

## New records on the distribution of the Spanish sand racer species (Squamata, *Psammodromus*) in Spain, Iberian Peninsula

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Received: 14 January 2020; returned for review: 18 February 2020; accepted X 2020.

The genus *Psammodromus* (Lacertidae) comprises six species. Three of the species are morphologically similar, phylogenetically closely related, and are distributed in the Iberian Peninsula (besides *P. algirus*): *P. occidentalis* (Western Iberia), *P. hispanicus* (Central Spain), and *P. edwardsianus* (Eastern Spain). Previous studies have shown these three species to have allopatric distributions in Iberia, though there are still many areas such as Andalucía where it is unclear which species of *Psammodromus* occurs. In this study we present nine new records of *Psammodromus* from key places in southern Spain. Since the three Iberian species are difficult to differentiate by morphology, specimens were genetically identified using mitochondrial DNA data and compared to all six species of the genus. Here we present an updated distribution map of *Psammodromus* specimens identified with molecular methods, increasing the ranges of all three species. We also propose wider regions of potential sympatry between *P. occidentalis* and *P. hispanicus*, and *P. edwardsianus* and *P. hispanicus* than previously recorded.

**Key words:** Biogeography; Cytochrome *b*; Gallotiinae; Range expansion; Southern Spain.

The genus *Psammodromus* belongs to the subfamily Gallotiinae in the Lacertidae family (ARNOLD *et al.*, 2007). Currently, *Psammodromus* comprises six species: *P. algirus* (LINNAEUS, 1758), distributed across the Iberian Peninsula and the Maghreb; *P. blanci* (LATASTE, 1880), distributed across north-eastern Morocco, Algeria and north-western Tunisia; *P. microdactylus* (BOETTGER, 1881) endemic to Morocco; *P. occidentalis* (FITZE *et al.*, 2012) from western

Iberia; *P. hispanicus* FITZINGER, 1826, from central Iberia; and *P. edwardsianus* (DUGÈS, 1829) from eastern Iberia and the Languedoc region. The phylogeny of *Psammodromus* includes three main clades: the first to diverge was the clade of *P. algirus* (comprised of the highly divergent western and eastern Iberian lineages; CARRANZA *et al.*, 2006), followed by the separation of two reciprocally monophyletic sister clades, one from

North Africa comprised of *P. blanci* and *P. microdactylus*, and another one from Iberia, comprised of *P. edwardsianus*, *P. hispanicus* and *P. occidentalis* (MENDES *et al.*, 2017).

The Iberian clade included recent taxonomic changes. The three species were initially considered as a single species, *P. hispanicus*, comprised of two subspecies: *P. h. hispanicus* and *P. h. edwardsianus*. Recently, FITZE *et al.* (2011) applied molecular methods and recognized three different independent evolutionary units: *P. h. hispanicus* western lineage, *P. h. hispanicus* central lineage and *P. h. edwardsianus*. These three evolutionary units correspond to the currently accepted species: *P. occidentalis*, *P. hispanicus* and *P. edwardsianus*, respectively (FITZE, 2012a,b,c; FITZE *et al.*, 2012). Previous studies of the distribution of the Iberian clade of *Psammotromus* have been carried out. In the paper by FITZE *et al.* (2011) (see FITZE *et al.*, 2012 for the same data according to the current taxonomy), a total of 265 specimens from

