

## Patterns of phenotypic variation reveal substantial differentiation in sexual dimorphism of three *Psammodromus* (Squamata, Lacertidae) species

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### Abstract

The Spanish sand racer (*Psammodromus hispanicus*) has been recently split into three distinct species: *P. hispanicus*, *P. edwardsianus*, and *P. occidentalis*. Some morphological differences have been reported but there is as yet no description allowing unambiguous identification of the three species. Here, we describe differentiation in body measurements, scalation traits, and colour traits as well as in the degree of sexual dimorphism. Our results show that *P. edwardsianus* can be easily distinguished by the presence of a supralabial scale below the subocular scale, which is absent in the other two species. *Psammodromus hispanicus* and *P. occidentalis* can be distinguished by the number of femoral pores, throat scales and ocelli, and the relative width of the anal scale. The degree of sexual size dimorphism and sexual colour dimorphism substantially differs among species, suggesting that different scenarios of sexual and natural selection may exist for each species. Moreover, sexually selected traits (nuptial colouration, ocelli, and femoral pores) significantly differ among species, suggesting that visual and chemical communication may also differ among species. Such differences could prevent reproduction and gene flow at secondary contact zones, potentially reinforcing isolation and speciation within this group of lizards.

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### Introduction

The Spanish sand racer, *Psammodromus hispanicus* Fitzinger, 1826 (Squamata, Lacertidae), is a small ground-dwelling lizard that inhabits the Iberian Peninsula and the French Mediterranean coast. It lives in sandy open habitats scattered with small bushes that it uses as shelters (Blasco, 1974; Carrascal *et al.*, 1989; Carretero and Llorente, 1991a). Early descriptions suggested the existence of two subspecies, *P. hispanicus hispanicus* (Fitzinger, 1826) on the Western Iberian Peninsula and *P. hispanicus edwardsianus* (Dugès, 1829) in the Eastern Iberian Peninsula and south of France (Boulenger, 1921; Hellmich, 1962; Perez-Mellado, 1998). Initial molecular studies provided evidence for important genetic differentiation between these two subspecies (Carranza *et al.*, 2006). However, recent studies based on wider sampling and using both mitochondrial and nuclear data support the existence of three distinct species: *P. edwardsianus* (Dugès, 1829), *P. hispanicus* Fitzinger, 1826, and *P. occidentalis* Fitze *et al.*, 2012 (see Fitze *et al.*, 2011, 2012). *Psammodromus edwardsianus* inhabits the Eastern Iberian Peninsula and Southern France, and split 4.8 (1.5-8.7) million years ago (Mya) from *P. hispanicus*, which inhabits the centre of the Iberian Peninsula. *Psammodromus occidentalis*, inhabiting the Western Iberian

Peninsula, diverged 8.3 (2.9–14.7) Mya from the ancestor of *P. hispanicus* and *P. edwardsianus*. Additionally, genetic differentiation between northern and southern populations has been found within *P. edwardsianus* and *P. occidentalis*, suggesting northern range expansions after the last glaciation. In contrast, no clear geographic structure has been found in *P. hispanicus* (Fitze *et al.*, 2011).

To date, the few existing phenotypic studies on *P. hispanicus* focused on the description of the two subspecies suggested by Boulenger (1921) and, thus, only on the differences between western and eastern populations of Spanish sand racers. However, this west-east scenario yielded contradictory phenotypic descriptions. For instance, whereas some authors claimed that traits such as the number of femoral pores or supralabial scales differ between western and eastern populations, other authors reported no differentiation (Boulenger, 1921; Blasco, 1974; Perez-Mellado, 1998). The existence of a third genetically distinct group in the centre of the Iberian Peninsula as recently demonstrated by Fitze *et al.* (2011) likely underlies these contradictory findings. Fitze *et al.* (2011, 2012) present the formal description of the three species, provided a preliminary and succinct phenotypic description of *P. edwardsianus*, *P. hispanicus*, and *P. occidentalis*. Yet, differences among species in important phenotypic characters such as colouration and colour patterns as well as in the degree of sexual dimorphism in colour traits, body measurements, and scalation traits have not been studied so far. Differentiation in colour traits and sexual dimorphism may occur rapidly during spe-

ciation (Lande, 1981). Interspecific differences in sexual dimorphism may indicate differences in the intensity of sexual and natural selection (Anderson, 1994; Stuart-Fox and Ord, 2004). Similarly, variation between sister species in colour traits may indicate that species are subjected to distinct selective pressures for example, owing to differences in ecological conditions (Stuart-Fox *et al.*, 2003; Stuart-Fox and Ord, 2004).

In the present study we studied phenotypic differentiation among the three Spanish sand racer species, combining the phenotypic traits studied in Fitze *et al.* (2011) with sixteen new external traits concerning colouration and colour pattern. We further investigated which phenotypic traits are sexually dimorphic and whether the degree of sexual dimorphism differs among species. We also tested if the genetic differences found between northern and southern populations of *P. edwardsianus* and *P. occidentalis* are associated with phenotypic differences. Finally, we developed a dichotomous key to distinguish between the three species, which is particularly useful for field classification and conservation purposes.

## Material and methods

### Population sampling and field measurements

In spring 2006, we sampled 21 Spanish populations that had been previously delineated using genetic markers as *Psammodromus hispanicus*, *P. edwardsianus* (northern and southern clade), and *P. occidentalis*

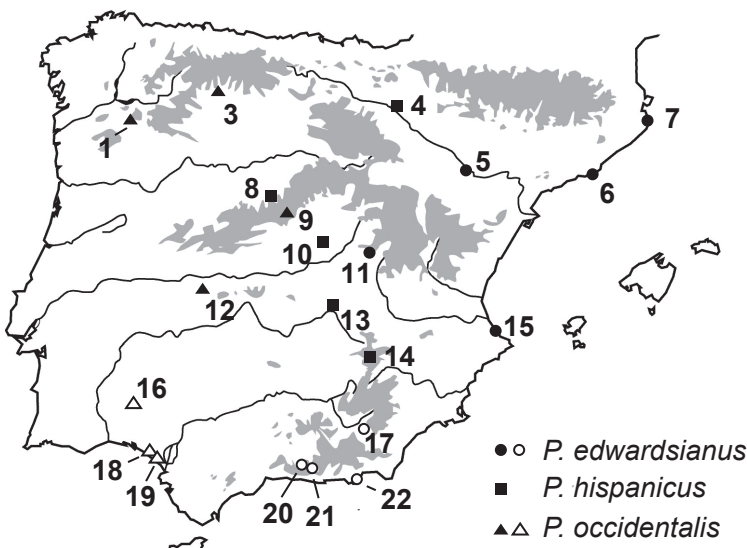


Fig. 1. Locations sampled of the three Spanish sand racer species (referenced in Table S1). Northern (●) and southern (○) populations of *Psammodromus edwardsianus* and northern (▲) and southern (△) populations of *P. occidentalis* are also indicated.

Table 1. Summary of measured variables. Numbers in brackets correspond to numbers in Fig. 2. Variables directly used in the statistical analyses are plotted in bold, whereas variables not used in the statistical analyses, but used to derive other variables (e.g. shape descriptors) or principal components (PC) are italicized.

