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Phylogeography and character evolution of *Euphorbia* sect. *Aphyllis* subsect. *Macaronesicae* (Euphorbiaceae)

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Abstract

The Macaronesian species of *Euphorbia* sect. *Aphyllis* subsect. *Macaronesicae* are distributed in four of the five archipelagos of Macaronesia and two mainland enclaves in Portugal and Morocco. The aims of this study are to investigate the biogeographic history of this group with AFLP and cpDNA markers, and to identify taxonomic entities within subsect. *Macaronesicae* based on genetic data, characterize them morphologically and infer the evolution of their diagnostic characters based on the reconstruction of ancestral character states. A continuous spatial diffusion analysis of AFLP data implicated Tenerife (central Canary Islands) as the area of origin of the group, followed by colonization of other Canarian islands and other Macaronesian archipelagos. Two dispersal events back to the mainland were also inferred. Our phylogenetic network, neighbour-joining clustering and Structure analyses of AFLP data demonstrated that species are genetically well delimited and suggested that they may have originated from a combination of allopatric speciation at broad scales (among islands) and fine scales (within islands), or possibly sympatric ecological speciation followed by more recent inter-island dispersal events. Ancestral character state reconstructions of morphological characters suggested that the ancestor of subsect. *Macaronesicae* was adapted to arid or mesic habitats, and traits associated with adaptation to humid habitats were acquired later. The central Canary Islands harbour the highest species diversity of this group in the

Archipelago, and the highest nuclear and plastid genetic diversity. With regards to taxonomy, phylogenetic analyses and neighbour-joining clustering analyses based on AFLPs showed two clearly differentiated genetic groups, sister to each other, which correspond to the *E. atropurpurea* and *E. lamarckii* complexes formerly recognised based on morphology. *Euphorbia aphylla* is recovered as sister to the rest of the species, supporting its exclusion from the two complexes. *Euphorbia tuckeyana* is excluded from the *E. lamarckii* complex.

Keywords

AFLP; ancestral character state reconstruction; Canary Islands; Macaronesia; spatial diffusion; *trnL-trnF*

Short title

Phylogeography of *Euphorbia* subsect. *Macaronesicae*

INTRODUCTION

The Macaronesian biogeographic region comprises five volcanic archipelagos situated between 14.5° N and 39.5° N latitude in the Atlantic Ocean: the Azores, Canary Islands, Cape Verde, Madeira and Selvagen Islands (Sunding, 1979); and two related mainland enclaves, one on the Atlantic coast of Morocco (Sunding, 1979) and the other at Cape Espichel in Portugal (Pedro, 1942). The Macaronesian archipelagos, like all oceanic islands, are considered a model system to study speciation, migration and extinction processes (Whittaker & Fernández-Palacios, 2007), due to their *de novo* origin after land emergence from the sea and their endemic species richness and diversity. The Macaronesian flora harbours high percentages of endemic plants, ranging from an estimated 13% in Madeira and Selvagen Islands (Jardim & Sequeira, 2008) to 40% in the Canary Islands (Santos-Guerra, 1999).

Phylogenetic and phylogeographic studies of Macaronesian endemic plant groups have tested several speciation processes and have revealed several patterns of oceanic island colonization and diversification. Most Macaronesian endemic plant clades have originated from a single colonization event (e.g. Helfgott & al., 2000), but some have originated from multiple colonization events (Park & al., 2001; Fuertes-Aguilar & al., 2002; Carine & al., 2004; Martín-Bravo & al., 2007; Díaz-Pérez & al., 2008). Niche pre-emption has been hypothesized to explain a higher rate of diversification in monophyletic groups that trace back to a single colonization event than in groups with multiple

colonization events (Silvertown & al., 2005). Generally, colonization of islands from the mainland has been considered to be a one-way journey, but back-colonization to the mainland (boomerang events) have also been documented (e.g. Carine & al., 2004). Diversification through adaptive radiation can be facilitated by heterogeneity of habitats (e.g. García-Maroto & al., 2009). The role of vicariance and dispersal in the radiation of endemic plant groups has also been investigated (Sanmartín & al., 2008).

Euphorbia L. sect. *Aphyllis* Webb & Berthel. has been demonstrated to be monophyletic based on molecular genetic evidence (Barres & al., 2011; Riina & al., 2013), and two subsections are diagnosable using morphological characters (Molero & al., 2002): subsect. *Macaronesicae* Molero & Barres comprises the Macaronesian species, and subsect. *Africanae* Molero & Barres comprises the east/south African and south Arabian species. *Euphorbia tuckeyana* Steud., endemic to Cape Verde, is sister to the rest of sect. *Aphyllis* (Barres & al., 2011), but was still included in subsect. *Macaronesicae* based on morphological similarities (Riina & al., 2013). Phylogenetic relationships within the two subsections were poorly resolved and incongruence between chloroplast and nuclear markers was detected, presumably due to hybridization and rapid diversification (Barres & al., 2011).

The present study focuses on subsect. *Macaronesicae*, which comprises 11 species distributed in four of the five oceanic archipelagos in the Atlantic Ocean that constitute Macaronesia—the Canary Islands, Cape Verde, Madeira and Selvagen Islands—and in the two mainland enclaves (Fig. 1). These species are mostly semi-succulent dendroid shrubs but also include succulent "pencil-like" shrubs (*E. aphylla* Brouss. ex Willd.), and are often dominant in such Macaronesian communities as the cardonal-tabaibal, a Canarian xerophilous lowland shrub community.

Species of subsect. *Macaronesicae* have been suggested to have dispersed to Macaronesia at least twice (Barres & al., 2011), one colonization giving rise to *E. tuckeyana* in Cape Verde, and the other clade arising from a common ancestor of the rest of the species present in the other archipelagos. Based on the phylogenetic reconstructions available to date, which showed little resolution and included only one individual per species, little more could be said on the diversification of the group, the colonization of the different archipelagos and islands, and the origin of the mainland populations. However, Barres & al. (2011) hypothesized that diversification of the group entailed both allopatric speciation (speciation caused by reproductive isolation of populations due to geographic barriers among oceanic islands) and adaptive, ecological speciation

(speciation caused by adaptation of populations to different habitats within islands). Biogeographic questions such as the origins of the entire clade diversification and of the two mainland enclaves, and the direction of island colonization are still unresolved.

Molero & al. (2002) defined two taxonomic complexes within subsect. *Macaronesicae* based on morphology and ecology (Table 1): the *E. atropurpurea* complex, comprising *E. atropurpurea* Brouss. ex Willd., *E. bourgaeana* J. Gay ex Boiss. and *E. bravoana* Svent.; and the *E. lamarckii* complex, comprising *E. anachoreta* Svent., *E. berthelotii* Bolle ex Boiss., *E. lamarckii* Sweet, *E. pedroi* Molero & Rovira, *E. piscatoria* Aiton, *E. regis-jubae* J. Gay and *E. tuckeyana*. *Euphorbia aphylla* was not included in either of these two complexes, but it also belongs to subsect. *Macaronesicae* according to recent phylogenetic analyses (Barres & al., 2011; Riina & al., 2013). In those studies, *E. tuckeyana* was excluded from the main clade composed of all species of the two complexes. Molecular markers used in Molero & al. (2002) and Barres & al. (2011) failed to confirm or reject the monophyly of each of these two complexes, presumably due to rapid radiation of the group and consequent low resolution of DNA sequences.

In the current study, we investigate population genetic structure and species boundaries using amplified fragment length polymorphism (AFLP) DNA fingerprinting data (Vos & al., 1995), which often provides more detailed information on patterns of genetic variation than DNA sequence data (Meudt & Clarke, 2007). We integrate the AFLP data with cpDNA and morphological data to reconstruct the history of island colonization and dispersal routes in Macaronesia, focusing on two questions: (i) Where did the lineage originate, and what is the pattern of diversification among islands and archipelagos? (ii) Are species from the mainland sister to the remainder of the taxa, or derived from archipelago lineages? In addition, our study aims to identify taxonomic entities within subsect. *Macaronesicae* based on genetic data, characterize them morphologically and infer the evolution of their diagnostic characters, based on the reconstruction of ancestral character states.

MATERIAL AND METHODS

Sampling.– The study includes specimens from 35 populations of the 11 species of subsect. *Macaronesicae* (Fig. 1; Table 2). Fresh leaves from up to six individuals from each field locality were collected and dried in silica gel. Sampling was designed to represent the global distribution of all species (Table 2). Field localities of previously reported hybrid specimens (Molero & Rovira, 2005a) and specimens with intermediate

morphological characters newly detected in the field were not sampled. A voucher specimen from each locality is deposited at BC or BCN (Table 2).

DNA extraction, AFLP fingerprinting and plastid DNA sequencing.– Total genomic DNA was extracted from 10 mg of silica gel dried leaves using the commercial NucleoSpin Plant kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the manufacturer's protocol. For the AFLP procedure, total genomic DNA was digested using the restriction enzyme pair *Eco*RI / *Mse*I. Nineteen selective primer pairs were surveyed in three individuals of three different species of which four were selected prioritizing maximum polymorphism and reproducibility of alleles scored: (1) *Eco*RI-AAG / *Mse*I-CTA, (2) *Eco*RI-AGC / *Mse*I-CCA, (3) *Eco*RI-AGC / *Mse*I-CTT, and (4) *Eco*RI-ACA / *Mse*I-CCA. We followed the AFLP protocol by Vos & al. (1995) as modified by Berres (2001). Selective amplifications were performed using the *Eco*RI primer marked with the fluorescent dye 6-FAM. Fragment analyses were done with an ABI PRISM 3730 capillary sequencer genetic analyzer (Applied Biosystems, Foster City, CA, USA) in the Pritzker Laboratory of the Field Museum Chicago using a 6-carboxyl-x-rhodamine (ROX) labeled internal lane standard (GeneFlo 625; CHIMERx, Madison, WI, USA). Alignment, binning, and scoring of fragments between 60 and 500 bp were performed with GeneMarker v1.85 (Softgenetics, State College, PA, USA). Reproducibility was checked for each primer pair with 13 randomly chosen replicated individuals from different species, and an error rate was calculated (Bonin & al., 2004). AFLP loci that were ambiguous, non-reproducible or scored as present for fewer individuals than the error rate were excluded from the dataset. The final scoring was exported as an absence/presence binary matrix.

To analyze the cpDNA markers, we selected the *trnL* intron and *trnL-trnF* spacer because this region showed a large number of polymorphism in previous studies (Barres & al., 2011). The PCR reactions were performed following Barres & al. (2011). PCR products were purified with ExoSAP-IT (USB Corp., Cleveland, Ohio, USA), and the amplified DNA segments were sequenced using BigDye Terminator Cycle Sequencing v3.1 (Applied Biosystems), following the manufacturer's protocol, at the University of Florida ICBR Core Facility on an ABI 3730xl capillary sequencer (Applied Biosystems). Nucleotide sequences were edited using BioEdit v7.0.5.3 (Hall, 1999) and were aligned manually. A region of 97 positions from the original matrix was deleted because of its ambiguous alignment. Up to four individuals per population were selected, obtaining a

total of 158 sequences. One sequence for each haplotype was deposited in the Genbank database with accession numbers KT799781-793.

AFLPs analyses.– The AFLPs binary data matrix was used to calculate a pairwise genetic distance matrix using Nei & Li (1979) restriction site distances. We used SplitsTree4 v4.11.3 (Huson & Bryant, 2006) to construct split networks of the total dataset with a neighbour-net (NN) algorithm using the absence/presence matrix. Neighbour-joining (NJ) and UPGMA clustering analyses of the genetic distance matrix were computed with Treecon v1.3b (Van de Peer & De Wachter, 1994). Branch support was estimated with 2000 nonparametric bootstrap (BS) replicates using the same program. In the NJ analyses, the trees were rooted with *E. tuckeyana*, based on previous analyses (Barres & al., 2011). A Principal Coordinate Analysis (PCO) was conducted with NTSYSPC v2.0 (Rohlf, 1997) for a dataset excluding the outgroup.

We carried out a Bayesian clustering method implemented in Structure v2.3.3 (Pritchard & al., 2000) to identify genetically disjunct groups in the complete dataset (excluding *E. tuckeyana*). To answer our biogeographic questions, we also carried out additional partial clustering analyses of clades obtained in the NJ tree containing species distributed across several islands. For the analyses of *E. aphylla*, *E. bourgaeana*, and *E. piscatoria* we used a dataset that excluded the primer pair *Eco*RI-ACA / *Mse*I-CCA to increase the number of specimens analysed (see Table 3). Analyses were conducted under an admixture model and allele frequencies correlated among individuals with no prior information on the individual's sampling location. This was not done for the *E. lamarckii* dataset that showed limited assignment power and for which we included the locprior option implemented in the Structure software (Hubisz & al., 2009), using island as the location prior. Ten independent runs for each K value were performed in all analyses. The number of groups (K) was set from $K = 1$ to $K = 15$ for the complete dataset and from $K = 1$ to the maximum number of populations in the partial analyses (see Table 3). Initial burn-in of 10^5 generations was followed by 10^6 additional Markov chain Monte Carlo (MCMC) generations. To determine the optimal number of K, we used the ΔK approach (Evanno & al., 2005) using Structure Harvester v0.6.93 (Earl & vonHoldt, 2012). Results from different runs were summarized by Clumpp v1.1.2b (Jakobsson & Rosenberg, 2007). Structure results were represented using Distruct v1.1 (Rosenberg, 2004).

