ORIGINAL ARTICLE

Biogeographical crossroad across the Pillars of Hercules: Evolutionary history of *Psammodromus* lizards in space and time

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Abstract

Aim: To infer the biogeographical and evolutionary history of the Western Mediterranean *Psammodromus* lizards with the aim of assessing the role of vicariance and dispersal on the cladogenetic events within the palaeogeological dynamics of the Strait of Gibraltar.

Location: North Africa and Western Europe.

Methods: We built a dataset including all six species of *Psammodromus* using mitochondrial (*12S, cytb, nd4*) and nuclear (*acm4, mc1r, pomc*) gene fragments. Species tree and concatenation methods were used to infer phylogenetic relationships and divergence times. Phylogenies were used for biogeographical inference using S-DIVA, DEC and BBM.

Results: *Psammodromus* probably originated in Iberia, with *P. algirus* diverging early. The ancestor of the African *P. blanci* and *P. microdactylus* dispersed to Africa through the Betic-Rif massif, *c.* 10 Ma. The cladogenetic events within Africa and Iberia were probably due to vicariance mediated by habitat and climatic changes at the end of the Miocene (*P. blanci* and *P. microdactylus*) and during the Pliocene (*P. occidentalis*, *P. hispanicus* and *P. edwardsianus*). *Psammodromus algirus* shows three lineages, two in Iberia and one in Africa, the latter originated following a transmarine dispersal during the Middle Pleistocene (1.5 Ma).

Main conclusions: Over-sea dispersal has played a major role in intercontinental exchange and divergence in *Psammodromus*, with two dispersal events towards Africa that occurred 10 and 1.5 Ma resulting in the African lineages. This study, combined with previous literature, provides compelling evidence that major biotic exchanges took place across the Strait of Gibraltar well before or long after the land connection during the Messinian Salinity Crisis (MSC, 5.9–5.33 Ma). These findings suggest caution in the application of the relatively short event of Atlantic flooding at the end of the MSC as cause for divergence in molecular clock calibrations, which is a popular approach in literature.

KEYWORDS
dispersal, Iberian Peninsula, Lacertidae, Messinian Salinity Crisis, North Africa, phylogeography, species tree, Strait of Gibraltar, transmarine colonization, vicariance

1 | INTRODUCTION

Biotic exchange between continents through vicariance and dispersal has classically been the focus of many biogeographical studies. The main faunal exchanges of North Africa have been with the Arabian Peninsula and the Levant through the Sinai in the east and with south-western Europe across the Strait of Gibraltar in the west. While dispersal has played a major role in the stable land bridge at the eastern crossroad (e.g Carranza, Arnold, Geniez, Roca, & Mateo, 2008; Metallinou et al., 2015; Tamar et al., 2016) the history of intermittent connections at the Strait of Gibraltar has created opportunities for both vicariance and dispersal to contribute to the biotic exchange between North Africa and Western Europe (e.g. Carranza et al., 2008; García-Vázquez et al., 2016; Jaramillo-Correa et al., 2010).

The Pillars of Hercules, as the Strait of Gibraltar has been known since ancient times, is an excellent setting for biogeographical inference, due to its well-known complex geological history (Figure 1). Briefly, in the middle Oligocene, the Hercynian belt detached from Iberia and broke into smaller blocks, including the Betic-Rif, which drifted southwards until reaching its final position in the Strait of Gibraltar, 10 Ma. Here, from the Middle Miocene, tectonic uplift caused progressive land emergence and reduction in seaways (Braga, Martin, & Quesada, 2003; Duggen, Hoernie, van den Bogaard, Rupke, & Morgan, 2003; Rosenbaum, Lister, & Duboz, 2002a), until the connection between the Mediterranean and the Atlantic was maintained by the Betic, Guadalhorce and the Rif corridors. The Betic corridor closed approximately 10 Ma and re-opened 8–9 Ma. As the sea level gradually decreased, this corridor closed definitively (Krijgsman et al., 2000), followed by the Guadalhorce (Pérez-Asensio, Aguirre, Schmiedl, & Civis, 2012) and the Rif corridors, creating a land bridge between Africa and Iberia (Krijgsman & Langereis, 2000), setting in motion the Messinian Salinity Crisis (MSC), from 5.96 to 5.33 Ma. The MSC ended abruptly with the formation of the Strait of Gibraltar, which remained open until the present (Duggen et al., 2003), although with varying extent. During the Pleistocene glaciations the sea level regressions shortened the distance between Africa and Iberia from the current 14 km to 4–5 km, with newly-formed islands available as stepping-stones for transcontinental colonizations of terrestrial species (Zazo, 1999).

As a consequence of this complex geological history, multiple dispersal–vicariance events took place across the Strait of Gibraltar, profoundly shaping genetic and demographic patterns of a wide number of taxa. Before the formation of the Strait of Gibraltar, the Betic-Rif Massif acted as an agent for dispersal–vicariance for taxon differentiation. Both the first closure of the Betic corridor, 10 Ma, and the MSC, 5.33 Ma, led to the dispersal of taxa in the area of the sub-Betic region and between Iberia and North Africa, respectively, whereas the re-opening of the Betic-corridor and the Strait of Gibraltar triggered the divergence of populations on both sides of the Strait (e.g. Carranza et al., 2008; Martínez-Solano, Gonçalves, Arntzen, & García-París, 2004; Velo-Antón et al., 2012). Transmarine colonizations long after the MSC had finished have also been
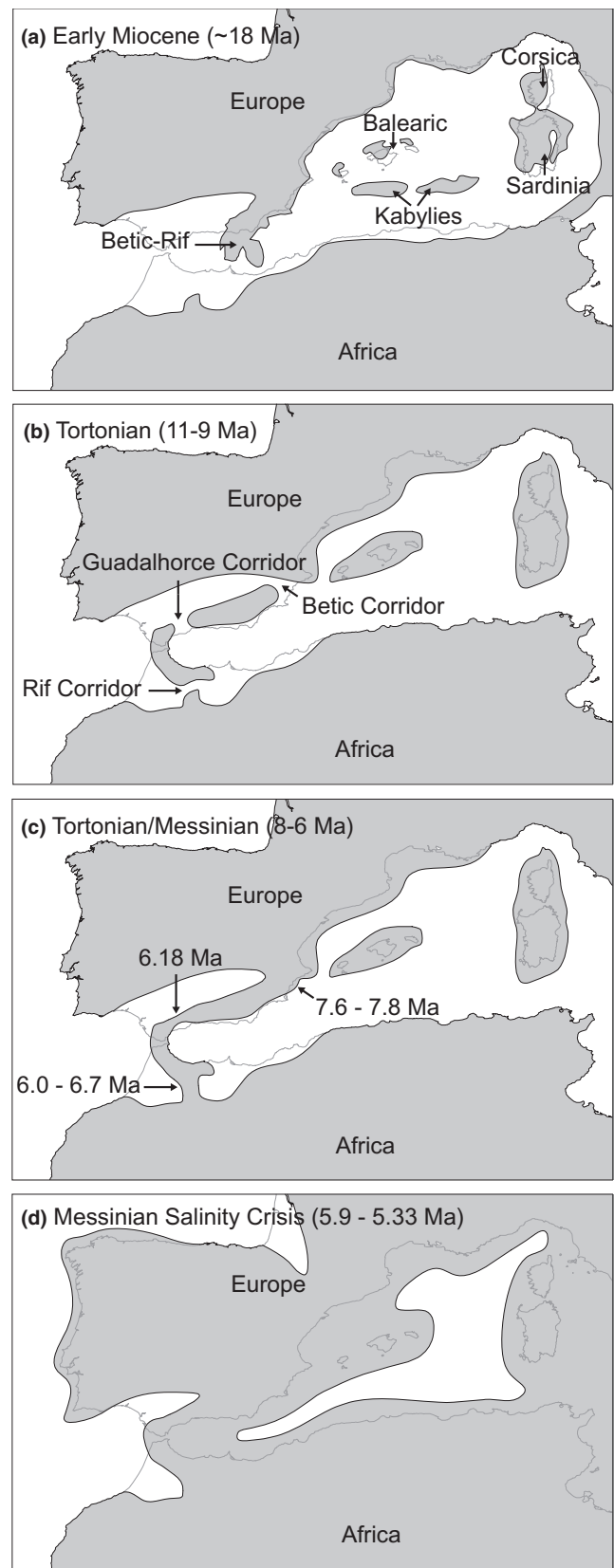