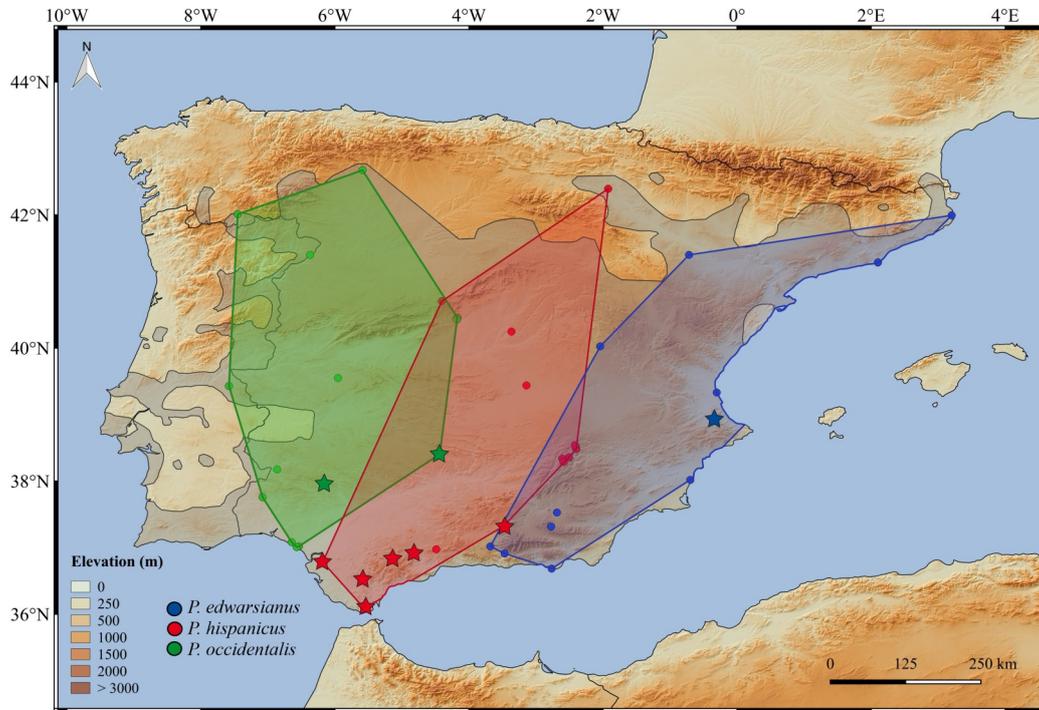
22 different localities were sampled across Iberia and molecular phylogenetic analyses were used to genetically identify the collected specimens. The results suggested allopatry between the three species, being *P. occidentalis* distributed across western Iberia, *P. hispanicus* across central Spain, and *P. edwardsianus* across eastern Spain. Nevertheless, as a result of the sparse sampling in FITZE *et al.* (2011), the size of the Iberian Peninsula, and the difficulty to key out these species without the aid of molecular methods (SAN-JOSE *et al.*, 2012; though *P. edwardsianus* is relatively easier to identify), there are still many areas in Iberia where it is still not clear which species of *Psammotromus* are present. Some of these areas include the southern limits of the distribution range in Andalucía, Spain.

In the present study, we report a total of nine new localities of *Psammotromus* from key locations in southern Spain and use molecular data to classify them to the

**Table 1.** Details of the material collected in this study and used in the phylogenetic analyses. Localities are shown in Fig. 1.

Specimen code	Species	Province	Latitude	Longitude	GenBank Accession Code
CN11269	<i>P. edwardsianus</i>	Valencia	38.92936	-0.34706	MT080173
CN11256	<i>P. occidentalis</i>	Ciudad Real	38.40388	-4.43542	MT080165
CN11258	<i>P. occidentalis</i>	Badajoz	37.95695	-6.15976	MT080166
CN11259	<i>P. hispanicus</i>	Málaga	36.90700	-4.81669	MT080167
CN11261	<i>P. hispanicus</i>	Granada	37.32472	-3.48191	MT080168
CN11263	<i>P. hispanicus</i>	Cadiz	36.83955	-5.13789	MT080172
CN11264	<i>P. hispanicus</i>	Cadiz	36.08676	-5.56196	MT080169
CN11266	<i>P. hispanicus</i>	Cadiz	36.78427	-6.18190	MT080170
CN11271	<i>P. hispanicus</i>	Málaga	36.52902	-5.58621	MT080171

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**Figure 1.** Updated distribution map of *P. occidentalis* (green), *P. hispanicus* (red) and *P. edwardsianus* (blue) overlaid on the distribution range in the Iberian Peninsula (grey). Coloured stars highlight the records reported in this study. Minimum convex polygons encompass the populations of each species. Exact localities from each population and GenBank accession codes of samples recorded in the present study are listed in Table 1. Map created using QGIS v.2.18.15 Las Palmas (QGIS Development Team, 2018).

species level. We provide an updated mitochondrial phylogeny and distribution map of the three Iberian *Psammodromus* species. The distribution and biogeography of these species are briefly discussed.

