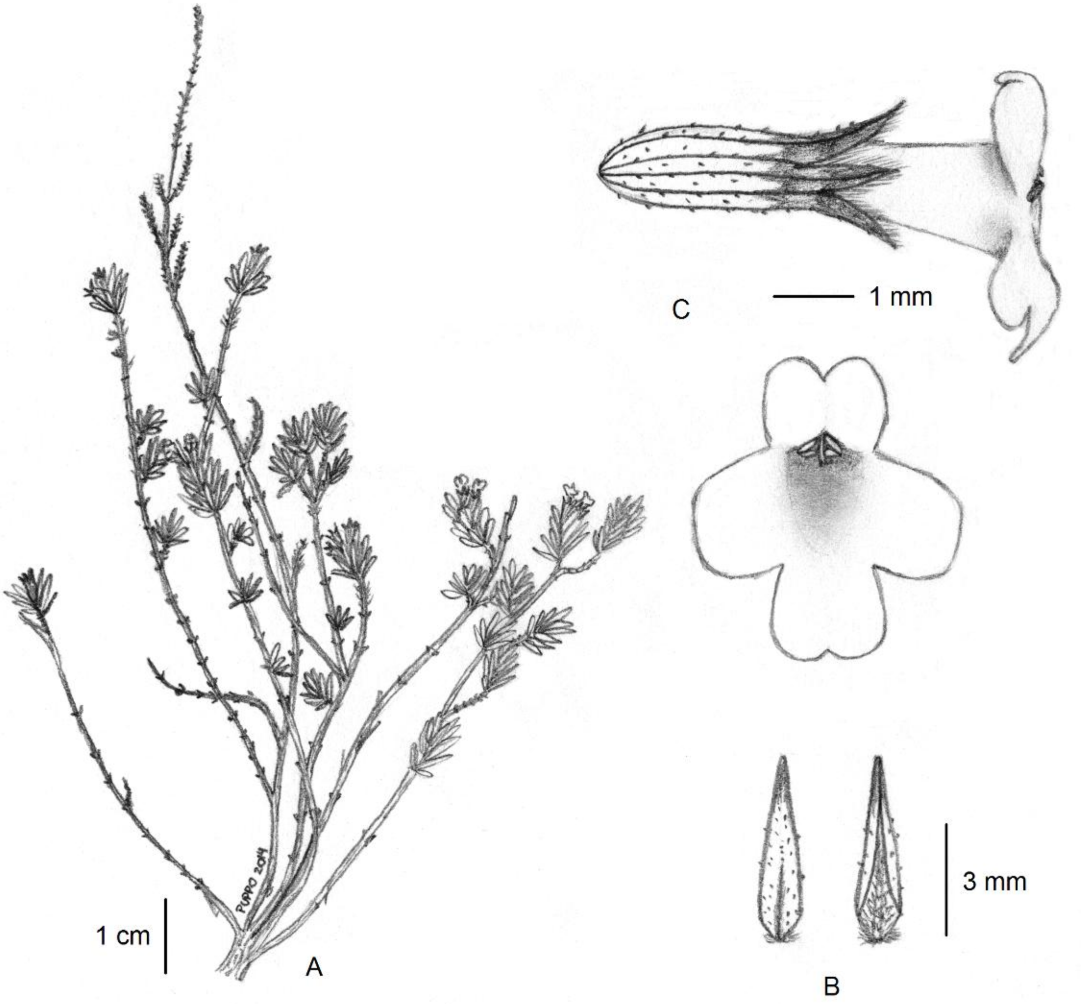


Fig. 8 *Micromeria pedro-luisii* flowering branch (A), leaf axial and abaxial (B) and flower, frontal and side view (C). Drawn by P. Puppo from the holotype.

Notes:—This species was formerly identified as *M. varia* subsp. *varia* in the revision of Pérez de Paz (1978) but molecular data shows that this taxon is different from *M. varia* from Tenerife so a new name is given.

In the higher regions, *M. pedro-luisii* can be found growing in sympatry with the other species present in the island: *M. gomerensis*, *M. lepida* subsp. *lepida* and subsp. *bolleana.* Intermediate specimens have been observed in these areas between some of these species and *M. pedro-luisii*. These hybrids are being formally described below.

Micromeria* × *garajonayii Puppo & P. Pérez, *nothosp. nov*. Fig. 9–A, B.

Type:—SPAIN. La Gomera: Parque Nacional del Garajonay, cruce de la Zarcita, 28º06’38,3’’N, 17º13’01,9’’W, Alt. 1170 m, 17 July 2012, *P. Puppo & P. Pérez 585.1* (holotype: TFC).

Hybrid between *Micromeria lepida* subsp. *lepida* and *M. pedro-luisii* presenting intermediate characteristics from both parents: indumentum and pedunculated cymes from the former, secondary ramification and pauciflorous cymes from the latter.

Subshrub, 30–40 cm high, highly branched from the base; secondary branching present; branches semi prostrated or ascending, velutinous or lanuginose, basal part of branches shedding bark. Leaves subsessile, ericoids, arranged in young branches; blades 4–8 × 0.8–1.2 mm, sublinear, revolute, sparsely velutinous or lanuginose adaxially, abaxially lanose. Cymes pedunculated, loosely arranged on younger branches; peduncles 2–5 mm long; bracts up to 3 mm long, linear; flowers subsessile or shortly pedicelated, pedicels less than 1 mm long; bracteoles 1.5–2 mm long, linear. Calyx tubular, green tinged with violet, sparsely velutinous, 3–4 mm long, calyx apices subulate, straight. Corolla white to lilac, exerted, lower lip projected outwards. Anthers lilac, included. Style included.

Etymology:—The epithet of this new hybrid alludes the area it inhabits, Garajonay National Park.

Paratype:—SPAIN. La Gomera: Parque Nacional del Garajonay, Cruce de la Zarcita, 28º06’38,3’’N, 17º13’01,9’’W, 17 July 2012, *P. Pérez 2012-G-13* (TFC).

Geographical Distribution:—This hybrid has been observed only in Cruce de la Zarcita in Garajonay National Park. It grows in rocky slopes, in the clearings of the forest, and border of roads above 1100 m.

Notes:—*M.* × *garajonayii* is easily recognized in the field because it shows intermediate morphological characteristics from both parents. Its habit is very similar to *M. pedro-luisii* (Fig. 8) presenting secondary branching, semi prostrated branches and leaves densely arranged in younger branches. Unlike *M. pedro-luisii*, this hybrid presents velutinous or lanuginose indumentum, pedunculated cymes, calyx apices subulate and inferior lip of the corolla projected outwards, characteristics that the hybrid takes from the other parent, *M. lepida* subsp *lepida* (Fig. 9–C, D). *Micromeria* ×

garajonayii presents normally developed stamens and styles. The hybrids were found growing next to specimens of *M. lepida* subsp. *lepida*. Even though specimens from *M. pedro-luisii* were not observed on the same locality where the hybrids grow, they were observed in nearby areas such as Mirador de El Rejo in the same National Park. Furthermore, small black bees were observed visiting the flowers from the parents and the hybrids, easily transporting pollen from one plant to the other among localities.

Micromeria × ayamosnae Puppo & P. Pérez, nothosp. nov. Fig. 10–A, B.

Type:—SPAIN. La Gomera: sobre Ayamosna, camino entre Ayamosna y Tagamiche, 28º05´56,4’’N, 17º10’22,5’’W, Alt. 786 m, 16 July 2012, P. Puppo, P. Pérez & F. Faure 570.4 (holotype: TFC).

Hybrid between Micromeria lepida subsp. bolleana and M. pedro-luisii presenting intermediate characteristics from both parents: pedunculated cymes and terebinthinaceous smell from the former, fewer leaves and exerted style from the latter.

Subshrub, 15–20 cm high, basal stem thick, highly branched from the base; branches ascendant or erect, sparsely velutinous, basal part of branches shedding bark. Leaves subsessile, ericoids, arranged in the upper parts of young branches; blades 5–8 × 0.8–1 mm, sublinear, revolute, sparsely velutinous or puberulous adaxially, abaxially strigose or densely velutinous. Cymes pedunculated, abundant, arranged on the upper parts of younger branches; peduncles 2.5–4 mm long; bracts up to 3 mm long, subulate, puberulous or velutinous. Flowers subsessile; bracteoles 1.5 mm long, linear, subulate, puberulous or velutinous. Calyx tubular, green tinged with violet, sparsely velutinous, 3–4 mm long, calyx apices acute, slightly projected outwards. Corolla white, exerted, lower lip projected downwards. Anthers white or lilac, included. Style slightly exerted.

Etymology:—The name of this new hybrid makes reference to the locality where the type was found, Ayamosna.

Paratypes:—SPAIN. La Gomera: sobre Ayamosna, camino entre Ayamosna y Tagamiche, 28º05´56,4’’N, 17º10’22,5’’W, 786 m, 16 July. 2012, P. Puppo, P. Pérez & F. Faure 570.1 (TFC); P. Puppo et al. 570.2 (TFC); P. Puppo et al. 570.3 (TFC); P. Puppo et al. 570.5 (TFC); P. Puppo et al. 570.6 (TFC).

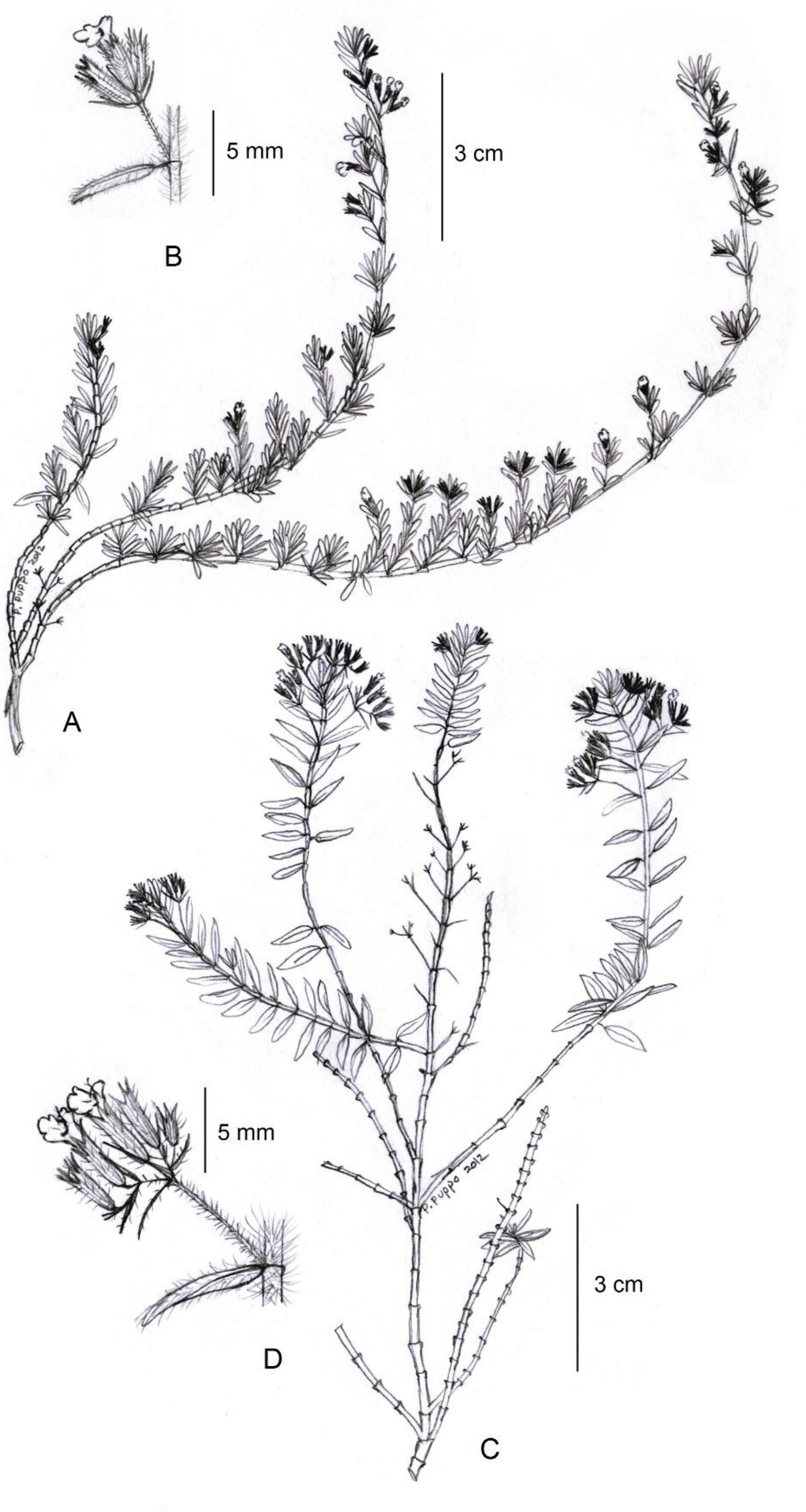


Fig. 9 *Micromeria* × *garajonayii* flowering branch (A) and inflorescence (B). Drawn by P. Puppo from the holotype. *Micromeria lepida* subsp. *lepida* flowering branch (C) and inflorescence (D). Drawn by P. Puppo from *Puppo et al. 584.1* (TFC).

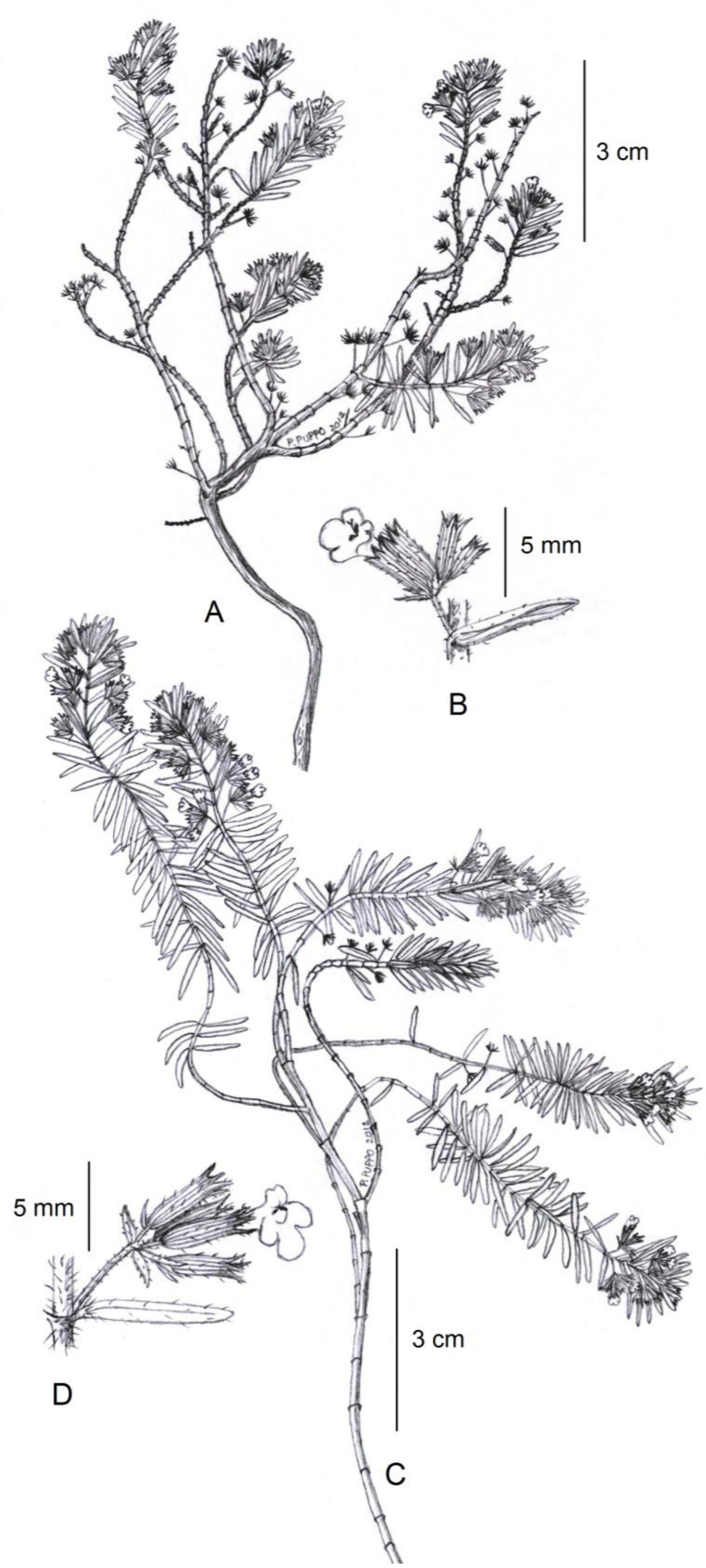


Fig. 10 *Micromeria* × *ayamosnae* flowering branch (A) and inflorescence (B). Drawn by P. Puppo from the holotype. *Micromeria lepida* subsp. *bolleana* flowering branch (C) and inflorescence (D). Drawn by P. Puppo from *Puppo et al. 571.3* (TFC).

Other Specimens Examined:—SPAIN. La Gomera: Barranco de Benchijigua, 28º04’48,3’’N, 17º12’33’’W, 758 m, 16 July 2012, P. Puppo, P. Pérez & F. Faure 575.1 (TFC); P. Puppo et al. 575.2 (TFC).