Variable	Description (unit)
<b>a. Body measures and scalation</b>	
<b>SVL</b> [1]	Snout to vent length (mm) measured on the alive animal
<i>Total length</i>	Snout to tail tip length (mm) measured on the alive animal
<b>SVL ratio</b>	Total length / SVL
<b>Body mass</b>	Lizard weight (g)
<i>Snout width</i> [2]	Distance (mm) between the left and right foremost intersection point of the first supraocular and the first supraciliar scale
<i>Snout length</i> [3]	Distance (mm) between the borders of the outermost left and right supraocular scales (located behind the eyes)
<b>Snout shape</b>	Degree of snout sharpness. Snout length / snout width
<i>Head length</i> [4]	Distance (mm) between the tip of the snout and the occipital edge
<i>Head width</i> [5]	Distance (mm) between the borders of the last left and right supraocular scale
<b>Head shape</b>	Degree of head sharpness. Head width / head length
<i>Anal scale width</i> [6]	Distance (mm) between the posterior borders of the anal scale
<b>Relative anal scale width</b>	Anal scale width / SVL
<b>Femoral pores</b> [7]	Mean number of right and left femoral pores
<b>Ventral scales</b> [8]	Number of transverse ventral scale rows
<b>Subocular scales</b> [9]	Number of supralabial scales below subocular scale
<b>Throat scales</b> [10]	Number of throat scales
<b>Collar scales</b> [11]	Number well-differentiated collar scales
<b>b. Colour traits</b>	
<i>Anal scale colouration</i> [12]	mean hue (°), mean saturation (%), and mean brightness (%) measured in four quadrants of the anal scale
<i>First ventral scale row colour</i> [13]	mean hue (°), mean saturation (%), and mean brightness (%) of the 4 scales of the first transverse ventral scale row
<i>Mid ventral scale row colour</i> [13]	mean hue (°), mean saturation (%), and mean brightness (%) of the 4 scales of the mid transverse ventral scale row
<i>Last ventral scale row colour</i> [13]	mean hue (°), mean saturation (%), and mean brightness (%) of the 4 scales of scales of the last transverse ventral scale row
<i>Ventral scale colour</i>	mean hue (°), mean saturation (%), and mean brightness (%) of the anterior, middle and posterior ventral scales
<b>Ventral colouration PC1, PC2, PC3</b>	principal components of anal scale and all ventral scale HSB values
<b>Neck colour</b> [14]	mean hue (°), mean saturation (%), and mean brightness (%) of the first 8 scales of the left and right outermost longitudinal lines
<b>Background colour</b> [15]	mean hue (°), mean saturation (%), and mean brightness (%) of the brown background colouration
<i>Black transverse line colour</i> [16]	mean hue (°), mean saturation (%), and mean brightness (%) of the central scale of the first 5 black dorsal transverse lines
<i>White longitudinal line colour</i> [17]	mean hue (°), mean saturation (%), and mean brightness (%) of the central scale of the first 5 white dorsal transverse lines
<b>Dorsal colour PC1, PC2</b>	principal components of the HSB values of the dorsal black transverse lines and white longitudinal lines
<b>c. Colour pattern traits</b>	
<b>Dorsal pattern index</b>	See Methods and S - Figure 1 of the Supporting information for further details
<b>Proportion of black colouration</b>	Percentage of dorsal black colouration (further details see methods)
<b>Proportion of white colouration</b>	Percentage of dorsal white colouration (further details see methods)
<b>Dorsal line thickness</b> [18]	Mean number of longitudinal scale rows forming the longitudinal lines
<b>Nuptial colouration</b>	Extent of the green nuptial colouration along the lizard's body (further details see methods)
<b>Number of ocelli</b> [19]	Mean number of right and left hand sided ocelli

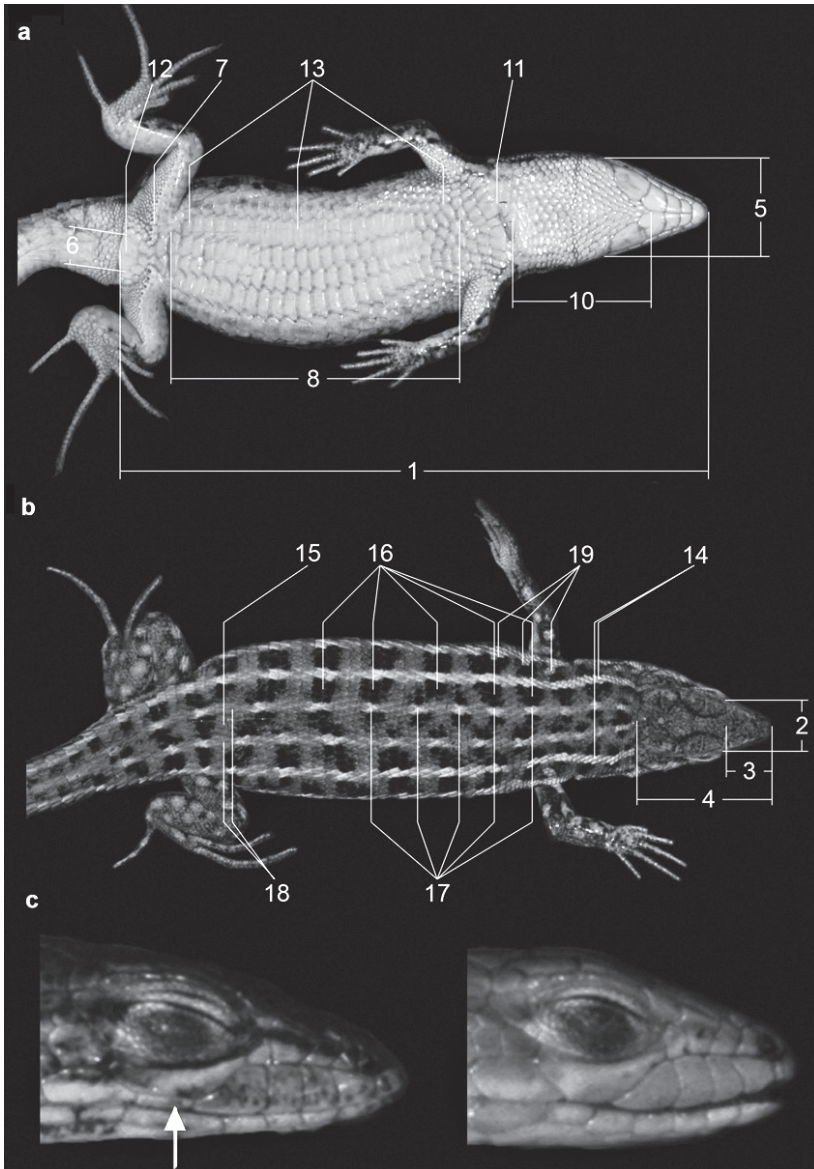


Fig. 2. Phenotypic measurements taken on Spanish sand racers. Numbers correspond to the traits summarized in Table 1. **a.** Ventral view of a lizard. **b.** Dorsal view of a lizard. **c.** Head detail showing the absence of a scale under the subocular scale (on the right; *P. hispanicus* and *P. occidentalis*) and the presence of a scale under the subocular scale (on the left; *P. edwardsianus*).

(northern and southern clade). We captured a total of 211 adult lizards from these populations (Fig. 1, Table S1; for further details see Fitze *et al.*, 2011). Genetic markers indicated that none of the species occurred in sympatry in the sampled populations (Fitze *et al.*, 2011) and no differences in the latitude and altitude of the sampled populations existed between species (all  $P > 0.16$ ). Immediately after capture, we took a standardized photograph of the belly, back, and flanks of every lizard following Fitze and Richner (2002). Lizards were carefully placed into an opaque box filled with foam material. A photographic filter lens (Hoya UV-

filter) was slid over the lizards to immobilize them. The box was placed in a standard position inside a larger opaque photographic chamber that maintained a fixed distance (40 cm) to a digital camera (Nikon D70S with a 105 mm f/2.8 Nikkor objective). Light was provided by two flashes (Nikon SB-600) fixed at both sizes of the chamber at an angle of  $13^\circ$  to the optical axis. Standard white patches (10×5 mm, Kodak Colour Control Patches, red = 255, green = 255, blue = 255) were fixed on each side of the filter lens for light calibration. No difference in light exposure was detected ( $P > 0.5$ ).

### *Body measures and scalation traits*

Snout-to-vent length (SVL), total length (both measured with a ruler to the nearest 1 mm), number of femoral pores, and body mass (to the nearest 1 mg) were determined in the field. Head and snout shape and the relative anal scale width were measured by importing the photographs into IMAGEJ program (National Institute of Science, USA) (Table 1a, Fig. 2). The number of ventral scale rows, the number of throat scale rows, the number of collar scales, and the presence/absence of a supralabial scale under the subocular scale were determined from the photographs (Table 1a, Fig. 2).

### *Colour traits*

We used Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, California, USA) to calculate hue, saturation, and brightness (hereafter referred to as HSB) of different ventral and dorsal colour traits (Table 1b). We measured colour of the anal scale and ventral scales (Fig. 2a [12, 13]). The anal scale was divided into four quadrants referenced by the anal scale's longitudinal and transversal axes. In each quadrant, we measured mean HSB values of the 10×10 pixel area closest to the intersection using the average filter of Adobe Photoshop®. Ventral scale colouration corresponds to the mean HSB values measured on the first, middle, and last transverse ventral scale rows. HSB values were measured from 10×10 pixel areas located in the middle of each of the four central scales in each row and the average per row was used in the analysis.

Dorsal colouration was characterized using four variables (Table 1b): neck colour, background colour, black transverse line colour, and white longitudinal line colour. Neck colour corresponds to the average HSB values measured on the left and right outermost, green-yellow longitudinal lines (Fig. 2b [14]). Average HSB values of a 6×6 pixel area were measured on the first eight scales of each longitudinal line and used to derive the average HSB values per line. Background colour measured the dominant brown colouration. Specifically, we measured mean HSB values of an 8×8 pixel area located between the black transversal line located between the hind legs and the subsequent black transversal line located closer towards the lizard's head (Fig. 2b [15]). Starting from the anterior legs and moving towards the hind legs, we measured the colour of the first five black transverse lines and white intersection points (colour of the white longitudinal lines; Fig. 2b [16, 17]). We measured mean HSB values of an

8×8 pixel area in the middle of each dark transverse line and intersection point. Means of the five black transverse lines and white intersection point measurements were used for the analyses.

