

## SYSTEMATICS OF THE NOSE-HORNED VIPER (*VIPERA AMMODYTES*, LINNAEUS, 1758)

LJILJANA TOMOVIC

*Institute of Zoology, Faculty of Biology, University of Belgrade, Belgrade, Serbia*

Geographic variability of the nose-horned viper (*Vipera ammodytes*) was analysed using multivariate techniques in order to clarify the taxonomic status and geographic ranges of the subspecies. Analyses included samples ranging from central northern Italy and southern Austria to easternmost Turkey, Georgia and Armenia, and hence, all described taxa. In total, 14 morphometric, five meristic and nine qualitative traits of 922 specimens (451 males and 471 females) were recorded and analysed using different multivariate statistics. The results showed the validity of four subspecies: one inhabiting the western and central parts of the species' range (from Italy, via Austria, to the western and central parts of the Balkans), the second occurring in the southernmost part of the Balkan peninsula, the third distributed from the southern and eastern Balkans to western Turkey and the fourth inhabiting eastern Turkey, Georgia and Armenia.

*Key words:* Balkans, multivariate analyses, snake, subspecies

### INTRODUCTION

The nose-horned viper (*Vipera ammodytes*, L. 1758) is one of the most widespread vipers of southern Europe. Its range extends from central northern Italy, southern Austria, through the Balkans and southern Romania to north-eastern Turkey and Transcaucasia (Arnold & Ovenden, 2002). According to some of the relevant literature (Eiselt & Baran, 1970; Biella, 1983; Biella & Blättler, 1989; Crnobrnja-Isailovic & Haxhiu, 1997) seven different subspecies have been described so far: *V. a. ammodytes*, Linnaeus, 1758; *V. a. illyrica*, Laurenti, 1768; *V. a. meridionalis*, Boulenger, 1903; *V. a. montandoni*, Boulenger, 1904; *V. a. transcaucasiana*, Boulenger, 1913; *V. a. ruffoi*, Bruno, 1968; and *V. a. gregorwallneri*, Sochurek, 1974. *V. a. transcaucasiana* is sometimes treated as a full species (Obst, 1983; Nilson *et al.*, 1999). However, the taxonomic status of some subspecies (*V. a. illyrica*, *V. a. ruffoi*, *V. a. gregorwallneri* and even *V. a. montandoni*) remains unclear. Generally, *V. a. illyrica* is treated as a synonym of the nominate subspecies (see Tomovic & Dzukic, 2003 and references therein). Golay *et al.* (1993) recognized only three subspecies: *V. a. ammodytes* (including *gregorwallneri* and *ruffoi*), *meridionalis* (including *montandoni*) and *transcaucasiana*. In contrast, Ulber (1994) recognized five subspecies: *V. a. ammodytes*, *meridionalis*, *montandoni*, *transcaucasiana* and *gregorwallneri*. In some of the latest references (e.g. McDiarmid *et al.*, 1999) only synonyms are listed and the taxonomic status of the subspecies is not considered. Although this species has been the subject of intense interest concerning its systematics and distribution several times in the past century (Fuhn & Vancea, 1961; Vozenilek, 1971; Sochurek, 1972, 1976, 1985;

Vozenilek & Cizek, 1978; Biella, 1983; Biella & Blättler, 1989; Brodmann, 1987), comprehensive studies using multivariate statistics to define subspecies from the whole species range have never been conducted. Since a previous study of *Vipera ammodytes* (Tomovic & Dzukic, 2003) had been carried out on populations from the central and eastern parts of the species' range only and had failed to show clear differentiation of the subspecies, additional analyses of populations from the rest of the range were needed for a complete taxonomic consideration of the nose-horned vipers.

Multivariate statistics were used to define taxonomic units up to the species level, as has recently been done by numerous authors for different snake taxa (Wüster & Thorpe, 1987; Wüster *et al.*, 1995; Wüster & Broadley, 2003; Zuffi & Bonnet, 1999; Zuffi, 2002). The advantage of these techniques is that they analyse the pattern of variation in all characters used simultaneously (Thorpe, 1987; Wüster *et al.*, 1992).

The aims of this study were: (1) to elucidate the pattern of geographic variation in the nose-horned viper across its entire range; (2) to test the validity of the conventional subspecies; (3) to take into consideration possible geographic ranges of re-defined subspecies.

### MATERIALS AND METHODS

#### STUDY AREA AND SAMPLES

The study area includes the whole range of the species, from central northern Italy and southern Austria, through the Balkans to Turkey, Georgia and Armenia (Fig. 1). I examined preserved specimens from the collections of the Naturhistorisches Museum in Vienna; the Museum of Natural History in Ljubljana; the Land Museum – Natural History Department in Sarajevo; the Institute for Biological Research in Belgrade; the Museum of Natural History in Belgrade; the Museum of Natural History in Skoplje; the Institute of Biology,

*Correspondence:* L. Tomovic, Institute of Zoology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia.