We used a continuous spatiotemporal Bayesian approach (Lemey & al., 2009) to reconstruct the spatial dynamics of subsect. *Macaronesicae.* This approach uses BEAST v1.8.2 (Drummond & al., 2012) with a lognormal relaxed random walk (RRW) model,

which is a time heterogeneous approach that allows for variation in diffusion rates across the branches of the phylogeny (Lemey & al., 2009), to infer the geographic location of ancestors and the diffusion of lineages continuously in space and time while allowing for genealogical uncertainty. We ran two independent MCMC analyses of 100 million generations each, sampling every 10,000 trees with BEAST v1.8.2 using a simple substitution model with estimated state frequencies for phylogenetic inference. A Bayesian skyline coalescent prior with a piecewise-linear skyline model was set as the model of population growth. The diffusion process was modelled by a lognormal relaxed random walk process with a lognormal distribution centred on 1. We specified a prior exponential distribution on the standard deviation of the lognormal distribution with a mean of five. We added random jitter with a window size of 1.0 to the tips, as several individuals from the same location were analysed. We used a strict molecular clock and evaluated MCMC mixture and convergence by requiring effective sample sizes (ESS) of at least 200 as estimated in Tracer v1.6.0 (Rambaut & al., 2013). We combined trees obtained from the two independent runs with LogCombiner (part of the BEAST package) after removing 10% of trees as burn-in (as suggested by Tracer, and supported by ESS calculations of >200). A maximum clade credibility (MCC) tree was produced and summarized with TreeAnnotator (part of the BEAST package) and visualized in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). The MCC tree obtained under the diffusion model was analysed with the Continuous Tree module of SPREAD v1.0.6 (Bielejec & al., 2011), and a visual representation of lineage diffusion over time and space was generated with Google Earth v6.0.1 (Google Inc.). SPREAD uses Bayes factors to evaluate the support for alternative hypotheses of historical diffusion among pairs of discrete locations based on Bayesian stochastic search variable selection estimates, accommodating the uncertainty of the original phylogenetic inference (Bielejec & al., 2011), and maps phylogenies annotated with continuous spatial information also allowing to export the high-dimensional posterior summaries to keyhole markup language (KML). Visualization allows for generating maps at different times with the time slicer function, using light colours to indicate more ancient diffusion events and dark colours to indicate recent events.

To assess the distribution of genetic variation, analyses of molecular variance (AMOVA) were carried out based on Euclidean distances among samples using Arlequin v3.1 (Excoffier & al., 2005) with 1000 random permutations. Different nested analyses were performed (Table 4).

Analyses of haplotypes.– A statistical parsimony haplotype network was constructed with the cpDNA sequences using TCS v1.21 (Clement & al., 2000). Indels were coded for this analysis using the simple coding algorithm (Simmons & Ochoterena, 2000) implemented in the program SeqState v1.4.1 (Müller, 2005). Then, in the TCS analysis, indels were treated as missing data.

Phylogenetic analyses of the haplotypes were performed using maximum parsimony (MP) and Bayesian inference (BI) methods, implemented in the programs PAUP* v4.0b10 (Swofford, 2002) and MrBayes v3.2 (Ronquist & Huelsenbeck, 2003), respectively. Six species from subsect. *Africanae* and one from sect. *Pachycladae* (Boiss.) Tutin were included as outgroups following Barres & al. (2011). MP analyses used heuristic search with 1000 replicates of random taxon addition with mulpars in effect and tree-bisection-reconnection (TBR) branch swapping. All most parsimonious trees were saved. Parsimony uninformative positions were excluded. After the strict consensus tree was computed, a nonparametric bootstrapping analysis (Felsenstein, 1985) was performed following Lidén & al. (1997), using 1000 replicates of heuristic search, 10 random taxon additions with 10 replicates per each BS replicate, multrees option not in effect, and no branch swapping.

The Akaike information criterion (Akaike, 1973), as implemented in MrModeltest v2.3 (Nylander, 2004), was used to select the best-fit model of substitution (GTR for the sequence partition and F81 for the indel partition). Two independent analyses of four Metropolis-coupled Markov chains were run for five million generations in MrBayes, saving one of every 500 trees until they reached stationary frequencies (final split frequency between the two runs, $P < 0.01$). A 50% majority rule consensus tree was computed from the posterior distribution after discarding the first 25% of trees as burn-in.

Analyses of character evolution.– Ancestral character state reconstructions for morphological characters were performed with Mesquite v2.74 (Maddison & Maddison, 2010). Species were represented by one terminal individual except for *E. regis-jubae*, for which two individuals were retained due to polymorphism in the Moroccan populations. *Euphorbia tuckeyana* was excluded from the analyses because the lack of members of subsect. *Africanae* could bias the results (see Barres & al., 2011). Maximum Likelihood (ML) was used to reconstruct the evolution of six selected characters on a NJ tree inferred from Nei and Li distances calculated with PAUP from the AFLP data under the single-rate (Mk1) model. Discrete characters used for ancestral state reconstruction were

selected because they are diagnostic of the two complexes previously recognized within the subsection (Table 1), diagnostic of a taxon (like subtruncate nectaries for *E. bourgaeana*, rugose seed surface for *E. piscatoria*, scrobiculariate seed surface for *E. bravoana* or mitriform caruncle and obnavicular-elongate caruncle for *E. pedroi*), or diagnostic of a regional group of populations within a taxon (horned nectaries for the Canarian populations of *E. regis-jubae*). Characters and their states were defined as follows: A. pleochasial organization (of the sympodial synflorescence branching pattern: simple, 0; double, 1); B. nectaries morphology (truncate, 0; subtruncate, 1; dentate, 2; horned, 3); C. sub-cyathial bracts persistence (deciduous before fructification, 0; deciduous just after fructification, 1; persistent, 2); D. sub-cyathial bracts union (free, 0; connate, 1); E. seeds surface (smooth-rugulose, 0; rugose, 1; excavate, 2; scrobiculariate, 3); F. caruncle morphology (obnavicular-truncate, 0; obnavicular-elongate, 1; mitriform, 2). Additionally, some of these attributes are related to ecological preferences and were specifically used to interpret the adaptation of the ancestral populations to the environment during the colonization of Macaronesia. These are double pleochasium and connate, persistent bracts, which imply the maintenance of large foliar structures during all year, contrary to the strategy adopted by drought tolerant species, which have small and/or deciduous leaves, in order not to lose water by evapotranspiration. Information on all the character states studied was obtained from our own observations and from the literature (Boissier, 1862; Press & Short, 1994; Molero & Rovira, 1996; 1998; Benedí & al., 1997; Bramwell & Bramwell, 2001; Molero & al., 2002; Acevedo & al., 2003; Mesa & al., 2007; Mesa, 2009).

RESULTS

AFLP analyses.– A total of 346 bands were scored for 189 individuals, of which 332 (95.95%) were polymorphic. The AFLP replicates we performed showed a genotyping error rate of 1.16% which we considered negligible in a study of this scale. Three fixed private alleles were found, one in *E. anachoreta* and two in *E. bravoana*. The NN diagram produced (Fig. 2) is highly congruent with the results obtained with NJ (Fig. 3), showing species as monophyletic with BS support between 78% and 100% (Fig. 3), except for *E. regis-jubae.* This species was recovered as paraphyletic, with a clade composed of all samples of *E. pedroi* embedded in it (BS=100%). Three main clusters were recovered (Fig. 3): the first included all populations of *E. aphylla* supported with 100% BS, the second included all species from the *E. atropurpurea* complex (*E. atropurpurea, E.*

306 *bravoana* and *E. bourgaeana*) with 94% of BS, and the third included all species from
307 the *E. lamarckii* complex (*E. anachoreta, E. berthelotii, E. lamarckii* var. *lamarckii*, *E.*
308 *lamarckii* var. *broussonetti* (Willd. ex Link) Molero & Rovira*, E. pedroi, E. piscatoria*
309 and *E. regis-jubae*), except for *E. tuckeyana,* with 95% BS support. *Euphorbia tuckeyana*
310 populations were grouped in a non-supported clade (Figs. 2 and 3). The same results were
311 recovered with the UPGMA tree (not shown). In the PCO analysis (Electr. Suppl.: Fig.
312 S1), 21.50% of the variability was explained by the first axis, 17.67% by the second axis
313 and 11.65% by the third axis, for a sum of 50.84% of the total variability. The specimens
314 were grouped in a similar way as in the NN analysis (Fig. 2). In the Structure analyses of
315 the whole dataset excluding *E. tuckeyana*, the optimal value of *K* was seven (Fig. 4A;
316 Table 3). The green group was found in *E. lamarckii*, which is in general genetically
317 uniform and in *E. anachoreta;* the yellow group was mainly found in the group composed
318 by *E. regis-jubae* and *E. pedroi* but also in *E. anachoreta* and *E. bravoana*; the brown
319 group was mainly found in *E. aphylla* but also in *E. bravoana;* the pink group was mainly
320 found in the genetically uniform *E. atropurpurea* but also in *E. bravoana* and *E.*
321 *lamarckii*; the blue group was mainly found in *E. berthelotii* but also in *E. aphylla* and *E.*
322 *bravoana*; the orange group was mainly found in the genetically uniform *E. piscatoria*
323 but also in *E. berthelotii.* The red group corresponded to *E. bourgaeana*. The partial
324 Structure analyses of clades analysed showed the following results: for *E. bourgaeana*
325 the optimal number of groups was three (Fig. 4B; Table 3); the first group (green group)
326 included all populations from La Gomera; the second group (light blue group) included
327 one population from west Tenerife (mauve group) and the third group included two
328 populations from east Tenerife. Another population of west Tenerife showed a high level
329 of genetic admixture of the mauve and the light blue groups (Fig. 4B). *Euphorbia*
330 *lamarckii* and *E. anachoreta* were clearly recovered as two different entities (Table 3;
331 Figure not shown). Considering this result, and also the results of all previous analyses
332 (Figs. 2 and 3; Electr. Suppl.: Fig. S1), we only performed the Structure analyses of the
333 most widely distributed species, *E. lamarckii*. This showed an optimal number of $K = 4$
334 (Fig. 4C; Table 3), with three different groups geographically structured (the pink group
335 was only present in Tenerife*,* the blue group was present in La Gomera and Tenerife, and
336 the orange group was present in La Palma and El Hierro; Fig. 4C) and one group scattered
337 in few individuals from La Gomera and Tenerife (green colour, Fig. 4C). The Structure
338 analysis detected two genetic groups as the optimal *K* for *E. piscatoria* (Fig. 4D), which
339 broadly corresponded to two different islands in the Madeiran archipelago, except for one

individual from Porto Santo that was assigned to the Madeiran main island population, and two additional individuals from Porto Santo that showed mixed ancestry of the two groups. In the *E. tuckeyana* partial analysis, four genetic groups were recovered. The green group characterized all individuals from the southern islands populations (Fogo and Santiago) but it is also present in a much lesser degree in all the specimens from other islands. The salmon group characterizes the only two specimens from São Vicente, which are genetically almost uniform, but this group is also present in other specimens, especially from Fogo. The orange group is especially represented in all the specimens from São Nicolau, but is also found in some specimens from Fogo and Santiago. Finally, the blue group characterizes all the specimens from Santo Antão, which are genetically almost uniform, but this group is also present in few individuals from all the other islands (Fig. 4E). The analyses of the cluster *E. regis-jubae* + *E. pedroi* (Fig. 4F; Table 3) recovered two groups, one found mainly in the Gran Canarian populations of *E. regis-jubae* (green group) and the other present in all populations of both species with a little level of mixture with some individuals from Gran Canaria island (purple group). Finally, one group (no population structure) was detected for *E. aphylla* (Table 3).

Spread diffusion analyses argue for an origin of diversification in Tenerife (Canary Islands, Fig. 5A), followed by several dispersal events to other Canarian Islands and Madeira (Fig. 5B). A first back-colonization of the mainland by a dispersal event from the east Canarian Islands (Fuerteventura and Lanzarote) to Morocco gave rise to the mainland *E. regis-jubae* populations (Fig. 5C). A second back-colonization of the mainland by an independent dispersal event from the east Canarian Islands to Portugal resulted in the origin of *E. pedroi*. Finally, *E. anachoreta* arose from the colonization of Selvagen Islands from Tenerife (Fig. 5D).