### *Colour pattern traits*

We distinguished four colour pattern traits (Table 1c). The dorsal pattern was classified with an index (dorsal pattern index) ranging from 0 to 7.5 and based on the presence, form (continuous, broken or spotted), and thickness (number of scale rows) of the black transverse lines as well as on the presence and form (continuous or dotted) of the whitish longitudinal lines (Fig. S2). For example, the dorsal pattern design in Fig. 2b has an index score of 5.75. It shows continuous external longitudinal lines (+1), dotted internal longitudinal lines (+0.75), and broken black transverse lines (+2) formed by two scale rows (+2). Additionally, we measured the thickness of the internal white longitudinal lines by counting on a standard position the number of transverse scale rows forming each line (Fig. 2b [18]). Mean number of scales of left and right lines was used for the analyses.

We measured the proportion of black and white colouration present on the lizards' back. We first selected a standard dorsal area between the hind and forelegs and between the outermost longitudinal lines. Within this area, we selected and calculated the number of black pixels using as black reference the mean HSB values measured on the black transverse lines (Table 1 [16]). The same procedure was used to calculate the number of white pixels but using as reference the mean HSB values of the white transverse lines (Table 1 [16]). Finally, we calculated the proportion of black and white colouration by dividing the counted black and white pixels by the number of pixels within the standard dorsal area.

The extent of the green nuptial colouration was measured using a nuptial colouration index (Fitze *et al.*, 2011) considering the presence (1)/absence (0) of green colouration on the head, neck, belly, supralabial scales, and dorsal skin. When lizards showed dorsal nuptial colouration, we additionally added 0.5 for every coloured longitudinal line ( $N = 4$  longitudinal lines) if colour did not reach the middle of the body and one point for every longitudinal line if colour extended further than the middle of the body. Thus, the maximum score was 9. We also counted the number of ocelli on left and right sides and used the mean for the analyses (Table 1b, Fig. 2b [19]).

### Statistical analysis

Statistical analyses were conducted using R 2.7.0 (R development core team, Vienna, Austria). Two repeated measurements taken on 12 lizards showed high repeatability of the variables (mean  $r$  of all variables  $\pm$  SE =  $0.79 \pm 0.03$ ). Lowest repeatability was found for the number of throat scales:  $F_{10,11} \geq 4.13$ ,  $P \leq 0.014$ ,  $r \geq 0.61$ ; Lessells and Boag, 1987).

Prior to analysing differences between species, we ran separate principal component analyses (PCA) on ventral and dorsal colour traits because they were highly correlated within trait sets (Table 1). For subsequent analyses, we retained PC axes with eigenvalues greater than one (Quinn and Keough, 2002).

To investigate differences between species, we fitted a permutational MANOVA (PERM-MANOVA; Adonis function, Vegan package) based on Euclidean distances and 9999 permutations (Anderson, 2001; McArdle and Anderson, 2001). To correct for scale differences, we standardized all variables (including the derived PCs) by dividing mean-centred observations by the standard deviation of each variable (Quinn and Keough, 2002). In *P. edwardsianus*, differences between southern and northern populations were also tested using a similar PERM-MANOVA. Differences between northern and southern populations of *P. oc-*

*cidental*s could not be tested due to the low sample size ( $N = 5$ ) in southern populations. We derived linear discriminant functions (LDF) to assess the relative importance of each variable for species differentiation and for differentiation between northern and southern population of *P. edwardsianus*. We ran univariate ANOVAs to specifically investigate which traits differed among species and between northern and southern populations of *P. edwardsianus* (Quinn and Keough, 2002).

To test for differences between species in sexual dimorphism, we included sex and its interaction with species in the PERM-MANOVA and ANOVA models. We controlled for non-independence of animals captured in the same population and for interpopulation variation by including population as a factor nested within species. For brevity, population effects are not shown. Models were simplified by backward elimination of the non-significant terms. ANOVA assumptions of normality and homoscedasticity and PERM-MANOVA assumption of homogeneity of multivariate dispersion were verified and log and power transformations were applied when necessary. Statistical significance was set at  $\alpha = 0.05$  and adjusted after multiple testing following Hochberg (1988).

To provide a method to distinguish the species under field conditions, we additionally derived LDF on

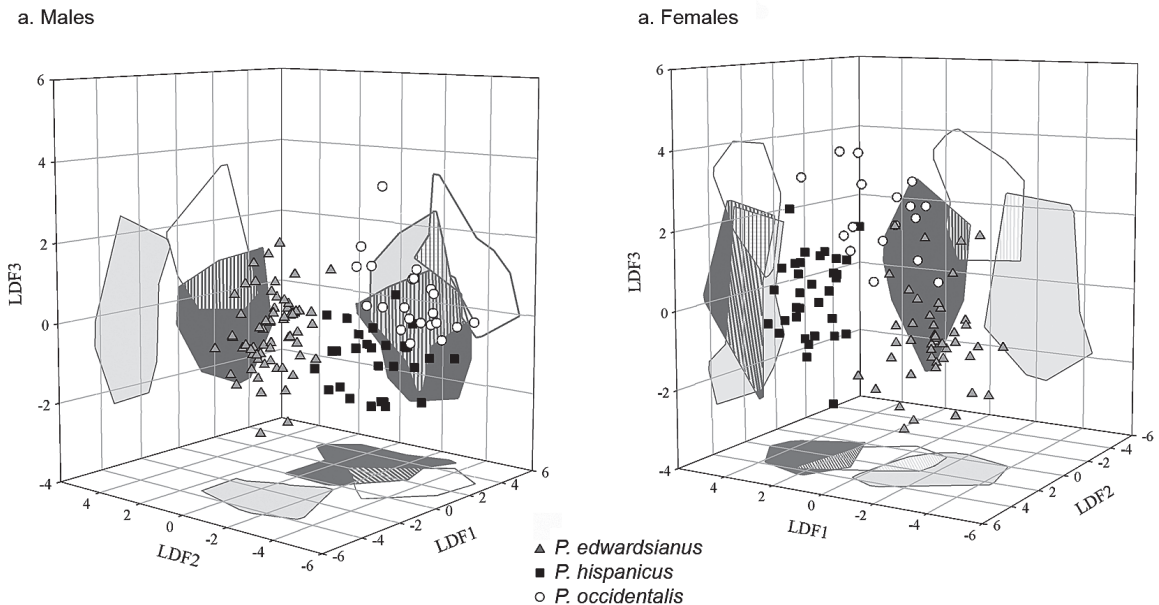


Fig. 3. Three-dimensional plot of the first three LDF yielded by the discriminant analysis on **a.** males and **b.** females of *P. edwardsianus*, *P. hispanicus*, and *P. occidentalis*. The area for *P. hispanicus* (dark grey), *P. edwardsianus* (light grey), and *P. occidentalis* (clear area) are plotted on each two-dimensional panel.

traits that are easy to measure in the field (femoral pores, throat scales, number of ocelli, and the relative anal scale width). Validation of species classifications was accomplished with leave-one-out cross-validation methods using the proportion of individuals sampled per species as prior probabilities (Venables and Ripley, 1999).

## Results

Three PCs explained 88% of the variance in ventral and anal scale colouration. Ventral colouration PC1 mainly reflected the hue of ventral and anal scales whereas PC2 mainly reflected brightness and PC3, saturation. For dorsal colouration, two PCs explained 72.5% of the variance (Table S3). Dorsal colouration PC1 mainly reflected saturation and brightness of the black transverse lines and brightness of the white longitudinal lines whereas PC2 mainly reflected hue and saturation of the white longitudinal lines.

### Differences among species

A PERM-MANOVA including all measured traits (variables indicated in bold in Table 1) showed statistically significant differences among the three *Psammodyromus* species ( $F_{2, 182} = 29.83$ ,  $P < 0.001$ ). Post-hoc tests showed that there were significant differences between all three species pairs (all contrasts  $t_{182} \geq 2.79$ ,  $P < 0.001$ ). There were significant differences between sexes ( $F_{1, 182} = 21.87$ ,  $P < 0.001$ ) and the interaction between species and sex was also significant ( $F_{2, 182} = 3.14$ ,  $P < 0.001$ ).

