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A new species of sand racer, *Psammodromus* (Squamata: Lacertidae), from the Western Iberian Peninsula

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Abstract

A new species of lacertid lizard of the genus *Psammodromus* is described from the Iberian Peninsula. Genetic and recently published phenotypic data support the differentiation of *Psammodromus hispanicus* into three, and not as previously suggested two, distinct lineages. Age estimates, lineage allopatry, the lack of mitochondrial and nuclear haplotype sharing between lineages, ecological niche divergence, and the current biogeographic distribution, indicated that the three lineages correspond to three independent species. Here, we describe a new species, *Psammodromus occidentalis* **sp. n.**, which is genetically different from the other sand racers and differentiated by the number of femoral pores, number of throat scales, snout shape, head ratio, green nuptial coloration, and number of supralabial scales below the subocular scale. We also propose to upgrade the two previously recognized subspecies, *Psammodromus hispanicus hispanicus* Fitzinger, 1826 from central Spain and *Psammodromus hispanicus edwardsianus* (Dugès, 1829) from eastern Spain, to the species level: *Psammodromus hispanicus* stat. nov. and *Psammodromus edwardsianus* stat. nov. Given that the holotype of *Psammodromus hispanicus* was lost, we designate a neotype. We also analysed museum specimens of *P. blanci*, *P. microdactylus* and *P. algirus* to describe differentiation of the *Psammodromus hispanicus* lineages/species from their closest relatives.

Key words: *Psammodromus hispanicus*, *Psammodromus edwardsianus*, *Psammodromus occidentalis* **sp. n.**, *Psamodromus blanci*, *Psammodromus microdactylus*, mitochondrial and nuclear differentiation, phenotypic differentiation

Introduction

The *Psammodromus* genus consists of four species: the Spanish Sand Racer *P. hispanicus* (Fitzinger 1826), *P. blanci* (Lataste, 1880), which is closest to *P. hispanicus* (estimated split at 20 ± 0.2 Mya; Carranza *et al.* 2006), *P. algirus* (Linneaeus, 1758) (estimated split at 25 ± 0.27 Mya; Carranza *et al.* 2006), and *P. microdactylus* (Boettger, 1881), whose phylogenetic relationship is unknown. The Spanish Sand Racer, *P. hispanicus* consists of two subspecies, *P. hispanicus hispanicus* (Fitzinger 1826) and *P. hispanicus edwardsianus* (Dugès 1829), that can be easily distinguished by the absence or presence of a supralabial scale below the subocular scale, respectively (Boulenger 1921, Mertens 1925, Pérez-Mellado 1998). Described formerly as separate species, they were considered a single species by Duméril & Bibron (1839). Boulenger (1921) was the first to consider two subspecies based on their distribution and morphological differences (e.g. presence/absence of supralabial scale below subocular scale), but he discarded this possibility by concluding "I […] do not deem it advisable, for the present at least, to separate *P. edwardsianus* as a variety or subspecies, although I have felt tempted to do so". Mertens (1925) definitively split *P. hispanicus* into two subspecies; *P. hispanicus edwardsianus* (type locality: "South of France"; Dugès, 1829) was reported from the Spanish east coast and its northern distribution reaches Saint Raphaël, France (crossing the Rhone River; Bons 1989), whereas *P. hispanicus hispanicus* (Terra typica restricta: south of Spain; 'Restricta' indi-

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cates here that Mertens & Müller (1928) attributed a more precise location than the originally described one) was cited from central Spain and Portugal (Boulenger 1921, Mertens 1925). Recent molecular work estimated their split at 9.6 ± 0.11 Mya (Carranza *et al.* 2006). Our recent genetic and phenotypic analyses showed that *P. hispanicus* consists of three different lineages (Fitze *et al.* 2011). Two lineages corresponded to *P. hispanicus hispanicus* and were named Central lineage and Western lineage, respectively, and the third lineage corresponded to *P. hispanicus edwardsianus*, referred to as *edwardsianus* lineage (Fitze *et al.* 2011). Molecular clock dating indicated that the Western lineage diverged 8.3 (2.9–14.7) Mya from the ancestor of the Central lineage and the *edwardsianus* lineage, whereas the other two lineages split 4.8 (1.5–8.7) Mya (Fitze *et al.* 2011). Analyses of phenotypic traits demonstrated that the three lineages could be optically distinguished. All individuals belonging to the *edwardsianus* lineage showed a supralabial scale below the subocular scale, whereas those belonging to the other two lineages showed no scale below the subocular scale. All three lineages differed in the number of femoral pores, number of throat scales, and in snout shape. The number of ocelli differed between the Central lineage and the other two lineages, but no differences in this trait were present between the *edwardsianus* and the Western lineages. Snout-to-vent length (SVL), SVL ratio, body mass, number of ventral scales, and number of collar scales differed between the *edwardsianus* and the Central lineage and there were no differences in these traits between the Western lineage and the two other lineages. The *edwardsianus* lineage differed from the other two lineages in head ratio and the nuptial coloration, and no differences existed in these traits between the Central and Western lineage (Fitze *et al.* 2011).

Here, we discuss phenotypic and genetic differences between *P. hispanicus edwardsianus*, from the Iberian East coast, *P. hispanicus hispanicus* Central lineage from central Spain, and *P. hispanicus hispanicus* Western lineage from the Western Peninsula, to clarify the taxonomy of the *P. hispanicus* group. We provide additional molecular evidence and where necessary we formally designate a holotype, a neotype, and paratypes. We also compared the *P. hispanicus* group with the other members of the genus *Psammodromus*, namely *P. blanci*, *P. microdactylus*, and *P. algirus*.