ϕ_{ST} values obtained ranged from 0.32 to 0.84 (Table 4).

Analyses of haplotypes.– We detected 13 haplotypes that differed from each other by one to six substitutions (Fig. 6). The relationships among these haplotypes are shown in Fig. 6B. Four haplotypes were shared among different species (Fig. 6A; Table 2). The most common haplotype was IV, present in 25 populations of nine species, and was the only haplotype sampled in *E. anachoreta*, *E. bravoana*, *E. pedroi* and *E. regis-jubae*. Six haplotypes were exclusive for one population (III, V, VII, X, XI and XIII). *Euphorbia lamarckii* exhibited the greatest haplotype diversity of any species (five haplotypes). The three populations of *E. atropurpurea* showed three different haplotypes. The populations of *E. aphylla* showed the same haplotype in Tenerife and La Gomera but

374 the population of Gran Canaria had an additional haplotype. In *E. bourgaeana*, more
375 cpDNA differences were found between populations within Tenerife than between
376 populations from Tenerife and La Gomera. The islands of Gran Canaria, Tenerife and La
377 Gomera (central Canary Islands) presented a higher number of different haplotypes (11)
378 than the western Canary Islands (three haplotypes) or the eastern Canary Islands + the
379 mainland (Portugal and Morocco), where only one haplotype was found.
380 Phylogenetic analyses of cpDNA resulted in low resolution (Electr. Suppl.: Fig.
381 S2). The only supported clade included three haplotypes (I, II and III), which are present
382 in the southern population of *E. atropurpurea*, most populations of *E. lamarckii* and one
383 individual of *E. piscatoria*.
384 **Analyses of character evolution.–** The ancestral state for pleochasial
385 organization was inferred to be simple, with acquisition of double organization in the *E.*
386 *atropurpurea* complex (Fig. 7A). For nectary morphology, the ancestral state was
387 reconstructed to be truncate. Dentate nectaries were inferred to have appeared twice
388 independently: in *E. piscatoria* and with some ambiguity in the ancestor of the *E. pedroi*
389 + *E. regis-jubae*, which was inferred to have dentate, horned or truncate nectaries with
390 the same probability (Fig. 7B). Subtruncate nectaries are an autopomorphy of *E.*
391 *bourgaeana* and horned nectaries are an autopomorphy of the Canarian populations of *E.*
392 *regis-jubae*. The sub-cyathial bracts appear to have been ancestrally deciduous before
393 fructification (Fig. 7C). Under this reconstruction, the ancestor of the *E. atropurpurea*
394 complex would have acquired persistent sub-cyathial bracts secondarily, and some
395 members of the *E. lamarckii* complex would have shifted to deciduous bracts just after
396 fructification. Free sub-cyathial bracts were inferred as ancestral, with a shift to connate
397 bracts in the *E. atropurpurea* complex (Fig. 7D). Seed surface was reconstructed to be
398 smooth-rugulose in the ancestor of subsect. *Macaronesicae*. Rugose seeds are an
399 autopomorphy of *E. piscatoria*, and excavate seeds were inferred to appear in the ancestor
400 of the *E. atropurpurea* complex (Fig. 7E). *Euphorbia bravoana* was reconstructed to have
401 later acquired scrobiculariate seeds as an autopomorphy. The obnavicular-truncate
402 caruncle was inferred to be the ancestral condition for caruncle morphology (Fig. 7F).
403 Two later shifts were reconstructed: one to obnavicular-elongate state in *E. pedroi* and
404 the other to mitriform state in *E. atropurpurea*.
405
406 **DISCUSSION**

Origin, diversification and dispersal routes in the Macaronesia.– Our analyses suggest that the clade originated in Tenerife, from which the group diversified by several dispersal to nearby islands and archipelagos (Fig. 5). These dispersals and resultant allopatry produced numerous single-island endemic species during the early diversification of the group. Subsequent inter-island dispersal has contributed to range expansion of several species that range across two or more islands. The high inter-island migration of subsect. *Macaronesicae* is attributable to numerous stochastic dispersal vectors: wind, driftwood, and endozoochory by birds, given that rock pigeons (*Columba livia canariensis* Bannerman) and migratory turtle doves (*Streptopelia turtur turtur* L.) have been recorded as *Euphorbia* seed feeders (Nogales, 1985; Berg, 1990).

Inter-archipelago dispersal.– Contrary to other Macaronesian endemic plant groups such as *Argyranthemum* Webb, for which a dispersal route north to south in the Madeira-Desertas-Selvagen Islands has been proposed (Francisco-Ortega & al., 1996), our study reveals a northward dispersal from the Canary Islands to Madeira Archipelago, as shown by the spatial diffusion analysis (Fig. 5B). One of the first dispersals of the group from Tenerife resulted in the origin of *E. piscatoria* in Porto Santo (age: 14.3 Ma; Geldmacher & Hoernle, 2000; Fig. 5B), and from there this species later colonized Madeira (age: 4.6 Ma; Geldmacher & Hoernle, 2000). In the past, the trade winds associated with the presence of higher mountains in earlier developmental stages of Porto Santo may have favoured the existence of more mesic habitats (Fernández-Palacios & al., 2011) and establishment of the ancestor of *E. piscatoria*. This species acquired two morphological characters after the colonization of this archipelago and the isolation from its ancestor: dentate nectaries and rugose seeds (Figs. 7B, 7E). *Euphorbia piscatoria* populations from both islands may have been isolated for a long time, as they exhibit strong geographic structure between Madeira and Porto Santo (Figs. 2–4; Electr. Suppl.: Fig. S1), except for one individual from Roche de Nossa Senhora in Porto Santo which is grouped with the specimens from Madeira (PIS34; Fig. 4D). Placement of this single individual might be due to lab error or recent establishment of seeds from Madeiran specimens in Porto Santo. Porto Santo populations are scarce and especially threatened by fragmentation of natural habitats and introduced grass-feeding animals (Faria & al., 2008). In either case, to conserve genetic diversity within *E. piscatoria,* our data support recognition of two Evolutionary Significant Units (ESU; Moritz, 1994) in the two different islands. Further studies regarding this species should include populations from

440 the Desertas Islands, 25 km disjunct, to understand their genetic affinities and possible
441 origin.
442 Colonization of the Selvagen Islands, an archipelago of islets that originated 12
443 Ma (Bogaard, 2013), is inferred to have occurred by dispersal from Tenerife (Fig. 5D)
444 before the arrival to Morocco or to the older Madeira (14.3 Ma; Geldmacher & Hoernle,
445 2000). *Euphorbia anachoreta,* an endemic from Ilhéu de Fora islet in the Selvagen
446 Archipelago, originated by allopatric differentiation from a shared common ancestor with
447 *E. lamarckii* (Fig. 4A) from Tenerife. Although we only included three *E. anachoreta*
448 individuals in the analyses, this represents about 12% of the single population of this
449 species (Carvalho, personal communication), which is among the 100 most threatened
450 species in Macaronesia (Jardim & al., 2008).
451 *Back-colonization to the mainland*.– Back-dispersal events of Macaronesian
452 organisms to the mainland have been reported for several plant groups (Mes & Hart, 1996;
453 Park & al., 2001; Carine & al., 2004). During the Quaternary glaciations, Macaronesian
454 islands acted as a biodiversity refuge, providing a source of genetic diversity that later
455 have contributed to mainland biodiversity (Patiño & al., 2015). An exchange of flora
456 between Macaronesia and the Atlantic coasts of the African and European continents
457 could have occurred repeatedly in the Quaternary during glacial times, when volcanic
458 marine seamounts emerged and could have acted as stepping stones facilitating the arrival
459 of several species at the mainland (García-Talavera, 1997; Fernández-Palacios & al.,
460 2011). As shown by the spatial diffusion analysis, back-colonization to the mainland from
461 the eastern Canary Islands explains the presence of *E. regis-jubae* on the Atlantic coast
462 of Morocco (Fig. 5C). Ecological conditions in Fuerteventura and Lanzarote have been
463 similar to those on the west coast of Morocco since the Pliocene (Caujapé-Castells, 2011),
464 favouring this establishment.
465 An independent back-colonization event from the eastern Canary Islands to
466 Portugal gave rise to *E. pedroi* (Fig. 5D), probably through the same dispersal pattern and
467 at approximately the same time as the origin of the Moroccan populations of *E. regis-*
468 *jubae*. The presence of other several plant species (e.g. *Convolvulus fernandesii* Pinto da
469 Silva and Teles, *Davallia canariensis* (L.) Sm., *Woodwardia radicans* (L.) Sm.) with
470 related lineages having a disjunct distribution in Macaronesia and Cape Espichel and
471 nearby mountains such as Serra da Arrábida and Serra de Sintra reinforces the
472 consideration of this area of Portugal as a second mainland Macaronesian enclave. The
473 influence of trade winds maintaining similar climate conditions would have facilitated

the establishment and permanence of these lineages. AFLP analyses (Figs. 2–4; Electr. Suppl.: Fig. S1) suggest that *E. pedroi* –which is recovered as a monophyletic entity embedded in a paraphyletic *E. regis-jubae*– could have originated by peripatric speciation (founder effect; Futuyma, 2005) from the more widely distributed and genetically more diverse *E. regis-jubae*. *Euphorbia pedroi* and the Moroccan populations of *E. regis-jubae* share a common character, dentate nectaries, which is inferred to be most probably present in their common ancestor from the Canary islands (Fig. 7B). The relatively low genetic diversity in the *E. regis-jubae - E. pedroi* complex (Fig. 6; Table 2) suggest a very recent origin of this group. Biased allele frequencies caused by the new population establishment by a few individuals often produces rapid genetic and morphological differentiation from the original populations by genetic drift (Futuyma, 2005). Indeed, *E. pedroi* is morphologically readily distinguishable from *E. regis-jubae* by its extremely elongate seed caruncle (Fig. 7F) and dentate nectaries (Fig. 7B) sometimes showing horns up to 0.3 mm long (in the Canarian *E. regis-jubae* these horns are 0.3 – 1 mm long). As the analysis of ancestral states shows, this type of caruncle would have developed after the colonization of Portugal, given that it is not inferred for the common ancestor of *E. pedroi* and *E. regis-jubae* (Figs. 7B and 7F). Because of its morphological distinctness, monophyly found in the NN and the NJ analyses (Figs. 2 and 3), and geographical isolation, we recommend maintaining *E. pedroi* as a separate species.

Euphorbia tuckeyana.– This species from Cape Verde was recovered as an independent clade from the rest of species from subsect. *Macaronesicae* (Barres & al., 2011). Results from the AFLP and the cpDNA analyses here performed confirm its independent origin as it is genetically isolated from the other species (Figs. 2, 3 and 6). With regards to the geographic structure of genetic variation inferred from the AFLP, a notable degree of admixture of most populations is observed (Fig. 4E). These results, the low cpDNA variation (Fig. 6), and the lack of further speciation in this archipelago, may suggest a recent colonization of the archipelago and recent divergence between populations from different islands, or alternatively the existence of gene flow between them. However, despite the genetic admixture, the four genetic groups detected by Structure show some degree of geographic structure: in populations from the southern islands (Fogo and Santiago) the predominant group is the green one, whereas in populations from the northern islands (Santo Antão, São Vicente and São Nicolau) the other three groups predominate. Similar patterns of genetic differentiation of northern from southern elements in Cape Verde have been found in other plant lineages (Romerias

& al., 2015), and this pattern has also correspondence with two of the main floristic or phyoteographic elements defined by Brochmann & al. (1997) for Cape Verde, who classified the islands in three main groups (see Fig. 1): the northern islands (Santo Antão, São Vicente and São Nicolau), the eastern islands (Maio, Sal and Boa Vista) and the Southern islands (Brava, Fogo and Santiago). The eastern islands, where *E. tuckeyana* is only found in Sal, were not represented in our study.

General patterns of diversification and genetic variation in the Canary Islands.– The central Canary Islands and specifically Tenerife were shown to be the centre of origin of the group (Fig. 5A), agreeing with the Sanmartín & al. (2008) model. The central Canary Islands harbour the highest diversity in the Archipelago. This can be explained as a consequence of the topological, climatic and habitat heterogeneity of these islands (Caujapé-Castells, 2011). Seven of the eight Canarian species (87.5%) of subsect. *Macaronesicae* are found in the central Canary Islands, three of them being endemics to Tenerife. Species from the central and western islands also show stronger inter-population nuclear genetic differentiation (Fig. 4), and a higher number of haplotypes (nine) was detected in the central islands than in the eastern islands and on the mainland (one; Fig. 6A). Most of the central-western island species (*E. atropurpurea, E. bourgaeana* and *E. lamarckii*), including the single-island endemic *E. atropurpurea* show striking intraspecific haplotype diversity, suggestive of incipient population differentiation (Fig. 6A). These observations and the coexistence of ecologically different species on some of the islands suggest that fine scale allopatric speciation or sympatric speciation due to ecological differentiation may have played a role in the differentiation of this group of species.