FIGURE 1 Simplified palaeogeological history of the Strait of Gibraltar area from the Middle Miocene (18 Ma) until the Messinian Salinity Crisis (adapted from Rosenbaum et al., 2002a and Paulo et al., 2008)

reported (Carranza, Arnold, & Pleguezuelos, 2006; Carranza, Arnold, Wade, & Fahd, 2004; Carranza, Harris, Arnold, Batista, & Gonzalez de la Vega, 2006; Carranza et al., 2008; Harris, Batista, Carretero, & Ferrand, 2004).

Given the intermittent land connections between Iberia and North Africa during the Miocene and varying extent of sea channels due to marine sea level oscillations during the Pleistocene, biogeographical inference across the Strait of Gibraltar has been documented as if it were a puzzle through time. The legacy of ancient biogeographical events is currently visible in distribution patterns at high taxonomic levels, generally at the genus level (Carranza et al., 2008; García-Vázquez et al., 2016; Sampaio, Harris, Perera, & Salvi, 2014), whereas more recent processes underlie the phylogeographical patterns of intraspecific groups (Carranza, Harris, et al., 2006; Jaramillo-Correa et al., 2010). While studies at the genus level are scarce, in most studies the genetic lineage distribution on both sides of the Strait is intraspecific, thus providing information for biogeographical events restricted to a relatively recent time-frame. A comprehensive vision of the biogeographical events across the Strait of Gibraltar might be enhanced by using as model a single genus, comprising taxa demonstrating both intra and interspecific genetic distributions on both sides of the Strait, such as the sand racer lizard genus *Psammodromus*.

Psammodromus, together with the Canary Islands endemic *Gallotia*, form the Gallotiinae subfamily, an ancient lineage of the Lacertidae lizard family (Arnold, Arribas, & Carranza, 2007). Six species are distributed across the Strait of Gibraltar: two North African endemic species, *P. blanci* (Lataste, 1880) and *P. microdactylus* (Boettger, 1881); three species in the Iberian Peninsula and Languedoc region, *P. edwarsianus* (Dugès, 1829), *P. occidentalis* (Fitze, Gonzalez-Jimena, San-Jose, Mauro, & Zardoya, 2012) and *P. hispanicus* (Fitzinger, 1826); and *P. algirus* (Linnaeus, 1758) distributed across the Iberian Peninsula and North Africa. Previous studies have proposed biogeographical hypotheses for the evolution of *Psammodromus* species. In the Iberian Peninsula, divergence due to geographical barriers and environmental differences following the closure of the Betic corridor may have led to the evolution of the three extant species (Fitze et al., 2011). Within *P. algirus*, mtDNA data indicate the existence of two main lineages: an eastern Iberian lineage and a western lineage including specimens from western Iberia and North Africa (Busack & Lawson, 2006; Carranza, Harris, et al., 2006; Verdú-Ricoy, Carranza, Salvador, Busack, & Díaz, 2010). These results could indicate that *P. algirus* first diverged in Iberia and later colonized North Africa. Molecular dating places the colonization of Africa by *P. algirus* approximately 1.9 Ma, long after the end of the MSC (Carranza, Harris, et al., 2006). The same study also puts the divergence between the African *P. blanci* and the Iberian clade long before the MSC, at approximately 20 Ma, thereby suggesting two transmarine colonizations to explain the distribution of *Psammodromus* lineages.

In this study, we generate a comprehensive molecular dataset for all the species of *Psammodromus*, with novel taxa and nuclear markers compared to previous studies. We apply both concatenation and coalescent species tree approaches, and estimate divergence times of the main lineages and their ancestral geographical distribution. Our main aim is to frame the temporal and spatial patterns of the evolutionary history of *Psammodromus* within the biogeographical dynamics of the Strait of Gibraltar in order to contribute to the understanding of the faunal exchange across the Pillars of Hercules.

2 | MATERIALS AND METHODS

2.1 | Sampling

Twenty-seven *Psammodromus* specimens were employed in the phylogenetic analyses, including all six species currently described (Figure 2). Lizards were captured and handled under permit of relevant authorities. Six samples of *Gallotia caesaris caesaris*, *G. c. gomerae* and *G. atlantica* were used as outgroups. Information regarding the sample codes and sampling localities is given in Table 1.

2.2 | DNA extraction, amplification and sequencing

Genomic DNA was extracted from alcohol-preserved tail muscle following standard high-salt protocols (Sambrook, Fritsch, & Maniatis, 1989). We amplified fragments of three mitochondrial (mtDNA) genes, NADH Dehydrogenase 4 plus flanking tRNAs (*nd4*), ribosomal 12S rRNA gene (*12S*) and cytochrome *b* (*cytb*), and of three nuclear (nucDNA) genes, Acetylcholinergic Receptor M4 (*acm4*), Melanocortin 1 Receptor (*mc1r*) and Proopiomelanocortin (*pomc*). These markers have been shown to be highly variable in lacertid lizards (e.g. Mendes, Harris, Carranza, & Salvi, 2016; Salvi, Schembri, Sciberras, & Harris, 2014). Primers and conditions of polymerase chain reactions (PCR) are reported in Table S1.

2.3 | Phylogenetic inference

Nucleotide sequences were aligned using the MUSCLE algorithm (Edgar, 2004) in GENEIOUS (Biomatters Ltd.). Haplotype reconstruction for nuclear markers was performed in PHASE 2.1 (Stephens, Smith, & Donnelly, 2001). PHASE was run three times to assure consistency of results, with a phase probability threshold of 0.7. The occurrence of recombination events in nucDNA was evaluated using the Pairwise Homoplasy Index (phi) test implemented in SPLITSTREE4 4.13.1 (Huson & Bryant, 2006).