Material and methods

Specimens analysed. In April and May 2006 we captured 265 specimens in twenty-two different localities in Spain (Figure 1; Fitze *et al.* 2011, Additional File 3 Table S2), in the provinces of Albacete, Almería, Barcelona, Ciudad Real, Cuenca, Cáceres, Galicia, Girona, Granada, Huelva, León, Navarra, Madrid, Segovia, Valencia, and Zaragoza. In addition, a holotype and paratypes were collected at Colmenar del Arroyo (Madrid, 40° 27’ 22.14’’N, 4° 10’ 28.36’’W) and a neotype and additional individuals of *P. hispanicus hispanicus* were captured at Perales de Tajuña (Madrid, 40° 14’ 52.24’’N, 3° 22’ 0.98’’W).

To compare *P. hispanicus* with the closest relatives, we analysed museum specimens of *P. algirus* from Spain (Salamanca: MNCN 36760, 36757-36758; Cáceres: MNCN 36924-36925; Teruel: MNCN 36736), of *P. blanci* originating from Melilla (Spain MNCN 7883, 7884; Zulueta 1909), and of *P. microdactylus* originating from Morocco (Tanger: MNCN 7879-7881; Morocco: SMF-SMF 13534 - lectotype). The species of eighteen non-classified specimens from North Africa belonging to either *P. blanci* or *P. microdactylus* (MNCN 7889 to 7881) and located in the collections of the Natural History Museum in Madrid were independently determined by PSF and LMSJ using the key provided by Schleich *et al.* (1996). The collection locality of the specimens analysed is given as precise as possible. However in some cases (North Africa), the description includes a big geographic area, given that the exact capture location and country are unknown.

Phenotypic measurements. Eleven phenotypic measures were taken (Fitze *et al.* 2011, Table 7) based on standardized digital photographs (for details see Fitze *et al.* 2011, Fitze & Richner 2002). The used photographic setup made sure that all photographs received a standard light exposure and that the photographic scale was identical among photos. Permutational MANOVA were used to investigate differences between lineages and univariate ANOVAs were used to understand which traits differed.

Molecular methods. Genomic DNA was extracted from ethanol-preserved tissues using ChargeSwitch gDNA Micro Tissue Kit (Invitrogene). A subsample including 59 representative specimens of *P. hispanicus* and 16 specimens of *P. algirus* were sequenced for mitochondrial cytochrome *b* (co*b*) and *NADH dehydrogenase subunit* 4 (nad4) partial genes and two nuclear loci: the suppressor of SWI4 1 described in *Anolis carolinensis* and an

unknown fragment named as clone 17 (see Fitze *et al.* 2011: additional file 4 Table S3 for specimen number and GenBank accession numbers).

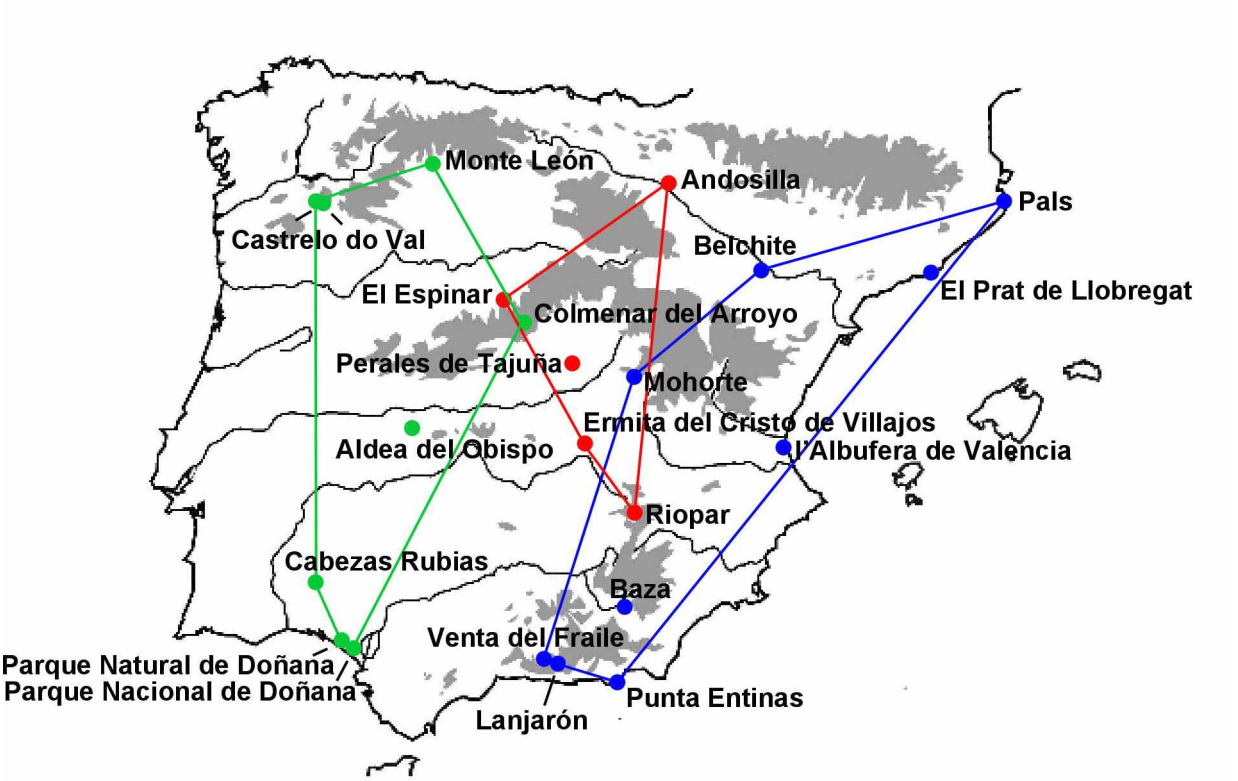

FIGURE 1. Sampling localities. Populations of *Psammodromus occidentalis* **sp. n.** are coloured in green, populations of *Psammodromus hispanicus hispanicus* are coloured in red, and those of *Psammodromus hispanicus edwardsianus* are coloured in blue. More details about the sampled populations can be found in Fitze *et al.* 2011 (Additional File 3 - Table S2). Minimum convex polygons encompass the sampled populations of each group.