Geographical Distribution:—Other than the type locality, this hybrid has been found in Barranco de Benchijigua, where both parents grow in sympatry. It grows in rocky slopes between 750–800 m.

Notes:—The habit of this hybrid resembles that of *M. lepida* subsp. *bolleana* (Fig. 10–C, D): thick basal stem, erect, ascending branches, and terebinthinaceous smell. On the other hand, *M.* × *ayamosnae* presents shorter branches with fewer leaves, and less dense pubescence, characteristics coming from the other parent *M. pedro-luisii* (see Fig. 8). Regarding the floral parts, the cymes are pedunculated as in *M. lepida* subsp. *bolleana* though the pedicels and flowers are not as long (Fig. 10). The calyx apices are acute and the style is exerted as in *M. pedro-luisii.* The hybrid specimens present normally developed stamens and styles.

Micromeria × *ayamosnae* was found growing among individuals from both parents in two localities in the SE of the island though their distribution might be wider. In the locality of Benchijigua, mosquitoes were observed visiting the flowers of the hybrid. Similarly, in the locality of Ayamosna bees were found going through the flowers of the parents and hybrids alike. Some individuals from *M. gomerensis* were observed as well in Ayamosna though no morphological characters were observed from this species in the hybrids.

Micromeria madeirensis Puppo & Bräuchler, *nom. nov*. Fig. 11. *Micromeria thymoides* (Sol. ex Lowe) Webb & Berthel., Hist. Nat. Iles Canaries 3: 71. [Dec.] 1844, nom. illeg., non De Not. Repert. Fl. Ligust.: 319–320. 1844. *Satureja thymoides* Sol. ex Lowe, Trans. Cambridge Philos. Soc. iv. 19. 1831.

Type: —PORTUGAL. “Hab. in Maderâ et Portu $^{\mathrm{Sto}}$, vulgaris”, *Sol. MSS.* (MB!).

Erect subshrub 10–35 cm height, highly branched; branches straight or ascending, basal parts of branches glabrous shedding bark, younger parts white tomentose. Leaves sessile or subsessile, petioles when present less than 1 mm long; blades herbaceous, 6–10 × 1–3 mm, green, basal blades lanceolate, flat, glabrate or minutely pubescent adaxially, abaxially tomentose on the midrib, upper blades linear, revolute, adaxially glabrous, velutinous or minutely strigose, abaxially tomentose. Cymes shortly pedunculated or almost sessile. Calyx shortly pubescent to pilose, less than 3 mm long, calyx apices lanceolate to subulate, white-ciliate. Corolla white to pink or pink to purple, up to 4,5 mm long, exerted. Anthers included or barely exerted. Style included or exerted.

Etymology:—The new name of this species alludes to the island of Madeira where this species grows.

Specimens Examined:—PORTUGAL. Madeira: Fayal-bresal, bajo Eira do Serrado, July 1976, *J. R. Arcebes & P. L. Pérez de Paz s.n.* (TFC); Ribeira Brava, 100 m, July 1976, *P. L. Pérez de Paz & J. R. Arcebes s.n.* (TFC); Juxta “Pico Arrieiro”, 1750 m, July 1976, *P. L. Pérez de Paz s.n.* (TFC); Ribeira do Inferno, 27 July 1977, *I. La Serna & M. del Arco s.n.* (TFC).

Geographical Distribution:—This species occurs in all islands of Madeira archipelago (Madeira, Porto Santo and Desertas). In Madeira, it is more abundant in the eastern half of the island from the sea-level up to the highest points between Pico do Arrieiro and Pico Ruivo, between 1600–1800 m.

Notes:—The epithet “thymoides” Webb & Berthel. is not used because De Notaris published *Micromeria thymoides* before Webb and Berthelot based on a specimen collected in Italy. *Micromeria thymoides* De Not. is a synonym of *M. graeca,* widely distributed in the Mediterranean region while *M. thymoides* Webb & Berthel. has been frequently cited as synonym of *M. varia*. Both names have been widely used in the literature, especially during the 19th century (see e.g. Bentham 1848, Otto & Dietrich 1852, Hartung & Bronn 1860, Christ 1885, 1888, Bolle 1892, Jackson 1893 for *M. thymoides* Webb & Berthel.; Grisebach 1843, Bertolini 1854, Durand & Jackson 1886–1895, Parlatore & Caruel 1884, Penzig 1897–1898 for *M. thymoides* De Not.) therefore the need to rename the taxon from Madeira.

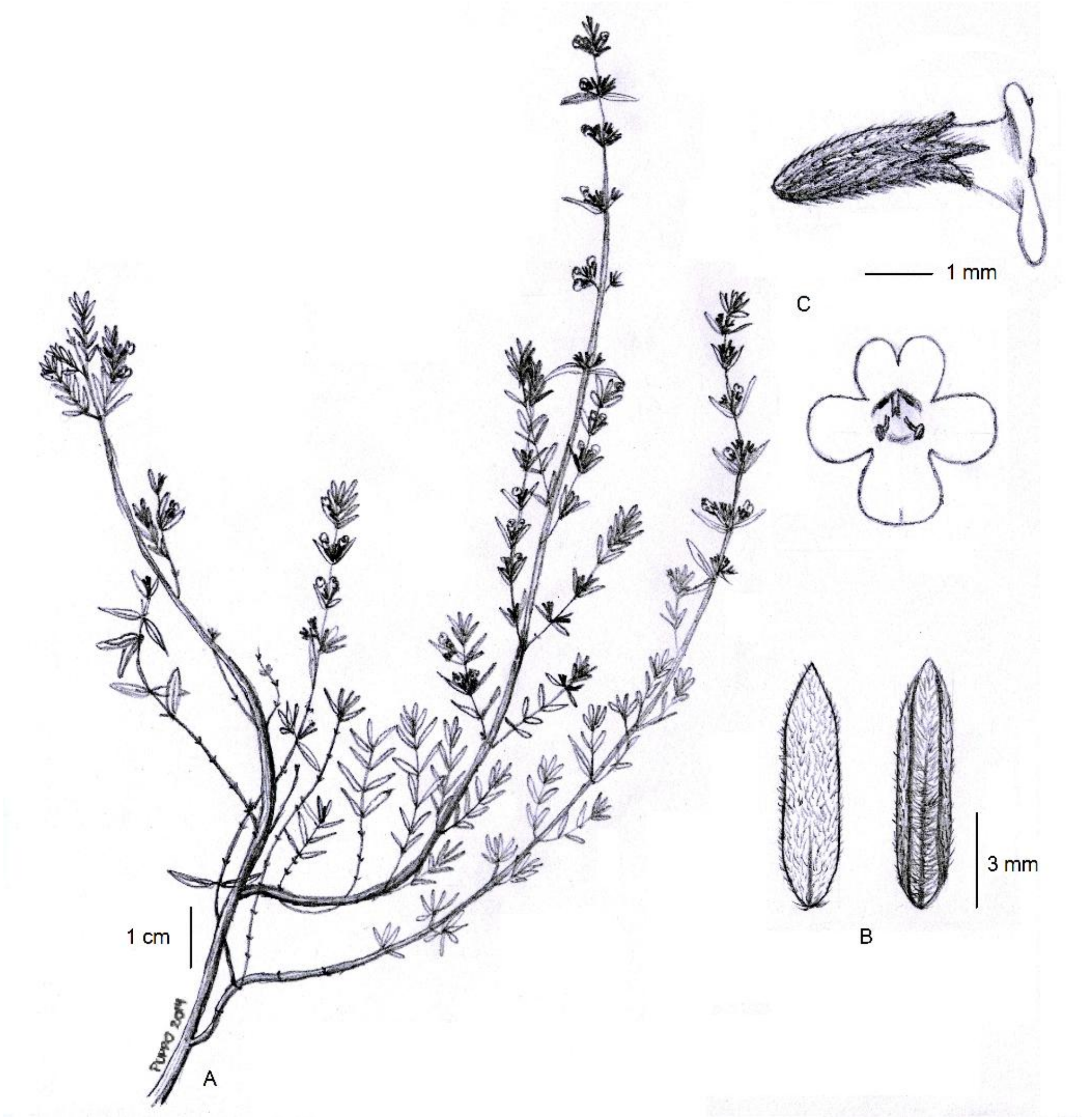


Fig. 11 *Micromeria madeirensis* flowering branch (A) leaf axial and abaxial (B) and flower, frontal and side view (C). Drawn by P. Puppo from *P. L. Pérez & J. R. Acebes s.n.* (TFC).

KEY TO ALL THE SPECIES OF *MICROMERIA* PRESENT IN THE CANARY ISLANDS (modified from Pérez de Paz 1978)

1. Leaves ovate, lanceolate or oblong-lanceolate, coriaceous or subcoriaceous, generally glabrous, margins straight or slightly revolute………………………………….. 2
- Leaves linear to lanceolate, not coriaceous, glabrate to hairy, tomentose or wooly, margins revolute……………………………………………………………………………… 6

2. Leaves with glands in the abaxial side; flowers less than 5 mm long…... *M. teneriffae*
- Leaves lacking glands; flowers larger than 10 mm long………………………………… 3

3. Leaves subcoriaceous, lanceolate, 2 cm long.; Gran Canaria…… *M. helianthemifolia*
- Leaves coriaceous, not lanceolate, less than 1.5 cm long.; Tenerife………………….. 4

4. Leaves ericoid, less than 2 mm width; Teno………………………………. *M. densiflora*
- Leaves not ericoid, wider than 3 mm; Anaga…………………………………………….. 5

5. Leaves oblong, apex acuminate; corolla pink-violet; style included……. *M. glomerata*
- Leaves obovate, apex acute; corolla white or slightly pink; style exerted…………………………………………………………………... *M. rivas-martinezii*

6. Leaves up to 3 cm long.; flowers larger than 12 mm long…………………………….. 7
- Leaves shorter than 2 cm long.; flowers smaller than 9 mm long……………………… 8

7. Subshrub larger than 60 cm tall; leaves lanceolate; corolla violet; restricted to NW Gran Canaria……………………………………………………………….. *M. pineolens*
- Subshrub less than 20 cm tall; leaves oblong; corolla white; restricted to SW Gran Canaria………………………………………………………………………… *M. leucantha*

8. Plants densely white-lanate or grey-tomentose; Gran Canaria……………………….. 9
- Plants glabrate, strigose, pilose, or tomentose; Gran Canaria or other islands…….. 11

9. Plants grey-tomentose; upper lip of the corolla conspicuous……………….. *M. tenuis*
- Plants white-lanate; upper lip of the corolla reduced…………………………………... 10

10. Inflorescence pedicelated; calyx ovate; corolla barely exerted…………….. *M. lanata*
- Inflorescence sessile; calyx tubular; corolla exerted……………………… *M. benthamii*

11. Corolla 1.5–2 times larger than the calyx; La Palma…………….. *M. herpyllomorpha*
- Corolla 1–1.5 times larger than the calyx; other islands………………………………. 12

12. Leaves up to 16 mm long, strigose, abaxial mid-vein sericate……… *M. hyssopifolia*
- Leaves less than 12 mm long, glabrate to tomentose throughout……………………. 13

13. Leaves ericoid, upper leaves arranged in fascicles…………………………………. 14
- Leaves ovate, lanceolate or linear, not arranged in fascicles…………………………. 18

14. Tenerife……………………………………………………………………………... 15
- La Gomera…………………………………………………………………………….. 17

15. Leaves glabrate or puberulent; corolla soft pink; from Teno to Anaga below 1000 m……………………………………………………………………………………... *M. varia*
- Leaves tomentose or pilose; corolla white; Las Cañadas above 2000 m…………… 16

16. Leaves strigose on the adaxial side, abaxial side lanuginose; calyx tubular; inferior corolla lip projected upwards……………………………………………..… *M. lachnophylla*
- Leaves pilose on the adaxial side, abaxial side tomentose; calyx campanulated; inferior corolla lip slightly folded backguards……………………………... *M. lasiophylla*

17. Plants glabrate to velutinous; inflorescence sessile; corolla clearly exerted…………………………………………………………………….. *M. pedro-luisii*
- Plants strigose to tomentose; inflorescence pedicelated; corolla barely exerted…………………………………………………………………………….. *M. lepida*

18. Corolla white; El Hierro……………………………………………………… *M. ferrensis*
- Corolla light pink to purple; other islands…………………………………………... 19

19. Leaves sparsely strigose; calyx less than 2.5 mm long.; La Gomera………………………………………………………………………… *M. gomerensis*
- Leaves glabrate, densely puberulent, or pubescent; calyx 2.5–3.5 mm long.; Gran Canaria or Lanzarote and Fuerteventura………………………………………………. 20

20. Leaves pubescent; Lanzarote and Fuerteventura……………………….. *M. rupestris*
- Leaves glabrate to densely puberulent; Gran Canaria………………….. *M. canariensis*

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Pedro Luis Pérez de Paz for providing helpful comments throughout this work. Christian Bräuchler helped especially with the code for nomenclature. Francisco Faure helped during fieldwork. We thank the staff at the TFC herbarium for facilitating the revision of the *Micromeria* collection. This research was supported by the Fundação para a Ciência e Tecnología (FCT) with a research grant to H.M. (PTDC/BIA-BEC/108866/2008) and a Ph.D. fellowship to P.P. (SFRH/BD/74747/2010).

REFERENCES

Bentham, G (1829) *Micromeria* Benth., gen. nov. In: Lindley, J (Ed.) Edward's Botanical Register, London, v. 15, 1282.

Bentham, G (1834) Labiatarum Genera et Species, London, 783 pp.

Bentham, G (1848) *Micromeria* Benth., gen. nov. In: De Candolle, A (Ed.) Prodromus Systematis Naturalis Regni Vegetabilis, Paris, v. 12, 27-603.

Bertoloni, A (1854) Flora Italica, Bononiae, v. 10, 639 pp.

Bolle, C (1860) Addenda ad floram Atlantidis, praecipue insularum Canariensium Gorgadumque. In: Seemann, WEG & B Seemann (Eds.) Bonplandia, Hannover, v. 8, 277-287.

Bolle, C (1892) Botanische Rückblicke auf die Inseln Lanzarote und Fuertaventura. In: Engler, A (Ed.) Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie, Leipzig, v. 16, 224-261.

Bornmüller, J (1909) Eine neue *Micromeria* der Kanarischen Inseln. In: Fedde, F (Ed.) Repertorium Specierum Novarum Regni Vegetabilis, Berlin, v. 9, 1-2.

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

Bräuchler, C, O Ryding and G Heubl (2008) The genus *Micromeria* (Lamiaceae), a synoptical update. Willdenowia 38, 363-410.

Christ, H (1885) Vegetation und Flora der Canarischen Inseln. In: Engler, A (Ed.) Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie, Leipzig, v. 6, 458-527.

Christ, H (1888) Spicilegium canariense. In: Engler, A (Ed.) Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie, Leipzig, v. 9, 86-172.

De Notaris, G (1844) Repertorium florae Ligusticae, Taurini, 495 pp.

Durand, T and BD Jackson (1886–1895) Index Kewensis, Bruxellis, suppl. 1, 519 pp.

Engler, A and K Prantl (1896) Naturlichen Pflanzenfamilien, Leipzig, v. 4(3a), 380 pp.

Grisebach, A (1843) Bericht über die Leistungen in der Pflanzengeographie, Berlin, 98 pp.

Hansen, A and P Sunding (1993) Flora of Macaronesia. Checklist of vascular plants. 4. revised edition. Sommerfeltia 17, 1-295.

Hartung, G and HG Bronn (1860) Die Azoren in ihrer äusseren erscheinung und nach ihrer geognostischen natur, Leipzig, 350 pp.

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK.

Jackson, BD (1893) Index Kewensis, Oxford, v. 2, pt. 2, 657-1299.

Lid, J (1968) Contributions to the flora of the Canary Islands. Skrifter utgitt av det Norske Videnskaps-akademi i Oslo ser. 2, v. 23, 1-212.

Lowe, RT (1831) Primitiae Faunae et Florae Maderae et Portus Sancti. Transactions of the Cambridge Philosophical Society v. 4, 1-70.

Meimberg, H, T Abele, C Bräuchler, JK McKay, PL Pérez de Paz and G Heubl (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.

Otto, F and A Dietrich (1852) Madeira. Allegemeine Gartenzeitung v. 20, 69-71.

Parlatore, F and T Caruel (1884) *Flora Italiana*, Firenze, v. 6, 656 pp.

Penzig, O (1897–1898) Florae Ligusticae Synopsis. Annali del Museo Civico di Storia Naturale di Genova ser. 2, v. 38, 423-531.

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

Puppo, P, M Curto, G Velo-Antón, PL Pérez de Paz and H Meimberg (2014) The influence of geological history on diversification in insular species: genetic and morphological patters of *Micromeria* Benth. (Lamiaceae) in Tenerife (Canary archipelago). Journal of Biogeography 41, 1871-1882.

Puppo, P, M Curto, J Gusmão-Guedes, J Cochofel, PL Pérez de Paz, C Bräuchler and H Meimberg (2015) Molecular phylogenetics of *Micromeria* (Lamiaceae) in the Canary Islands and Madeira, diversification and inter-island colonization patterns inferred from nuclear genes. Molecular Phylogenetics and Evolution 89, 160-170.

Webb, PB and S Berthelot (1844) Histoire Naturelle des Iles Canaries, Paris, t. 3, ptie 2, sect. 3, 1-479 pp.

