

Updates to the ranges of *Psammodromus edwardsianus*, *P. hispanicus*, and *P. occidentalis* in the Iberian Peninsula based on citizen science and new genetic data

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The genus *Psammodromus* comprises six species of lacertid lizards: *P. microdactylus* (Boettger, 1881) and *P. blanci* (Lataste, 1880) are endemic to North Africa; *P. algirus* (Linnaeus, 1758) is found in North Africa, Iberia, and southern France; *P. edwardsianus* (Dugès, 1829) is found in Iberia and southern France; and *P. occidentalis* Fitzze et al., 2012 and *P. hispanicus* Fitzinger, 1826 are only found in Iberia. Of these, *P. edwardsianus*, *P. hispanicus*, and *P. occidentalis* were only recently recognised as distinct species based on genetic, morphological, and ecological niche divergence data (Fitzze et al., 2011, 2012).

Psammodromus edwardsianus had long been recognised as a valid subspecies of *P. hispanicus* and can easily be differentiated from the two other species by the presence of a supralabial scale that separates its subocular scale from the lip (vs. subocular reaches the lip in the other two species) and other differences in head shape and coloration. Its range has been broadly described (as a subspecies of *P. hispanicus*) to include southern France and the Spanish autonomous communities of Catalonia, Aragon, Valencia, and Murcia (Böhme, 1981), with its southernmost location in Granada Province, Andalusia, added by Fitzze et al. (2011; see Faria et al., 2021). However, in spite of these general statements, the range of *P. edwardsianus* remains imperfectly known and, as far as we know, no

attempt has been made to properly map its distribution.

Psammodromus occidentalis and *P. hispanicus* are very similar morphologically with no diagnostic features separating the two (Fitzze et al., 2011, 2012) and multivariate classification correctly identifies only around 80% of individuals (San-Jose et al., 2012). Safe field identification is thus impossible based on current knowledge, and genetic identification is currently needed to confirm new localities of these two species. Both species occur in the Spanish autonomous communities of Castilla-León, Castilla-La Mancha, Madrid, Extremadura, and Andalusia, in Navarra Province, as well as in Portugal (Böhme, 1981; Fitzze, 2012a–c; Faria et al., 2021). Due to difficulties with their identification, very few specific localities have been confirmed for these two species (16 for *P. occidentalis*, 20 for *P. hispanicus*) and their ranges are poorly known as a consequence. It appears although that they are mostly parapatric, with *P. occidentalis* in the west of the range and *P. hispanicus* in the east (Fitzze et al., 2011, 2012; Mendes et al., 2017; Molina et al., 2020; Faria et al., 2021; Fig. 1).

We here present an updated range map for these three species (Fig. 1), using data compiled from published localities confirmed using molecular data, images from iNaturalist.org and Observado.org, specimens held in the collection of the Biogéographie et Ecologie des Vertébrés team (BEV) in CEFE (Montpellier), and the photos in the photographic collection of Philippe Geniez (PG). Specimens in the BEV collection or photos in the PG collection (see individual codes in the Appendix) are available from the authors on request. Records based on photos or on specimens for which genetic data were not available were considered to be *P. edwardsianus* if they possessed a supralabial scale below the subocular scale. All other specimens were retained as a single *P. occidentalis/hispanicus* grouping. Photos that did not show this feature clearly were discarded. For *P. edwardsianus*, we only report Spanish localities here because its range in France is already well known (Geniez

