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The relevance of Evolutionary Significant Units for the conservation of island-restricted reptiles: *Tarentola boettgeri bischoffi* as a case study

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Abstract. Within vertebrates, reptiles are good island colonisers, often leading to considerable levels of intraspecific diversity among populations inhabiting different islands/archipelagos. This study explores the mitochondrial phylogeographic structure of *Tarentola boettgeri*, a gecko species endemic to the Macaronesian archipelagos of Selvagens and the Canary Islands. Our research introduces a novel monophyletic group, comprising the populations from the islands of Selvagem Pequena and Ilhéu de Fora. Furthermore, we confirm the previously identified genetic clusters associated with Selvagem Grande, Gran Canaria and El Hierro. We estimate that the origin of *T. boettgeri* dates to the upper Miocene (ca. 6.4 Mya), and that the separation of *T. boettgeri bischoffi* on Selvagem Grande, Selvagem Pequena, and Ilhéu de Fora, occurred ca. 0.5 Mya. The absence of genetic differences between Selvagem Pequena and Ilhéu de Fora suggests recent gene flow or founder events, possibly facilitated by land connections during major glaciations. Conversely, the geographic barriers between Selvagem Grande and Selvagem Pequena likely persisted, preventing genetic admixing. The significant genetic distances observed among all populations underscore the necessity of an integrative taxonomic revision for *T. boettgeri*. In light of our findings, and with particular consideration of the small population sizes of *T. boettgeri bischoffi* on Selvagem Pequena and Ilhéu de Fora, we propose that the identified monophyletic groups should be managed as Evolutionarily Significant Units (ESUs). Accordingly, our study highlights the importance of recognizing ESUs in island-restricted reptile populations for targeted conservation efforts, especially given their unique intraspecific diversity and the vulnerability of their habitats.

Keywords: geckos, Macaronesia, mtDNA, oceanic islands, phylogenetic analysis, Selvagens archipelago.

Introduction

The human-induced defaunation wave swiping the planet is comparable in extent and magnitude to prehistoric mass extinctions (Dirzo et al., 2014). Extinction rates are now orders of magnitude above pre-human background levels, and this defaunation crisis is compounded by widespread declines in species abundance

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and extinctions of local populations (De Vos et al., 2015). As the conservation of intraspecific diversity critically maximises the adaptive and evolutionary potential of extant taxa (Crozier, 1997; Reed and Frankham, 2003; Jump et al., 2009; Bruford et al., 2017), the identification of conservation management units with distinct evolutionary histories is key for the efficient allocation of limited conservation resources.

Evolutionarily Significant Units (ESUs) – sets of populations with unique morphological/genetic features that separate them from similar populations or sets of populations – were devised to offer a unit for conservation that goes beyond traditional taxonomic classifications and allows for the identification of "significant" or adaptive molecular and ecological divergence (Ryder, 1986; Crandall et al., 2000). The concept has undergone substantial modifications since its development, resulting in the formulation of multiple definitions over time (see e.g., Hoelzel, 2023). Yet, a key feature of ESUs is the preservation of the ecological sustainability of populations and the underlying evolutionary processes in conservation strategies (Moritz, 1999). This entails safeguarding crucial genetic components within a species to ensure that the evolutionary process remains unhindered (Waples, 1995). Furthermore, ESUs increasingly acknowledge that both adaptive divergence and historical geographic isolation should be considered, each representing distinct points along a spectrum, reflecting the varying impacts of evolutionary forces in generating genetic differentiation (Hoelzel, 2023).

In small, isolated populations, as commonly observed on islands, the interplay between genetic and demographic factors significantly amplifies extinction risk (Frankham, 1997). Islands are frequently subject to disruptive events like droughts or volcanic activity, which can further exacerbate populational bottlenecks (Whittaker and Fernández-Palacios, 2007). Furthermore, invasive species frequently lead to ecological harm, population decline or even extinction of native species in island ecosystems (Bellard et al., 2017). Consequently, the assessment and conservation of unique island populations and their genetic variability, encompassing all ESUs, hold notable importance.