Discriminant analyses yielded five LDF that successfully discriminated between species (Fig. 3). The presence/absence of a supralabial scale below the subocular scale (Fig. 2c) distinguished *P. edwardsianus* (present) from both *P. hispanicus* and *P. occidentalis* (absent) in 100% of the cases. Given the lack of variance, this trait could not be included in the discriminant analyses. LDF1 explained 55% of the variance and mainly reflected neck hue and saturation, the extent of the nuptial colouration, and the number of femoral pores and throat scales (Table S4). This function separated *P. edwardsianus* males from *P. hispanicus* and *P. occidentalis* males (Fig. 3a). LDF1 also separated *P. edwardsianus* females from *P. hispanicus* females whereas *P. occidentalis* females occupied an intermediate position (Fig. 3b). LDF2 explained 31% of the variance and reflected SVL, the number of ven-

tral scales, ventral colouration PC3, and relative anal scale width (Table S4). LDF2 separated *P. occidentalis* males from *P. hispanicus* males and males and females of all species (most males and females had negative and positive LDF2 scores, respectively; Fig. 3). LDF3, which explained 7.1% of the variance, separated *P. occidentalis* males and females from the other species. This function reflected the number of ocelli and ventral scales, the dorsal colouration PC2, and the proportion of white colouration. The remaining two LDFs only explained 6% and 1% of the variance, respectively (Table S4).

Univariate ANOVAs showed significant differences between species in body measures, scalation, colouration, and colour pattern (Table 2-3). All species differed significantly in the number of femoral pores and throat scales, which were higher in *P. edwardsianus*, intermediate in *P. occidentalis*, and lowest in *P. hispanicus* (see contrasts in Table 2). In all species, females showed a lower number of femoral pores than males, but no sex differences existed in the number of throat scales. *Psammodyromus edwardsianus* showed a smaller SVL than *P. hispanicus* and *P. occidentalis*, which showed no significant differences between them. In all species, males were significantly smaller than females. SVL ratio (Table 1a) was significantly greater in *P. edwardsianus* than in *P. occidentalis*, whereas only a marginal trend was found between *P. edwardsianus* and *P. hispanicus*. No significant differences in SVL existed between *P. occidentalis* and *P. hispanicus*. SVL ratio was higher in males than females of all species. Head shape (Table 1a) was more pointed (*i.e.* lower head shape values) in *P. edwardsianus*, whereas no differences existed between *P. hispanicus* and *P. occidentalis*. In all species, females showed more pointed heads than males. Snout shape did not differ between species but significantly differed between sexes, being more pointed (*i.e.* lower snout shape values) in females than in males of the three species. The number of collar scales was higher in *P. hispanicus* than in the other two species, between which no significant differences existed. Sexes did not significantly differ for this trait. Relative anal scale width was larger in *P. occidentalis* than in *P. edwardsianus* but was not larger than in *P. hispanicus*. In all species, females had significantly smaller anal scales than males.

Hue and saturation of the background colour significantly differed between species (Table 3a). In *P. edwardsianus*, background hue was higher (*i.e.* more yellow) and saturation lower (*i.e.* greyer) than in *P.*

*occidentalis*. No differences were found between *P. hispanicus* and the other two species. Background brightness did not significantly differ among species and no significant sex differences existed in background hue, saturation, and brightness. Dorsal colour PC1 was higher in *P. hispanicus* than in *P. edwardsianus* and *P. occidentalis*, between which no differences existed, indicating that *P. hispanicus* showed lighter dorsal transverse and longitudinal lines than the other two species. Independent of the species, males showed higher dorsal PC1 values than females. Dorsal colour PC2 was higher (*i.e.* white longitudinal lines were less white and saturated) in *P. edwardsianus* than in *P. occidentalis* while no significant differences existed between these two species and *P. hispanicus*. Dorsal colour PC2 was not significantly different between sexes. The dorsal pattern index was higher in *P. occidentalis* than in *P. edwardsianus*, indicating that the dorsal pattern was more reticulated in the former species (Fig. S1). Males of all species showed more reticulated dorsal patterns than females. Significant differences existed between species in the proportion of dorsal black colour, although such differences were only marginally significant after adjusting for multiple testing. Thus, the proportion of black colour tended to be higher in *P. occidentalis* compared to the other species (Table 3). Independent of the species, black colour proportion was higher in males than in females. No differences in the proportion of white colour existed between species or sexes. Dorsal lines in *P. edwardsianus* were significantly thicker than in *P. occidentalis* and tended to be thicker than in *P. hispanicus*. In this latter species, dorsal lines tended to be thicker than in *P. occidentalis*. No sex difference existed for the dorsal line thickness.

Sexual dimorphism differed between species as revealed by significant species  $\times$  sex interactions in Table 2 and 3. Females were heavier than males in *P. hispanicus* and *P. occidentalis* but not in *P. edwardsianus* (Table 4). Post-hoc analyses further showed that female body mass differed between species (Table 2) whereas no significant interspecific differences existed for males ( $t_{182} < 2.01$ ,  $P > 0.09$ ). The number of ventral scales was significantly larger in females than in males of all species (Table 4). However, sexual dimorphism was more marked in *P. hispanicus* and *P. occidentalis* than in *P. edwardsianus*, given that females of the first two species showed a higher number of ventral scales than *P. edwardsianus* females (Table 2) whereas interspecific males did not differ in this trait ( $t_{182} < 1.62$ ,  $P > 0.11$ ).

The number of ocelli was higher in males than in females of *P. edwardsianus*, whereas no significant differences existed between sexes in *P. occidentalis* and *P. hispanicus* (Table 4). In *P. occidentalis*, males and females showed numerous ocelli, presenting as many as *P. edwardsianus* males ( $t_{182} < 0.22$ ,  $P > 0.82$ ). In contrast, *P. hispanicus* males and females showed a reduced number of ocelli, with males showing fewer ocelli than males of the other species ( $t_{182} > 4.04$ ,  $P < 0.01$ ), and females showing as few as *P. edwardsianus* females (Table 3). The extent of the nuptial colouration significantly differed between sexes in *P. occidentalis* and *P. hispanicus* but not in *P. edwardsianus* (Table 4), where it was significantly less extended than in the other two species (Table 3). Neck hue was sexually dimorphic in all species although sex differences were less pronounced in *P. edwardsianus* than in *P. occidentalis* and *P. hispanicus* (Table 3–4). Neck brightness was sexually dimorphic only in *P. occidentalis* but not in the other two species (Table 4). Neck saturation was different between species and sexes but no significant species  $\times$  sex interaction was found. Necks of *Psammodromus occidentalis* were more saturated than necks of *P. hispanicus* and *P. edwardsianus*, between which were found no differences in this trait. Independently of the species, males showed less saturated necks than females.

Ventral colour PC2 was significantly lower in *P. hispanicus* males than in *P. hispanicus* females, whereas no significant differences between sexes were observed in *P. edwardsianus* and *P. occidentalis* (Table 4). Among species, *P. hispanicus* females showed higher values (*i.e.* showed brighter ventral colours) than females belonging to the other two species (Table 3) whereas *P. occidentalis* males showed significantly higher values for ventral colouration PC2 than the other two species ( $t_{182} > 2.60$ ,  $P < 0.03$ ). Ventral colour PC1 and PC3 were significantly different between sexes but not between species. Males had lower values in the ventral colour PC1 and higher values in the ventral colour PC3 (*i.e.* more yellow and saturated bellies) than females.

#### *Differences between northern and southern populations of P. edwardsianus*

The PERM-MANOVA revealed significant differences between northern and southern *P. edwardsianus* populations ( $F_{1,96} = 5.17$ ,  $P = 0.001$ ), between sexes ( $F_{1,96} = 9.82$ ,  $P = 0.001$ ), but no significant interaction between sex and geographic location ( $F_{1,96} = 1.23$ ,  $P = 0.2$ ). The



Table 2. Differences among species and sexes in body measures and scalation traits. a) results from univariate ANOVAs, b) means  $\pm$  standard errors, and ranges of each trait per sex and species (PE: *P. edwardsianus*, PH: *P. hispanicus*, PO: *P. occidentalis*), and c) individual contrasts between species in the case of significant differences between species. For significant interactions between species and sex, individual contrasts between species are shown only for the sex where differences between species were significant.