### MATERIAL AND METHODS

Nine specimens of *Psammodromus* from southern Spain were included in the analyses (Table 1). To genetically identify these individuals and evaluate their

phylogenetic position we sequenced and compared them to all available sequences of *Psammodromus* species (i.e., *P. algerus*, *P. blanci*, *P. edwardsianus*, *P. hispanicus*, *P. microdactylus* and *P. occidentalis*) retrieved from GenBank (mainly from the studies of CARRANZA *et al.*, 2006; FITZE *et al.*, 2011; SAN-JOSE *et al.*, 2012; MENDES *et al.*, 2017). We included as outgroup four specimens of *Gallotia*, its phylogenetic sister genus (Fu, 2000; ARNOLD *et al.*, 2007). The new sequences obtained in this study were

deposited in GenBank (accession numbers MT080165-MT080173). Information of the samples collected in this study is presented in Table 1 and localities are shown in Fig. 1; information on the other sequences retrieved from GenBank is in Table S1.

Genomic DNA of alcohol-preserved tissue samples was extracted using the SpeedTools Tissue DNA Extraction kit (Biotools, Madrid, Spain). A fragment of 300 bp of the mitochondrial gene Cytochrome *b* (*cytb*) was amplified with Cytb1 and Cytb2 primers (KOCHER *et al.*, 1989). Purification and bi-directional sequencing were carried out by Macrogen (Macrogen Inc., Madrid, Spain). Chromatographs were checked, assembled and edited using Geneious v.7.1.9 (Biomatter Ltd.). Sequences were aligned using MAFFT v.7.3 (KATO & STANDLEY, 2013) with default parameters and no stop codons were detected when translated to amino acids.

Phylogenetic analyses were performed under Maximum likelihood (ML) and Bayesian inference (BI) frameworks. The ML analysis was conducted in RAxML v.8.1.2 as implemented in raxmlGUI v.1.5 (SILVESTRO & MICHALAK, 2012). The analysis was performed with the GTR+G model of sequence evolution and 100 random addition replicates. Nodal support was assessed with 1000 bootstrap replicates. The BI analysis was conducted with BEAST v.1.8.4 (DRUMMOND *et al.*, 2012). We used jModelTest v.2.1.7 (GUINDON & GASCUEL, 2003; DARRIBA *et al.*, 2012) to select the best model of nucleotide substitution under the Bayesian information criterion (BIC). We carried out the BEAST analysis

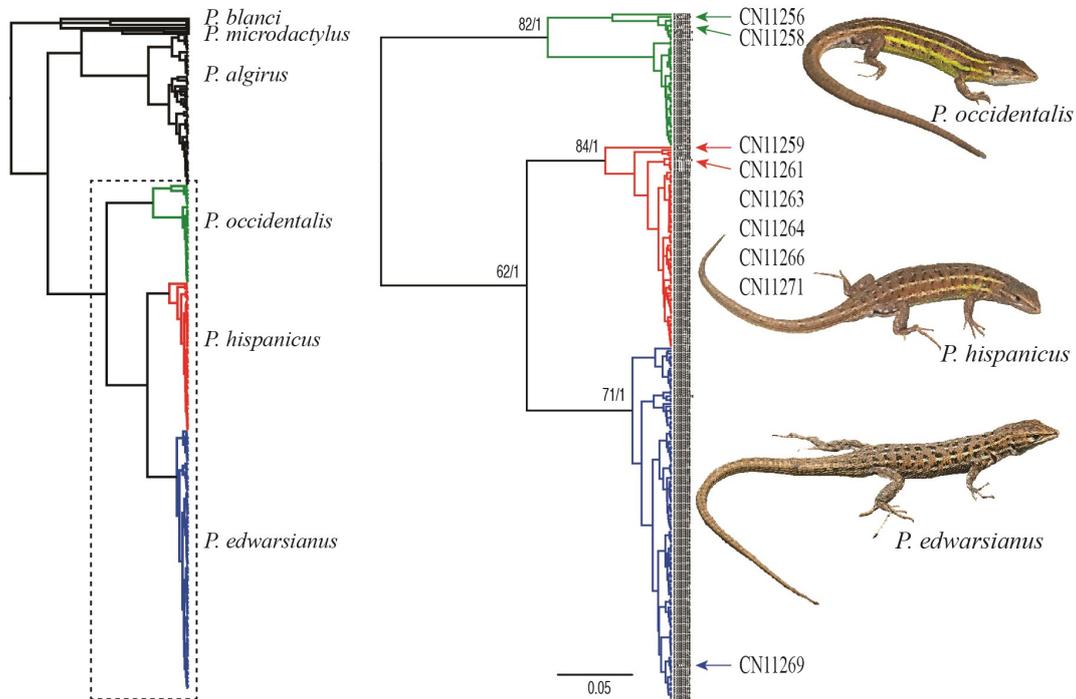
with the following priors (otherwise by default): TrN+G model; Coalescent: Constant size tree model; random starting tree; alpha prior uniform (0–10); uncorrelated relaxed clock (uniform distribution; 0–1). Three individual runs of  $5 \times 10^7$  generations were carried out, with sampling at intervals of every  $5 \times 10^4$  generations. Convergence, posterior trace plots, effective sample sizes (ESS > 200), and burn-in were evaluated with Tracer v.1.6 (RAMBAUT *et al.*, 2014). The runs were combined in LogCombiner discarding the first 10% of trees as burn-in, and the ultrametric tree was generated with TreeAnnotator (both available in the BEAST package). Phylogenetic trees were visualized with FigTree v.1.4.3 (RAMBAUT & DRUMMOND, 2010).

