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Morphological and genetic diversity of the Balearic lizard, *Podarcis lilfordi* (Günther, 1874): Is it relevant to its conservation?

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Abstract

Aims: To characterize the genetic and morphological diversification of the endangered Balearic lizard *Podarcis lilfordi* and to assess the relevance of this diversity, and how it is described, to conservation measures.

Location: This study covers all the populations of the Balearic lizard, *Podarcis lilfordi*, present in its range of distribution at coastal islets of Menorca, Mallorca and Cabrera Archipelago.

Methods: We analysed genetic and morphological variation across the 43 known extant populations of the Balearic lizard, using mitochondrial and nuclear markers. We examined morphometric and scalation characters using, in some cases, phylogenetically independent contrasts. We also incorporated the study of dorsal coloration and dorsal colour pattern including the analysis of melanism in several populations.

Results: We detected clear genetic divergence between Menorcan populations and populations from Mallorca and Cabrera, in both nuclear and mtDNA markers, but genetic divergence is relatively low among different insular populations within these groups. In contrast, morphological divergence was substantial both between Menorcan and remaining populations and within these groups. Morphological traits, such as dorsal coloration, body size and the number and size of scales, seemed to be linked with differences in climatic conditions between populations. In addition, some traits, as melanism, showed a strong phylogenetic signal.

Main conclusions: The morphological and genetic diversity of the Balearic lizard is incongruent with the subspecies described in the classical taxonomic literature. Moreover, current populations differ not only in some genetic and morphological features, but also in several ecological and ethological characteristics, in many cases unique to one population. Based on our results, we propose abandoning the use of subspecies to describe the extraordinary morphological diversity of the Balearic lizard and its replacement with the concept of evolutionary significant units (ESUs).

ESUs are particularly suitable to describe and recognize such diversity and, especially, to ensure the continuity of the evolutionary process.

KEYWORDS

Balearic Islands, conservation, Lacertidae, morphology, phylogeny, *Podarcis lilfordi*, scalation

1 | INTRODUCTION

For a variety of taxa, islands make a very important contribution to biodiversity, out of proportion to their land area in comparison with continents. Island contribution to global diversity is mainly in terms of endemic forms, instead of species diversity (Whittaker & Fernández-Palacios, 2007). This is the case of Balearic Islands, with only three species of autochthonous terrestrial vertebrates, one midwife toad and two lacertid lizards, all of them endemic to Balearic Islands.

According to IUCN assessment, the Balearic lizard, *Podarcis lilfordi* (Günther, 1874), is an endangered species (Pérez-Mellado & Martínez-Solano, 2009) of Mediterranean wall lizard endemic from the Balearic Islands (Western Mediterranean, Spain). *P. lilfordi* is a member of the first post-Messinian faunal assemblage reported from Menorca Island (Bover, Quintana, & Alcover, 2008; Bover et al., 2014). During the Holocene, around 2,000 years ago, the species became extinct on the main islands of Mallorca and Menorca, probably as a consequence of the introduction of terrestrial predators by Romans (Pérez-Mellado, 2009 and references therein). It now survives only on the small islets found around the coasts of Menorca and Mallorca, together with Cabrera archipelago and a small introduced population in Colonia Sant Jordi, in Southern Mallorca (Figure 1).

Phylogeographical studies indicate that the Balearic lizard separated from its sister species, *Podarcis pityusensis* (Boscá, 1883), after the refilling of the Mediterranean basin, at the end of the Messinian Salinity Crisis (Brown et al., 2008; Terrasa, Pérez-Mellado, et al., 2009; Terrasa, Rodríguez, et al., 2009). Menorcan and Mallorcan populations appear to have diverged at the beginning of the Quaternary period, 2.6 my ago (Brown et al., 2008). Sea levels were higher during the Late Pliocene than during the present day (Emig & Geistdoerfer, 2004), precluding a land connection between Mallorca and Menorca. The genetic separation of lizards from Mallorca, including Cabrera archipelago, and Menorca was maintained during later periods of the Pleistocene, despite potential for secondary contact that could have taken place during eustatic Pleistocene sea level changes (Emig & Geistdoerfer, 2004). Consequently, during more than 2.5 my, the reciprocally monophyletic clades of Mallorca, Cabrera and Menorca evolved with little or no introgression. Another major divergence occurred around 2 my in Western Mallorca and the rest of populations around Mallorca Island (Brown et al., 2008).

In addition to these major divergences, within the groups of Mallorca and Menorca there has been a subsequent differentiation of isolated populations forming one of the most surprising arrays of allopatric populations. Thus, the Balearic lizard was recognized as a polytypic species (Huxley, 1940 in Mayr, 1963). Taxonomic studies to provide formal recognition of this variation began with Von Bedriaga who defined three infraspecific taxa (Bedriaga, 1879). During the first third of the 20th century, several subspecies of the Balearic lizard were described in a few years by Müller and Eisentraut (Eisentraut, 1928a, 1928b; Müller, 1927; Wetsttein, 1937). The remaining subspecies were described during the second half of the 20th century (Hartmann, 1953; Pérez-Mellado & Salvador, 1988; Salvador, 1979, 1980; Table 1).

However, the validity of this subspecific arrangement is largely debatable, especially if we take into account the genetics of these insular populations (Terrasa, Pérez-Mellado, et al., 2009). The construction of taxonomies based on phylogenetic species concept could remove the need for subspecific descriptions (Haig et al., 2006; Terrasa, Pérez-Mellado, et al., 2009). An alternative solution would be the use of evolutionary significant units (ESUs) that initially were defined as units that should be reciprocally monophyletic for mtDNA alleles and that show a significant divergence of allele frequencies at nuclear loci (Moritz, 1994). We will explore the arrangement of these ESUs in the case of the Balearic lizard and its consistence with the traditional separation of subspecies.

The temporal process of separation during lineage divergence can accumulate genetic, ecological and morphological changes, resulting in a better adaptation to local environmental conditions. The occupation and use of different habitats by lizards would lead to a divergent selection on traits that define several morphological characteristics as body size, body shape, coloration patterns or scalation characters (Hu, Wu, Ma, Chen, & Ji, 2019; Muñoz et al., 2013; Wollenberg, Wang, Glor, & Losos, 2013). We analyse, within the frame of genetic variability, these morphological characteristics in all extant populations of the Balearic lizard. Nuclear DNA markers are analysed for the first time, and we describe the morphometry, scalation and colour patterns of populations. Morphological traits are also related to different climatic conditions at different geographical locations (Mayr, 1963). In Balearic Islands, due to their latitudinal situation, climatic conditions can be different for the two main clades of Mallorca and Menorca populations. Thus, we tested the potential influence of main climatic conditions of Mallorca and Menorca, that is, rainfall and environmental temperatures, on morphological characters of lizards.

Our goal is to answer the following questions: 1. How valid is the current taxonomic arrangement of populations if we consider their genetic and morphological variability? and 2. What is the relationship between the observed morphological diversity and the evolutionary history of populations (phylogeny) or their current climatic conditions?

2 | METHODS

2.1 | Specimens

We studied all known populations (Figure 1) of *P. lilfordi*, although we did not obtain genetic, morphometric or coloration information from all of them. Samples from Cabrera Island were obtained from four sites: Cabrera Port (the area around the Cabrera Bay), Cabrera far (the Ansiola Peninsula lighthouse, at the south-western corner of the Island), Morro den Tià (another Western Peninsula of the Island) and Miranda (central part of Cabrera Island, Figure 1). Note that the population studied from the Bay of Palma (Mallorca), at Porrassa Islet, is known to have been introduced, as well as the small population of Colonia de Sant Jordi in Southern Mallorca.

2.2 | Climatic characteristics

The aridity of the Balearic Islands was estimated with the I_q index (Sahin, 2012). In this index, the ratio of annual precipitation to annual mean specific humidity (Sh) is employed. The mean specific humidity can be easily computed with mean temperature, relative humidity and local pressure which are the most commonly measured meteorological data (Sahin, 2012). Due to the limited geographical distribution of *P. lilfordi*, we were only able to compare climatic data from three weather stations: the Port of Palma de Mallorca (Palma

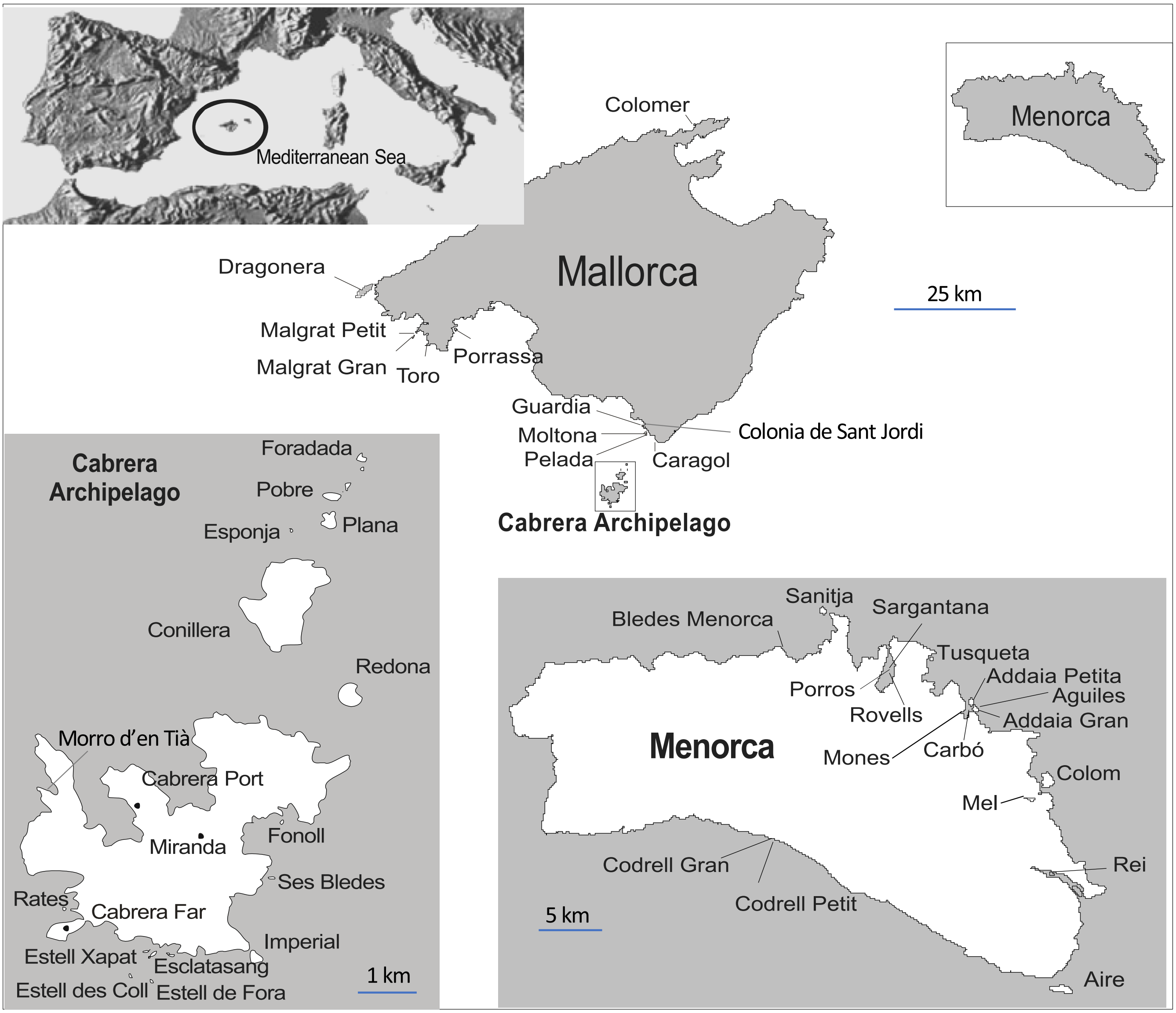

FIGURE 1 The distribution of major clades of *Podarcis lilfordi* in Balearic Islands (Ibiza Island is not represented in the map). We showed all known populations of the Balearic lizard in Menorca, Mallorca and Cabrera Archipelago (see Table 1 for a list of described subspecies and their distribution)

