

*Amphibia-Reptilia*

Sources of intraspecific morphological variation in *Vipera*  
*seoanei*: Allometry, sex, and colour phenotype

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**Supplementary material**

**Table S1****Table S1.1.** Number of individuals used for linear biometric analyses assigned to each group.

SEX	AGE	bilineata	cantabrica	classic	melanistic	uniform	total
F	Juveniles	-	3	5	3	-	11
	Subadults	-	4	2	1	-	7
	Adults	15	56	92	23	5	173
M	Juveniles	1	15	15	1	-	32
	Subadults	2	14	20	4	-	40
	Adults	13	63	102	21	1	128

**Table S1.2.** Number of individuals used for geometric morphometric analyses assigned to each group.

SEX	AGE	bilineata	cantabrica	classic	melanistic	uniform	total
F	Juveniles	-	1	3	-	-	4
	Subadults	-	1	1	-	-	2
	Adults	4	20	25	3	-	52
M	Juveniles	-	8	6	-	-	14
	Subadults	-	3	8	-	-	11
	Adults	3	15	19	4	-	41

**Table S1.3.** Total and by group number of missing values replaced for each trait: SVL (snout-vent length), TAIL (tail length), HL (head length), ML (mouth length), JL (jaw length), HW (head width), HH (head height), SH (snout height), TOTAL (total sample size).

	Total	Juveniles		Subadults		Adults	
		M	F	M	F	M	F
SVL	-	-	-	-	-	-	-
TAIL	11	-	-	1	-	4	6
HL	5	2	-	2	-	1	-
ML	20	4	-	4	-	7	5
JL	24	4	1	3	-	7	9
HW	55	2	1	7	-	23	22
HH	73	2	2	12	1	31	25
SH	50	2	2	8	1	21	16
TOTAL	391	32	11	40	7	128	173

**Table S1.4.** Minimum (Min), mean, maximum (Max) and standard deviation (SD) values for linear morphometric traits: SVL (snout-vent length), TAIL (tail length), HL (head length), ML (mouth length), JL (jaw length), HW (head width), HH (head height), SH (snout height).

	M				F			
	Min	Mean	Max	Sd	Min	Mean	Max	Sd
SVL	145	342.876	490	86.274	145	396.068	535	64.752
TAIL	20	54.842	85	14.774	21	51.117	79	8.710
HL	9.060	15.535	20.790	2.467	9.350	16.872	20.520	2.001
ML	9.730	16.343	21.840	2.668	10.160	17.760	22.400	1.947
JL	10.920	19.088	25.640	3.215	11.620	20.918	25.500	2.328

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<b>HW</b>	5.500	12.386	20.620	2.216	6.560	13.625	18.880	1.918
<b>HH</b>	4.200	7.419	10.350	1.274	4	8.241	13.050	1.152
<b>SH</b>	1.360	2.856	3.980	0.492	1.740	3.043	4.300	0.377

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## Text S1

Evaluation of measurement error during digitizing landmarks to study variation in head shape in *Vipera seoanei*.

## Methods

In order to examine the effect of digitizing error in studying intraspecific head shape variation in *Vipera seoanei*, one of us (GA) digitized all specimens twice. To test for the presence of measurement error and evaluate its magnitude as compared to the total shape variation present in our sample, we first superimposed all original and repeated landmark configurations. and then we fit a linear model on Procrustes residuals with individual (ind) and digitizing repetition (rep) as explanatory variables. Linear models were fitted using the function *procD.lm* of *geomorph* R-package (Adams, Collyer and Kaliontzopoulou, 2019) and evaluated using residual randomization procedures as implemented in the R-package *RRPP* (Collyer and Adams, 2018, 2019).

To further verify the consistency of both digitizations, we used a two-block partial least-squares analysis (PLS: Rohlf and Corti, 2000), to quantify the multivariate association between shape variables produced in each of them. The significance of the correlation coefficient between PLS vectors was evaluated through 1000 data permutations. All PLS procedures were implemented using the function *two.b.pls* of *geomorph* R-package (Adams, Collyer and Kaliontzopoulou, 2019).

Finally, to investigate whether specimen origin (museum collection vs. alive vs. road killed) had an effect on the precision of shape data collection, we first quantified individual measurement error (indME) as the Procrustes distance between the two digitizations available for each specimen. Using these individual values, we then fit a linear model with indME as the response variable and specimen origin as the predictor, the significance of which was also evaluated using *RRPP* as described above.

## Results

ANOVA statistics derived from the linear model fit to evaluate digitizing error indicated the occurrence of significant shape variation both among individuals and between the two digitization rounds (supplementary table S1). Examination of  $R^2$  values related to different effects suggests that 91.5% of shape variation is attributable to differences among individuals, while the rest is due to the effect of measurement error. Of this, about 3.4% is systematic error (repetition effect). By contrast, about 5.1% is random error, where digitization effects vary among individuals, as indicated by the residual term, which in this case corresponds to the ind×rep interaction. This means that systematic error, which may bias downstream shape analysis, represents – in our case – less than 5% of the total shape variation in our sample.