The Selvagens Islands are a small Portuguese archipelago in the North Atlantic Ocean, 280 km south of Madeira and 165 km north of the Canary Islands (fig. 1A). The archipelago is composed of multiple islets and three main islands: Selvagem Grande (4.5 km^2; maximum altitude of 154 m a.s.l.), Selvagem Pequena (20 ha; maximum altitude of 49 m a.s.l.), and Ilhéu de Fora (8 ha, maximum altitude of 18 m a.s.l.). Selvagem Pequena and Ilhéu de Fora are located ca. 15 km southwest of Selvagem Grande and are ca. 1.5 km apart. The archipelago has terrestrial formations more than 29 million years old (Geldmacher et al., 2001), making these islands the oldest in Macaronesia (Mateo et al., 2023). The age of this archipelago, along its proximity to currently submerged but previously emerged archipelagos during the glaciations which are closer to the African coast, suggests that it may have acted as a potential gateway for the colonisation of other Macaronesian islands (Rebelo, 2008).

The herpetofauna of Selvagens is restricted to one lacertid, the Madeiran wall lizard *Teira dugesii selvagensis*, and one gekkonid, the Boettger's wall gecko *Tarentola boettgeri bischoffi*, both considered endemic subspecies and present in the three main islands of the archipelago (Oliveira et al., 2005; Penado et al., 2015). However, the dynamics of colonisation and biogeography within the two species remain unresearched in the case of *Teira dugesii* and contentious and undetermined in the case of *Tarentola boettgeri*.

Boettger's wall gecko (*T. b. bischoffi*) from Selvagens, also locally knowns as Selvagens gecko, was initially proposed as a full species by Joger (1984) based on immunological and biochemical data. Yet, although its phylogeography is still under debate, the latest molecular results suggest that it should instead be

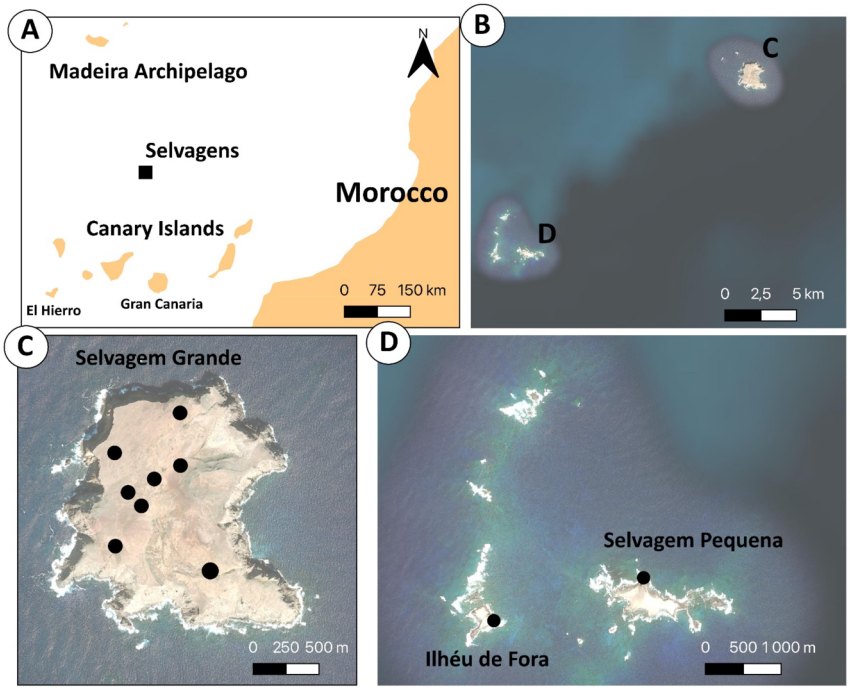

Figure 1. Maps depicting (A) the geographic location of the Selvagens Archipelago within Macaronesia and highlighting the islands of Gran Canaria and El Hierro, part of the Canary Islands; and (B) the organisation of the Selvagens Archipelago. The black dots denote the sampling localities in (C) Selvagem Grande, and (D) Selvagem Pequena and Ilhéu de Fora.