Willemse, RH (1991) New Combinations and Names for Macaronesian *Satureja* Taxa (Labiatae). Willdenowia 21 (1-2), 81-85.

Chapter 5:

General Discussion

5.1 How island's geology influences the diversification of insular taxa: the example of *Micromeria* in Tenerife

About one quarter of all known extant species is restricted to islands (Kreft et al. 2008). Among the factors responsible for the high number of insular species, area and geographical isolation have been identified as the variables with the strongest effect followed by geology and elevation (Kreft et al. 2008). In the case of geologically complex islands, successive volcanic activity, landslides, caldera collapses, etc, have a strong impact on the diversification of insular species. Such is the case in Tenerife, where the complexity of its geological history has shaped the diversification of its taxa.

Tenerife's palaeoislands harbor a unique biodiversity. In the Canarian archipelago, Teno has been identified as the region presenting the highest endemic plant richness with ca. 140 taxa (Reyes-Betancort et al. 2008). The palaeoislands of Anaga and Adeje also show high levels of plant species richness with ca. 110 and 100 taxa each (Reyes-Betancort et al. 2008) In Tenerife, several species groups present a similar phylogeographic pattern with sister taxa inhabiting one of the palaeoislands (Juan et al. 2000). This genetic structure may result from the secondary connection of the palaeoislands by the uprising of the Teide volcano, but also from more recent catastrophic events that re-isolated parts of the island. Two huge landslides isolated Anaga from Teno: one ca. 650-370 ka, gave origin to La Orotava valley, and a second, more recent (ca 170 ka), formed Las Cañadas Caldera and was later filled by the Teide volcano (Ancochea et al. 1990; Watts and Masson 1995; Juan et al. 2000). A third massive landslide ca. 800-600 ka isolated Anaga from Adeje and gave origin to Güímar valley (Ancochea et al. 1990; Juan et al. 2000). While the central part of the island is still in its building stage, the remnants of the palaeoislands are in the last stage of their life cycle with areas significantly reduced due to volcanism and erosion. Thus, following the general dynamic model of oceanic island biogeography (Whittaker et al 2007, 2008), the speciation curve in the central part of the island would still be high while species richness in the older areas is decreasing due to habitat loss.

Several genera with taxa endemic to at least one palaeoisland have been included in phylogenetic analyses. Most of these palaeoisland endemics are not early diverging lineages but are part of crown groups in their respective phylogenies suggesting a relatively recent origin. This pattern has been observed in ca. 85% of the cases (Trusty et al. 2005) including several genera in the Asteraceae (Francisco-

Ortega et al. 1996, 2001a; Susanna et al. 1999; Moore et al. 2002; Jones et al. 2014), Crassulaceae (Mort et al. 2002), Fabaceae (Percy and Cronk 2002; Allan et al. 2004), Lamiaceae (Barber et al. 2000; Trusty et al. 2005), Boraginaceae (Böhle et al. 1996), and Brassicaceae (Francisco-Ortega et al. 2002) (See Appendix for a list of genera).

Contrary to this, few genera present early diverging lineages restricted to the palaeoislands. In *Convolvulus* (Convolvulaceae), one species (*C. volubilis*) is present in all three palaeoislands and constitutes the first branching lineage of endemic suffrutescences with climbing habit (Carine et al. 2004). Likewise, in *Lavatera* (Malvaceae), *L. phoenicea* is endemic to Anaga and Teno and is sister to the other Macaronesian taxa (Fuertes-Aguilar et al. 2002). *Sonchus tuberifer* (Asteraceae) is endemic to Teno and is probably the sister taxon to the rest of Macaronesian species (Kim et al. 1996). These studies support the idea that, at least for some groups, the palaeoislands of Tenerife indeed harbor old endemic lineages.

Molecular studies focusing at a population level have also found similar patterns where the genetic structure of the populations coincides with the geological history of Tenerife. Dlugosch and Parker (2007) identified one variety of *Hypericum canariense* (Clusiaceae) restricted to the Anaga massif. Rumeu et al. (2014) found that the populations of *Juniperus cedrus* (Cupressaceae) from Tenerife are different from the other islands. van Hengstum et al. (2012) and Jones et al. (2014) reported a differentiation between the populations from Adeje and Teno in two species of *Pericallis* (*P. lanata* and *P. echinata*; Asteraceae). Several examples like these exist in animals as well. In *Dysdera* spiders, *D. verneaui* constitutes and old, isolated lineage in Teno that probably preceded the formation of the Teide volcano (Macías-Hernández et al. 2013). In ground beetles, there is differentiation among Anaga populations presumably due to ancient habitat discontinuities (Moya et al. 2004). In *Gallotia* lizards the Güímar landslide had a major impact in the genetic structure of its populations (Brown et al. 2006). And in many other groups there is a genetic structure associated with Tenerife’s palaeoislands, e.g. *Tarentola* geckos (Gübitz et al. 2000), *Gallotia* and *Chalcides* lizards (Thorpe et al. 1996; Brown et al. 2000), *Pimelia* darkling beetles (Juan et al. 1996), *Steganecarus* mites, *Loboptera* cockroaches (Juan et al. 2000), among others (see Appendix).

Phylogenetic results on the species of *Micromeria* in Tenerife (paper I, Chapter 2) suggest that a first diversification event occurred ca. 6.7 Ma in the palaeoisland of Anaga giving origin to *M. teneriffae* and later (2.7 Ma) to *M. glomerata* and *M. rivas-martinezii*. According to the phylogeny, a second diversification event occurred ca. 4.5

Ma in Teno giving origin to the other palaeoisland endemic, *M. densiflora*. The palaeoislands of Teno and Anaga emerged ca. 7 and 6 Ma and their secondary connection probably occurred about 3.5 Ma (Ancochea et al. 1990; Fernández-Palacios et al. 2011) thus *M. teneriffae* and *M. densiflora* precede the emergence of the central shield. It is possible that some *Micromeria* species occurred in the palaeoisland of Adeje as well but got extinct when erosion significantly reduced the size of this palaeoisland. A distribution of species in the three palaeoislands is known for at least three other groups supporting this theory: *Convolvulus* (Convolvulaceae) (Carine et al. 2004), *Pericallis* (Asteraceae) (van Hengstum et al. 2012; Jones et al. 2014), and *Pimelia* beetles (Juan et al. 1996). Morphometric analyses (paper I, Chapter 2) show that the palaeoisland endemics *M. densiflora*, *M. glomerata* and *M. rivas-martinezii* are different among them and from the remaining species. *Micromeria teneriffae* appears slightly overlapping with the central taxa. Overall the palaeoisland species have larger flowers and leaves, and present a broader range of variation in the morphometric space. The morphological characteristics of these species seems to be related to the basaltic or salic rocks where they grow, which are much different in age and geological nature than those present in the younger parts of the island.

A third diversification event ca. 3.9 Ma gave origin to the *Micromeria* species inhabiting the central part of Tenerife. The age of this subclade roughly coincides with the fusion of the three palaeoislands (3.5 Ma). It is probable that the central part was colonized from Teno given that *M. densiflora* appears as the sister taxon to the central species. Relations among the species within the central subclade are poorly supported, probably because of introgression or incomplete lineage sorting. Morphometric analyses show that the central species segregate in roughly two groups when analyzed independently from the palaeoisland species: *M. varia* including the samples of *M. lasiophylla*, and *M. hyssopifolia* including the sample from *M. lachnophylla.* These species share some morphological characteristics, e.g. pink corollas in the first group and white corollas in the second, but are overall very plastic phenotypically, especially *M. varia* and *M. hyssopifolia*. These results, i.e. low genetic divergence together with morphological differentiation, have also been reported in other groups of Macaronesian plants such as *Echium* (Boraginaceae) (Böhle et al. 1996), *Androcymbium* (Colchicaceae) (Caujapé-Castells et al. 1999), *Dactylis* (Poaceae) (Sahuquillo and Lumaret 1999), *Sideritis* (Lamiaceae) (Barber et al. 2002), *Teline* and *Lotus* (Fabaceae) (Percy and Cronk 2002; Allan et al. 2004), and in *Pimelia* beetles (Juan et al. 1996).

Genetic diversity levels calculated using microsatellite markers in *Micromeria* from Tenerife (paper II, Chapter 2) suggest that the three species narrowly restricted to the palaeoislands of Anaga and Teno: *M. glomerata*, *M. rivas-martinezii* and *M. densiflora*, are genetically less diverse than the common species *M. hyssopifolia*. This might be explained by bottleneck events or an increase of inbreeding since the populations of these species have been presumably reduced as a consequence of the reduction of the palaeoislands' area. Though preliminary, these results agree with the findings of García-Verdugo et al. (2015) that insular narrow endemics show lower genetic diversity levels than widely distributed island endemics. The geological history of Tenerife is undoubtedly related to these differences in genetic variability since geological changes in islands (erosion, subsidence, volcanism, etc) are the primary factors affecting genetic diversity in insular endemic species (Stuessy et al. 2012, 2013).

In summary, the species of *Micromeria* in Tenerife constitute and interesting example of how the geological history of an island can shape the evolution of its taxa. The palaeoisland species *M. densiflora*, *M. glomerata* and *M. rivas-martinezii* are early diverging lineages persisting in small areas in the palaeoislands and will presumably get extinct when the continuous erosion causes the remnants of Anaga and Teno to disappear. The central species *M. varia*, *M. hyssopifolia*, *M. lachnophylla* and *M. lasiophylla* can be regarded as examples of speciation to newly available habitats after the formation of the Teide volcano and the secondary connection of the palaeoislands. An increase in genetic differentiation would be expected in time in these central species. Finally, this secondary connection of previously isolated parts might have also given the opportunity to some other species, such as *M. teneriffae*, to extend its range and survive outside its original distribution.

5.2 Colonization patterns of *Micromeria* in the Canary Islands

Most studies show that the colonization of oceanic archipelagos follows a progression rule, from the older islands towards the younger ones (see e.g. Juan et al. 2000; Cowie and Holland 2006). Numerous examples of this have been reported in Hawaiian taxa, see e.g.: Nepokroeff et al. 2003, Percy et al. 2008, Dubar-Co et al. 2008, Givnish et al. 2009, Applehans et al. 2014 for plants, and Roderick and Gillespie 1998, Hormiga et al. 2003, Holland and Hadfield 2004 for animals. And a similar pattern has been observed for numerous plant groups in the Canary Islands, e.g.: *Olea* (Oleaceae) (Hess et al. 2000), *Inulaeae* (Asteraceae) (Francisco-Ortega et al. 2001b), *Dendrocrambe* (Brassicaceae) (Francisco-Ortega et al. 2002), *Lotus* (Fabaceae) (Allan et al. 2004), and several groups of animals (see Juan et al. 2000) (see Appendix).

Previous studies in *Micromeria* using ISSR fingerprinting (Meimberg et al. 2006) does not support an east to west colonization but revealed a split between samples from the eastern islands (Gran Canaria, Lanzarote and Fuerteventura) and the western islands (Tenerife, La Palma and El Hierro). Samples from La Gomera form two clades one in each group of islands. Meimberg et al. (2006) study suggested that each of the Canary Islands were colonized one time and La Gomera was colonized twice, once from the eastern islands and another from the western islands. The phylogenetic analyses presented in this thesis (Paper III, Chapter 3) also support the East/West split and, in addition, reveal that the species of *Micromeria* in the Canary Islands probably colonized the archipelago from two islands: Gran Canaria and Tenerife. Similar patterns have been observed in few other groups in the Canarian archipelago as well, e.g.: *Echium* (Boraginaceae) (Böhle et al. 1996), the woody *Sonchus* alliance (Asteraceae) (Kim et al. 1996; Lee et al. 2005), *Gonospermum* alliance (Asteraceae) (Francisco-Ortega et al. 2001a), *Hypericum* (Clusiaceae) (Dlugosch and Parker 2007), lizards (Thorpe et al. 1994), and *Meladena* beetles (Ribera et al. 2003).

Within the eastern clade, *Micromeria* species from Gran Canaria form two groups apparently not correlated to the geological history of the island. Gran Canaria presented different phases of volcanic activity that resulted in two geological zones, the SW half of the island (“palaeocanaria”) composed of Miocene substrates, and the NE half (“neocanaria”) rejuvenated by eruptions during the Pliocene (Del-Arco et al. 2002). The two groups of *Micromeria* species observed in Gran Canaria do not correspond to these geological zones but might be the result of other events, also related to volcanic activity such as massive extinctions or vicariance (Juan et al. 2000). This differentiation could also be caused by incomplete lineage sorting or continuous gene flow among the species. Similar within-island variation has been reported in *Tarentola* geckos (Juan et al. 2000). Samples from Lanzarote and La Gomera are nested within the Gran Canaria clade suggesting colonization from this island (Paper III, Chapter 3). It is possible that *Micromeria* initially arrived to the Canary Islands via Lanzarote and Fuerteventura since these easternmost islands are in closer proximity to the continent (less than 100 km). It is also probable that the original *Micromeria* lineages disappeared due to the severe erosion and changes in sea level that affected Lanzarote and Fuerteventura during the late Pliocene (Fernández-Palacios et al. 2011), making a back colonization from Gran Canaria possible. La Gomera was probably colonized several times from Gran Canaria.

The composition of the western clade seems related to the geological evolution of Tenerife and it generally agrees with the results obtained in paper I (Chapter 2) discussed in the previous section. The phylogeny presented in paper III (Chapter 3) suggests that other westernmost islands were colonized from Tenerife: La Palma, El Hierro and La Gomera, as well as the archipelago of Madeira. La Palma was probably colonized from Teno since samples from that island group together with this palaeoisland's taxon, *M. densiflora*. This would be the most likely scenario taking into account a stepping stone model since Teno is geographically closer to La Palma. El Hierro on the other hand, seems to have been colonized from central Tenerife. La Gomera was probably colonized more than once from Anaga and from Teno or central Tenerife. La Gomera together with the three palaeoislands of Tenerife formed a small archipelago in the Miocene (Fernández-Palacios et al. 2011) and a disjoint distribution of species in this palaeoarchipelago has been reported for several plant genera (see Trusty et al. 2005) and some animals (e.g. Cox et al. 2012).

Previous studies have suggested the importance of Tenerife as a center of dispersal in the Canary Islands (Francisco-Ortega et al. 2002; Allan et al. 2004). And at least in *Echium* (Boraginaceae) (Böhle et al 1996), *Crambe* (Brassicaceae) (Francisco-Ortega et al. 2002), and *Lotus* (Fabaceae) (Allan et al. 2004), Tenerife appears as the source for the colonization of the remaining islands that compose the Canarian archipelago. In *Hypericum* (Clusiaceae), taxa from Tenerife colonized La Gomera coinciding with trade wind direction and water currents (Dlugosch and Parker 2007). In *Gonepteryx* butterflies (Brunton and Hurst 1998), *Meladema* beetles (Ribera et al. 2003), and *Gallotia* lizards (Cox et al. 2012), Tenerife probably colonized La Gomera and La Palma. As in the case of *Micromeria*, Tenerife's palaeoislands seem to have played a key role in the colonization of other westernmost islands. In *Hypericum canariense* (Clusiaceae) taxa from Anaga probably colonized El Hierro (Dlugosch and Parker 2007). In *Gallotia* lizards, the palaeoisland of Adeje is suggested as the colonization source for two different paths: Anaga and subsequently La Palma, and La Gomera and from there to El Hierro (Thorpe et al. 1994; Cox et al. 2012). In *Dysdera* spiders, La Gomera and El Hierro were probably colonized from Teno (Macías-Hernández et al. 2013).

Madeira archipelago was probably colonized from Tenerife as well, concretely from Anaga or Central Tenerife. Given the results obtained in the Tenerife phylogeny (paper I, Chapter 2), it is most likely that the latter case is true. Madeira is ca. 450 km north of the Canary Islands and a stepping stone connection between the two archipelagos has been suggested via the Savage Islands (Fernández-Palacios et al. 2011). Evidence for the colonization of Madeira from Tenerife has been reported in at least two other plant groups: *Sonchus* (Asteraceae) (Lee et al. 2005) and *Pericallis* (Asteraceae) (Panero et al. 1999; Jones et al. 2014). But more examples of colonizations from other islands of the archipelago are also known, e.g. *Echium* (Boraginaceae) (Böhle et al. 1996; Kim et al. 2008) from La Palma, *Sideritis* (Lamiaceae) (Barber et al. 2002; Kim et al. 2008) from El Hierro, *Crambe* and *Dendrocrambe* (Brassicaceae) (Francisco-Ortega et al. 2002; Kim et al. 2008) from Lanzarote. And in several other groups colonization from the Canarian archipelago is confirmed but no further information is available as to which island, e.g.: *Bystropogon* (Lamiaceae) (Trusty et al. 2005), *Convolvulus* (Convolvulaceae) (Carine et al. 2004), *Aeonium* (Crassulaceae) (Mort et al. 2002; Kim et al. 2008), and *Teline* and *Genista* (Fabaceae) (Percy & Cronk 2002) (see Appendix).