Phylogenetic relationships among the *Psammodromus* species were inferred by maximum likelihood (ML), Bayesian inference (BI) and the Bayesian species tree approach based on the multi-species coalescent model (Heled & Drummond, 2010). For the ML and BI analyses, the mitochondrial and (unphased) nuclear DNA sequence data (mt-nucDNA) were concatenated. The ML analyses were performed in RAXML 7.4.2 (Stamatakis, 2006). ML searches included 10 random addition replicates and 1,000 nonparametric bootstrap replicates, applying the general time-reversible models with a gamma model of rate heterogeneity (GTRGAMMA) for each of the six gene
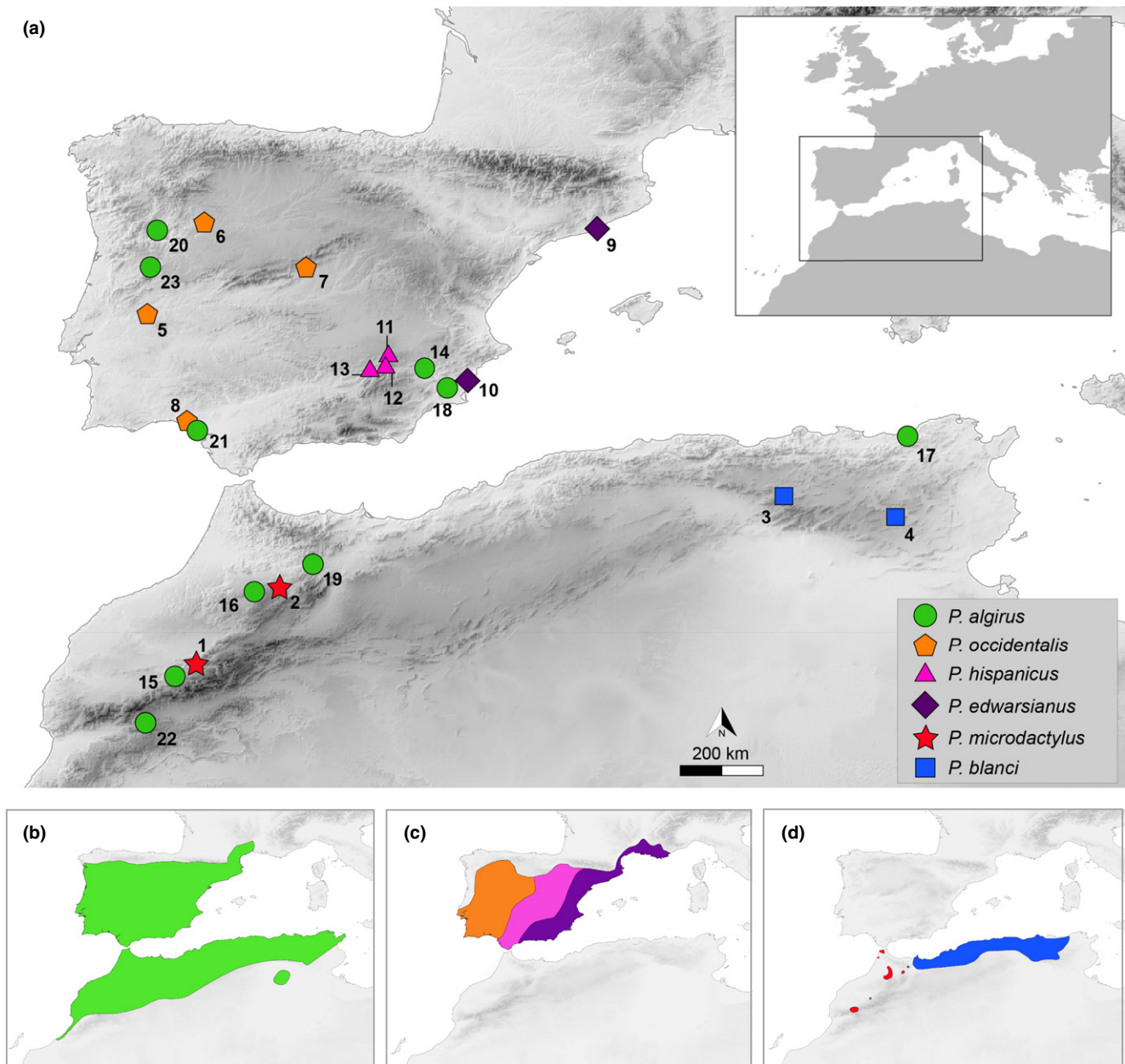
FIGURE 2 Map showing the location in the Iberian Peninsula and North Africa of the *Psammodromus* specimens used in this study (a). Detailed information on sampling localities and specimens is reported in Table 1. Distribution of *Psammodromus* species displayed in smaller maps: (b) *P. algirus*, (c) *P. occidentalis*, *P. hispanicus* and *P. edwarsianus*, (d) *P. microdactylus* and *P. blanci*

partitions. Bayesian analyses were performed in BEAST 1.8.0 (Drummond, Suchard, Xie, & Rambaut, 2012) The best model of nucleotide substitution for each gene was assessed in jMODELTEST 2.1.7 (Posada, 2008) under the Bayesian information criterion (Table S2). Models and prior specifications applied in BEAUTi were as follows (otherwise by default): tree model of all gene partitions linked, nucleotide substitution and clock models unlinked; model of nucleotide substitution for *12S* set as HKY, because preliminary runs with the GTR model presented low effective sample size (ESS) values. A relaxed uncorrelated lognormal clock was set for all genes, Yule process of speciation as tree prior, random starting tree, alpha uniform (0, 10), ucld.mean uniform and operator kappa

(2.0). BEAST was run three times, with 50 million generations, sampling every 5,000 generations. The use of the Yule process of speciation prior requires only one sequence per species, whereas our concatenated dataset included multiple samples per species. Therefore, to inspect the sensitivity of our estimates to the choice of tree prior, we confirmed that identical results were obtained in an additional run applying the same settings as above but using only one representative sequence for each species (Figure S1).

The species tree was inferred using the *BEAST extension of the BEAST software. We used the mtDNA sequences and the phased alignments of the nuclear genes and their relative models of

TABLE 1 *Psammodromus* species, code, map location code in Iberia and North Africa represented in Figure 2, locality, coordinates and GenBank accession number of the specimens used in this study