*E-mail:* lili@bf.bio.bg.ac.yu

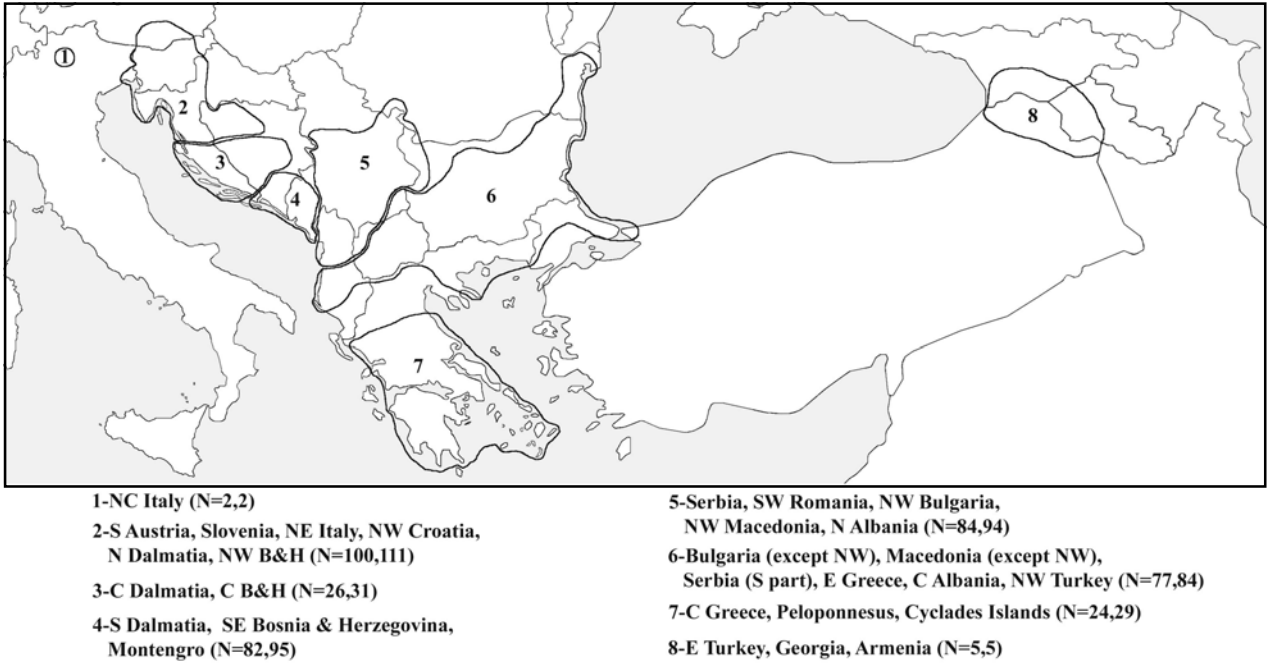


FIG. 1. Map of geographic groups analysed. Numbers of specimens per group of males are given in parentheses (first number = sample size used for analyses of morphometric characters, second number = sample size used for analyses of qualitative traits).

University of Priština; the National Museum of the Bulgarian Academy of Sciences in Sofia; and the private collections of I. Krizmanic, R. Ajtic, J. Crnobrnja-Isailovic and myself.

In total, 922 specimens (451 males and 471 females) were analysed. For morphometric analyses, only adult

and subadult animals (799 specimens – 400 males and 399 females) were examined.

Since the number of specimens per locality was insufficient for performing statistical analyses, several localities were a priori pooled into compound geographic groups, based on collecting gaps and

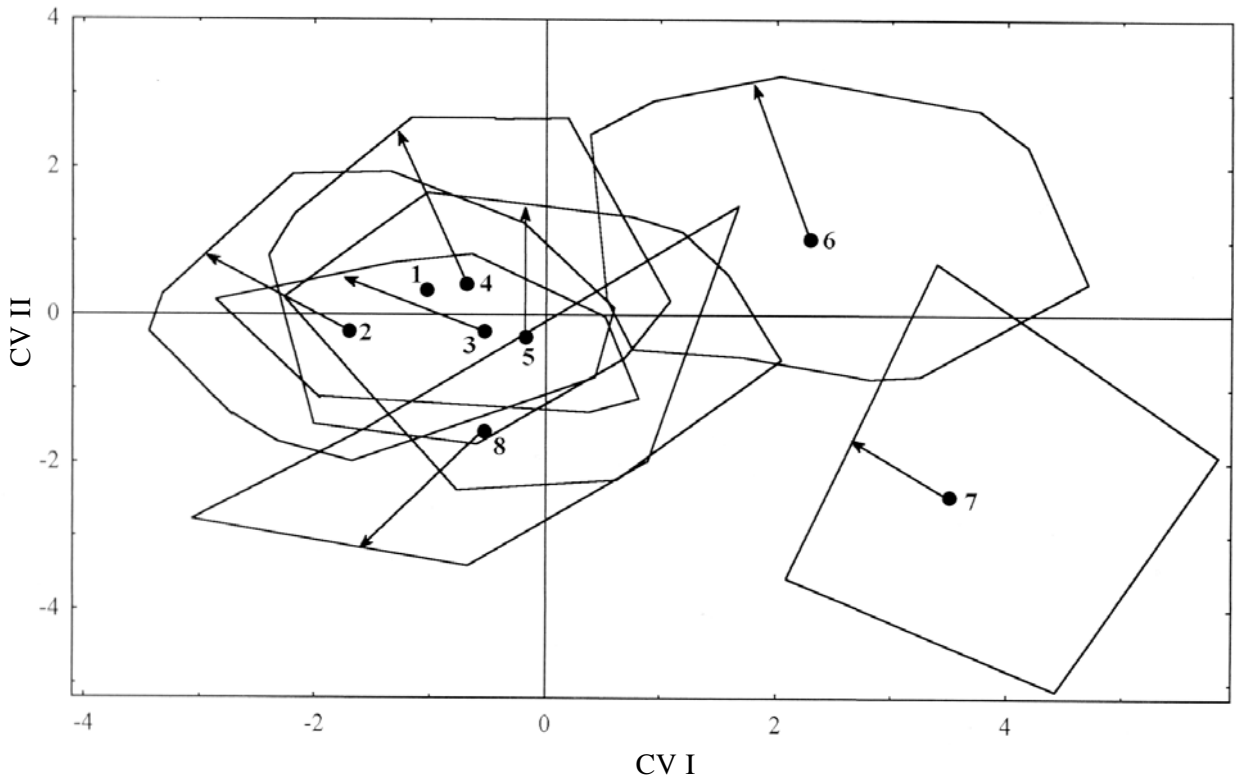


FIG. 2. Relative position of individual male specimens in the projection of the first and the second canonical variates (size-adjusted morphometric and meristic data). Points denote means of geographic groups, lines indicate the position of far-distant individual specimens (outliers were removed upon 95% confidence level).

TABLE 1. Character coefficients for three canonical variates from the discriminant function analysis of the nine groups of males. Significant coefficients are in bold.

