Distribution and morphological variation of *Vipera berus nikolskii* Vedmederja, Grubant et Rudaeva, 1986 in Western Ukraine, The Republic of Moldova and Romania

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Abstract. Morphological variation of vipers of the *Vipera berus* complex in Eastern Romania, the Republic of Moldova and Western and Central Ukraine was studied using multivariate statistics. Discriminant analysis, based on ten meristic characters in 89.7% of cases (males) and in 92.0% cases (females), was able to separate reference samples of subspecies *Vipera berus berus* and *Vipera berus nikolskii* and was conducted to classify snakes from the studied territory. According to these results, *V. b. nikolskii* inhabits the broad-leaved forests in the forest-steppe zone in the Republic of Moldova, the hilly part of Eastern Romania and Central Ukraine. Specimens from a contact zone between *V. b. berus* and *V. b. nikolskii* have intermediate morphology and, thus, could represent the result of introgression. Populations of the Nikolsky's viper from the western part of its range combine high level of morphological differentiation from *V. b. berus* with the presence of non-black specimens and even include populations without melanistic specimens, previously thought not to occur in this taxon. A morphological description of the largest samples is given and determination of *V. b. nikolskii* is discussed.

Keywords: Eastern Europe, subspecies, systematics, *Vipera berus* complex.

Introduction

The common adder *Vipera berus* L., 1758 is one of most studied snake species in the world. However, problems still exist in the systematics of the *Vipera berus* complex (Joger et al., 2007), which contains several taxa with predominantly European distributions. *Vipera nikolskii* Vedmederja, Grubant et Rudajeva, 1986 was initially included in *V. b. berus* as the black colour morph and then described as a separate species with a range situated south of an imaginary line through Kaniv (Ukraine)–Kursk–Tambov–Buzuluk (Russia) (Vedmederja, Grubant and Rudajeva, 1986). Current data remain controversial and are not yet able to clarify

the phylogenetic position and systematic (specific or subspecific) status of this taxon. The first attempt to solve this problem by using molecular markers showed that *V. b. nikolskii* has a sequence of cytochrome b identical to that of Russian *V. b. berus* (Joger et al., 1997). Later, diverged haplotypes, sister to *Vipera barani* Böhme et Joger, 1983 from Northern Turkey were found in two localities from the southern border of its range in Russia. However, most of the *V. b. nikolskii* samples were confirmed to have *V. b. berus* haplotype (Kalyabina-Hauf et al., 2004; Joger et al., 2007). Both haplotype groups of *V. b. nikolskii* share the same morphology and ecology, rather distinct from *V. b. berus*, but have a wide transition zone in the north (Milto and Zinenko, 2005; Zinenko, 2006b). Therefore, the Nikolsky's viper was treated as a subspecies according to the biological species concept (Milto and Zinenko, 2005) or was still considered as a taxon with uncertain status (Bakiev, Böhme and Joger, 2005; Joger et al., 2007).

This uncertainty regarding taxon status and the complexity of morphology-based subspecies recognitions led to a lack of data about the dis-

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tribution of *V. b. nikolskii*. In spite of new data published in recent years, the western limits of the range of *V. b. nikolskii* remain unclear (Bakiev, Böhme and Joger, 2005; Milto and Zinenko, 2005). Data on morphological variation of viper populations in the forest-steppe zones of Western Ukraine and the Moldova Republic, which are regarded as *V. b. nikolskii* in several papers, remain scarce and are limited to short descriptions dispersed throughout many publications in local journals. Kotenko (1992) and, later, Tabachishin and Zavjalov (2003) reported populations of *V. b. nikolskii* in the Odesa region in Western Ukraine. The contact zone between *V. b. berus* and *V. b. nikolskii* was located in the vicinity of Kaniv (Zinenko and Ruzhilenko, 2003; Zinenko, 2004). Dotsenko (2004) discussed determination of the adder specimens from Moldova in the museum collections but failed to draw any conclusions, except for noting the similarities of some specimens to *V. b. berus* and of others to *V. b. nikolskii*. In the redescription of *V. b. nikolskii*, the same specimens from the Republic of Moldova were classified as *V. b. nikolskii* (Milto and Zinenko, 2005). Clear morphological differences of snakes in Iaşi County (Romania) from neighboring *V. b. berus* populations in the Carpathians were noticed even before the initial description of the Nikolsky's viper (Fuhn and Vancea, 1961; Vancea, Fuhn and Borcea, 1982). Further extension of the range of *V. b. nikolskii* was suggested by Milto and Zinenko (2005) and was based on the morphological descriptions given in the previously cited papers. An extensive study on snakes from the Republic of Moldova showed that local populations were morphologicaly similar to *V. b. nikolskii* but noted a high level of color polymorphism, a feature not characteristic for melanistic *V. b. nikolskii* populations from Ukraine and Russia (Ţurcanu, 2006).

In most of the previously mentioned papers, local populations were not properly compared with populations of *V. b. nikolskii* from other localities within its range. On the other hand, extensive description of geographical variation of meristic characters across the known ranges of both taxa and employment of multivariate statistics allow a more precise determination of populations and delimitation of their ranges in previously poorly studied regions.

The territory of the forest-steppe zone located to the West of the Dnieper River is continuously covered with similar habitats as those found in the range of *V. b. nikolskii* in Russia and Eastern Ukraine (Milto and Zinenko, 2005; Zinenko, 2006a), potentially representing one-fourth of its range.

The aims of the present study were to verify, using multivariate statistics, the assumptions about the existence of *V. b. nikolskii* in the region located between the Carpathians and the Dnieper River and describe morphological variation of local populations.