A 275–418 bp region of *cob* gene was amplified in all individuals using either forward primer L14841 (Kocher *et al.* 1989) or MNCN-Glu F (San Mauro *et al.* 2004) and reverse primer H15149 (Kocher *et al.* 1989). A fragment of 865 bp of the *nad4* gene was amplified using primers *nad4* and LEU (Arevalo *et al.* 1994) for most specimens. We designed two primers (forward: L11162, reverse: tRNA-His H11749, see Fitze *et al.* 2011 Table 6) in conserved regions of the *nad4* gene to amplify a shorter and fully overlapping region (504 bp) in the remaining individuals. Primers for the two nuclear regions (suppressor of SWI4 1 and clone17) were obtained by cloning (for further details see Fitze *et al.* 2011). Further detail about PCR conditions can be found in Fitze *et al.* 2011.

Phylogenetic analyses. We conducted two types of analyses that complement those of Fitze *et al.* 2011. First, we compiled a data set, hereafter referred to as mitochondrial data set that included partial sequences of *cytb* and *nad4* of 54 *P. hispanicus* individuals.

Second, we compiled a nuclear data set consisting of partial sequences of the suppressor of SWI4 1 and of nuclear clone 17 of the same 54 individuals.

Sequences were aligned using Clustal X version 1.83 (Thompson *et al.* 1997) with default penalties for gap opening and gap extension. Alignments were verified by eye. For each molecular marker, independent alignments were prepared, and the best-fit models of nucleotide substitution were inferred using the Akaike information criterion (AIC (Aikaike 1973) as implemented in Modeltest version 3.7 (Posada & Crandall 1998). The substitution models selected were: HKY + Γ for *cytb*, TrN + I for *nad4*, HKY + Γ + I for suppressor of SWI4 1, and TrNef + Γ for nuclear clone 17. Both combined data sets were analyzed using maximum likelihood (ML; Felsenstein 1981), and Bayesian inference (BI; Huelsenbeck & Ronquist 2000). ML analyses were performed with RAxML version 7.2.6 (Stamatakis 2006) using the rapid hill-climbing algorithm (Stamatakis *et al.* 2007) and starting from 100 distinct randomized maximum-parsimony starting trees. For BI analyses, we used MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2000; Ronquist & Huelsenbeck 2003). We run four simultaneous Markov chains for 20 million

generations, sampling every 2000 generations (10,000 trees), and discarding the first 10% of generations (1,000 trees) as burn-in to prevent sampling before reaching stationarity. Adequate convergence of the Bayesian Markov chain Monte Carlo runs was assessed by low standard deviation of split frequencies (as implemented in MrBayes) as well as using Tracer version 1.5 (http://tree.bio.ed.ac.uk/software/tracer/). Two independent BI runs were performed to increase the chance of adequate mixing of the Markov chains, and to give some chance of spotting failure to converge. For the BI analysis, independent best-fit models were used for each of the employed molecular markers. For ML analyses, the GTR + Γ model was used for all partitions due to software (RAxML) constraints, and model parameters were unlinked and estimated separately among partitions. Statistical support for internal branches in the ML analyses was evaluated by non-parametric bootstrapping (Felsenstein 1985) with 2,000 replicates and using posterior probabilities in the BI analyses.

Results

Genetic analyses: Phylogenetic Relationships within *Psammodromus hispanicus*

Phylogenetic analyses of the mt dataset were based on a 846 bp alignment with 279 variable positions, and 263 parsimony-informative sites. Phylogenetic analyses of the nuclear dataset were based on a 1168 bp alignment with 135 variable positions, and 88 parsimony-informative sites. The ML reconstructed trees are shown in Figure 2. The BI trees were identical in topology to the ML tree regarding basal nodes and differed only in the arrangement of terminal nodes, which did not received statistical support in either analysis.

Both analyses show that *P. hispanicus* split into two well-supported lineages one representing the Western lineage, and the other one formed by the Central and *edwardsianus* lineage (Figure 2). The date of the split was estimated at 8.3 (2.9–14.7) Mya (Fitze *et al.* 2011). The monophyly of both the Central and *edwardsianus* lineage was supported in both datasets (Figure 2), which supports previous findings (Fitze *et al.* 2011). The split between the Central and the *edwardsianus* lineage has been estimated at 4.8 (1.5–8.7) Mya (Fitze *et al.* 2011).

None of the *cytochrome b* (*cytb*), *nad 4*, suppressor of SWI4 1, or clone 17 haplotypes was shared between *P. hispanicus* lineages (Fitze *et al.* 2011, Figure 4) and minimum-spanning networks, spatial haplotype distributions (Fitze *et al.* 2011, Figure 4), and lineage specific minimum convex polygons (Figure 1) showed current allopatry. These analyses show that the *edwardsianus* lineage inhabits Eastern Spain and Southern France; the Central lineage inhabits central Spain, and the Western lineage Western Spain.

Previous analyses showed that *P. hispanicus* and *P. algirus* form two sister lineages (Fitze *et al.* 2011), which may have split approximately 17.25 (5.19–30.59) Mya (Fitze *et al.* 2011, Figure 3).

Phenotypical analyses. There were significant differences among *P. hispanicus* lineages in the number of femoral pores, supralabial scales below the subocular scale, throat scales, ventral scales, collar scales, and ocelli, (Fitze *et al.* 2011, Table 3). They further differed in SVL, SVL ratio, snout shape, anal scale width, head ratio, body mass, and green nuptial coloration (Fitze *et al.* 2011, Table 3).

Post – hoc analyses showed significant differences among the three lineages (Fitze *et al.* 2011, Table 3). Univariate analyses revealed that the Central and Western lineage differed in the number of femoral pores, throat scales, ocelli, and snout shape, but not in SVL ratio, anal scale width, body mass, number of ventral, collar, and supralabial scales below the subocular scale, and in head ratio, SVL, and green nuptial coloration.

The *edwardsianus* and Western lineage differed in the number of femoral pores, throat scales, snout shape, head ratio, the number of supralabial scales below the subocular scale, and green nuptial coloration, but not in the number of ocelli, ventral scales, and collar scales, and in anal scale width, body mass, SVL ratio, and SVL.