We calculated inter- and intra-specific uncorrected *p*-distances of the *cytb* mitochondrial data in MEGA v.7.0.14 (KUMAR *et al.*, 2016).

## RESULTS

Our dataset for the phylogenetic analyses comprised 367 sequences of the *cytb* gene with a total length of 300 bp: nine newly sequenced individuals of *Psammodromus* from southern Spain, 354 available sequences of *Psammodromus* from GenBank, and four specimens of *Gallotia* (Tables 1 and S1). The ML and BI phylogenetic analyses present a structure of six clades corresponding to the known six species of the genus, though with unsupported relationships between them (Figs. 2 and S1). In both the ML and BI analyses, the nine individuals from southern Spain were nested in three clades belonging to *P. edwardsianus* (CN11269), *P.*

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**Figure 2.** Positioning of the specimens from this study in a phylogeny derived from ML and BI analyses using the models explained in the text, based on 300 bp cytochrome *b* sequences (nine from this study + 354 retrieved from GenBank). On the left, the entire tree of the genus *Psammodromus*, including all six species. On the right, an enlargement of the three clades to which our specimens belong too. Numbers above the nodes correspond to bootstrap support and posterior probability from the ML and BI analyses, respectively. Specimen codes referring to the nine newly sequenced individuals are shown in Table 1. Coloured arrows indicate the location of each specimen within its corresponding clade. The complete ML and BI trees are presented in Figure S1.

*hispanicus* (CN11259, CN11261, CN11263, CN11264, CN11266, CN11271) and *P. occidentalis* (CN11256, CN11258). Two deep lineages were recovered in three species: *P. blanci*, *P. algirus*, and in one of our focal species, *P. occidentalis*. Within the latter species, specimens from one lineage range in southern Spain (specimens from Huelva, southern Badajoz and southern Ciudad Real), and the other specimens occur in the rest of the distribution range.

The inter-specific genetic divergence among *Psammodromus* taxa/lineages (Table S2) ranged between 6.4% (between the two lineages of *P. algirus*) to 18.3% (between *P. blanci* A and *P. microdactylus*). Among our three focal species distances ranged between 7.1% (*P. edwarsianus* and *P. hispanicus*) to 12.9% (*P. hispanicus* and *P. occidentalis* B). Intra-specific distance ranged between 0% (*P. blanci* A) to 3.78% (*P. microdactylus*). Within

our three focal species distances ranged between 0.1–1% (*P. occidentalis*).

### DISCUSSION

Our phylogenetic analyses have identified the nine newly sequenced *Psammodromus* individuals from southern Spain as belonging to the Iberian clade of the genus and within it to the three species occurring in Iberia: *P. edwardsianus* (n=1), *P. hispanicus* (n=6), and *P. occidentalis* (n=2).

