Origin and diversification of the genus *Echium* (Boraginaceae) in the Cape Verde archipelago

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Abstract Much work has been done on the endemic floras of Madeira and the Canary Islands but little is known about the origin and diversification of endemic plants of the Cape Verde archipelago. In this study we focus on the three endemic species of the genus *Echium* L. (Boraginaceae) in Cape Verde. Phylogenetic analyses based on nuclear (ITS1, ITS2) and plastid (*trnT-L*, *trnL-F*, *rps16*, *psaI-accd*) DNA markers produced similar topologies that suggest a recent single colonization event in the Cape Verde archipelago, with subsequent diversification during the Pleistocene (<1.8 Ma). All datasets recovered a split between the "southern" (*E. hypertropicum*, *E. vulcanorum*) and "northern" (*E. stenosiphon* s.l.) island species, and suggest that habitat adaptation and inter-island colonization played a prominent role in the evolution of *Echium* in Cape Verde. Implications of the results are discussed in the context of the biogeography of the Macaronesian Islands. The northern island species are classified into three distinct subspecies, one for each of the northern islands: *E. stenosiphon* subsp. *stenosiphon* endemic to São Vicente, subsp. *lindbergii* endemic to Santo Antão, and subsp. *glabrescens* endemic to São Nicolau.

Keywords Cape Verde Islands; Macaronesia; phylogeny; systematics

■ INTRODUCTION

The Macaronesian region, comprising the archipelagos of the Azores, Madeira, Selvages, Canary, and Cape Verde Islands, is part of the Mediterranean hotspot, one of the 34 world biodiversity hotspots (Myers & al., 2000, and updates at http://www.biodiversityhotspots.org). The region is one of the most important floristic areas in terms of conservation within the Mediterranean (Médail & Quézel, 1997) and its flora exhibits many distinctive characteristics including a high degree of endemism (ca. 900 species) and a distinctive growth form spectrum with a high incidence of woodiness, particularly in the endemic floras of the Canary Islands (ca. 72%) and the Cape Verdes (ca. 63%) (Caujapé-Castells & al., 2010). The Macaronesian flora also includes several spectacular examples of evolutionary radiations that have long attracted attention (e.g., Kim & al., 1996; Francisco-Ortega & al., 2002; Mort & al., 2002; Barber & al., 2007).

The Cape Verde archipelago encompasses the southernmost islands of Macaronesia, and is located ca. 500 km west of Senegal (West Africa) and 1500 km south of the Canary Islands. This archipelago has nine main islands: Santo Antão, São Vicente, and São Nicolau constitute a northern group of islands; Santiago, Fogo, and Brava form a southern group; and Sal, Boavista, and Maio form an eastern group that is low in altitude. In keeping with the other Macaronesian archipelagos, the Cape Verde Islands are all volcanic and oceanic in origin. According to Patriat & Labails (2006), almost all of the emerging volume of the Macaronesian Islands was due to Tertiary volcanism, with the peak activity dating from the Middle Miocene. Published data for the Cape Verdes (e.g., Day & al., 1999; Scheidegger, 2002; Doucelance & al., 2003) are not always precise enough to provide a detailed chronology for the geological succession of islands, but there does appear to be an East to West decrease in age (Eastern Islands: ~25.6 and ~21.1 million years for Sal and Maio respectively; Western Islands: ~5.9 and ~7.57 million years for Brava and Santo Antão; Duarte & Romeiras, 2009). The Cape Verdes belong to the African region of semi-arid sahelian climate with only one to three months of humid climate (Brochmann & al., 1997). However, in the northeast-exposed slopes, the trade winds often cause fogs which are of considerable importance for the supply water to the natural vegetation, during the long dry season (Duarte & al., 2008).

The endemic flora of the Cape Verdes comprises 82 taxa (Brochmann & al., 1997) and, as already noted, most of the endemics are woody perennial species. Three of the twenty-seven Macaronesian endemic species of *Echium* L. (Boraginaceae) are endemic to the Cape Verde Islands (Bramwell, 1972), including two species on the southern islands: *E. hypertropicum* Webb on Santiago and the Brava islands, and *E. vulcanorum* A. Chev. in the volcanic mountain regions of Fogo. The third species, *E. stenosiphon* Webb, is found in the northern islands: São Nicolau, São Vicente, and Santo Antão.

Echium hypertropicum and *E. vulcanorum* (the two southern taxa) are shrubs up to 2.5 m high that display dense inflorescences, bearing flowers with five stamens exserted from the corolla tube. *Echium stenosiphon* is a sub-shrub up to 1.5 m high with lax inflorescences bearing flowers with only two exserted stamens (Romeiras & al., 2008).

Within the northern island species *Echium stenosiphon* there is considerable morphological variation. Pettersson (1960) actually recognized three different species, one on each of the three northern islands (*E. lindbergii* on Santo Antão, *E. glabrescens* on São Nicolau, *E. stenosiphon* on Sao Vicente). Bramwell (1972) recognised two subspecies, subsp. *lindbergii* (Pett.) Bramwell in the mountains of Santo Antão and subsp. *stenosiphon* on the semiarid coastal slopes of Santo Antão and on São Nicolau and São Vicente. Martins (1995) and Romeiras & al. (2008), finally, recognised only a single, broadly circumscribed and variable species, but noting differences in leaf indumentum and shape between plants on the three northern islands. A population genetic analysis based on RAPD data (Romeiras & al., 2007) also revealed low levels of gene flow between *E. stenosiphon* populations from São Nicolau, São Vicente, and Santo Antão, suggesting genetic isolation between the populations on each of the northern islands.

The relationships of the endemic *Echium* species of Cape Verde remain unclear. Molecular phylogenetic studies on Macaronesian *Echium* (Böhle & al., 1996 and Kim & al., 2008 based on the *trnT-L* spacer, *trnL-F* intron and spacer, and nrITS 1; García-Maroto & al., 2009 based on *D6DES-p*) suggested a single colonization of the region from a continental herbaceous ancestral species. Initial colonization and diversification occurred on the Canary Islands, followed by a secondary colonization of Madeira and the Cape Verde group. Low genetic differentiation was reported by Böhle & al. (1996), making it difficult to establish robust phylogenetic relationships among Macaronesian *Echium* species. However, using *D6DES-p*, García-Maroto & al. (2009) showed much higher levels of genetic variation and suggested that the three woody Cape Verde taxa form a well-supported clade, being sister to a clade of two annual herbaceous species (*E. bonnetii* Coincy, *E. pitardii* A. Chev.) endemic to the Canary Islands. Whilst supported by moderate posterior probability and bootstrap support values, this grouping is at odds with morphology which would suggest a closer relationship between the Cape Verdean species and perennial woody species from the Canary Islands.

This paper explores the relationships and evolution of Cape Verde *Echium* species using nrITS and plastid DNA (*trnT-L*, *trnL* intron, *trnL-F*, *rps16*, *psaI-accd*) sequences, complemented with previously published data on the morphology and ecology of the species (Romeiras & al., 2007, 2008). The aims of this paper are to (1) test the monophyly and infer the sister group of Cape Verde's endemic *Echium* species; (2) assess patterns of island colonization and diversification within the archipelago; (3) clarify the classification of the *E. stenosiphon* complex of the northern islands using molecular phylogenetic data.