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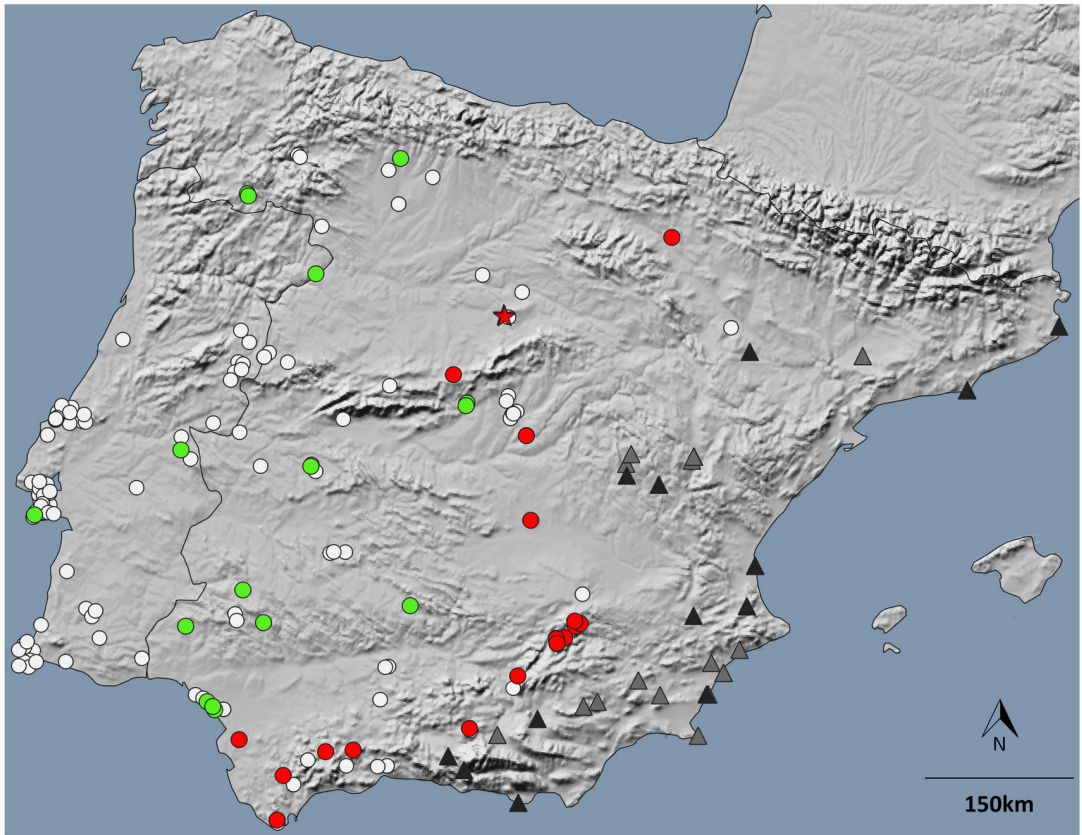


Figure 1. Ranges of three *Psammodromus* species in the Iberian Peninsula. Symbols represent *P. edwardsianus* (black triangles = genetically confirmed, grey triangles = morphologically confirmed), *P. hispanicus* (red circles = genetically confirmed), *P. occidentalis* (green circles = genetically confirmed), and *P. occidentalis/hispanicus* (white circles = morphologically confirmed). The red star identifies the locality of the new genetically confirmed *P. hispanicus* population.

and Cheylan, 2012). All data used to produce the map are given in the Appendix. Note that many localities on iNaturalist, notably all localities for *P. occidentalis*, are given with imprecise locations (accuracy of 28 km) due to the policy of this database on threatened species (see <https://www.inaturalist.org/pages/help#geoprivacy>). At the scale of the Iberian Peninsula, this only minimally affects the map provided here.

We also present new localities for *P. hispanicus* based on three adult females collected near Carrascal del Rio, Segovia, Spain on 25 May 2016 (41.3647°N, 3.9028°W, WGS84; specimen BEV.14627 / tissue sample T11267) and 26 May 2016 (41.3530°N, 3.8896°W; specimens BEV.14628–29 / tissue samples T11268–69). The first specimen was collected in a grassy clearing between dry open forests and cultivated land. The other specimens were found in a dry, steppe-like habitat on uncultivated slopes of small limestone

hills. The head scalation of these individuals excluded *P. edwardsianus* but, since they were collected close to the known range limits of the two other species (Fig. 1), genetic data were needed to identify them. We therefore barcoded these individuals, assuming that mitochondrial DNA is species-diagnostic in these species, as suggested by previous results, although the lack of cyto-nuclear discordance near contact zones has not been verified (Fitze et al., 2011, 2012).