Variable	Parameter	Statistics	P	Species	b) Means $\pm$ SE and (range) per sex and species		Contrasts	Statistics	P
					Female	Male			
SVL	Species	$F_{2,184} = 13.91$	<0.001	PE	48.24 $\pm$ 0.5 (39 – 56)	45.1 $\pm$ 0.3 (34 – 51)	PE vs PH	$t_{184} = 4.29$	<0.001
	Sex	$F_{1,184} = 54.28$	<0.001	PH	51.27 $\pm$ 0.5 (43 – 57)	48.3 $\pm$ 0.6 (42 – 57)	PE vs PO	$t_{184} = 4.45$	<0.001
	Interaction	$F_{2,182} = 0.37$	0.691	PO	50.76 $\pm$ 1.1 (44 – 58)	46.4 $\pm$ 0.7 (38 – 55)	PH vs PO	$t_{184} = 0.17$	0.865
SVL ratio	Species	$F_{2,184} = 5.08$	0.007	PE	2.3 $\pm$ 0.05 (1 – 2.8)	2.6 $\pm$ 0.1 (1.8 – 3.4)	PE vs PH	$t_{184} = 2.96$	0.010
	Sex	$F_{1,184} = 38.83$	<0.001	PH	2.1 $\pm$ 0.05 (1.2 – 2.6)	2.3 $\pm$ 0.1 (1.7 – 2.7)	PE vs PO	$t_{184} = 2.22$	0.055
	Interaction	$F_{2,182} = 0.36$	0.698	PO	2.2 $\pm$ 0.08 (1.4 – 2.6)	2.4 $\pm$ 0.1 (1.8 – 2.8)	PH vs PO	$t_{184} = 0.84$	0.402
Body mass	Species	$F_{2,182} = 8.79$	<0.001	PE	1.74 $\pm$ 0.05 (0.99 – 2.47)	1.66 $\pm$ 0.04 (0.96 – 2.51)	PE vs PH <sup>1</sup>	$t_{182} = 3.46$	0.003
	Sex	$F_{1,182} = 21.59$	<0.001	PH	1.98 $\pm$ 0.06 (1.37 – 2.89)	1.77 $\pm$ 0.05 (1.39 – 2.62)	PE vs PO <sup>1</sup>	$t_{182} = 4.57$	<0.001
	Interaction	$F_{2,182} = 3.74$	0.026	PO	2.07 $\pm$ 0.12 (1.20 – 2.75)	1.71 $\pm$ 0.07 (1.08 – 2.30)	PH vs PO <sup>1</sup>	$t_{182} = 1.23$	0.222
Snout shape	Species	$F_{2,182} = 2.93$	0.056	PE	1.05 $\pm$ 0.01 (0.95 – 1.15)	1.08 $\pm$ 0.01 (0.98 – 1.34)			
	Sex	$F_{1,182} = 14.36$	<0.001	PH	1.04 $\pm$ 0.01 (0.8 – 1.12)	1.05 $\pm$ 0.01 (0.96 – 1.11)			
	Interaction	$F_{2,182} = 1.48$	0.230	PO	1.07 $\pm$ 0.01 (0.93 – 1.11)	1.12 $\pm$ 0.01 (1.06 – 1.23)			
Head shape	Species	$F_{2,207} = 8.75$	<0.001	PE	0.47 $\pm$ 0.005 (0.42 – 0.59)	0.49 $\pm$ 0.003 (0.41 – 0.56)	PE vs PH	$t_{207} = 3.63$	0.001
	Sex	$F_{2,207} = 12.14$	<0.001	PH	0.49 $\pm$ 0.002 (0.47 – 0.52)	0.50 $\pm$ 0.006 (0.34 – 0.55)	PE vs PO	$t_{207} = 3.12$	0.004
	Interaction	$F_{2,182} = 0.62$	0.539	PO	0.49 $\pm$ 0.004 (0.46 – 0.51)	0.50 $\pm$ 0.01 (0.41 – 0.57)	PH vs PO	$t_{207} = 0.04$	0.968
Relative anal scale width	Species	$F_{2,207} = 3.75$	0.025	PE	0.059 $\pm$ 0.001 (0.048 – 0.092)	0.066 $\pm$ 0.001 (0.046 – 0.095)	PE vs PH	$t_{207} = 1.12$	0.264
	Sex	$F_{1,207} = 78.47$	<0.001	PH	0.056 $\pm$ 0.001 (0.045 – 0.068)	0.067 $\pm$ 0.001 (0.049 – 0.079)	PE vs PO	$t_{207} = 2.73$	0.021
	Interaction	$F_{2,182} = 2.2$	0.114	PO	0.061 $\pm$ 0.001 (0.052 – 0.069)	0.069 $\pm$ 0.002 (0.058 – 0.083)	PH vs PO	$t_{207} = 2.02$	0.089
Femoral pores	Species	$F_{2,184} = 34.64$	<0.001	PE	11.7 $\pm$ 0.2 (9.5 – 15)	12.5 $\pm$ 0.1 (10 – 14.5)	PE vs PH	$t_{184} = 8.24$	<0.001
	Sex	$F_{1,184} = 30.15$	<0.001	PH	9.7 $\pm$ 0.1 (8 – 12)	10.2 $\pm$ 0.1 (9 – 11.5)	PE vs PO	$t_{184} = 4.85$	<0.001
	Interaction	$F_{2,182} = 1.32$	0.270	PO	10.9 $\pm$ 0.3 (8.5 – 13)	11.5 $\pm$ 0.2 (10 – 13)	PH vs PO	$t_{184} = 2.87$	0.005
Ventral scales	Species	$F_{2,182} = 2.99$	0.053	PE	25.8 $\pm$ 0.2 (22 – 29)	23.8 $\pm$ 0.2 (20 – 27)	PE vs PH <sup>1</sup>	$t_{184} = 2.84$	0.005
	Sex	$F_{1,182} = 156.4$	<0.001	PH	27.1 $\pm$ 0.3 (20 – 30)	24.5 $\pm$ 0.3 (21 – 29)	PE vs PO <sup>1</sup>	$t_{184} = 3.64$	<0.001
	Interaction	$F_{2,182} = 6.94$	0.001	PO	27.2 $\pm$ 0.4 (23 – 29)	23.6 $\pm$ 0.3 (21 – 26)	PH vs PO <sup>1</sup>	$t_{184} = 0.9$	0.369
Throat scales	Species	$F_{2,184} = 18.88$	<0.001	PE	20.2 $\pm$ 0.3 (15 – 26)	20.5 $\pm$ 0.3 (15 – 25)	PE vs PH	$t_{184} = 6.05$	<0.001
	Sex	$F_{1,184} = 0.7$	0.404	PH	18 $\pm$ 0.3 (15 – 23)	17.8 $\pm$ 0.2 (16 – 21)	PE vs PO	$t_{184} = 2.81$	0.005
	Interaction	$F_{2,182} = 0.55$	0.578	PO	19.5 $\pm$ 0.4 (16 – 22)	18.5 $\pm$ 0.3 (15 – 23)	PH vs PO	$t_{184} = 3.28$	0.002
Collar scales	Species	$F_{2,184} = 7.37$	<0.001	PE	0.2 $\pm$ 0.1 (0 – 3)	0.3 $\pm$ 0.1 (0 – 3)	PE vs PH	$t_{182} = 3.81$	<0.001
	Sex	$F_{1,184} = 0.06$	0.814	PH	1.4 $\pm$ 0.2 (0 – 3)	1.2 $\pm$ 0.2 (0 – 3)	PE vs PO	$t_{182} = 1.03$	0.304
	Interaction	$F_{2,182} = 0.24$	0.787	PO	0.6 $\pm$ 0.3 (0 – 3)	0.7 $\pm$ 0.2 (0 – 3)	PH vs PO	$t_{182} = 2.65$	0.017

<sup>1</sup> Contrasts on females only

Table 3. Differences among species and sexes in colouration and colour pattern traits. a) results from ANOVAs, b) means  $\pm$  standard errors and ranges per sex and species (PE: *P. edwardsianus*, PH: *P. hispanicus*, PO: *P. occidentalis*), and c) individual contrasts between species.