Material and methods

The studied material consists of specimens which were processed in the field in the Republic of Moldova and the adjacent territory of Chernivtsi region, Ukraine during 2006; snakes from Republic of Moldova and Ivano-Frankivs'k region of Ukraine which were processed by the second author in previous years; the third authors' data on morphology of populations in Iaşi County, Mt. Ceahlău (Neamţ County) Câmpulung-Moldovenesc (Suceava County) and Piatra Craiului (Alba County) in Romania, collected in 2006 and 2007; collections of the Natural History Museum of Iaşi, (NHMI, Iaşi, Romania), "Grigore Antipa" National Museum of Natural History, (GANMNH, Bucharest, Romania); "Ion Borcea" Natural Sciences Museum Complex (IBNSMC, Bacau, Romania), the Museum of Nature at V. N. Karazin Khakiv National University, (MNKNU, Kharkiv, Ukraine), the Zoological Museum of National Museum of Natural History (ZM NMNH, Kyyiv, Ukraine), live specimens from Kaniv Nature reserve, snakes from scientific collection of "Roztochchja" Nature reserve (RNR, Lviv region, Ukraine) and "Medobory" Natural reserve (MNR, Ternopil region, Ukraine) (table 1).

Ten meristic characters which are widely used for vipers systematics were recorded: Ventralia, (Ventr., including Praeventralia); Subcaudalia, (S.cd.); Squamae dorsalis, (Sq.); Labialia, (Lab.); Sublabialia, (S.lab.); Circumocularia, (C.oc.); Subocularia rows, (S.oc.); Lorealia, (Lor.); Intercanthalia, (Ic.); Parafrontalia, (Pf.). We used the sum of both sides for bilateral traits. All characters are easy to count and were accurately adjusted by coauthors, therefore minimizing any possible observer bias. Additionally, in some of the studied specimens we recorded the number of Gularia, (Gul.), the number of zigzag windings in nonmelanistic specimens, the proportion of completely black

Table 1. Material list and posterior probabilities of discrimination in DA for specimens or populations.

Localities and region	Coordinates		Subspecies and mean of posterior probability of discrimination for single specimens	*n* and place of deposition or data origin
	N	E		
Romania				
"Cluj-Faget", Făgetu Ierii (Cluj)	46°46′	23°36′	*V. b. berus*, 0.98	1, MNHI
Firiza, Săpânţa river Basin, "Tăul lui Dumitru" (Maramures)	47°45′	23°36′	*V. b. berus*, 1	1, GANMNH
Sacel, Iza river basin, «Izvorul Albastru al Izei» (Maramures)	47°38′	24°26′	*V. b. berus*, 1	1, GANMNH
Brezoi (Vîlcea)	45°20′	24°16′	*V. b. nikolskii*, 0.84	1, MNHI
Piatra-Craiului (Braşov and Argeş)	45°28′	25°02′	*V. b. berus*, 0.88	1, MNHI; 2 (own data)
Gheboaia (Dîmboviţa)	44°48′	25°44′	*V. b. nikolskii*, 0.99	1, MNHI
Doftonei river valey – «Valea Doftonei» (Prahova)	45°08′	25°46′	*V. b. berus*, 1	1, GANMNH
Baraolt (Covasna)	46°05′	25°36′	*V. b. nikolskii*, 0.70	1, MNHI
Ceahlău (Neamţ)	47°02′	25°58′	*V. b. berus*, 0.96	4 (own data)
Lacu Roşu (Neamţ)	46°48′	25°48′	*V. b. berus*, 0.87	1, MNHI; 4, GANMNH
Suceava (SUC) group				
Cîmpulung-Moldovenesc (Suceava)	47°31′	25°34′	*V. b. berus*	1, MNHI; 12 (own data)
Deia (Suceava)	47°35′	25°36′	*V. b. berus*	1, MNHI
Rarău (Suceava)	47°27′	25°33′	*V. b. berus*	4, MNHI
Giumalău (Suceava)	47°25′	25°25′	*V. b. berus*	1, MNHI
Bacău (BAC) group				
Pîngăraţi (Neamţ)	46°55′	26°12′	*V. b. nikolskii*	1, MNHI
Brusturoasa (Bacău)	46°31′	26°11′	*V. b. berus*	2, IBNSMC
Apa Rosie, Nemira Mountains (Bacău)	46°15′	26°21′	*V. b. berus*	1, IBNSMC
Poiana Uzului (Bacău)	46°19′	26°20′	*V. b. berus*	1, IBNSMC
Dofteana; Trotus River Valley (Bacău)	46°19′	26°30′	*V. b. nikolskii*	1, IBNSMC
Căiuţi (Bacău)	46°09′	26°55′	*V. b. berus*	1, IBNSMC
Adjud, Padurea Cioara (Vrancea)	46°05′	27°11′	*V. b. berus*	1, IBNSMC
Repedea (Vrancea)	45°54′	27°05′	*V. b. nikolskii*	1, MNHI
"Valea Girlei", Gârleni (Bacău)	46°39′	26°46′	*V. b. berus*	1, MNHI
Gîdinţi and Roman (Neamţ)	46°56′	27°00′	*V. b. berus*	1, MNHI;
	46°55′	26°55′		1, IBNSMC
Romanian Moldova (RMOL) group				
Dobrovăţ (Iaşi)	46°58′	27°42′	*V. b. nikolskii*	9, MNHI; 1, GANMNH
Vlădiceni (Iaşi)	47°06′	27°41′	*V. b. nikolskii*	1, MNHI
Bârnova (Iaşi)	47°03′	27°36′	*V. b. nikolskii*	1, GANMNH; 2, MNHI; 15 (own data)
Mironeasa (Iaşi)	46°57′	27°25′	*V. b. nikolskii*	7, MNHI
Epureni (Iaşi)	47°17′	27°25′	*V. b. nikolskii*	1, MNHI
(Iaşi)	47°10′	27°35′	*V. b. nikolskii*	1, MNHI
Bârlad (Vaslui)	46°13′	27°40′	*V. b. nikolskii*	1, MNHI
Stuhuleţ (Vaslui)	46°24′	28°03′	*V. b. nikolskii*	1, IBNSMC
Sofroneşti (Vaslui)	46°50′	27°24′	*V. b. nikolskii*	1, GANMNH

Table 1. (Continued).