**Table S2.1.** ANOVA statistics from a linear model fit to shape data to evaluate the effect of individual (ind) and digitizing repetition (rep) on shape variation. df: degrees of freedom, SS: Sums of Squares, MS: Mean Squares,  $R^2$ : coefficient of determination, F: F-value, Z: standardized z-score, p: corresponding p-value based on 1000 permutations of residuals.

	df	SS	MS	$R^2$	F	Z	p
<b>ind</b>	139	1.439	0.010	0.915	17.958	22.370	0.001
<b>rep</b>	1	0.054	0.054	0.034	93.463	6.426	0.001
<b>Residuals</b>	139	0.080	5.77*10 <sup>-4</sup>	0.051			
<b>Total</b>	279	1.573					

The partial least-squares analysis implemented to examine the multivariate association between the shape variables produced by both digitizing repetitions confirms this observation. Indeed, the two-blocks PLS provided a significant vector of association between shape variable blocks, where the correlation among vectors was  $r_{PLS} = 0.939$  ( $p = 0.001$ ).

Finally, our test of whether individual measurement error depended on the origin of specimen collection, including 98 specimens from museum collections, 35 collected alive in the field, and 8 specimens found as road kills, indicated no significant effect (supplementary table S2.2).

**Table S2.2.** ANOVA statistics from a linear model fit to individual measurement error (indME) to evaluate the effect of specimen origin (ori). df: degrees of freedom, SS: Sums of Squares, MS: Mean Squares,  $R^2$ : coefficient of determination, F: F-value, Z: standardized z-score, p: corresponding p-value based on 1000 permutations of residuals.

	<b>Df</b>	<b>SS</b>	<b>MS</b>	<b>R<sup>2</sup></b>	<b>F</b>	<b>Z</b>	<b>p</b>
<b>ori</b>	2	0.000	0.000	0.010	0.713	0.182	0.477
<b>Residuals</b>	137	0.033	0.000	0.990			
<b>Total</b>	139	0.033					

Given these results, we consider that, despite the existence of digitizing error, which is practically unavoidable in GM studies (Arndt and Mårtensson, 1998), the magnitude of systematic error is very reduced as compared to the total shape variation present in our data, and that the origin of the specimens is unlikely to influence substantially the shape patterns observed here.

## References

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**Table S2**

Post-hoc comparisons for morphological variables on which ANOVA comparisons indicated a significant effect of colour morph (table 2). Pairwise comparisons based on 1000 random permutations of Euclidean distances were performed across pattern  $\times$  sex groups, when both variables had a significant effect (i.e. SVL, TAIL, ML); or between pattern groups only, when the effect of sex was not significant (i.e. HL). Significant pairwise differences (at  $\alpha = 0.05$ ) are highlighted in boldface.

<b>SVL</b>	bilineata F	cantabrica F	classic F	melanistic F	bilineata M	cantabrica M	classic M	melanistic M
bilineata F								
cantabrica F	0.571							
classic F	0.922	<b>0.043</b>						
melanistic F	0.448	0.368	0.068					
bilineata M	0.157	0.120	0.370	0.093				
cantabrica M	0.171	0.060	0.444	0.066	0.405			
classic M	0.629	0.639	0.990	0.484	0.077	0.199		
melanistic M	0.783	0.095	0.722	0.091	0.479	0.588	0.983	
<b>TAIL</b>	bilineata F	cantabrica F	classic F	melanistic F	bilineata M	cantabrica M	classic M	melanistic M
bilineata F								
cantabrica F	0.532							
classic F	0.127	0.2795						
melanistic F	0.468	0.758	0.553					
bilineata M	0.446	0.724	0.776	0.876				
cantabrica M	0.456	0.230	<u>0.051</u>	0.290	0.383			
classic M	0.345	0.186	<b>0.013</b>	0.242	0.347	0.958		
melanistic M	0.435	0.652	0.827	0.827	0.91	0.374	0.339	
<b>ML</b>	bilineata F	cantabrica F	classic F	melanistic F	bilineata M	cantabrica M	classic M	melanistic M
bilineata F								
cantabrica F	0.255							
classic F	0.368	0.274						
melanistic F	0.185	0.102	0.956					
bilineata M	0.281	0.182	0.610	0.637				
cantabrica M	0.447	0.632	0.279	0.103	0.211			
classic M	0.297	0.186	0.606	0.626	0.929	0.216		
melanistic M	0.886	<b>0.029</b>	0.797	0.674	0.818	0.554	0.895	