Variable	Parameter	Statistic	P	b) Means $\pm$ SE and (range) per sex and species		c) Individual contrasts between species	
				Species	Female	Male	Contrasts
<b>Ventral colouration PC1</b>							
	Species	$F_{2,184} = 1.59$	0.206	PE	0.92 $\pm$ 0.25 (- 3.23 - 5.35)	- 0.03 $\pm$ 0.21 (- 3.8 - 4.56)	
	Sex	$F_{1,184} = 10.84$	0.001	PH	- 0.39 $\pm$ 0.22 (- 3 - 1.59)	- 0.61 $\pm$ 0.27 (- 4.6 - 1.55)	
	Interaction	$F_{2,182} = 1.84$	0.162	PO	- 0.09 $\pm$ 0.49 (- 4.21 - 2.36)	- 0.61 $\pm$ 0.4 (- 4.18 - 2.32)	
<b>Ventral colouration PC2</b>							
	Species	$F_{2,182} = 5.99$	0.003	PE	- 0.32 $\pm$ 0.22 (- 3.66 - 2.71)	- 0.15 $\pm$ 0.15 (- 2.83 - 3.54)	$t_{182} = 2.98$
	Sex	$F_{1,182} < 0.01$	0.987	PH	0.67 $\pm$ 0.16 (- 1.44 - 2.55)	0 $\pm$ 0.17 (- 1.32 - 2.81)	$t_{182} = 0.62$
	Interaction	$F_{2,182} = 5.01$	0.008	PO	- 0.03 $\pm$ 0.22 (- 2.29 - 1.58)	0.45 $\pm$ 0.17 (- 1.24 - 2.01)	$t_{182} = 3.45$
<b>Ventral colouration PC3</b>							
	Species	$F_{2,184} = 0.29$	0.746	PE	- 0.62 $\pm$ 0.17 (- 3.33 - 1.68)	0.23 $\pm$ 0.12 (- 1.89 - 2.5)	
	Sex	$F_{1,184} = 84.49$	<0.001	PH	- 0.5 $\pm$ 0.09 (- 1.5 - 0.65)	0.49 $\pm$ 0.17 (- 1.54 - 2.45)	
	Interaction	$F_{2,182} = 1.84$	0.162	PO	- 0.4 $\pm$ 0.17 (- 1.99 - 0.45)	1.04 $\pm$ 0.17 (- 1.02 - 2.05)	
<b>Neck hue</b>							
	Species	$F_{2,182} = 64.71$	<0.001	PE	36.28 $\pm$ 0.66 (27.57 - 47.63)	38.37 $\pm$ 0.63 (26.27 - 59.81)	$t_{182} = 9.63$
	Sex	$F_{1,182} = 40.17$	<0.001	PH	54.04 $\pm$ 1.62 (33.76 - 69.86)	63.72 $\pm$ 2.4 (31.43 - 94.71)	$t_{182} = 8.21$
	Interaction	$F_{2,182} = 5.48$	0.005	PO	43.09 $\pm$ 1.24 (32.85 - 51.3)	56.84 $\pm$ 2.01 (36.73 - 69.43)	$t_{182} = 3.31$
<b>Neck saturation</b>							
	Species	$F_{2,184} = 18.83$	<0.001	PE	32.18 $\pm$ 1.21 (16.38 - 49.37)	30.44 $\pm$ 0.76 (19.3 - 45.8)	$t_{184} = 5.59$
	Sex	$F_{1,184} = 10.04$	0.002	PH	46.8 $\pm$ 1.64 (33.07 - 67.04)	43.91 $\pm$ 1.57 (29.51 - 61.42)	$t_{184} = 4.46$
	Interaction	$F_{2,182} = 1.33$	0.267	PO	40.82 $\pm$ 2.77 (21.14 - 59.06)	42.71 $\pm$ 2.44 (25.61 - 64.6)	$t_{184} = 1.36$
<b>Neck brightness</b>							
	Species	$F_{2,182} = 1.46$	0.235	PE	43.09 $\pm$ 0.61 (33.44 - 54.44)	44.21 $\pm$ 0.43 (34 - 54)	$t_{182} = 1.57$
	Sex	$F_{1,182} = 3.34$	0.069	PH	42.6 $\pm$ 0.63 (36.27 - 50.5)	40.83 $\pm$ 0.77 (33.83 - 50.72)	$t_{182} = 3.22$
	Interaction	$F_{2,182} = 5.6$	0.004	PO	44.46 $\pm$ 0.9 (39.15 - 53.44)	41.44 $\pm$ 0.55 (36.62 - 46.44)	$t_{182} = 1.32$
<b>Background colour hue</b>							
	Species	$F_{2,184} = 4.25$	0.016	PE	27.16 $\pm$ 0.67 (19 - 40)	28.33 $\pm$ 0.9 (16 - 60)	$t_{184} = 1.84$
	Sex	$F_{1,184} = 2.14$	0.145	PH	24.03 $\pm$ 0.8 (14 - 35)	24.68 $\pm$ 0.74 (15 - 33)	$t_{184} = 2.79$
	Interaction	$F_{2,182} = 0.1$	0.905	PO	24.29 $\pm$ 1 (18 - 35)	24.86 $\pm$ 0.89 (19 - 32)	$t_{184} = 0.69$
<b>Background colour saturation</b>							
	Species	$F_{2,184} = 5.55$	0.005	PE	42.02 $\pm$ 1.75 (14 - 65)	37.84 $\pm$ 1.71 (7 - 64)	$t_{184} = 1.76$
	Sex	$F_{1,184} = 1.13$	0.289	PH	48.27 $\pm$ 2.34 (20 - 76)	49.26 $\pm$ 2.44 (23 - 78)	$t_{184} = 3.15$
	Interaction	$F_{2,182} = 1.18$	0.310	PO	50.24 $\pm$ 2.74 (30 - 68)	51.18 $\pm$ 2.7 (27 - 73)	$t_{184} = 1.23$

<b>Background colour brightness</b>										
Species	$F_{2,184} = 0.57$	PE	29.22 ± 0.88 (19 - 47)	30.06 ± 0.74 (18 - 51)					$t_{184} = 3.82$	<0.001
Sex	$F_{1,184} = 1.12$	PH	28.03 ± 1 (15 - 37)	27.26 ± 1.24 (15 - 44)					$t_{184} = 1.23$	0.220
Interaction	$F_{2,182} = 0.01$	PO	27.47 ± 1.4 (16 - 38)	28.73 ± 1.26 (16 - 40)					$t_{184} = 2.7$	0.015
<b>Dorsal colour PCI</b>										
Species	$F_{2,184} = 8.23$	PE	- 0.17 ± 0.29 (- 4.06 - 4.27)	0.07 ± 0.24 (- 5.66 - 5.54)						
Sex	$F_{1,184} = 4.92$	PH	0.19 ± 0.24 (- 3.8 - 3.18)	0.36 ± 0.29 (- 3.62 - 3.2)						
Interaction	$F_{2,182} = 0.11$	PO	- 0.44 ± 0.39 (- 3.09 - 1.66)	0.22 ± 0.28 (- 2.31 - 2.06)						
<b>Dorsal colour PC2</b>										
Species	$F_{2,184} = 3.36$	PE	0.23 ± 0.18 (- 2.88 - 3.13)	0.17 ± 0.11 (- 1.68 - 2.42)						
Sex	$F_{1,184} = 3.03$	PH	- 0.42 ± 0.2 (- 2.63 - 1.92)	0.26 ± 0.21 (- 2.73 - 2.24)						
Interaction	$F_{2,182} = 2.31$	PO	- 0.73 ± 0.2 (- 2.59 - 0.39)	- 0.4 ± 0.27 (- 2.95 - 2.09)						
<b>Dorsal pattern index</b>										
Species	$F_{2,184} = 4.24$	PE	4.5 ± 0.2 (3.25 - 6.25)	4.85 ± 0.13 (3.25 - 6.25)						
Sex	$F_{1,184} = 5.65$	PH	4.7 ± 0.2 (3.25 - 6.25)	5.03 ± 0.19 (3.25 - 6.25)						
Interaction	$F_{2,182} = 0.72$	PO	4.9 ± 0.2 (3.25 - 6.25)	4.75 ± 0.2 (3.25 - 6.25)						
<b>Proportion of black colouration</b>										
Species	$F_{2,184} = 3.31$	PE	0.198 ± 0.013 (0.054 - 0.443)	0.258 ± 0.013 (0.129 - 0.67)						
Sex	$F_{1,184} = 31.14$	PH	0.212 ± 0.02 (0.037 - 0.503)	0.287 ± 0.022 (0.058 - 0.513)						
Interaction	$F_{2,182} = 1.1$	PO	0.24 ± 0.025 (0.049 - 0.443)	0.287 ± 0.022 (0.11 - 0.473)						
<b>Proportion of white colouration</b>										
Species	$F_{2,184} = 2.6$	PE	0.034 ± 0.002 (0.011 - 0.075)	0.035 ± 0.002 (0.014 - 0.11)						
Sex	$F_{1,184} = 1.75$	PH	0.03 ± 0.002 (0.012 - 0.058)	0.032 ± 0.003 (0.01 - 0.07)						
Interaction	$F_{2,182} = 0.44$	PO	0.023 ± 0.002 (0.013 - 0.042)	0.024 ± 0.002 (0.013 - 0.043)						
<b>Dorsal line thickness</b>										
Species	$F_{2,184} = 9.69$	PE	3.6 ± 0.2 (1 - 5)	3.8 ± 0.1 (1 - 5)						
Sex	$F_{1,184} = 0.85$	PH	3.2 ± 0.2 (1 - 5)	3.1 ± 0.2 (1 - 5)						
Interaction	$F_{2,182} = 0.03$	PO	2.8 ± 0.3 (1 - 5)	2.7 ± 0.2 (2 - 4)						
<b>Nuptial colouration</b>										
Species	$F_{2,182} = 32.93$	PE	0.78 ± 0.19 (0 - 4)	0.4 ± 0.12 (0 - 4)						
Sex	$F_{1,182} = 12.6$	PH	3.27 ± 0.38 (0 - 8)	5.48 ± 0.43 (0 - 9)						
Interaction	$F_{2,182} = 9.35$	PO	2.88 ± 0.7 (0 - 8)	7.05 ± 0.45 (0 - 9)						
<b>Number of ocelli</b>										
Species	$F_{2,182} = 11.77$	PE	1.22 ± 0.14 (0 - 3)	2.51 ± 0.13 (0 - 5)						
Sex	$F_{1,182} = 34.73$	PH	0.58 ± 0.12 (0 - 2)	0.95 ± 0.17 (0 - 2.5)						
Interaction	$F_{2,182} = 7.95$	PO	1.82 ± 0.19 (0 - 3)	2.16 ± 0.2 (0.5 - 4.5)						