In most Macaronesian plant groups, inter-island colonization between similar ecological zones seems to be the primary mode of speciation (Francisco-Ortega et al. 2001a) (see Appendix for a list of taxa). In few genera however, monophyletic groups of species are restricted to single islands suggesting an insular diversification after a single colonization event, e.g. *Aeonium* (Crassulaceae) (Mort et al. 2001), *Crambe* (Brassicaceae) (Francisco-Ortega et al. 1996), *Tanacetum* (Gonosperminae) (Francisco-Ortega et al. 2001a), *Sonchus* and *Pericallis* (Asteraceae) (Kim et al. 1996; Panero et al. 1999). In *Micromeria*, it seems that species diversified into different habitats after a single colonization event in most islands. In the case of La Gomera, multiple introductions from Gran Canaria and Tenerife gave origin to the three different lineages observed in that island. These lineages occupy different habitats than their related taxa so inter-island dispersal between similar ecological zones do not seem to explain the observed pattern. It is also possible on the other hand, that the inter-island colonization occurred in fact between similar ecological zones but these changed with time making the current habitats different from the original ones.

5.3 Taxonomic Implications

In the last revision for Canarian *Micromeria*, Pérez de Paz (1978) identified 16 species with numerous subspecies or varieties. The different molecular studies presented in this thesis suggest the revision of the species status of several *Micromeria* taxa.

As explained in the previous section, the species of *Micromeria* present in each island resulted from a single colonization event (except for La Gomera) (paper III, Chapter 3). This means that the species from each island are single island endemics except for the taxon growing in Lanzarote and Fuerteventura.

In Tenerife, phylogenetic and morphometric results (paper I, Chapter 2; paper III Chapter 3) clearly distinguished the palaeoisland endemics: *M. glomerata*, *M. rivas-martinezii* and *M. densiflora*, from the rest of species. All three species present coriaceous leaves and large flowers (ca. 10 mm). *Micromeria glomerata* is characterized by its ovate, acuminate leaves ca. 8 mm long, and white flowers and is restricted to salic rocks in Taganana, Anaga (Fig. 1A, B). *Micromeria rivas-martinezii* presents ovate-lanceolate leaves ca. 11 mm long, and white flowers and is restricted to phonolite rocks in Juan Bay, Anaga (Fig. 1C-E). *Micromeria densiflora* is restricted to basaltic and phonolite rocks in Buenavista, Teno, and is characterized by small, narrowly lanceolate leaves ca. 5 mm long and lilac flowers (Fig. 1F, G). The central species distributed in the higher parts of the island, *M. lachnophylla* and *M. lasiophylla*, are also recognized as monophyletic in the phylogenies. *Micromeria lachnophylla* occurs all around the island being more abundant between 2000-2400 m (Fig. 2A, B). *Micromeria lasiophylla* is restricted to the fissures of the cliffs in the SE part of the Circo de Las Cañadas, always above 2000 m (Fig. 2C, D). The remaining three species: *M. teneriffae*, *M. hyssopifolia*, and *M. varia* are different morphologically as evidenced by morphometric analyses but appear polyphyletic in the phylogenies. *Micromeria teneriffae* is characterized by its coriaceous, obovate, acuminate leaves ca. 6 mm long and small white-lilac flower ca. 6 mm long (Fig. 2E, F). This species is probably and old lineage that originated in the palaeoisland of Anaga and then expanded its distribution to the SE of the island up to Güímar and Fasnia. Two varieties have been described in *M. teneriffae*: var. *teneriffae* and var. *cordifolia* (Pérez de Paz 1978); these are not supported in the phylogeny since the samples of both varieties do not group together. *Micromeria varia* and *M. hyssopifolia* present high levels of phenotypical variation which has made their taxonomy complicated in the past (see paper IV, Chapter 4). Typical individuals of *M. varia* present ericoid, revolute leaves arranged in fascicles and

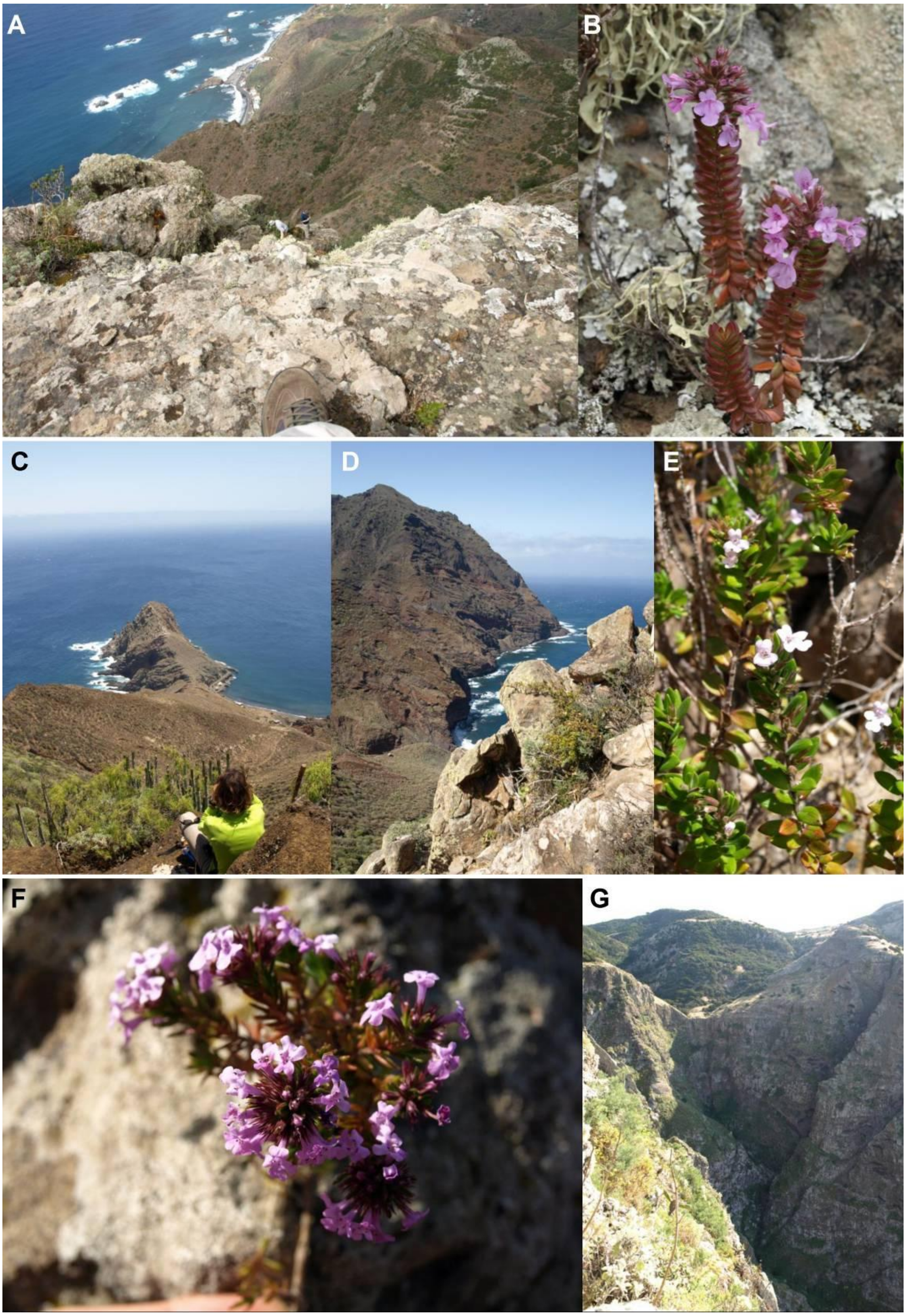


Fig. 1 *Micromeria glomerata*, habitat in Buenavista, Teno (A), flowering individual (B). *Micromeria rivas-martinezii*, habitat in Roque de Juan Bay, Anaga (C, D), flowering branches (E). *Micromeria densiflora*, flowering individual (F), habitat in Buenavista, Teno (G). All photos taken by P. Puppo.

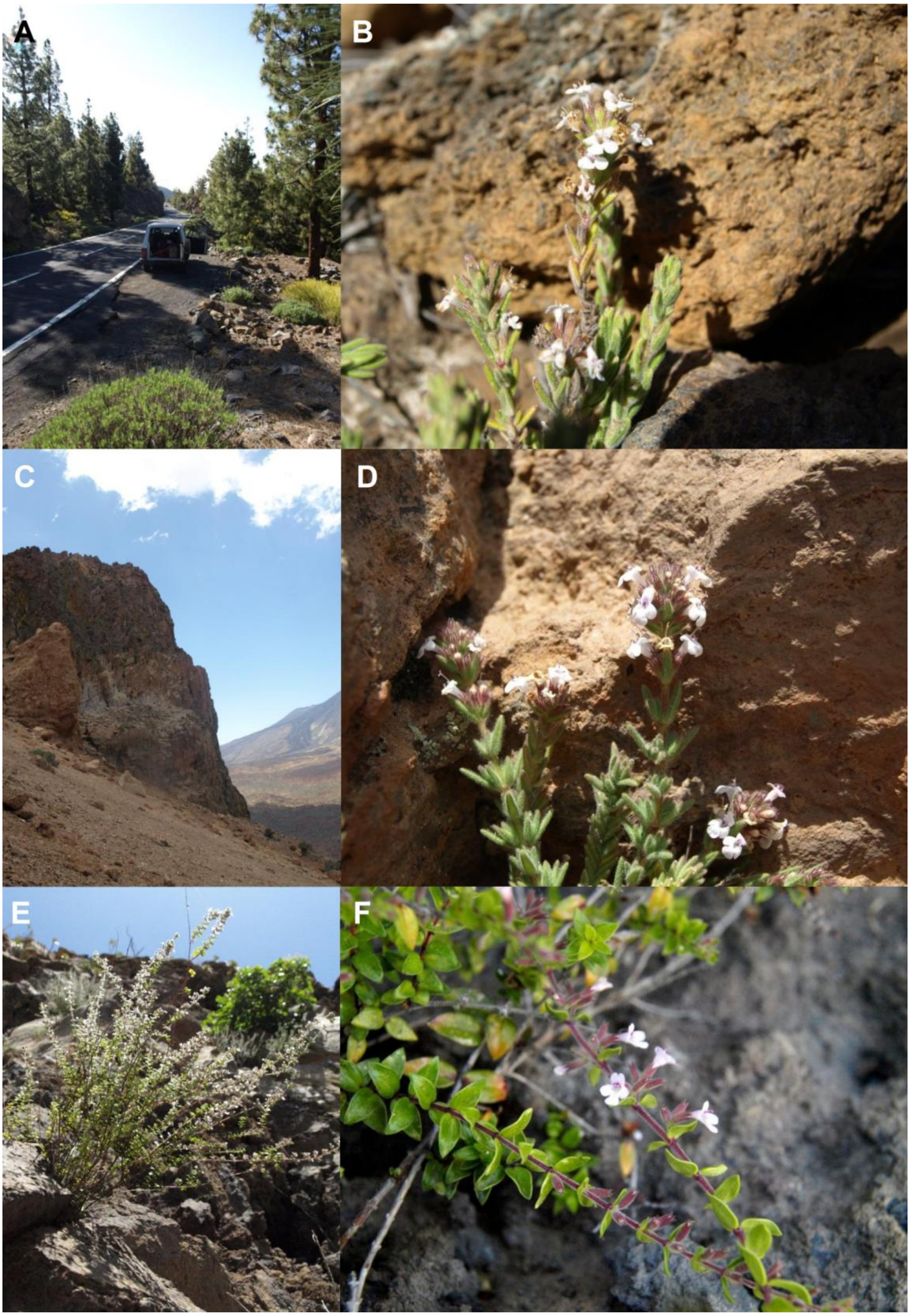


Fig. 2 *Micromeria lachnophylla*, habitat in El Portillo (A), flowering branches (B). *Micromeria lasiophylla*, habitat in Teide National Park (C), flowering branches (D). *Micromeria teneriffae*, habit (E), flowering branch (F). All photos taken by P. Puppo.

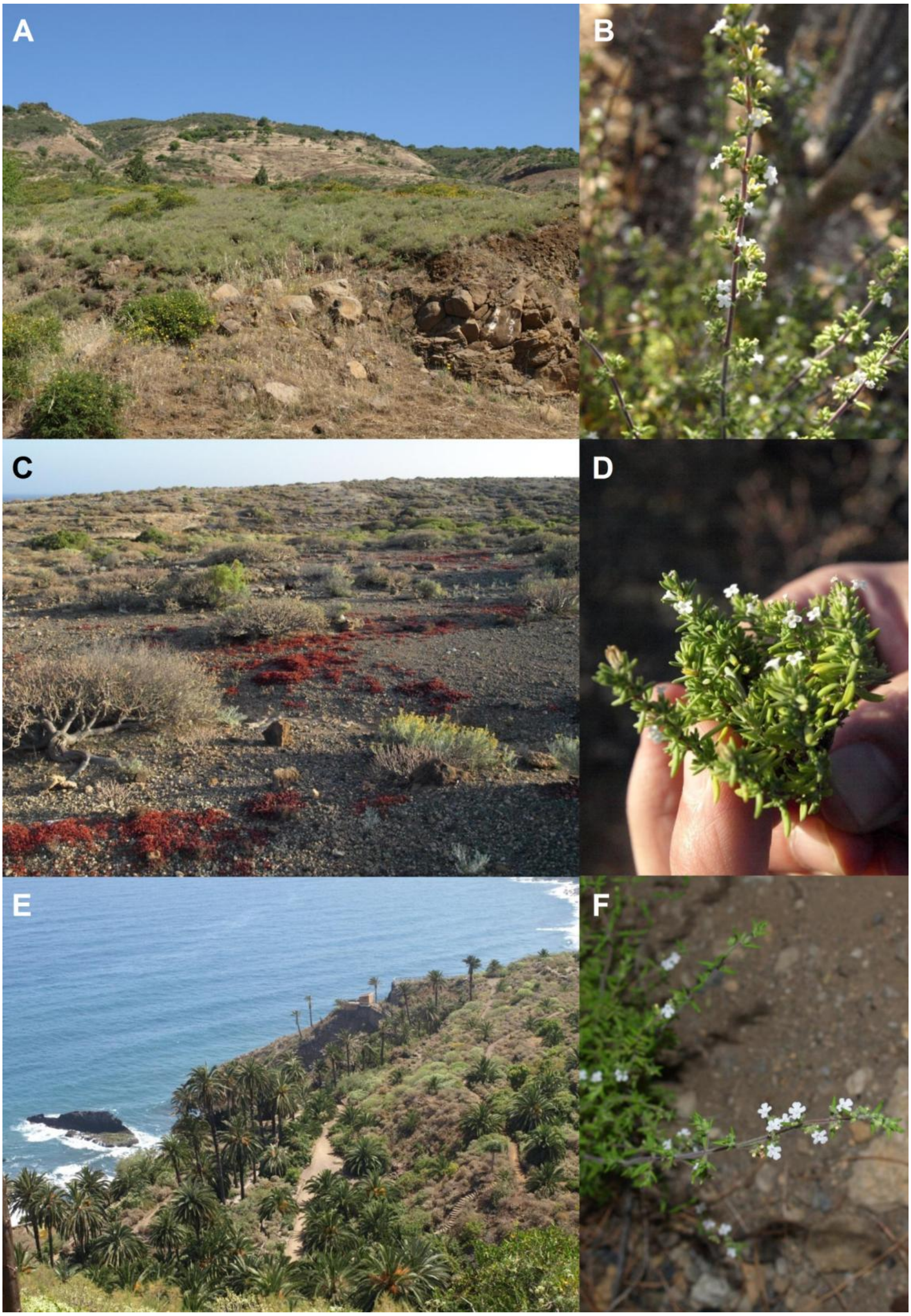


Fig. 3 *Micromeria hyssopifolia* var. *hyssopifolia*, “tomillar” in Arafo (A), flowering branches (B); var. *kuegleri*, habitat in Los Abades (C), flowering individual (D); var. *glabrescens*, habitat in Rambla de Castro (E), flowering branch (F). All photos taken by P. Puppo.

pink flowers. The most common phenotype of *M. hyssopifolia* presents strigose indumentum giving the plant a grayish appearance, lanceolate leaves and white flowers (Fig. 3A, B). Three varieties have been described in *M. hyssopifolia*: var. *hyssopifolia*, var. *kuegleri*, and var. *glabrescens* (Pérez de Paz 1978; Fig. 3). These are not supported in the phylogeny since the arrangement of the samples appears aleatory disregarding of variety or geographical distribution. The lack of monophyly of these species should be taken cautiously since relations within the central species clade are not conclusive and are poorly supported. On the other hand, the polyphyly in these species could be explained by introgression events since *M. varia* hybridizes with both *M. teneriffae* and *M. hyssopifolia* in zones of sympatry. Furthermore, it seems that hybridization is fairly common in groups with a single colonization event (Carine et al. 2004) and it has been well documented in other Canarian taxa (see Brochman 1984; Francisco-Ortega et al. 1996; Barber et al. 2000; Jorgensen and Olesen 2001; Jones et al. 2014; Scheunert and Heubl 2014).

Micromeria varia was the only species reported to occur in all islands of the archipelago (except for La Palma) and was thought to be the progenitor of *Micromeria* in the Canary Islands (Peréz de Paz 1978). The phylogenetics results obtained here do not support this view. Rather, the different *M. varia* described for each island constitute different lineages and so they have been elevated to species status (paper V, Chapter 4). Furthermore, Pérez de Paz (1978) described different subspecies of *M. varia* for each island evidencing the existing morphological differentiation of these lineages. *Micromeria varia* as circumscribed here is a single island endemic of Tenerife since the type specimen was collected in that island (paper IV, Chapter 4).