Localities and region	Coordinates		Subspecies and mean of posterior probability of discrimination for single specimens	n and place of deposition or data origin
	N	E		
Republic of Moldova				
Kodry (KOD) group				
Voynovo	47°15′	28°30′	*V. b. nikolskii*	4, ZM NMNH
Vulkaneshty (Nisporeny)	47°08′	28°11′	*V. b. nikolskii*	5 (own data)
Meresheny	46°46′	28°32′	*V. b. nikolskii*	1 (own data)
Other localities in Kodry			*V. b. nikolskii*	1, ZM NMNH; 8 (own data)
Northern Moldova (NMOL) group				
Sakharna	47°40′	28°57′	*V. b. nikolskii*	1, ZM NMNH
Rud', Arioneshty, Tatarushi-Nouy	48°22′	27°51′	*V. b. nikolskii*	11 (own data)
Ukraine				
Various localities in Carpathians (Zakarpats'ka and Lviv regions)			In reference sample of *V. b. berus*	43, ZM NMNH; 1, MNKNU
"Roztochchya" Nature Reserve (Lviv region)	49°55′	23°45′	*V. b. berus*, 0.68	1, RNR
Bystritsa (Verkhovyna district, Ivano-Frankivs'k region)	48°24′	24°13′	*V. b. berus*, 0.53	2, ZM NMNH
Zelene (Verkhovyna district, Ivano-Frankivs'k region)	48°02′	24°45′	*V. b. berus*, 1	1, ZM NMNH
Bukivka (Galych district, Ivano-Frankivs'k region, Ukraine)	48°57′	24°57′	*V. b. berus*, 0.58	2 (own data)
"Medobory" Nature reserve (Grymayliv district, Ternopil' region, Ukraine)	49°18′	26°13′	*V. b. berus*, 0.89	1, MNR
Poljana (Khotyn district, Chernivtsi region, Ukraine)	48°28′	26°14′	*V. b. nikolskii*, 0.51	2, ZM NMNH; 5 (own data)
Kaniv (KAN) group				
Kaniv; the Ros' river mouth (Cherkassy region, Ukraine)	49°43′ 49°39′	31°31′ 31°34′	*V. b. nikolskii*	14, MNKNU; 2, ZM NMNH
Chornyy lis (CLIS) group				
Znamenka, Kirovohrad; "Kherson" (Southern part of Central Ukraine)	48°45′	32°35′	*V. b. nikolskii*	3, ZM NMNH; 1, ZIN; 6 (oun data)
Literature data (Romania)				
Broşteni (Suceava)	47°14′	25°41′	*V. b. berus*, 0.62	2, Bačescu, 1933
Holdiţa (Suceava)	47°16′	25°42′	*V. b. berus*, 0.56	1, Bačescu, 1933
Liteni (Suceava)	47°30′	26°32′	*V. b. nikolskii*, 0.99	1, Bačescu, 1933
Ceahlău (Neamţ)	47°02′	25°58′	*V. b. nikolskii*, 0.73	1, Bačescu, 1933
Valea Sabasci (Neamţ)	47°13′	25°51′	*V. b. berus*, 0.78	1, Bačescu, 1933
Grajduri (Iaşi)	46°57′	27°31′	*V. b. berus*, 0.66	1, Bačescu, 1933
Tomeşti (Iaşi)	47°07′	27°42′	*V. b. nikolskii*, 0.94	1, Bačescu, 1933
Chicerea (Iaşi)	47°05′	27°44′	*V. b. nikolskii*, 0.98	1, Bačescu, 1933
Bârnova (Iaşi)	47°03′	27°36′	*V. b. nikolskii*, 0.93	1, Bačescu, 1933
Dobrovăţ (Iaşi)	46°58′	27°42′	*V. b. berus*, 0.66	1, Bačescu, 1933

("melanistic"), black with light elements on Labialia and Ventralia ("not completely melanistic") and non-melanistic adult specimens and, when possible, the color of the venom. In order to compare the number of zigzag windings in our matherial with *V. b. berus* and *V. b. nikolskii*, we counted this character in 32 juvenile *V. b. nikolskii* specimens from Kharkiv vicinities, Ukraine and 19 specimens of *V. b. berus* from Western Ukraine (Zakarpats'ka and Volyn regions, Ukraine).

As the values of most meristic characters did not present a normal distrubtion (K-S test), differences between groups were tested with the Mann-Whitney U Test and were used for multivariate analyses, since all of them have displayed significant differences between samples (see also Milto and Zinenko, 2005). We ran Discriminant Analysis (DA) based on ten previously mentioned meristic traits (except for Gul.) in order to clarify the relative importance of characters as discriminators between taxa and to render the classification

of individual specimens from the studied populations. In DA, we used comparative reference samples of *V. b. berus* (Zakarpats'ka, Volyn and the Northern part of Sumy regions of Ukraine, Leningrad, Novgorod, Pskov, Moscow, Rjazan regions of Russia, Bielorussia; $n = 192$) and *V. b. nikolskii* (Kharkiv region, Ukraine and Voronezh region, Russia; $n = 167$), consisting of unambiguous populations of both subspecies located far from contact zones.

In order to estimate the capacity of DA to determine subspecies correctly and the possible effect of overfitting we performed a hold-out cross-validation procedure, as described by Golay et al. (2008). The samples of both subspecies and sexes were divided equally into training and testing subsamples. The former category was used to establish the discriminant functions, while the latter was employed to check the predictive accuracy of the previously derived discriminant functions. To create subsamples we ordered specimens of different sexes and subspecies separately and assigned binary codes to them. The specimens with code "1" were treated as a training group and the ones with "zero" as a testing group.