The Central and *edwardsianus* lineage differed in the number of femoral pores, throat scales, ocelli, ventral scales, and collar scales, and in SVL ratio, snout shape, body mass, head ratio, SVL, and green nuptial coloration, but not in anal scale width (Fitze *et al.* 2011).

The *edwardsianus* lineage had more femoral pores, more throat scales, more ocelli, a higher SVL ratio, less pointed snouts, lower body mass, less ventral scales, lower head ratio, smaller SVL, less collar scales, and less green nuptial coloration than the Central lineage. The Western lineage showed intermediate values, except for number of ocelli and snout shape where values were bigger than in the Central linage, and in snout shape and green nuptial coloration where values were bigger than in the *edwardsianus* lineage. The *edwardsianus* lineage can be

easily distinguished from the two other lineages by the presence of a supralabial scale below the subocular scale, which is absent in the other lineages (Fitze *et al.* 2011).

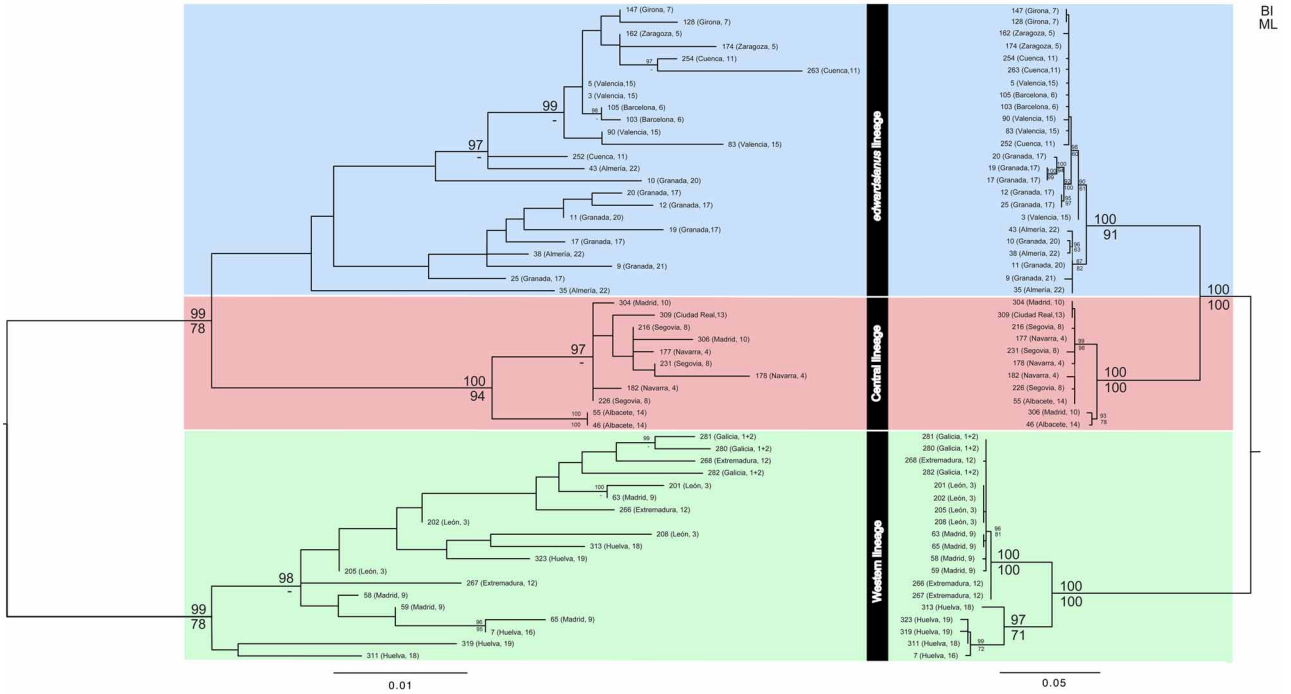

FIGURE 2. Maximum likelihood phylogeny of *Psammodromus hispanicus* based on the nuclear (left phylogeny) and on the mt data set (right phylogeny). The number above each branch refers to the Bayesian posterior probability (shown as percentage) of the node. Bootstrap values for ML are shown below branches. The lineage name, and the specimen's reference number are given. The sample location and the population number are indicated in brackets.

Ten of the 18 non-classified specimens from North Africa were identified as *Psammodromus blanci* (MNCN 7890, 7891, 7893, 7895, 7897, 7898, 7901 - 7903, 7905, 7906) and two as *Psammodromus microdactylus* (MNCN 7895, 7899), while six specimens could not be unequivocally attributed using Schleich *et al.*'s (1996) criteria. None of the *Psammodromus microdactylus* had a gular fold and 100% *P. blanci* had a more or less distinct gular fold (Table 1). All *P. blanci* showed kind of a collar, i.e. collar scales that clearly differed from throat and ventral scales were absent in all cases, but all showed a clear size difference between the last throat row and the ventral scales. In most *P. microdactylus* specimens throat scales continuously increased in size towards the ventral scale and no clear size distinction existed between the last throat row and the first ventral scale row. Lateral stripes were absent in *P. microdactylus*, maybe due to the specimen's age, and could be seen in 58% of *P. blanci*. Only unequivocally attributed individuals were used in between species comparisons.