TABLE 1 Known populations of the Balearic lizard and described subspecies (see Figure 1 for locations of populations under study)

Location	Population	Subspecies	MtDNA (*N*)	COI (*N*)
Mallorca	Colomer	*P. l. colomi* (Salvador, 1979)	4	2
	Dragonera	*P. l. gigliolii* (Bedriaga, 1879)	4	2
	Malgrat Petit	*P. l. hartmanni* (Wetsttein, 1937)	2	2
	Malgrat Gran	*P. l. hartmanni* (Wetsttein, 1937)	2	2
	Toro	*P. l. toronis* (Hartmann, 1953)	3	1
	Caragol	*P. l. jordansi* (Müller, 1927)	2	2
	Guardia	*P. l. jordansi* (Müller, 1927)	2	2
	Moltona	*P. l. jordansi* (Müller, 1927)	2	2
	Pelada	*P. l. jordansi* (Müller, 1927)	2	2
	Colonia de Sant Jordi	Undescribed		2
	Porrassa	Introduced	2	2
Menorca	Aire	*P. l. lilfordi* (Günther, 1874)	2	2
	Addaia Gran	*P. l. addayae* (Eisentraut, 1928)	5	2
	Addaia Petita	*P. l. addayae* (Eisentraut, 1928)	3	2
	Colom	*P. l. brauni* (Müller, 1927)	3	2
	Bledes Menorca	*P. l. sargantanae* (Eisentraut, 1928)	2	2
	Codrell Gran	*P. l. codrellensis* (Pérez-Mellado & Salvador, 1988)	2	2
	Codrell Petit	*P. l. codrellensis* (Pérez-Mellado & Salvador, 1988)	3	2
	Carbó	*P. l. carbonerae* (Pérez-Mellado & Salvador, 1988)	3	2
	Mel	Undescribed	4	2
	Porros	*P. l. porrosicola* (Pérez-Mellado & Salvador, 1988)	2	2
	Rovells	*P. l. sargantanae* (Eisentraut, 1928)	3	2
	Sargantana	*P. l. sargantanae* (Eisentraut, 1928)	3	2
	Tusqueta	*P. l. sargantanae* (Eisentraut, 1928)	5	2
	Sanitja	*P. l. fenni* (Eisentraut, 1928)	4	2
	Aguiles	Undescribed	1	4
	Mones	Undescribed		2
	Rei	*P. l. balearica* (Bedriaga, 1879)	2	4
Cabrera	Cabrera Far	*P. l. kuligae* (Müller, 1927)	2	2
	Cabrera Port	*P. l. kuligae* (Müller, 1927)	4	2
	Morro d'en Tià	*P. l. kuligae* (Müller, 1927)		2
	Miranda	*P. l. kuligae* (Müller, 1927)	3	2
	Foradada	*P. l. fahrae* (Müller, 1927)	3	2
	Pobre	*P. l. pobrae* (Salvador, 1979)	2	2
	Plana	*P. l. planae* (Müller, 1927)	3	1
	Esponja	*P. l. espongicola* (Salvador, 1979)	3	2
	Redona	*P. l. conejerae* (Müller, 1927)	2	2
	Conillera	*P. l. conejerae* (Müller, 1927)	1	2
	Rates	*P. l. kuligae* (Müller, 1927)	2	2
	Ses Bledes	*P. l. nigerrima* (Salvador, 1979)	4	2
	Fonoll	*P. l. kuligae* (Müller, 1927)	4	1
	Imperial	*P. l. imperialensis* (Salvador, 1979)	3	2
	Estell des Coll	*P. l. estelicola* (Salvador, 1979)	2	2
	Estell de Fora	*P. l. estelicola* (Salvador, 1979)	2	1
	Estell Xapat	*P. l. xapaticola* (Salvador, 1979)	2	2
	Esclatasang	*P. l. xapaticola* (Salvador, 1979)	3	2

(Continues)

TABLE 1 (Continued)

Location	Population	Subspecies	MtDNA (*N*)	COI (*N*)
Pitiusic Islands (outgroup)	Na Gorra	*P. pityusensis*		1
	Espardell	*P. pityusensis*	1	1
	Espalmador	*P. pityusensis*	1	1
	Caragoler	*P. pityusensis*	1	1
	Alga	*P. pityusensis*	1	1
	Negra Nord	*P. pityusensis*	1	1
	Porcs	*P. pityusensis*		1
	Eivissa	*P. pityusensis*	1	1
	Vedrà	*P. pityusensis*	1	1
	Formentera (Sant Francesc Xavier)	*P. pityusensis*	1	1
	Formentera (Punta Trocadors)	*P. pityusensis*	1	1

Note: We include subspecific assignation for each population and sample sizes of gene fragments studied. In the case of Mel, Colonia de Sant Jordi, Porrasa, Aguiles and Mones there is no subspecific assignation. In Formentera Island, we include the location of studied samples.

Puerto, B228, Mallorca Island), Palma de Mallorca International Airport (Palma Aeropuerto, B278, Mallorca Island) and Menorca International Airport (Menorca Aeropuerto, B894, Menorca Island). The two weather stations on Mallorca Island are very close. We employed data from these weather stations freely provided by AEMET Open Data (Agencia Estatal de Meteorología, Spain). Aridity indices and rainfall were calculated with annual data from 1981 to 2018.

2.3 | Genetic study

2.3.1 | DNA extraction, amplification and sequencing

Total genomic DNA was extracted from tail tips following standard protocols (González et al., 1996). A total of 104 (Table 1) individuals of *P. lilfordi* have been studied for four non-overlapping mtDNA fragments: (a) partial 12S rRNA, (b) two partial fragments of cytochrome *b* (CYTB), (c) partial control region (CR) and (d) two partial subunits of the NADH dehydrogenase gene and associated tRNAs (referred to as ND1, ND2, tRNA_{Ile}, tRNA_{Gln} and tRNA_{Met}). Primers and amplification conditions are the same as those used in our previous studies of *Podarcis* (Rodríguez et al., 2013; Terrasa, Pérez-Mellado, et al., 2009). Additionally, 92 (Table 1) individuals were sequenced for COI fragment using primers LCO-1490: GGT CAA CAA ATC ATA AAG ATA TTG G and HCO-2198: TAA ACT TCA GGG TGA CCA AAA AAT CA (Folmer, 1994). Specimens of *Podarcis pityusensis* were used as outgroup. One nuclear gene was amplified and sequenced for 45 samples: melanocortin-1 receptor gene (MC1R) (720 bp). Primers and amplification conditions are the same as those used in Buades et al. (2013).

Both strands of the PCR products were sequenced and carried out on an automated ABI 3130 sequencer (Applied Biosystems) using a BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) and edited using CodonCode Aligner software (CodonCode Corporation). Nuclear data were phased using the PHASE algorithm (Stephens, Smith, & Donnelly, 2001) within DnaSP v.6 (Rozas et al., 2017). All GenBank accession numbers are indicated in Table S8.

2.3.2 | Divergence and phylogenetic analyses

Distance-based method (p-distance) based on mitochondrial data (mtDNA and COI fragment) between subspecies was calculated in MEGA 7 (Kumar, Stecher, & Tamura, 2016) to stablish the level of sequence divergence. We used a dataset including 46 *P. lilfordi* populations for genetic analyses (Table 1). Three different datasets were used: (1) concatenated mitochondrial four DNA fragments (12S, cytb, CR and NADH) providing an alignment of 2,382 bp length, (2) COI fragment alignment with a length of 656 bp and (3) phased nuclear alignment (MC1R) with a length of 720 bp. Sequences were aligned in the MAFFT v7.423 online server (Katoh, Rozewicki, & Yamada, 2017) using the iterative refinement method (FFT-NS-i). For the protein-coding genes, alignments were verified by translating nucleotide sequences to amino acids. Best-fit nucleotide substitution models and partitioning scheme were chosen simultaneously using PartitionFinder V1.1.1 (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016) under the corrected Akaike Information Criterion (AICc), due to the small sample sizes under analysis. The partitioning schemes were defined by hand with the "user" option, with branch lengths of alternative partitions "unlinked" to search for the best-fit scheme, which consisted of one partition for the mtDNA dataset: [HKY + G+I] and 2 partitions for the COI dataset: 1st and 2nd position [HKY + I] and 3rd position [GTR + G].

Bayesian analyses were performed with MrBayes 3.2.1 (Ronquist et al., 2012). The analyses were run for 10^6 generations with sampling frequency every 10^3 generations. Numbers of runs and chains were left as default, two and four, respectively. Sufficient number of generations was confirmed by examining the stationarity of the log-likelihood (lnL) values of the sampled trees and the value of average standard deviations of the split frequencies being lower than

0.01. Results were analysed in TRACER v1.6 (Rambaut, Suchard, Xie, & Drummond, 2014) to assess convergence and effective sample sizes (ESS) for all parameters. A burnin of 25% was applied, and the phylogenetic trees were visualized and edited using FIGTREE v1.4.2 (Rambaut, 2012).

The genealogical relationships between the haplotypes of the phased nuclear gene were inferred using the TCS statistical parsimony network approach (Clement, Posada, & Crandall, 2000) with 95% connection limit implemented in the program POPART 1.7 (http://popart.otago.ac.nz; Clement, Snell, Walker, Posada, & Crandall, 2002; Leigh & Bryant, 2015).

2.4 | Morphological analysis

The morphology of 5,755 specimens (3,455 males and 2,300 females) was studied (Tables S1–S6). Six body dimensions, as well as body mass (weight), were included in this study: snout–vent length (SVL), intact tail length (TL), pileus length (PL), head width (HW), head height (HH) and hindleg length (HLL). All measurements were made with a digital calliper to the nearest 0.01 mm, with the exception of SVL and intact tail length, which were measured with a steel rule to the nearest 1 mm. Weight was obtained with a spring scale Pesola®. Males and females, as in all lacertid lizards, were separated according with the size of femoral pores of males and the presence of hemipenial structures (Pérez-Mellado & Gosá, 1988). We considered as adult individuals, males and females with SVL above 45 mm. Six scalation characters were studied: gularia, collaria, dorsalia, ventralia, left femoralia and left fourth digit lamellae (see Pérez-Mellado & Gosá, 1988 for methodological details of body measurements and scalation counts). Obviously, not all characters were recorded from all individuals and sample sizes are very variable, according with availability of adult lizards from each population (see Tables S1–S6). All body measurements were done by the same researcher (VPM).