Total genomic DNA was extracted from tail tips using the Dneasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) following recommended procedures. Negative extraction blanks were made by processing tubes without tissue in exactly the same way as tissue samples and were used in all PCR reactions to ensure a lack of contamination. As an 879 bp fragment of the *ND4* gene was already available for all *Psammodromus* species (Fitze et al., 2011, 2012), this locus was amplified for our samples us-

ing primers ND4F (TTACTTTTACTTGGAGTTGCAC-CA) and ND4R (CACCTATGACTACCAAAAGCT-CATGTAGAAGC; Arévalo et al., 1994). PCR reactions were conducted in 20 µl volumes with 2 µl of DNA, 10 µl of Taq Polymerase [1×] (REExtract-N-Amp PCR ReadyMix, Sigma-Aldrich, Saint Louis, Missouri, USA), 0.5 µl of each primer [10 µM], and 7 µl of purified water. Amplifications were performed using the following protocol: initiation of 5 min at 94°C; 40 cycles of 95°C for 30 s, 58°C for 1:30 min, 72°C for 1 min; and final elongation of 10 min at 72°C. To test the success of the PCR, 3 µl of PCR product was migrated on a 1% agarose gel for 30 min at 100 V and 80 mA. Successfully amplified DNA fragments were sequenced by Eurofins Genomics (Ebersberg, Germany) using the same primers as for amplification. Sequences have been deposited in GenBank under accession numbers OM974543, OM974544, and OM974545 for specimens BEV.14627, BEV.14628, and BEV.14629, respectively.

The *ND4* sequences from the three samples were compared to reference samples of *P. microdactylus* (MF684964–66), *P. blanci* (MF684968–70), *P. algirus* (FJ587983–85), *P. edwardsianus* (FJ587814–16), *P. occidentalis* (FJ587833–35), and *P. hispanicus* (FJ587823–24, FJ587826). A phylogenetic tree was constructed from the alignment of *ND4* sequences using the Maximum Likelihood algorithm implemented in Mega-X (Kumar et al., 2018) with 1000 bootstraps and the HKY+G substitution model, which was selected as the best-fitting substitution model using defaults settings implemented in Mega-X.

The three individuals from Carrascal del Rio were identified as *P. hispanicus* (Fig. 2) and constitute a new locality for this species (Fig. 1). The two localities are 1.7 km distant (by air) from each other, 85 km and 200 km from the two closest *P. hispanicus* localities at El Espinar and Andosilla, and 103 km and 202 km from the closest *P. occidentalis* localities at Colmenar del Arroyo and Monte León (Fitze et al., 2011, 2012). They constitute the first indication of the occurrence of the species in the plateau of Castilla-Léon north of the Sistema Central mountains. At the northern edge of these plateaux, *P. occidentalis* has been identified just north of León (Fitze et al., 2011, 2012), indicating that these two species must meet somewhere on the northern plateau of Castilla-Léon. The large geographic distance between known *P. hispanicus* and *P. occidentalis* localities illustrates the incomplete knowledge of these species and the need to find and publish new confirmed localities. Genetic identification is currently

the only reliable method to confirm such records, but morphological criteria to separate *P. hispanicus* and *P. occidentalis* should be investigated to assist in determining the exact ranges of these two species.

Our study also helps refine the range limits for *P. edwardsianus* and *P. hispanicus*. Under the assumption that *P. occidentalis* and *P. hispanicus* are entirely parapatric, the range of *P. edwardsianus* only comes close to that of *P. hispanicus* (Fig. 1). Most of our additional, morphology-based localities fall inside the distribution polygons of previous genetic samples, which suggests that populations of the *P. hispanicus* complex are scarce or absent in many areas where information on species identity is currently lacking. In the Ebro depression, a photo of *P. hispanicus/occidentalis* from Zaragoza (iNat/80749918; Appendix) considerably reduces the gap between *P. edwardsianus* and *P. hispanicus* and may indicate the existence of a potential contact zone which could be studied to evaluate the presence and extent of gene flow between *P. edwardsianus* and *P. hispanicus*. Further dedicated fieldwork in the areas between mapped localities (Fig. 1) is necessary to refine the western range limit of *P. edwardsianus* and the eastern range limit of *P. hispanicus*, which can be established by simple visual examination of the specimens.