All specimens from Western Ukraine, Moldova and Romania had no priory classification in DA. For Canonical Variate Analysis (CVA) and, later, for morphological description, specimens were pooled into groups according to natural geographic regions, collecting gaps and uniformity of classification in DA. Geographical samples of *V. b. nikolskii* were collected within the naturally outlined regions separated by the Siret, Prut, Reut and Dniester rivers. Therewith, several groups in forest-steppe zone are restricted to island massifs of forests on heights and are also naturally isolated by flat, forest-free landscapes (KAN and CLIS, KOD and NMOL). Six groups with prevailing characters of *V. b. nikolskii* were organized: Romanian Moldova (RMOL) – Central Moldavian plateau, Iași and Vaslui regions, Romania; Kodry (KOD) – Kodry height, Republic of Moldova; Northern Moldova (NMOL) – Dniester plateau, the North-East of Republic of Moldova, separated from another group by the Reut river; two populations from Central Ukraine: Chornyy lis (CLIS) – Znamenka vicinities, Kirovohrad region and Kaniv (KAN) – the right bank of the Dnieper in Kaniv, Cherkassy region. Two extra groups were formed from closely situated locations in Eastern Carpathians in Romania: Suceava (SUC) – mountainous habitats in Suceava County, classified predominantly as *V. b. berus*; Bacău (BAC) – the snakes without any certain discrimination from numerous localities in more southern regions than

SUC group, from eastern macroslope in Central Carpathians, Neamţ, Bacău and Vrancea counties. Localities and exact numbers within groups are given in table 1.

An extensive description of scalation (Ventr.; S.cd.; Lab.; C.oc.; Ic.; Pf.) given by Bačescu (1933) has allowed us to run another Discriminant Analysis with a restricted number of characters, and on the basis of the same reference samples, to calculate posterior probabilities of discrimination of several additional snakes from Romania. This DA has made it possible to enlarge the data on the distribution and borders of subspecies in Romania.

Incomplete individual data sets were excluded from multivariate analyses, resulting in small differences between size of samples used in morphological description and DA. All the analyses were made separately for males and females to avoid effects of sexual dimorphism observed in both subspecies (Milto and Zinenko, 2005), an exception being made with the number of zigzag windings. We used STATISTICA 6.0 for Windows for all the analyses.

Results

The means of posterior probabilities of discrimination together with the ratios of discriminated as one of the subspecies in groups are listed in table 2. Individual posterior probabilities of discrimination for non-pooled specimens are given in table 1. Specimens of reference samples of *V. b. berus* and *V. b. nikolskii* in the DA were correctly classified in 95.6% and 87.2% of cases in males ($r = 0.77$; Wilk's $\lambda = 0.404$; $F(10, 174) = 25.693$; $p < 0.0001$) and 92.1% and 89.2% of cases in females ($r = 0.79$; Wilk's $\lambda = 0.378$; $F(10, 174) = 26.941$; $p < 0.0001$).

Cross-validation procedure of DA revealed high discrimination ability both for training and testing groups (table 3) and similiarities to general DA. Therefore, we continued to use only the results of DA, without cross-validation.

Table 2. Discrimination results for the geographical samples of *V. berus* from Romania, Republic of Moldova and Ukraine.

	SUC, Romania	BAC, Romania	RMOL, Romania	KOD, Moldova	NMOL, Moldova	KAN, Ukraine	CLIS, Ukraine
Prevailing discrimination, *Ssp.*	*berus*	*berus*	*nikolskii*	*nikolskii*	*nikolskii*	*nikolskii*	*nikolskii*
Discrimination (%)	$n = 19$ 84.2%	$n = 12$ 58.3%	$n = 41$ 92.7%	$n = 19$ 89.5%	$n = 12$ 100%	$n = 16$ 81.3%	$n = 10$ 100%
Mean of posterior probability	$n = 19$ 0.80	$n = 12$ 0.58	$n = 41$ 0.85	$n = 19$ 0.79	$n = 12$ 0.98	$n = 16$ 0.77	$n = 10$ 0.93

Table 3. Correctness of subspecies discrimination in different subgroups in cross-validation procedure of DA.

	V. b. berus		V. b. nikolskii	
	Training	Testing	Training	Testing
Males	95.7% (n = 46)	88.9% (n = 45)	93.6% (n = 47)	85.1% (n = 47)
Females	94.9% (n = 51)	86.0% (n = 50)	83.8% (n = 37)	91.9% (n = 37)
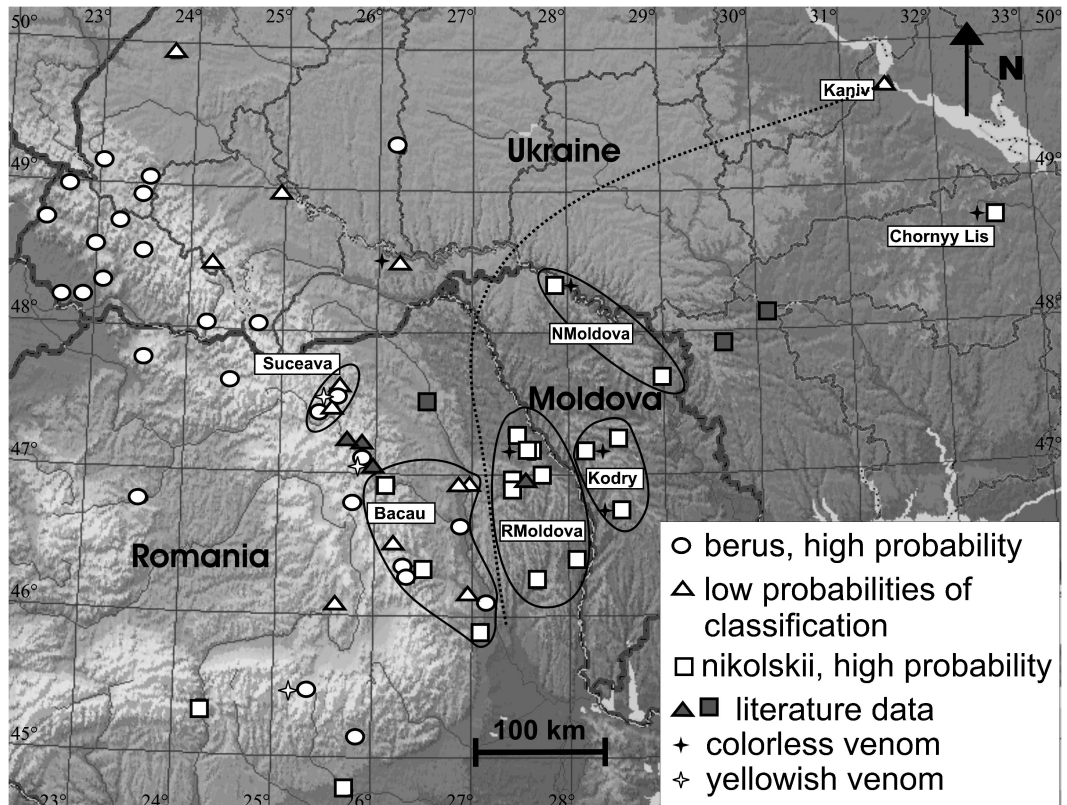
Figure 1. Map of the studied region. Localities, discrimination of specimens, geographical groups (solid lines) and *V. b. nikolskii* range (broken line) are shown.