Morphometric and scalation characters were compared among the three groups of populations (Mallorca, Cabrera and Menorca) using ANOVA type, Lawley–Hotelling type, Bartlett–Nanda–Pillai type and Wilks' lambda type test statistics, as implemented in the R-package "npmv" (Burchett, Ellis, Harrar, & Bathke, 2017). These statistics were then used as the basis for permutation or randomization tests. Nonparametric relative effects of each morphometric and scalation character were also tested. These effects give an indication of stochastic superiority; that is, they measure the probability that a value obtained from one group is larger than a value randomly selected from the whole dataset. In agreement with default settings of npmv, we employed the results of Wilks' lambda in pairwise comparisons, and because $N > 30$, we used F approximation (Burchett et al., 2017).

In addition, we employed a non-metric multidimensional scaling (NMDS) to establish morphological divergence, separately for adult females and males, among 43 populations of *P. lilfordi*. The method aims to depict the inherent pattern of a dissimilarity matrix in a geometric picture with a minimum number of dimensions (Clover, 1979). We used the metaMDS function from the "vegan" R-package (Oksanen et al., 2018). This function runs NMDS several times from random starting configurations, compares results and stops after detecting two similar minimum stress solutions (Oksanen et al., 2018). The goodness-of-fit of the ordination was assessed by the coefficients of determination (R^2) for the linear and nonlinear regressions of the NMDS distances on the original ones (Borcard, Gillet, & Legendre, 2011). Finally, we recorded the stress values of NMDS (Zuur, Ieno, & Smith, 2007). All calculations were done within R environment (R Core Team, 2019).

Dorsal colouration of lizards was analysed with the "colordistance" R-package (Weller, 2019; Weller & Westneat, 2019). This package is an objective comparative tool to colour profiling and comparison of digital images. The standard RGB image analysis cannot provide the wavelength resolution of reflectance spectrophotometry. However, the use of digital images can reflect the visual sensitivities of several species (Losey et al., 2003; Weller & Westneat, 2019).

Our colour analysis was restricted to 30 populations of adult males and 27 populations of adult females of the Balearic lizard from which we had good JPEG dorsal colour images. We employed 2–15 individual images per population. Non-background pixels were binned to read images into the R environment as 3D arrays. We used colour histograms as the binning method in a 5 × 2 × 3 hue–saturation–value (HSV) colour space. HSV is the most suitable method when different digital cameras or variable light conditions were employed to obtain images (Weller & Westneat, 2019). For calculating the distance between one binned image and another, we employed the earth mover's distance or Wasserstein metric (EMD) method. In this way, colour histograms from each image or from a group of images (i.e., from all individuals of a given population) are compared with a symmetrical distance matrix that is plotted as its corresponding heatmap. In the heatmap, we used default colours ranging from yellow (least similar) to blue (most similar).

2.5 | Comparative analysis

We did comparative analyses of morphological traits using the mtDNA tree (Figure 2). Due to the low levels of divergence and consequently the high number of polytomies, we were unable to obtain an ultrametric and fully dichotomous tree for most comparative analyses. Thus, we restricted our analysis to the comparison of morphological continuous variables with the "caper" package (Orme et al., 2018), using the method of Pagel (1992) to calculate contrasts at polytomies. We compared in this way all continuous variables of morphometry and scalation using SVL as covariate (Orme et al., 2018). In addition, to study the presence/absence of melanic coloration, we employed the D statistics (Fritz & Purvis, 2010) for binary variables of the "caper" package. In order to standardize the effects of phylogeny size and prevalence, "phylo.d" uses two simulated null models: a model of phylogenetic randomness, where trait values are randomly shuffled relative to the tips of the phylogeny, and the Brownian threshold model, where a continuous trait is evolved along the phylogeny under a Brownian process and then converted to a binary trait using a threshold that reproduces the relative prevalence of the observed trait. D typically varies between 0 and 1. A D value close to zero indicates that the binary trait evolves on a tree following the Brownian model (i.e., strong phylogenetic signal).

3 | RESULTS

3.1 | Climatic characteristics in the Balearic Islands

We found significantly lower aridity (I_q index) in Menorca ($F_{2,111}$ = 12.59, p = 1.18 × 10^{-5}), but no differences between Mallorca port and airport (Tukey test, p > .05; Mallorca port, $\bar{x}$ = 49.13 ± 2.0, n = 38, min: 23.25, max: 75.83; Mallorca airport, $\bar{x}$ = 50.07 ± 1.8, n = 38, min: 28.15, max: 70.3; and Menorca airport, $\bar{x}$ = 62.68 ± 2.52, n = 38, min: 32.4, max: 93.2). Average annual rainfall was also significantly higher in Menorca ($F_{2,111}$ = 12.91, p = 9.13 × 10^{-6}), but again, there were no differences between Mallorca airport and port (Tukey test, p > .05; Mallorca port, $\bar{x}$ = 455.2 ± 19.08 mm, n = 38, min: 201.3, max: 702.2; Mallorca airport, $\bar{x}$ = 415.78 ± 15 mm, n = 38, min: 227.2, max: 559.6; and Menorca airport, $\bar{x}$ = 548.03 ± 21.95, n = 38, min: 272.2, max: 811.7).

3.2 | Genetic analysis

We have sequenced four fragments plus COI of mtDNA genome. The major genetic differentiation has been detected between Menorca and Mallorca + Cabrera populations, as can be seen in the Bayesian analysis based on COI fragment (Figure 2) and mtDNA concatenated dataset (Figure 3). The latter shows a better resolution of the cladogenesis of the Balearic lizard and its relationship with subspecific assignation. In all analyses, there is a large amount of polytomies, especially in the case of Menorca clade (Figures 2 and 3). Only populations from Western Mallorca (Dragonera, Malgrat islets and Toro) exhibit a clear monophylia of subspecies (*P. l. gigliolii*, *P. l. hartmanni* and *P. l. toronis*, respectively, Figure 3). Genetic distances estimated between different subspecies based on concatenated mtDNA fragments (Table 2) show levels of differentiation range between 0.001 and 0.035, and confirm these conclusions. Genetic distances based on COI fragment (not included) show similar results.

Overall, the TCS network built from phased nuclear gene MC1R (Figure 4) exhibits a low variability (it is necessary to say that a minor number of samples are used). It also shows high gene flow between populations from Mallorca and Cabrera and confirmed the clear separation between Menorca and the remaining populations.

In Porrassa Island inhabits an introduced population, it is included in phylogenetic trees and exhibits a high proximity to Cabrera archipelago. Thus, the most probable origin could be Cabrera Island, as in the case of Colonia Sant Jordi lizards.

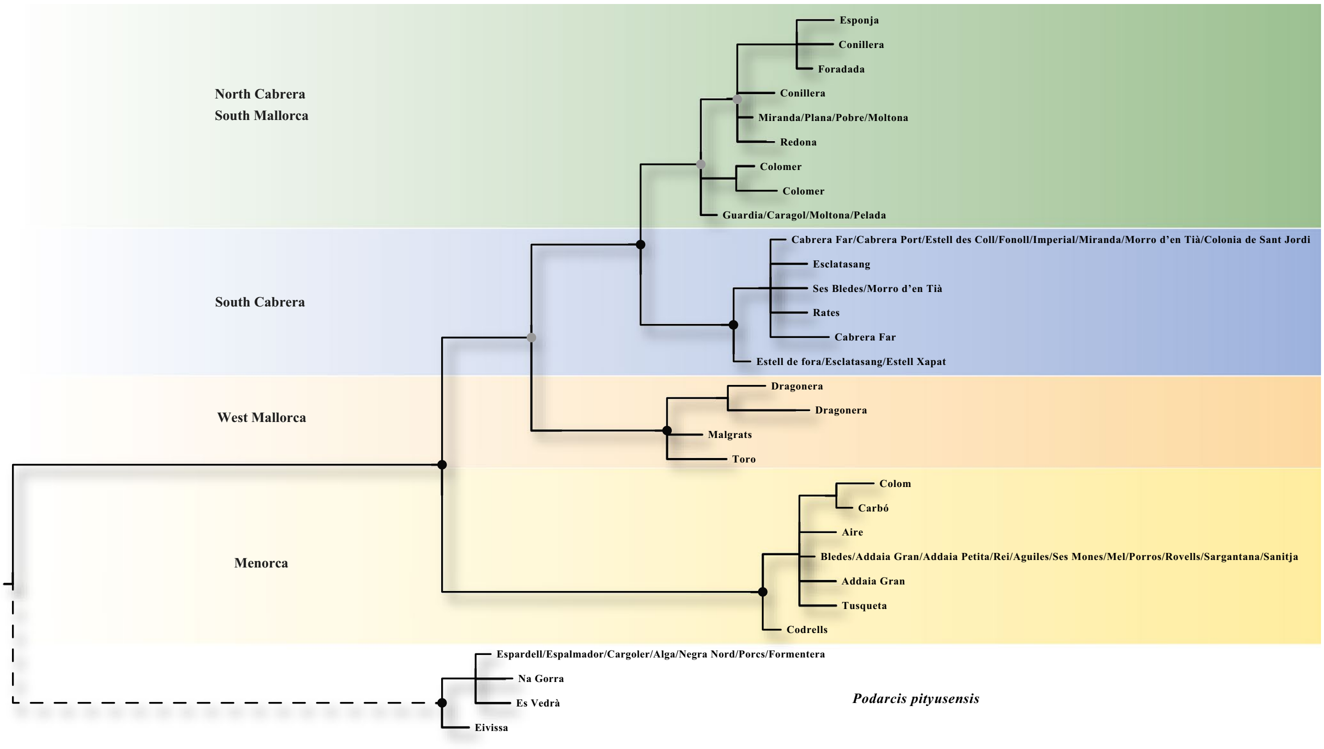

FIGURE 2 Bayesian analysis based on COI fragment (656 bp). Codrells include Codrell Petit and Codrell Gran, and Malgrats included Malgrat Petit and Malgrat Gran. Black dots represent a posterior probability (PP) greater than 90 and grey dots a PP ranged between 80 and 90

3.3 | Morphometry and scalation

With the exception of lamellae ($F_{1,1592} = 1.835$, $p = .176$), all morphological traits are sexually dimorphic in *P. lilfordi* (Table S7). Thus, all analyses were done separately for adult males and females. Morphology shows a transition from Menorca to Mallorca and Cabrera characterized by increased body size (see Figures 5 and 6 for SVL, and Tables S1–S6), and a decrease in adjusted head dimensions (Tables S1–S6), both in adult males and females. Body size (SVL) differs among the three major clades of Mallorca, Menorca and Cabrera (one-way ANOVA, $F_{2,\ 3,452} = 35.34$, $p = 6.39 \times 10^{-16}$; Menorca, $\bar{x} = 68.5 \pm 0.1$ mm, 45–86 mm, $n = 2,628$; Mallorca, $\bar{x} = 69.72 \pm 0.23$ mm, 55–81 mm, $n = 383$; and Cabrera, $\bar{x} = 70.44 \pm 0.23$ mm, 57.4–83 mm, $n = 444$), with larger lizards in Mallorca and Cabrera than in Menorca (Tukey, $p = .00002$ and $p < .001$, respectively), but similar sizes between Mallorca and Cabrera (Tukey, $p = .093$). Interestingly, apart the lizards from Mel Islet (Menorca), the largest lizards are from three unrelated and melanistic populations of Menorca (Aire Island), Mallorca (Colomer) and Cabrera (Estell des Coll, Figures 5 and 6).