Character	CV I	CV II	CV III
Lt cor	0.050	0.009	0.163
Alt cor	-0.385	-0.126	-0.142
L cd	-0.219	-0.038	-0.122
L cap	-0.165	0.223	-0.004
Lt cap	0.174	0.344	<b>0.587</b>
Alt cap	0.058	0.125	0.216
D o	0.247	0.201	-0.440
Dols	-0.183	0.370	-0.455
Alt corni	0.118	<b>-0.553</b>	-0.065
Alt r	-0.082	-0.329	0.194
L scr	<b>0.612</b>	0.404	-0.247
Lt scr	-0.463	-0.254	0.166
D	-0.110	0.064	0.370
V	<b>-0.856</b>	0.446	-0.362
S	0.059	0.290	<b>0.619</b>
Eigenvalue	2.375	0.699	0.431
Cum. prop.	60.2%	77.9%	88.8%

physiographic barriers. By using principal component analysis (PCA) the groups were tested for geographic heterogeneity. If analyses showed heterogeneity of the samples they were split into separate groups, and if the variability of the samples overlapped, they were pooled together.

TABLE 2. Mean values, standard deviations and ranges of significant discriminant morphometric (size-adjusted data) and meristic (original data) characters for the four re-defined subspecies (males).

	Mean	Min	Max	SD
<i>V.a.ammodytes</i> (n=294)				
Lt cap	17.9	11.8	23.0	1.69
Alt corni	4.1	2.6	6.6	0.57
L scr	3.3	2.2	4.5	0.37
V	152.0	139.0	161.0	4.13
S	35.9	25.0	45.0	2.78
<i>V.a.meridionalis</i> (n=24)				
Lt cap	17.9	12.7	21.8	2.14
Alt corni	4.3	3.2	5.3	0.56
L scr	3.6	3.0	4.2	0.38
V	138.8	130.0	150.0	4.57
S	31.4	23.0	34.0	2.60
<i>V.a.montandoni</i> (n=77)				
Lt cap	19.2	15.0	23.5	1.54
Alt corni	3.6	2.3	5.4	0.68
L scr	4.0	3.1	4.8	0.39
V	146.8	137.0	156.0	4.23
S	34.8	30.0	38.0	1.87
<i>V.a.transcaucasiana</i> (n=5)				
Lt cap	16.1	15.6	16.7	0.44
Alt corni	4.5	2.8	5.5	1.06
L scr	3.0	2.4	4.3	0.76
V	151.2	145.0	156.0	4.32
S	36.0	33.0	38.0	2.12

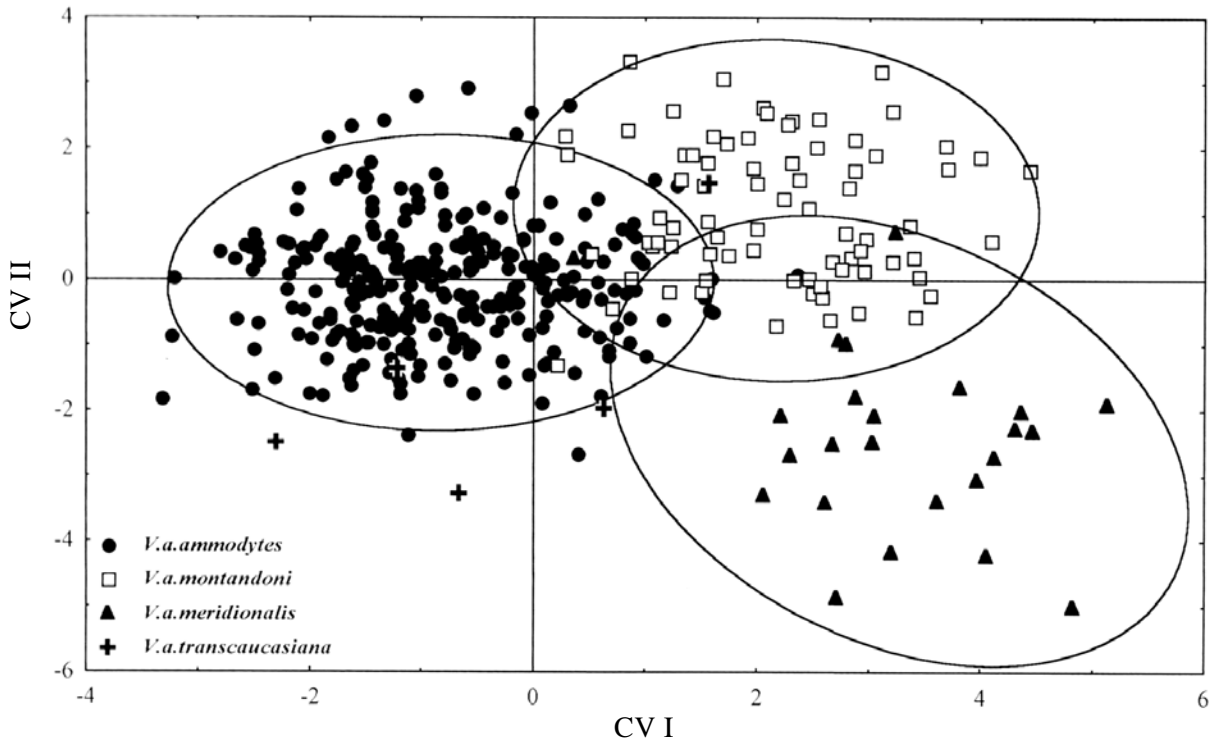


FIG. 3. Relative position of individual male specimens of re-defined subspecies in the projection of the first and the second canonical variates (size-adjusted morphometric and meristic data). Lines indicate 95% confidence level.

TABLE 3. Results of *post hoc* tests (Spjotvoll/Stoline test for unequal *n*) of comparison of means for significant discriminant morphometric (size-adjusted data) and meristic (original data) characters for the four redefined subspecies (males). n.s.,  $P>0.05$ ; \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ .