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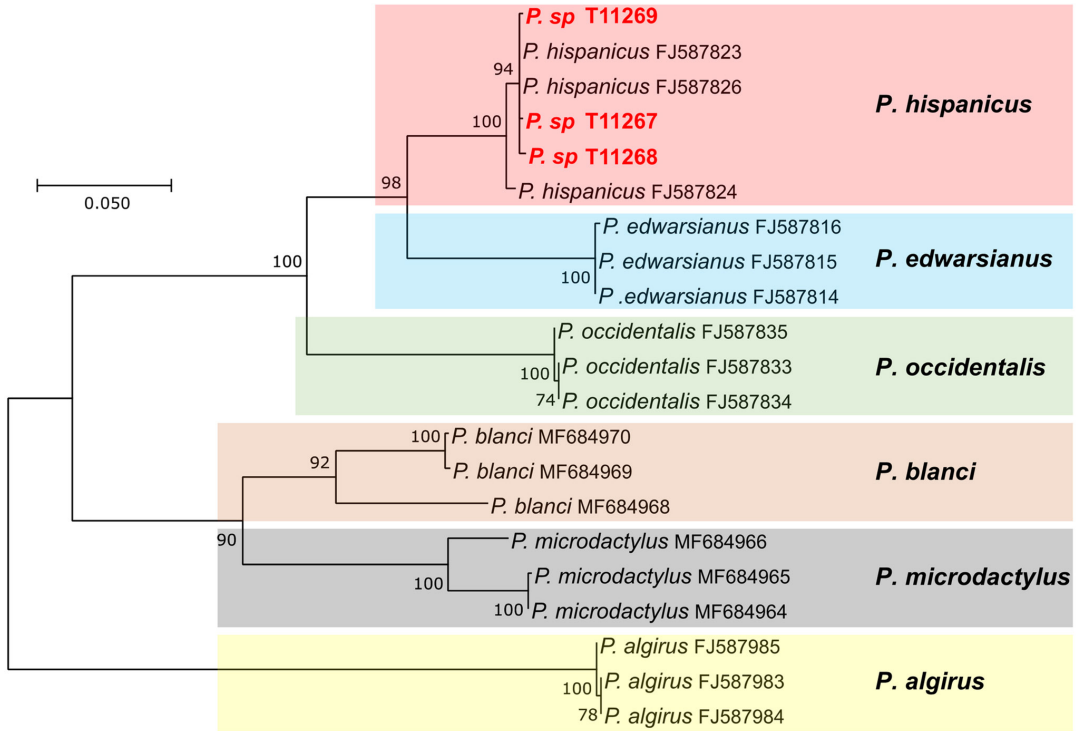


Figure 2. Maximum-likelihood tree inferred from an 879 bp fragment of the *ND4* gene. The three new samples are labelled in red.

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Appendix. Records used to map the distribution of the *Psammotromus hispanicus* complex (Fig. 1). In the Source column, references are abbreviated as follows: Fitze et al. = Fitze et al. (2011, 2012); Mendes et al. = Mendes et al. (2017); Molina et al. = Molina et al. (2020); Faria et al. = Faria et al. (2021); iNat/00000000 = iNaturalist.org, with each record available online by replacing the eight zeros with the numeric code in the link <https://www.inaturalist.org/observations/00000000>; Obs/000000000 = Observation.org, with each record available online by replacing the nine zeros with the numeric code in the link <https://www.observation.org/observations/000000000>. BEV numbers are voucher specimens in the BEV collection and PG numbers refer to photographs in the collection of Philippe Geniez. Other abbreviations are used for genetic data (G), morphological data (M), Spain (ES), and Portugal (PT). Records for which accuracy information is unavailable are indicated by a black circle (•).