Using phylogenetically independent contrasts, PL, PW, HLL, lamellae and ventralia traits are significantly different among adult males of the populations under study (results in Table 3). PL, HH, HLL, gularia, dorsalia and collaria are different in adult females (Table 4).

The nonparametric multivariate analysis (ANOVA type test, see above), rejects the hypothesis of equality among male lizards from Cabrera, Mallorca and Menorca (Table 5, $p < .001$ in all tests). There is an overall significant difference in body dimensions and scalation between Mallorca and Menorca lizards ($p < .05$) and Cabrera and Menorca lizards ($p < .05$), but not between Mallorca and Cabrera archipelagos ($p > .05$). In adult females, we also reject the hypothesis of equality among Cabrera, Mallorca and Menorca (Table 6, $p < .001$). We find significant differences in body dimensions and scalation between Mallorca and Menorca lizards ($p < .05$) and Cabrera and Menorca lizards ($p < .05$), but again, we do not find significant differences between Mallorca and Cabrera ($p > .05$).

The analysis of relative effects shows higher values in terms of probabilities for all morphometric and scalation characters of male lizards from Cabrera, with lowest values for all characters from male lizards from Menorca (Table 7). Similar tendencies are observed in females (Table 8). In males, higher probabilities correspond to the length of intact tails, body mass, head height and pileus length of lizards from Cabrera. In females, all probabilities of effects of each variable are lower than in males, indicating a closer distance of their morphologies among the three groups of islands. Only tail lengths of Cabrera females showed a value above 0.80 (Table 8).

NMDS analysis of populations of adult males confirms the morphological divergence of lizards from Menorca, situated in the left side of the diagram (Figure 7), with a central overlap of Cabrera

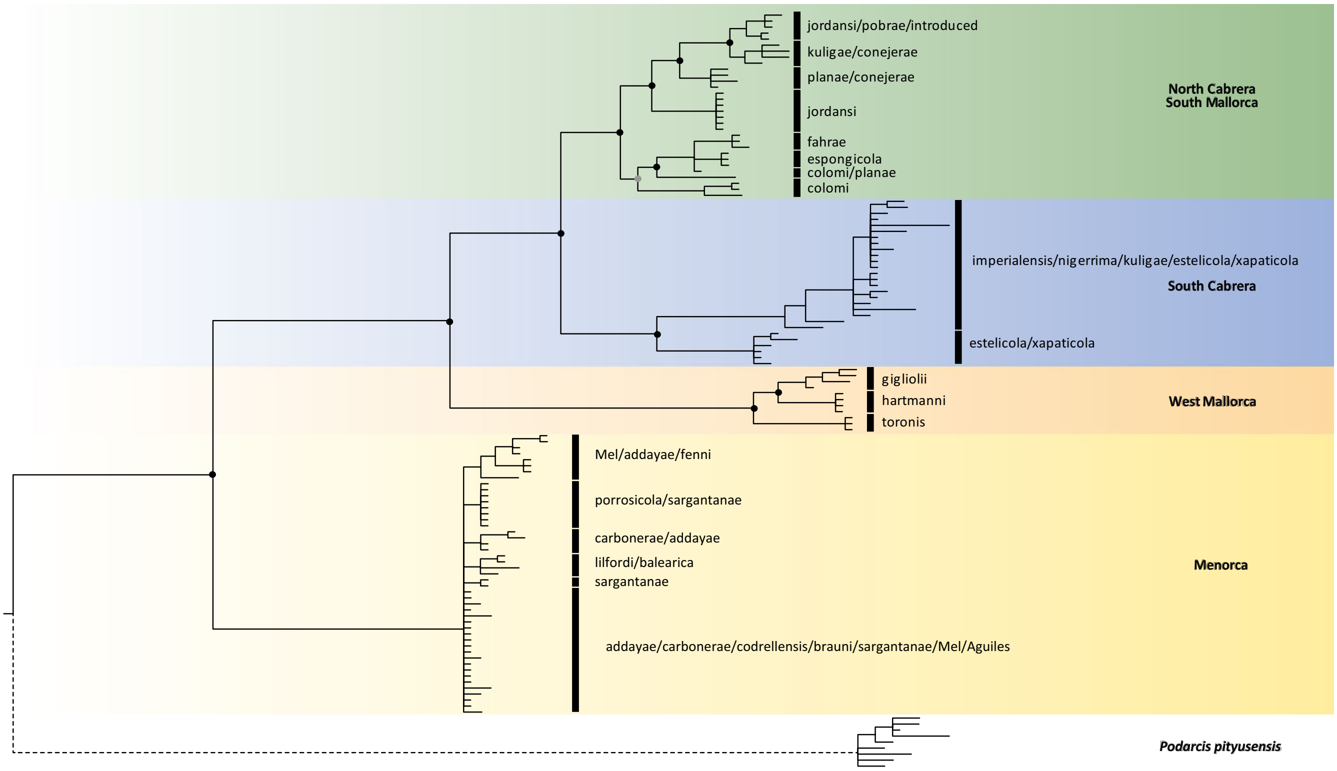

FIGURE 3 Bayesian analysis based on mtDNA concatenated dataset (2,382 bp). Clades are arranged by described subspecies, excluding "introduced" corresponding to the population of Porrassa Islet. Black dots represent a posterior probability (PP) greater than 80 and grey dot a PP smaller than 80

TABLE 2 Evolutionary divergence over sequences using p-distances among defined subspecies of *P. lilfordi* based on the four mtDNA fragments

	jordansi	introduced	colomi	fahrae	pobrae	planae	espongicola	conejerae	kuligae	xapaticola	estelicola	imperialensis	nigerrima	gigliolii	hartmanni	toronis	addayae	lilfordi	carbonerae	codrellensis	brauni	porrosicola	balearica	fenni	sargantanae
jordansi																									
introduced	0,005																								
colomi	0,007	0,008																							
fahrae	0,007	0,008	0,006																						
pobrae	0,005	0,001	0,007	0,007																					
planae	0,005	0,005	0,006	0,004	0,004																				
espongicola	0,007	0,008	0,006	0,002	0,007	0,003																			
conejerae	0,005	0,003	0,007	0,006	0,003	0,003	0,005																		
kuligae	0,012	0,012	0,013	0,013	0,012	0,012	0,013	0,012																	
xapaticola	0,014	0,014	0,013	0,013	0,013	0,013	0,013	0,013	0,010																
estelicola	0,014	0,014	0,014	0,014	0,014	0,014	0,013	0,014	0,008	0,006															
imperialensis	0,015	0,016	0,016	0,015	0,016	0,015	0,015	0,015	0,005	0,009	0,006														
nigerrima	0,014	0,015	0,015	0,015	0,015	0,015	0,014	0,015	0,004	0,009	0,006	0,001													
gigliolii	0,023	0,024	0,023	0,023	0,023	0,022	0,022	0,023	0,025	0,024	0,025	0,026	0,026												
hartmanni	0,024	0,025	0,024	0,024	0,024	0,023	0,024	0,024	0,026	0,025	0,026	0,026	0,026	0,004											
toronis	0,024	0,025	0,024	0,024	0,024	0,024	0,024	0,024	0,026	0,025	0,026	0,027	0,026	0,006	0,006										
addayae	0,026	0,027	0,027	0,027	0,027	0,026	0,027	0,026	0,028	0,028	0,029	0,030	0,030	0,032	0,033	0,033									
lilfordi	0,026	0,028	0,028	0,027	0,028	0,027	0,027	0,027	0,029	0,029	0,030	0,031	0,030	0,033	0,034	0,034	0,001								
carbonerae	0,027	0,028	0,028	0,028	0,028	0,027	0,027	0,027	0,029	0,029	0,030	0,031	0,030	0,033	0,034	0,035	0,002	0,003							
codrellensis	0,026	0,027	0,027	0,027	0,027	0,026	0,026	0,026	0,028	0,028	0,028	0,030	0,029	0,032	0,033	0,034	0,001	0,001	0,001						
brauni	0,026	0,027	0,027	0,027	0,027	0,026	0,026	0,026	0,028	0,028	0,029	0,030	0,029	0,032	0,033	0,033	0,001	0,001	0,002	0,000					
porrosicola	0,026	0,028	0,027	0,027	0,028	0,027	0,027	0,027	0,028	0,028	0,029	0,030	0,029	0,033	0,034	0,034	0,001	0,002	0,002	0,000	0,001				
balearica	0,026	0,028	0,028	0,027	0,028	0,027	0,027	0,027	0,029	0,029	0,030	0,031	0,030	0,032	0,033	0,034	0,002	0,001	0,003	0,002	0,002	0,002			
fenni	0,027	0,028	0,028	0,028	0,028	0,027	0,027	0,027	0,029	0,029	0,030	0,031	0,031	0,032	0,033	0,034	0,002	0,002	0,003	0,002	0,002	0,002	0,003		
sargantanae	0,026	0,027	0,027	0,027	0,027	0,026	0,026	0,026	0,028	0,028	0,029	0,030	0,030	0,032	0,033	0,034	0,001	0,001	0,002	0,001	0,001	0,001	0,002	0,002	

Island, Guardia and Caragol from Cabrera and Mallorca clades, respectively, and Aguiles and Aire Island from Menorca group. The right side of the diagram is occupied by lizard populations from Cabrera and Mallorca. The picture is less clear in adult females, with a right position of Mel and Mones populations from Menorca (Figure 8). Thus, NMDS diagrams only partially reflect the geographical arrangement of populations, with a better picture in adult males.

3.4 | Patterns of lizard coloration

The comparative analysis of the presence/absence of melanism indicates that this trait has a strong phylogenetic signal ($D = 0.1267$, a value significantly different from a fully random evolution, $p < .001$, and with a high probability to be the result of a Brownian evolution, $p = .98$). For males (Figure 9) and females (Figure 10), we find clear groups of melanistic and non-melanistic populations. In adult males, non-melanistic lizard from Dragonera and Cabrera are grouped with some populations from Menorca, while the remaining non-melanistic populations of Menorca are grouped together in another clade (Figure 9). Conversely, the only melanistic Menorcan population (Aire) is grouped with the remaining populations of Cabrera and Mallorca. In adult females, the grouping of populations according to dorsal colour is even clearer. A first large group separates all melanistic or almost melanistic females from Menorca (Aire Island), Mallorca and Cabrera, with the remaining non-melanistic females mixed in the second clade (Figure 10 and Tables S1–S8).