Specimens of *P. hispanicus* can be distinguished from *P. algirus* by the smaller body size, a shorter tail length that rarely exceeds 2 x SVL, absence of pterygoid teeth, presence of a gular fold and distinct collar scales, and presence of two central ventral rows of clearly narrower scales compared to scales of adjoining rows. The comparison of the *P. hispanicus* lineages with *P. blanci* and *P. microdactylus* from known localities showed that *P. microdactylus* can be distinguished from the other species by the absence of a gular fold (Table 1). In 40 % of *P. microdactylus* kind of a collar could be detected, while a collar was present in 100% of the other species (Table 1). All *P. hispanicus* lineages show rectangular and non-overlapping ventral scales and two lateral stripes. *Psammodromus blanci* showed one or two lateral stripes in 58.3% of the individuals and *P. microdactylus* showed no lateral stripes. In both species the ventral scales are pointed or rounded and imbricated. While in the *P. hispanicus* lineages the central ventral rows were smaller than the adjoining rows, those of *P. blanci* and *P. microdactylus* were only slightly smaller or in some cases even bigger than the adjoining ventral scales. The first supraocular scale was small with respect to the second supraocular scale in all members of *P. hispanicus* and it was very tiny and by eye almost non-visible in most individuals of *P. blanci* and *P. microdactylus*. Our analyses of *P. blanci* and *P. microdactylus* showed, that the only character that allows distinguishing between the two species (at least in old museum specimens) is the presence/absence of the diffuse gular fold. Six specimens showed intermediate characters and did not

allow for species distinction. These results clearly show, that a robust taxonomic description of North African species is lacking and that further research is needed.

TABLE 1. Comparison of the *P. hispanicus* lineages with the closest relatives, namely *P. blanci* and *P. microdactylus*. Shown are differences between lineages and species measured on alive and museum specimens in the presence/absence of gular fold, collar, and lateral stripes and differences in ventral scale and ocular scale size.

species/lineage	N	gular fold present	collar present	lateral stripes
P. hispanicus hispanicus Western lineage	29	100%	100%	100%
P. hispanicus hispanicus Central lineage	20	100%	100%	100%
P. hispanicus edwardsianus lineage	51	100%	100%	100%
P. blanci	12	100%	100%	58.3%
P. microdactylus	5	0%	40%	0%

continued.

species/lineage	ventral scales		range of proportion of central ventral rows	1st supraocular scale
Western lineage	rectangular	not overlapping	70–50% of adjoining row	small in 100%
Central lineage	rectangular	not overlapping	70–50% of adjoining row	small in 100%
edwardsianus lineage	rectangular	not overlapping	70–50% of adjoining row	small in 100%
P. blanci	pointed or round	imbricated	110–75% of adjoining row	very small in 64.3%, small in 35,7%
P. microdactylus	pointed or round	imbricated	138–85% of adjoining row	very small in 71.4%, small in 28.6%

Designation of Neotype for *Psammodromus hispanicus*

In 1826, Fitzinger mentioned for the first time a specimen attributed to *Psammodromus hispanicus*. In his book he wrote 'Eine schöne neue Art aus Spanien, *Psammodromus hispanicus* Mihi, gab Veranlassung zur Gründung einer neuen Gattung…' or translated to English 'a nice new species from Spain, *Psammodromus hispanicus* Mihi, was the reason for a new genus' (Fitzinger 1826, page 22). Given that *P. hispanicus* was listed as being part of the Reptile collection of the Imperial-Royal Zoology Museum in Vienna (k. k. zoologisches Museum zu Wien; see page 52), it is not clear whether Fitzinger or somebody else collected the specimen. In Fitzinger's Classification, no collection date nor a type locality were described. We contacted Dr. H. Grillitsch, head of the herpetology collection at the Natural History Museum in Vienna (NHMW), who confirmed that there exist two specimens of *P. hispanicus* at the NHMW and that the collection of the k. k. zoologisches Museum zu Wien is nowadays part of the NHMW. According to Grillitsch, none of the two specimens can be attributed to the specimen described by Fitzinger, for the following reasons. First, no collection date and no collector name are indicated on the specimen labels. Second, the writing is not Fitzinger's hand writing. Third, on the label it is written '*Psammodromus Edwardsii* D&B = *P. hispan.* Spanien??'. Given that Dugès used the name *L. edwarsiana* (Lézard d'Edwards) for the first time in 1829 (the specimen was later reclassified as belonging to *Psammodromus*), it is likely that the two specimens at the NHMW were collected or named after 1829, given that they were already named on the label as *P. edwardsii*. Due to these facts and according to Grillitsch, the type specimen of *P. hispanicus* must have been lost or is untraceable. According to ICZN's (2000) Article 75.3.4, the conditions for delimitating a neotype are thus fulfilled.

Neotype: The original description states that *P. hispanicus* is a nice new species from Spain (see above). To be consistent with this description we designate a specimen as neotype of *P. hispanicus* that belongs to the lineage, which only exists in Spain (Fitze *et al.* 2011).

The neotype (Figure 3) is deposited at the Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, Spain (MNCN/ ADN 41745, field number PF_09_670). It is conserved in pure alcohol and frozen at -80°C.

It is an adult male from Perales de Tajuña (Madrid), captured on 15 October 2010 by Patrick S. Fitze and Luis M. San-Jose.