Source	ID type	Country	Latitude (°N)	Longitude (°W)	Accuracy
<i>Psammotromus edwardsianus</i>					
Faria et al.	G	ES	39.9854	1.6287	•
Faria et al.	G	ES	38.7648	0.9825	•
Molina et al.	G	ES	38.9294	0.3471	•
Mendes et al.	G	ES	41.29	2.1	•
Mendes et al.	G	ES	38.02	0.70	•
Fitze et al.	G	ES	41.4011	0.7185	•
Fitze et al.	G	ES	41.2867	2.09893	•
Fitze et al.	G	ES	41.9949	3.20075	•
Fitze et al.	G	ES	40.0254	2.0431	•
Fitze et al.	G	ES	39.3326	0.3053	•
Fitze et al.	G	ES	37.5291	2.6882	•
Fitze et al.	G	ES	37.0194	3.6818	•
Fitze et al.	G	ES	36.9124	3.4682	•
Fitze et al.	G	ES	36.6857	2.7674	•
BEV.14060	M	ES	40.1386	2.0718	50 m
BEV.15339	M	ES	38.497	0.3706	50 m
BEV.1790	M	ES	40.2398	2.0287	100 m
iNat/6048301	M	ES	40.2673	1.2599	50 m
iNat/21791145	M	ES	41.5047	0.72434	50 m
iNat/21643654	M	ES	38.0204	0.6792	50 m
iNat/42766810	M	ES	37.7222	2.1600	50 m
iNat/69575894	M	ES	40.3077	1.2411	50 m
iNat/64455556	M	ES	40.3077	1.2463	50 m
iNat/75596142	M	ES	39.3357	0.3077	50 m
iNat/79005962	M	ES	37.6056	0.7391	50 m
Obs/212675112	M	ES	38.2504	0.5286	50 m
Obs/212764253	M	ES	38.3310	0.6922	50 m
iNat/82000608	M	ES	37.7896	2.0017	50 m
iNat/68600826	M	ES	37.9453	1.2584	50 m
PGe.3186	M	ES	37.3086	3.1373	500 m
PGe.3187	M	ES	38.0579	1.5458	500 m
<i>Psammotromus hispanicus</i>					
BEV.14627	G	ES	41.3647	3.9028	80 m
BEV.14628	G	ES	41.3530	3.8896	200 m
BEV.14629	G	ES	41.3530	3.8896	200 m
Fitze et al.	G	ES	42.3944	1.9225	•
Fitze et al.	G	ES	40.7052	4.4047	•
Fitze et al.	G	ES	40.2478	3.3669	•
Fitze et al.	G	ES	39.4388	3.1438	•
Fitze et al.	G	ES	38.4876	2.4002	•
Mendes et al.	G	ES	38.36	2.51	•
Mendes et al.	G	ES	38.34	2.61	•
Mendes et al.	G	ES	38.29	2.59	•
Molina et al.	G	ES	36.9070	4.8167	•
Molina et al.	G	ES	37.3247	3.4819	•
Molina et al.	G	ES	36.8395	5.1379	•
Molina et al.	G	ES	36.0868	5.5620	•
Molina et al.	G	ES	36.7842	6.1819	•
Molina et al.	G	ES	36.5290	5.5862	•
Molina et al.	G	ES	38.5217	2.3467	•
Faria et al.	G	ES	38.3593	2.5097	•
Faria et al.	G	ES	38.3372	2.6071	•
<i>Psammotromus hispanicus</i> (cont.)					
Faria et al.	G	ES	38.2933	2.5912	•
Faria et al.	G	ES	38.5362	2.4168	•
Faria et al.	G	ES	37.9133	3.0032	•
<i>Psammotromus hispanicus/occidentalis</i>					
PGe.3176	M	ES	40.4974	6.4953	500 m
PGe.3177	M	PT	40.6052	7.023	500 m
PGe.3178	M	PT	38.528	8.945	1000 m
PGe.3179	M	PT	37.4426	8.7941	500 m
PGe.3180	M	ES	37.1239	6.7976	500 m
PGe.3188	M	ES	37.0474	6.5665	25 m
PGe.3181	M	ES	37.921	6.484	5000 m
PGe.3182	M	ES	37.1064	6.7003	2000 m
PGe.3183	M	ES	41.6207	3.7219	1000 m
PGe.3184	M	ES	41.3563	3.8400	250 m
PGe.3185	M	ES	41.3563	3.8400	250 m
iNat/1631030	M	PT	37.3558	7.5186	28 km
iNat/14663617	M	ES	39.7266	6.9102	10 m
iNat/5417896	M	ES	42.5382	5.7113	28 km
iNat/14828393	M	PT	39.4730	8.8267	28 km
iNat/14792409	M	PT	39.4730	8.8625	28 km
iNat/15110133	M	ES	42.5565	5.1286	28 km
iNat/14829104	M	PT	39.4753	8.8152	28 km
iNat/14828453	M	PT	39.5428	8.8547	28 km
iNat/16253979	M	PT	40.3926	7.1087	28 km
iNat/16229208	M	PT	40.2905	7.1254	28 km
iNat/15471016	M	PT	37.2156	8.9422	28 km
iNat/15470515	M	PT	39.7574	7.2565	28 km
iNat/17347943	M	PT	40.2056	7.1543	28 km
iNat/16628166	M	PT	40.3841	7.0442	28 km
iNat/16393479	M	PT	40.3292	7.0484	28 km
iNat/21118662	M	PT	38.7723	9.2969	28 km
iNat/20346273	M	PT	38.7966	5.3367	10 m
iNat/17652585	M	PT	37.1804	8.8204	28 km
iNat/25702108	M	ES	42.4923	6.9141	10 m
iNat/37534609	M	ES	41.8646	6.4183	10 m
iNat/30413660	M	ES	38.7578	5.5162	10 m
iNat/29571548	M	ES	37.0427	6.4344	10 m
iNat/42701021	M	ES	40.5490	6.7515	10 m
iNat/42701021	M	PT	39.5536	7.6091	28 km
iNat/40903190	M	PT	38.6557	9.1642	28 km
iNat/40876657	M	PT	37.7039	8.3054	28 km
iNat/40037027	M	PT	38.7844	9.1676	28 km
iNat/43321230	M	ES	37.0427	6.4344	56 km
iNat/46418190	M	PT	38.7504	9.1151	28 km
iNat/46647160	M	PT	38.6115	9.1369	28 km
iNat/46766272	M	PT	38.7832	9.1413	28 km
iNat/46887375	M	PT	38.6873	9.1400	28 km
iNat/47284450	M	PT	38.6733	9.1763	28 km
iNat/47387517	M	PT	38.7909	9.1062	28 km
iNat/61707512	M	PT	38.9701	8.0187	28 km
iNat/59271509	M	PT	40.7005	7.1581	28 km
iNat/59058424	M	PT	37.1936	8.9313	28 km