The new distribution map based on the new localities presented here further extends the ranges of these three species in southern Iberia. Although our new records do not exhibit large differences in the species' distributions when compared with previous studies (e.g., CARRANZA *et al.*, 2006; FITZE *et al.*, 2011; SAN-JOSE *et al.*, 2012; MENDES *et al.*, 2017), we confirm the presence of *P. hispanicus* in the south (from Málaga, Granada and Cadiz), and detect a wider distribution of *P. occidentalis* (from Badajoz and Ciudad Real), and *P. edwardsianus* (from Valencia). The two deep geographic lineages recovered within *P. occidentalis* are congruent with previous studies (FITZE *et al.*, 2011; MENDES *et al.*, 2017) and necessitate further investigation, including additional specimens.

The biogeographical history of *Psammodromus* suggests that diversification within Iberia is most probably the result of vicariance (FITZE *et al.*, 2011; MENDES *et al.*, 2017). During the Miocene, the final closure of the Betic corridor and the formation of the Guadalquivir basin led to a vicariance event and the divergence of *P. occidentalis* from *P. hispanicus* and *P. edwardsianus* (FITZE *et al.*, 2011; MENDES *et*

*al.*, 2017). Subsequently, the split between the latter two species has occurred due to the uplift of the Spanish Central System which led to the restructure of the main river drainages in the Iberian Peninsula (GOMEZ & LUNT, 2007; FITZE *et al.*, 2011; MENDES *et al.*, 2017). Post-glacial northward expansion from a southern refugia has been suggested for the three species following a latitudinal gradient of mitochondrial diversity (FITZE *et al.*, 2011; HERRERO & FITZE, 2015). The vicariant speciation events likely favoured allopatry among the three species in Iberia - *P. edwardsianus*, *P. hispanicus* and *P. occidentalis* ranging in the east, central, and west of the peninsula, respectively (FITZE *et al.*, 2011).

In the present study we show two potentially overlapping regions between the estimated distribution areas of *P. occidentalis* and *P. hispanicus*, and of *P. edwardsianus* and *P. hispanicus* (Fig. 1), extending the overlapping regions found in FITZE *et al.* (2012). Potential sympatry between *P. occidentalis* and *P. hispanicus* may agree with the niche modelling analyses from FITZE *et al.* (2011), although the two species almost did not show overlapping habitat suitability (contrary to the relatively wider overlapping habitat suitability of *P. edwardsianus* with the two species), suggesting niche divergence may have played a role in their cladogenesis. However, when taking into account that the centre of the Iberian Peninsula was considered a suitable habitat for both *P. edwardsianus* and *P. hispanicus*, our findings of a possible overlap are here supported. Interestingly, our new records of *P. hispanicus* contradict the niche modelling analyses of the species in FITZE *et al.* (2011),

which did not predict its occurrence in southern Spain.

### CONCLUSIONS

Geological and climatic events have had and probably still have an impact on the evolution and distribution of *Psammodromus* lizards. The new records included in this study for *P. occidentalis*, *P. hispanicus* and *P. edwardsianus* in southern Spain improve our current knowledge regarding their distribution in this region. The expansion of the distribution ranges of the three species allowed us to detect possible areas of sympatry among them. Overall, these results suggest that further fieldwork is necessary to broaden our knowledge of the distribution, ecology, and evolution of the *Psammodromus* species in the Iberian Peninsula.

### Acknowledgements

We wish to thank J. A. M. Barnestein, D. Donaire Barroso, R. Carmona González, A. Fraile Molina, J. C. Fraile Molina, S. Gómez Ramírez, F. Jiménez Cazalla, H. Moreno Salguero, P. Toscano Díaz-Galiano, and P. A. Verdejo Díaz for his/her help in the field. Samples were obtained with authorisation from the Consejería de Medio Ambiente y Ordenación del Territorio, Dirección de General de Gestión del Medio Natural y Espacios Protegidos, Junta de Andalucía, Spain to J.P.G.V. The work was supported by grant 2017-SGR-00991 from the Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement de la Generalitat de Catalunya to SC. BB-C is supported by FPU grant FPU18/04742.

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