	<i>V. a. ammodytes</i>	<i>V. a. meridionalis</i>	<i>V. a. montandoni</i>
Lt cap			
<i>V. a. meridionalis</i>	n.s.		
<i>V. a. montandoni</i>	***	*	
<i>V. a. transcaucasiana</i>	n.s.	n.s.	*
Alt corni			
<i>V. a. meridionalis</i>	n.s.		
<i>V. a. montandoni</i>	***	**	
<i>V. a. transcaucasiana</i>	n.s.	n.s.	n.s.
L scr			
<i>V. a. meridionalis</i>	**		
<i>V. a. montandoni</i>	***	***	
<i>V. a. transcaucasiana</i>	n.s.	n.s.	***
V			
<i>V. a. meridionalis</i>	***		
<i>V. a. montandoni</i>	***	***	
<i>V. a. transcaucasiana</i>	n.s.	***	n.s.
S			
<i>V. a. meridionalis</i>	***		
<i>V. a. montandoni</i>	*	***	
<i>V. a. transcaucasiana</i>	n.s.	*	n.s.

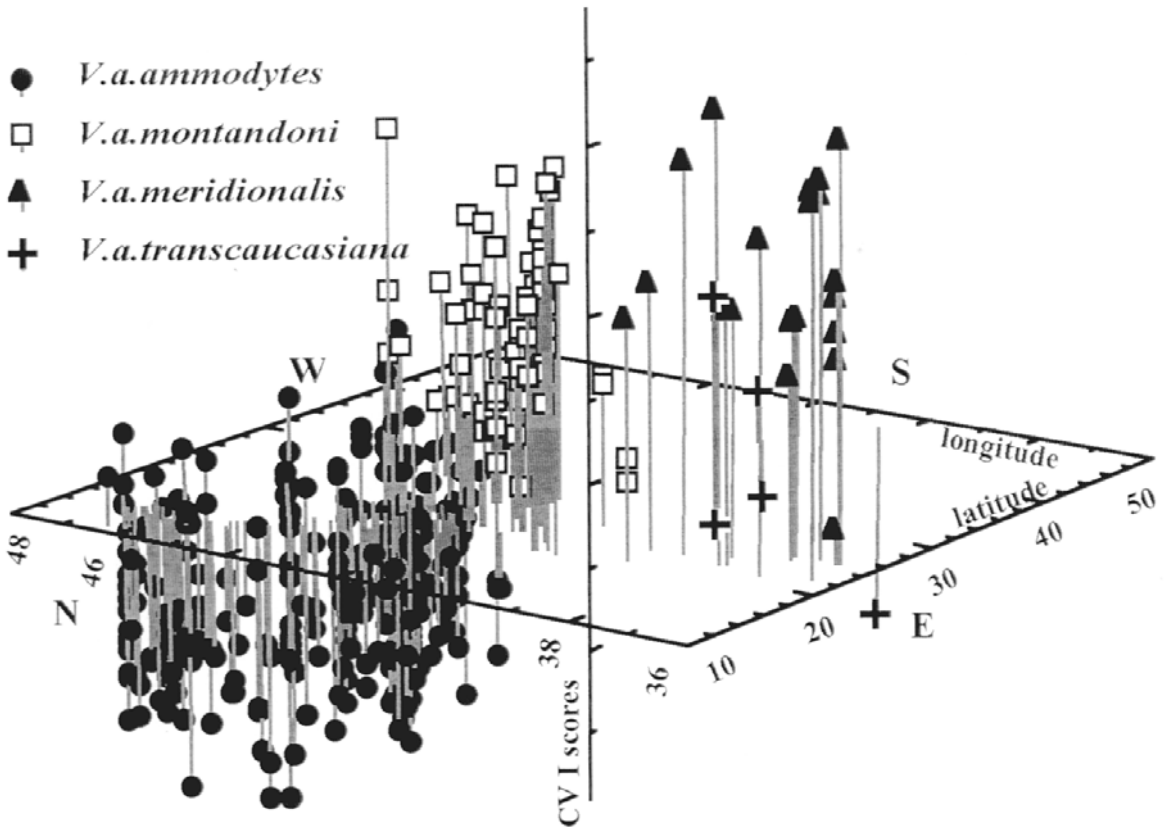


FIG. 4. Three-dimensional scatter plot of individual CV I scores against geographic positions (longitude and latitude) for the re-defined taxa.