Since incorrect determination in DA was observed even in the reference *V. b. berus* and *V. b. nikolskii* samples, only specimens or samples with posterior probabilities of discrimination above the lower border of 95% of confidence interval for reference samples (0.79 for *V. b. nikolskii* and 0.85 for *V. b. berus*) were considered to have been ascertained. The others were proven to lack reliable determination.

Most vipers collected from the forest steppe zone to the east from the Carpathians (RMOL, KOD, NMOL and CLIS) were determined as *V. b. nikolskii* (table 2, fig. 1). The snakes from the KAN group had slightly lower probability of determination as *V. b. nikolskii*. Among specimens from the Carpathians, the discrimination of *V. b. berus* prevailed. However, in the BAC group, from the Eastern slopes of the Carpathians (from Neamţ, Bacău and Vrancea Counties), the majority of the studied specimens had low posterior probabilities of discrimination. The specimens with different subspecies affiliation here have a mosaic distribution (fig. 1).

Table 4. Character standardized coefficients and their percentages of eigenvalues for discriminant function of *V. b. berus* and *V. b. nikolskii* reference samples.

	Males	Females
Ventr.	0.83	0.62
S.cd.	0.06	0.25
Sq.	0.21	0.29
Lab.	−0.01	0.17
S.lab.	0.06	0.07
C.oc.	−0.05	−0.22
S.oc.	0.07	0.57
IC.	−0.21	−0.12
Pf.	0.12	−0.02
Lor.	0.44	0.25
Eigenval	1.48	1.64

The most significant contributions in discrimination function represented (in descending order) Ventr., S.oc., Sq., Lor., S.cd., C.oc. in females and Ventr., Lor., Sq., IC., ISO. in males analysis (table 4). These characters are valuable for determination of subspecies (table 5; also see Milto and Zinenko, 2005).

Correct discriminations in reference samples of separate DA with Bačescu's (1933) published data had 86.1% and 79.7% of females and 86.8% and 84.7 % of males *V. b. berus* and *V. b. nikolskii* respectively (females analysis Wilk's λ = 0.54710, $F(6, 173)$ = 23.869, $p < 0.0001$, males analysis Wilk's λ = 0.47709, $F(6, 182) = 33.246$, $p < 0.0001$).

The canonical discriminant analysis has shown a relatively strong separation of reference samples (fig. 2). All *V. b. nikolskii* samples overlapped substantially (fig. 2). Canonical coefficients values between morphological characters and the first root (table 6) are similar to character standardized coefficients with the discriminant function (table 4).

Within *V. b. nikolskii* samples (RMOL, KOD, NMOL, CLIS, KAN) only a limited variation of meristic traits exists. However, distribution of the main types of coloration (which was previously one of the most easily recorded and frequently used diagnostic features of the Nikolsky's viper) differs considerably between samples (tables 2 and 5). In SUC (*V. b. berus*), melanistic specimens are quite rare. Further to

the south (BAC) the number of black adults grows and becomes higher in *V. b. nikolskii* groups in Romania and Moldova (RMOL and KOD). In Ukraine, (CLIS, KAN) the majority of adults are black. However, even here (KAN) single normally colored specimens are known (table 5; see also Zinenko and Ruzhilenko, 2003; Zinenko, 2004). The presence of non-melanistic adult specimens in populations with pronounced morphology of *V. b. nikolskii* distinguishes all its populations to the west of the Dnieper. Most remarkable is the population from northern Moldova, which has the most evidently expressed scalation characters of *V. b. nikolskii*, but without any recorded black specimens.

The number of zigzag windings differed significantly among groups ($F(6, 101)$ = 21.7, $p < 0.00001$). The mean number of zigzag windings is larger in *V. b. berus* ($76.37 \pm 3.84, n = 19$) than in *V. b. nikolskii* reference samples ($58.69 \pm 6.05, n = 32$). Post-hoc comparison with Bonferroni test showed existance of two homogenous groups ($p < 0.05$) with SUC and *V. b. berus* reference sample in one joint group with a higher zigzag number and *V. b. nikolskii* reference sample and the rest of analyzed samples with fewer zig-zag windings in the second (table 5).