considered a subspecies of *T. boettgeri* (Carranza et al., 2000). Apart from the Selvagens, *T. boettgeri* is found in two islands of the Canary archipelago: Gran Canaria (the archipelago's second nearest island to Selvagens, possibly above sea level since the middle Miocene, ca. 14 million ago) and El Hierro (the youngest island of the Canary Islands, originated in the Pleistocene, ca. 1.7 million years ago, and the more distant of the Canarian islands to Selvagens). Carranza et al. (2000) corroborated previous findings by Nogales et al. (1998), demonstrating that the populations of El Hierro (*T. b. hierrensis*) are more closely related to individuals from Selvagem Grande (*T. b. bischoffi*) than to conspecifics from Gran Canaria (*T. b. boettgeri*). Considering that El Hierro is much younger than Gran Canaria and Selvagens (Jérémine, 1950; Carracedo, 1984), Nogales et al. (1998) suggested that the most parsimonious explanation was that El Hierro was colonized by geckos from the Selvagens. Yet, Carranza et al. (2000) found that two specimens of *T. b. boettgeri* from the south-west of Gran Canaria were more closely related to *T. b. hierrensis* and *T. b. bischoffi* than to another individual from the north-east of the island. Moreover, Gübitz et al. (2005) also found that *T. b. bischoffi* clustered with the haplotypes of *T. b. boettgeri* from North-western Gran Canaria. Hence, the recognition of *T. b. bischoffi* as a full species would render *T. boettgeri* as paraphyletic. So, these authors proposed as most appropriate to regard all three forms as subspecies of *T. boettgeri* (Carranza et al., 2000). These findings pose a challenge regarding the most likely colonisation route of the ancestor of *T. boettgeri* from the mainland to Macaronesia; one hypothesis is that *T. boettgeri* from Gran Canaria colonised Selvagens and from here dispersed to El Hierro; an alternative scenario considers that both Gran Canaria and Selvagens were colonised via Madeira archipelago at different

times, with the ancestor of *T. b. hierrensis* coming from the Selvagens (Carranza et al., 2000).

The phylogenetic position of *T. b. bischoffi* derived from the previous molecular studies was based on three mitochondrial genetic fragments (12S and two cytb fragments), using two and three specimens from Selvagem Grande. In this study, apart from an increased number of individuals from Selvagem Grande, we also added specimens from Selvagem Pequena and Ilhéu de Fora. Hence, our primary goals are to (i) reassess the phylogenetic relationships between all subspecies of *T. boettgeri*; (ii) quantify the genetic diversity and isolation among the three populations of Selvagens; and (iii) determine whether they constitute different evolutionary significant units, warranting different conservation management.

Material and methods

Sampling and laboratory procedures

A total of 22 *T. boettgeri bischoffi* were collected in May 2023 from the three islands of the Selvagens, namely Selvagem Grande (N = 8), Selvagem Pequena (N = 7) and Ilhéu de Fora (N = 7) (fig. 1C, D). Tissue from the tail tip muscle was collected from each specimen and preserved in 96% ethanol. Genomic DNA was extracted using the E.Z.N.A® Tissue DNA Kit. The cytochrome *b* (cyt*b*) and 12S rRNA mitochondrial (mtDNA) genes were amplified by Polymerase Chain Reaction (PCR). The primers used in both amplification and sequencing were 12Sa and 12Sb for the 12S rRNA, cyt *b*1 and cyt *b*2 (Kocher et al., 1989) for the first fragment (cytb1), and cyt *b*2_*F* and CB3-3' (Palumbi, 1996) for the second fragment (cytb2) of the cyt*b* gene. Amplification of all mtDNA markers was carried out in a 10 μl volume, comprised of 5 μl of QUIAGEN Multiplex PCR Master Mix (Quiagen, Crawley, UK), 0.3 μl of each primer, 3.4 μl of ultra-pure water, and 2 μl of DNA extract. Thermocycling conditions were performed following Carranza et al. (2000). All amplified fragments were sequenced in a Sanger sequencer and deposited in GenBank (12S from PP910310 to PP910330; cytb1 from PP910127 to PP910147; and cytb2 from PP910148 to PP910161).

Phylogenetic analysis

A total of 11 cyt*b* and 12S sequences of *Tarentola* sp. were retrieved from GenBank (Nogales et al, 1998; Carranza et al., 2000) and added to the dataset. The obtained sequences were imported into Geneious Prime® (v.2022.2.2 Biomatters Ltd.) where the alignment was performed using MAFFT v.7.490 (Katoh et al., 2002; Katoh and Standley, 2013), under default parameters. Phylogenetic analysis based on the two mitochondrial fragments was performed under Maximum Likelihood (ML) and Bayesian Inference (BI) methods, using *Hemidactylus turcicus* and *Chondrodactylus laevigatus* as outgroups (following Carranza et al., 2000). To determine the best fitting nucleotide model, we used ModelFinder (Kalyaanamoorthy et al., 2017) from the IQ-TREE Web server (Trifinopoulos et al., 2016).