■ MATERIALS AND METHODS

Taxon sampling. — A total of 27 accessions corresponding to 14 species of *Echium* were collected in Cape Verde (*E. hypertropicum*, *E. vulcanorum*, *E. stenosiphon* s.l.), Madeira (*E. candicans* L. f., *E. nervosum* Dryand), and Portugal (*E. lusitanicum* L.), or obtained from the Botanical Gardens of Madrid and Cordova (seven endemic Canary Island species). A representative voucher of each specimen was deposited in herbarium LISC. Sequence data of another 23 species (13 continental species and 10 Canary Island endemics) were obtained from GenBank (see Appendix). Five samples of *E. stenosiphon* were included from each island on which it occurs (São Nicolau, São Vicente, Santo Antão; see Appendix). For all other *Echium* species used in this study, a single accession was included in the analysis.

Molecular methods. — Total DNA was extracted from ~0.3 g of silica gel-dried leaf material using a modified CTAB method of Doyle & Doyle (1987) and purified, using QIAquick columns (Qiagen, Valencia, California, U.S.A.) according to the manufacturer's protocols. Polymerase chain reaction (PCR) amplifications, using 20–30 ng of genomic DNA, were performed to amplify the cpDNA regions. The $trnT_{UGU}$-$trnL_{UAA}$, $trnL_{UAA}$ intron, and $trnL_{UAA}$-$trnF_{GAA}$ regions were amplified, using primers described by Taberlet & al. (1991) (trnT a; trnL b; trnL c; trnL d; trnL e; trnF f). The *rps16* intron was amplified using the primers rps16-F/rps16-R (Oxelman & al., 1997) and the *psaI-accd* region was amplified using the primers accd-769F/psaI-75R (Grivet & al., 2001). To amplify the ITS region (ITS1, 5.8S rRNA, ITS2), the primers ITS4 and ITS5 were used as described by White & al. (1990).

PCR reactions were carried out, using a 2720 Thermal Cycler (Perkin-Elmer, Applied Biosystems, Foster City, California, U.S.A.) and performed in a final volume of 50 µl (2 µl of DNA, 50 pmol of each primer, 1.25 mM of each dNTPs, in a reaction buffer of 50 mM KCl, 20 mM Tris-HCl [pH 8.4], 2 mM $MgCl_2$ with 10 µg BSA and 1 unit *Taq* DNA polymerase [GibcoBRL]). PCR conditions were the following: an initial denaturation step of 94°C for 2 min, followed by 28 cycles consisting of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 3 min, and a final extension step of 72°C for 7 min. Amplification products were purified, using the QIAquick purification kit (Qiagen) following the manufacturer's protocols. An aliquot of 25–50 ng of purified DNA template was used with the BigDye Terminator v.3.1 Cycle Sequencing Kit components (Applied Biosystems), following the manufacturer's instructions.

Phylogenetic analyses. — Sequences were verified with BioEdit v.7.0.9 (Hall, 1999), and alignments were performed in ClustalX v.2.0.10 (Thompson & al., 1997) using default parameters. The sequences reported in this study are available from GenBank; accession numbers are provided in the Appendix. In order to clarify the relationships among the insular *Echium* species, two datasets were compiled. The first included sequences of 27 samples of *Echium* (26 from the Macaronesian region and the continental *E. lusitanicum* as outgroup) that were sampled and sequenced specifically for this study. The second, larger dataset included GenBank sequences and

comprised representatives of 37 taxa from Macaronesia and the Continent (see Appendix) with *E. russicum* J.F. Gmel., native in Russia and Western Asia, as outgroup. This dataset was used to infer wider phylogenetic relationships within Macaronesian *Echium* species.

Both datasets were analyzed under the same conditions after being tested with a chi-square (χ^2) test of homogeneity of base frequencies across the taxa. Three methods of phylogenetic inference were used: maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI). The trees generated by these methods were checked for congruence. The ML analyses were conducted with a heuristic search using a TBR algorithm option in PAUP* v.4.0.b10 (Swofford, 2002). Modeltest v.3.7 software (Posada & Crandall, 1998) associated with PAUP* was used to select the most appropriate evolutionary model for the different datasets, based on the Akaike information criterion. The most appropriate model for each case was then used to infer the ML tree (Felsenstein, 1988) with a heuristic search with 100 replicates of random-addition sequence and a tree-bisection-reconnection (TBR). This calculation involved the following parameters: a shape parameter of the gamma distribution used to set the relative size of four rate categories, the proportion of invariable sites, and the proportion of different types of transition and transversions (or the transition/transversion ratio in simpler models). The data was resampled 1000 times using the bootstrap technique to evaluate the robustness of the nodes of the phylogenetic trees. Parsimony analyses were conducted with a heuristic search using the TBR algorithm option in PAUP*. Initial trees were obtained via stepwise addition with 100 replicates of random-addition sequence. Ensemble indices—consistency index (Kluge & Farris, 1969), retention index (Farris, 1989), and homoplasy index (Archie, 1989)—were calculated to describe the homoplasy of the tree. The support of the recovered nodes was calculated using 1000 non-parametric bootstrap replicates (Felsenstein, 1985). Gaps were treated as missing data in all analyses. Bayesian phylogenetic analyses were performed using MrBayes v.3.1 (Ronquist & Huelsenbeck, 2003). Bayesian posterior probabilities (PP) were estimated by a Metropolis-Coupled Markov Chain Monte Carlo sampling algorithm (MCMCMC). The Markov Chain Monte Carlo (MCMC) procedure ensures that trees are sampled in proportion to their probability of occurrence under the given model of gene-sequence evolution while the MCMCMC approach ensures that the Markov chain does not become trapped in local optima. The conditions for the BI analysis were previously set up in relation to stationarity and model selection. We set up a total of 1.6×10^6 generations, so the likelihood scores of the trees would reach stationarity within the generation's time, and sampled every 100 generations with a "burn-in" of the first 5% of the trees. For each partition, the model selection was carried out with MrModeltest v.2.2 (Nylander, 2004) and implemented according to the authors' recommendations. A combined matrix of nrITS and of cpDNA datasets was constructed, and previously selected models were used to analyse the data (Nylander & al., 2004). Different partitions were allowed to evolve at different rates, with unlinked topology and unlinked parameters of the nucleotide models across all partitions. For each model, four runs were carried out using a different random starting seed to assess congruence of the likelihood values (Huelsenbeck & al., 2002). The model likelihood values for each of the analyses were compared and the best were used to determine topology, branch length, and clade robustness through the clade credibility values obtained from a 50% majority-rule consensus tree of the retained 95% of trees. Furthermore, to assess whether the sequences evolved in a clock-like manner, a likelihood ratio test (Huelsenbeck & Crandall, 1997) was performed. The log likelihood value of a tree with the same topology and evolutionary model was calculated with and without enforcing a molecular clock. Twice the difference between the likelihoods was then compared with a χ^2 distribution with $n - 2$ degrees of freedom, where n was the number of sequences used in the analysis (Huelsenbeck & Crandall, 1997).

For each of the two datasets and phylogenetic analyses, the nrITS and the cpDNA regions were analysed separately and combined analyses after testing for incongruence length difference test (ILD) (Farris & al., 1995; Cunningham, 1997), as implemented in PAUP* with all invariant characters removed. Concatenation and data preparation for the different analytical software's were carried out using Concatenator v1.1.0 (Pina-Martins & Paulo, 2008).