In El Hierro, Pérez de Paz (1978) identified two taxa, one highly plastic morphologically and widely distributed throughout the island which he called *M. hyssopifolia*, and the other confined to the two northern-most tips of the island which he identified as *M. varia* subsp. *hierrensis*. As discussed above, the names *M. hyssopifolia* and *M. varia* refer to taxa from Tenerife. Thus, *M. ferrensis* is the new name given to the lineage identified in El Hierro (paper IV, Chapter 4). Phylogenetic analyses do not support the distinction of more than one taxa in the island since specimens previously assigned to *M. hyssopifolia* and *M. varia* appear intermixed in the tree. Rather, the specimens previously assigned to *M. varia* subsp. *hierrensis* constitute an extreme form of the already variable *M. ferrensis*. *Micromeria ferrensis* is characterized by ovate, lanceolate or linear leaves, not arranged in fascicles and white flowers 2-3 mm long.

In a similar way, Pérez de Paz (1978) described two taxa for La Palma: *M. herpyllomorpha*, widely distributed throughout the island and highly plastic morphologically, and *M. lasiophylla* subsp. *palmensis*, restricted to the higher parts of the island above 2000 m. Molecular analyses presented in this thesis do not support a link between the taxon identified as *M. lasiophylla* from La Palma and the one described from Tenerife. *Micromeria lasiophylla* is a taxon endemic to Tenerife. On the other hand, phylogenetic evidence does not support the differentiation of these two La Palma lineages either so subsp. *palmensis* has been placed as a subspecies of *M. herpyllomorpha* (paper V, Chapter 4). *Micromeria herpyllomorpha* is a subshrub highly variable in pubescence: glabrate, strigose, pilose, or tomentose, with leaves up to 15 mm long, and flowers white to lilac up to 8 mm long and densely arranged in the upper parts of the branches.

In Gran Canaria, *M. pineolens* and *M. helianthemifolia* form monophyletic, highly supported groups in the phylogeny. *Micromeria pineolens* is perhaps the most strikingly different taxon in the genus. It is a shrub up to 1 m tall with densely pubescent leaves larger than 1 cm and pink flowers up to 1.5 cm long and is restricted to the humid pine forest in the NW part of the island (Fig. 4A, B). *Micromeria helianthemifolia* is characterized by lanceolate leaves up to 2 cm long and large pink and white flowers up to 2 cm long. This species is restricted to the central part of Gran Canaria between 500-1400 m (Fig. 4C, D). These two species are the most different among the *Micromeria* species perhaps because they grow in geologically older substrates. Samples assigned to *M. varia* subsp. *canariensis* and subsp. *meridialis* are grouping together in the phylogeny forming a monophyletic group with high support. Since *M. varia* is a taxon restricted to Tenerife, a new combination has been proposed for the taxa in Gran Canaria including the two subspecies: *M. canariensis* subsp. *canariensis* and subsp. *meridialis*. *Micromeria canariensis* presents lanceolate leaves, glabrate to densely pubescent, not arranged in fascicles and pink to purple flowers up to 5 mm long. The remaining four species recognized in Gran Canaria: *M. leucantha*, *M. tenuis*, *M. lanata*, and *M. benthamii* are not monophyletic in the phylogeny though relations among them are not conclusive and poorly supported. As explained above, introgression might be responsible for this observed polyphyly since these four species have ample zones of sympatry and hybrids between them have been observed in the field. Furthermore, each of these species presents a unique combination of features that differentiate them morphologically. *Micromeria leucantha* presents large leaves up to 3 cm arranged in the tip of the branches, and white, large flowers up to 1.5 cm long

Fig. 4 *Micromeria pineolens*, flowering branches (A, B). *Micromeria helianthemifolia*, habitat in Fataga clif (C), flowering branches (D). *Micromeria leucantha*, habitat in San Nicolás (E), flowering branch (F). Photos A and B taken by P.L. Pérez de Paz, C-F taken by P. Puppo.

(Fig. 4E, F). *Micromeria tenuis* is characterized by its pedunculated cymes and small whitish flowers 5-6 mm (Fig. 5A, B). *Micromeria lanata* is differentiated mainly by the white-wooly pubescence that covers the plant and its small lilac or whitish flowers 2-4 mm long (Fig. 5C, D). These three species grow in cliffs with trachyte and syenite rocks of volcanic origin (Pérez de Paz 1978). Lastly, *M*. *benthamii* is a shrublet with erect, ascending branches with white or lilac flowers 7-10 mm growing in deeper soils throughout the island above 700 m (Fig. 5E, F).

In Lanzarote and Fuerteventura, only one species was recognized, *M. varia* subsp. *rupestris* (Pérez de Paz 1978). As for the other *M. varia* subspecies, a new combination has been proposed for this taxon, *M. rupestris*. This species is characterized by its sessile or subsessile puberulent leaves up to 7 mm long, dark purple flowers 3-4 mm long densely arranged in the tip of the branches. Contrary to what is observed in other *Micromeria* species, *M. rupestris* is rarely forming large populations ("tomillares") in these islands. Rather, it grows scattered in both islands above 500 m.

La Gomera seems to be the only island in the archipelago where more than one colonization event occurred in *Micromeria*. These multiple introductions gave origin to at least three different lineages: *M. lepida*, and what were identified as two subspecies of *M. varia*, subsp. *gomerensis*, and subsp. *varia*. *Micromeria lepida* is characterized by the conspicuous internodes in the branches, long peduncles and pedicels, flowers white or lilac ca. 7 mm long (Fig. 6A). This species is distributed in the humid areas in the SE of the islands mainly between 300 and 700 m, and in the forest clearings above 800 m. A new combination has been proposed for the former *M. varia* subsp. *gomerensis*: *M. gomerensis*. This species presents a subshrub habit, highly branched, leaves not arranged in fascicles, and flowers lilac to purple 3-4 mm long (Fig. 6B). *Micromeria gomerensis* is distributed throughout the island and is especially abundant between 200 and 800 m. The former taxon identified as *M. varia* subsp. *varia* needed a new name so it is now *M. pedro-luisii*. This species is characterized by its leaves arranged in fascicles and white flowers 4-6 mm long (Fig. 6C). *Micromeria pedro-luisii* is distributed throughout the island being especially frequent in the northern part where it grows from sea level up to the higher parts of the island.

With the present revision, the number of species recognized in the Canary Islands increases from 16 to 21, all of them single island endemics except for *M. rupestris* which grows in Lanzarote and Fuerteventura. Tenerife and Gran Canaria are the islands with the higher number of *Micromeria* species, eight and seven species

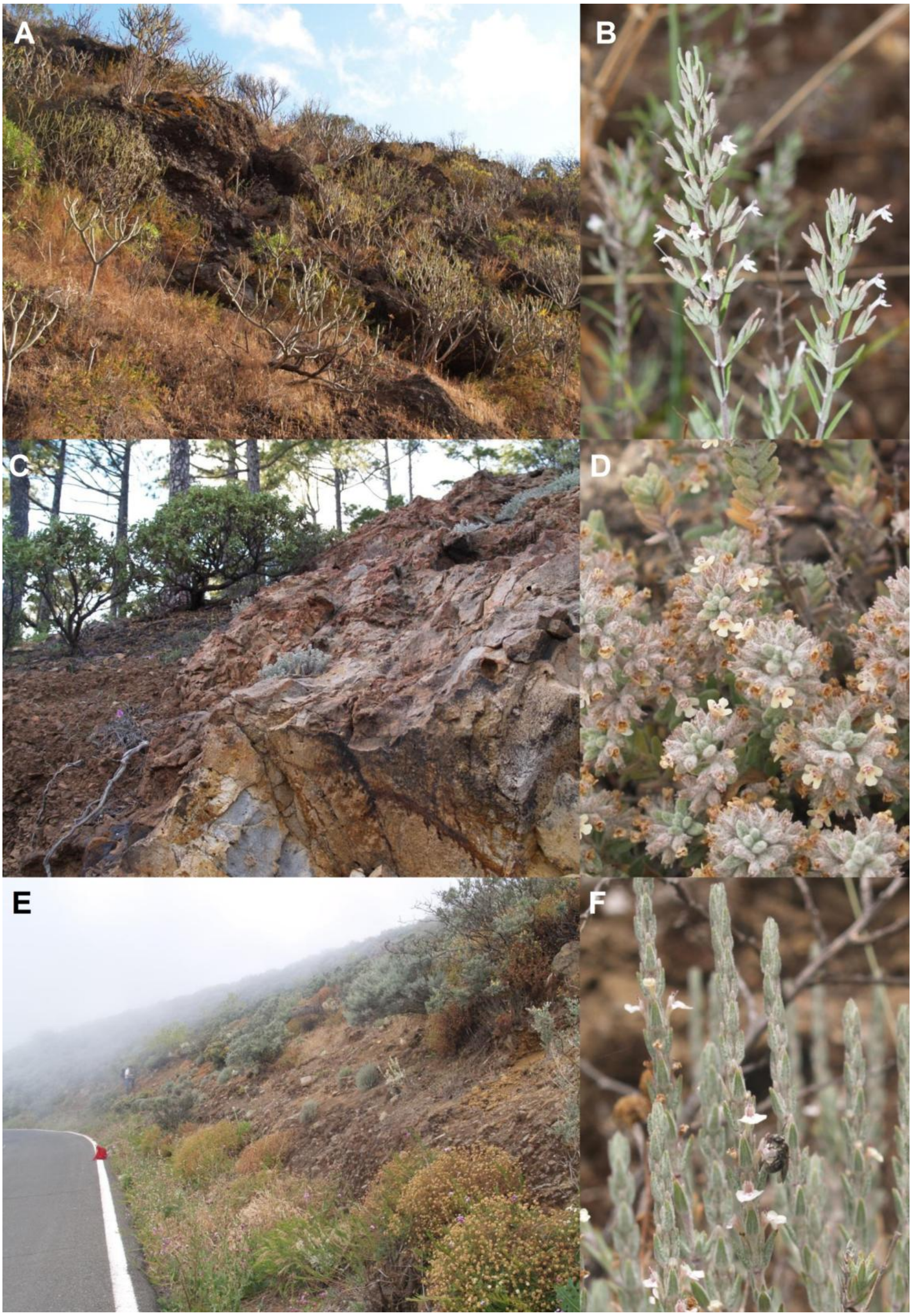


Fig. 5 *Micromeria tenuis*, habitat in Degollado de Venguera (A), flowering branches (B). *Micromeria lanata*, habitat in Antenara-Tamadaba (C), flowering branches (D). *Micromeria benthamii*, habitat in Caldera de los Marteles (E), flowering branches (F). All photos taken by P. Puppo.

Fig. 6 *Micromeria lepida* (A). *Micromeria gomerensis* (B). *Micromeria pedro-luisii* (C). All photos taken by P. Puppo.

respectively. La Gomera presents three species, and the remaining islands, La Palma, El Hierro, Lanzarote and Fuerteventura, present one species each.

5.4 Future work

This thesis has made an important contribution to the knowledge of how *Micromeria* species diversified in the Canary Islands. In a broad sense, this work has also contributed to the understanding of how the geological history of the islands as well as inter-island colonizations affected the diversification processes of insular taxa on oceanic archipelagos. Further work in several aspects would be desirable to complement the findings of the present study.

Morphometric analyses were conducted on the species present in Tenerife. These analyses helped distinguish the two groups of species present in the island: palaeoisland endemics and central species. Furthermore, it revealed that the morphological differences between the groups were mainly found in the floral characters. Similar studies with the species from Gran Canaria would be desirable especially to see if the two groups of species recovered in the phylogeny also present morphological differences in the morphospace. Also, in order to survey the overall morphological variation of the genus in the archipelago, it would be necessary to include all the remaining species in these analyses. It would also be interesting to see if the samples from the different islands segregate from each other in the morphospace.

The phylogeny including *Micromeria* samples from all islands of the Canary archipelago revealed interesting patterns of inter-island colonization. In the future, it would be desirable to increase the sampling for those islands where few samples were available for this study, i.e. Lanzarote and Fuerteventura. This could allow a better understanding of the role of the older islands in the colonization of the archipelago (if any) or the identification of an older, residual lineage in these islands. It would also be interesting to increase the samples from the Madeiran archipelago, Madeira and Porto Santo, in order to improve our understanding of the colonization of this archipelago. Likewise, including a comprehensing sampling from the mainland species would allow a better understanding of the relations of Macaronesia *Micromeria* with other regions where it is distributed: Mediterranean, Africa, Asia, and Middle East.

A set of microsatellite markers were developed as part of this thesis and were tested in some species from Tenerife revealing that the palaeoisland endemics present lower levels of genetic diversity than the common *M. hyssopifolia*. Using this set of markers and Bayesian clustering algorithms (i.e. STRUCTURE) it would be interesting to further investigate the genetic structure of the *Micromeria* species present in Tenerife as well as in the remaining islands of the archipelago and compare these results with the ones obtained in the phylogenetic analyses.

5.5 Literature cited

Allan, GJ, J Francisco-Ortega, A Santos-Guerra, E Boerner and EA Zimmer (2004) Molecular phylogenetic evidence for the geographic origin and classification of Canary Island *Lotus* (Fabaceae: Loteae). Molecular Phylogenetics and Evolution 32, 123-138.

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

Applehans, MS, J Wen, KR Wood, GJ Allan, EA Zimmer and WL Wagner (2014) Molecular phylogenetic analysis of Hawaiian Rutaceae (*Melicope*, *Platydesma* and *Zanthoxylum*) and their different colonization patterns. Botanical Journal of the Linnean Society 174, 425-448.

Barber, JC, J Francisco-Ortega, A Santos-Guerra, A Marrero and RK Jansen (2000) Evolution of endemic *Sideritis* (Lamiaceae) in Macaronesia: Insights from a chloroplast DNA restriction site analysis. Systematic Botany 25, 633-647.

Barber, JC, J Francisco-Ortega, A Santos-Guerra, KG Turner and RK Jansen (2002) Origin of Macaronesian *Sideritis* L. (Lamioideae: Lamiaceae) inferred from nuclear and chloroplast sequence datasets. Molecular Phylogenetics and Evolution 23, 293-306.

Böhle, U-R, HH Hilger and WF Martin (1996) Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). Proceedings of the Natural Academy of Sciences of Philadelphia 93, 11740-11745.

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

Brown, RP, R Campos-Delgado and J Pestano (2000) Mitochondrial DNA evolution and population history of the Tenerife skink *Chalcides viridanus.* Molecular Ecology 9, 1061-1067.

Brown, RP, PA Hoskisson, J-H Welton and M Báez (2006) Geological history and within-island diversity: a debris avalanche and the Tenerife lizard *Gallotia galoti*. Molecular Ecology 15, 3631-3640.

Brunton, CFA, and GDD Hurst (1998) Mitochondrial DNA phylogeny of brimstone butterflies (genus *Gonepteryx*) from the Canary Islands and Madeira. Biological Journal of the Linnean Society 63, 69-79.

Carine, MA, SJ Russell, A Santos-Guerra and J Francisco-Ortega (2004) Relationships of the Macaronesian and Mediterranean Floras: Molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Convolvulus* (Convolvulaceae). American Journal of Botany 91, 1070-1085.

Caujapé-Castells, J, RK Jansen, J Pedrola-Monfort and N Membrives (1999) Chloroplast DNA restriction site phylogeny of the genus *Androcymbium* (Colchicaceae). Systematic Botany 24, 581-597.

Cowie, RH, and BS Holland (2006) Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. Journal of Biogeography 33, 193-198.

Cox, SC, S Carranza and RP Brown (2012) Divergence times and colonization of the Canary Islands by *Gallotia* lizards. Molecular Phylogenetics and Evolution 56, 747-757.

Del-Arco, M, M Salas, JR Acebes, M del C Marrero, JA Reyes-Betancort, and PL Pérez de Paz (2002). Bioclimatology and climatophilous vegetation of Gran Canaria (Canary Islands). Annales Botanici Fennici 39, 15-41.

Dlugosch, KM, and IM Parker (2007) Molecular and quantitative trait variation across the native range of the invasive species *Hypericum canariense*: evidence for ancient patterns of colonization via pre-adaptation? Molecular Ecology 16, 4269-4283.

Dubar-Co, S, AM Wieczorek and CW Morden (2008) Molecular phylogeny and adaptive radiation of the endemic Hawaiian *Plantago* species (Plantaginaceae). American Journal of Botany 95, 1177-1188.

Fernández-Palacios, JM, L de Nascimento, R Otto, JD Delgado, E García-del-Rey, JR Arévalo and RJ Whittaker (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, RK Jansen and A Santos-Guerra (1996) Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. Proceedings of the Natural Academy of Sciences of Philadelphia 93, 4085-4090.

Francisco-Ortega, J, JC Barber, A Santos-Guerra, R Febles-Hernández and RK Jansen (2001a) Origin and evolution of the endemic genera of Gonosperminae (Asteraceae: Anthemideae) from the Canary Islands: evidence from nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA. American Journal of Botany 88, 161-169.

Francisco-Ortega, J, SJ Park, A Santos-Guerra, A Benabid and RK Jansen (2001b) Origin and evolution of the endemic Macaronesian Inuleae (Asteraceae): evidence from the internal transcribed spacers of nuclear ribosomal DNA. Biological Journal of the Linnean Society 72, 77-97.