In lizard populations from Menorca, we find only one (5.88%) or two (11.76%, if we include melanistic individuals from Aguiles Islet) melanistic populations. In populations of Mallorca and Cabrera, melanism is dominant among populations, with 7 of 9 extant native populations (77%) in Mallorca coastal islands and 10 of 14 populations in Cabrera archipelago (71.43%). In fact, in Cabrera Island, the subpopulations of Morro den Tià and Cabrera far show also a clear tendency for melanism and were grouped by colour analysis together with melanistic populations in the case of adult females (Figure 10), and in a lesser measure in the case of adult males (Figure 9), where there is a group of melanic lizards (Esclatasang, Malgrat Gran and Guardia) and a group of very dark lizards (Cabrera far, Morro den Tià and Toro islets). This result indicates a clear tendency for melanism linked with the isolation in small islets and concomitant demographic effects of that isolation.

4 | DISCUSSION

4.1 | The evolutionary framework of the Balearic lizard

The Balearic lizard was a member of the first post-Messinian faunal assemblage reported from Menorca Island (Bover et al., 2008, 2014). This fauna spread during the Early and Middle Pliocene, including a lizard (*Podarcis* sp., Bailón, 2004) which could be the ancestor of

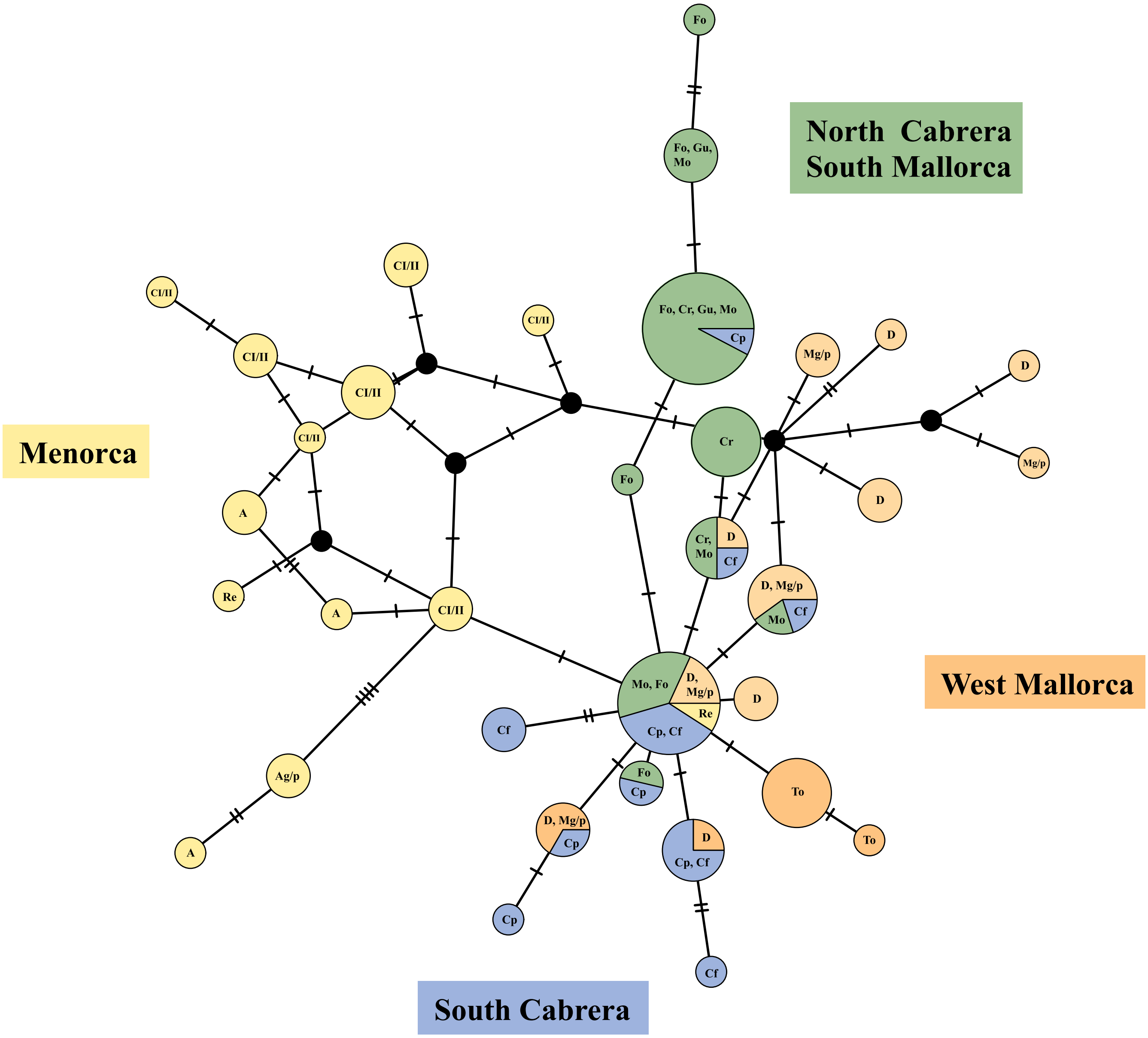

FIGURE 4 TCS network based on phased nuclear gene MC1R for some populations of the four well-delimited clades: (1) North Cabrera and South Mallorca (green), (2) South Cabrera (blue), (3) West Mallorca (orange) and (4) Menorca (yellow). Every population is represented by its abbreviation (Cr: Colomer, Fo: Foradada, Gu: Guardia, Mo: Moltona, Cf: Cabrera_Far, Cp: Cabrera_Port, To: Toro, D: Dragonera, Mp/g: Malgrat Petit (Mp) and Malgrat Gran (Mg), A: Aire, Re: Rei, CI/II: Codrell Gran (CI) and Codrell Petit (CII) and Ag/p: Addaia Gran and Addaia Petita)

Podarcis lilfordi. In Mallorca Island, some remains of a lizard have been found in deposits from the Early Pleistocene to the Holocene and were described as *Podarcis* sp. Some of the additional remains from Middle Pleistocene to Holocene were then ascribed to *Podarcis lilfordi* (Bailón, 2004; Bover et al., 2014). Hence, fossil remains of the Balearic lizard were found from a more recent period in Mallorca than in Menorca. These results are in agreement with the cladogenesis that separated Menorca and Mallorca + Cabrera clades some 2.6 ma ago, that is, during the beginning of the Quaternary, at a significantly cooling period (Brown et al., 2008 and references therein). During that period, many cladogenetic events occurred around the world (Guo, He, & Zhang, 2005; Paulo, Dias, Bruford, Jordan, & Nichols, 2001; Veith, Kosuch, & Vences, 2003).

Even if there is a large variability among microinsular populations at the whole range of the geographical distribution of the Balearic lizard, we found significant differences between Menorca and Mallorca and between Menorca and Cabrera populations, while less clear differences are present in morphology between Cabrera and Mallorca. This pattern is in agreement with genetic information. Non-metric multidimensional scaling (NMDS) showed a similar picture, with most of the populations from Menorca coastal islets separated from those of Mallorca islets and Cabrera archipelago

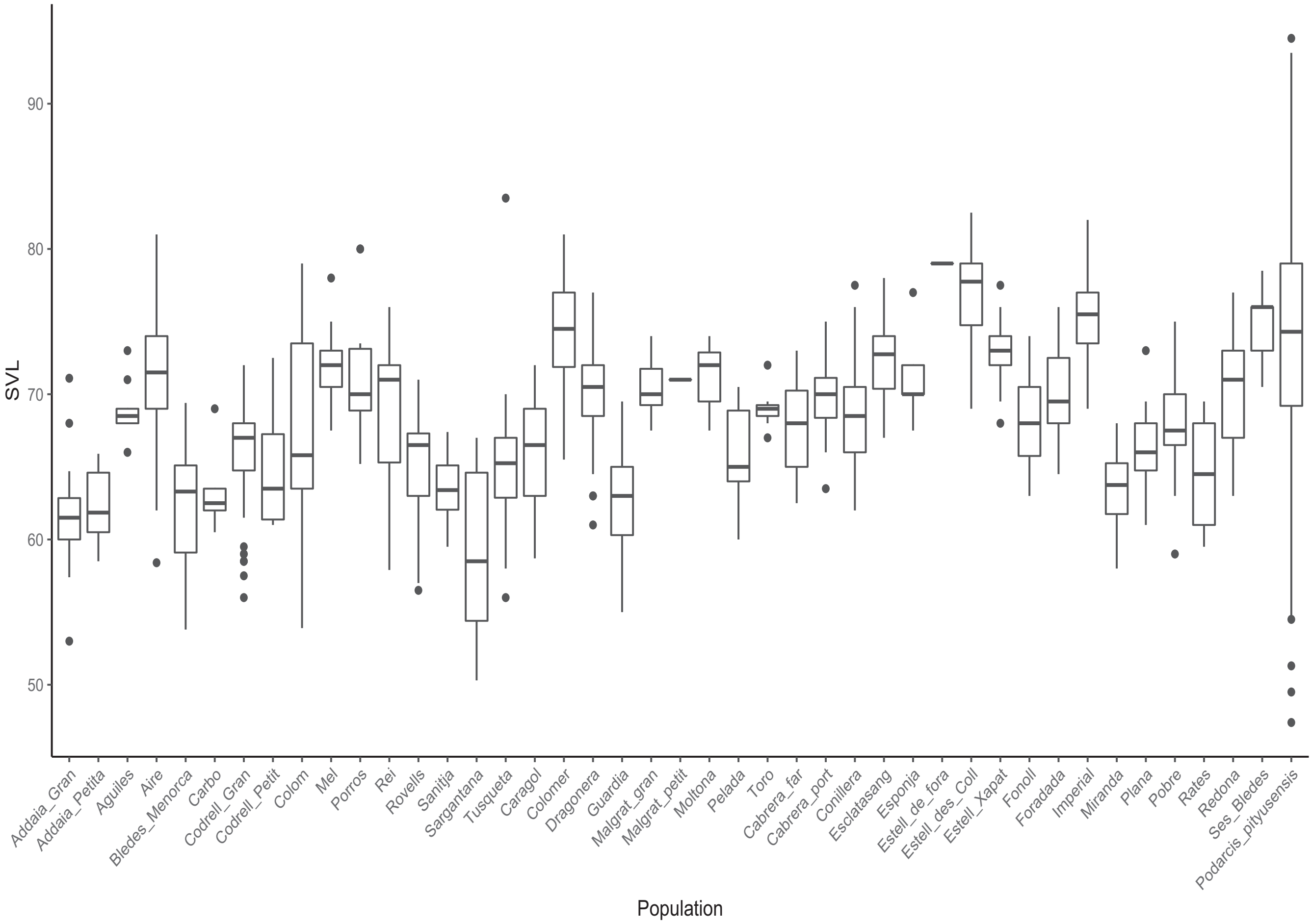

FIGURE 5 Boxplots of SVL of adult males from Balearic lizard populations ranged by archipelagoes. From the left to the right, Menorca, Mallorca and Cabrera. See the increase of body size from Menorca to Cabrera

(Figure 7). That separation is lower in the case of adult females (Figure 8), even if also in females the NMDS analysis showed that the majority of Menorca populations are ranged in the left part of the NMDS diagram.