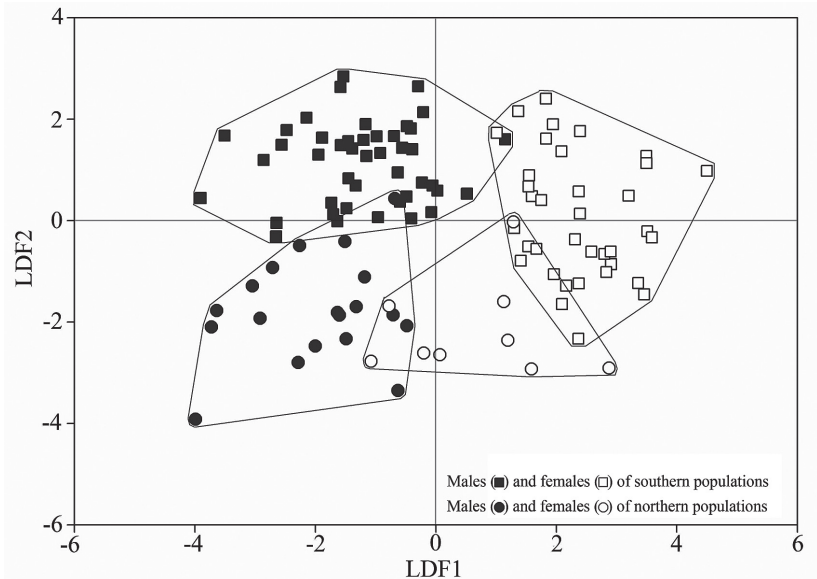
<sup>1</sup> Contrasts on females only

<sup>2</sup> Contrasts on males and females

Table 4. Differences in sexual dimorphism among *P. edwardsianus*, *P. hispanicus*, and *P. occidentalis*. Positive and negative  $t$  values indicate that sexual dimorphism is biased toward males and females, respectively. Statistical significance (after corrections for multiple testing) contrasts are indicated with asterisks (\*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ ).

	<i>P. edwardsianus</i>	<i>P. hispanicus</i>	<i>P. occidentalis</i>
Body mass	$t_{182} = -1.22$	$t_{182} = -2.70^*$	$t_{182} = -3.65^{**}$
Ventral scale rows	$t_{182} = -6.83^*$	$t_{182} = -7.58^{**}$	$t_{182} = -7.75^{**}$
Number of ocelli	$t_{182} = 8.60^{***}$	$t_{182} = 1.98$	$t_{182} = 1.88$
Nuptial colouration	$t_{182} = -1.34$	$t_{182} = 3.18^*$	$t_{182} = 2.72^*$
Neck hue	$t_{182} = 3.48^{**}$	$t_{182} = 2.72^*$	$t_{182} = 5.04^{**}$
Neck brightness	$t_{182} = 1.83$	$t_{182} = -1.26$	$t_{182} = -2.53^*$
Ventral colouration PC2	$t_{182} = 0.68$	$t_{182} = -2.91^*$	$t_{182} = 1.41$

Fig. 4. Plot of first (LDF1) and second (LDF2) linear discriminant function scores of the discriminant analysis on males and females from northern and southern populations of *P. edwardsianus*. LDF1 allowed distinguishing between males (filled symbols) and females (open symbols) and LDF2 between northern (circles) and southern (squares) populations of *P. edwardsianus*.



LDF analysis yielded three discriminant functions (Table S4). LDF1 (65% of the explained variance) reflected the number of ocelli, SVL, SVL ratio, and the number of ventral scales and it separated males from females (Fig. 4). LDF2 (29.9% of the explained variance) separated northern and southern populations of *P. edwardsianus* and it mainly reflected the number of throat scales and dorsal colour PC1 (Fig. 4).

Posterior univariate ANOVAs showed that northern populations had fewer throat scales (northern populations:  $19.9 \pm 0.2$  scales, southern populations:  $21.7 \pm 0.2$  scales,  $F_{1,97} = 5.72$ ,  $P = 0.019$ ), higher dorsal PC1 values (northern populations:  $0.41 \pm 0.2$ , southern populations:  $-1.53 \pm 0.3$ ,  $F_{1,97} = 10.39$ ,  $P = 0.002$ ), and a greener neck colouration (northern populations:  $38.46 \pm 0.81^\circ$ , southern populations:  $31.99 \pm 1.45^\circ$ ,  $F_{1,97} = 5.72$ ,  $P = 0.019$ ) than southern populations. Additionally, females had more ventral scales than males in northern populations (sex  $\times$  geographic location:  $F_{1,96} = 4.34$ ,  $P = 0.039$ , females:  $26.03 \pm 0.21$  rows, males:  $23.03 \pm 0.21$  rows,  $t_{96} = 7.41$ ,  $P < 0.0001$ ),

but no significant sex differences existed in southern populations (females:  $24.89 \pm 0.39$  rows, males:  $23.84 \pm 0.31$ ,  $t_{96} = 1.67$ ,  $P = 0.098$ ).

#### Key for the three *Psammodromus* species

*Psammodromus edwardsianus* can be distinguished from the other two species by the presence of a supralabial scale below the subocular scale (see Appendix). Since none of the measured traits allowed for unequivocal distinction between *P. hispanicus* and *P. occidentalis* we ran an additional discriminant analysis based on four easy-to-measure variables; the number of femoral pores, the number of ocelli, the relative anal scale width, and the number of throat scales (see Appendix and Table 1a). Cross-validation showed that the derived LDFs correctly attributed 89% of *P. hispanicus* and 79.5% of *P. occidentalis* individuals. The percentage of males and females correctly attributed to these species was 85% and 86%, respectively.

## Discussion

### *Phenotypic differentiation*

Recent molecular studies in Spanish sand racers demonstrated the existence of three genetically differentiated lineages that are now recognized as distinct species (Fitze *et al.* 2011, 2012). We show that these three species, *P. hispanicus*, *P. edwardsianus*, and *P. occidentalis* exhibit substantial differentiation in phenotypic traits and in the degree of sexual dimorphism. As first pointed out by Boulenger (1921), the presence of a supralabial scale below the subocular scale is a good trait to categorically differentiate *P. edwardsianus* from *P. hispanicus* and *P. occidentalis*, whose subocular scale directly touches the mouth. The presence of this scale and the relative position of the subocular scale in relation to the mouth are also accurate diagnostic traits in other genera of the family Lacertidae (*e.g.* *Acanthodactylus*; Bons and Geniez, 1995; Harris and Arnold, 2000). However, this scale is absent and the subocular scale always touches the mouth in other species of the genus *Psammodromus* and in the sister genus *Gallotia*, indicating that the presence of this scale in *P. edwardsianus* is unique and derived within the entire subfamily Gallotiinae (Salvador, 1998; Hernández *et al.*, 2000; Galewski *et al.*, 2001). As revealed by the discriminant function analysis, *P. edwardsianus* can be also distinguished by a higher number of femoral pores and throat scales, and by a less extended nuptial colouration and a less green and less saturated neck colour. In addition, *P. edwardsianus* presents a smaller SVL, a higher SVL ratio (*i.e.* a larger relative tail length), and a more pointed head. *Psammodromus hispanicus* and *P. occidentalis* could not be distinguished on the basis of a single qualitative trait. However, discriminant functions allowed successful discrimination between the two species (~80%). Main differences between *P. hispanicus* and *P. occidentalis* were observed in the number of femoral pores, throat scales, and collar scales, which were smaller in the former species. Further, *P. hispanicus* showed fewer ocelli than *P. occidentalis* and greener and more saturated neck colourations.