In contrast, despite the proximity to the mainland and the older age of the eastern group of islands, the total number of Macaronesian endemic species in these islands is lower than in the rest (Reyes-Betancort & al., 2008). In accordance with this pattern, there is only one subsect. *Macaronesicae* eastern islands endemic, *E. regis-jubae*, which presents a low level of inter-population genetic differentiation (Figs. 4F and 6). The flat topology and the ecological homogeneity of these islands could have allowed gene flow between populations, eroding any genetic differentiation of possibly distinct colonizing genotypes which previously had been isolated on the mainland (Caujapé-Castells, 2011). In the eastern populations, genetic variation is noticeably higher within than between populations (Table 4).

Systematic considerations.– Although the radiation of the group is hypothesised to have been relatively recent (Barres & al., 2011), there have been time and isolation enough to generate genetic differentiation between species and maintain them as genetically isolated and morphologically distinguishable entities. The combination of AFLP and cpDNA markers together with previous morphological data allow us to provide some insights on the systematics of the group.

We propose to exclude *E. tuckeyana* from the *E. lamarckii* complex considering its independent origin already detected in a previous work (Barres & al., 2011) and its current genetic isolation, evidenced by both the AFLP (Figs. 2 and 3) and cpDNA analyses (Fig. 6).

Euphorbia aphylla, a fleshy aphyllous pencil-like shrub found in saline habitats in the central Canary Islands, was resolved as sister to the rest of subsect. *Macaronesicae* ingroup with 100% BS support in the NJ analyses (Fig. 3), in agreement with Molero & al.'s (2002) taxonomic treatment, that excluded this species from the two main taxonomic complexes recognized in the group based on morphology. The isolation of *E. aphylla* from the rest is confirmed by the haplotype network, as it has two different exclusive haplotypes (IX and X; Fig. 6A). We found no genetic differentiation within the populations of *E. aphylla* (not shown), although it grows on three different islands of the Canarian archipelago (Fig. 1).

The AFLP analyses supported the two taxonomic complexes (Figs. 2 and 3; Electr. Suppl.: Fig. S1) as sister clades with strong BS support (BS = 95%; Fig. 3). Both complexes are genetically differentiated and some of the morphological differences between them may be ecological adaptations (Fig. 7A, C, D; Table 1). Species from the *E. lamarckii* complex, as well as *E. aphylla*, are adapted to arid and mesic habitats not affected by trade winds, such as pine forest and arid lowland scrub in Madeira and the Canary Islands, and the two mainland enclaves in Morocco and Portugal. They have free, deciduous bracts (Fig. 7C, D) and a simple synflorescence (Fig. 7A), which are reconstructed as the ancestral condition for the *E. lamarckii* complex and for the whole subsect. *Macaronesicae* and in our analyses. These structures would contribute to reduce water loss in the dry season, and would have allowed the establishment in similar habitats when the group first colonized the islands. Species from the *E. atropupurea* complex, on the other hand, are found in mesic to humid habitats affected by trade winds where laurel forests grow in Tenerife and La Gomera. They have double synflorescences (Fig. 7A) and connate, semi-persistent bracts (Fig. 7C, D), as they do not need to save water by reducing

their structures. These traits, as inferred by our analyses, would have been acquired secondarily by the common ancestor of this complex, together with excavate seeds instead of smooth-rugulose seeds (Fig. 7E). Plastid DNA markers showed very low resolution (Electr. Suppl.: Fig. S2) but the only supported clade contained *E. atropurpurea* and *E. lamarckii*, belonging to the two different taxonomic complexes recognized in all AFLPs analyses. This incongruence supports the hypothesis of introgression by the maternal lineage as suggested by Barres & al. (2011).

Euphorbia bourgaeana shows strong genetic differentiation between La Gomera and Tenerife (Fig. 4B), presenting the highest ϕ_{ST} values (0.83; Table 4) in our study, in accordance with their geographic isolation. Populations from La Gomera were considered an independent species, *E. lambii* Svent., in the past (Sventenius, 1960). However, the degree of genetic differentiation of *E. lambii* from populations of Tenerife is not higher than that found between genetic groups detected within Tenerife (Fig. 4B; Table 4), and more cpDNA differences were found between populations within Tenerife than between populations from Tenerife and La Gomera (Fig. 6). The higher genetic variation found within the whole of *E. bourgaeana* and especially within Tenerife and the lack of a clear pattern of macro- and micromorphological differentiation between the two island groups of populations (Molero & Rovira, 2005b; Molero, Barres & Rovira, in prep.) suggest that *E. lambii* should be considered part of the variation of *E. bourgeana*. In terms of conservation, we recommend the recognition of three different ESUs (Moritz, 1994) for the vulnerable *E. bourgaeana* species (Bañares & al., 2010): one in La Gomera and two in east and west Tenerife, corresponding with the two main genetic groups detected in this island. Recognizing three ESUs (Moritz, 1994) in this species would help to preserve its total genetic diversity and guide conservation strategies.

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Figure Captions

Fig. 1. Sampling localities of 53 populations of the 11 *Euphorbia* species included in the study. Details on localities and number of specimens sampled are given in Table 2.

Fig. 2. Neighbour-net diagram of the whole AFLPs dataset constructed with Splitstree v4.11.3. BS values above 80% from a NJ analysis of the same dataset are shown only on main clades. Species names are labelled as: ANA, *E. anachoreta*; APH, *E. aphylla*; BER, *E. berthelotii*; BOU, *E. bourgaeana*; LAMB, *E. lamarckii* var. *broussonetti;* LAML, *E. lamarckii* var. *lamarckii*; PED, *E. pedroi*; PIS, *E. piscatoria*; REG, *E. regis-jubae*; TUC, *E. tuckeyana*. The population codes as shown in Fig. 1.

Fig. 3. Neighbour Joining tree of 189 individuals from 53 populations used in the AFLPs analyses. Numbers above branches indicate BS support values > 50%. Species names are labelled as: ANA, *E. anachoreta*; APH, *E. aphylla*; BER, *E. berthelotii*; BOU, *E. bourgaeana*; LAM, *E. lamarckii*; PED, *E. pedroi*; PIS, *E. piscatoria*; REG, *E. regis-jubae*; TUC, *E. tuckeyana*. Population numbers follow Fig. 1.

Fig. 4. Bar plots from the genetic structure analyses obtained with Structure v2.3.3. In all panels, vertical bars estimate the proportion of each individual's genome that comes from the *K* postulated genetic groups. An admixture model was used for all analyses presented. (A) $K = 7$ for the whole dataset excluding *E. tuckeyana.* (B) $K = 3$ for 28 *E. bourgaeana* individuals. (C) $K = 4$ for 45 *E. lamarckii* individuals. (D) $K = 2$ for 30 *E. piscatoria* individuals. (E) $K = 4$ for 35 *E. tuckeyana* individuals. (F) $K = 2$ for 37 individuals of *E. regis-jubae* and *E. pedroi*. Species names and populations numbers are labelled as in Fig. 1. Geographic origin is labelled as: ET, east Tenerife; F, Fuerteventura; G, La Gomera; GC, Gran Canaria; H, El Hierro; L, Lanzarote; M, Madeira; Mo, Morocco; P, La Palma; PS, Porto Santo; S, Santiago; SA, Santo Antão; SV, São Vicente; T, Tenerife; WT, west Tenerife.

Fig. 5. Spatial diffusion of subsect. *Macaronesicae* populations based on the MCC tree analysed with BEAST in an RRW model at four times intervals. The red lines represent the branches of the MCC tree. The blue regions represent the 80%-HPD uncertainty in the location of ancestral branches with a gradient between light and dark representing older vs. younger diffusion events.

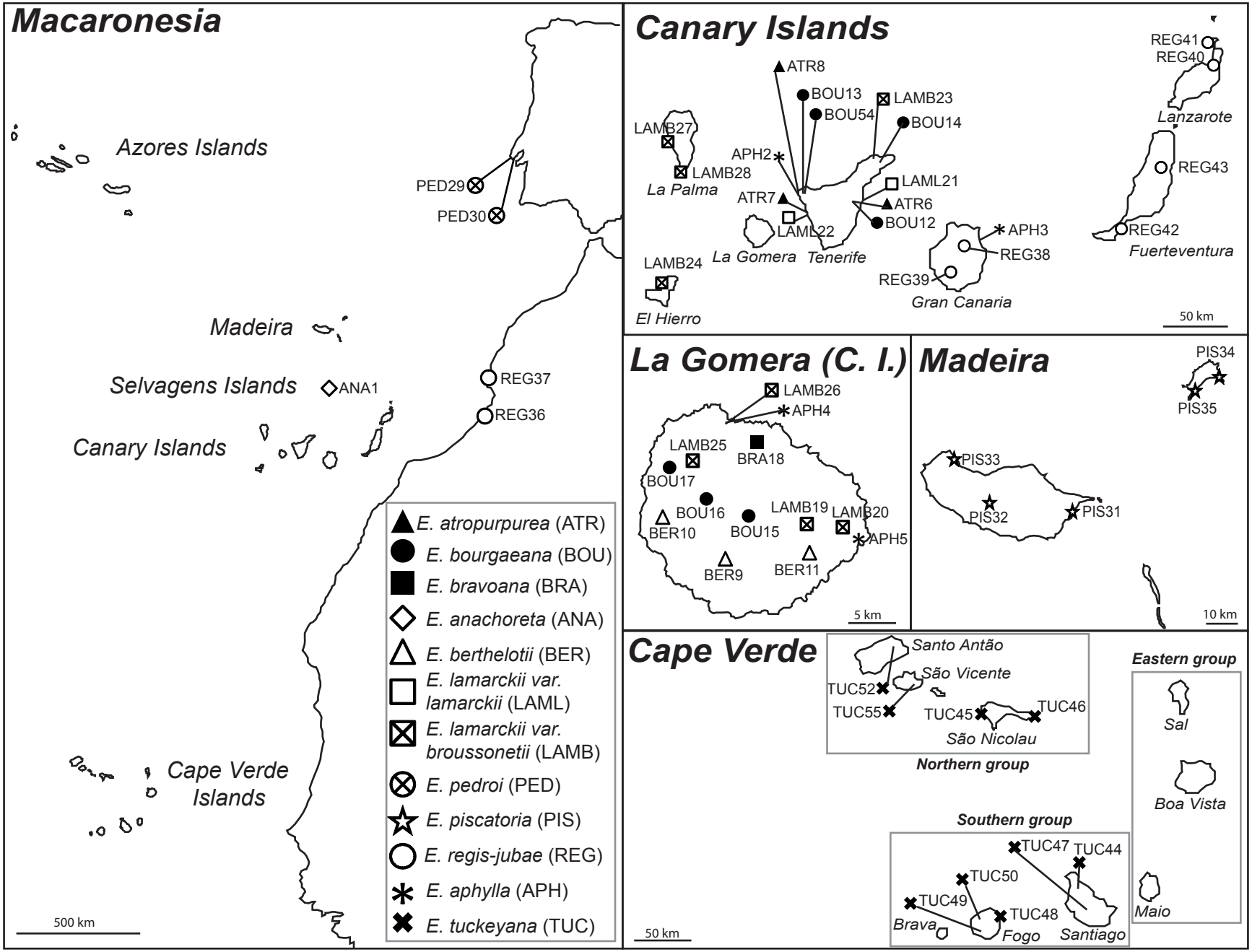
Fig. 6. Distribution and relationships of cpDNA haplotypes. (A): Geographic distribution of the 13 haplotypes detected. Donut chart slices indicate proportions of haplotypes in each population sampled. (B): Statistical parsimony network of cpDNA haplotypes. Circles are proportional to the number of individuals containing each