4.2 | Influence of climate in morphological features

Bergmann's rule (Bergmann, 1847) proposes that in birds and mammals, body size is greater in high latitudes for adaptive reasons: the lower surface–volume ratio in endotherms of greater body size confers thermoregulatory advantages, as heat losses are minimized in colder environments. The issue is more complex in ectotherms. In Squamata, the existence of an inverse Bergmann's rule has been proposed (Oufiero, Gartner, Adolph, & Garland, 2011 and references therein), with larger body sizes in lower latitude individuals, that is, in warmer environments (Ashton & Feldman, 2003). But there are also data that demonstrate an opposite trend in some groups of reptiles (Sears & Angilletta, 2004). In *P. lilfordi*, there is a clear trend to larger body sizes in lower latitude populations of Mallorca and Cabrera, confirming the expectations of an inverse Bergmann's rule. However, several factors that we do not address here can also influence body size variation, as dietary shifts, intraspecific competition or reduced predation pressure (Uller et al., 2019).

Numerous studies have addressed the adaptive value of the size and number of scales in relation to climatic variables (Arnold, 1973; Bogert, 1949; Calsbeek, Knouft, & Smith, 2006; Hellmich, 1951; Soulé, 1966; Soulé & Kerfoot, 1972). In some cases, lizards from larger islands showed fewer and larger scales, a trend interpreted as an adaptive response to the higher average temperatures on larger islands. Thus, in localities where climate is warmer, we can expect larger scales. In addition, the experimental work of Regal (1975) supported the hypothesis that enlarged scales of lizards inhabiting warmer habitats may function as heat shields. In this way, scales can be considered a protection against abrasion and water loss (Alibardi, 2003; Walker & Liem, 1994). Thus, populations from arid habitats tend to have fewer and larger scales to reduce skin exposure and the amount of evaporative water loss (see, e.g., Hu et al., 2019 and references therein). A fewer number of scales reduce skin exposure, while a larger number of smaller scales increase the exposed skin surface and, consequently, the rate of dehydration (Calsbeek et al., 2006; Neilson, 2002).

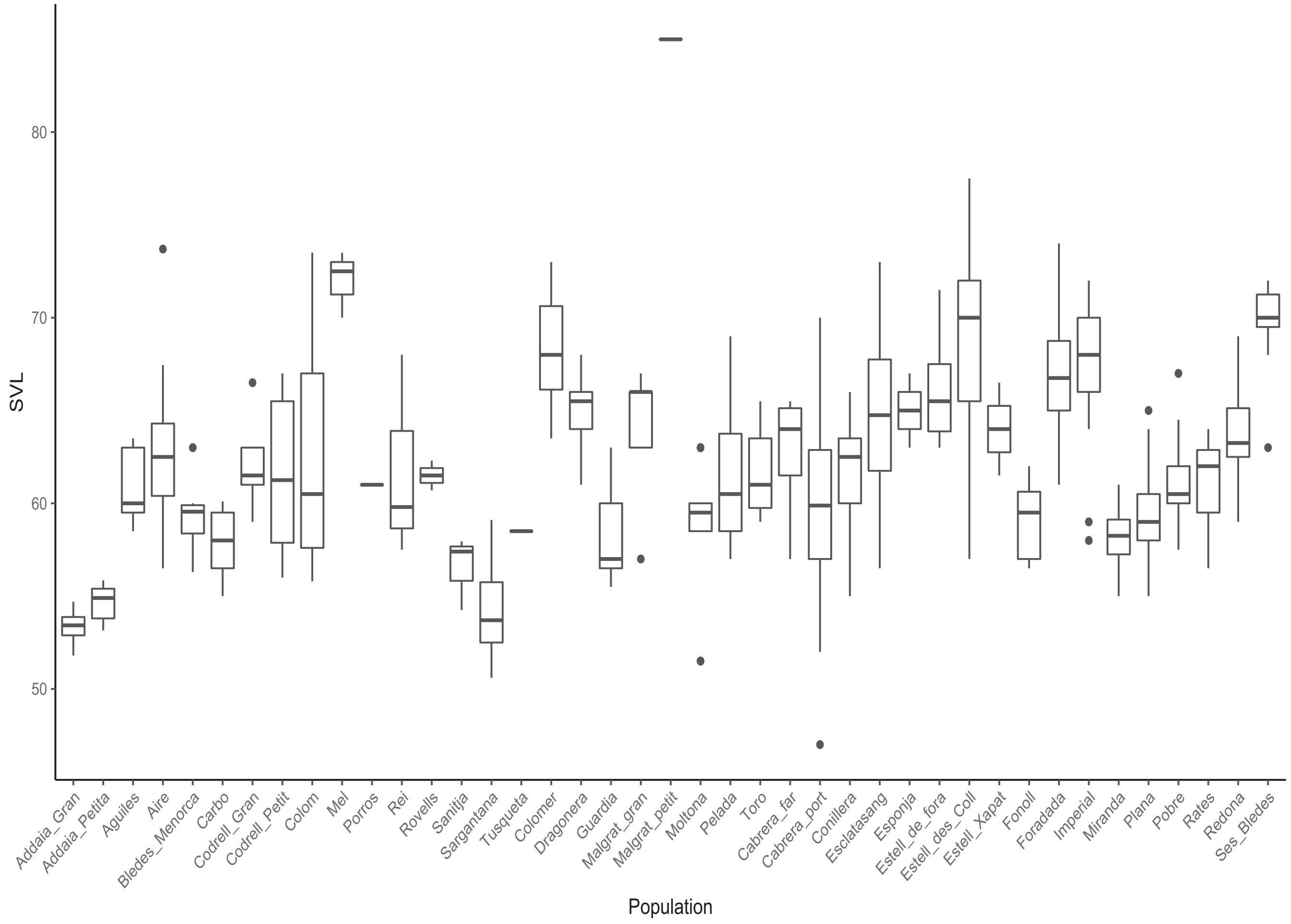

FIGURE 6 Boxplots of SVL of adult females from Balearic lizard populations ranged by archipelagoes. From the left to the right, Menorca, Mallorca and Cabrera

TABLE 3 Results of linear models comparing all Balearic lizard populations, with phylogenetically independent contrasts, each log-transformed morphometric trait, using SVL as covariate. Adult males

Trait	Model	Adjusted R^2	$F_{1,24}$-value	p-Value
Pileus length	logPL ~ logSVL	.3426	14.03	.001
Head height	logHH ~ logSVL	.02081	1.531	.2279
Pileus width	logPW ~ logSVL	.3632	15.26	.000667
Hindleg length	logHLL ~ logSVL	.6372	44.9	6.246×10^{-7}
Lamellae	loglam ~ logSVL	.1977	7.16	.01322
Femoralia	logfem ~ logSVL	.06596	2.765	.1093
Gularia	loggul ~ logSVL	.09029	3.481	.07434
Ventralia	logven ~ logSVL	.1721	6.196	.02013
Collaria	logcol ~ logSVL	.03918	2.019	.1682
Dorsalia	logdorsalia ~ logSVL	−.01892	0.5358	.4713

In *P. lilfordi*, all populations from Menorca share a significantly higher number of dorsal, ventral and gular scales than lizards from Mallorca and Cabrera. Mallorca and Cabrera lizards are under a significantly more arid climate than those from Menorca, confirming this expectation. However, this is only the present-day situation. The separation of Menorca and Mallorca + Cabrera took place 2.6 m.y. ago, coinciding with the transition from Pliocene to Pleistocene periods (Walker, Geissman, Bowring, & Babcock, 2018). Since the Late Pliocene/Early Pleistocene, the climatic conditions of Balearic Islands were predominantly humid-Mediterranean (Wagner, Eckmeier, Skowronek, & Günster, 2014). Then, during the transition from Late Pleistocene to the Holocene, when took place the final isolation of lizards from Cabrera populations, it was a rising of temperatures, with a warm-temperate climate and dry summer/humid winter seasons (Wagner et al., 2014), coinciding with the decrease of the number of dorsal scales in Mallorca and Cabrera populations.

TABLE 4 Results of linear models comparing all Balearic lizard populations, with phylogenetically independent contrasts, each log-transformed morphometric trait, using SVL as covariate. Adult females

Trait	Model	Adjusted R^2	$F_{1,24}$-value	*p*-Value
Pileus length	logPL ~ logSVL	.1406	5.091	.03344
Head height	logHH ~ logSVL	.5368	29.97	1.296×10^{-5}
Pileus width	logPW ~ logSVL	.1021	3.844	.06163
Hindleg length	logHLL ~ logSVL	.153	5.515	.02743
Lamellae	loglam ~ logSVL	−.00801	0.8013	.3796
Femoralia	logfem ~ logSVL	.002392	1.06	.3135
Gularia	loggul ~ logSVL	.1874	6.765	.01567
Ventralia	logven ~ logSVL	−.03289	0.204	.65555
Collaria	logcol ~ logSVL	.1533	5.527	.02727
Dorsalia	logdorsalia ~ logSVL	.3042	11.93	.002063

TABLE 5 Nonparametric inference of multivariate samples comparing adult male lizards from Menorca, Mallorca and Cabrera groups

Results	Test statistic	*df*1	*df*2	*p*-Value	Permutation test *p*-value
ANOVA type test *p*-value	6.185	6.279	44.7713	0	.002
McKeon approximately for the Lawley–Hotelling test	5.413	26.000	81.5385	0	.000
Muller approximately for the Bartlett–Nanda–Pillai test	4.314	27.167	102.8553	0	.000
Wilks' lambda	4.878	26.000	98.0000	0	.000

Note: For each test statistic, we give its statistical value, numerator (*df*1) and denominator (*df*2) degrees of freedom and *p*-values using both, *F* approximation and permutation (randomization) methods (see more details in the text).

TABLE 6 Nonparametric inference of multivariate samples comparing adult female lizards from Menorca, Mallorca and Cabrera groups

Results	Test statistic	*df*1	*df*2	*p*-Value	Permutation test *p*-value
ANOVA type test *p*-value	2.101	6.953	46.0228	.063	.103
McKeon approximately for the Lawley–Hotelling test	3.961	26.000	81.5385	.000	.000
Muller approximately for the Bartlett–Nanda–Pillai test	3.303	27.788	52.0683	.000	.000
Wilks' lambda	3.657	26.000	48.0000	.000	.000

Note: For each test statistic, we give its statistical value, numerator (*df*1) and denominator (*df*2) degrees of freedom and *p*-values using both, *F* approximation and permutation (randomization) methods.

4.3 | Melanism of the Balearic lizard

Melanistic lizards appeared in several population of the Balearic lizard, and our comparative analysis of dorsal colouration grouped all melanistic populations, in spite of their geographical situation. We can speculate that melanistic colouration confers relevant adaptive advantages to its holders that would explain its appearance in different lineages of the Balearic lizard. It is interesting to note that in the clade of *P. lilfordi* from Menorca, only Aire Island, the oldest coastal islet (Pérez-Mellado, 2009), has a melanistic population, while in supposedly derived populations of Mallorca lizards, melanism has been probably an adaptive response fairly common, both on the coast of Mallorca and in Cabrera archipelago. In fact, our results indicate that melanism is an evolutionary trait with a strong phylogenetic signal.