Appendix (cont.)

Source	ID type	Country	Latitude (°N)	Longitude (°W)	Accuracy
<i>Psammmodromus hispanicus/occidentalis</i> (cont.)					
iNat/57583624	M	PT	40.3447	8.5918	28 km
iNat/49986316	M	PT	38.6047	9.0165	28 km
iNat/48820289	M	PT	37.0799	8.8284	28 km
iNat/70341370	M	PT	38.7122	9.0520	28 km
iNat/65493447	M	ES	40.0662	5.6700	10 m
iNat/65381828	M	PT	38.7354	9.1834	28 km
iNat/64812495	M	ES	40.4783	5.1725	10 m
iNat/63955434	M	PT	38.6760	9.1153	28 km
iNat/72023588	M	PT	37.0499	8.8894	28 km
iNat/71966252	M	PT	38.5142	9.0808	28 km
iNat/71610643	M	PT	37.6409	8.2153	28 km
iNat/71422948	M	PT	38.7306	9.0521	28 km
iNat/70455709	M	PT	38.4467	9.1808	28 km
iNat/70455614	M	PT	38.5958	9.1087	28 km
iNat/70403536	M	PT	38.5210	9.0623	28 km
iNat/73436041	M	PT	38.5083	9.1617	28 km
iNat/73635252	M	ES	42.2337	5.4988	10 m
iNat/74686237	M	PT	38.0123	8.6310	28 km
iNat/72225250	M	PT	38.5703	9.1160	28 km
iNat/72205801	M	PT	38.5275	9.0110	28 km
iNat/72187527	M	ES	39.5713	5.9499	6500 m
iNat/72184810	M	ES	40.4971	6.8012	1800 m
iNat/79460210	M	PT	37.7040	8.1866	28 km
iNat/84159304	M	PT	37.1521	8.4076	28 km
iNat/78720592	M	PT	40.4968	6.8029	1500 m
iNat/78718987	M	ES	40.4970	6.8018	3300 m
iNat/78706306	M	ES	40.4984	6.8003	1900 m
iNat/74861306	M	PT	38.7997	9.2055	28 km
iNat/90867783	M	PT	39.2604	9.2389	28 km
iNat/90321322	M	PT	37.0124	8.8289	28 km
iNat/90197253	M	PT	37.0803	8.7546	28 km
iNat/87966606	M	PT	38.7760	5.4777	52 km
iNat/87093602	M	ES	37.0430	6.4344	56 km
iNat/99814926	M	PT	39.4247	9.0057	28 km
iNat/99807718	M	PT	39.5788	9.0264	28 km
iNat/98544720	M	PT	37.1521	8.9895	28 km
iNat/97165871	M	PT	39.3645	7.4386	28 km
iNat/94332748	M	PT	39.5117	5.8844	10 m
iNat/90868386	M	PT	39.5260	9.0591	28 km
iNat/90868209	M	PT	39.5010	9.1512	28 km
iNat/90868018	M	PT	39.4408	9.1738	28 km
iNat/100614632	M	ES	42.2343	5.4982	31 m
iNat/100614633	M	ES	42.2340	5.4988	31 m
iNat/101413852	M	PT	37.4539	8.0705	28 km
iNat/99814930	M	PT	39.4196	9.1850	28 km
iNat/99814929	M	PT	39.4953	9.1826	28 km
iNat/99814928	M	PT	39.5729	9.1503	28 km
iNat/99814927	M	PT	39.5267	9.0334	28 km