The ML analysis was done using RaxML-NG v.1 (Kozlov et al., 2019), considering a partitioned model, with the GTR + F + G4 and the HKY + F + I nucleotide models for the 12S, and cytb, respectively. The analysis started with a total of 200 trees (100 random and 100 parsimony) and 3000 bootstraps for branch support, estimated after checking for tree convergence with the *bsconverge* function. Computation of branch support was performed using the Transfer Bootstrap Expectation support metric (TBE, Lemoine et al., 2018), which is supposedly more appropriate for very large trees.

The software BEAST v.2.7.5 (Bouckaert et al., 2019) was used to estimate a calibrated BI genealogy. We set the 12S mean clock rate to 0.00827 as in Rato et al. (2012), based on the mean substitution rates for exactly the same 12S region, derived from a fully-calibrated phylogeny of *Tarentola* from the Canary Islands (Carranza et al., 2000; Carranza et al., 2002).

Analyses were run twice for 20×10^6 generations with a sampling frequency of 1000. Models and prior specifications applied were as follows (otherwise by default): Strict Clock, Coalescent with Constant Population Size, the GTR + F + G4 nucleotide model for the 12S alignment and the HKY + F + I for the cytb fragment, and gammaShape uniform (0, 10). Convergence for all model parameters was assessed by examining trace plots and histograms in Tracer v.1.7.1 (Rambaut et al., 2018) after obtaining an adequate sample size (ESS) > 200. The initial 10% of samples were discarded as burn-in. Runs were combined using LogCombiner, and maximum credibility trees with divergence time means and 95% highest probability densities (HPDs) were produced using Tree Annotator. Trees were visualized using FigTree v.1.4.4 (Rambaut, 2009).

Haplotype networks and genetic differentiation

To investigate haplotype diversity, we have built haplotype networks for each mtDNA fragment and for the concatenated mtDNA dataset. A median-joining haplotype network was constructed using the software PopArt (Bandelt et al., 1999; Leigh and Bryant, 2015), with the parameter epsilon set to 0.

Calculation of uncorrected genetic *p*-distances between the previously obtained phylogenetic groups was performed using Mega v.11 (Tamura et al., 2021).

We conducted a species delimitation analysis on the concatenated mtDNA alignment of only the *T. boettgeri* specimens, using the Assemble Species by Automatic Partitioning (ASAP) software (Puillandre et al., 2021). This particular software was selected due to its specialization in

handling single-locus data and its capacity to operate without needing any prior assumptions regarding the number of species or phylogenetic relationships. Crucially, it generates an adhoc score, facilitating an objective evaluation and ranking of species partitions (Puillandre et al., 2021). A lower ASAP score indicates a better species partition. For distance computation, the Simple Distance (*p*-distance) model was employed, while all other parameters remained at their default settings. ASAP delimitation was interpreted by evaluating both the partitions with first- and second-best ASAP score following Puillandre et al. (2021).

Results

A total of 1104 bp for 35 individuals representing all the taxa concerned in this study were used to assess the phylogeography of *T. boettgeri*. Out of these, 681 bp were derived from the cytochrome *b* gene (primers cytochrome *b*1 and cytochrome *b*2) and 423 bp from the 12S rRNA gene.

The obtained relationships among the different subspecies of *T. boettgeri* were identical using the two phylogenetic approaches (ML and BI). However, the Bayesian analysis provided a better supported genealogy for most nodes (fig. 2, supplementary fig. S1). Phylogenetic topology and node ages agree with previous studies (Nogales et al., 1998; Carranza et al., 2000; Gübitz et al., 2005): *T. b. boettgeri* is paraphyletic with some specimens more closely related to the individuals from El Hierro and Selvagens than with the other specimen from Gran Canaria (specimen CA11GC). The lineage represented by CA11GC diverged from the common ancestor of all *T. boettgeri* about 6.4 Mya. The Gran Canarian subspecies diverged from the ancestor of both *T. b. bischoffi* and *T. b. hierrensis* during the Pliocene around 4.0 Mya, and these two subspecies got separated ca. 1.4 Mya (as in Carranza et al., 2000). The populations of *T. b. bischoffi* from Selvagem Grande, and Selvagem Pequena/Ilhéu de Fora appear as two monophyletic groups, separated during the Middle Pleistocene, ca. 0.5 Mya. There is no clear divergence between the Selvagem Pequena and Ilhéu de Fora populations.