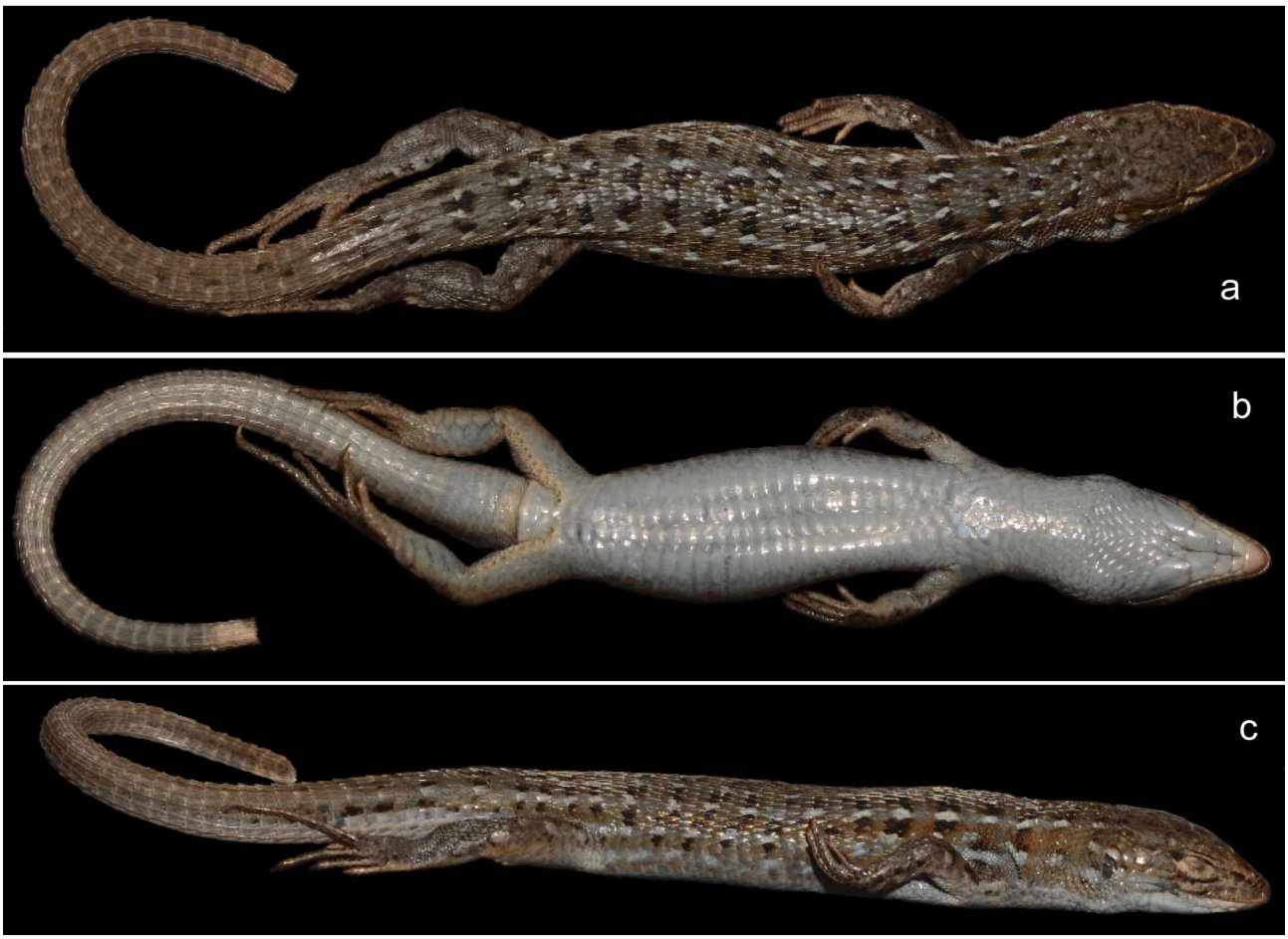

FIGURE 3. Photographs of the neotype of *Psammodromus hispanicus* voucher number MNCN/ ADN 41745; a) dorsal view, b) ventral view, c) lateral view (right side).

Description of the neotype: Adult male: SVL 49 mm, total length 124 mm, belly colour white, body mass 1.941 g, 10 femoral pores on both hind limbs, head length 11.06 mm, snout length 4.27 mm, snout width 3.71 mm, anal scale width 3.35 mm, 25 ventral scale rows, no supralabial scale below the subocular scale, 18 throat scales, four collar scales, three ocelli on each flank, nuptial coloration score 0.

Other specimens collected at the neotype location: MNCN/ ADN 41746 (field number PF09_669), first-year male; MNCN/ ADN 41747 (field number PF09_671), adult male; MNCN/ ADN 41748 (field number PF09_672), adult female; MNCN/ ADN 41749 (field number PF09_673), adult female; MNCN/ ADN 41750 (field number PF09_674), first-year male; All specimens were captured in the neotype locality (Perales de Tajuña, Madrid) on 15 October 2010 by Patrick S. Fitze and Luis M. San-Jose. They are conserved in pure alcohol and frozen at -80°C. The characteristics of the collected individuals are summarized in Table 2.

Psammodromus occidentalis new species

Holotype: MNCN/ ADN 34516 (field number PF_08_2001, GenBank accession number FJ587677), adult male from Colmenar del Arroyo (Madrid, Spain, 40° 27’ 22.14’’N, 4° 10’ 28.36’’W) captured on 24 October 2008 by Patrick S. Fitze and Virginia Gonzalez-Jimena. It is conserved in pure alcohol and frozen at -80°C.

TABLE 2. Variation of specimens of the *Psammodromus hispanicus hispanicus* Central lineage collected on 15 October 2010 at the Neotype locality.

Voucher number	MNCN/ ADN				
	41746	41747	41748	41749	41750
age	1st year	adult	adult	adult	1st year
sex	male	male	female	female	male
SVL (mm)	45	48	50	47	40
total length (mm)	113	96	91	109	103
tail characteristics	intact	re-grown, cut at 57 mm[1]	re-grown, cut at 56 mm[1]	re-grown, cut at 71 mm[1]	intact
belly coloration	white	white	yellowish	yellowish	yellowish
body mass (g)	1.48	1.87	1.99	1.48	1.05
# femoral pores (left/right)	10/10	10/10	10/10	10/11	10/11
head length (mm)	10.4	11.4	10.3	9.9	9.7
snout length (mm)	3.8	4.1	3.9	3.8	3.5
snout width (mm)	3.4	3.6	3.6	3.4	3.2
anal scale width (mm)	3.0	3.6	3.3	2.5	2.4
# ventral scale rows	24	24	28	29	24
# supralabial scales below subocular scale	0	0	0	0	0
# number throat scales	17	17	19	21	17
# collar scales	0[2]	6	5	3	5
# ocelli (left/right)	2/2	2/2	1/1	2/2	2/2
nuptial coloration score	0		0	0	0

[1]measured from snout tip; [2]not differentiated.

Description of the holotype. Adult male (Figure 4) with detached original tail: SVL 43 mm, total length 63 mm, belly colour white, body mass 1.497 g, 12 femoral pores on both hind limbs, head length 10.7 mm, head width 5.5 mm, snout length 3.5 mm, snout width 4.1 mm, anal scale width 2.9 mm, 24 ventral scales, no supralabial scale below the subocular scale, 18 throat scales, one collar scale, two ocelli on each flank, nuptial coloration score 0.