TABLE 7 Estimated nonparametric relative effects, showing intergroup differences as probabilities for adult males (see more details in the text)

Group	SVL	Tail	Weight	PL	HH	HW	HLL	LAM	FEM	GUL	DOR	VENT	COLL
Cabrera	0.88542	0.89062	0.96094	0.88281	0.86198	0.85156	0.96615	0.48958	0.79948	0.19531	0.27604	0.44531	0.42188
Mallorca	0.74062	0.84766	0.78750	0.65781	0.24062	0.63203	0.66641	0.47969	0.40547	0.51094	0.50391	0.63984	0.54766
Menorca	0.43015	0.40885	0.41651	0.44654	0.52956	0.45343	0.43995	0.50460	0.50092	0.51578	0.51241	0.47580	0.49525

TABLE 8 Estimated nonparametric relative effects, showing intergroup differences as probabilities for adult females

Group	SVL	Tail	Weight	PL	HH	HW	HLL	LAM	FEM	GUL	DOR	VENT	COLL
Cabrera	0.52137	0.80342	0.76496	0.50427	0.45726	0.50000	0.59402	0.65385	0.55556	0.13248	0.27778	0.38034	0.47863
Mallorca	0.71635	0.79487	0.68109	0.64423	0.29808	0.68750	0.67628	0.49679	0.34936	0.46795	0.52724	0.67308	0.52724
Menorca	0.43590	0.38324	0.41987	0.45833	0.56227	0.44643	0.43956	0.48443	0.53709	0.54853	0.51603	0.46337	0.49451

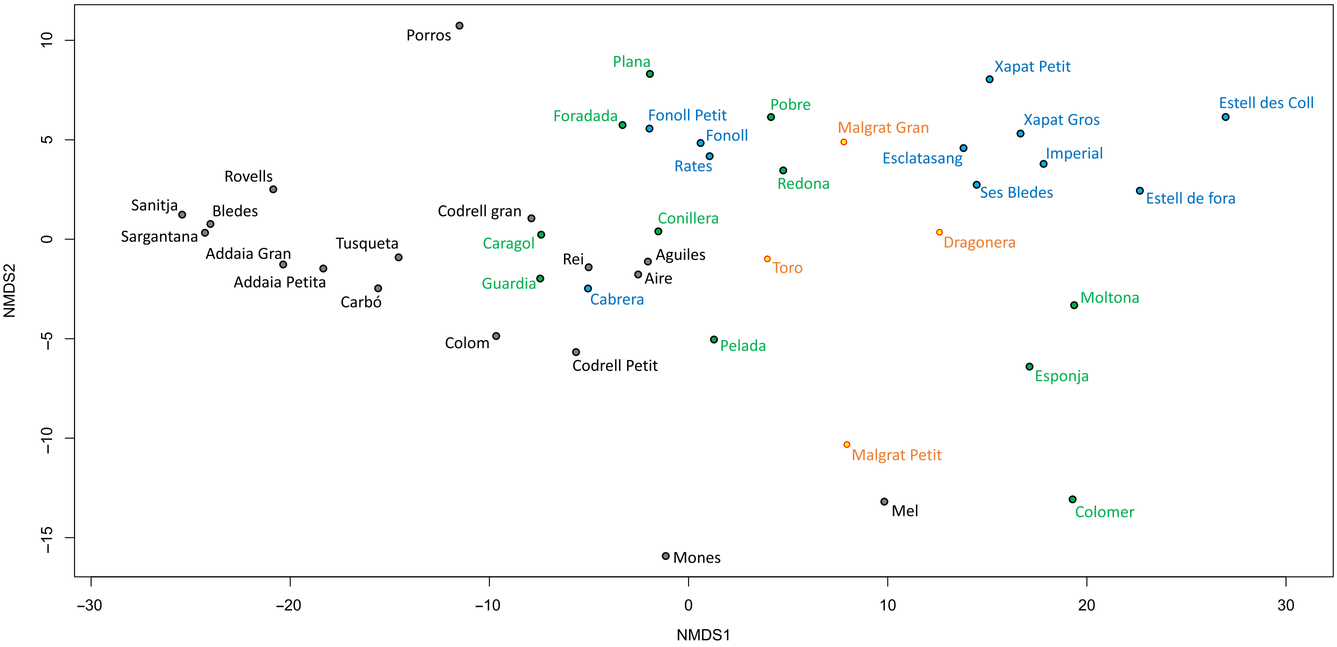

FIGURE 7 Non-metric multidimensional scaling biplot of a Euclidean dissimilarity matrix of log-transformed morphometric data from 43 populations of adult males of the Balearic lizard. Black: populations from Menorca, green: populations from Northern Cabrera and Southern Mallorca, blue: populations from Southern Cabrera and orange: populations from Western Mallorca. Colours of major clades according to phylogeny of *P. lilfordi* from Figure 3

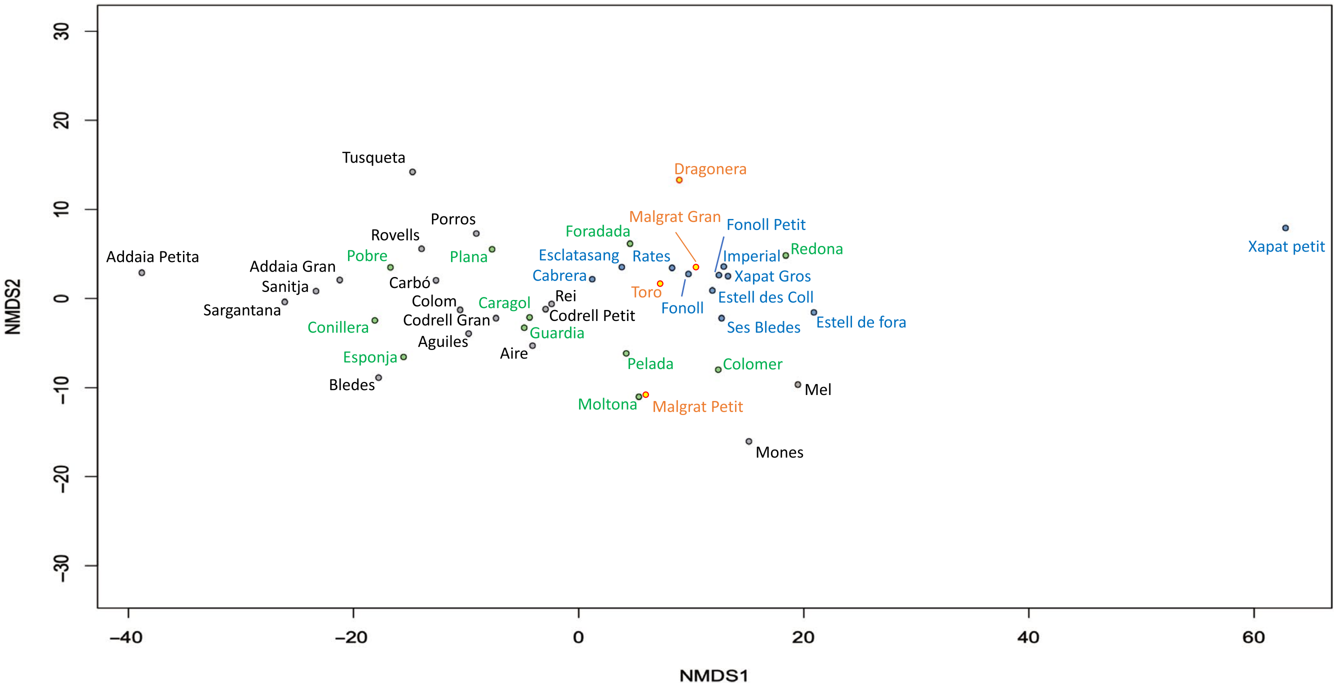

FIGURE 8 Non-metric multidimensional scaling biplot of a Euclidean dissimilarity matrix of log-transformed morphometric data from 43 populations of adult females of the Balearic lizard. Colours as in Figure 7

4.4 | Taxonomic arrangement of *P. lilfordi* according to genetic and morphological traits

As expected, if we compare the arrangement of described subspecies of the Balearic lizard with genetic, morphological and colouration results, it is clearly a lack of congruence of present-day accepted subspecies (Table 1). In this study, we only employed mtDNA and one nuclear gene and we were unable to find a congruent arrangement of lizard populations. It could be interesting in future studies to explore small-scale genetic differentiation among populations with more robust and faster evolving genomic markers. In any case, most of the described subspecies are not supported by genetic

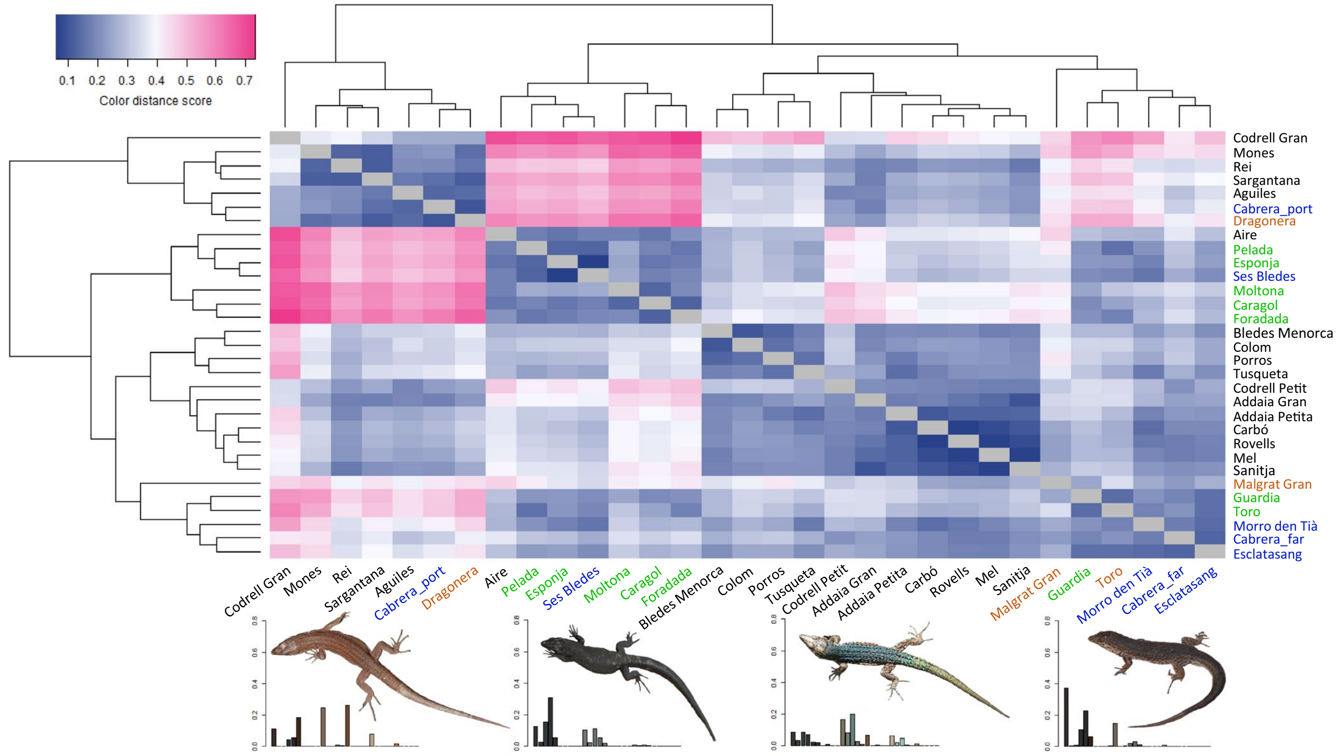

FIGURE 9 Heatmap of adult males of *P. lilfordi* obtained from dorsal digital images. The dorsal image pixels have been grouped into a 5 × 2 × 3 HSV colour space. Lower values (in blue) indicate greater dorsal colouration similarity of populations and higher values (in pink), a greater distance among them. At the base of the figure, we show four histograms and pictures of males from Aguiles, Aire, Porros and Toro populations, as a representation of each of the main clades of the heatmap. The histograms show the percentage of pixels of each of the 5 × 2 × 3 = 30 colour categories employed in the analysis. The first five bars of each histogram correspond to the black colour (see, e.g., its dominance in Aire and Toro populations). In turn, bars 6, 11, 16, 21 and 26 correspond to brown colours, from the darkest to the lightest (present, for example, in the case of Aguiles). The rest of intermediate bars of the histograms correspond to green and blue tones, clearly important in males from Porros Islet (see more details in the text). Colours of population's labels as in Figure 7