The haplotype network for the concatenated dataset revealed the existence of two mitochondrial haplotypes in El Hierro and three in Gran Canaria (fig. 3). In Selvagem Grande, all individuals share the same haplotype, and a similar pattern occurs in Selvagem Pequena/Ilhéu de Fora, apart from one individual from Selvagem Pequena having a distinct haplotype. The specimens from Selvagens are separated by 11 mutation steps from El Hierro and by 38 from Gran Canaria. Furthermore, the analysis predicts the existence of two missing haplotypes, either extinct or unsampled. Similar patterns are found when analysing the haplotype networks for each separate gene (supplementary fig. S3).

The maximum genetic *p*-distance (table 1) is observed between the individual CA11GC from Gran Canaria and the population from El Hierro (11.1%), and the minimum between the two phylogenetic lineages from Selvagens (0.6%). Indeed, the highest values are observed in the comparisons between CA11GC and the other islands, ranging from 10.5% to 11.1%. The differentiation between *T. b. boettgeri* and the other subspecies is around 7%, and between El Hierro and Selvagens is 2.5%.

The results from the two best ASAP scores (1.5 and 3.0) suggest that *T. boettgeri* could be partitioned into four or five distinct species, respectively (fig. 4). In both partition scenarios, the specimen TarGrande (from Selvagem Grande), the individual CA11GC, and the specimens from Gran Canaria, should be considered as different taxa. In the second-best score, the individuals from El Hierro would constitute a distinct species. Although the scoring scheme from ASAP does not support the divergence between the populations of Selvagem Grande from the ones in Selvagem Pequena and Ilhéu de Fora, the probability of this partition is low (<0.05), indicating that this group is unlikely (i.e., that the groups within this node probably correspond to different species).

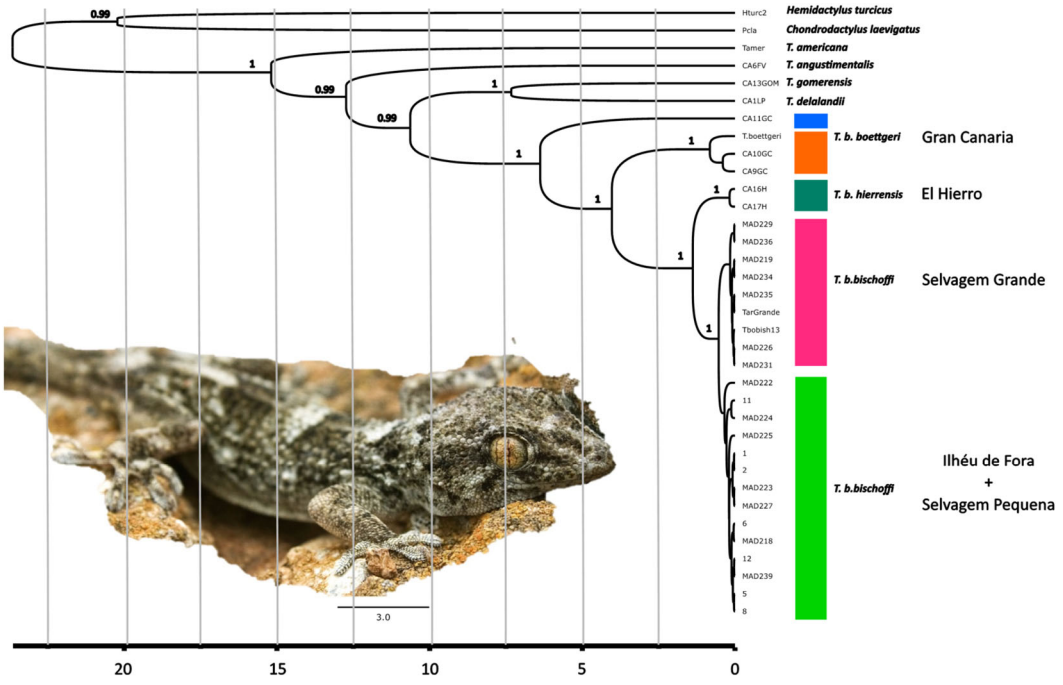

Figure 2. Calibrated Bayesian phylogenetic topology using three mitochondrial markers (12S + cytb1 + cytb2). The scale below the tree is in millions of years. Next to the nodes are represented the ML bootstrap support followed by the posterior probability from the BI. The ML topology is available in supplementary fig. S1.