Species	Code	Map code	Locality	Geographic coordinates	GenBank accession number					
					12S	cytb	nd4	acm4	mc1r	pomc
P. microdactylus	25232	1	Azilal, Morocco	31.92 N, −6.66E	MF684874	MF684919	MF684964	MF684898	MF684938	MF684987
P. microdactylus	25250	1	Azilal, Morocco	31.92 N, −6.66 E	MF684875	MF684920	MF684965	MF684899	MF684939	
P. microdactylus	25384	2	Ait Khalifa, Sefrou, Morocco	33.69 N, −4.84 E	MF684876	MF684921	MF684966		MF684940	
P. blanci	11219	3	Belezma National Park, Algeria	35.58 N, 6.08 E	MF684877	MF684922	MF684967		MF684941	
P. blanci	11220	3	Belezma National Park, Algeria	35.58 N, 6.08 E	MF684878	MF684923	MF684968		MF684942	
P. blanci	T31-47a	4	Bou Chebka, Tunisia	35.12 N, 8.49 E	MF684879	MF684924	MF684969	MF684900	MF684943	MF684988
P. blanci	T31-47b	4	Bou Chebka, Tunisia	35.12 N, 8.49 E	MF684880	MF684925	MF684970			
P. occidentalis	14	5	Castelo de Vide, Portugal	39.43 N, −7.58 E				MF684901	MF684944	MF684989
P. occidentalis	4213	6	Miranda do Douro, Portugal	41.40 N, −6.37 E				MF684902	MF684945	
P. occidentalis	25453	7	Colmenar del Arroyo, Spain	40.43 N, −4.18 E	MF684881		MF684971	MF684903	MF684946	MF684990
P. occidentalis	25454	8	Doñana National Park, Spain	37.01 N, −6.57 E	MF684882	MF684926	MF684972	MF684904	MF684947	MF684991
P. edwarsianus	1869	9	El Prat de Llobregat, Spain	41.29 N, 2.10 E				MF684905	MF684948	MF684992
P. edwarsianus	14359	10	La Mata y Torrevieja Natural Park, Spain	38.02 N, −0.70 E	MF684883		MF684973	MF684906	MF684949	MF684993
P. hispanicus	1723	11	La Hueta, Spain	38.36 N, −2.51 E	KX080629		KX081066	KX080992	KX080782	
P. hispanicus	1784	11	La Hueta, Spain	38.34 N, −2.61 E	MF684884		MF684974	MF684907	MF684950	MF684994
P. hispanicus	1850	12	Segura de la Sierra, Spain	38.29 N, −2.59 E	KX080630		KX081067	KX080993	KX080783	
P. hispanicus	1856	13	Cortijos de Tortas, Spain	38.54 N, −2.42 E	MF684885		MF684975		MF684951	MF684995
P. algirus	1291	14	Embalse de Camarillas, Spain	38.34 N, −1.65 E	MF684886		MF684976		MF684952	MF684996
P. algirus	2347	15	Imi n'Ifri, Morocco	31.72 N, −6.97 E	KX080631		KX081068		KX080784	
P. algirus	2356	16	Azrou, Morocco	33.54 N, −5.32 E	KX080632		KX081069	KX080994	KX080785	MF684997
P. algirus	3784	17	Tabarka, Tunisia	36.95 N, 8.76 E	MF684887	MF684927	MF684977	MF684908	MF684953	MF684998
P. algirus	3854	18	Múrcia, Spain	37.93 N, −1.10 E	MF684888	MF684928	MF684978	MF684909	MF684954	MF684999
P. algirus	4235	19	Taza, Morocco	34.13 N, −4.03 E	MF684889	MF684929	MF684979	MF684910	MF684955	MF685000
P. algirus	16794	20	São Lourenço, Portugal	41.29 N, −7.37 E	MF684890	MF684930	MF684980	MF684911	MF684956	MF685001
P. algirus	21567	21	Matalascañas, Spain	37.00 N, −6.56 E	MF684891	MF684931	MF684981	MF684912	MF684957	MF685002
P. algirus	23902	22	Jbel Siroua, Morocco	30.74 N, −7.61 E	MF684892	MF684932	MF684982	MF684913	MF684958	
P. algirus	24987	23	Gouveia, Portugal	40.51 N, −7.53 E	MF684893	MF684933		MF684914	MF684959	MF685003
Gallotia atlantica	1244	−	Lanzarote, Canary Islands, Spain	29.05 N, −13.56 E	KX080625		KX081062	KX080988	KX080778	MF685004

(Continues)

TABLE 1 (Continued)

Species	Code	Map code	Locality	Geographic coordinates	GenBank accession number					
					12S	*cytb*	*nd4*	*acm4*	*mc1r*	*pomc*
Gallotia atlantica	1341	–	Lanzarote, Canary Islands, Spain	28.95 N, −13.77 E	KX080626		KX081063	KX080989	KX080779	MF685005
Gallotia caesaris gomerae	19355	–	La Gomera, Canary Islands, Spain	28.12 N, −17.16 E	MF684894	MF684934	MF684983	MF684915	MF684960	MF685006
Gallotia caesaris gomerae	19375	–	La Gomera, Canary Islands, Spain	28.10 N, −17.13 E	MF684895	MF684935	MF684984	MF684916	MF684961	MF685007
Gallotia caesaris caesaris	19413	–	El Hierro, Canary Islands, Spain	27.81 N, −17.91 E	MF684896	MF684936	MF684985	MF684917	MF684962	MF685008
Gallotia caesaris caesaris	19470	–	El Hierro, Canary Islands, Spain	27.73 N, −18.12 E	MF684897	MF684937	MF684986	MF684918	MF684963	MF685009

nucleotide evolution (Table S2). The settings applied in *BEAST for the species tree reconstruction were similar to the concatenated BEAST analyses, except the tree model of the mitochondrial genes *12S*, *cytb* and *nd4* was linked. *BEAST was run three times with 400 million generations, sampling every 40,000 generations.

We used TRACER 1.6 to check all BEAST runs for convergence (burn-in = 10%) and that all ESS parameters were higher than 200. All runs were combined with LOGCOMBINER and TREEANNOTATOR was used to calculate the maximum clade credibility (MCC) tree summarizing the posterior distribution of tree topologies and branch lengths. Trees were visualized in FIGTREE 1.4 (available at http://tree.bio.ed.ac.uk/software/figtree/). BEAST analyses were performed on the CIPRES Science Gateway 3.3 (Miller, Pfeiffer, & Schwartz, 2010).

2.4 | Molecular dating

In order to infer the time of cladogenetic events within Gallotiinae we used the estimated rates of evolution of *12S* and *cytb* of lacertid lizards (Carranza & Arnold, 2012). We implemented a normal distribution prior for the ucld.mean parameters of the *12S* (mean: 0.00553, stdev: 0.00128) and *cytb* (mean: 0.0164, stdev: 0.00317) gene fragments in *BEAST. To infer divergence time within species we also applied the same priors in BEAST on the concatenated mt-nucDNA dataset (all gene partitions linked).

We cross-validated the substitution rates used by performing (1) an estimate of *12S* and *cytb* rates based on the procedure and the dataset of Carranza and Arnold (2012) but excluding the MSC vicariance between *Podarcis* species as calibration point (since the use of the MSC in molecular clock calibrations is under debate; see Hewitt, 2011 and also the Discussion section), and (2) a time tree estimate applying on our dataset the priors on *Gallotia* nodes used by Carranza and Arnold (2012): a uniform prior from 14.5 to 0 Ma for the

root of *Gallotia* and a prior constraining the split between *Gallotia c. caesaris* and *Gallotia c. gomerae* soon after the emergence of the El Hierro Island 1.12 Ma (Normal prior: mean = 1, *SD* = 0.1, upper limit truncated to 1.12). Rates and time estimates resulting from all molecular dating analyses were almost identical (Figure 3 and Figure S2). Additionally, the *12S* rate used in this study is in agreement with the *12S* rate estimated in other lacertid species (Salvi, Pinho, & Harris, 2017).

2.5 | Ancestral area reconstruction

Biogeographical reconstructions were performed in RASP 3.0 (Yu, Harris, Blair, & He, 2015) using the statistical dispersal–vicariance analysis (S-DIVA) (Yu, Harris, & He, 2010), dispersal-extinction cladogenesis (DEC) (Ree & Smith, 2008) and Bayesian binary MCMC (BBM) (Yu, Harris, & Xingjin, 2011) methods. We used all the post-burnin trees and the MCC tree resultant from the concatenated BEAST run as input. To evaluate the effect of changing the number of areas in the reconstruction of the ancestral geographical distribution, we performed two sets of analyses: with two areas: North Africa (A) and Iberian Peninsula (B) (Figure 3), and with three areas: North Africa (A), Western Iberia (B) and Eastern Iberia (C) (Figure S3).

