

## ARE THERE MORPHOLOGICAL DIFFERENCES BETWEEN TWO GENETICALLY DIFFERENTIATED CLADES IN THE ADDER *Vipera berus berus*?

S. Ursenbacher,<sup>1</sup> I. Sasu,<sup>1</sup> M. Rossi,<sup>1</sup> and J.-C. Monney<sup>2</sup>

The morphologies of 164 adders (*Vipera berus berus*) were analyzed in order to assess if morphological differences occur between the Northern and the Italian clades. Pholidosis and some corporal proportions were measured, corresponding to 17 parameters. Several parameters show significant differences between clades (e.g., subcaudals, labials, sublabials, parietal scales). Moreover, discriminant analyses separate both clades with high degrees of accuracy of a correct classifying.

**Keywords:** *Vipera berus*, morphology, discriminant analysis.

### INTRODUCTION

The adder, *Vipera berus* (Linnaeus, 1758), has the most widespread terrestrial snake in the world (Saint Girons, 1978). Despite this large distribution area, only three sub-species are recognised: *V. b. berus*, *V. b. bosniensis* (Boettger, 1880), *V. b. sachalinensis* (Zarevsky, 1917). Moreover, the nominal subspecies is present from France to middle Russia, with only a low level of morphological variation over approximately 10,000 km<sup>2</sup> (Saint Girons, 1978). Homogeneity within this subspecies was also confirmed by 2 different studies based on genetic markers (Joger et al., 2003; Ursenbacher et al., submitted), except adders in Italy, Northern Slovenia, southern Austria and extreme southeastern Switzerland (the Italian clade). Given the high degree of genetic differentiation (the split between the two clades occurred more than 1 Million years ago), the aim of this short note is to compare the morphology of these two clades.

### MATERIAL AND METHODS

**Measurements.** We measured 164 *Vipera berus berus* deposited in the Natural History Museum of Geneva, Switzerland (MHNG): 101 (54 females and 47 males) from the Northern clade and 63 (35 females and 28 males) from the Italian clade described in Ursenbacher et al. (submitted) and Joger et al. (2003). Clade was assigned according to the location of the collected animal. The number of ventrals (V), subcaudals (C), apicals (A), parietals (P) intercanthals scales (I), as well as, on both sides, the

total number of loreals (L), canthals (CA), peri-oculars (PO), supralabials (SUP), sublabials (SUB) and parafrontals scales (PA) were counted according to the method used by Saint Girons (1978).

The number of rows between the peri-oculars and the supralabials was also counted on both sides (RO). Altogether, 12 parameters describing head scale patterns were measured. Moreover, the total length (LOT.C), the length of the tail (LO.Q), the snout-vent length (SVL), and head length (LO.T) and width (LR.T) were measured; from these, the proportion of length composed of the tail (%TAIL = LO.Q/LOT.C) and the head (%HEAD = LO.T/LOT.C) and the relative width of the head (%W.T = LR.T/LO.T) were calculated. Only sub-adults and adults, defined by a total length >300 mm were included in analyses.

**Statistical analyses.** All statistical analyses were done using SPSS 11.0. 1 for MacOSX (SPSS Inc.). Analyses were conducted with data for males and females together, as well as for each sex separately. First the SVL were compared between clades. Subsequently, comparisons between clades for all parameters were done using Student's

**TABLE 1.** Probabilities of *t*-Tests or the Wilcoxon Tests for Parameters with at Least One Significant Value

	Female and male probability		Female probability		Male probability	
V	0.020	*	0.062	NS	0.064	NS
C	0.000	***	0	***	0	***
L	0.001	***	0.029	*	0.009	**
SUP	0.012	*	0.122	NS	0.003	***
SUB	0.002	***	0.017	*	0.012	*
I	0.001	***	0.005	***	0.089	NS
P	0	***	0.002	***	0.013	*
%TAIL	0.119	NS	0.036	*	0.358	NS

NS,  $P > 0.05$ ; \* $p = 0.05$ ; \*\* $p = 0.01$ ; \*\*\* $p = 0.005$ .

<sup>1</sup> Laboratoire de Biologie de la Conservation, Institut d'Ecologie, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland; E-mail: sylvain.ursenbacher@ie-zea.unil.ch.

<sup>2</sup> Centre de Coordination pour la Protection des Amphibiens et des Reptiles de Suisse (KARCH), CH-3005 Bern, Switzerland.

*t*-test, Welch's *t*-test and Wilcoxon's test. Scale pattern measurements and ratios (%TAIL, %HEAD, %W.T) were analyzed with discriminant.