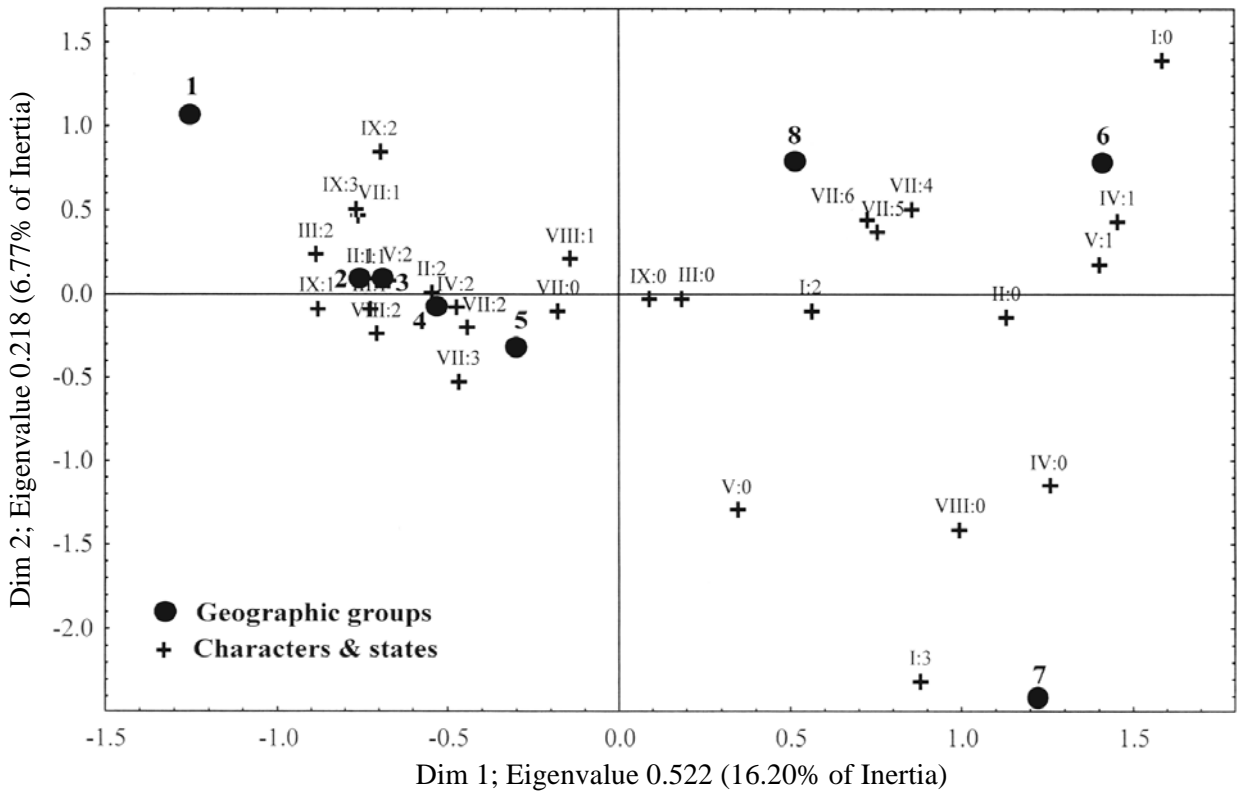


FIG. 5. Scatter-plot of coordinates of the columns (geographic group) and rows (character states) on the first and the second correspondence axes (qualitative data), for males.

CHARACTERS

*Morphometric traits.* For each specimen, 14 morphometric measures were recorded with a string (to 1 mm precision) and with digital callipers (to 0.01 mm precision): SVL – snout-vent length; L cor – body length (without head); Lt cor – body width (at mid-body point); Alt cor – body height (at mid-body point); L cd – tail length; L cap – head length (from the tip of the snout to the articulation point of the lower jaw and quadrate); Lt cap – head width (across the widest part of the head); Alt cap – head height (at the highest point of the head);

D o – eye diameter (mean value of the both sides); Dols – distance between the eye and upper labial (mean value of the both sides); Alt corni – horn height (from the rostral plate to the top of the horn); Alt r – snout height (from edge of the upper lip to the canthus rostralis); L scr – height of rostral plate; Lt scr – width of rostral plate.

*Meristic traits.* Five meristic characters were counted: number of dorsal scale rows (D), number of ventral (V) and subcaudal scales (S), number of gular scales (G) and number of sublabials contacting one inframaxillary scale (IM/SL).

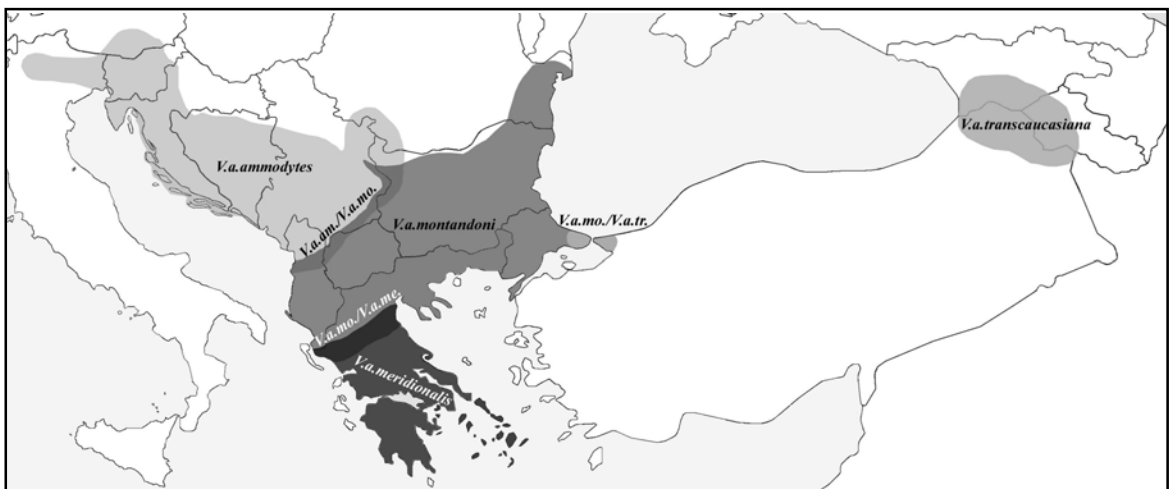


FIG. 6. Distribution ranges of redefined subspecies and transition zones.

TABLE 5. Frequencies of qualitative character states of re-defined subspecies (males).

	<i>V. a. ammodytes</i>	<i>V. a. meridionalis</i>	<i>V. a. montandoni</i>	<i>V. a. transcaucasiana</i>
I:0	3	1	32	1
I:1	238	1	5	4
I:2	75	14	42	0
I:3	17	13	5	0
II:0	58	28	83	5
II:1	244	0	0	0
II:2	31	1	1	0
III:0	183	29	83	4
III:1	33	0	1	0
III:2	33	0	0	1
IV:0	9	4	6	0
IV:1	8	15	58	1
IV:2	316	10	20	4
V:0	35	7	7	0
V:1	15	21	75	2
V:2	283	1	2	3
VI:0	19	0	0	0
VI:1	12	0	3	0
VI:2	6	3	1	0
VI:3	151	15	48	0
VI:4	79	4	0	0
VI:5	60	5	23	0
VI:6	6	2	9	0
VI:7	0	0	0	5
VII:0	25	0	3	0
VII:1	39	0	1	0
VII:2	75	4	4	0
VII:3	135	14	1	0
VII:4	21	4	27	4
VII:5	34	7	43	0
VII:6	4	0	5	1
VIII:0	15	20	15	1
VIII:1	315	9	69	4
VIII:2	3	0	0	0
IX:0	290	28	82	5
IX:1	23	0	0	0
IX:2	3	0	0	0
IX:3	17	1	2	0
Total	333	29	84	5