■ RESULTS

Phylogenetic reconstructions. — A fragment of 669 base pairs (bp) of nrITS, and of 2658 bp for the five cpDNA regions (*trnT-L*, *trnL* intron, *trnL-F*, *rps16*, *psaI-accd*) was sequenced for the 27 *Echium* specimens (corresponding to 14 taxa). For the large dataset of 37 *Echium* samples (including sequences obtained from GenBank) the length of the cpDNA regions (nrITS1, *trnT-L*, *trnL-F*) was ca. 1539 bp and that of the ITS1 region ca. 238 bp (Table 1).

Alignment of ITS and cpDNA sequences was relatively straightforward due to little length variation. Two ingroup indels for the ITS region and three ingroup indels for the cpDNA were removed from the analyses. Three different partitions of the two datasets were analysed: ITS only, cpDNA only, and a concatenated matrix of the two partitions.

Table 1 summarises the characteristics and evolutionary model selected for each combination of datasets analysed. The ILD test showed that the fragments were congruent for both combinations (ILD $P = 0.25$; ILD $P = 0.10$). The chi-square test of homogeneity of base frequencies across taxa showed no significant difference for the two concatenated datasets ($\chi^2 = 0.3590$, df = 78, $P = 1.00$; $\chi^2 = 5.4324$, df = 108, $P = 1.00$). For ITS and cpDNA the chi-square test of homogeneity of base frequencies across taxa showed no significant difference for the three datasets (ITS $\chi^2 = 0.8304$, df = 78, $P = 1.00$; cpDNA $\chi^2 = 0.2336$, df = 78, $P = 1.00$ [data from 27 *Echium* specimens]; ITS $\chi^2 = 12.3367$, df = 108, $P = 1.00$; cpDNA $\chi^2 = 2.8967$, df = 108, $P = 1.00$ [data from 37 *Echium* taxa]).

Only the concatenated trees are shown since they produced the most robust results. The Bayesian phylogenetic tree for the concatenated dataset of the 27 *Echium* samples sequenced for

this study is shown in Fig. 1. Two basal clades were resolved: one including *E. candicans* and *E. nervosum* from Madeira, and *E. aculeatum* Poir. from the Canary Islands; and the other comprising the remaining ingroup species from Macaronesia. The latter clade comprised a basal subclade with strong maximum likelihood bootstrap support, which included all but three of the Canary Islands species and a second subclade wherein *E. hierrense* Webb ex Bolle was sister to the well-supported Cape Verde clade, indicating a single colonization of this archipelago (Fig. 1).

The Cape Verde *Echium* species formed two clades, one distributed on the northern islands (*E. stenosiphon* accessions) and the other on the southern islands (86% PP, 85% MP BS, 100% ML BS; 98% PP, 99% MP BS, 100% ML BS, respectively). Within the *E. stenosiphon* clade, three subclades were resolved which corresponded to each of the islands on which this species occurs (Fig. 1). No variation was observed between *E. stenosiphon* species sampled from the same island.

When the results of the concatenated dataset were compared to the ITS or cpDNA results alone, a less resolved pattern was observed in the partitioned analyses. *Echium hierrense* was resolved as sister to the Cape Verde clade in the cpDNA analysis but not for the ITS analysis, where it was resolved as sister species to all other Macaronesian taxa (results not shown).

The Bayesian tree obtained for the large dataset of 37 *Echium* taxa is shown in Fig. 2. Inferred phylogenies from the BI, MP, and ML analyses of the combined datasets all produced the same overall topology. All analyses clearly supported a deep divergence between the continental and island species, with the continental Mediterranean species *E. parviflorum* Moench and *E. sabulicola* Pomel forming a sister clade to the Macaronesian species. Within this clade, basal relationships within the Macaronesian clade were not resolved and several Canary Island species appeared as basal, unresolved lineages. Species from Madeira and Cape Verde were resolved as derived groups and clustered with the Canary Island species. This general pattern was consistent across the inference methods, but with variable support (Fig. 2).

The monophyly of the Cape Verde *Echium* species and the relationships within the northern and southern clades in the expanded dataset (Fig. 2) were comparable to the other dataset that included fewer species sampled for more gene regions (Fig. 1). In the expanded dataset however, *E. hierrense* was resolved in a polytomy with the Cape Verdean clade and two other clades of Canarian taxa (Fig. 2).

Divergence times. — The likelihood ratio test showed no statistically significant difference between the log likelihoods of phylogenetic trees, with or without the molecular clock assumed for the concatenated smaller dataset ($\chi^2 = 21.85$, df = 13, $P > 0.05$) for the ITS ($\chi^2 = 19.44$, df = 13, $P > 0.05$), and for the cpDNA datasets ($\chi^2 = 9.81$, df = 13, $P > 0.05$). Based on the estimations of Richardson & al. (2001) and Koch & al. (2006)

Table 1. Variability and phylogenetic model details for each gene and gene combination analysed: fragment size in base pairs, numbers of variable and parsimony informative sites, number of maximum parsimony trees and their respective length, consistency index (CI), retention index (RI), Homoplasy index (HI), selected evolutionary model, shape parameter of the gamma distribution (Γ) proportion of invariable sites, and (I) individual substitution rates.

	Data from 27 *Echium* accessions			Data from 37 *Echium* accessions		
Sequence characteristics	cpDNA [*trnT-L*, *trnL* intron, *trnL-F*, *rps16*, *psaI-accd*]	ITS [ITS1, 5.8S rRNA, ITS2]	cpDNA+ITS [*trnT-L*, *trnL* intron, *trnL-F*, *rps16*, *psaI-accd*] + [ITS1, ITS2]	cpDNA [*trnT-L*, *trnL* intron, *trnL-F*]	ITS [ITS1]	cpDNA+ITS [*trnT-L*, *trnL* intron, *trnL-F*] + [ITS1]
Size [bp]	2658	669	3327	1539	238	1777
Variable sites	51	51	102	87	118	205
Informative sites	17	13	30	44	46	90
No. MP trees	1	9	10	165	30	4096
Tree length	53	55	159	95	167	279
CI	1	0.927	0.929	0.947	0.856	0.835
RI	1	0.826	0.871	0.976	0.902	0.899
HI	0.000	0.073	0.071	0.053	0.144	0.165
Model	TVM+I	GTR+I	TIM+I	TVM+G	SYM+G	GTR+G+I
Γ	–	–	–	0.2916	1.1081	0.8567
I	0.8143	0.6715	0.8735	0	0	0.5975
A-C	1.0718	1.1754	1.0000	1.5702	0.2303	0.7366
A-G	1.9047	1.9101	1.6646	1.5624	1.6238	1.5486
A-T	0.3213	1.3792	0.4912	0.5868	0.8736	0.4862
C-G	1.6428	0.1291	0.4912	2.3131	0.4123	1.4819
C-T	1.9047	3.7347	2.8418	1.5624	2.6615	2.2376

for the ITS rate of sequence evolution of $6.9 \pm 0.5 \times 10^{-9}$ substitutions/site/year and for cpDNA a rate of $2.2 \pm 0.1 \times 10^{-9}$ substitutions/site/year, the origin of the Cape Verde *Echium* species is estimated to have occurred during the Pleistocene (<1.8 Ma). Divergences between the two biogeographical groups (northern and southern taxa) fall within the interval 0.5–1.3 Ma. The diversification within the *E. stenosiphon* species complex (Northern Islands) as well as within the southern taxa is dated to the Middle Pleistocene (~0.3–0.5 Ma).