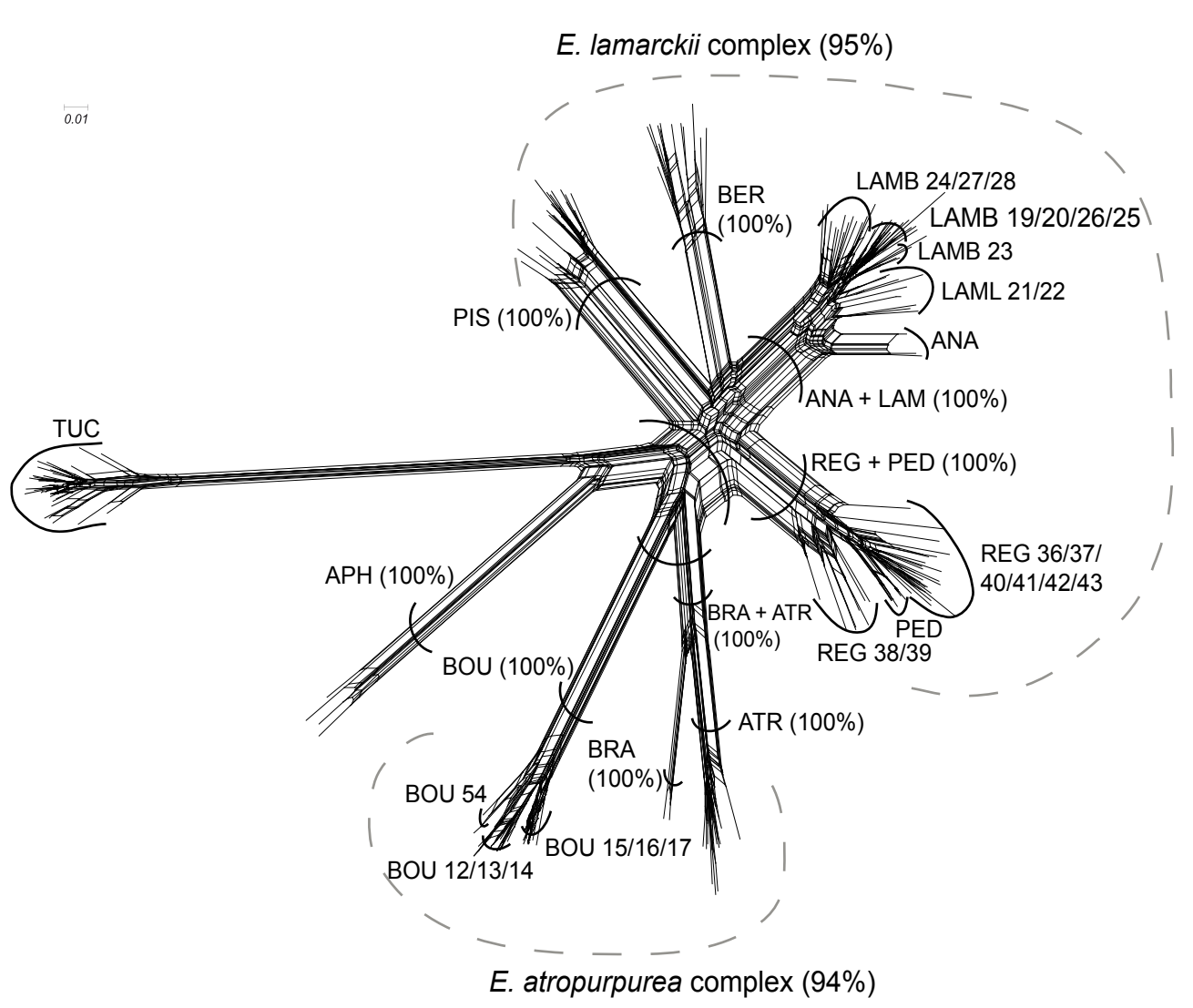
haplotype, black circles indicate unsampled intermediate haplotypes. Discontinuous lines represent uncertainty about the order of character changes and are shown to indicate alternative relationships in the diagram. Species names and populations numbers are labelled as in Fig. 1.

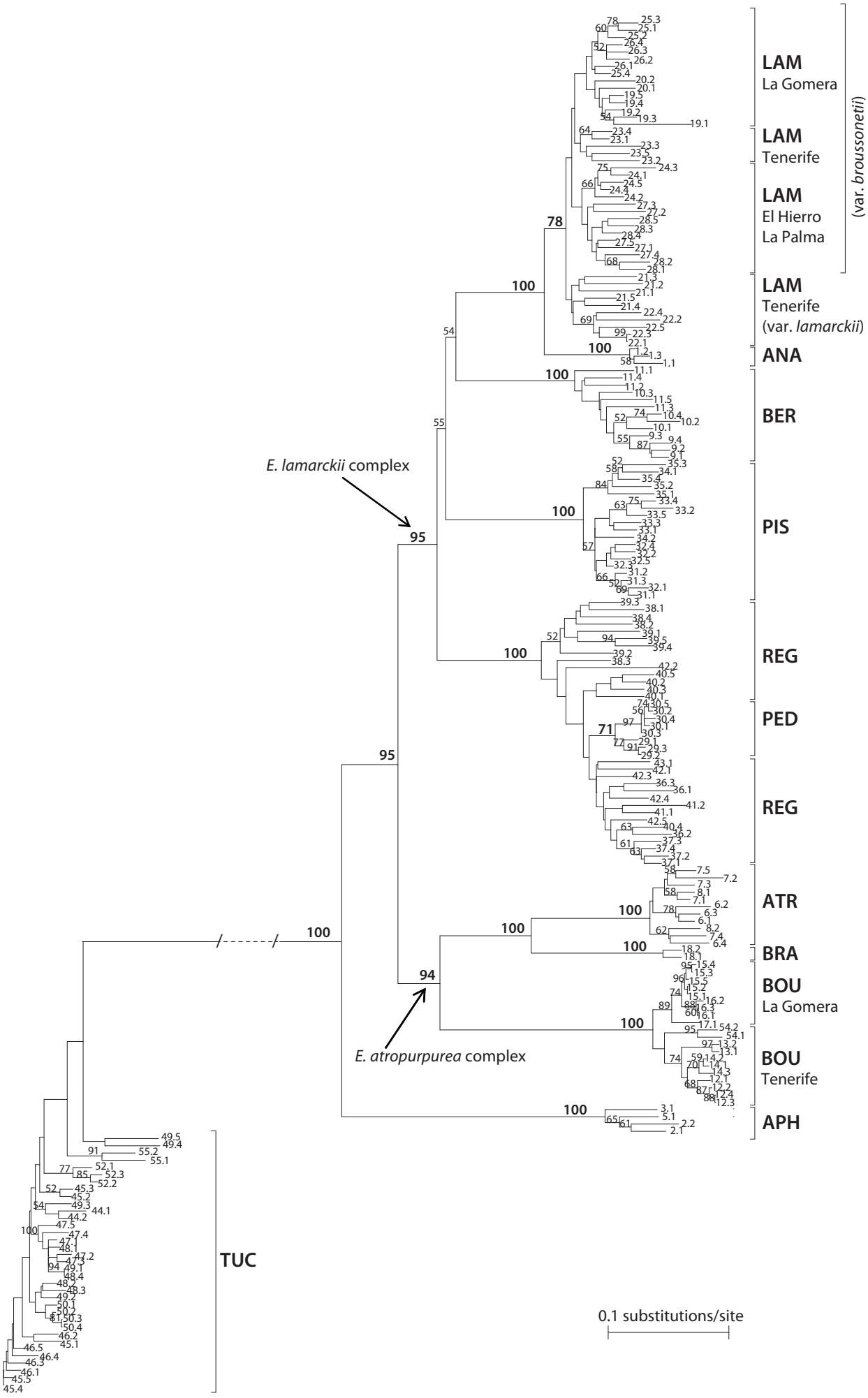
Fig. 7. Ancestral character state reconstruction for each morphological character studied based on the maximum likelihood NJ tree inferred from Nei and Li distances from the AFLP data reconstructed with Mesquite v2.74.

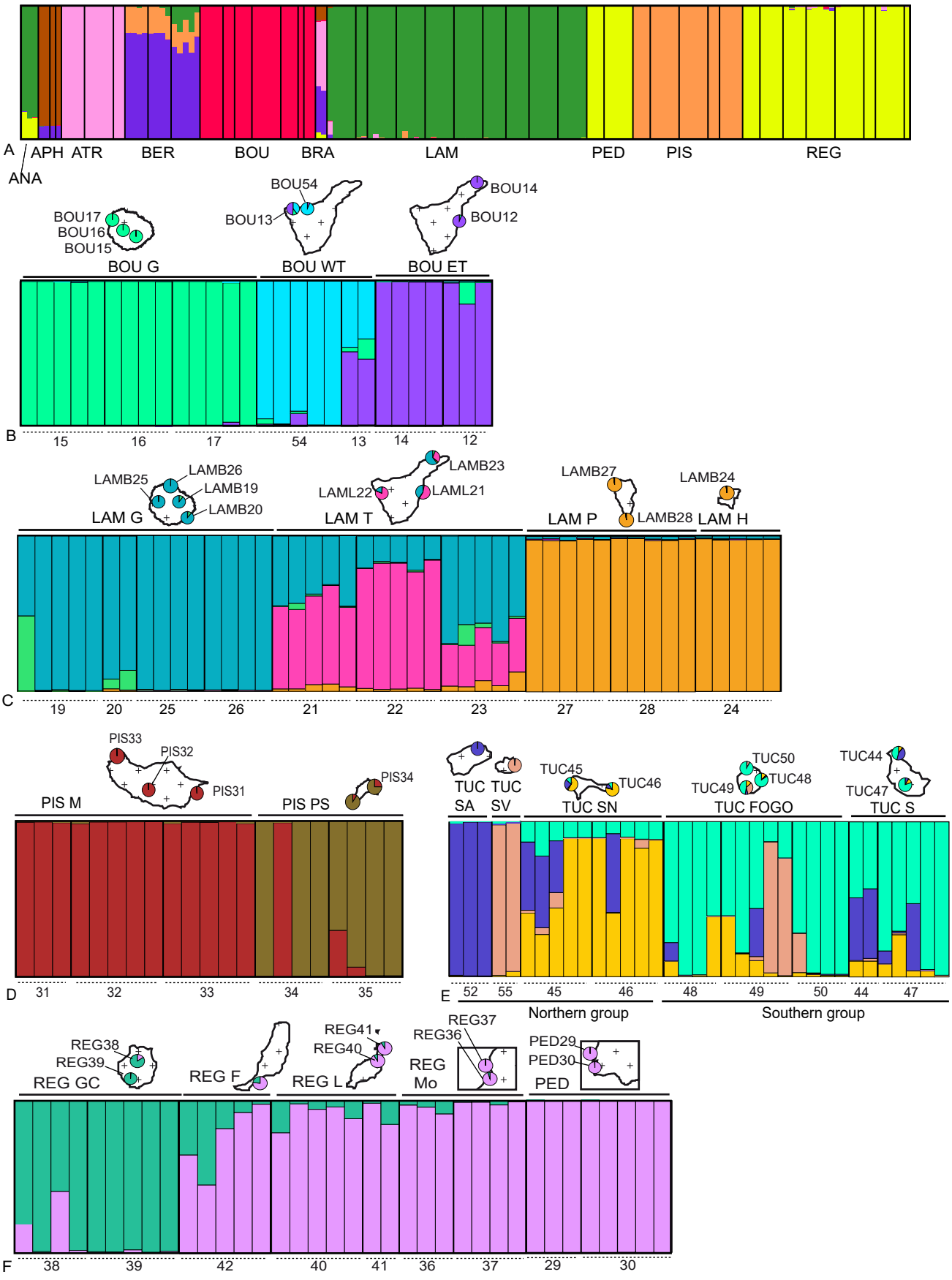
Fig. S1. Principal Coordinate Analysis of 346 AFLP markers for the whole dataset excluding *E. tuckeyana* based on Dice's similarity coefficient.

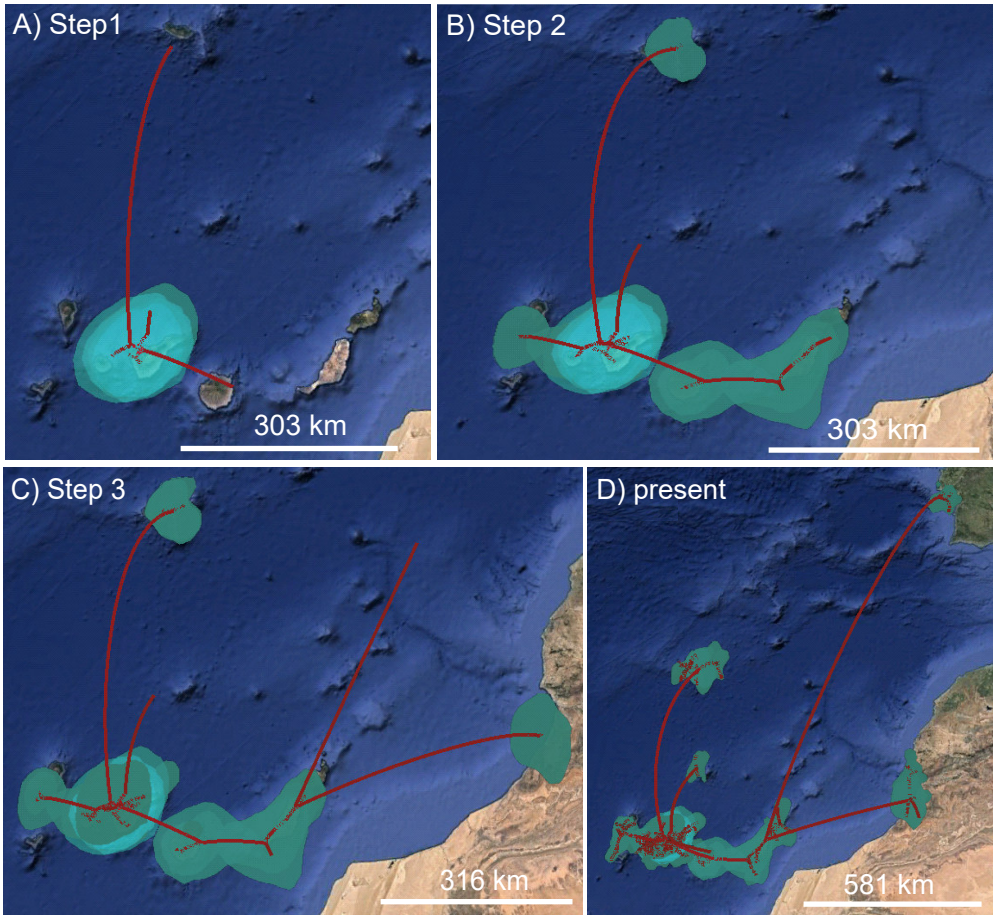
Fig. S2. Phylogenetic relationships among plastid DNA haplotypes of subsect. *Macaronesicae*, six species of subsect. *Africanae* and one species of sect. *Pachycladae* based on Bayesian inference. Bayesian posterior probabilities (≥ 0.50) and bootstrap values (≥ 50%) are indicated above branches.