*Qualitative traits.* For analysis of qualitative traits, I used the following nine, with taxonomic significance (see Tomovic & Dzukic, 2003 and references therein): I – number of suprarostal plates (with states 0: suprarostal lacking – joined with rostral plate, 1: one suprarostal plate, 2: two suprarostal plates, 3: three suprarostal plates); II – connection of nasorostral plates with canthus rostralis (with states 0: neither nasorostral plate in contact with canthus rostralis, 1: both nasorostral plates in contact with canthus rostralis, 2: asymmetry – one nasorostral plate in contact with canthus rostralis, the other not); III – nasorostral plates (with states 0: undivided, 1: one plate divided, 2: both plates divided); IV – rostral height/width ratio (with states 0: equal height and width, 1: greater height, 2: greater width); V – the relation between the rostral and nasorostral plates (with states 0: heights of rostral and

nasorostral plates equal, 1: height of rostral plate greater, 2: height of nasorostral plates greater); VI – type of dorsal trunk pattern (with types 0, 1, 2, 3, 4, 5, 6, 7; see Tomovic & Dzukic, 2003, Fig. 2A); VII – type of head pattern (with types 0, 1, 2, 3, 4, 5, 6; see Tomovic & Dzukic, 2003, Fig. 2B); VIII – the type of lower lip spots (with states 0: undivided spot, 1: divided spot, 2: spot lacking); and IX – presence of large scales on the dorsal side of the head (with states 0: large scales absent, 1: parietal present, 2: frontal present, 3: both parietal and frontal scales present). Since scoring of characters VI and VII could be subjective, I compared variability of all specimens first and then defined the categories. The colour of the ventral surface of the end of the tail was considered a significant taxonomic character among nose-horned vipers. In this study, this character could not be scored due to the poor condition of older alcohol-preserved specimens.

#### STATISTICAL METHODS

Both morphometric and meristic characters were checked for between-group differences by means of ANOVA (meristic) and ANCOVA (morphometric, with SVL as the independent variable) and only those that displayed significant between-group differences (15 characters) were then used for multivariate analyses. In order to assess the variation across the geographic groups free of the effects of body size, each individual's linear measurement was regressed to the mean snout-vent length of 502 mm, using the pooled within-group regression coefficient obtained by ANCOVA. Discriminant analyses were run on meristic traits and size-adjusted morphometric data together. Since the sexual dimorphism of morphological traits had previously been confirmed (Tomovic *et al.*, 2002), analyses for males and females were done separately. Due to similar discrimination patterns between the sexes, the results are given for males only.

Discriminant function analysis was performed on size-adjusted morphometric and meristic data, in order to clarify the relative importance of characters as discriminators between a priori groups and the relative positions of the centroids and individual specimens of geographic groups (Manly, 1986). In order to avoid pooling of specimens from the possible intergradation zones, they were excluded from the canonical analyses of pre-defined geographic groups consisting of unambiguous specimens; the analysis placed specimens from possible intergradation zones without any a priori assignment to any geographic group. Those specimens were entered into the analyses after the canonical variates/discriminant functions had been established and were placed into previously defined groups a posteriori. Thus, eight final geographic groups were established (Fig. 1).

With the results of the multivariate techniques, subspecies were re-defined and univariate statistics (descriptive statistics and *post hoc* Spjotvoll/Stoline test

for unequal *n*) were employed only for the characters that significantly contributed to discrimination of the samples. Mean values, ranges and standard deviations of discriminative morphometric and meristic characters for the re-defined subspecies are given for comparison with previously published data (Table 2). Also, the results of *post hoc* tests (Table 3) were applied in order to evaluate the taxonomic value of previously used characters for identification of re-defined subspecies.

Correspondence analysis (Rohlf, 1988) was used in order to clarify which qualitative characters (and states) define the taxonomic units. The output of such an analysis was the coordinates of the row (subspecies) and column (character states) on correspondence axes displayed on the scatter plot. Frequencies of qualitative traits of re-defined taxa are given in Table 5 (note: for character III, some specimens were not included in analyses due to lack of data).

Statistical analyses were performed with statistical package Statistica (version 5.1) for Windows 95 (StatSoft Inc., 1997).

## RESULTS

### MORPHOMETRIC AND MERISTIC DATA

*Discriminant function analysis.* Discriminant function analysis on size-adjusted morphometric and meristic data of males showed that five characters – height of rostral plate (L scr), number of ventral plates (V), height of horn (Alt corni), width of head (Lt cap) and number of subcaudal plates (S) – contributed significantly to the discrimination of geographic groups (Table 1). The first discriminant function canonical variate clearly separated the southern and eastern groups from the groups inhabiting the central and north-western parts of the Balkan peninsula (Fig. 2). The second discriminant function canonical variate additionally separated the samples from the southernmost part of the range (central Greece with Peloponnesus and Cyclades Islands) from the groups inhabiting the eastern and southern parts of the Balkans (central Albania, southern Serbia, Former Yugoslav Republic of Macedonia (excluding NW part), Bulgaria (excluding NW part), SE Romania, E Greece and NW Turkey). The geographic group from the easternmost part of the range (8 – E Turkey, Georgia, Armenia) was also separated from the groups inhabiting the north-western and central part of the range by the second discriminant function. The scatter plot of scores of individual male specimens in the projection of the first two discriminant

axes (Fig. 2), showed that variability of group 7 – central Greece with Peloponnesus and Cyclades Islands – only slightly overlapped with the rest of the samples. In addition, variability of the sample from the southern and eastern part of the Balkans overlapped, but was considerably more prominent than that exhibited by the geographic groups from the central and western parts of the range. The geographic groups from the wide-ranging area, including central northern Italy, southern Austria, Slovenia, Croatia, Bosnia & Herzegovina, Montenegro, Serbia (except the southernmost part), central Albania, north-western Bulgaria, north-western Macedonia and south-western Romania (1–5) as well as from the easternmost part of the species range (eastern Turkey, Georgia and Armenia – 8) showed lack of clear discrimination. In general, the geographic groups from the southern and eastern parts of the Balkan peninsula exhibited a higher degree of morphological differentiation than the groups from the central and western part of the species range. The area where variability of the samples overlapped could denote the position of the specimens belonging to the transitional populations.