■ DISCUSSION

Origin of the Cape Verde endemic *Echium* species. — Despite the low level of sequence divergence found in our study, and reported also for other Macaronesian endemics (e.g., Caujapé-Castells & al., 1999; Percy & Cronk, 2002; Allan & al., 2004; Fairfield & al., 2004; Archibald & al., 2006), the phylogenetic analyses unequivocally place the Cape Verde endemic *Echium* species into one well-supported clade (Figs. 1–2). The

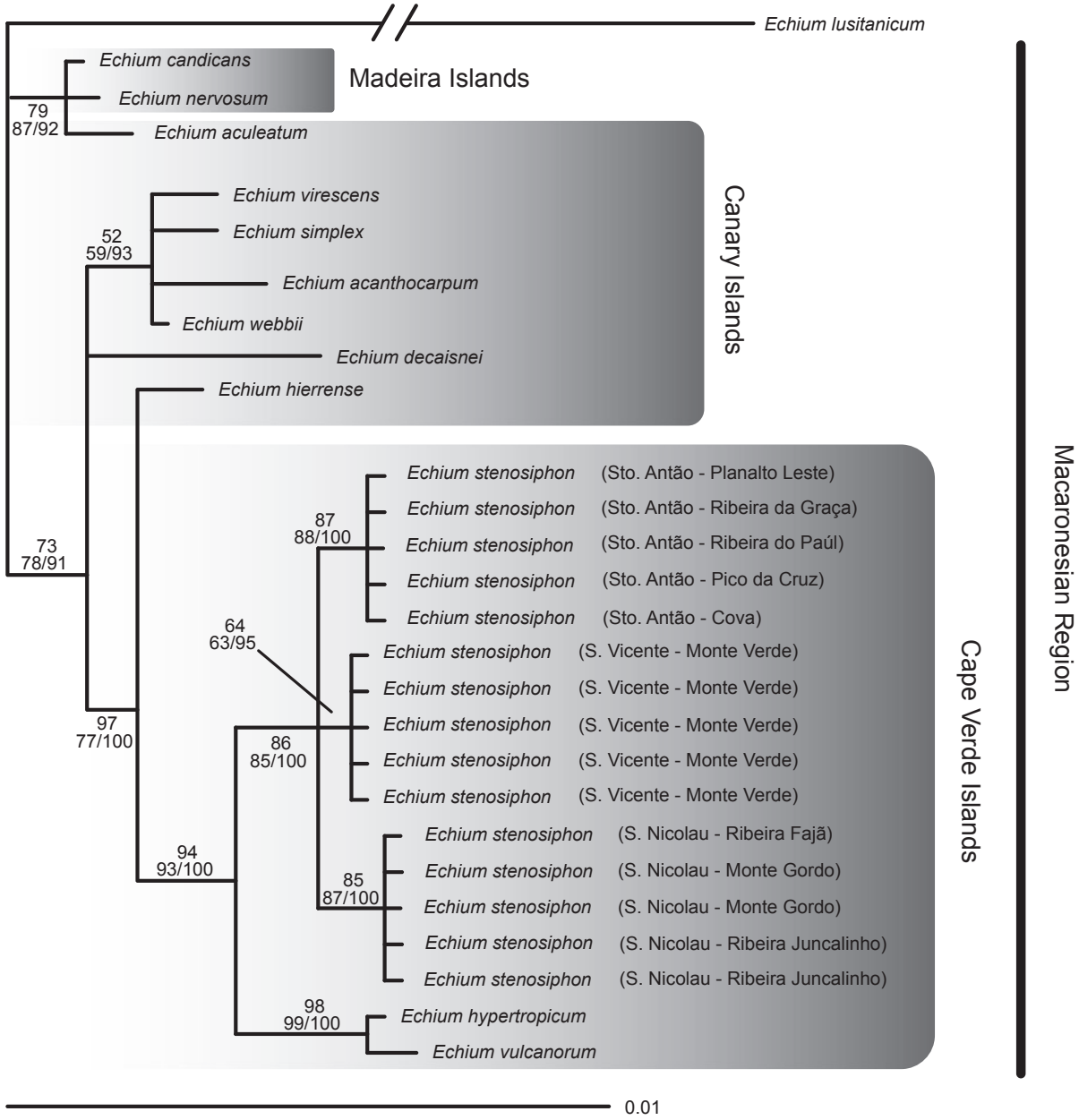

Fig. 1. Bayesian phylogenetic tree of 3327 bp of the concatenated dataset of ITS and cpDNA sequences of 27 *Echium* samples. Names in the terminal nodes refer to species listed in the Appendix. Main geographic divisions between Madeira, Canary and Cape Verde Islands are indicated. Bayesian posterior probability values (PP) are shown in the top value next to the corresponding branch, bootstrap values (BS) obtained from 1000 pseudo-replicates from maximum parsimony (left) and maximum likelihood (right) are below the posterior probability values.