S-DIVA was conducted using 1,000 trees randomly sampled from the input trees. DEC analyses were run either with the default connectivity value (equal to 1.0) between ancestral ranges along the time trees or implementing the known palaeogeographical model of the Strait of Gibraltar (detailed settings in Appendix S1). Results were identical between the simple and the stratified models (likely because of the low number of cladogenetic events in each time slice, see Ree & Sanmartín, 2009), so only results from the default connectivity model are provided here. BBM analyses were run with the Jukes-Cantor model, site variation set to equal and maximum number of areas set to two and three, respectively. We performed two
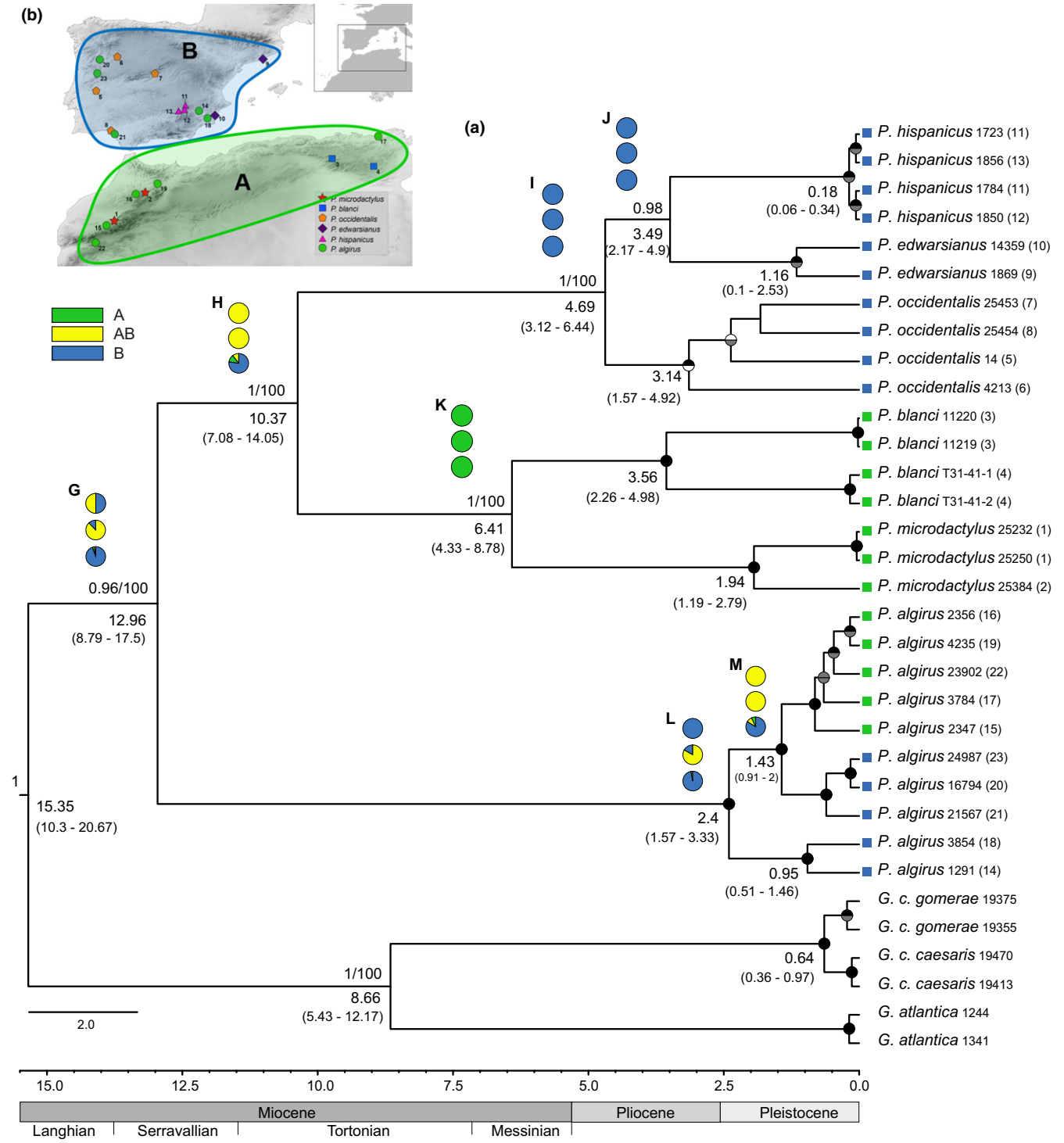
FIGURE 3 (a) Bayesian tree depicting the phylogenetic relationships between *Psammodromus* lizards based on concatenated mitochondrial (*12S*, *cytb*, *nd4*) and nuclear (*acm4*, *mc1r*, *pomc*) DNA sequence. Numbers beside species names indicate specimen codes (Table 1) and numbers between brackets indicate locality codes as shown in Figure 2 and (b). Values above nodes represent Bayesian posterior probabilities (BPP) ≥0.9 (left) and maximum likelihood bootstrap (BS) values ≥70 (right). Node support for intra-specific clades is represented by black circles: BPP≥0.98 (upper half) and BS≥95 (bottom half); grey circles: 0.9 ≤ BPP<0.98 (up) and 70 ≤ BS < 95 (bottom) and empty circles for nodes with lower support. Values below nodes represent the estimated age of the node with associated 95% highest posterior density interval (in parentheses) according to molecular dating analyses. Coloured squares represent the geographic origin of each tip sample in either Iberia or Africa according to the inset (b). Coloured pie charts in correspondence of nodes (which are named from G to M) represent results from ancestral range reconstructions based on S-DIVA (top), DEC (middle) and BBM (bottom) methods. Colour legend of ancestral areas represented in pie charts is at the top left. (b) Map of the study area with sampling localities and the areas used for ancestral range reconstruction: (A) Africa (green) and (B) Iberia (blue)

simultaneous runs with five million generations, sampling each 100 generation, without outgroup species.

3 | RESULTS

3.1 | Molecular data, *Psammodromus* phylogeny and divergence time

A total of 159 new sequences were obtained (GenBank accession numbers in Table 1). In order to concatenate only sequences from the same individual we avoided including published sequences from GenBank. The length of the gene fragments and the number of polymorphic sites are reported in Table S2. The translation of these genes into amino acid sequences did not contain stop codons. The phi test did not find statistically significant evidence for recombination in the nuclear gene fragments ($p > .05$).