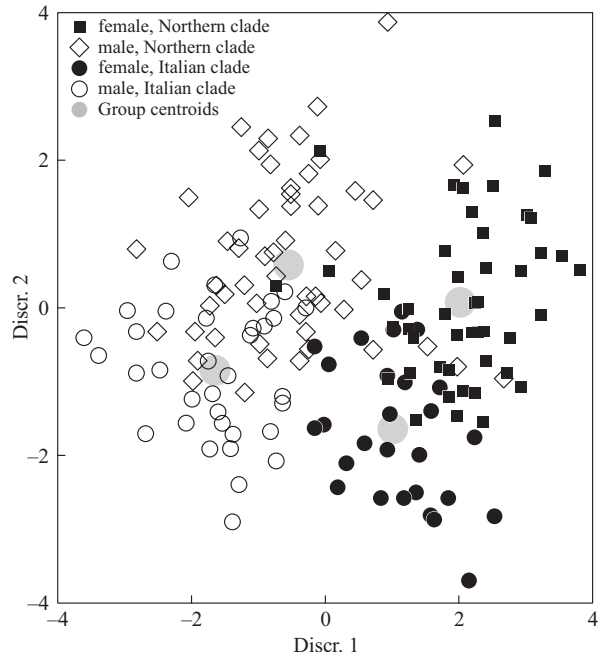
**RESULTS**

**Comparison between clades.** SVL did not differ between clades either when males and females were analyzed together ( $Z = -0.507, p = 0.612$ ) or when considered separately ( $Z = -0.269, p = 0.788$  for females,  $Z = -0.991, p = 0.321$  for males). All significant differences between the two clades are shown in Table 1. Significant differences for both sexes both combined and split were observed for the number of subcaudals (C), loreals (L), sublabials (SUB) and parietals (P). Only males showed a difference in supralabials (SUP), while only females differed in intercanthals scales (I) and the proportion of the tail (%TAIL). When males and females were combined, the number of ventral scales (V) differed significantly between clades although neither males nor females alone showed a significant difference.

**Discriminant analyses.** The result of the discriminant analysis grouping males and females is shown in Fig. 1. Group membership was predicted correctly in 68.5 to 85.7% of cases. When the same analyses were done on females and males separately, the predicting accuracy was 83.3 – 88.6% for females and 91.5 – 92.9% for males.

**DISCUSSION**

The Northern and Italian clades seem to have several morphological differences as shown above, some of which



**Fig. 1.** Discriminant analysis with all animals (Discr. 1: 77.8% of the variance; Discr. 2: 20.8% of the variance; Wilks'  $\lambda = 0.1836, p < 0.0005$ ).

might be useful to morphologically determine the clade of an animal. Adders from the Italian clade have a lower number of subcaudals (about 3 subcaudals less than the Northern clade), higher number of loreals and sublabials, a lower number of intercanthals, and the parietals are less split (Table 2). The number of ventral scales also seems to be lower in the Italian clade, but this difference is not significant when the analysis is conducted on males and females separately. Saint Girons (1978) has already ob-

**TABLE 2.** Measurements on Females and Males of Each Clade, with the Mean, Standard Deviation, Minimum and Maximum Observed

Group		V	C	L	SUP	SUB	I	P
<b>Females</b>								
Italian clade <i>n</i> = 35	Mean	145.77 ± 3.23	27.63 ± 2.13	6.71 ± 2.47	17.63 ± 0.84	20.89 ± 1.37	6.91 ± 2.62	1.97 ± 0.38
	Minimum	137	23	2	16	19	2	0
	Maximum	153	34	11	19	24	14	3
Northern clade <i>n</i> = 54	Mean	147.15 ± 3.55	30.50 ± 3.59	5.54 ± 2.40	17.17 ± 1.61	20.00 ± 2.06	8.70 ± 3.26	2.46 ± 0.86
	Minimum	139	21	1	12	15	4	2
	Maximum	155	39	11	20	25	18	7
<b>Males</b>								
Italian clade <i>n</i> = 28	Mean	142.43 ± 3.73	34.36 ± 2.39	5.89 ± 2.60	17.93 ± 1.15	21.21 ± 2.17	6.82 ± 2.63	2.00 ± 0.27
	Minimum	134	29	2	16	15	3	1
	Maximum	149	39	12	22	26	12	3
Northern clade <i>n</i> = 47	Mean	144.11 ± 3.54	36.81 ± 2.71	4.47 ± 1.99	17.11 ± 1.03	19.96 ± 1.77	7.91 ± 2.69	2.47 ± 0.95
	Minimum	135	30	0	15	16	2	2
	Maximum	152	42	9	19	23	16	6

served a lower number of ventrals in adders belonging to the Italian clade. Scali and Gentilli (1999) also showed a lower number of ventrals in specimens from the Po plane (northern Italy).

However, the number of analyzed animals is not very high and the majority of adders of the Italian clade come from the Graubunden region (extreme southeastern Switzerland), and might be different from adders from the rest of the distribution of the Italian clade. Due to a low number of animals per location, it is not possible to analyze the present dataset in detail. The number of samples of each clade, especially animals from Russia, Scandinavia, Italy, Slovenia and Austria should also be increased for better resolution of the local morphological differences. Finally, adding more morphological characters such as dorsal pattern (see Nilson and Andrén, 2001, for *V. ursinii* group) might also reveal more differences within *V. b. berus*. Nevertheless, these preliminary analyses suggest morphological differentiation in *V. b. berus* following genetic splitting.

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