Venom from specimens from Bârnova (Iaşi, Romania), Meresheny (Khencheshti district, Republic of Moldova), Vulkaneshty (Nisporeny, Republic of Moldova), Rud (Yedintsy, Republic of Moldova), Chornyy lis (Znamenka district, Kirovohrad region, Ukraine) was transparent, like in other *V. b. nikolskii* populations to the East of the Dnieper (Milto and Zinenko, 2005; Bakiev et al., 2008). Yellowish venom was observed in all Carphathian populations morphologically identified as *V. b. berus*: Câmpulung-Moldovenesc, (Suceava, Romania), Piatra-Craiului (Argeş, Romania) and Ceahlău, (Neamţ, Romania) (fig. 1). Specimens from Khotin heights (Chernivtsi region, Ukraine) represented an exception, where col-

Table 5. Morphological variability for the geographical samples of *V. berus* from Romania, Republic of Moldova and Ukraine: *n*, mean ± SD, min-max.

	SUC, Romania, females	BAC, Romania, females	RMOL, Romania, females	KOD, Republic of Moldova, females	NMOL, Republic of Moldova, females	KAN, Ukraine, females	CLIS, Ukraine, females
Ventr.	8 149.00 ± 3.66 142-154	7 150.86 ± 2.73 147-154	15 154.73 ± 3.20 150-161	9 153.11 ± 3.48 147-157	8 159.00 ± 1.60 157-161	9 154.00 ± 2.50 150-158	3 154.33 ± 1.53 153-156
S.cd.	8 27.88 ± 1.89 25-31	7 30.57 ± 2.88 26-33	15 35.40 ± 1.18 33-41	8 33.50 ± 3.81 27-38	8 34.75 ± 2.76 30-39	9 31.56 ± 3.78 23-35	3 34.00 ± 1.00 33-35
Sq.	8 20.50 ± 0.93 19-21	7 20.14 ± 1.07 19-21	15 21.93 ± 1.03 21-24	9 21.22 ± 0.67 21-23	8 21.00 ± 0 21	9 21.00 ± 0 21	3 21.67 ± 1.15 21-23
Lab.	8 17.75 ± 0.71 16-18	7 17.57 ± 0.98 16-19	15 17.80 ± 0.77 16-19	9 17.78 ± 0.44 17-18	8 18.38 ± 1.50 16-21	9 18.22 ± 0.44 18-19	3 18.00 ± 0 18
S.lab.	8 21.13 ± 1.25 19-23	7 20.43 ± 1.13 19-22	15 21.60 ± 1.72 18-24	9 21.11 ± 1.83 19-24	8 22.13 ± 1.13 21-24	9 20.44 ± 1.33 18-22	3 22.33 ± 1.53 21-24
C.oc.	8 19.00 ± 2.33 16-21	7 19.00 ± 2.45 15-23	15 19.67 ± 1.59 16-22	9 19.44 ± 1.33 18-22	8 19.75 ± 1.67 17-22	9 18.89 ± 1.45 16-20	3 20.67 ± 1.15 20-22
S.oc.	8 1.17 ± 0.20 2:2* – 0%	7 1.09 ± 0.19 2:2 – 0%	15 1.30 ± 0.36 2:2 – 13.3%	9 1.17 ± 0.25 2:2 – 0%	8 1.38 ± 0.33 2:2 – 12.5%	9 1.39 ± 0.40 2:2 – 22.2%	3 1.92 ± 0.14 2:2 – 66.7%
Ic.	8 8.51 ± 2.33 5-12	7 6.71 ± 1.98 5-10	15 8.13 ± 2.77 4-12	9 7.67 ± 1.73 5-9	8 8.50 ± 2.78 6-13	12 8.83 ± 2.62 5-13	3 10.00 ± 2.00 8-12
Pf.	8 10.50 ± 2.00 8-14	7 8.57 ± 1.72 5-10	15 10.00 ± 3.85 5-22	9 6.78 ± 2.39 2-10	8 8.13 ± 1.73 6-11	12 6.83 ± 1.90 4-9	3 9.33 ± 4.04 5-13
Lor.	8 6.50 ± 1.69 4-10	7 7.14 ± 2.48 4-10	15 8.93 ± 2.05 6-13	9 7.78 ± 2.28 4-12	8 8.63 ± 1.30 7-11	12 8.58 ± 2.02 4-12	3 10.33 ± 0.58 10-11

Table 5. (Continued).

|	SUC, Romania, females	BAC, Romania, females	RMOL, Romania, females	KOD, Republic of Moldova, females	NMOL, Republic of Moldova, females	KAN, Ukraine, females	CLIS, Ukraine, females
Gul.	7 8.29 ± 1.80 6-11	7 9.00 ± 2.16 6-13	13 10.00 ± 1.73 8-13	6 8.33 ± 0.82 8-10	7 8.43 ± 1.51 6-10	6 9.17 ± 1.47 8-12	3 8.33 ± 1.53 7-10
Zigzag number (males and females together)	12 69.33 ± 9.47 54-83	5 57.60 ± 8.76 50-68	26 56.12 ± 8.95 40-76	5 55.80 ± 6.50 47-64	9 55.55 ± 4.39 46-62	1 60	–
Ratio of melanistic/not completely melanistic among adults (%)	8 0/25	7 14.3/14.3	10 20/10	6 0/50	8 0	7 14.3/85.7	3 33.3/66.7