Paratypes: MNCN/ ADN 34515 (field number PF_08_2000, GenBank accession number FJ587676), first-year female. BMNH 2008.271 (field number PF_08_2002; DNA sample MNCN/ ADN 34517; GenBank accession number FJ587678), adult male. BMNH 2008.272 (field number PF_08_2003; DNA sample MNCN/ADN 34518, GenBank accesion number: FJ587679), first year male. All specimens were captured in the type locality (Colmenar del Arroyo, Madrid 40° 27' 22.14''N, 4° 10' 28.36''W) by Patrick S. Fitze and Virginia Gonzalez-Jimena on October 24th, 2008. They are conserved in pure alcohol and frozen at -80°C. The characteristics of the collected individuals are summarized in Table 3.

Diagnosis. *Psammodromus occidentalis* **sp. n.** (former name: *P. hispanicus hispanicus* Western lineage; Fitze *et al.* 2011) shows 20–29 ventral scale rows, no supralabial scale below the subocular scale, 15–26 throat scales, 0–3 collar scales, 9–15 femoral pores, a snout shape of 0.96–2.51, 0–5 ocelli, and a nuptial coloration score of 0–4. It can be distinguished from the *edwardsianus* lineage by the absence of a supralabial scale below the subocular scale, lower femoral pore numbers, more extended nuptial coloration, and slightly bigger snout shape values, and from the Central lineage (*P. hispanicus*) by bigger snout shape values, corresponding to a less pointed snout, higher number of femoral pores, and higher number of ocelli. Molecular differentiation based on mitochondrial and the nuclear datasets shows that *P. occidentalis* **sp. n.** is more distant from the *edwardsianus* and Central lineage than the later two from each other. *Psammodromus occidentalis* **sp. n.** can be easily distinguished from *P. algirus* by the smaller body size, a shorter tail length that rarely exceeds 2 x SVL (in adult individuals with intact tail: mean = 1.5 x ± 0.02; range = 0.7 – 2.4; *P. algirus* > 2 x SVL, Böhme 1981), absence of pterygoid teeth (Arnold 1989), presence of a gular fold and distinct collar scales, absence of imbricated and pointed ventral scales, and presence of two

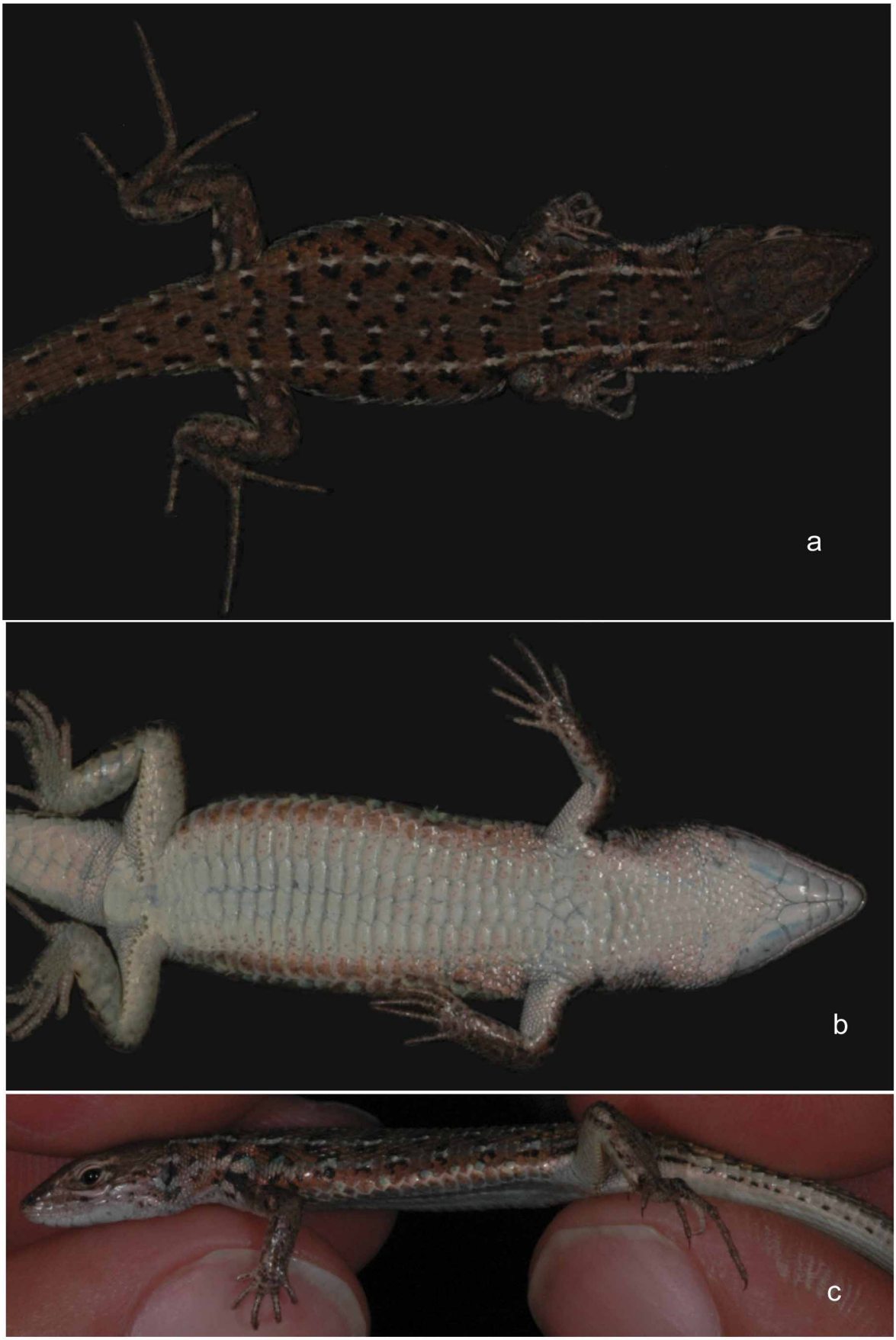

FIGURE 4. Photographs of the holotype *Psammodromus occidentalis* **sp. n.** voucher number MNCN/ADN 34516; a) dorsal view, b) ventral view, c) lateral view (left side).