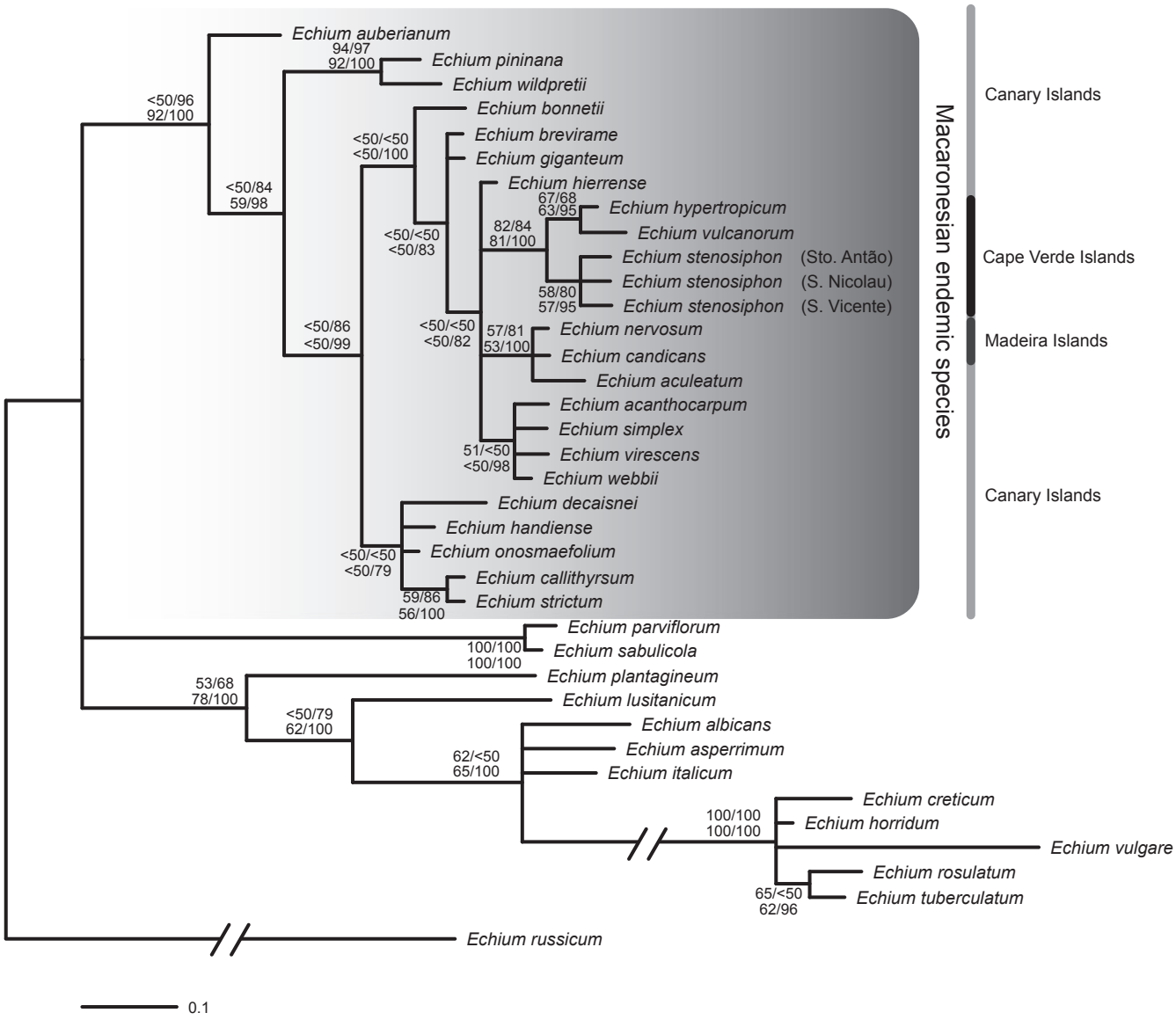

Fig. 2. Bayesian phylogenetic tree of 1977 bp of the concatenated dataset of ITS and cpDNA sequences of 37 *Echium* samples. For further explanation see Fig. 1.

similar topologies obtained from the ITS and cpDNA datasets strongly argue for monophyly of this genus in the Cape Verde archipelago and indicate that colonization of the archipelago probably occurred during the Pleistocene (< 1.8 Ma). Moreover, the Bayesian analyses of the cpDNA data suggest that *E. hierrense*, endemic to El Hierro, may share a common ancestor with the Cape Verde endemic species (Fig. 1). The latter result requires further confirmation due to incongruence of the cpDNA and ITS trees and the unresolved placement of *E. hierrense* and the Cape Verde clade in the more widely sampled analysis (Fig. 2). Close relationship between Cape Verde endemics and endemics from the western Canary Islands was also found in *Sonchus* (Lee & al., 2005) wherein *S. daltonii* Webb, the only endemic *Sonchus* species in the Cape Verde Islands, appeared to be closely related to *S. hierrensis* (Pit.) Boulos, *S. bornmuelleri* Pit., and *S. gandogeri* Pit. from La Palma and El Hierro.

The recent study of García-Maroto & al. (2009) agrees with the monophyly of the Cape Verde species but suggests a different sister-group relationship, involving *E. bonnetii* and *E. pitardii*. The widespread *E. bonnetii* is common in the eastern Canary Islands whereas *E. pitardii* is endemic to Lanzarote. *Echium bonnetii* and *E. pitardii* are both annual herbs, up to 25 cm. These two species were not analysed in our reduced dataset, but in the larger dataset *E. bonnetii* was resolved as sister to a group of Canary, Cape Verde, and Madeira species, albeit with weak support.

The *D6DES-p* region used by García-Maroto & al. (2009) is involved in the synthesis of polyunsaturated fatty acids, such as acid γ-linoleic (GLA, 18:3 n-6). A survey of the fatty acid

composition in *Echium* seeds revealed that the Macaronesian endemic species contain an unusually high level of GLA which could have represented a selective advantage during adaptation to the island's environments (Guil-Guerrero & al., 2003). Moreover, Gunstone (1992) concluded that ecological factors, such as soil type and temperature, can affect the contents of fatty acids (including GLA) in the seeds of Boraginaceae. The woody endemic Cape Verde taxa and the two annual Canarian herbaceous species (*E. bonnetii*, *E. pitardii*), occur in similar ecological conditions. Convergent mutations in *D6DES-p* driven by selection in response to similar environmental pressures in these two lineages cannot be ruled out and may explain the markedly different relationships for the Cape Verde species resolved using this marker.

Molecular clock dating suggests a recent origin for *Echium* in the Cape Verde archipelago. The endemic *Echium* species of the archipelago do not appear to be Tertiary relicts as postulated by authors such as Bramwell (1972) since the data suggest they arrived to Cape Verde Islands less than 1.8 Ma from a Canary Island ancestral *Echium* species. Our results corroborate recent findings of Kim & al. (2008), which suggested recent dispersals from Canary Islands to Madeira and Cape Verde (1.33 ± 1.05 Ma).

Patterns of island colonization and diversification in Cape Verde. — Whereas the analysis of García-Maroto & al. (2009) using *D6DES-p* placed the Cape Verde species in an unresolved polytomy, both datasets analysed in this paper (Figs. 1–2) recovered a split between the "southern" (*E. hypertropicum*, *E. vulcanorum*) and the "northern" (*E. stenosiphon* s.l.) species. This is consistent with the study of Romeiras & al. (2008), which recovered significant differences in habit and floral morphology between the southern and northern species. The results of molecular clock analysis suggest recent speciation in the archipelago with the divergence between the southern and the northern clades, falling within the interval of 0.5–1.3 Ma.

The congruence between gene trees, together with ecomorphological data, suggests the existence of two different patterns of diversification within the *Echium* species of Cape Verde. *Echium hypertropicum* and *E. vulcanorum* are sister taxa that differ in ecology, *E. hypertropicum* occurring up to 1000 m on Santiago on old volcanic soils and *E. vulcanorum* in Fogo between 1600–2400 m and preferring recent volcanic soils. This suggests that speciation of these two southern species was associated with adaptation to different habitats. In addition, given the allopatric distributions of these two sister species, geographic isolation may have played a role. Adaptive speciation has also been inferred for other plant groups from the Macaronesian region (e.g., Francisco-Ortega & al., 1996; Kim & al., 2008).

In the northern islands, *E. stenosiphon* s.l. appears to have diversified through the isolation of lineages following the colonization of similar ecological zones on different islands. All populations of *E. stenosiphon* exhibit broadly similar ecological preferences for range of altitude, exposition, and soil types. However, each island is reciprocally monophyletic in the analysis (Fig. 1), suggesting the absence of gene flow between plants on different islands. It thus appears that local adaptations to different habitats (in the southern taxa) as well as isolation following inter-island colonization between similar ecological zones (in the northern taxa) have contributed to the evolution of endemic diversity in *Echium* of Cape Verde. Inter-island colonization and isolation has also played an important role in the evolution of the endemic Canarian flora (e.g., *Adenocarpus* DC.: Fabaceae, Percy & Cronk, 2002; *Argyranthemum* Webb ex Sch. Bip.: Asteraceae, Francisco-Ortega & al., 1996; *Bystropogon* L. Hér.: Lamiaceae, Trusty & al., 2005; and *Lotus* L.: Fabaceae, Allan & al., 2004).