Source	ID type	Country	Latitude (°N)	Longitude (°W)	Accuracy
<i>Psammmodromus hispanicus/occidentalis</i> (cont.)					
iNat/104544420	M	PT	39.4475	9.1748	28 km
iNat/104479131	M	PT	39.4266	9.1781	28 km
iNat/104402817	M	PT	37.2497	8.9153	28 km
iNat/99815911	M	PT	39.4320	9.1859	28 km
Obs/210069056	M	ES	42.4773	6.8800	10 m
Obs/209333286	M	ES	39.4478	6.5689	10 m
Obs/228007328	M	PT	37.0012	8.9478	10 m
Obs/225931703	M	ES	37.9816	6.5196	10 m
iNat/72363899	M	ES	36.7244	5.3292	10 m
iNat/14288196	M	ES	38.8083	2.3716	10 m
iNat/10529690	M	ES	36.8176	4.3855	10 m
iNat/9439973	M	ES	36.4609	5.4444	10 m
iNat/9439972	M	ES	36.4610	5.4449	10 m
iNat/9439971	M	ES	36.4608	5.4447	10 m
iNat/72349609	M	ES	40.4253	3.5881	10 m
iNat/72056529	M	ES	40.3862	3.5984	10 m
iNat/72056528	M	ES	40.3862	3.5996	10 m
iNat/71899533	M	ES	36.7428	4.8647	10 m
iNat/70956118	M	ES	37.4440	4.6001	10 m
iNat/80214970	M	ES	40.4634	3.5362	10 m
iNat/79631006	M	ES	40.5992	3.6740	28 km
iNat/77740887	M	ES	40.5464	3.6860	10 m
iNat/73908304	M	ES	40.4385	3.5781	10 m
iNat/73375028	M	ES	36.7934	4.4942	10 m
iNat/72402619	M	ES	40.4360	3.5722	10 m
iNat/99135600	M	ES	41.7149	4.2621	15 km
iNat/81341382	M	ES	37.3062	3.4637	10 m
iNat/103357559	M	ES	36.0602	5.5490	10 m
iNat/80749918	M	ES	41.6079	0.9970	10 m
Obs/208670692	M	ES	37.777	4.5705	10 m
Obs/211060799	M	ES	37.7653	4.6068	10 m
BEV.7409	M	ES	37.7879	3.035	50 m
<i>Psammmodromus occidentalis</i>					
Fitze et al.	G	ES	42.0130	7.4491	•
Fitze et al.	G	ES	41.9953	7.4303	•
Fitze et al.	G	ES	42.6735	5.5885	•
Fitze et al.	G	ES	40.4561	4.1745	•
Fitze et al.	G	ES	39.5503	5.9543	•
Fitze et al.	G	ES	37.7587	7.0786	•
Fitze et al.	G	ES	37.0826	6.6447	•
Fitze et al.	G	ES	37.0202	6.5374	•
Mendes et al.	G	PT	39.43	7.58	•
Mendes et al.	G	PT	41.4	6.37	•
Mendes et al.	G	ES	40.43	4.18	•
Mendes et al.	G	ES	38.4039	4.4354	•
Molina et al.	G	ES	37.9569	6.1598	•
Faria et al.	G	ES	38.2270	6.483	•
Faria et al.	G	PT	38.4718	9.1708	•
Faria et al.	G	ES	37.0478	6.5673	•