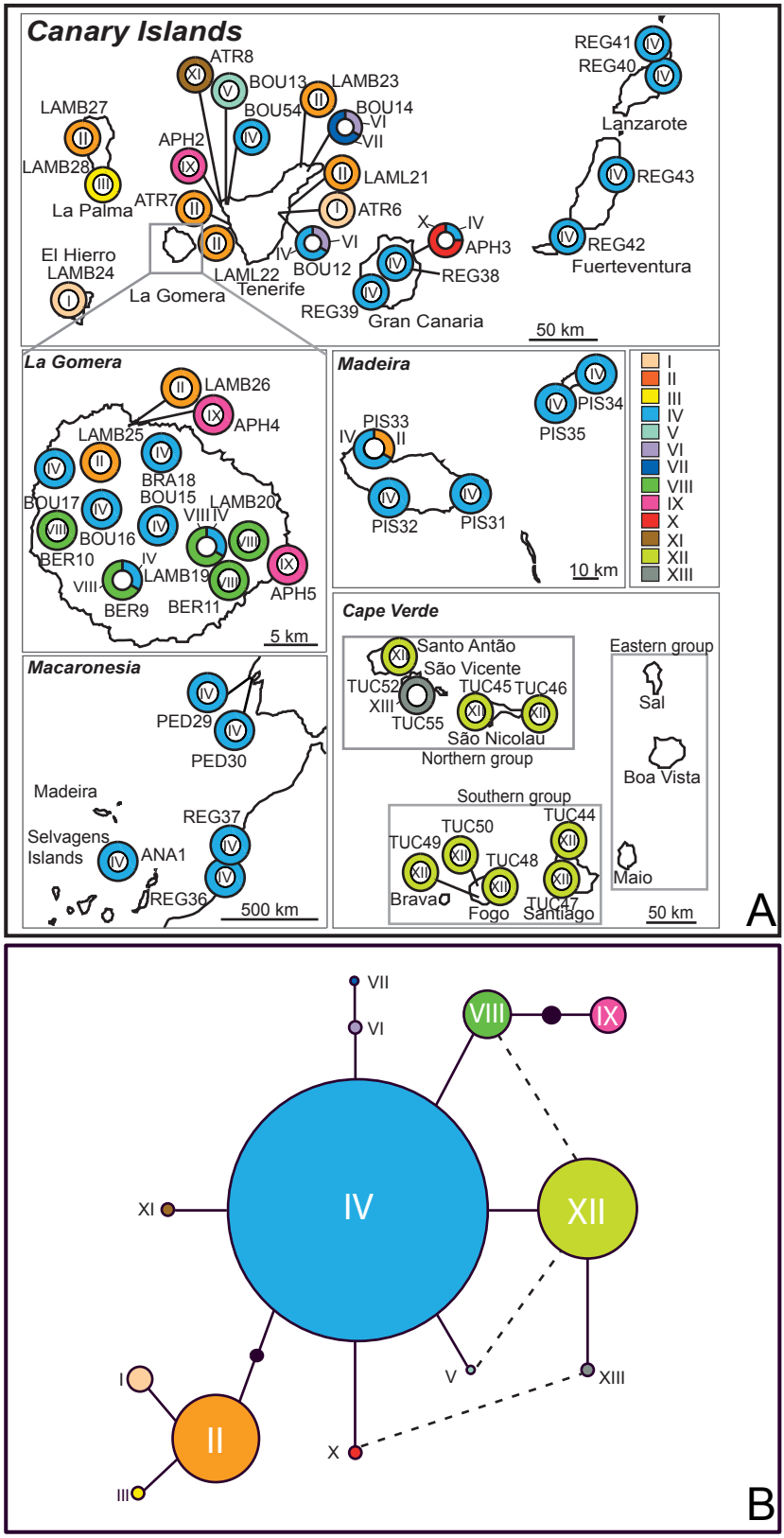

Macaronesia
Azores Islands
Madeira
Selvagens Islands
Canary Islands
Cape Verde Islands
PED29
PED30
ANA1
REG37
REG36
500 km
E. atropurpurea (ATR)
E. bourgaeana (BOU)
E. bravoana (BRA)
E. anachoreta (ANA)
E. berthelotii (BER)
E. lamarckii var. lamarckii (LAML)
E. lamarckii var. broussonetii (LAMB)
E. pedroi (PED)
E. piscatoria (PIS)
E. regis-jubae (REG)
E. aphylla (APH)
E. tuckeyana (TUC)
Canary Islands
ATR8
BOU13
LAMB23
BOU54
BOU14
LAMB27
APH2
LAMB28
La Palma
LAML21
ATR7
ATR6
BOU12
LAML22
APH3
La Gomera
Tenerife
REG38
LAMB24
REG39
El Hierro
Gran Canaria
REG41
REG40
Lanzarote
REG43
REG42
Fuerteventura
50 km
La Gomera (C. I.)
LAMB26
APH4
LAMB25
BRA18
BOU17
BOU16
LAMB19
LAMB20
BER10
BOU15
APH5
BER9
BER11
5 km
Madeira
PIS34
PIS35
PIS33
PIS32
PIS31
10 km
Cape Verde
Santo Antão
São Vicente
TUC52
TUC55
TUC45
TUC46
São Nicolau
Northern group
Eastern group
Sal
Boa Vista
Maio
Southern group
TUC47
TUC44
TUC50
TUC49
TUC48
Brava
Fogo
Santiago
50 km


E. lamarckii complex (95%)
0.01
BER (100%)
LAMB 24/27/28
LAMB 19/20/26/25
LAMB 23
LAML 21/22
ANA
PIS (100%)
ANA + LAM (100%)
TUC
REG + PED (100%)
REG 36/37/ 40/41/42/43
APH (100%)
BRA + ATR (100%)
PED
REG 38/39
BOU (100%)
ATR (100%)
BRA (100%)
BOU 54
BOU 15/16/17
BOU 12/13/14
E. atropurpurea complex (94%)

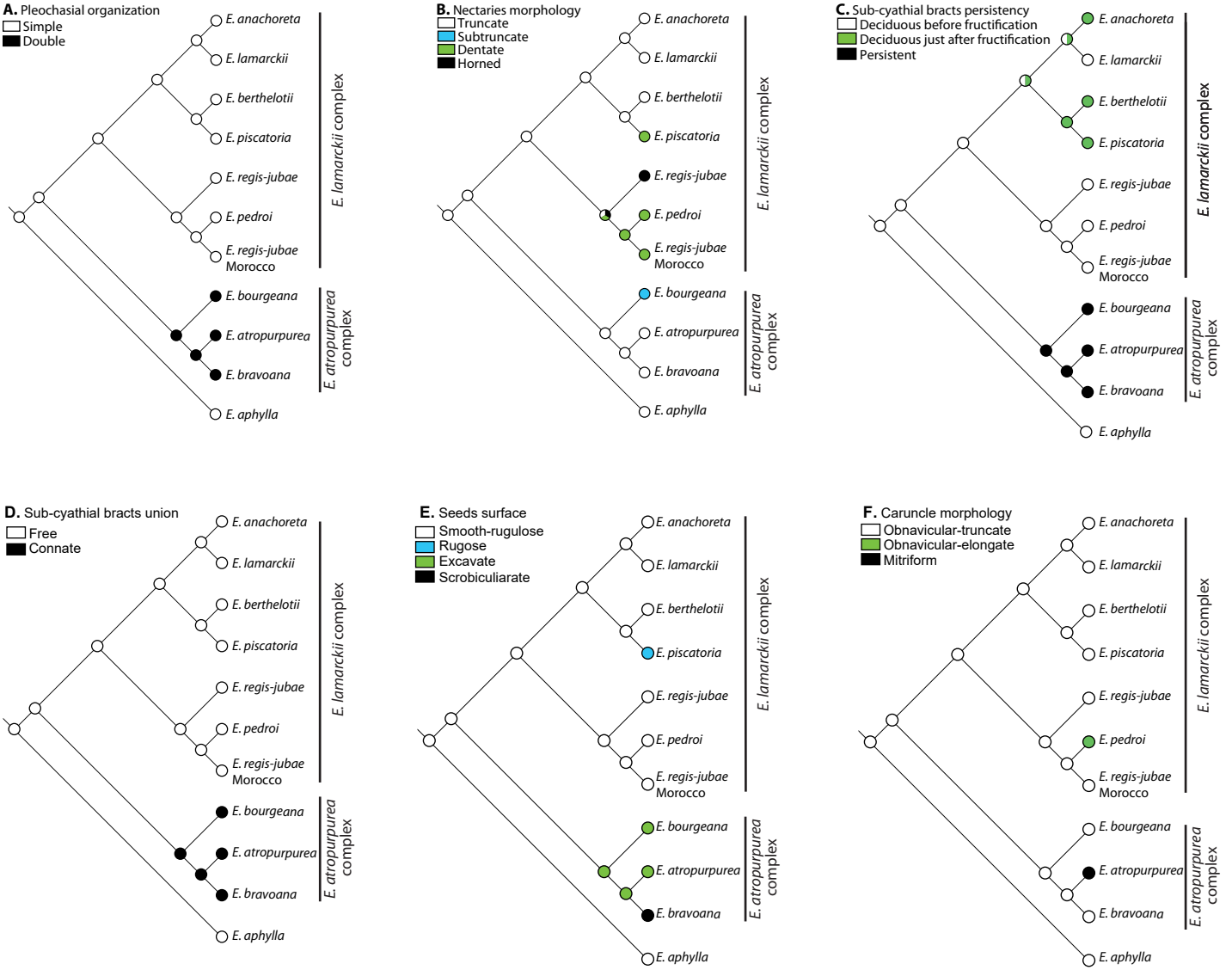

LAM
La Gomera
LAM
Tenerife
LAM
El Hierro
La Palma
(var. broussonetii)
LAM
Tenerife
(var. lamarckii)
ANA
BER
PIS
REG
PED
REG
ATR
BRA
BOU
La Gomera
BOU
Tenerife
APH
TUC
E. lamarckii complex
E. atropurpurea complex
0.1 substitutions/site