Phylogenetic relationships between *Psammodromus* species recovered by species tree and concatenated dataset were identical (Figures 3a & 4). Maximum likelihood and BI analyses based on the concatenated dataset resulted in identical relationships between species with overall high bootstrap (BS) and Bayesian posterior probabilities (BPP) values (Figure 3a). The divergence time estimates were similar between concatenated (Figure 3a) and species trees (Figure 4), therefore from here on we will refer only to time estimates of the concatenated tree (Figure 3a). The genera *Gallotia* and *Psammodromus* are reciprocally monophyletic with divergence between them estimated at 15 Ma (95% highest posterior densities interval (95% HPD): 10.3–20.67). All *Psammodromus* species are monophyletic (Figures 3a & 4; BPP≥0.9, BS≥70). *Psammodromus algirus* was the first species to diverge within the *Psammodromus* clade (Figures 3a & 4; BPP = 1, BS = 100), approximately 13 Ma (95% HPD: 8.79–17.5), and includes three lineages: the Eastern Iberia lineage,
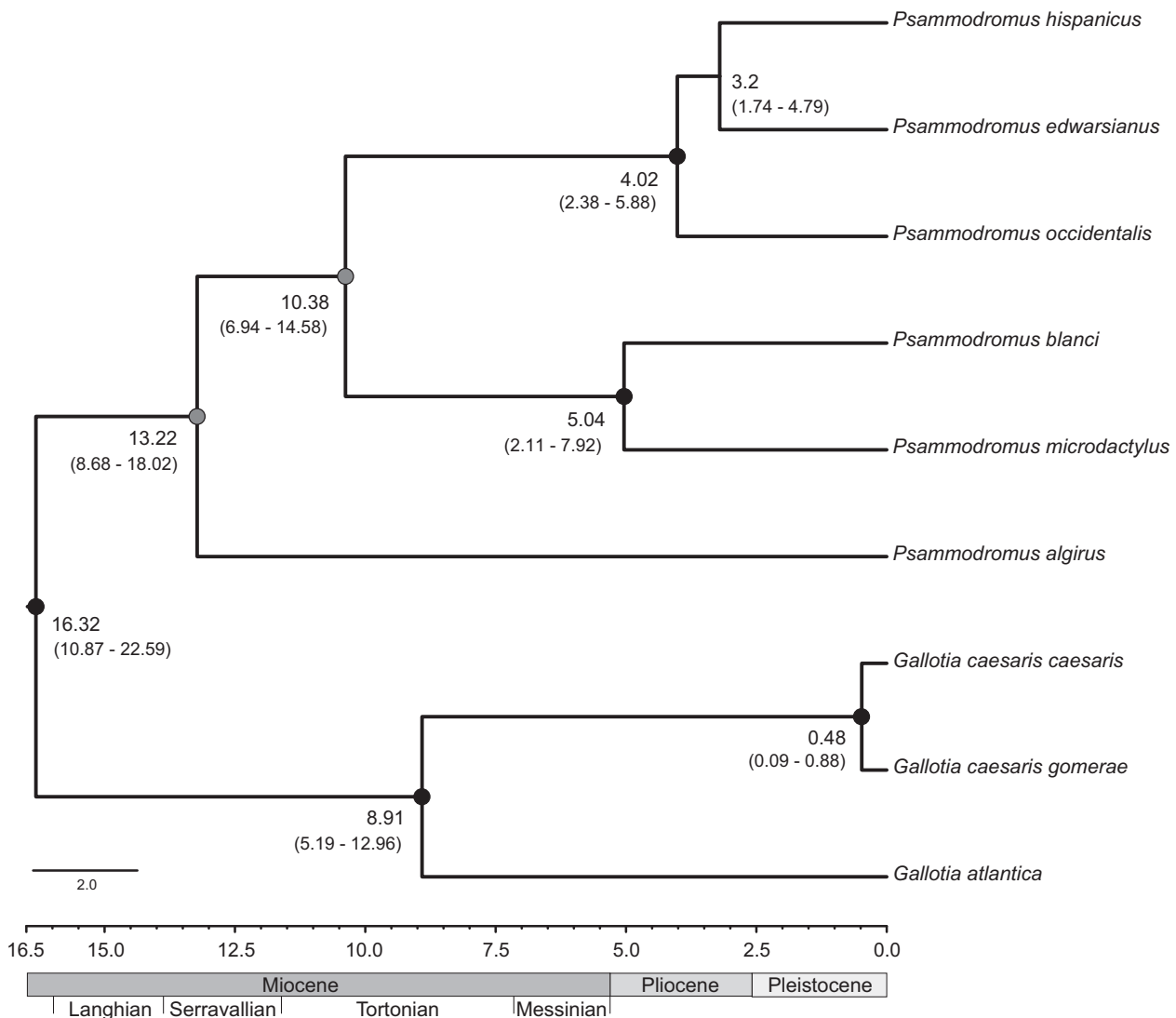

FIGURE 4 Species tree of *Psammodromus* lizards inferred from mitochondrial (*12S, cytb, nd4*) and nuclear (*acm4, mc1r, pomc*) DNA sequences using the multispecies coalescent model in *BEAST. Black circles represent Bayesian posterior probabilities (BPP) = 1 and grey circles represent 0.98 ≤ BPP<1. Values below nodes represent the age of the node and the associated 95% highest posterior density interval (in parentheses)

which diverged circa 2.5 Ma (95% HPD: 1.57–3.33) from the Western Iberia lineage and the North African lineage. The latter two diverged about 1.4 Ma (95%HPD: 0.91–2.0) (Figures 2 & 3). The North African endemic *P. blanci* and *P. microdactylus* are sister species (Figures 3a & 4; BPP = 1, BS = 100) and diverged from the Iberian clade (*P. occidentalis*, *P. hispanicus* and *P. edwarsianus*) approximately 10 Ma (95% HPD: 7.08–14.05) and between each other 6 Ma (95% HPD: 4.33–8.78) (Figures 3a & 4). *Psammodromus blanci* shows a deep differentiation between two lineages in the Aurès Mountains, estimated at 3.5 Ma (95% HPD: 2.26–4.98), (Figures 2 & 3a). *Psammodromus microdactylus* presents a comparatively lower, but still notable, differentiation between the two populations sampled (Figures 2 & 3a). Diversification within the Iberian clade started approximately 4.7 Ma (95% HPD: 3.12–6.44) with the split of *P. occidentalis* followed by the split between the sister species *P. hispanicus* and *P. edwarsianus* at 3.5 Ma (95% HPD: 2.17–4.9) (Figures 3a & 4).

3.2 | Ancestral area reconstruction

All biogeographical analyses, based on either two areas (Africa and Iberia) or three areas (Africa, Western Iberia and Eastern Iberia), inferred the same biogeographical scenario for the nodes K, I and J: the ancestor of the species *P. blanci* and *P. microdactylus* has an African origin (node K), and the ancestors of *P. occidentalis*, *P. hispanicus* and *P. edwarsianus* (nodes I and J) have an Iberian origin (Figure 3 & Figure S3). The ancestral areas inferred for the basal nodes within the genus (G and H) are either in Iberia (BBM: 95% in G, 77% in H; S-DIVA: 50% in G) or shared by Iberia and Africa (S-DIVA: 50% in G, 100% in H; DEC 87%: in G, 100% in H) in the two areas analyses (Figure 3). A similar inference is presented in node M for the most recent lineages within *P. algirus*, with BBM (80%) supporting with higher probability an Iberian ancestry, while S-DIVA and DEC support an Iberian and African origin. Finally, the origin of *P. algirus*, node L, is likely to be Iberian, as indicated by S-DIVA and BBM (Figure 3).

Regarding the nodes G, H, L and M the results from RASP analyses based on three areas are congruent with two areas analyses but show increased inference uncertainty. For example, S-DIVA and DEC infer a similar pattern in the two and three areas analyses for the nodes G, H, L and M, with the exception of S-DIVA in node L suggesting an Iberian origin in two areas and uncertain origin in three areas (Figure 3 & Figure S3). Increased inference uncertainty in the three areas compared to the two areas analyses is also apparent in the BBM results with higher probabilities for an Iberian origin of nodes G, H and M in the analyses based on two areas and comparable probabilities associated to either an Iberian or an African origin in the three areas analysis.

4 | DISCUSSION

The use of a multilocus dataset including all species allowed estimation of a fully resolved phylogeny of the genus *Psammodromus*.

Relationships between species received high statistical support (Figures 3a & 4) and are congruent with relationships previously inferred using mostly mtDNA data from incomplete taxon sets (Busack, Salvador, & Lawson, 2006; Carranza, Harris, et al., 2006; Fitze et al., 2011, 2012; Verdú-Ricoy et al., 2010). *Psammodromus algirus* represents an early branch within the genus and is sister to two clades endemic to Africa and Iberia, respectively. In the Iberian clade, the species *P. occidentalis* is sister to *P. hispanicus* and *P. edwarsianus*. Unlike previous studies, our phylogeny includes *P. microdactylus* which is sister to the other African species *P. blanci*. Both species of this African clade show two deeply divergent lineages separated by a relatively small geographical distance (Figures 2 & 3).