	SUC, Romania, males	BAC, Romania, males	RMOL, Romania, males	KOD, Republic of Moldova, males	NMOL, Republic of Moldova, males	KAN, Ukraine, males	CLIS, Ukraine, males
Ventr.	11 144.18 ± 4.07 134-148	5 149.60 ± 4.72 142-154	27 150.37 ± 4.67 141-160	12 151.42 ± 2.31 148-157	6 150.50 ± 1.76 149-153	11 149.27 ± 3.16 144-154	7 150.29 ± 2.92 147-156
S.cd.	10 35.30 ± 2.31 31-38	5 39.60 ± 7.57 27-45	27 42.33 ± 2.83 36-47	12 43.08 ± 2.07 41-47	4 44.25 ± 1.25 43-46	11 40.55 ± 2.77 35-44	7 41.57 ± 2.15 39-45
Sq.	11 20.18 ± 0.98 19-21	5 20.80 ± 1.10 19-22	27 21.28 ± 0.66 21-23	12 21.50 ± 0.90 21-23	6 21.00 ± 0	11 21.55 ± 0.82 21-23	7 21.57 ± 0.79 21-23
Lab.	11 17.36 ± 1.03 15-18	5 18.40 ± 0.55 18-19	27 17.48 ± 0.85 16-18	12 18.08 ± 0.90 16-20	6 18.33 ± 0.52 18-19	11 17.73 ± 0.47 17-18	7 18.00 ± 0
S.lab.	11 20.27 ± 1.10 19-22	5 22.00 ± 3.16 17-25	27 20.96 ± 1.87 18-24	12 21.83 ± 1.03 20-23	6 22.83 ± 1.94 21-26	11 20.82 ± 1.47 18-23	7 23.00 ± 1.63 21-25

Table 5. (Continued).

	SUC, Romania, males	BAC, Romania, males	RMOL, Romania, males	KOD, Republic of Moldova, males	NMOL, Republic of Moldova, males	KAN, Ukraine, males	CLIS, Ukraine, males
C.oc.	11 19.82 ± 2.09 15-22	5 20.60 ± 2.19 18-24	27 19.19 ± 2.17 15-24	11 18.64 ± 1.36 17-22	6 20.00 ± 1.10 18-21	11 18.36 ± 1.50 17-22	7 20.29 ± 1.38 18-22
S.oc.	11 1.02 ± 0.08 2:2 – 0%	5 1.02 ± 0.06 2:2 – 0%	27 1.04 ± 0.17 2:2 – 0%	11 1.00 ± 0.00 2:2 – 0%	6 1.27 ± 0.39 2:2 – 16.7%	11 1.00 ± 0.00 2:2 – 0%	7 1.18 ± 0.17 2:2 – 0%
Ic.	11 9.18 ± 2.04 6-13	5 10.00 ± 1.58 8-12	27 7.52 ± 2.38 4-13	11 6.82 ± 2.14 4-10	6 7.83 ± 2.48 5-12	12 6.50 ± 2.11 3-9	7 7.29 ± 1.80 5-9
Pf.	11 9.55 ± 3.08 4-14	5 8.60 ± 1.52 7-10	27 10.04 ± 2.49 6-16	11 6.91 ± 4.11 0-12	6 7.33 ± 1.51 5-9	12 7.92 ± 1.44 6-10	7 7.00 ± 1.73 5-9
Lor.	11 6.45 ± 1.44 4-9	5 7.20 ± 3.03 4-10	27 6.85 ± 1.96 4-10	11 6.00 ± 1.61 4-8	6 7.67 ± 0.82 6-8	12 4.75 ± 1.06 4-7	7 6.86 ± 0.69 6-8
Gul.	10 8.90 ± 2.23 6-13	5 9.40 ± 1.52 7-11	17 9.47 ± 1.41 6-12	9 9.11 ± 1.54 6-11	6 10.83 ± 1.47 9-13	8 8.13 ± 1.13 6-10	6 8.67 ± 1.03 8-10
Ratio of melanistic/not completely melanistic among adults (%)	7 0/14.3	6 20/0	19 26.3/15.8	8 37.5/25	6 0	10 40/50	7 100/0

* Ration of specimens with two rows of S.oc.
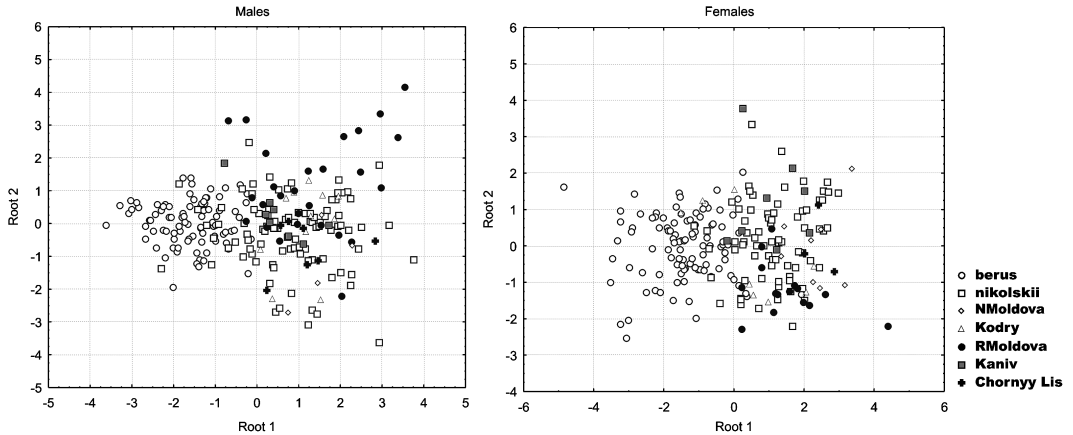
Figure 2. Scatter plot of canonical discriminant scores of the analysis of meristic characters of males and females *V. b. berus*, *V. b. nikolskii* and five geographical samples from Romania, Republic of Moldova and Western Ukraine.

Table 6. Character standardized coefficients and their percentages of eigenvalues for two first canonical variables from the Canonical Variate Analysis of the seven geographic groups of *V. b. berus* and *V. b. nikolskii*.