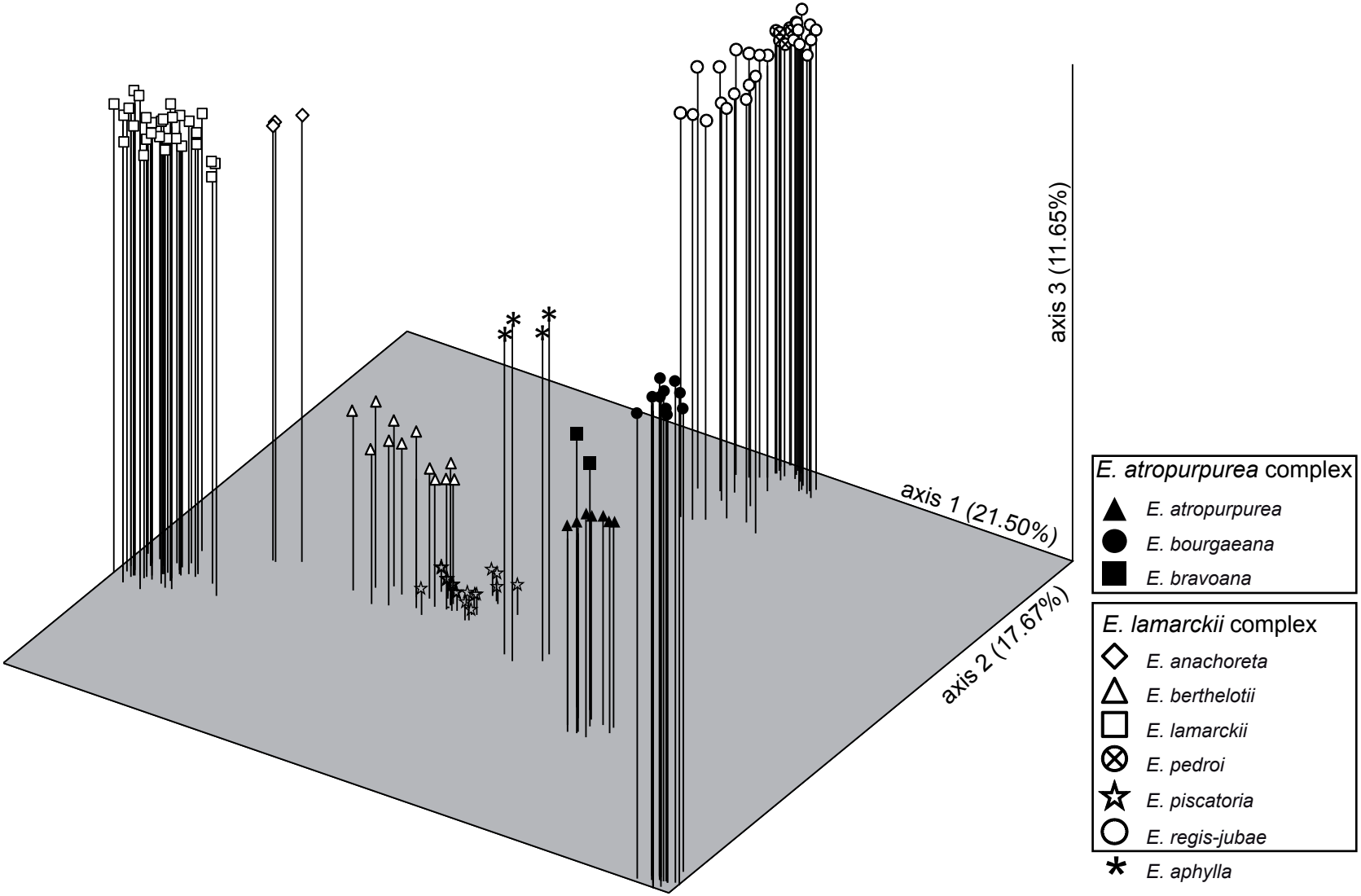

A
APH
ATR
BER
BOU
BRA
LAM
PED
PIS
REG
ANA
BOU17
BOU16
BOU15
BOU54
BOU13
BOU14
BOU12
BOU G
BOU WT
BOU ET
B
15
16
17
54
13
14
12
LAMB25
LAMB26
LAMB19
LAMB20
LAM G
LAML22
LAMB23
LAML21
LAM T
LAMB27
LAMB24
LAMB28
LAM P
LAM H
C
19
20
25
26
21
22
23
27
28
24
PIS33
PIS32
PIS31
PIS34
PIS M
PIS PS
D
31
32
33
34
35
TUC SA
TUC SV
TUC45
TUC46
TUC SN
TUC50
TUC49
TUC48
TUC FOGO
TUC44
TUC47
TUC S
E
52
55
45
46
48
49
50
44
47
Northern group
Southern group
REG38
REG39
REG GC
REG F
REG41
REG40
REG L
REG37
REG36
REG Mo
PED29
PED30
PED
F
38
39
42
40
41
36
37
29
30


A) Step1
303 km
B) Step 2
303 km
C) Step 3
316 km
D) present
581 km


Canary Islands
ATR8
BOU13
LAMB23
BOU54
BOU14
LAMB27
APH2
LAML21
LAMB28
La Palma
ATR7
ATR6
El Hierro
LAMB24
La Gomera
LAML22
Tenerife
BOU12
APH3
REG38
REG39
Gran Canaria
REG41
REG40
Lanzarote
REG43
REG42
Fuerteventura
50 km
La Gomera
LAMB26
APH4
LAMB25
BRA18
BOU17
BOU15
LAMB20
BOU16
BER10
LAMB19
APH5
BER9
BER11
5 km
Madeira
PIS33
PIS34
PIS35
PIS32
PIS31
10 km
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
Macaronesia
PED29
PED30
Madeira
REG37
Selvagens
Islands
ANA1
REG36
500 km
Cape Verde
Santo Antão
São Vicente
TUC52
TUC45
TUC46
XIII
TUC55
São Nicolau
Northern group
Eastern group
Sal
Boa Vista
Southern group
TUC44
TUC50
TUC49
TUC48
Brava
Fogo
TUC47
Santiago
Maio
50 km
A

VII
VI
VIII
IX
XI
IV
XII
V
XIII
I
II
X
III
B


A. Pleochasial organization
Simple
Double
B. Nectaries morphology
Truncate
Subtruncate
Dentate
Horned
C. Sub-cyathial bracts persistency
Deciduous before fructification
Deciduous just after fructification
Persistent
D. Sub-cyathial bracts union
Free
Connate
E. Seeds surface
Smooth-rugulose
Rugose
Excavate
Scrobiculiarate
F. Caruncle morphology
Obnavicular-truncate
Obnavicular-elongate
Mitriform
E. anachoreta
E. lamarckii
E. berthelotii
E. piscatoria
E. regis-jubae
E. pedroi
E. regis-jubae Morocco
E. bourgeana
E. atropurpurea
E. bravoana
E. aphylla
E. lamarckii complex
E. atropurpurea complex


axis 3 (11.65%)
axis 1 (21.50%)
axis 2 (17.67%)
E. atropurpurea complex
E. atropurpurea
E. bourgaeana
E. bravoana
E. lamarckii complex
E. anachoreta
E. berthelotii
E. lamarckii
E. pedroi
E. piscatoria
E. regis-jubae
E. aphylla

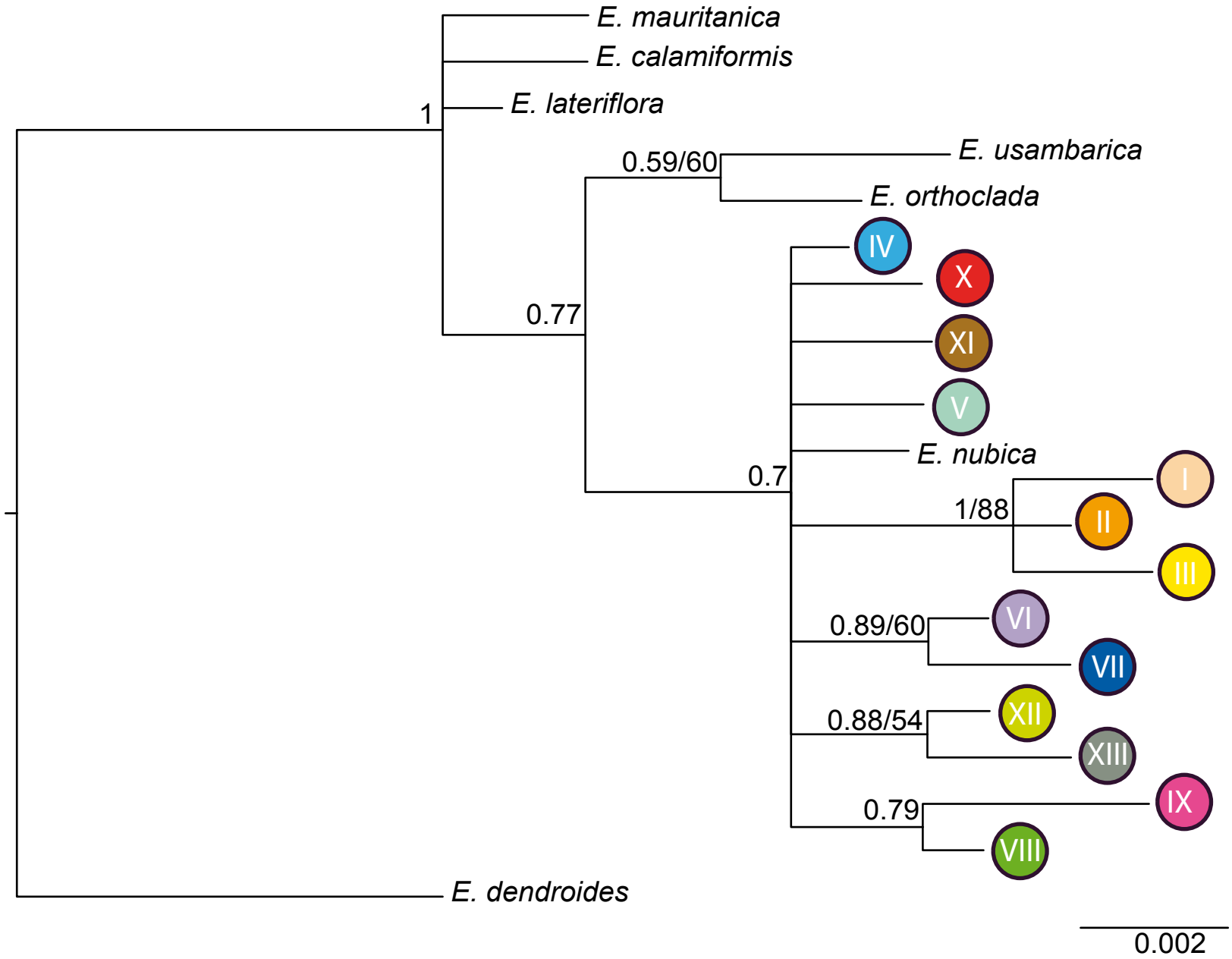

E. mauritanica
E. calamiformis
E. lateriflora
E. usambarica
E. orthoclada
E. nubica
E. dendroides
1
0.59/60
0.77
0.7
1/88
0.89/60
0.88/54
0.79
IV
X
XI
V
I
II
III
VI
VII
XII
XIII
IX
VIII
0.002

Table 1. Diagnostic morphological and ecological characters of the taxonomic complexes of *Euphorbia* sect. *Aphyllis* in Macaronesia (Molero & al., 2002).

	Leaves	**Synflorescence**	**Sub-cyathial bracts**	**Seeds**	**Ecology**
***E. atropurpurea* complex** including *E. atropurpurea*, *E. bourgaeana* and *E. bravoana*	Semi-persistent	Double	Large (10 – 20 mm) Connate Persistent	Excavate to scrobiculariate	Mesophilous and meso-hygrophilous habitats
***E. lamarckii* complex** including *E. anachoreta*, *E. berthelotii*, *E. lamarckii* var. *lamarckii*, *E. lamarckii* var. *broussonetti, E. pedroi*, *E. piscatoria*, *E. regis-jubae* and *E. tuckeyana*	Deciduous	Simple lax	Small (< 10 mm) Free to the base Deciduous	Smooth to rugose	Xerophilous and mesophilous habitats
E. aphylla	Absent	Simple congested	Small (< 2 mm) Free Deciduous	Smooth to rugulose	Xerophilous-halophilous habitats

Table 2. Taxa sampled, general distribution, population codes as shown in Fig. 1, localities, voucher number and number of individuals used for the study of AFLPs and plastid DNA haplotypes. N AFLP is the number of individuals used in the AFLP study, in parentheses is the number of individuals used in some Structure analyses including only three primer pairs. In the haplotypes column the number of individuals for each haplotype is indicated in parentheses.