*Post hoc* tests of significant discriminative characters (Table 3) showed that the most prominent differences among re-defined subspecies were displayed by the following characters: number of ventral (V) and subcaudal scales (S) and height of rostral plate (L scr). Thus, *V. a. meridionalis* was characterised by the presence of significantly lower numbers of ventral and subcaudal plates as compared to all other subspecies. On the other hand, *V. a. montandoni* was characterized by the higher values of rostral plate height and head width comparing to all other re-defined taxa. The two above-mentioned taxa differed in the number of subcaudal and ventral plates as well as in the values of horn height (Table 2).

### QUALITATIVE DATA

*Correspondence analyses.* Unexpectedly, analyses of qualitative traits showed a quite similar differentiation pattern of geographic groups (Fig. 5). When character VI (type of dorsal trunk pattern) was excluded from the analyses due to possible subjectivity of the coding, samples were differentiated into three groups defined by the specific frequencies of characters and states. The first group consists of the samples from the eastern (and partly southern) part of the Balkans (6) and was defined by the following characters and combination of states: I:0 (suprarostal plate lacking); II:0 (neither nasorostral plate in contact with canthus

TABLE 4. Classification of cases of re-defined subspecies.

	% of exact classification	<i>V. a. ammodytes</i>	<i>V. a. montandoni</i>	<i>V. a. meridionalis</i>	<i>V. a. transcaucasiana</i>
<i>V. a. ammodytes</i>	97.62	287	7	0	0
<i>V. a. montandoni</i>	88.31	9	68	0	0
<i>V. a. meridionalis</i>	87.50	1	2	21	0
<i>V. a. transcaucasiana</i>	20.00	3	1	0	1
Total	94.25	300	78	21	1

rostralis); IV:1 (greater height of rostral plate); V:1 (greater height of rostral than nasorostral plates) and VII: 4, 5 and 6 (types of head pattern). The second group consist of samples from the southernmost part of the Balkans (7) and was characterized by the specific frequencies of characters and states: I:3 (three suprarostal plates); II:0 (neither nasorostral plate in contact with canthus rostralis); IV:0 (equal height and width of rostral plate); V:0 (heights of rostral and nasorostral plates equal) and VIII:0 (lower lip spot undivided). The third group contains all of the samples from the central and the western part of the range (1–5) and is defined by a numerous specific characters and states. The geographic group from the easternmost part of the range (8) was characterized by the mixture of state frequencies defining the above-mentioned three groups. Geographic groups from the southern and the eastern part of the Balkans displayed a considerably higher degree of differentiation upon analyses of qualitative traits.

## DISCUSSION

### REDEFINED SUBSPECIES AND DISTRIBUTION RANGES

As confirmed by a previous study (Tomovic & Dzukic, 2003), only a combination of morphometric, meristic and qualitative characters could be taxonomically informative in analyses of morphological differentiation and systematics of nose-horned vipers.

Results of both discriminant and correspondence analyses showed a lack of clear morphological differentiation among the samples belonging to the geographic groups conventionally classified as *Vipera ammodytes ammodytes*, *V. a. gregowallneri*, *V. a. ruffoi* and *V. a. illyrica*. Variability of all analysed morphological characters corresponded to the description of the nominate subspecies, and hence the former taxa should be regarded as synonyms of *V. a. ammodytes*, at least at the morphological level. Thus, the distribution of *V. a. ammodytes* would include central northern Italy, southern Austria, Slovenia, Croatia, Bosnia & Herzegovina, Montenegro, Serbia (excluding the southernmost part), northern Albania and north-western Macedonia as well as north-western Bulgaria and western Romania (Fig. 6). Contrary to the situation in the central and western parts of the range, morphological differentiation of geographic groups from the eastern and southern parts of the range was more complicated. Results of discriminant and correspondence analyses showed a considerably higher degree of differentiation of geographic groups from the southern and the eastern parts of the Balkans.

The highest degree of differentiation was displayed by the geographic groups from the southernmost part of the Balkan peninsula – central Greece, Peloponnesus and Cyclades. Since morphological characteristics corresponded to the subspecies *V. a. meridionalis*, I suppose that the “typical” populations of this subspecies most probably inhabit only the areas mentioned (Fig. 6).

Our previous studies revealed lack of clear morphological differentiation among the samples from the northern and the southern parts of Bulgaria, FYR of Macedonia and the southernmost part of Serbia (Tomovic & Dzukic, 2003). Morphological analyses and hybridization experiments with the samples from the Bulgarian part of the range also rejected the validity of subspecies *V. a. montandoni* (Christov *et al.*, 1997; Christov & Beshkov, 1999; Beshkov & Nanev, 2002) and treated it as a synonym of *V. a. meridionalis*. Morphological analyses of the specimens from Istanbul, Adapazari, Bursa and Kusadasi demonstrated (Tok & Kumlutas, 1996; Kutrup, 1999) that the European and the western part of Asiatic Turkey could be inhabited either by *V. a. meridionalis* or by *V. a. montandoni*. Golay *et al.* (1993) included *V. a. montandoni* in the list of synonyms of *V. a. meridionalis*. In addition, recent phylogenetic analyses of two samples from Bulgaria (assigned as belonging to *V. a. meridionalis* and *V. a. montandoni*) revealed that their distance divergence was very small – 0.4% (Garrigues *et al.*, 2005). Based upon this, they also suggested that *V. a. montandoni* should be synonymous with *V. a. meridionalis*. However, in the experimental studies of Christov & Beshkov (1999), Beshkov & Nanev (2002) and Garrigues *et al.* (2005), different populations of *V. a. meridionalis* (from Greece and/or western Turkey) were not compared, and they assumed southern Bulgarian populations to be *V. a. meridionalis*.