Francisco-Ortega, J, J Fuertes-Aguilar, S-C Kim, A Santos-Guerra, DJ Crawford, and RK Jansen (2002) Phylogeny of the Macaronesian endemic *Crambe* section *Dendrocrambe* (Brassicaceae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. American Journal of Botany 89, 1984-1990.

Fuertes-Aguilar, J, MF Ray, J Francisco-Ortega, A Santos-Guerra and RK Jansen (2002) Molecular evidence from chloroplast and nuclear markers for multiple colonizations of *Lavatera* (Malvaceae) in the Canary Islands. Systematic Botany 27, 74-83.

García-Verdugo, C, M Sajeva, T La Mantia, C Harrouni, F Msanda and J Caujapé-Castells (2015) Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. Molecular Ecology. DOI: 10.1111/mec.13060

Givnish, TJ, KC Millam, AR Mast, TB Paterson, TJ Theim, AL Hipp, JM Henss, JF Smith, KR Wood and KJ Sytsma (2009) Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). Proceedings of the Royal Society B 276, 407-416.

Gübitz, T, RS Thorpe and A Malhotra (2000) Phylogeography and natural selection in the Tenerife gecko *Tarentola delalandii*: testing historical and adaptive hypothesis. Molecular Ecology 9, 1213-1221.

Hess J, JW Kadereit and P Vargas (2000) The colonization history of *Olea europaea* L. in Macaronesia based on internal transcribed spacer 1 (ITS-1) sequences, randomly amplified polymorphic DNAs (RAPD), and intersimple sequence repeats (ISSR). Molecular Ecology 9, 857-868.

Holland, BS, and MG Hadfield (2004) Origin and diversification of the endemic Hawaiian tree snails (Achatinellinae: Achatinellidae) based on molecular evidence. Molecular Phylogenetics and Evolution 32, 588-600.

Hormiga, G, M Arnedo and RG Gillespie (2003) Speciation on a conveyor belt: sequential colonization of the Hawaiian Islands by *Orsonwelles* spiders (Araneae, Linyphiidae). Systematic Biology 52, 70-88.

Jones, KE, JA Reyes-Betancort, SJ Hiscock and MA Carine (2014) Allopatric diversification, multiple habitat shifts, and hybridization in the evolution of *Pericallis* (Asteraceae), a Macaronesian endemic genus. American Journal of Botany 101, 637-651.

Jorgensen, TH and JM Olesen (2001) Adaptive radiation of island plants: evidence from *Aeonium* (Crassulaceae) of the Canary Islands. Perspectives in Plant Ecology, Evolution and Systematics 4, 29-42.

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

Juan, C, BC Emerson, P Oromí, and GM Hewitt (2000) Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. Trends in Ecology and Evolution 15, 104-109.

Kim, SC, DJ Crawford, J Francisco-Ortega and A Santos-Guerra (1996) A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. Proceedings of the Natural Academy of Sciences of Philadelphia 93, 7743-7748.

Kim, S-C, MR McGowen, P Lubinsky, JC Barber, ME Mort and A Santos-Guerra (2008) Timing and Tempo of Early and Successive Adaptive Radiations in Macaronesia. PLoS ONE 3(5): e2139. doi:10.1371/journal.pone.0002139

Kreft, H, W Jetz, J Mutke, G Kier and W Barthlott (2008) Global diversity of island floras from a macroecological perspective. Ecology Letters 11, 116-127.

Lee, C, S-C Kim, K Lundy and A Santos-Guerra (2005) Chloroplast DNA phylogeny of the woody *Sonchus* alliance (Asteraceae: Sonchinae) in the Macaronesian Islands. American Journal of Botany 92, 2072-2085.

Macías-Hernández, N, L Bidegaray-Batista, BC Emerson, P Oromí and M Arnedo (2013) The imprint of geologic history on within-island diversification of woodlouse-hunter spiders (Araneae, Dysderidae) in the Canary Islands. Journal of Heredity 104, 341-356.

Meimberg, H, T Abele, C Bräuchler, JK McKay, PL Pérez de Paz and G Heubl (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.

Moore, MJ, J Francisco-Ortega, A Santos-Guerra and RK Jansen (2002) Chloroplast DNA evidence for the roles of island colonization and extinction in *Tolpis* (Asteraceae: Lactuceae). American Journal of Botany 89, 518-526.

Mort, ME, DE Soltis, PS Soltis, J Francisco-Ortega and A Santos-Guerra (2001) Phylogenetic relationships and evolution of Crassulaceae based on matK sequences. American Journal of Botany 88, 76-91.

Mort, ME, DE Soltis, PS Soltis, J Francisco-Ortega and A Santos-Guerra (2002) Phylogenetics and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. Systematic Botany 27, 271-288.

Moya, O, HG Contreras-Díaz, P. Oromí and C Juan (2004) Genetic structure, phylogeography and demography of two ground-beetle species endemic to the Tenerife laurel forest (Canary Islands). Molecular Ecology 13, 3153-3167.

Nepokroeff, M, KJ Sytsma, WL Wagner and EA Zimmer (2003) Reconstructing Ancestral patterns of colonization and dispersal in the Hawaiian understory tree genus *Psychotria* (Rubiaceae): a comparison of parsimony and likelihood approaches. Systematic Biology 52, 820-838.

Panero, JL, J Francisco-Ortega, RK Jansen and A Santos-Guerra (1999) Molecular evidence for multiple origins of woodiness and a New World biogeographic connection of the Macaronesian Island endemic *Pericallis* (Asteraceae: Senecioneae). Proceedings of the Natural Academy of Sciences of Philadelphia 96, 13886-13891.

Percy, DM and QCB Cronk (2002) Different fates of island brooms: contrasting evolution in *Adenocarpus*, *Genista*, and *Teline* (Genisteae, Fabaceae) in the Canary Islands and Madeira. American Journal of Botany 89, 854-864.

Percy, DM, AM Garver, WL Wagner, HF James, CW Cunningham, SE Miller and RC Fleischer (2008) Progressive island colonization and ancient origin of Hawaiian Metrosideros (Myrtaceae). Proceedings of the Royal Society B 275, 1479-1490.

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

Reyes-Betancort, JA, A Santos-Guerra, IR Guma, CJ Humphries and MA Carine (2008) Diversity, rarity and the evolution and conservation of the Canary Islands endemic flora. Anales del Jardín Botánico de Madrid 65, 25-45.

Ribera, I, DT Bilton and AP Vogler (2003) Mitochondrial DNA phylogeography and population history of *Meladema* diving beetles on the Atlantic Islands and in the Mediterranean basin (Coleoptera, Dytiscidae). Molecular Ecology 12, 153-167.

Roderick, GK, and RG Gillespie (1998) Speciation and phylogeography of Hawaiian terrestrial arthropods. Molecular Ecology 7, 519-531.

Rumeu, B, P Vargas, R. Jaén-Molina, M Nogales and Juli Caujapé-Castells (2014) Phylogeography and genetic structure of the threatened Canarian *Juniperus cedrus* (Cupressaceae). Botanical Journal of the Linnean Society 175, 376-394.

Sahuquillo, E, and R Lumaret (1999) Chloroplast DNA variation in *Dactylis glomerata* L. taxa endemic to the Macaronesian islands. Molecular Ecology 8, 1797-1803.

Scheunert, A, and G Heubl (2014) Diversification of *Scrophularia* (Scrophulariaceae) in the Western Mediterranean and Macaronesia -Phylogenetic relationships, reticulate evolution and biogeographic patterns. Molecular Phylogenetics and Evolution 70, 296-313.

Stuessy, TF, K Takayama and P López Sepúlveda (2012) Founder effects are invisible in endemic species of oceanic islands. Journal of Biogeography 39, 1565-1566.

Stuessy, TF, K Takayama, P López-Sepúlveda and DJ Crawford (2013) Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. Botanical Journal of the Linnean Society

Susanna, A, T Garnatje and N García-Jacas (1999) Molecular phylogeny of *Cheirolopus* (Asteraceae-Centaureinae) based on ITS sequences of nuclear ribosomal DNA. Plant Systematics and Evolution 214, 147-160.

Thorpe, RS, DP McGregor, AM Cumming and WC Jordan (1994) DNA evolution and colonization sequence of island lizards in relation to geological history: mtDNA RFLP, cytochrome B, cytochrome oxidase, 12S ribosomal RNA sequence, and nuclear RAPD analysis. Evolution 48, 230-240.

Thorpe, RS, H Black and A Malhotra (1996) Matrix correspondence tests on the DNA phylogeny of the Tenerife Lacertid elucidate both historical causes and morphological adaptation. Systematic Biology 45, 335-343.

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

van Hengstum, T, S Lachmuth, JGB Oostermeijer, H(J)CM den Nijs, PG Meirmans and PH van Tienderen (2012) Human-induced hybridization among congeneric endemic plants on Tenerife, Canary Islands. Plant Systematics and Evolution 298, 1119-1131.

Watts, AB, and DG Masson (1995) A giant landslide on the north flank of Tenerife, Canary Islands. Journal of Geophysical Research 100, 24487-24498.

Whittaker, RJ, RJ Ladle, MB Araújo, JM Fernández-Palacios, JD Delgado and JR Arévalo (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., KA Triantis, and RL Ladle (2008) A general dynamic theory of oceanic island biogeography. Journal of Biogeography 35, 977-994.

APPENDIX

Examples of genera and their corresponding reference(s) for the different topics discussed in Chapters 1 and 5.

Topic	Genus (Family)	Reference
MACARONESIA		
Single origin / introduction followed by diversification in the islands	Adenocarpus (Fabaceae)	Percy & Cronk 2002
	Aeonium (Crassulaceae)	Mes et al. 1996; Mort et al. 2001
	Allagopappus (Asteraceae)	Francisco-Ortega et al. 2001b
	Androcymbium (Colchicaceae)	Caujapé-Castells et al. 1999
	Argyranthemum (Asteraceae)	Francisco-Ortega et al. 1997b
	Bencomia (Rosaceae)	Helfgott et al. 2000
	Bystropogon (Lamiaceae)	Trusty et al. 2005
	Cheirolopus (Asteraceae)	Susanna et al. 1999
	Crambe (Brassicaceae)	Francisco-Ortega et al. 2002
	Echium (Boraginaceae)	Böhle et al. 1996
	Genista (Fabaceae)	Percy & Cronk 2002
	Gonospermum (Asteraceae)	Francisco-Ortega et al. 2001a
	Isoplexis (Scrophulariaceae)	Bräuchler et al. 2004
	Ixanthus (Gentianaceae)	Thiv et al. 1999
	Limonium (Plumbaginaceae)	Lledó et al. 2005
	Lotus (Fabaceae)	Allan et al. 2004
	Pericallis (Asteraceae)	Panero et al. 1999; Swenson & Manns 2003
	Saxifraga (Saxifragaceae)	Vargas et al. 1999a
	Schizogyne (Asteraceae)	Francisco-Ortega et al. 2001b
	Sideritis (Lamiaceae)	Barber et al. 2000; 2002
	Sonchus (Asteraceae)	Kim et al. 1996
	Tolpis (Asteraceae)	Park et al. 2001; Moore et al. 2002
	Viera (Asteraceae)	Francisco-Ortega et al. 2001b
	See also:	Silvertown 2004; Goodson et al. 2006; Kim et al. 2008
Multiple introductions	Astericus (Asteraceae)	Goertzen et al. 2002
	Dracaena (Dracaenaceae)	Marrero et al. 1998
	Euphorbia (Euphorbiaceae)	Molero et al. 2002; Barres et al. 2011
	Hedera (Araliaceae)	Vargas et al. 1999a, 1999b; Ackerfield & Wen 2003; Valcarcel et al. 2003
	Helichrysum (Asteraceae)	Galbany-Casals et al. 2009
	Ilex (Aquifoliaceae)	Cuénoud et al. 2000; Manen et al. 2002
	Isothecium mosses	Draper et al. 2015
	Juniperus (Cupressaceae)	Rumeu et al. 2014
	Lavatera (Malvaceae)	Ray 1995; Fuertes-Aguilar et al. 2002
	Matthiola (Brassicaceae)	Jaen-Molina et al. 2009
	Micromeria (Lamiaceae)	Bräuchler et al. 2005
	Olea (Oleaceae)	Hess et al. 2000; Medail et al. 2001
	Plantago (Scrophulariaceae)	Rønsted et al. 2002
	Pulicaria (Asteraceae)	Francisco-Ortega et al. 2001a
	Solanum (Solanaceae)	Bohs & Olmstead 2001
	Teline (Fabaceae)	Percy & Cronk 2002
	See also:	Barber et al. 2000; Carine et al. 2004; Silvertown 2004; Goodson et al. 2006

(Continued)

PROBLABLE multiple introductions	Artemisia (Asteraceae)	Torrell et al. 1999
	Campanula (Campanulaceae)	Cited in Carine et al. 2004, no further ref
	Daucinae (Apiaceae)	Spalik & Downie 2007
	Dracaena (Dracaenaceae)	Marrero et al. 1998
	Helianthemum (Cistaceae)	Cited in Carine et al. 2004, no further ref
	Hypericum (Clusiaceae)	Robson 1977
	Limonium (Plumbaginaceae)	Fuertes-Aguilar et al. 2002
	Salvia (Lamiaceae)	Fuertes-Aguilar et al. 2002
	Senecio (Asteraceae)	Fuertes-Aguilar et al. 2002
	Viola (Violaceae)	Fuertes-Aguilar et al. 2002
Evidence for **adaptive radiation**	Argyranthemum (Asteraceae) in Madeira	Kim et al. 1996; Francisco-Ortega et al. 1997a
	Bystropogon (Lamiaceae) in Canary Is.	Trusty et al. 2005
	Gonospermum (Asteraceae) in Canary Is.	Francisco-Ortega et al. 2001b
	Micromeria (Lamiaceae)	Meimberg et al. 2006; present study (paper III)
	Sideritis (Lamiaceae) in the Canary Is.	Barber et al. 2000
	Sonchus (Asteraceae) in Madeira	Kim et al. 1996; Francisco-Ortega et al. 1997a
	Teline (Fabaceae) in the Canary Is.	Percy & Cronk 2002
Links to the **Mediterranean**	Adenocarpus (Fabaceae)	Käs & Wink 1995, 1997; Percy & Cronk 2002
	Aeonium (Crassulaceae)	Mort et al. 2001, 2002
	Androcymbium (Colchicaceae)	Caujapé-Castells et al. 1999
	Arbutus (Ericaceae)	Hileman et al. 2001
	Argyranthemum (Asteraceae)	Francisco-Ortega et al. 1995, 1997b
	Artemisia (Asteraceae)	Torrell et al. 1999
	Avena (Poaceae)	Alicchio et al. 1995
	Bellis (Asteraceae)	Fiz et al. 2002
	Bencomia (Rosaceae)	Helfgott et al. 2000
	Beta (Chenopodiaceae)	Santoni & Bervillé 1992
	Chamaecytisus (Fabaceae)	Käs & Wink 1995, 1997; Badr et al. 1994
	Cheirolopus (Asteraceae)	Susanna et al. 1999
	Echium (Boraginaceae)	Böhle et al. 1996
	Erucastrum (Brassicaceae)	Warwick & Black 1993
	Euphorbia (Euphorbiaceae)	Barres et al. 2011.
	Genista (Fabaceae)	Percy & Cronk 2002
	Gonospermum (Asteraceae)	Watson et al. 2000; Francisco-Ortega et al. 2001a
	Hypochaeris (Asteraceae)	Cerbah et al. 1998
	Isoplexis (Scrophulariaceae)	Carvalho & Culham 1998
	Ixanthus (Gentianaceae)	Thiv et al. 1999
	Micromeria (Lamiaceae)	Bräuchler et al. 2005; Meimberg et al. 2006
	Pinus (Pinaceae)	Krupkin et al. 1996; Liston et al. 1999; Geada-López et al. 2002
	Plocama (Rubiaceae)	Andersson & Rova 1999
	Ranunculus (Ranunculaceae)	Johansson 1998
	Reichardia (Asteraceae)	Kim et al. 1996
	Sambucus (Caprifoliaceae)	Eriksson & Donoghue 1997; Trusty et al. 2001
	Scrophularia (Scrophulariaceae)	Scheunert & Heubl 2014
	Sideritis (Lamiaceae)	Barber et al. 2002
	Sinapidendron (Brassicaceae)	Warwick & Black 1993
	Spartocytisus (Fabaceae)	Cubas et al. 2002
	Tinguarra (Apiaceae)	Downie et al. 2000a
	Todaroa (Apiaceae)	Downie et al. 2000b
	Tolpis (Asteraceae)	Park et al. 2001; Moore et al. 2002
	Vaccinium (Ericaceae)	Powell & Kron 2002
	Vierea (Asteraceae)	Francisco-Ortega et al. 2001b
	See also:	Juan et al. 2000; Carine et al. 2004

(Continued)