The biogeographic pattern found in *Echium*, with no taxa co-occurring on the same island of Cape Verde and clear morphological differences existing between the northern and southern species (Fig. 3), has also been reported for other endemic plant groups. For instance, the endemic genus *Diplotaxis* DC. shows complex patterns of morphological variation with some endemic species restricted to the northern islands and other species occurring only in the southern islands (Brochmann & al., 1997). *Diplotaxis varia* Rustan occurs in Santiago and Brava and resembles *D. hirta* Rustan & L. Borgen, which is restricted to the volcanic areas of Fogo Island, a similar pattern to that observed in the two southern *Echium* species. Phylogenetic analyses of other Cape Verde endemic plant groups should be carried to substantiate the findings of this study and to improve our understanding of the evolution and biogeography of the flora of the archipelago.

Taxonomy of the *Echium stenosiphon* complex in the northern islands. — The results presented in this paper highlight differences between populations of the taxonomically problematic *E. stenosiphon* from the islands of São Nicolau, São Vicente, and Santo Antão. These differences are consistent with the morphological differences observed by Pettersson (1960) and Romeiras & al. (2008), and support the recognition of distinct taxa on each of the three northern islands, treated here as subspecies: *E. stenosiphon* subsp. *stenosiphon* endemic to São Vicente, subsp. *lindbergii* endemic to Santo Antão, and subsp. *glabrescens* endemic to São Nicolau. The three subspecies can be easily distinguished based on indumentum characteristics (e.g., type, density, and distribution of pustular trichomes on adaxial and abaxial leaf surfaces) and leaf shape (Fig. 3C). The revised classification of *E. stenosiphon* s.l. is presented below.

Key to the subspecies of *Echium stenosiphon* of Cape Verde

1. Leaves lanceolate, pustular trichomes with long-pointed tips and a prominent base (3–4 rows of epidermal cells), abundant …… ***E. stenosiphon*** subsp. ***lindbergii***
1. Leaves ovate to elliptical, pustular trichomes with medium to short-pointed tips, scarce…… 2
2. Leaves mainly ovate, pustular trichome with a very large base (4–5 rows of epidermal cells), ± uniformly distributed ……***E. stenosiphon*** subsp. ***stenosiphon***
2. Leaves elliptical, pustular trichome with large base (4 rows of epidermal cells), adaxially ± uniform, abaxially only at veins …… ***E. stenosiphon*** subsp. ***glabrescens***

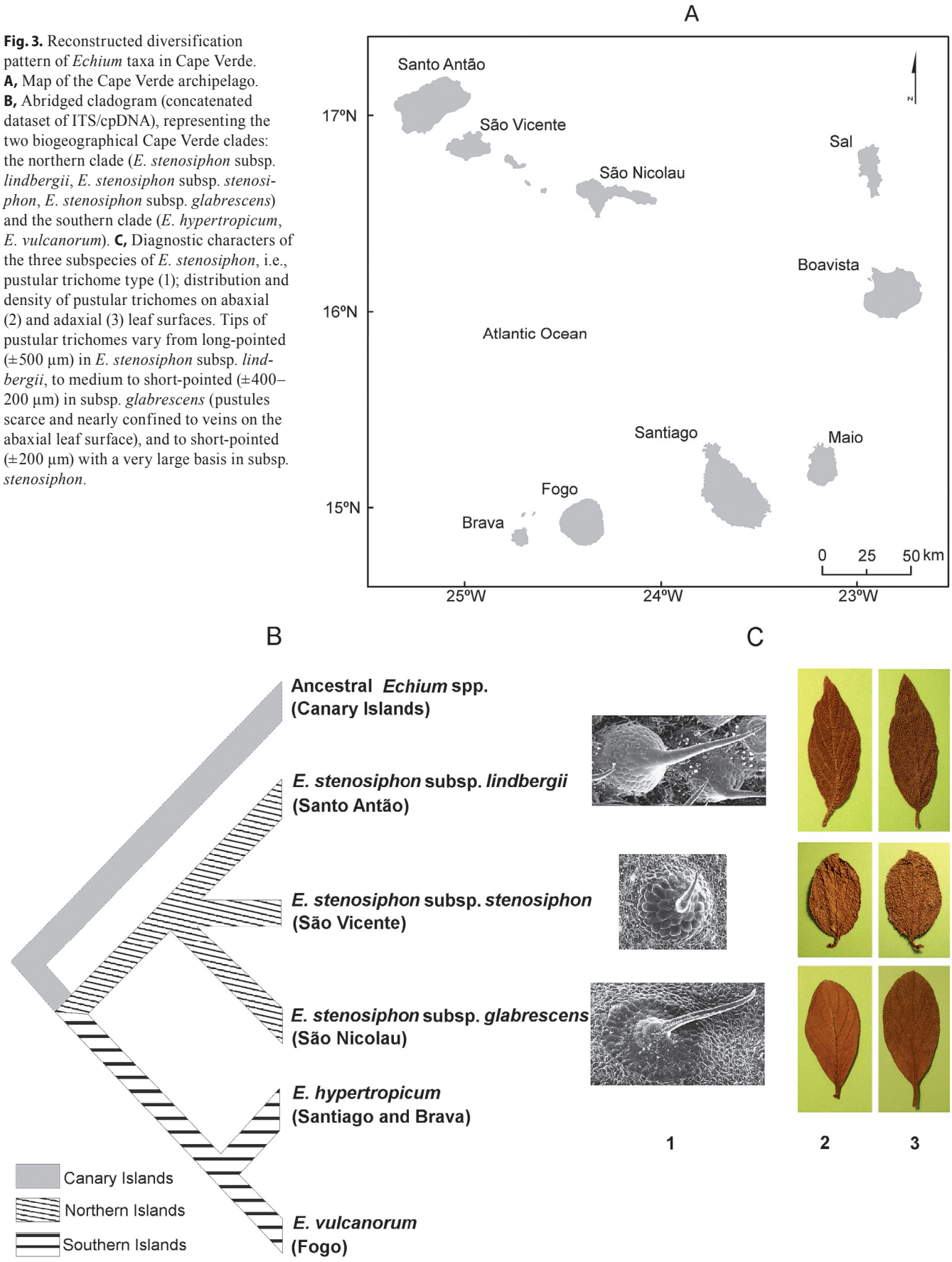

Fig. 3. Reconstructed diversification pattern of *Echium* taxa in Cape Verde. **A,** Map of the Cape Verde archipelago. **B,** Abridged cladogram (concatenated dataset of ITS/cpDNA), representing the two biogeographical Cape Verde clades: the northern clade (*E. stenosiphon* subsp. *lindbergii*, *E. stenosiphon* subsp. *stenosiphon*, *E. stenosiphon* subsp. *glabrescens*) and the southern clade (*E. hypertropicum*, *E. vulcanorum*). **C,** Diagnostic characters of the three subspecies of *E. stenosiphon*, i.e., pustular trichome type (1); distribution and density of pustular trichomes on abaxial (2) and adaxial (3) leaf surfaces. Tips of pustular trichomes vary from long-pointed (±500 µm) in *E. stenosiphon* subsp. *lindbergii*, to medium to short-pointed (±400–200 µm) in subsp. *glabrescens* (pustules scarce and nearly confined to veins on the abaxial leaf surface), and to short-pointed (±200 µm) with a very large basis in subsp. *stenosiphon*.