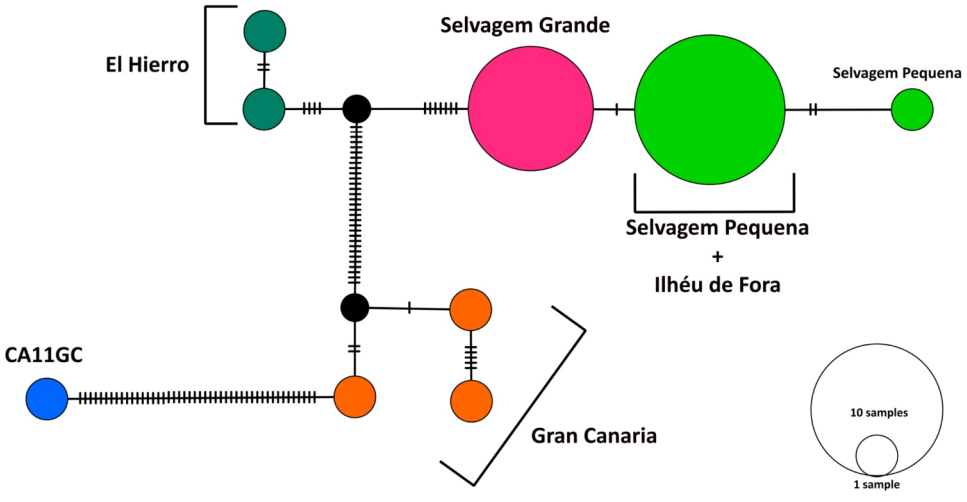

Figure 3. Median-joining haplotype network for the combined mitochondrial dataset (12S + cytb1 + cytb2). Black circles represent hypothetical missing haplotypes, and the mutation steps are shown as transversal lines in the branches. The colours match with the lineages obtained in fig. 2.

Table 1. Uncorrected genetic *p*-distances based on the combined mtDNA fragments (12S and cytb), between the different lineages of *Tarentola boettgeri* obtained from the phylogenetic analyses.

Clade	Clade			
	Selvagem Pequena and Ilhéu de Fora	Selvagem Grande	El Hierro	Gran Canaria
Selvagem Pequena and Ilhéu de Fora				
Selvagem Grande	0.006			
El Hierro	0.025	0.025		
Gran Canaria	0.071	0.072	0.072	
CA11GC lineage	0.105	0.107	0.111	0.090

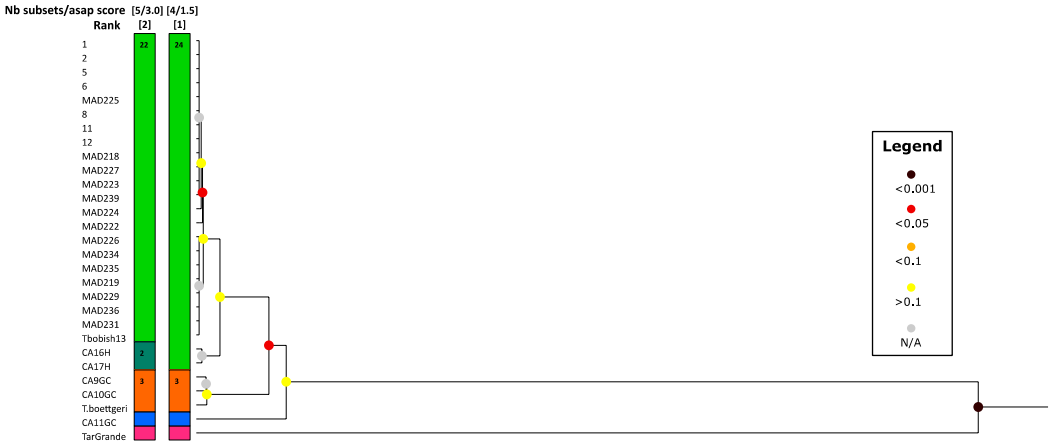

Figure 4. ASAP's species partition of the *T. boettgeri* specimens, considering the first- (1.5) and second-best (3.0) scores. These correspond to 4 and 5 partitions, respectively. The different partitions are represented by distinct colours on the bar, and the number inside corresponds to the number of assigned specimens. The colours of the dendrogram's nodes correspond to different probabilities, with darker colours matching with lower probabilities, while a grey dot meaning that the probability was not computed. When a probability is very low (dark colour), it suggests that the groups within the node likely correspond to different species.