In contrast, this study clearly shows that the geographic groups from the eastern and partly southern parts of the Balkans were rather different from those inhabiting the southernmost part of the Balkan peninsula, and hence, might be classified as *V. a. montandoni*. Thus, the range of this subspecies might include: SE Romania, most of Bulgaria (excluding NW part), western (European) Turkey, NE, N, NW Greece, FYR Macedonia (excluding NW part), the southernmost part of Serbia and southern and central Albania (Fig. 6). It should be noted that populations of the nose-horned viper inhabiting these areas (with exception of SE Romania and NE Bulgaria) were previously treated as populations belonging to *V. a. meridionalis* (Buresch & Zonkow, 1934; Karaman, 1939; Radovanovic, 1951; Biella, 1983; Dzukic, 1995; Crnobrnja-Isailovic & Haxhiu, 1997; Beshkov & Nanev, 2002; Vozenilek, 2002).

The geographic groups from the eastern part of Turkey, Georgia and Armenia were generally treated as subspecies *V. a. transcaucasiana* (Eiselt & Baran, 1970; Tok & Kumlutas, 1996; Kutrup, 1999 and references therein). On the other hand, this subspecies has been raised to specific level by some authors (Obst, 1983; Nilson *et al.*, 1999; Herrmann *et al.*, 1987). Although this taxon was originally described on the basis of a few qualitative traits only, and one of the “diagnostic” characters was the broken zig-zag band (e.g. “dorsal zig-zag band... is replaced by a series of nar-



row, transverse bars... which, on some parts of the body, break up into pairs forming two alternating series...”, see Boulenger, 1913), the dorsal trunk pattern was not included in my analysis due to possible subjectivity of the scoring (see Tomovic & Dzukic, 2003). Additionally, during inspection of all 922 specimens from different (mostly southern and eastern Balkans) areas, I found specimens having both partly (39) or completely (nine) broken dorsal bands (not presented here). The presence of a specific type of dorsal band may have resulted from “ecogenesis, i.e. different current selection pressures leading to adaptive modifications of morphological characters” (Wüster *et al.*, 1992). Since the results of multivariate analyses of both quantitative and qualitative traits showed that there was no specific morphological differentiation of the geographic groups from the easternmost part of the range, this could imply that there is no justification for raising *V. a. transcaucasiana* to the species level based on apparent differences in a single character and/or distribution gap. These findings are consistent with the results of the phylogenetic analyses of Garrigues *et al.* (2005), who showed that the sample of *V. a. transcaucasiana* was less distant than *V. a. ammodytes*, and that the “*V. ammodytes* complex” represented a monophyletic group.

#### TRANSITIONAL ZONES

Since these analyses made it possible to re-define the subspecies and propose new geographic ranges, transitional zones had to be modified as well. Specimens of re-defined subspecies having canonical scores within overlapping zones (Fig. 3) and that were misclassified (Table 4) could belong to transitional populations. Intergradation zones between *V. a. ammodytes* and *V. a. montandoni* would include western Bulgaria, eastern, south-eastern and southern Serbia, the north-western part of FYR of Macedonia and central Albania (Fig. 6). These zones were quite precisely defined, but pertained to *V. a. ammodytes* and *V. a. meridionalis* (Buresch & Zonkow, 1934, see figure 38; Karaman, 1939). The northern continental part of Greece and probably the southernmost part of Albania might represent intergradation zones between *V. a. meridionalis* and *V. a. montandoni* (Fig. 6). Specimens from the Bosphorus region could belong to transitional populations between *V. a. montandoni* and *V. a. transcaucasiana*.

#### NW TO SE GRADIENT OF DEGREE OF DIFFERENTIATION

The most intriguing result of this study is the pronounced increasing north-west to south-east gradient of morphological differentiation. It was demonstrated that a similar pattern of increasing gradient of morphological degree of differentiation NW to SE was displayed by populations of the Eastern grass snake (*Natrix natrix natrix*) and populations from Greece and Turkey were

assigned as “those near the root of the eastern phylogenetic tree” (Thorpe, 1984). Recently, a lot of integrative studies of molecular phylogeography have shown that animal and plant taxa from southern areas of Europe display significantly higher degrees of genetic differentiation and diversity than those from central and northern part of Europe (e. g. Hewitt, 1996, 1999; Taberlet *et al.*, 1998 and references therein). These “large-scale” studies designated the Iberian, Apennine and Balkan peninsulas as the main refugia of the European biota during the Pleistocene glaciations. Some hypotheses suggest that “a reduction in diversity from southern to northern Europe in the degree of allelic variation and species subdivision... is attributed to rapid expansion northward and the varied topography of southern refugia allowing populations to diverge through several ice ages” (Hewitt, 1996, 1999). As was shown for the grasshopper (see Hewitt, 1999), populations of the Balkans (probably western or central parts) whose northern populations formed the leading edge of postglacial expansion, display less haplotype diversity than those from Greece and Turkey. To my knowledge, “medium-scale” studies that analysed genetic (or morphological) patterns of diversity of amphibian and/or reptile species from one of the refugia exclusively (especially the Balkans), have rarely been conducted (e.g. *Rana latastei* from Italy and Slovenia – east-to-west gradient of genetic diversity shown by Garner *et al.*, 2004; Iberian Peninsula – Gómez & Lunt, 2004, and references therein).

#### CONCLUSIONS

Phenotypic variation could sometimes reflect genotypic variation, but could also be simply the result of environmental variation, or both. Despite the fact that these analyses provide evidence for the validity of four subspecies, some may argue that they should not be recognized as separate taxa, as morphological differentiation of geographic groups does not appear to be categorical, but clinal (Fig. 4). Without phylogeographic analysis, we can only assume that the aforementioned “large-scale” pattern of genetic and morphological diversity could be introduced into “medium-scale” genetic and/or morphological differentiation schemes for the nose-horned viper. In any case, for a systematic overview of this species, further phylogenetic studies are required.

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