■ TAXONOMIC SYNOPSIS

Echium stenosiphon Webb in Hooker, Niger Fl.: 155, tab. XV. 1849 – Type: "in Monte Verde, ins. S. Vicentii ultra 1000 ped. alt., 06.1841", *Vogel 81* (lectotype: K photo!, designated by Bramwell, 1972[1]).

Echium stenosiphon subsp. ***stenosiphon***

Morphology. – Leaves ovate to elliptical; apex obtuse. Pustular trichomes, uniformly distributed on both leaf surfaces, but more numerous on the adaxial surface, with short-pointed tips (±200 µm) and very large basis, with 4–5 rows of epidermal cells involved in pustule formations.

Habitat and distribution. – São Vicente Island. This very rare species is found in the semi-arid elevational zones (500–750 m) of Monte Verde Mt., on the northern-eastern exposed slopes.

Nomenclatural remarks. – When Webb (1849) described *Echium stenosiphon*, he cited two syntypes, one from São Vicente (*Vogel 81*) and the other from São Nicolau (*Forbes 32*), both mounted on a single herbarium sheet kept in Royal Botanic Gardens Kew (K000419077 and K000419078, respectively; see http://plants.jstor.org/specimen/k000419078, accessed May 2011). Bramwell (1972) designated the right-hand specimen on the sheet, labelled "*Forbes 32*", as the lectotype of *E. stenosiphon*. However, based on the morphology of the two syntypes it appears that the two labels on the sheet were incorrectly applied. The left-hand specimen matches the São Nicolau endemic subsp. *glabrescens*, suggesting it was collected by Forbes on that island. The right-hand specimen (i.e., the lectotype), on the other hand, matches subsp. *stenosiphon* restricted to São Vicente and was most likely collected by Vogel, considered here to be the collector of the lectotype designated by Bramwell (1972).

Echium stenosiphon subsp. ***lindbergii*** (Pett.) Bramwell in Lagascalia 2(1): 97. 1972 ≡ *Echium lindbergii* Pett. in Commentat. Biol. 22(9): 36. 1960 – Type: Cape Verde, Santo Antão, Cova, 31.12.1953, *H. Lindberg s.n.* (holotype: H).

Morphology. – Leaves mainly lanceolate; apex acute. Adaxial surface of young leaves covered by a dense indumentum of simple (unicellular) and pustular trichomes. Conical unicellular trichomes, directly inserted on the epidermis, are especially abundant on young leaves. Pustular trichomes with prominent base and long-pointed tips (±500 µm), abundant on both leaf sides, tend to develop a hispid surface.

Habitat and distribution. – Santo Antão. This species occurs in different populations (e.g., Cova; Ribeira do Paul; Pico da Cruz) along the mountain slopes exposed to the trade winds, up to 1500 m, e.g., Pico da Cruz (*M. Romeiras & Matos 72*, LISC); sporadically it occurs at low altitudes (200–400 m) on the northern slopes.

Notes. – *Echium stenosiphon* subsp. *lindbergii* (Pett.) Bramwell, is restricted to the high altitudes (800–1400 m) of Santo Antão. It was described by Bramwell (1972) as having indumentum of small, white, pustular-based trichomes, calyx segments narrowly lanceolate and corolla shape more or less campanulate. However, in all studied specimens of *E. stenosiphon* the corolla shape was always narrowly infundibuliform to campanulate, excluding this characteristic in determining any intraspecific taxa. Specimens collected below 400 m on Santo Antão, and considered by Bramwell as *E. stenosiphon* subsp. *stenosiphon*, cannot be distinguished from specimens from higher altitude zones of this island, as all plants from the island show a consistent phenotype in trichome types, calyx and corolla shape. The plants described by Bramwell (1972) as *Echium stenosiphon* subsp. *stenosiphon* from Santo Antão fall well within the range of subsp. *lindbergii* and all plants of *E. stenosiphon* from Santo Antão are referable to subsp. *linbergii* as circumscribed here.

Echium stenosiphon subsp. ***glabrescens*** (Pett.) Romeiras & M.C. Duarte, **comb. et stat. nov.** ≡ *Echium glabrescens* Pett. in Commentat. Biol. 22(9): 39. 1960 – Type: Cape Verde, São Nicolau, Monte Gordo, 15.12.1953, *H. Lindberg s.n.* (holotype: H).

Morphology. – Leaves elliptical; apex acute to obtuse. Pustular trichomes with large basis, with 4 rows of epidermal cells, and medium to short-pointed tips (±400–200 µm). Pustular trichomes scarce and nearly confined to the lower surface of the veins.

Habitat and distribution. – São Nicolau. This species is found on northern/eastern mountain slopes in semi-arid to subhumid zones (600–1100 m) in Monte Gordo and Ribeira da Fajã; sporadically it occurs at low altitudes on the northern slopes of Ribeira do Juncalinho (*Romeiras & Matos* 53, LISC).

Notes. – The morphological distinction of this subspecies was first recognized by Pettersson (1960), who stressed the importance of the leaf indumentum (pustular trichomes scarce and nearly confined to the veins) for distinguishing the specimens from São Nicolau, described as *Echium glabrescens* Pett., from those of the other northern islands.

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1 Bramwell (1972) mistakenly referred to this specimen as "*Forbes 32, In insula S. Nicolai*" because of an apparent error in the labelling of plants on the herbarium sheet, as explained in the discussion.

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Appendix. List of *Echium* species sampled and GenBank accession numbers. Fresh leaves were collected in Cape Verde Islands, Madeira Island, and Portugal; leaves from Canary taxa were collected in the Botanical Garden of Córdoba (BG Córdoba) and in Royal Botanical Garden of Madrid (RBG Madrid), Spain. An asterisk indicates sequences obtained from GenBank, and published in Böhle & al. (1996); a dash indicates missing sequences.

Taxon: geographic origin; voucher information; GenBank accession numbers for *trnT-L*, *trnL intron*, *trnL-F*, *psaI-accd*, *rps16*, and ITS.