Here we integrate the information of the main cladogenetic events within *Psammodromus*, and their estimated time and ancestral geographical distribution, within the context of the palaeogeographical evolution of the Strait of Gibraltar to trace the biogeographical history of *Psammodromus* across North Africa and Western Europe. The biogeographical inference from the Bayesian binary MCMC (BBM) is in agreement with the fossil and palaeogeographical evidence and with the timing and pattern of cladogenetic events inferred for *Psammodromus* from molecular data (Figures 1 & 3). Biogeographical inferences obtained with the S-DIVA and DEC methods show higher uncertainty and wider ancestral ranges at deep nodes. Such a pattern has been observed before (Liu et al., 2016; Perea, Cobo-Simon, & Doadrio, 2016) and is likely due to the underlying assumptions of these methods (Buerki et al., 2011). Both DIVA and DEC models are based on the assumption of "vicariance-mediated allopatry", in which dispersal is modelled as wide ancestral range divided by vicariance (or also peripheral isolate speciation in DEC) at speciation nodes (Sanmartín, 2007), so speciation following early dispersal events in the phylogeny imply wide ancestral ranges at deep nodes (see e.g. node H in Figure 3). Additionally, in DEC analyses widespread terminals subtended by a long branch, such as *P. algirus* in our case, may have the effect of increasing the uncertainty at deeper nodes (Buerki et al., 2011; Ree, Moore, Webb, & Donoghue, 2005).

4.1 | Historical biogeography of *Psammodromus*

The origin of the genus *Psammodromus* was likely in Western Europe according to previous knowledge. The European origin of lacertids, and of the Gallotiinae in particular, is supported by (1) fossil evidence (Augé, 2005; Čerňanský, Klembara, & Smith, 2016; Figure S4, Table S3), (2) the mainly European distribution of the first genera to branch off in the lacertid phylogeny (Arnold et al., 2007) and (3) a high species diversity in Europe compared to Africa (Arnold et al., 2007; Pavlicev & Mayer, 2009).

The origin of the *Psammodromus* radiation is estimated to have started in the Middle Miocene (Fitze et al., 2011; present study) or even in the Early Miocene (Carranza, Harris, et al., 2006). At that time, the westernmost Mediterranean was characterized by intense geological activity, with the southward drift of the Betic-Rif block across the Gibraltar region. The exact position of the Betic-Rif after

the breaking of the Hercynian belt until it reached its final position in the Strait of Gibraltar remains debatable. While some authors advocate that the Betic-Rif was attached to Iberia until a marine transgression separated them, 16–14 Ma (Lonergan & White, 1997), others suggest that, separated from Iberia, it formed a continuous block with the Balearic Islands and Kabylies until 15 Ma (Rosenbaum, Lister, & Duboz, 2002b; Rosenbaum et al., 2002a) or with the Balearic Islands alone, until 10 Ma (Fontboté et al., 1990). Given the absence of remains referable to *Psammodromus* within the well-studied fossil record of the Balearic Islands (Bailon, Boistel, Bover, & Alcover, 2014; Pinya & Carretero, 2011 and references therein) it is likely that the genus would have arrived to Africa by dispersal from the palaeo-Iberia through the Betic-Rif, after the detachment of the latter from the other blocks. The alternative hypotheses that the ancestor of *P. blanci* and *P. microdactylus* would have reached Africa by vicariance following the detachment of either the Hercynian belt (Betic-Rif+Balearics+Kabylies+Corsica-Sardinia+Calabria) from Iberia (30 Ma) or the Betic-Rif block from the other Hercynian blocks (16–14 Ma) is less plausible not only for the lack of *Psammodromus* fossils in these blocks but also considering the estimated time of divergence between the Iberian and the African clades, although the latter cannot be definitively rejected. According to our estimates, the cladogenetic event that gave rise to the ancestor of *P. blanci* and *P. microdactylus* occurred during the Middle to Late Miocene, or even earlier in the Early to Middle Miocene (Carranza, Harris, et al., 2006). Assuming no undocumented extinctions, this time estimate provides an approximate upper limit for the colonization of Africa. Whether the cladogenesis between the ancestors of the Iberian clade (*P. occidentalis*, *P. edwarsianus* and *P. hispanicus*) and of the

African clade (*P. blanci* and *P. microdactylus*) was due to a dispersal event between these areas, or if the divergence of the ancestor of the African clade slightly predated its dispersal, the colonization of Africa took place during a period when the Betic-Rif massif was fragmented and separated from mainland by sea corridors to the north and south (Figure 1b). The Northern Betic sea corridor closed approximately 10 Ma, originating a temporary land bridge between Iberian and the sub-Betic massif that disappeared when this sea corridor re-opened 9–8 Ma. Therefore, the arrival into North Africa of the ancestor of *P. blanci* and *P. microdactylus* was likely achieved through stepping stones across the Betic-Rif (Figure 5a).

The ancestor of *P. blanci* and *P. microdactylus* underwent considerable diversification after it reached North Africa. The divergence between the two species is dated at the end of the Miocene (Figure 5a). Climatic conditions, shifting towards aridity in North Africa during the Miocene and the Pliocene, may have played a role in their divergence, as has been described for other reptile species during this period (Amer & Kumazawa, 2005; Gonçalves et al., 2012). We identified two main evolutionary lineages within each species. In *P. blanci*, the divergence between the two lineages is as deep as between the species *P. hispanicus* and *P. edwarsianus* (3.5 Ma). Such a level of divergence between two sampling localities closely located in the Eastern Maghreb (Figure 2) is suggestive of undescribed diversity, especially considering that the range of *P. blanci* extends to Eastern Morocco. The high genetic differentiation within *P. microdactylus* and its fragmented distribution are indicative of a relictual pattern possibly resultant of widespread extinction mediated by climatic or ecological changes. Additional studies are necessary to fully understand the diversity
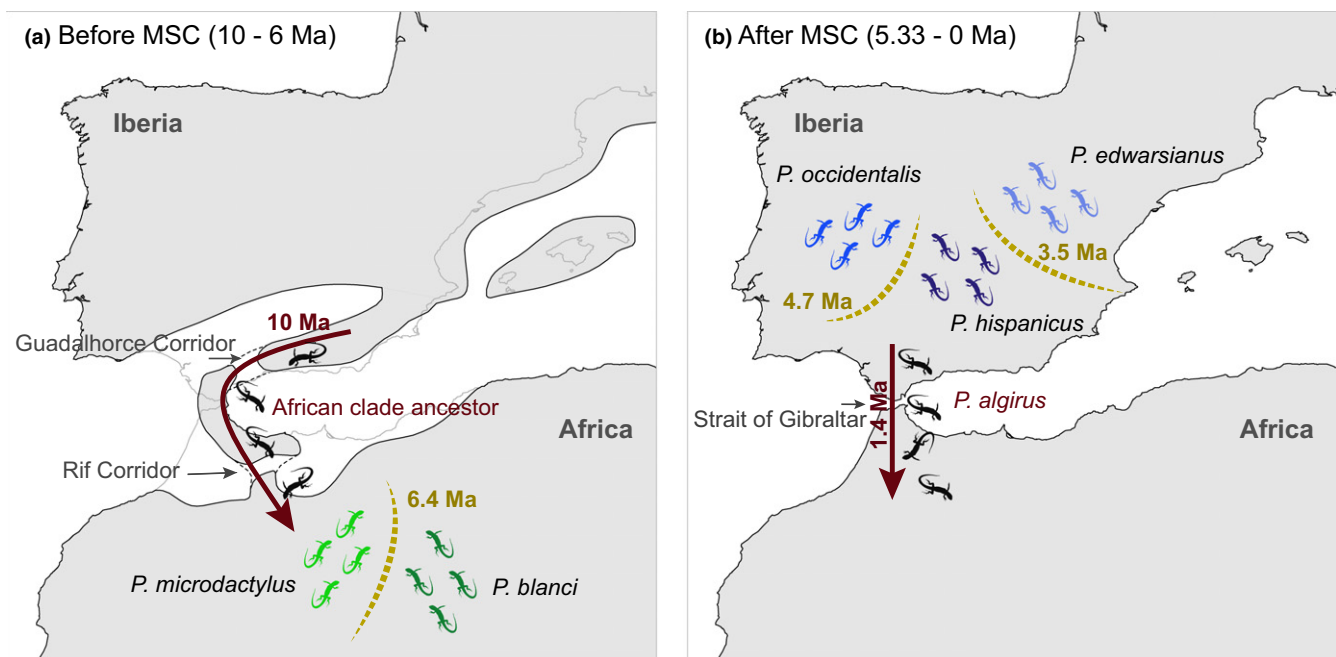