Species	Distribution	Pop.	Locality, Collection and Voucher Number	N AFLP	Haplotypes
Euphorbia anachoreta Svent.	Selvagens Islands	ANA1	Portugal, Madeira, Ilhas Selvagens, *National Park s.n.* (BC)	3	IV (4)
Euphorbia aphylla Brouss. ex Willd	Gran Canaria, La Gomera and Tenerife (Canary Islands)	APH2	Spain, Canary Islands, Tenerife, Teno, Punta del Fraile, *Barres 74 & Vilatersana* (BC 873330)	2 (4)	IX (3)
		APH3	Spain, Canary Islands, Gran Canaria, La Isleta, close to the military installations, road Las Coloradas, *López-Pujol 9 & Caujapé-Castells* (BC 944367)	1 (2)	X (3), IV (1)
		APH4	Spain, Canary Islands, La Gomera, Vallehermoso, playa de Vallehermoso, *Barres 97 & Vilatersana* (BC 873340)	- (2)	IX (3)
		APH5	Spain, Canary Islands, La Gomera, San Sebastián de la Gomera, Puntallana Natural Reserve, Nuestra Señora de Guadalupe Chapel, *Barres 109 & Vilatersana* (BC 873348)	1 (3)	IX (3)
Euphorbia atropurpurea Brouss.	Tenerife (Canary Islands)	ATR6	Spain, Canary Islands, Tenerife, Güímar, old Güímar road (Güímar Viewpoint), *Barres 63 et al.* (BC 873324)	4	I (3)
		ATR7	Spain, Canary Islands, Tenerife, Guía de Isora, *Barres 66 & Vilatersana* (BC 873325)	5	II (3)
		ATR8	Spain, Canary Islands, Tenerife, Teno, Punta del Fraile, *Barres 75 et al.* (BC 873331)	2	XI (3)
Euphorbia berthelotii Bolle ex Boiss.	La Gomera (Canary Islands)	BER9	Spain, Canary Islands, La Gomera, road from El Pajarito to Alajeró, *Barres 99 & Vilatersana* (BC 873342)	4	IV (1), VIII (2)
		BER10	Spain, Canary Islands, La Gomera, Gran Rey Valley, near Cesar Manrique House, *Barres 112 & Vilatersana* (BC 873349)	4	VIII (3)
		BER11	Spain, Canary Islands, La Gomera, Santiago, between Sabinares turning and Santiago beach, *Barres 116 & Vilatersana* (BC 873351)	5	VIII (3)

Euphorbia bourgaeana J.Gay ex Boiss.	La Gomera and Tenerife (Canary Islands)	BOU12	Spain, Canary Islands, Tenerife, Güímar, Chamoco ravine, *Barres 61 et al.* (BC 873323)	4 (4)	IV (1), VI (2)
		BOU13	Spain, Canary Islands, Tenerife, Punta de Teno, El Charco ravine, *Barres 73 et al.* (BC 873329)	2 (2)	V (2)
		BOU14	Spain, Canary Islands, Tenerife, Anaga, Roque Negro, *Barres 78 et al.* (BC 873381)	3 (3)	VI (1), VII (2)
		BOU15	Spain, Canary Islands, La Gomera, Garajonay National Park, Los Noruegos, *Barres 94 et al.* (BC 873337)	5 (5)	IV (3)
		BOU16	Spain, Canary Islands, La Gomera, road from El Cercado to Las Hayas, *Barres 101 et al.* (BC 873344)	3 (4)	IV (3)
		BOU17	Spain, Canary Islands, La Gomera, Garajonay National Park, Chorros de Epina, *Barres 103 et al.* (BC 873346)	1 (5)	IV (3)
		BOU54	Spain, Canary Islands, Tenerife, Teno, Chajabe-Los Martínez, *Mesa et al. s.n.* (Personal Herbarium)	3 (5)	IV (3)
Euphorbia bravoana Svent.	Tenerife (Canary Islands)	BRA18	Spain, Canary Islands, La Gomera, road from Agulo to Las Rosas, *Barres 96 & Vilatersana.* (BC 873339)	2	IV (3)
Euphorbia lamarckii Sweet var. *lamarckii*	South Tenerife (Canary Islands)	LAM21	Spain, Canary Islands, Tenerife, Güímar, *Barres 54 & Vilatersana* (BC 873322)	5	II (3)
		LAM22	Spain, Canary Islands, Tenerife, Guía de Isora, *Barres 67 & Vilatersana* (BC 873326)	5	II (3)
Euphorbia lamarckii var. *broussonetii* (Willd. ex Link) Molero & Rovira	North Tenerife, La Gomera, La Palma and El Hierro (Canary Islands)	LAM19	Spain, Canary Islands, La Gomera, road from Hermigua to Las Casetas, Altos de Uteza, *Barres 110 & Vilatersana* (BC 873390)	5	IV (1), VIII (2)
		LAM20	Spain, Canary Islands, La Gomera, San Sebastián de la Gomera, Puntallana Natural Reserve, Riscos de Aluce, *Barres 115 & Vilatersana* (BC 873391)	2	VIII (2)
		LAM23	Spain, Canary Islands, Tenerife, Anaga, Punta de Hidalgo, *Barres 85 & Vilatersana* (BC 873387)	5	II (3)
		LAM24	Spain, Canary Islands, El Hierro, Frontera, Punta de la Dehesa, El Verodal beach, *Barres 86 & Vilatersana* (BC 873333)	5	I (3)
		LAM25	Spain, Canary Islands, La Gomera, between Epina and Vallehermoso, near Macayo, *Barres 113 & Vilatersana* (BC 873350)	4	II (3)
		LAM26	Spain, Canary Islands, La Gomera, Vallehermoso, *Barres 98 & Vilatersana* (BC 873341)	4	II (3)

		LAM27	Spain, Canary Islands, La Palma, Las Angustias ravine, Los Llanos, *Barres 119 et al.* (BC 873353)	5	II (3)
		LAM28	Spain, Canary Islands, La Palma, Fuencaliente, *Barres 124 et al.* (BC 873357)	5	III (3)
Euphorbia pedroi Molero & Rovira	Sesimbra Peninsula (Portugal)	PED29	Portugal, Sesimbra, Cabo Espichel, Chao dos Navegantes, *J. Molero 31/03/2010* (BCN 70795)	3	IV (3)
		PED30	Portugal, Sesimbra, Serra de Ares, between California beach and Cape Ares, *J. Molero 30/03/2010* (BCN 70791)	5	IV (3)
Euphorbia piscatoria Ait.	Madeira, Porto Santo & Desertas Islands	PIS31	Portugal, Madeira, Machico, Machico viewpoint, *Barres 126 et al.* (BC 873359)	3 (3)	IV (3)
		PIS32	Portugal, Madeira, Serra de Água, Pousada dos Vinhaticos, *Barres 130 et al.* (BC 873394)	5 (5)	IV (3)
		PIS33	Portugal, Madeira, Ribeira da Janela, *Barres 131 et al.* (BC 873395)	5 (5)	II (1), IV (2)
		PIS34	Portugal, Madeira, Porto Santo, Roche de Nosa Senhora, *Barres 159 et al.* (BC 873408)	2 (4)	IV (3)
		PIS35	Portugal, Madeira, Porto Santo, Pico Ana Ferreira south slope, *Barres 161 et al.* (BC 873374)	4 (4)	IV (3)
Euphorbia regis-jubae J. Gay	Fuerteventura, Lanzarote, Gran Canaria and west coast of Morocco	REG36	Morocco, road from Tiznit to Souk el Arba du Sahel, near Mirleft, *Barres 50 & López-Viñallonga* (BC 873320)	3	IV (3)
		REG37	Morocco, between Agadir and Essouira, Cape Ghir, *Barres 51 & López-Viñallonga* (BC 873321)	4	IV (3)
		REG38	Spain, Canary Islands, Gran Canaria, between Vega de San Mateo and Teror, *López-Pujol 1 & Caujapé-Castells* (BC 942715)	4	IV (3)
		REG39	Spain, Canary Islands, Gran Canaria, El Sao, Agaete Valley, *López-Pujol 2 & Caujapé-Castells* (BC 942850)	5	IV (3)
		REG40	Spain, Canary Islands, Lanzarote, Lomo de En medio, Los Valles, *López-Pujol 4 & Olangua* (BC 943147)	5	IV (3)
		REG41	Spain, Canary Islands, Lanzarote, Graciosa Island, between Agujas and Morro de las Pedreras, *López-Pujol 6 & Olangua* (BC 943764)	2	IV (2)
		REG42	Spain, Canary Islands, Fuerteventura, Jandía, Los Canarios ravine, *López-Pujol 7 & Olangua* (BC 943135)	5	IV (3)

		REG43	Spain, Canary Islands, Fuerteventura, La Asomada, *López-Pujol 8 & Olangua* (BC 943867)	1	IV (3)
Euphorbia tuckeyana Steud.	Boa Vista, Brava, Fogo, Sal, Santiago, Santo Antao, Sao Nicolau, Sao Vicente (Cape Verde)	TUC44	Cape Verde, Santiago, Sierra Malagueta, *Galbany-Casals 2100 & Molero* (BCN 67400)	2	XII (3)
		TUC45	Cape Verde, São Nicolau, between Barril and Praia Branca, Covadinha ravine, *Galbany-Casals 2104 & Molero* (BCN 67404)	5	XII (3)
		TUC46	Cape Verde, São Nicolau, Alto das Cabaças, *Galbany-Casals 2107 & Molero* (BCN 67407)	5	XII (3)
		TUC47	Cape Verde, Santiago, Pico de Antonia mountains, *Galbany-Casals 2121 & Molero* (BCN 67421)	5	XII (3)
		TUC48	Cape Verde, Fogo, between Achada Grande and Corvo, *Galbany-Casals 2125 & Molero* (BCN 67425)	4	XII (3)
		TUC49	Cape Verde, Fogo, Cha das Caldeiras, *Galbany-Casals 2128 & Molero* (BCN 67428)	5	XII (3)
		TUC50	Cape Verde, Fogo, Ribeira Felipe after Lomba, *Galbany-Casals 2133 & Molero* (BCN 67433)	4	XII (3)
		TUC52	Cape Verde, Santo Antâo, Cova, Agua das Caldeiras, *Molero s n. & Rovira* (BCN 58767)	3	XII (3)
		TUC55	Cape Verde, São Vicente, Monte Verde, *Molero s n. & Rovira* (BCN 58754)	2	XIII (3)

Table 3. Optimal number of K obtained with Structure v2.3.3. ΔK values are given for each *K* considered. Numbers in bold indicate the values for K chosen as best in the different analyses. Species name codes as in Fig. 1.

	AFLP dataset excluding *E. tuckeyana* (4 primer pairs)	**ANA + LAM (4 primers pairs)**	**APH (3 primer pairs)**	**ATR+BRA (3 primer pairs)**	**BOU (3 primer pairs)**	**LAM (4 primer pairs)**	**PIS (3 primer pairs)**	**REG + PED (4 primer pairs)**	**TUC (4 primer pairs)**
K = 1	**-**	**-**	**-**	-	**-**	**-**	**-**	**-**	**-**
K = 2	0.28	**622.13**	0.20	**243.82**	14.16	0.01	**167.68**	**291.47**	14.06
K = 3	1.37	1.16	0.14	1.42	**168.73**	1.26	1.86	19.86	0.69
K = 4	1.51	11.13	**0.50**	-	1.40	**122.81**	2.21	2.43	**24.8**
K = 5	0.08	1.02	**-**		1.44	0.97	**-**	1.24	0.03
K = 6	1.81	2.81			0.31	0.55		0.37	1.18
K = 7	**61.04**	20.41			**-**	0.31		0.18	1.04
K = 8	0.59	0.84				0.47		3.16	2.99
K = 9	4.26	0.17				**-**		0.38	0.07
K = 10	0.37							**-**	**-**
K = 11	4.55								
K = 12	0.58								
K = 13	6.15								
K = 14	9.96								
K = 15	**-**								

Table 4. Analyses of molecular variance (AMOVA) with Euclidean pairwise distances of AFLP markers, using 346 (4 primer pairs) or 249 (3 primer pairs) individuals. In all cases *P*-values of ϕ_{ST} are < 0.0001. d.f. = degrees of freedom.

		d.f.	Sum of squares	Variance components	% of variation	ϕ_{ST}
*E. aphylla**	Among populations	2	30.54	2.86	32.76	0.33
	Within populations	7	39.67	5.67	67.24	
E. berthelotii	Among populations	2	46.06	3.57	31.80	0.32
	Within populations	10	76.55	7.65	68.20	
*E. bourgaeana**	Among populations	6	141.83	5.37	69.28	0.69
	Within populations	21	50.07	2.38	30.72	
*E. bourgaeana** by structure groups	Among groups	2	105.65	4.97	54.49	0.74
	Among populations	4	36.17	1.77	19.38	
	Within populations	21	50.07	2.38	26.13	
*E. bourgaeana** by islands	Among groups	1	51.94	3.91	54.45	0.84
	Among populations	4	36.17	2.09	29.10	
	Within populations	17	20.07	1.18	16.45	
E. lamarckii	Among populations	9	242.12	4.43	38.53	0.38
	Within populations	35	247.30	7.06	61.47	
E. pedroi	Among populations	1	15.51	3.69	69.21	0.69
	Within populations	6	9.87	1.64	30.79	
E. piscatoria	Among populations	4	82.75	3.85	37.66	0.38
	Within populations	14	89.35	6.38	62.34	
E. regis-jubae	Among populations	7	169.01	4.33	33.08	0.33
	Within populations	21	184.17	8.77	66.92	
E. regis-jubae + *E. pedroi*	Among populations	9	244.72	5.49	43.30	0.43
	Within populations	27	194.03	7.19	56.70	
E. regis-jubae + *E. pedroi* by structure groups	Among groups	1	83.31	4.39	28.83	0.53
	Among populations	8	161.41	3.66	24.03	
	Within populations	27	194.03	7.19	47.14	
E. regis-jubae + *E. pedroi* by islands or mainland enclaves	Among groups	4	169.104	3.37	25.92	0.45
	Among populations	5	75.62	2.46	18.88	

	Within populations	27	194.03	7.18	55.20	
E. tuckeyana	Among populations	8	113.79	2.69	41.07	0.41
	Within populations	26	100.55	3.87	58.93	