Discussion

Our results corroborate the previously identified genetic clusters identified of *Tarentola boettgeri* by Nogales et al. (1998) and Carranza et al. (2000) and add a new monophyletic group, corresponding to the populations inhabiting the small islands of Selvagem Pequena and Ilhéu de Fora. In light of the ages obtained for the node estimation, it is clear that the diversification of *T. boettgeri* dates back to the upper Miocene (ca. 6.4 Mya), with the separation of the Gran Canaria lineage represented by CA11GC from its common ancestor. The diversification of this species continued across the Pliocene and Pleistocene, with the last separation occurring between the populations from Selvagem Grande, and Selvagem Pequena and Ilhéu de Fora, ca. 0.5 Mya.

The lack of genetic separation between Selvagem Pequena and Ilhéu de Fora suggests the existence of recent gene flow or even founder events between the two islands. During major glaciations the polar ice sheets spread considerably, and the formation of large volumes of accumulated ice reduced sea levels worldwide by about 120 m, exposing vast tracts of land currently submerged (Rohling et al., 1998). The lower sea level during these periods likely created land connections between these two islands, which are only ca. 1.5 km apart. In fact, Ilhéu de Fora is part of the same shelf as Selvagem Pequena, with relatively shallow

waters between both islands (63 m maximum depth described in Santos et al., 2019). Selvagem Grande is 18.5 km away from Selvagem Pequena, and the seafloor between them is full of eruptive fissures (Santos et al., 2019). Hence, the exposed land mass during the glaciations may not have been enough to promote a connection between these two islands, leading to the diversification of their gecko populations. Interestingly, the accepted phylogeography of the Selvagens lizard, *Teira dugesii selvagensis*, suggests a Pliocene (2.5 Mya) colonisation of the archipelago from Madeira, and recent contacts between the populations of Selvagem Grande and Selvagem Pequena (Brehm et al., 2003).

The contact between different insular populations during glacial times is well documented for several Mediterranean reptile species, such as *Podarcis filfolensis* from the Maltese archipelago (Salvi et al., 2014), *P. lilfordi* from the Balearic islands (Brown et al., 2008), and *P. tiliguerta* from Corsica and Sardinia (Senczuk et al., 2019). The sea level oscillations during the Pleistocene have also shaped the genetic diversity and island biogeography of reptiles in other parts of the world, such as in the Galápagos lava lizards (Jordan and Snell, 2008) or the Komodo dragons from Lesser Sunda region of eastern Indonesia (Iannucci et al., 2021).

Our study could not unravel the most likely colonisation route of *T. boettgeri* from the mainland to Macaronesia. Nevertheless, our corroboration of the two distinct lineages of *T. boettgeri* in Gran Canaria (with one of them being the most ancient among all subspecies), along with the absence of this species in Madeira, suggests that the diversification of *T. boettgeri* likely occurred from Gran Canaria. Subsequently, the species may have colonised Selvagens and then spread to El Hierro from there, just as previously suggested (Nogales et al, 1998; Carranza et al., 2000).