FIGURE 5 Dispersal–vicariance events in *Psammodromus* in Iberia and North Africa before (a) and after (b) the end of the Messinian salinity crisis, 5.33 Ma. Dark red represents the direction, age and taxa in the dispersal events. Dashed yellow represent age and vicariance events in Africa and Iberia

and the evolutionary history of these species, with a possible novel taxonomical assessment.

A major role for vicariance processes behind the diversification of the Iberian clade has been previously suggested (Fitze et al., 2011) and is supported by our results. Under this biogeographical scenario, the final closure of the Betic corridor (7.6–7.8 Ma) caused the formation of the Guadalquivir basin, which acted as a vicariant factor driving the divergence between *P. occidentalis* and the remaining Iberian species following an East-West differentiation (Figure S3). This geographical pattern has been described for multiple Iberian species (e.g. Harris, Carranza, Arnold, Pinho, & Ferrand, 2002; Harris & Sá-Sousa, 2002; Martínez-Solano et al., 2004). Later, during the MSC, geological and climatic changes associated with the uplift of the Spanish Central System and the re-configuration of the main river drainages in Iberia would have ultimately led to the differentiation between *P. edwarsianus* and *P. hispanicus* (Fitze et al., 2011). The main difference between our results and those from Fitze et al. (2011) is that the inferred time is more recent in our study. This may be attributed to the use of different molecular data and rates of evolution. However, the uncertainty of such estimates overlap for the majority of the associated confidence intervals, while supporting the same pattern of evolution of these three species.

Finally, the pattern of diversification in the most widespread species of the genus, *P. algirus*, shows some striking parallels with the wall lizard *Podarcis vaucheri* (Kaliontzopoulou, Pinho, Harris, & Carretero, 2011). The fact that *P. algirus*, like *Podarcis vaucheri*, has an African lineage nested within two paraphyletic Iberian lineages is an indication that both species diverged in Iberia and then reached North Africa. According to our dated phylogeny, the divergence of the African lineage of *P. algirus* took place approximately 1.4 Ma, and therefore this species colonized North Africa through transmarine dispersal across the Strait of Gibraltar (Figure 5b). This colonization was likely associated with a sea level low-stand during a glacial stage, when the distance between Iberia and North Africa was reduced. Such a scenario has been described by Carranza, Harris, et al. (2006) and is concordant with our ancestral area reconstructions (Figure 3, nodes L and M). During the last five million years, several species have independently crossed in both directions the Strait of Gibraltar in different occasions after its opening (Carranza et al., 2004; Carranza, Arnold, et al. 2006; Carranza, Harris, et al., 2006; Carranza et al., 2008 Gaubert et al., 2011; Harris, Batista, Lymberakis, & Carretero, 2004; Harris, Batista, Carretero, et al., 2004).

The historical biogeography of *Psammodromus* indicates that the Strait of Gibraltar has been a permeable biogeographical barrier for these lizards since their early evolution during the Middle Miocene. While the MSC (5.9–5.33 Ma) is often seen as a major period for biotic exchanges between Africa and Europe, due to their wide land connection, this study shows that dispersal over narrow sea channels before and after the MSC (Figure 5) possibly had a major role in shaping the biogeographical patterns of *Psammodromus* across the Pillars of Hercules.

4.2 | Concluding remarks on biotic exchange between Western Europe and North Africa

In this study, our dated phylogeny and different ancestral area reconstructions provided a robust statistical framework for biogeographical inference, while the well-known geological history of this region provided an independent temporal (and spatial) framework for such an inference. This may compensate the intrinsic uncertainty associated with time estimates based on molecular clocks (Pulquério & Nichols, 2007) and the paucity of fossil data associated to the internal nodes of our phylogeny.

The historical biogeography of *Psammodromus* seems to have been predominantly a history of dispersals across the Strait of Gibraltar. Both interspecific and intraspecific diversification in this genus has been associated with dispersal from Iberia to North Africa through transmarine colonizations on different occasions (Figure 5). On the other hand, vicariance most likely played a role in the diversification of lineages within Iberia and North Africa (Figure 5), but more phylogeographical data, hopefully combined with fossil information, are needed to develop a stronger understanding of diversification patterns of the African taxa.

The cladogenetic events between Iberian and African lineages within *Psammodromus* are dated well before or long after the vicariant event at the end of the MSC (5.33 Ma). Indeed, none of the Bayesian analyses of divergence time (BEAST or *BEAST), which incorporated a wide range of molecular rates, provided 95% posterior intervals on these nodes that overlapped with the MSC. Therefore, in *Psammodromus*, range evolution and cladogenesis are clearly associated to overseas dispersals from Iberia toward Africa rather than with the vicariance at the end of the MSC. This pattern is not restricted to *Psammodromus* and is well documented in recent literature. Indeed, for many terrestrial organisms such as worm lizards and salamanders the high divergence of populations across the Strait of Gibraltar is associated to early crossing of the Strait since the Middle Miocene, likely through the Betic-Rif stepping stones, whereas in many other—such as shrews, larks, snakes, chameleons, skinks, salamanders, frogs and mongooses— shallow divergence underlie dispersal during the Late Pleistocene, possibly in correspondence of sea level low-stand in glacial maxima (reviewed in Hewitt, 2011; see also Gaubert et al., 2011; Santos, Rato, Carranza, Carretero, & Pleguezuelos, 2012). This emerging body of evidence has far reaching implications for molecular biogeography studies beyond the Strait of Gibraltar. Indeed, to date numerous studies have extensively assumed the end of the MSC as the main vicariant cause of species divergence across the Strait and many sea channels across the Mediterranean (e.g. Brown et al., 2008; Chueca, Gómez-Moliner, Forés, & Madeira, 2017; Prüser & Mossakowski, 1998) and used this timing point in phylogenies to calibrate rates of molecular divergence and speciation. However, this and other recent studies (references listed in Hewitt, 2011) demonstrate that the assumption of such a "vicariance-mediated allopatry" model may not be justified in many cases and a "dispersal-mediated allopatry" model of speciation fits most of the available data significantly better. Since the latter model implies either much older or more recent divergence compared with the

vicariance model used so far, this realization warrant a re-assessment of many available molecular rates inferred around the MSC paradigm.

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DATA ACCESSIBILITY

All sequences generated for this study are deposited in GenBank with accession numbers listed in the results section (see Table 1).

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BIOSKETCH

Joana Mendes is a PhD student at CIBIO (Portugal). This work is part of her dissertation, which main focus is the application of phylogenetic methods to understand the evolutionary history of squamate taxa in the Palearctic. The authors have a long-term collaboration history in phylogeography and phylogenetics of reptiles and amphibians from the Mediterranean basin.

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