divergence of clades (Figures 2 and 3), nor by morphometric and colouration traits. Moreover, in morphology we found striking differences among the three groups of populations, Menorca, Mallorca and Cabrera, but with an arrangement unrelated with described subspecies. In the case of colouration, a trait commonly employed in classical subspecific diagnoses and descriptions, the arrangement matched populations according with dominant dorsal colours, regardless of their geographical location or their subspecific membership. However, it is interesting to note that, at least in adult males (Figure 9), there is a clade of non-melanistic populations of Menorca, well separated from a mixture of Mallorca and Cabrera populations, melanistic and non-melanistic, in the right side of the diagram. In females, an isolated clade is formed by the bulk of melanistic populations of Cabrera, Mallorca and Aire Island (Figure 10).

Some cases merit a particular comment. In Menorca, the populations of Codrell Gran and Codrell Petit, described as *P. lilfordi codrellensis*, are very close to each other, barely separated by an arm of sea of no more than fifteen metres and widely isolated from the remaining populations of Menorca (Figure 1). However, in the case of males, their morphological distance is very noticeable. It is also the case of males from Malgrat Gran and Malgrat Petit islets, in the south-western coast of Mallorca. These populations were collectively described as *P. lilfordi hartmanni* and forming, from the genetic viewpoint, a clear monophyletic clade (Figure 3). Two lizard populations from Menorca remain undescribed, from Mones and Mel islets (Table 1). Both are morphologically well separated (Figures 7 and 8), but with a strong genetic similarity to populations apparently unrelated, as in the case of Addaia Gran and Mel (Figures 2 and 3). Some of the described subspecies of *P. lilfordi* are morphologically and genetically differentiated, as those from Dragonera, Malgrat islets and Toro in Mallorca coast (Figures 2 and 3). However, it is not the case of several populations of the Balearic lizard described as different subspecies, as *P. lilfordi lilfordi* from Aire Island and *P. lilfordi balearica* from Rei Island (Terrasa, Pérez-Mellado, et al., 2009 and this study).

Insular populations of terrestrial vertebrates encounter a physical impediment to gene flow between populations, and it is therefore expected that such populations may diverge in isolation (Mayr, 1963). Being frequently smaller in population size, the fixation of neutral genes is likely to take place in a faster way than in continents. However, use of neutral genes might reasonably yield no difference among some populations, even if these populations differ markedly in phenotype. It is useful to estimate the historical patterns of divergence among populations (O'Brien & Mayr, 1991), and population differences had a high

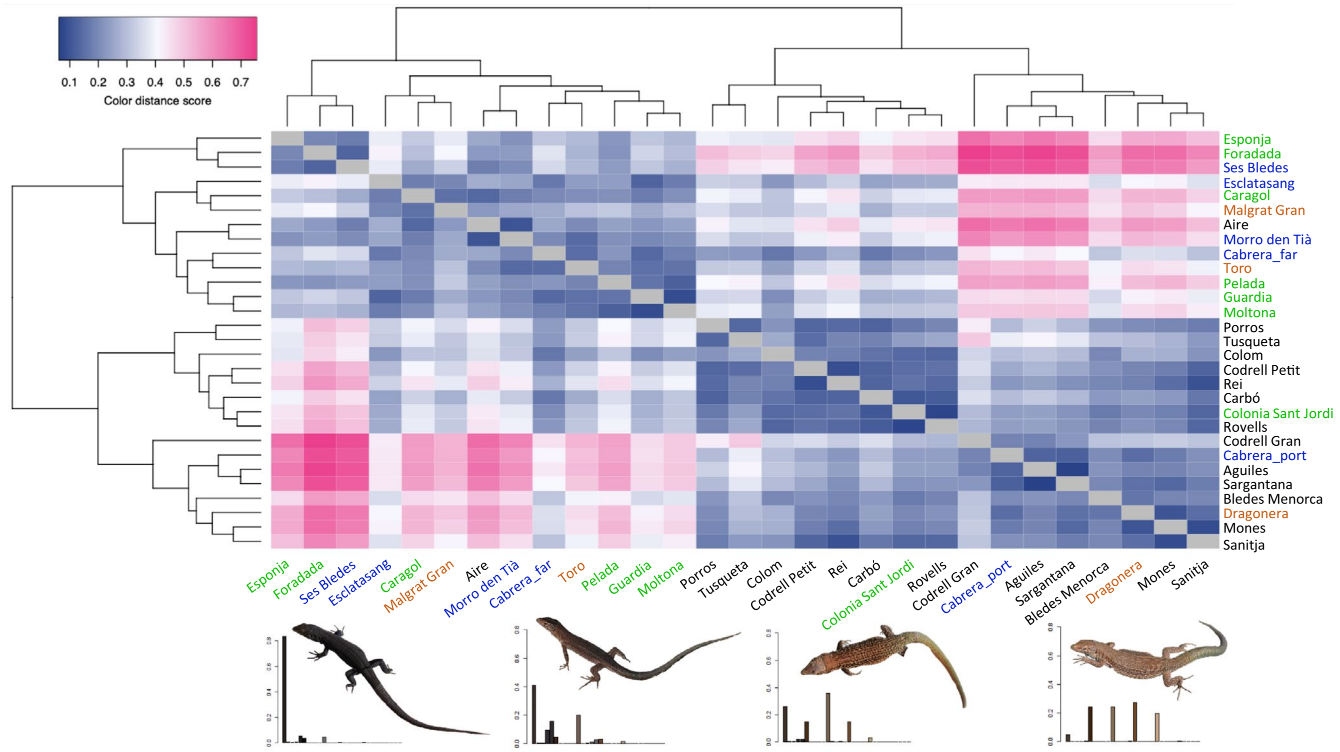

FIGURE 10 Heatmap of adult females of *P. lilfordi* obtained from dorsal images. As a representation of each of the main clades of the upper heatmap, we include four pictures from Malgrat Gran, Toro, Rovells and Cabrera Port populations, with their corresponding histograms. Colours of population's labels as in Figure 7

conservation utility, as proxies for the sub-structure found within species (Phillimore & Owens, 2006). However, from a cladistic viewpoint, the usefulness of subspecies is a controversial issue (Vinarski, 2015; Wilson & Brown, 1953; Winker, 2010). As classificatory units, subspecies are not useful in comparative studies, because subspecies are groups of populations defined by hypothetical biological relations or geographical distributions, rather than by homology, that is, shared derived characteristics (Ebach & Williams, 2009). In this context, the use of subspecific categories is extremely difficult.

Would it make sense to describe new subspecies for such populations, solely based on morphological differences? From our viewpoint, if that is the question, we should apply the same criteria to describe as different subspecies all the populations separated by morphological differences, without paying attention to genetic differences between them. On the contrary, if we apply strictly genetic criteria, the proximity of numerous populations would force us to invalidate numerous subspecies previously described only with morphological criteria, but unsustainable from a genetic perspective.

4.5 | The application of evolutionary significant units to Balearic lizards

Solving this dilemma can only be achieved abandoning the very concept of subspecies in the case of the Balearic lizard. As an alternative, we propose the use of evolutionary significant units (ESUs, Moritz, 1994, 2002). In addition to its original genetic definition (Moritz, 1994), an ESU was also defined as a group of organisms substantially reproductively isolated that represent an important component in the evolutionary legacy of the species (Waples, 1995). This is the case of every microinsular population of *P. lilfordi*, physically isolated even from closest islets. An ESU can often correspond to species or subspecies boundaries in classical taxonomy, but in some cases, it can be extended to isolated populations (Karl & Bowen, 1999).

The ESU has been proposed as a unit of conservation (Moritz, 1994; Vogler & DeSalle, 1992; Waples, 1995). We have to evaluate each population along two axes of diversity which might be described as molecular genetics and adaptive morphology. Adaptive diversity is the diversity of morphological and ecological traits we observe today in different populations of *P. lilfordi*. This diversity represents the raw material for future evolution. Consequently, we have to recognize every isolated population of the Balearic lizard as an ESU (Pérez-Mellado, 2008).

In this study, we have only addressed the description and possible origin of some of the morphological features of the Balearic lizard. However, it remains to elucidate the origin and causality of a myriad of ethological and ecological features that, in one way or another, characterize each population (Pérez-Mellado, 2009 and references therein). This is the task that implies the complete preservation of the microevolutionary process in the islets where the Balearic lizard survives for thousands of years. The potential taxonomic identity of each population is not its most relevant aspect, and it is paradoxical that it is the main aspect that has attracted the attention of experts and amateurs.

In short, in each population of *P. lilfordi* we observe an evolutionarily independent history linked to different ecological conditions, extremely variable population sizes and availability of trophic resources, or the presence of particular competitors and predators. This situation has led to adaptive responses, in many cases unique to each population, that are resolved in morphological, ethological and ecological features so far only observed in that populations. It is impossible to foresee what the future knowledge of numerous populations holds for which there is no reliable data on ecology and behaviour of their lizards.

Consequently, the recognition of all the populations as ESU implies that each and every one of the populations of the Balearic lizard is fully relevant to its conservation. We do not want to protect only the product of evolution, that is, the extraordinary variability of the polytypic Balearic lizard today observed. We want to preserve the future process of evolution of the Balearic lizard (Moritz, 2002).

The Balearic lizard is listed by IUCN as endangered (criteria: B1ab (ii) + 2ab (iii), Pérez-Mellado & Martínez-Solano, 2009). Paradoxically, the regional conservation managers of the Balearic Islands consider that the species is only vulnerable (Viada, 2006). Probably, this unrealistic assessment derives from the fact that some specific populations, such as those of the Dragonera or Cabrera Islands, have a large number of lizards. The complete preservation of all the extant populations will be the only guarantee of conservation of the unique evolutionary process of this species (Pérez-Mellado, 2008).

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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BIOSKETCH

Our research team come from two countries and three different universities. For the last 20 years, we have carried out research into the phylogeography, evolution, ecology and behavioural ecology of insular lizards from Western Mediterranean basin. Our approach is multidisciplinary, as we address the study of insular populations of reptiles using molecular techniques, morphological analyses and the study of the most relevant ecological and behavioural traits. We are especially interested in the adaptive features that could explain the survival of insular lizards in ecosystems that provide extreme environmental conditions and in which they appear to survive the pressure from human occupation that began 5,000 years ago.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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