MACARONESIAN TAXA. ***Echium acanthocarpum*** Svent.: Canary Islands, La Gomera; *M. Romeiras s.n.* (BG Córdoba); EU433634, EU433604, EU433619, EU433574, EU433589, EU048853. ***E. aculeatum*** Poir.: Canary Islands, Tenerife; *M. Romeiras s.n.* (BG Córdoba); EU433630, EU433600, EU433615, EU433570, EU433585, EU048849. ***E. auberianum*** Webb & Berth.: Canary Islands; *L43177, *L43178, *L43179, –, –, *L43180. ***E. bonnetii*** Coincy: Canary Islands; *L43181, *L43182, *L43183, –, –, *L43184. ***E. brevirame*** Sprague & Hutch.: Canary Islands; *L43185, *L43186, *L43187, –, –, *L43188. ***E. callithyrsum*** Webb ex Bolle: Canary Islands; *L43193, *L43194, *L43195, –, –, *L43196. ***E. candicans*** L. f.: Madeira Island; *M. Romeiras 90* (LISC); EU433637, EU433607, EU433622, EU433577, EU433592, EU048856. ***E. decaisnei*** Webb & Berth.: Canary Islands, Gran Canaria; *M. Romeiras s.n.* (RBG Madrid); EU433633, EU433603, EU433618, EU433573, EU433588, EU048852. ***E. giganteum*** L. f.: Canary Islands; *L43221, *L43222, *L43223, –, –, *L43224. ***E. handiense*** Svent.: Canary Islands; *L43217, *L43218, *L43219, –, –, *L43220. ***E. hierrense*** Webb ex Bolle: Canary Islands, El Hierro; *M. Romeiras s.n.* (BG Córdoba); EU433629, EU433599, EU433614, EU433569, EU433584, EU048848. ***E. hypertropicum*** Webb: Cape Verde Islands, Santiago, Serra da Malagueta; *M. Romeiras 3* (LISC); EU433639, EU433609, EU433624, EU433579, EU433594, EU048858. ***E. nervosum*** Dryand.: Madeira Island; *M. Romeiras 91* (LISC); EU433636, EU433606, EU433621, EU433576, EU433591, EU048855. ***E. onosmifolium*** Webb & Berth.: Canary Islands; *L43257, *L43258, *L43259, –, –, *L43260. ***E. pininana*** Webb & Berth.: Canary Islands; *L43265, *L43266, *L43267, –, –, *L43268. ***E. simplex*** DC.: Canary Islands, Gran Canaria; *M. Romeiras s.n.* (BG Córdoba); EU433632, EU433602, EU433617, EU433572, EU433587, EU048851. ***E. stenosiphon*** subsp. ***glabrescens*** (Pett.) Romeiras & M.C. Duarte comb. et stat. nov.: Cape Verde Islands, São Nicolau, Monte Gordo; *M. Romeiras & Matos 42* (LISC); EU433640, EU433610, EU433625, EU433580, EU433595, EU048859. *E. stenosiphon* subsp. *glabrescens*: Cape Verde Islands, São Nicolau, Monte Gordo; *M. Romeiras & Matos 43* (LISC); JF284672, JF284648, JF284660, JF284624, JF284636, JF284612. *E. stenosiphon* subsp. *glabrescens*: Cape Verde Islands, São Nicolau, Ribeira Faja; *M. Romeiras & Matos 40* (LISC); JF284673, JF284649, JF284661, JF284625, JF284637, JF284613. *E. stenosiphon* subsp. *glabrescens*: Cape Verde Islands, São Nicolau, Ribeira Juncalinho; *M. Romeiras & Matos 51* (LISC); JF284674, JF284650, JF284662, JF284626, JF284638, JF284614. *E. stenosiphon* subsp. *glabrescens*: Cape Verde Islands, São Nicolau, Ribeira Juncalinho; *M. Romeiras & Matos 52* (LISC); JF284675, JF284651, JF284663, JF284627, JF284639, JF284615. ***E. stenosiphon*** subsp. ***lindbergii*** (Pett.) Bramwell: Cape Verde Islands, Santo Antão, Cova; *M. Romeiras & Matos 73* (LISC); EU433642, EU433612, EU433627, EU433582, EU433597, EU048861. *E. stenosiphon* subsp. *lindbergii*: Cape Verde Islands, Santo Antão, Planalto Leste; *M. Romeiras & Matos 63* (LISC); JF284680, JF284656, JF284668, JF284632, JF284644, JF284620. *E. stenosiphon* subsp. *lindbergii*: Cape Verde Islands, Santo Antão, Ribeira da Garça; *M. Romeiras & Matos 66* (LISC); JF284681, JF284657, JF284669, JF284633, JF284645, JF284621. *E. stenosiphon* subsp. *lindbergii*: Cape Verde Islands, Santo Antão, Ribeira do Paul; *M. Romeiras & Matos 69* (LISC); JF284682, JF284658, JF284670, JF284634, JF284646, JF284622. *E. stenosiphon* subsp. *lindbergii*: Cape Verde Islands, Santo Antão, Pico da Cruz; *M. Romeiras & Matos 72* (LISC); JF284683, JF284659, JF284671, JF284635, JF284647, JF284623. ***E. stenosiphon*** subsp. ***stenosiphon*** Webb: Cape Verde Islands, São Vicente, Monte Verde; *M. Romeiras & Matos 59* (LISC); EU433641, EU433611, EU433626, EU433581, EU433596, EU048860. *E. stenosiphon* subsp. *stenosiphon*: Cape Verde Islands, São Vicente Monte Verde; *M. Romeiras & Matos 55* (LISC); JF284676, JF284652, JF284664, JF284628, JF284640, JF284616. *E. stenosiphon* subsp. *stenosiphon*: Cape Verde Islands, São Vicente, Monte Verde; *M. Romeiras & Matos 56* (LISC); JF284677, JF284653, JF284665, JF284629, JF284641, JF284617. *E. stenosiphon* subsp. *stenosiphon*: Cape Verde Islands, São Vicente, Monte Verde; *M. Romeiras & Matos 57* (LISC); JF284678, JF284654, JF284666, JF284630, JF284642, JF284618. *E. stenosiphon* subsp. *stenosiphon*: Cape Verde Islands, São Vicente, Monte Verde; *M. Romeiras & Matos 58* (LISC); JF284679, JF284655, JF284667, JF284631, JF284643, JF284619. ***E. strictum*** L. f.: Canary Islands; *L43293, *L43290, *L43289, –, –, *L43292. ***E. virescens*** DC.: Canary Islands, Tenerife; *M. Romeiras s.n.* (BG Córdoba); EU433631, EU433601, EU433616, EU433571, EU433586, EU048850. ***E. vulcanorum*** A. Chev.: Cape Verde Islands, Fogo, Bordeira; *M. Romeiras 17* (LISC); EU433638, EU433608, EU433623, EU433578, EU433593, EU048857. ***E. webbii*** Coincy: Canary Islands, La Palma; *M. Romeiras s.n.* (BG Córdoba); EU433635, EU433605, EU433620, EU433575, EU433590, EU048854. ***E. wildpretii*** H. Pearson ex Hook. f.: Canary Islands; *L43313, *L43314, *L43315, –, –, *L43316. **CONTINENTAL TAXA.** ***Echium albicans*** Lag. & Rodr.: *L43168, *L43170, *L43171, –, –, *L43172. ***E. asperrimum*** Lam.: *L43173, *L43174, *L43175, –, –, *L43176. ***E. creticum*** L.: *L43205, *L43206, *L43207, –, –, *L43208; ***E. horridum*** Batt.: *L43225, *L43226, *L43227, –, –, *L43228. ***E. italicum*** L.: *L43233, *L43234, *L43235, –, –, *L43236. ***E. lusitanicum*** L.: Portugal, Vila Real, *M. Romeiras 92* (LISC); EU433628, EU433598, EU433613, EU433568, EU433583, EU048847. ***E. parviflorum*** Moench: *L43261, *L43262, *L43263, –, –, *L43264. ***E. plantagineum*** L.: *L43269, *L43270, *L43271, –, –, *L43272. ***E. rosulatum*** Lange: *L43273, *L43274, *L43275, –, –, *L43276. ***E. russicum*** J.F. Gmel.: *L43277, *L43278, *L43279, –, –, *L43280. ***E. sabulicola*** Pomel: *L43285, *L43286, *L43287, –, –, *L43288. ***E. tuberculatum*** Hoffmanns. & Link: *L43297, *L43298, *L43299, –, –, *L43300. ***E. vulgare*** L.: *L43311, *L43310, *L43309, –, –, *L43312.