Links to **Africa**	Ceropegia (Aclepiadaceae)	Cited in Juan et al. 2000, no further ref
	Euphorbia (Euphorbiaceae)	Barres et al. 2011
	Helichrysum (Asteraceae)	Galbany-Casals et al. 2009
	Limonium (Plumbaginaceae)	Lledó et al. 2005
	Matthiola (Brassicaceae)	Jaen-Molina et al. 2009
	Micromeria (Lamiaceae)	Bräuchler et al. 2005
	Sideroxylon (Sapotaceae)	Cited in Juan et al. 2000, no further ref
	Solanum (Solanaceae)	Olmstead & Palmer 1997
	See also:	Francisco-Ortega et al. 2001a; Galbany-Casals et al. 2009
	*Danaus butterflies	Cited in Juan et al. 2000, no further ref
Links to **Asia**	Apollonias (Lauraceae)	Cited in Juan et al. 2000, no further ref
	Euphorbia (Euphorbiaceae)	Barres et al. 2011
	*Cyclyrius butterflies	Cited in Juan et al. 2000, no further ref
	*Vanessa butterflies	Cited in Juan et al. 2000, no further ref
Links to **America**	Bystropogon (Lamiaceae)	Cited in Juan et al. 2000, no further ref
	*Danaus butterflies	Cited in Juan et al. 2000, no further ref
Links to **Australia**	Picconia (Oleaceae)	Cited in Juan et al. 2000, no further ref
CANARY ISLANDS		
Colonization **from Eastern to Western** islands (from older to younger islands)	Dendrocrambe (Brassicaceae)	Francisco-Ortega et al. 2002
	Inulaeae (Asteraceae)	Francisco-Ortega et al. 2001b
	Lotus (Fabaceae)	Allan et al. 2004
	Olea (Oleaceae)	Hess et al. 2000
	See also:	Silvertown 2005; Dlugosch & Parker 2007
	*Gallotia lizards	Juan et al. 2000
	*Pimelia and Hegeter beetles	Juan et al. 2000
	*Parus birds	Kvist et al. 2005
Split between Eastern and **Western** islands	Gonospermum (Asteraceae)	Francisco-Ortega et al. 2001a
	Micromeria (Lamiaceae)	Meimberg et al. 2006; present study (paper III)
	Periploca (Euphorbiaceae)	Garcia-Verdugo et al. 2015
	Teline (Fabaceae)	Percy & Cronk 2002
Radiations from Tenerife and/or Gran Canaria	Echium (Boraginaceae)	Böhle et al. 1996
	Gonospermum alliance (Asteraceae)	Francisco-Ortega et al. 2001a
	Hypericum (Clusiaceae)	Dlugosch & Parker 2007
	Micromeria (Lamiaceae)	Present study (paper III)
	Sonchus alliance (Asteraceae)	Kim et al. 1996; Lee et al. 2005
	*lizards	Thorpe et al. 1994
	*Meladena beetles	Ribera et al. 2003
Monophyletic groups restricted to a single island	Aeonium (Crassulaceae)	Mort et al. 2001
	Crambe (Brassicaceae)	Francisco-Ortega et al. 1996
	Micromeria (Lamiaceae)	Meimberg et al. 2006; present study (paper III)
	Pericallis (Asteraceae)	Panero et al. 1999
	Sonchus (Asteraceae)	Kim et al. 1996
	Tanacetum (Gonosperminae)	Francisco-Ortega et al. 2001a
	See also:	Allan et al. 2004
Inter-island colonization between similar ecological zones	Adenocarpus (Fabaceae)	Percy & Cronk 2002
	Aeonium (Crassulaceae)	Mes & t'Hart 1996; Mort et al. 2002
	Argyranthemum (Asteraceae)	Francisco-Ortega et al. 1996
	Crambe (Brassicaceae)	Francisco-Ortega et al. 2002
	Lotus (Fabaceae)	Allan et al. 2004
	Pericallis (Asteraceae)	Panero et al. 1999
	Sonchus (Asteraceae)	Kim et al. 1996
	See also:	Francisco-Ortega et al. 2001a; Silvertown 2004; Goodson et al. 2006

(Continued)

Introduction of flora **TO Madeira**	Aeonium (Crassulaceae)	Mort et al. 2002; Kim et al. 2008
	Bystropogon (Lamiaceae)	Trusty et al. 2005
	Convolvulus (Convolvulaceae)	Carine et al. 2004
	Crambe, Dendrocrambe (Crassulaceae)	Francisco-Ortega et al. 2002; Kim et al. 2008
	Cryptotaenia (Apiaceae)	Spalik & Downie 2007
	Echium (Boraginaceae)	Böhle et al. 1996; Kim et al. 2008
	Micromeria (Lamiaceae)	Present study (papers I and III)
	Pericallis (Asteraceae)	Panero et al. 1999; Jones et al. 2014
	Sideritis (Lamiaceae)	Barber et al. 2002; Kim et al. 2008
	Sonchus (Asteraceae)	Kim et al. 1996; 2008; Lee et al. 2005
	Teline, Genista (Fabaceae)	Percy & Cronk 2002
	See also:	Barber et al. 2000; Dlugosch & Parker 2007
Introduction of flora **FROM Madeira**	Aeonium (Crassulaceae)	Mes & t'Hart 1996
	Argyranthemum (Asteraceae)	Francisco-Ortega et al. 1996
	See also:	Barber et al. 2000
Evidence for **ancient hybridization**	Aeonium (Crassulaceae)	Mes & t'Hart 1996; Jorgensen & Frydenberg 1999
	Argyranthemum (Asteraceae)	Francisco-Ortega et al. 1996
	Echium (Boraginaceae)	Böhle et al. 1996
	Pericallis (Asteraceae)	Jones et al. 2014
	Scrophularia (Scrophulariaceae)	Scheunert & Heubl 2014
	Sonchus (Asteraceae)	Kim et al. 1996
	See also:	Jorgensen & Olesen 2001
Low sequence divergence, high morphological differentiation	Androcymbium (Colchicaceae)	Caujapé-Castells et al. 1999
	Astericus (Asteraceae)	Francisco-Ortega et al. 1999
	Dactylis (Poaceae)	Sahuquillo & Lumaret 1999
	Echium (Boraginaceae)	Böhle et al. 1996
	Micromeria (Lamiaceae)	Present study (paper I)
	Sideritis (Lamiaceae)	Barber et al. 2002
	Teline, Lotus (Fabaceae)	Percy & Cronk 2002; Allan et al. 2004
	See also:	Allan et al. 2004
	*Pimelia beetles	Juan et al. 1996
TENERIFE		
Genera with endemic taxa to at least one palaeo-island that has been included in phylogenetic analyses. **CROWN GROUP (recent lineages)**	Aeonium (Crassulaceae)	Mort et al. 2002
	Argyranthemum (Asteraceae)	Francisco-Ortega et al. 1996
	Bystropogon (Lamiaceae)	Trusty et al. 2005
	Cheirolopus (Asteraceae)	Susanna et al. 1999
	Crambe (Brassicaceae)	Francisco-Ortega et al. 2002
	Echium (Boraginaceae)	Böhle et al. 1996
	Lotus (Fabaceae)	Allan et al. 2004
	Lugoa (Asteraceae)	Francisco-Ortega et al. 2001a
	Monanthes (Crassulaceae)	Mort et al. 2002
	Pericallis (Asteraceae)	Jones et al. 2014
	Sideritis (Lamiaceae)	Barber et al. 2000
	Teline (Fabaceae)	Percy & Cronk 2002
	Tolpis (Asteraceae)	Moore et al. 2002
OLD LINEAGES (early diverging)	Convolvulus (Convolvulaceae)	Carine et al. 2004
	Lavatera (Malvaceae)	Fuertes-Aguilar et al. 2002
	Micromeria (Lamiaceae)	Present study (papers I and III)
	Sonchus (Asteraceae)	Kim et al. 1996
UNKNOWN	Dicheranthus (Caryophyllaceae)	Oxelman et al. 2001; Smissen et al. 2002
	Hypochaeris (Asteraceae)	Cerbah et al. 1998
	Vierea (Asteraceae)	Francisco-Ortega et al. 2001b

(Continued)

Diversification / population s**tructure coinciding** with **Tenerife's geological history**	Argyranthemum coronopifolium (Asteraceae)	Brochman 1984
	Hypericum canariense (Clusiaceae)	Dlugosch & Parker 2007
	Juniperus cedrus (Cupressaceae)	Rumeu et al. 2014
	Micromeria (Lamiaceae)	Present study (papers I and III)
	Pericallis lanata, P. echinata (Asteraceae)	van Hengstum et al. 2012; Jones et al. 2014
	*Calathus abaxoides ground beetle	Emerson et al. 1999
	*Chalcides skinks	Brown et al. 2000
	*Dysdera spiders	Macías-Hernández et al. 2013
	*Eutrichopus canariensis ground beetle	Moya et al. 2004
	*Gallotia lizards	Thorpe et al. 1996; Brown et al. 2006
	*Loboptera cockroaches	Cited in Juan et al. 2000, no further ref
	*Pimelia darkling beetles	Juan et al. 1996
	*Steganecarus mites	Cited in Juan et al. 2000, no further ref
	*Tarentola geckos	Gübitz et al. 2000
Tenerife as a center for dispersal in the Canary Islands	Crambe (Brassicaceae)	Francisco-Ortega et al. 2002
	Echium (Boraginaceae)	Böhle et al 1996
	Hypericum canariense (Clusiaceae)	Dlugosch & Parker 2007
	Lotus (Fabaceae)	Allan et al. 2004
	*Dysdera spiders	Macías-Hernández et al. 2013
	*Gallotia lizards	Thorpe et al. 1994; Cox et al. 2012
	*Gonepteryx butterflies	Brunton & Hurst 1998
	*Meladema beetles	Ribera et al. 2003

*Indicates animal taxa.

References

Ackerfield, J and J Wen (2003) Evolution of *Hedera* (the ivy genus, Araliaceae): insights from chloroplast DNA data. International Journal of Plant Sciences 164, 593-602.

Alicchio, R, L Aranci and L Conte (1995) Restriction fragment length polymorphism based on phylogenetic analysis of *Avena* L. Genome 38, 1279-1284.

Allan, GJ, J Francisco-Ortega, A Santos-Guerra, E Boerner and EA Zimmer (2004) Molecular phylogenetic evidence for the geographic origin and classification of Canary Island *Lotus* (Fabaceae: Loteae). Molecular Phylogenetics and Evolution 32, 123-138.

Andersson, L and JHE Rova (1999) The rps16 intron and the phylogeny of the Rubiodeae (Rubiaceae). Plant Systematics and Evolution 214, 161-186.

Badr, A, W Martin and U Jensen (1994) Chloroplast DNA restriction site polymorphism in Genisteae (Leguminosae) suggests a common origin for European and American lupines. Plant Systematics and Evolution 193, 95-106.

Barber, JC, J Francisco-Ortega, A Santos-Guerra, A Marrero and RK Jansen (2000) Evolution of endemic *Sideritis* (Lamiaceae) in Macaronesia: Insights from a chloroplast DNA restriction site analysis. Systematic Botany 25, 633-647.

Barber, JC, J Francisco-Ortega, A Santos-Guerra, KG Turner and RK Jansen (2002) Origin of Macaronesian *Sideritis* L. (Lamioideae: Lamiaceae) inferred from nuclear and chloroplast sequence datasets. Molecular Phylogenetics and Evolution 23, 293-306.

Barres, L, R Vilatersana, J Molero, A Susanna and M Galbany-Casals (2011) Molecular phylogeny of Euphorbia subg. *Esula* sect. *Aphyllis* (Euphorbiaceae) inferred from nrDNA and cpDNA markers with biogeographic insights. Taxon 60, 705-720.

Böhle, U-R, HH Hilger and WF Martin (1996) Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). Proceedings of the Natural Academy of Sciences of Philadelphia 93, 11740-11745.

Bohs, L and RG Olmstead (2001) A reassessment of *Normania* and *Triguera* (Solanaceae). Plant Systematics and Evolution 228, 33-48.

Bräuchler, C, H Meimberg and G Heubl (2004) Molecular phylogeny of the genera *Digitalis* L. and *Isoplexis* (Lindley) Loudon (Veronicaceae) based on ITS- and trnLF sequences. Plant Systematics and Evolution 248, 111-128.

Bräuchler, C, H Meimberg, T Abele and G Heubl (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 - Anthemideae) in the Canary Islands. Nordic Journal of Botany 4, 729-736.

Brown, RP, R Campos-Delgado and J Pestano (2000) Mitochondrial DNA evolution and population history of the Tenerife skink *Chalcides viridanus.* Molecular Ecology 9, 1061-1067.

Brown, RP, PA Hoskisson, J-H Welton and M Báez (2006) Geological history and within-island diversity: a debris avalanche and the Tenerife lizard *Gallotia galoti*. Molecular Ecology 15, 3631-3640.

Brunton, CFA, and GDD Hurst (1998) Mitochondrial DNA phylogeny of brimstone butterflies (genus *Gonepteryx*) from the Canary Islands and Madeira. Biological Journal of the Linnean Society 63, 69-79.

Carine, MA, SJ Russell, A Santos-Guerra and J Francisco-Ortega (2004) Relationships of the Macaronesian and Mediterranean Floras: Molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Convolvulus* (Convolvulaceae). American Journal of Botany 91, 1070-1085.

Carvalho, JA and A Culham (1998) Conservation status and preliminary results on the phylogenetics of *Isoplexis* (Lindl.) Benth. (Scrophulariaceae), an endemic Macaronesian genus. Boletim do Museo Municipal do Funchal Suplemento 5, 109-127.

Caujapé-Castells, J, RK Jansen, J Pedrola-Monfort and N Membrives (1999) Chloroplast DNA restriction site phylogeny of the genus *Androcymbium* (Colchicaceae). Systematic Botany 24, 581-597.

Cerbah, M, T Souza-Chies, MJ Jubier, B Lejeune and S Siljakyakovlev (1998) Molecular phylogeny of the genus *Hypochaeris* using internal transcribed spacers of nuclear rDNA: inference for chromosomal evolution. Molecular Biology and Evolution 15, 345-354.

Cox, SC, S Carranza and RP Brown (2012) Divergence times and colonization of the Canary Islands by *Gallotia* lizards. Molecular Phylogenetics and Evolution 56, 747-757.

Cubas, P, C Pardo and H Tahiri (2002) Molecular approach to the phylogeny and systematics of *Cytisus* (Leguminosae) and related genera based on nucleotide sequences of nrDNA (ITS region) and cpDNA (trnLtrnF intergenic spacer). Plant Systematics and Evolution 233, 223-242.

Cuénoud, P, MAD Martinez, PA Loizeau, R Spichiger, S Andrews and JF Manen (2000) Molecular phylogeny and biogeography of the genus *Ilex* L. (Aquifoliaceae). Annals of Botany 85, 111-122.

Dlugosch, KM, and IM Parker (2007) Molecular and quantitative trait variation across the native range of the invasive species *Hypericum canariense*: evidence for ancient patterns of colonization via pre-adaptation? Molecular Ecology 16, 4269-4283.

Downie, SR, DS Katz-Downie and K Spalik (2000a) A phylogeny of Apiaceae tribe *Scandiceae*: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. American Journal of Botany 87, 76-95.

Downie, SR, MF Watson, K Spalik and DS Katz-Downie (2000b) Molecular systematics of Old Wold Apioideae (Apiaceae): relationships among some members of tribe *Peucedaneae* sensu lato, the placement of several island-endemic species, and resolution within the apioid superclade. Canadian Journal of Botany 78, 506-528.

Draper, I, L Hedenäs, M Stech, J Patiño, O Werner, JM González-Mancebo, M Sim-Sim, T Lopes and R M Ros (2015) How many species of *Isothecium* (Lembophyllaceae, Bryophyta) are there in Macaronesia? A survey using integrative taxonomy. Botanical Journal of the Linnean Society. DOI: 10.1111/boj.12250

Emerson, BC, P Oromí and GM Hewitt (1999) MtDNA phylogeography and recent intraisland diversification among Canary Island *Calathus* beetles. Molecular Phylogenetics and Evolution 13, 149-158.

Eriksson, T and MJ Donoghue (1997) Phylogenetic relationships of *Sambucus* and *Adoxa* (Adoxoideae, Adoxaceae) based on nuclear ribosomal ITS sequences and preliminary morphological data. Systematic Botany 22, 555-573.

Fiz, O, V Valcarcel and P Vargas (2002) Phylogenetic position of Mediterranean Astereae and character evolution of daisies (*Bellis*, Asteraceae) inferred from nrDNA ITS sequences. Molecular Phylogenetics and Evolution 25, 157-171.

Francisco-Ortega, J, RK Jansen, DJ Crawford and A Santos-Guerra (1995) Chloroplast DNA evidence for intergeneric relationships of the Macaronesian endemic genus *Argyranthemum* (Asteraceae). Systematic Botany 20, 413-422.

Francisco-Ortega, J, RK Jansen and A Santos-Guerra (1996) Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. Proceedings of the Natural Academy of Sciences of Philadelphia 93, 4085-4090.

Francisco-Ortega, J, DJ Crawford, A Santos-Guerra and RK Jansen (1997a) Origin and evolution of *Argyranthemum* (Asteraceae: Anthemideae) in Macaronesia. In TJ Givnish and KJ Sytsma (eds.), Molecular evolution and adaptive radiation, 407-431. Cambridge University Press, Cambridge, UK.

Francisco-Ortega, J, A Santos-Guerra, A Hines and RK Jansen (1997b) Molecular evidence for a Mediterranean origin of the Macaronesian endemic genus *Argyranthemum* (Asteraceae). American Journal of Botany 84, 1595-1613.

Francisco-Ortega, J, LR Goertzen, A Santos-Guerra, A Benabid and RK Jansen (1999) Molecular systematics of the *Asteriscus* alliance (Asteraceae: Inuleae) I: Evidence from the internal transcribed spacers of nuclear ribosomal DNA. Systematic Botany 24, 249-266.