TABLE 3. Variation of *Psammodromus occidentalis* **sp. n.** paratypes collected on 24 October 2008 at the holotype locality.

Voucher number	MNCN/ ADN 34515	BMNH 2008.271	BMNH 2008.272
age	1st year	adult	1st year
sex	female	male	male
SVL (mm)	39	44	37
total length (mm)	69	126	105
tail characteristics	tail cut previous to capture	intact	intact
belly coloration	white	white	yellowish
body mass (g)	0.98	1.55	1.00
# femoral pores (left/right)	10/10	11/11	11/11
head length (mm)	9.3	11.2	9.2
head width (mm)	4.7	5.9	4.8
snout length (mm)	3.0	3.5	3.6
snout width (mm)	3.3	3.9	2.9
anal scale width (mm)	2.4	3.4	2.2
# ventral scale rows	26	22	24
# supralabial scales below subocular scale	0	0	0
# number throat scales	19	20	20
# collar scales	1	1	1
# ocelli (left/right)	2/2	2/2	2/3
nuptial coloration score	0	0	0

central ventral rows of clearly narrower scales compared to scales of adjoining rows. *Psammodromus occidentalis* **sp. n.** differs from *P. blanci* by a clearly present gular fold, absence of imbricate or rounded ventral scales, two narrower central ventral rows (compared to the adjoining ventral rows), by rarely existing solid lateral lines, and by a brown grayish dorsal ground color (Schleich *et al.* 1996). It differs from *P. microdactylus* by the presence of a gular fold, distinct collar scales, a brown grayish dorsal ground color, two dashed lateral lines, absence of pointed or rounded central ventral rows, presence of two narrower central ventral rows, and absence of greenish or dark olive dorsal ground color (Schleich *et al*. 1996).

Etymology. The species epithet refers to the geographical distribution of this lizard. All described populations are located on the Western Iberian Peninsula.

Distribution. *P. occidentalis* **sp. n.** was captured on the Western Iberian Peninsula (Figure 1). The *edwardsianus* and Central lineages were found on the East coast and in the centre of the Iberian Peninsula, as previously described (Boulenger 1921; Fitze *et al.* 2011).

Discussion

Two subspecies of the Spanish Sand Racer, *P. hispanicus edwardsianus* and *P. hispanicus hispanicus* have been described and are accepted so far. They can be distinguish based on the presence or absence of a supralabial scale below the subocular scale, respectively (Boulenger 1921). Our analyses, which were based on a relatively large number of specimens, strongly supported this taxonomic hypothesis since the alternative states of the described trait perfectly distinguished between all analyzed *P. hispanicus hispanicus* and *P. hispanicus edwardsianus* individuals. Moreover, our analyses also revealed other important differences including that *P. hispanicus hispanicus* Central lineage is significantly bigger than *P. hispanicus edwardsianus*, and that *P. hispanicus edwardsianus* has more femoral pores and ocelli, less collar scales, and less extended nuptial coloration. In addition, haplotype distributions based on mitochondrial co*b* and *nad4* fragments and nuclear suppressor of SWI4 1 and clone 17 showed current allopatry (100%) of the two subspecies. Their split was estimated at 4.8 (1.5–8.7) Mya. All populations

consisted of specimens belonging to one genetic lineage only (Fitze *et al.* 2011). The strong phenotypic and genetic differences together with the age of the estimated split and allopatry allow proposing that the two subspecies should be upgraded to the species level: *Psammodromus hispanicus* and *Psammodromus edwardsianus* stat. nov. These results confirm those of Carranza *et al.* (2006) who also suggested that the two subspecies might be two independent lineages that split around 9.6 Mya years ago.

Our results also indicated that there exists a third lineage (*P. hispanicus hispanicus* Western lineage). Phenotypic analyses showed that this lineage differs from (1) the other two species in the number of femoral pores, the number of throat scales, and snout shape, (2) from *P. edwardsianus* in the extent of the green nuptial coloration, head ratio, and the absence of a supralabial scale below the subocular scale, and from (3) *P. hispanicus* in the number of ocelli (see Fitze *et al.* 2011 Table 3). Some traits are similar to those of *P. edwardsianus* (number of ocelli, anal scale width, number of ventral and collar scales), whereas others are similar to those of *P. hispanicus* (nuptial coloration, SVL, head ration, number of ventral, collar and supralabial scales below subocular scale), potentially explaining why this lineage has been overlooked until now. The molecular data (both mt and nuclear data), the estimated age of divergence (8.3 (2.9–14.7) Mya), allopatry of mt and nuclear haplotypes, and the phenotypic differences, indicate that this third lineage must correspond to a yet undescribed species, which we hereafter name as *Psammodromus occidentalis* **sp. n.** Previous work as well indicated that the three species inhabit different ecological niches with *P. hispanicus* showing the highest niche divergence (with respect to the other two species) and living in habitats characterized by lowest minimum winter temperatures and precipitation seasonality, and intermediate vegetation cover (Fitze *et al.* 2011). *P. occidentalis* **sp. n.** and *P. edwardsianus* showed smaller niche divergence. *P. occidentalis* **sp. n.** lived in habitats with the highest vegetation cover, winter precipitation, precipitation seasonality and winter minimum temperatures, while *P. edwardsianus* lived in habitats with low vegetation cover and precipitation, and generally higher temperatures (Fitze *et al.* 2011). The patterns of niche divergence are in line with the molecular and phenotypic data and the molecular dating and provides additional evidence that *P. hispanicus* consists of three different species: *P. hispanicus*, *P. edwardsianus*, and *P. occidentalis* **sp. n.**

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