The observed phenotypic differentiation may indicate important differences in performance and behaviour (van Damme *et al.*, 1998). For instance, between species differences in SVL have been observed to result in differences in sprint performance (Losos, 1990; Bauwens *et al.*, 1995), home range (Turner *et al.*, 1969;

Perry and Garland, 2002), thermoregulation, and daily activity patterns (Stevenson, 1985). The smaller SVL in *P. edwardsianus* may therefore indicate that this species may differ from other Spanish sand racers in some of the above-mentioned features. The more pointed head shape of *P. edwardsianus* may be associated with shorter bite cycles and reduced prey processing times in contrast to *P. hispanicus* and *P. occidentalis* (Verwajen and van Damme, 2007). Altogether, the phenotypic differences here determined may suggest that distinct selective pressures act on Spanish sand racer species (Losos *et al.*, 1997). Fitze *et al.* (2011) showed that the three species occupy different ecological niches (those of *P. occidentalis* and *P. edwardsianus* are more similar than that of *P. hispanicus*), but found no congruent association between niche differentiation and variation in morphological traits. Other ecological factors than those considered in Fitze *et al.* (2011) (18 climatic, one topographic and two vegetation index variables) may underlie phenotypic differentiation within Spanish sand racers. For instance, differences in selective pressures derived from community structure (*i.e.* predation, competitive interactions with other species, and prey spectra) may also result in species differentiation in body size (Blomberg and Shine, 2000), shape (Verwajen and van Damme, 2007), and colouration (Endler, 1983; Forsman and Appelqvist, 1998).

*Psammodromus edwardsianus* exhibited substantial phenotypic variation that corresponds to the north-south genetic structure reported by Fitze *et al.* (2011). Northern populations exhibited a lower number of throat scales, greener necks, and lighter dorsal colouration. Additionally, females in northern but not in southern populations were larger than males. Such geographic variation in phenotypic traits may be the consequence of postglacial range expansion during which *P. edwardsianus* may have confronted changing environmental conditions and, hence, different selective pressures as the species expanded toward the north of the Iberian Peninsula (Hewitt, 1996, 1999; Fitze *et al.*, 2011). Similar north-south patterns of phenotypic variation have been reported in other animal species of the Iberian Peninsula (*e.g.* Alexandrino *et al.*, 2007). Given the limited number of individuals captured in southern populations of *P. occidentalis*, we could not test whether geographic variation in phenotypic traits also exists within *P. occidentalis*. Such variation is expected given the observed north-south differentiation observed within *P. edwardsianus*, and it deserves further attention.

### Sexual dimorphism

Strong sexual dimorphism was observed in body measures, scalation traits, colour pattern, and colour traits in all species. We found that females were larger and heavier than males in *P. hispanicus* and *P. occidentalis*. Female-biased sexual size dimorphism is usually attributed to selective pressures favouring larger and, hence, more fecund females (Shine, 1989). Selection for female fecundity may be more important in species where small female body size strongly constrains egg carrying capacity (Maritz and Alexander, 2011). Similar selective pressures are likely to occur in Spanish sand racers, which are among the smallest species of the subfamily Gallotiinae. Moreover, larger species of the subfamily Gallotiinae commonly exhibit male-biased size dimorphism (Herrel et al., 1999; Hernández et al., 2000; Molina-Borja, 2003), which may further support this hypothesis. In *P. edwardsianus*, sexual size dimorphism was less marked given that body mass did not differ between sexes and that SVL was only different between males and females of northern but not of southern populations. Different, non-mutually exclusive hypotheses may underlie relaxed sexual dimorphism in *P. edwardsianus*. Enlarged female body size is known to impair locomotion and, in circumstances of high predation risk, such impairment may counteract potential fitness gains of enlarged body size (Seigel et al., 1987; Sinervo et al., 1991). In addition to body size enlargement, selection may favour other high-fecundity strategies (Sinervo et al., 2000). Thus, in certain circumstances, selection may favour small females that lay many clutches of few eggs instead of large females that lay many eggs in one single clutch. Both hypotheses may be supported in *P. edwardsianus*. First, *P. edwardsianus* shows a longer annual activity cycle, which may allow it to lay more than one clutch per year (Carretero and Llorente, 1991b). Second, it lives in more open habitats than, at least, *P. occidentalis* (Fitze et al., 2011), which may entail a higher predation risk (Carrascal et al., 1989).

The degree of sexual colour dimorphism differed among species in traits that may be under sexual selection (Diaz, 1992; Salvador and Veiga, 2008). Nuptial colouration was highly dimorphic in *P. hispanicus* and *P. occidentalis* but not in *P. edwardsianus*, where it was almost absent. In contrast, the number of ocelli was sexually dimorphic in *P. edwardsianus* but not in *P. hispanicus* and *P. occidentalis*. In Spanish sand racers, nuptial colouration and ocelli may play an important role in intra- and intersexual selection, as observed in

the sister species *P. algirus* (Linnaeus, 1758) (Martín and Forsman, 1999; Salvador and Veiga, 2001, 2008). The observed differences suggest that the mechanisms governing male-male interactions as well as female mate choice may differ among Spanish sand racer species. Such differences may further impose a barrier to reproduction, preventing gene flow in a scenario of secondary contact among Spanish sand racers (West-Eberhard, 1983; Jiggins et al., 2001). In line with these findings, we found important differences in the number of femoral pores, which may indicate that chemical communication strategies or the relative importance of chemical scent marks for communication may also differ among species (Martín and López, 2000; López and Martín, 2005). Altogether, these findings suggest that different evolutionary forces underlie visual and chemical signalling in Spanish sand racer species, which makes this species group a suitable model to test distinct evolutionary scenarios related with animal signalling and speciation (Maan and Seehausen, 2011).

The data presented here indicate that the degree of phenotypic differentiation is higher between *P. edwardsianus* and the other Spanish sand racers than between *P. hispanicus* and *P. occidentalis*. This pattern suggests that the degree of phenotypic differentiation is not correlated with genetic distances in Spanish sand racers, given that *P. edwardsianus* is genetically closer to *P. hispanicus* than to *P. occidentalis*, which diverged from the common ancestor of *P. edwardsianus* and *P. hispanicus*. Similarly, phenotypic differentiation was not correlated with inferred ecological niches since *P. occidentalis* and *P. edwardsianus* showed more similar ecological niches than *P. hispanicus*. However, this pattern is in line with previous morphological descriptions based on a lower number of traits and where differences between sexes were not considered, which support the robustness of our findings (Fitze et al., 2011). Comparisons among closely related groups often obscure the predicted association between phenotypic and genetic variation, given that differences in other determinant factors (e.g. ecology or behaviour) may strongly increase phenotypic variability (Wayne and O'Brien, 1986; Hackett and Rosenberg, 1990). Differences in the degree of sexual dimorphism suggest that substantial differences in reproductive and sexual behaviour may exist among species, which help to explain why phenotypic and genetic distances are uncoupled within Spanish sand racers. The key to Spanish sand racer species provided here allows field and experimental studies to be conducted that are necessary in order to completely understand the evolutionary mechanisms leading to

speciation in this group of animals, as well as helping in the conservation management of these species.

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## Appendix

### Key to the Spanish sand racers

- 1. Presence of a supralabial scale below the subocular scale ..... *P. edwardsianus*
- 1'. No supralabial scale below the subocular scale ..... 2
- 2. Value from E1 greater than value from E2 ..... *P. hispanicus*
- 2'. Value from E2 greater than value from E1 ..... *P. occidentalis*

#### Females:

$$e^{(9.03 + (0.13 \times \text{throat scales} - 0.6 \times \text{femoral pores} - 0.89 \times \text{ocelli} - 91.8 \times \text{relative anal scale width}))} \text{ [E1]}$$

$$e^{(-21.47 + (-0.26 \times \text{throat scales} + 1.17 \times \text{femoral pores} + 1.73 \times \text{ocelli} + 178.2 \times \text{relative anal scale width}))} \text{ [E2]}$$

#### Males:

$$e^{(7.9 + (0.06 \times \text{throat scales} - 0.86 \times \text{femoral pores} + 0.46 \times \text{ocelli} + 1.15 \times \text{relative anal scale width}))} \text{ [E1]}$$

$$e^{(-13.9 + (-0.08 \times \text{throat scales} + 1.21 \times \text{femoral pores} + 0.64 \times \text{ocelli} - 1.62 \times \text{relative anal scale width}))} \text{ [E2]}$$

### On-line supplementary information (SI)

- S1. Sampled localities and sample size.
- S2. Details of the method used for measuring the dorsal pattern
- S3. Component loadings of principal component analyses.
- S4. Factor loadings from the linear discriminant functions on the three Spanish sand racer species and on northern and southern populations of *P. edwardsianus*.