Francisco-Ortega, J, JC Barber, A Santos-Guerra, R Febles-Hernández and RK Jansen (2001a) Origin and evolution of the endemic genera of Gonosperminae (Asteraceae: Anthemideae) from the Canary Islands: evidence from nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA. American Journal of Botany 88, 161-169.

Francisco-Ortega, J, SJ Park, A Santos-Guerra, A Benabid and RK Jansen (2001b) Origin and evolution of the endemic Macaronesian Inuleae (Asteraceae): evidence from the internal transcribed spacers of nuclear ribosomal DNA. Biological Journal of the Linnean Society 72, 77-97.

Francisco-Ortega, J, J Fuertes-Aguilar, S-C Kim, A Santos-Guerra, DJ Crawford, and RK Jansen (2002) Phylogeny of the Macaronesian endemic *Crambe* section *Dendrocrambe* (Brassicaceae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. American Journal of Botany 89, 1984-1990.

Fuertes-Aguilar, J, MF Ray, J Francisco-Ortega, A Santos-Guerra and RK Jansen (2002) Molecular evidence from chloroplast and nuclear markers for multiple colonizations of *Lavatera* (Malvaceae) in the Canary Islands. Systematic Botany 27, 74-83.

Galbany-Casals, M, N Garcia-Jacas, L Sáez, C Benedí and A. Susanna (2009) Phylogeny, biogeography, and character evolution in Mediterranean, Asiatic and Macaronesian *Helichrysum* (Asteraceae, Gnaphalieae) inferred from nuclear phylogenetic analyses. International Journal of Plant Sciences 170, 365-380.

García-Verdugo, C, M Sajeva, T La Mantia, C Harrouni, F Msanda and J Caujapé-Castells (2015) Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. Molecular Ecology. DOI: 10.1111/mec.13060

Geada-López, G, K Kamiya and K Harada (2002) Phylogenetic relationships of *Diploxylon* pines (subgenus *Pinus*) based on plastid sequence data. International Journal of Plant Sciences 163, 737-747.

Goertzen, LR, J Francisco-Ortega, A Santos-Guerra, JP Mower, CR Linder and RK Jansen (2002) Molecular systematics of the *Asteriscus* alliance (Asteraceae: Inuleae) II: combined nuclear and chloroplast data. Systematic Botany 27, 815-823.

Goodson, BE, A Santos-Guerra and RK Jansen (2006) Molecular systematics of *Descurainia* (Brassicaceae) in the Canary Islands: biogeoraphic and taxonomic implications. Taxon 55, 671-682.

Gübitz, T, RS Thorpe and A Malhotra (2000) Phylogeography and natural selection in the Tenerife gecko *Tarentola delalandii*: testing historical and adaptive hypothesis. Molecular Ecology 9, 1213-1221.

Helfgott, DM, J Francisco-Ortega, A Santos-Guerra, RK Jansen and BB Simpson (2000) Biogeography and breeding system evolution of the woody *Bencomia* alliance (Rosaceae) in Macaronesia based on ITS sequence data. Systematic Botany 25, 82-97.

Hess J, JW Kadereit and P Vargas (2000) The colonization history of *Olea europaea* L. in Macaronesia based on internal transcribed spacer 1 (ITS-1) sequences, randomly amplified polymorphic DNAs (RAPD), and intersimple sequence repeats (ISSR). Molecular Ecology 9, 857-868.

Hileman, LC, MC Vasey and VT Parker (2001) Phylogeny and biogeography of the Arbutoideae (Ericaceae): implications for the Madrean-Tethyan hypothesis. Systematic Botany 26, 131-143.

Jaén-Molina, R, J Caujapé-Castells, JA Reyes-Betancort, H Akhani, O Fernández-Palacios, J Pérez de Paz, R Febles-Hernández and A Marrero-Rodríguez (2009) The molecular phylogeny of *Matthiola* R. Br. (Brassicaceae) inferred from ITS sequences, with special emphasis on the Macaronesian endemics. Molecular Phylogenetics and Evolution 53, 972-981.

Johansson, JT (1998) Chloroplast DNA restriction site mapping and the phylogeny of *Ranunculus* (Ranunculaceae). Plant Systematics and Evolution 213, 1-19.

Jones, KE, JA Reyes-Betancort, SJ Hiscock and MA Carine (2014) Allopatric diversification, multiple habitat shifts, and hybridization in the evolution of *Pericallis* (Asteraceae), a Macaronesian endemic genus. American Journal of Botany 101, 637-651.

Jorgensen, TH and J Frydenberg (1999) Diversification in insular plants: inferring the phylogenetic relationship in *Aeonium* (Crassulaceae) using ITS sequences of nuclear ribosomal DNA. Nordic Journal of Botany, 19, 613-621.

Jorgensen, TH and JM Olesen (2001) Adaptive radiation of island plants: evidence from *Aeonium* (Crassulaceae) of the Canary Islands. Perspectives in Plant Ecology, Evolution and Systematics 4, 29-42.

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

Juan, C, BC Emerson, P Oromí, and GM Hewitt (2000) Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. Trends in Ecology and Evolution 15, 104-109.

Käss, E and M. Wink (1995) Molecular phylogeny of the Papilionoideae (family Leguminoseae): rbcL gene sequences versus chemical taxonomy. Botanica Acta 108, 149-162.

Käss, E and M Wink (1997) Phylogenetic relationships in the Papilionoideae (family Leguminosae) based on nucleotide sequences of cpDNA (rbcL) and ncDNA (ITS1 and 2). Molecular Phylogenetics and Evolution 8, 65-88.

Kim, SC, DJ Crawford, J Francisco-Ortega and A Santos-Guerra (1996) A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. Proceedings of the Natural Academy of Sciences of Philadelphia 93, 7743-7748.

Kim, S-C, MR McGowen, P Lubinsky, JC Barber, ME Mort and A Santos-Guerra (2008) Timing and Tempo of Early and Successive Adaptive Radiations in Macaronesia. PLoS ONE 3(5): e2139. doi:10.1371/journal.pone.0002139

Krupkin, AB, A Liston and SH Strauss (1996) Phylogenetic analysis of the hard pines (*Pinus* subgenus *Pinus*, Pinaceae) from chloroplast DNA restriction site analysis. American Journal of Botany 83, 489-498.

Kvist, L, J Broggi, JC Illera and K Koivula (2005) Colonization and diversification of the blue tits (*Parus caeruleus* teneriffae-group) in the Canary Islands. Molecular Phylogenetics and Evolution 34, 501-511.

Lee, C, S-C Kim, K Lundy and A Santos-Guerra (2005) Chloroplast DNA phylogeny of the woody *Sonchus* alliance (Asteraceae: Sonchinae) in the Macaronesian Islands. American Journal of Botany 92, 2072-2085.

Liston, A, WA Robinson, D Pinero and ER Alvarez-Buylla (1999) Phylogenetics of *Pinus* (Pinaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. Molecular Phylogenetics and Evolution 11, 95-109.

Lledó, MD, MB Crespo, MF Fay and MW Chase (2005) Molecular Phylogenetics of *Limonium* and related genera (Plumbaginaceae): biogeographical and systematic implications. American Journal of Botany 92, 1189-1198.

Macías-Hernández, N, L Bidegaray-Batista, BC Emerson, P Oromí and M Arnedo (2013) The imprint of geologic history on within-island diversification of woodlouse-hunter spiders (Araneae, Dysderidae) in the Canary Islands. Journal of Heredity 104, 341-356.

Manen, J-F, MC Boulter and Y Naciri-Graven (2002) The complex history of the genus *Ilex* L. (Aquifoliaceae): evidence from the comparison of plastid and nuclear DNA sequences and from fossil data. Plant Systematics and Evolution 235, 79-98.

Marrero, A, AS Almeida and M Gonzalez-Martin (1998) A new species of the wild dragon tree, *Dracaena* (Dracaenaceae) from Gran Canaria and its taxonomic and biogeographic implications. Botanical Journal of the Linnean Society 128, 291-314.

Medail, F, P Quezel, G Besnard and B Khadari (2001) Systematics, ecology and phylogeographic significance of *Olea europaea* L. ssp *maroccana* (Greuter & Burdet) P. Vargas et al., a relictual olive tree in south-west Morocco. Botanical Journal of the Linnean Society 137, 249-266.

Meimberg, H, T Abele, C Bräuchler, JK McKay, PL Pérez de Paz and G Heubl (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.

Mes THM, J Van Brederode and H Hart (1996) Origin of the woody Macaronesian Sempervivoideae and the phylogenetic position of the East African species of*Aeonium*. Botanica Acta 109.477-491.

Mes, THM and HT Hart (1996) The evolution of growth forms in the Macaronesian genus *Aeonium* (Crassulaceae) inferred from chloroplast DNA RFLPs and morphology. Molecular Ecology 5, 351-363.

Molero, J, T Garnatje, A Rovira, N Garcia-Jacas and A Susanna (2002) Karyological evolution and molecular phylogeny in Macaronesian dendroid spurges (*Euphorbia* subsect. *Pachycladae*). Plant Systematics and Evolution 231, 109-132.

Moore, MJ, J Francisco-Ortega, A Santos-Guerra and RK Jansen (2002) Chloroplast DNA evidence for the roles of island colonization and extinction in *Tolpis* (Asteraceae: Lactuceae). American Journal of Botany 89, 518-526.

Mort, ME, DE Soltis, PS Soltis, J Francisco-Ortega and A Santos-Guerra (2001) Phylogenetic relationships and evolution of Crassulaceae based on matK sequences. American Journal of Botany 88, 76-91.

Mort, ME, DE Soltis, PS Soltis, J Francisco-Ortega and A Santos-Guerra (2002) Phylogenetics and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. Systematic Botany 27, 271-288.

Moya, O, HG Contreras-Díaz, P. Oromí and C Juan (2004) Genetic structure, phylogeography and demography of two ground-beetle species endemic to the Tenerife laurel forest (Canary Islands). Molecular Ecology 13, 3153-3167.

Olmstead, RG and JD Palmer (1997) Implications for phylogeny, classification and biogeography of *Solanum* from cpDNA restriction site variation. Systematic Botany 22, 19-29.

Oxelman, B, B Ahlgren and M Thulin (2002) Circumscription and phylogenetic relationships of *Gymnocarpos* (Caryophyllaceae - Paranychioideae). Edinburgh Journal of Botany 59, 221-237.

Panero, JL, J Francisco-Ortega, RK Jansen and A Santos-Guerra (1999) Molecular evidence for multiple origins of woodiness and a New World biogeographic connection of the Macaronesian Island endemic *Pericallis* (Asteraceae: Senecioneae). Proceedings of the Natural Academy of Sciences of Philadelphia 96, 13886-13891.

Park, SJ, EJ Korompai, J Francisco-Ortega, A Santos-Guerra and RK Jansen (2001) Phylogenetic relationships of *Tolpis* (Asteraceae: Lactuceae) based on ndhF sequence data. Plant Systematics and Evolution 226, 23-33.

Percy, DM and QCB Cronk (2002) Different fates of island brooms: contrasting evolution in *Adenocarpus*, *Genista*, and *Teline* (Genisteae, Fabaceae) in the Canary Islands and Madeira. American Journal of Botany 89, 854-864.

Powell, EA and KA Kron (2002) Hawaiian blueberries and their relatives-a phylogenetic analysis of *Vaccinium* sections *Macropelma*, *Myrtillus* and *Hemimyrtillus* (Ericaceae). Systematic Botany 27, 768-779.

Ray, MF (1995) Systematics of *Lavatera* and *Malva* (Malvaceae, Malveae) - a new perspective. Plant Systematics and Evolution 198, 29-53.

Ribera, I, DT Bilton and AP Vogler (2003) Mitochondrial DNA phylogeography and population history of *Meladema* diving beetles on the Atlantic Islands and in the Mediterranean basin (Coleoptera, Dytiscidae). Molecular Ecology 12, 153-167.

Robson, NBK (1977) Studies in the genus *Hypericum* L. (Guttiferae). 1. Infrageneric classification. Bulletin of the British Museum of Natural History (Botany) 5, 291-355.

Rønsted, N, MW Chase, DC Albach and MA Bello (2002) Phylogenetic relationships within *Plantago* (Plantaginaceae): evidence from nuclear ribosomal ITS and plastid trnL-F sequence data. Botanical Journal of the Linnean Society 139, 323-338.

Rumeu, B, P Vargas, R. Jaén-Molina, M Nogales and Juli Caujapé-Castells (2014) Phylogeography and genetic structure of the threatened Canarian *Juniperus cedrus* (Cupressaceae). Botanical Journal of the Linnean Society 175, 376-394.

Sahuquillo, E, and R Lumaret (1999) Chloroplast DNA variation in *Dactylis glomerata* L. taxa endemic to the Macaronesian islands. Molecular Ecology 8, 1797-1803.

Santoni, S and A Bervillé (1992) Characterization of the nuclear ribosomal DNA units and phylogeny of *Beta* L. wild forms and cultivated beets. Theoretical and Applied Genetics 83, 533-542.

Scheunert, A, and G Heubl (2014) Diversification of *Scrophularia* (Scrophulariaceae) in the Western Mediterranean and Macaronesia -Phylogenetic relationships, reticulate evolution and biogeographic patterns. Molecular Phylogenetics and Evolution 70, 296-313.

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

Silvertown, J, J Francisco-Ortega and M Carine (2005) The monophyly of island radiations: an evaluation of niche pre-emption and some alternative explanations. Journal of Ecology 93, 653-657.

Smissen, RD, JC Clement, PJ Garnock-Jones and GK Chambers (2002) Subfamilial relationships within Caryophyllaceae as inferred from 5′ ndhF sequences. American Journal of Botany 89, 1336-1341.

Spalik, K and SR Downie (2007) Intercontinental disjunctions in *Cryptotaenia* (Apiaceae: Oenantheae): an appraisal using molecular data. Journal of Biogeography 34, 2039-2054.

Susanna, A, T Garnatje and N García-Jacas (1999) Molecular phylogeny of *Cheirolopus* (Asteraceae-Centaureinae) based on ITS sequences of nuclear ribosomal DNA. Plant Systematics and Evolution 214, 147-160.

Swenson, U and U Manns (2003) Phylogeny of *Pericallis* (Asteraceae): a total evidence approach reappraising the double origin of woodiness. Taxon 52, 533-546.

Thiv, M, L Struwe and JW Kadereit (1999) The phylogenetic relationships and evolution of the Canarian laurel forest endemic *Ixanthus viscosus* (Aiton) Griseb (Gentianaceae): evidence from matK and ITS sequences, and floral morphology and anatomy. Plant Systematics and Evolution 218, 299-317.

Thorpe, RS, DP McGregor, AM Cumming and WC Jordan (1994) DNA evolution and colonization sequence of island lizards in relation to geological history: mtDNA RFLP, cytochrome B, cytochrome oxidase, 12S ribosomal RNA sequence, and nuclear RAPD analysis. Evolution 48, 230-240.

Thorpe, RS, H Black and A Malhotra (1996) Matrix correspondence tests on the DNA phylogeny of the Tenerife Lacertid elucidate both historical causes and morphological adaptation. Systematic Biology 45, 335-343.

Torrell, M, N Garcia-Jacas, A Susanna and J Valles (1999) Phylogeny in *Artemisia* (Asteraceae, Anthemideae) inferred from nuclear ribosomal DNA (ITS) sequences. Taxon 48, 721-736.

Trusty, JL, A Santos-Guerra, T Eriksson and J Francisco-Ortega (2001) Biodiversity, conservation, and molecular phylogenies in the laurel forest of the Canary Islands: the case of a critically endangered taxon of *Sambucus*. Botany 2001, Annual Meeting of the Botanical Society of America, August 12-16, 2001, Albuquerque, New Mexico. www.2001.botanyconference.org

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

Valcárcel, V, O Fiz and P Vargas (2003) Chloroplast and nuclear evidence for multiple origins of polyploids and diploids of *Hedera* (Araliaceae) in the Mediterranean basin. Molecular Phylogenetics and Evolution 27, 1-20.

van Hengstum, T, S Lachmuth, JGB Oostermeijer, H(J)CM den Nijs, PG Meirmans and PH van Tienderen (2012) Human-induced hybridization among congeneric endemic plants on Tenerife, Canary Islands. Plant Systematics and Evolution 298, 1119-1131.

Vargas, P, HA Mcallister, C Morton, SL Jury and MJ Wilkinson (1999a) Polyploid speciation in *Hedera* (Araliaceae): phylogenetic and biogeographic insights based on chromosome counts and ITS sequences. Plant Systematics and Evolution 219, 165-179.

Vargas, P, CM Morton and SL Jury (1999b) Biogeographic patterns in Mediterranean and Macaronesian species of *Saxifraga* (Saxifragaceae) inferred from phylogenetic analyses of ITS sequences. American Journal of Botany 86, 724-734.

Warwick, SI and LD Black (1993) Molecular relationships in subtribe *Brassicinae* (Cruciferae, tribe Brassiceae). Canadian Journal of Botany 71, 906-918.

Watson, LE, TM Evans and T Boluarte (2000) Molecular phylogeny and biogeography of tribe *Anthemideae* (Asteraceae), based on chloroplast gene ndhF. Molecular Phylogenetics and Evolution 15, 59-69.