The existence of two major mitochondrial clades from two seemingly allopatric, clearly geographically separated populations in Gran Canaria has already been documented in previous studies (Nogales et al., 1998; Carranza et al., 2000; Gübitz et al., 2005), and is hypothesised to result from volcanic activity on the island, which led to the separation of two populations of *T. b. boettgeri* by vicariance (Gübitz et al., 2005). The current gene flow between these two genetically distinct populations seems vestigial and is likely to be associated with the low vagility of the species and/or marked geographic ecological differences (Nogales et al., 1998; Gübitz et al., 2005). Noteworthy, the genetic distance obtained between the two monophyletic groups from Gran Canaria (9%) is higher or equivalent to what has been described between full species of *Tarentola* inhabiting the same Cape Verdean Island (e.g., *T. nicolauensis – T. maioensis*: 8.7%; *T. raziana – T. gigas*: 4.6% in Vasconcelos et al., 2012). Indeed, the genetic divergence is even higher when CA11GC is compared with *T. b. hierrensis* (11.1%) or *T. b. bischoffi* (ranging from 10.5% to 10.7%). When comparing *T. b. boettgeri* with the populations from El Hierro and Selvagens, the genetic distances range from 7.1% to 7.2%, higher than the observed values between different *Tarentola* species occurring in distinct Cape Verdean islands (e.g., *T. rudis – T. substituta*: 5.4%; *T. rudis – T. raziana*: 5.4% in Vasconcelos et al., 2012). Contrastingly, Vasconcelos et al. (2010) obtained a 2.2% mitochondrial genetic distance between *T. caboverdiana* and *T. substituta*, which is lower than what we observe between *T. boettgeri* from El Hierro and any of the groups from Selvagens.

Our results clearly suggest that an integrative taxonomic revision of *T. boettgeri* across its entire geographical range is paramount, starting with the two phylogenetic groups from Gran Canaria. This could be achieved by integrating morphological, immunological and biochemical traits with whole-genome sequencing (WGS). Adaptive differences indicated by immunological and biochemical data, led Joger (1984) to propose the Selvagens populations as

a full species. Moreover, the species delimitation results suggest that all identified monophyletic groups should be classified as distinct species, which would likely have implications for the IUCN Red List status of the species (*T. boettgeri* is currently classified as Least Concern). Nonetheless, for the time being, and considering our results and the geographic isolation of the different insular populations, we propose that, with the exception of the two phylogenetic groups found in Gran Canaria, the identified monophyletic groups should be defined (and managed) as ESUs (following Crandall et al., 2000; Fraser and Bernatchez, 2001). Even in the face of advancements in next-generation sequencing technologies, the use of single locus mitochondrial markers in conservation studies are still relevant and useful. In the particular case of *T. boettgeri*, the use of mitochondrial markers allows for comparisons with previous research (Nogales et al., 1998; Carranza et al., 2000; Gübitz et al., 2005), facilitating continuity and enabling us to build upon existing knowledge. Nevertheless, WGS information provides unmatched power and resolution for examining demographic trends, admixture and introgression, natural selection and species diversification (references in Theissinger et al., 2023), which should be implemented in future studies on *T. boettgeri*.

Although the two phylogenetic groups found in Gran Canaria are reciprocally monophyletic for the mitochondrial markers, more sampling is warranted to confirm that they are indeed genetically and geographically isolated, as this is one of the criteria defined by Crandall et al. (2000) for the classification of populations as ESUs. The recognition of the distinct monophyletic *T. boettgeri* groups as individual ESUs is essential to define future conservation strategies, in particular for narrow-range populations with small population sizes, such as the ones found in the Selvagens archipelago. *Tarentola b. bischoffi* is currently classified as Vulnerable by the Portuguese Red Data Book (Oliveira et al., 2005) based on its highly restricted distribution area and concentration in only three populations, two of which are on very low-lying islands vulnerable to sea-level rise. The entire distribution area is included within a Nature Reserve where access is restricted. Still, in the coming years, the Selvagem Grande population could face potential impacts from the increasing numbers of Madeiran wall lizards after successfully eradicating rabbits and mice. Despite differences in their activity patterns, the generalist diet and behaviour of the wall lizard (Matias et al., 2009; Neves et al., 2017; Neves et al., 2022; Rato et al., 2022), coupled with its greater aggressiveness and larger size, may result in an increase in predation incidents on the eggs, juveniles, and even adult individuals of the Selvagens gecko and competition for food resources and retreat sites (R. Rebelo, pers. obs.; Penado et al., 2015).

Island-restricted reptiles constitute a significant share of the total reptile diversity (Roll et al., 2017). Yet, they are a poorly understood group that inhabits some of the most vulnerable habitats worldwide (Fernández-Palacios et al., 2021). As exemplified here by the *T. boettgeri*, many of these populations have substantial unique intraspecific molecular divergence. The failure to recognize these as distinct ESUs can lead to an underestimation of their conservation relevance, compromising our capacity to delineate much-needed evidence-based conservation strategies.

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