	Males root 1	Males root 2	Females root 1	Females root 2
Ventr.	0.73	−0.01	0.68	0.11
S.cd.	0.23	0.25	0.25	−0.49
Sq.	0.25	0.05	0.24	−0.15
Lab.	0.00	−0.29	0.20	0.48
S.lab.	0.16	−0.32	0.01	−0.30
C.oc.	0.06	0.28	−0.15	−0.41
S.oc.	0.06	−0.52	0.51	0.48
IC.	−0.33	−0.45	−0.19	0.21
Pf.	0.22	0.77	−0.04	−0.46
Lor.	0.36	0.03	0.25	−0.14
Eigenvalues	1.52	0.25	1.92	0.17

orless venom was observed in morphologically intermediate snakes, more similar to *V. b. berus.*

Discussion

The southern position of *V. b. nikolskii* within the general *V. berus* distribution area, together with the known influence of temperature during embriogenesis on morphology and scalation raise the question of the validity of the morphologically based determination of subspecies. However, the well documented relationship between temperature and the number of ventral scales in snakes is not always direct, as observed in *Vipera aspis* (Lourdais et al.,

2004), but could also be flat, without a significant effect from different temperature regimes (*Thamnophis elegans* – Arnold and Peterson, 2002), or, as another experiment shows, U-shaped with enlarged number of ventral scales both with higher or lower temperatures of incubation (*Natrix fasciata* – Osgood, 1978). In studies where it was assayed, strong maternal effect and thus the hereditary nature of scalation characters were also confirmed (Arnold and Peterson, 2002; Lourdais et al., 2004). Obviously, the data concerning temperature influence on scalation collected in laboratory experiments are even less applicable to the natural conditions due to the buffering of temperature effects by maternal thermoregulation in viviparous species or temperature fluctuation between years.

Both the experimental and the natural effects of temperature on the number of ventrals do not exceed 3% of the number of scales (Osgood, 1978; Arnold and Peterson, 2002) or 4 ventral scales in *V. aspis* (Lourdais et al., 2004). Thus, theoretically observed inter-population differences in ventral scale numbers within *V. b. berus* and *V. b. nikolskii*, which do not exceed 3 scales (Milto and Zinenko, 2005) could be caused by differences in climatic conditions. However, no pronounced cline variation of ventral numbers is observed within *V. b. berus* specimens in Eastern Europe in spite of the climatic gradients (Zi-

nenko, unpublished data): for example, average temperatures of July change from 16 to 21°C from North to South. In addition, the difference in the number of ventrals between subspecies is more than 4-5 scales and reaches 8 scales between some populations of *V. b. berus* and *V. b. nikolskii* (Milto and Zinenko, 2005). Differences in scalation between subspecies remain obvious even in regions of parapatric distribution (Zinenko, 2004; Milto and Zinenko, 2005; this paper) and when populations inhabit atypical habitats for their subspecies (Zinenko, unpublished data).

The influence of various environmental factors on morphology in reptiles was shown in several recent studies (Osgood, 1978; Malhotra and Thorpe, 1997; Lourdais et al., 2004; Sanders et al., 2004). Thus, the importance of morphological variation and even applicability of external morphology for systematics or diagnostics sometimes became doubtful. It is generally accepted, that morphological variation has two sources: phylogenetic and ecological (Thorpe et al., 1991), with examples varying from the complete absence of phylogenetic signal in variation of morphological traits (Malhotra and Thorpe, 1997) to remaining considerable concordance between morphology and phylogeny (Sanders et al., 2004; Golay et al., 2008). Therefore, this allows the further use of morphological descriptions, if not in systematics (i.e., grounds for the description of new species or taxonomic level clarification), surely in determination of taxonomic affiliation of specimens but only when differences between taxa and characters variation are known. In the case of *V. b. berus* and *V. b. nikolskii* in Eastern Europe, differences in scalation characters between taxa and their geographical variation were previously studied and clear differences were found (Milto and Zinenko, 2005).

Another argument for the applicability of morphological characters for determination is the general congruence of the pattern of geographical variation of *V. b. nikolskii* in Eastern Europe. In the case of *V. b. nikolskii,* different

characters like scalation, coloration (both frequency of melanism and shape of zigzag band; Zinenko and Bondarenko, unpublished), reproductive characteristics (Zinenko et al., 2005), venom activity (Malenyov et al., 2007) and its composition (Bakiev et al., 2008; Ramazanova et al., 2008) and partly genetics (Kalyabina-Hauf et al., 2004; Joger et al., 2007) are congruent, corresponding to the main idea of the Procedure of Random resampling/congruence (Thorpe, 1991). If applied to this case, its principle supports the prevailing role of phylogenetics in the determination of scalation variation in *V. b. berus* and *V. b. nikolskii*: "If the geographic variation is caused by historical phylogenetic process then all characters systems would in general be subjected to the same processes and should be broadly congruent" – p. 333 (Thorpe, 1991). Thus, phylogenetically caused scalation variation of *V. b. nikolskii* allow us to determine subspecies affiliation using a set of morphological characters and multivariate analysis.

The vipers from Central Ukraine, Moldova and Central Moldavian plateau in Romania are almost morphologically identical to *V. b. nikolskii* from the Eastern part of its range. The border with *V. b. berus* stretches along the external eastern slopes of the Carpathian ridge, following in longitudinal direction (to the south) from Khotin heights and then presumably across the South-Eastern part of Chernivtsi region and the southern part of Vinnitsa and then goes across the known hybrid zone in Cherkassy regions in Ukraine. The issue of penetration of *V. b. nikolskii* to the West, along the southern slopes of the Carpathians remains under question. However, we consider this penetration improbable due to the fact that the belt of typical *V. b. nikolskii* habitats of broadleaved forests is very narrow here. Moreover, the rivers, which run down from the southern slopes of the Carpathians, form numerous corridors for migration from dense mountain populations of *V. b. berus*. So, *V. b. berus* could easily competitively exclude or dissolve *V. b. nikolskii* populations, as it happens along the Dniepers' left bank in Ukraine and the

Volgas banks' in Russia (Zinenko, 2004; Milto and Zinenko, 2005). Specimens with intermediate morphology between the subspecies are found in boundary regions between *V. b. berus* and *V. b. nikolskii*. Limited introgression apparently takes place, but it is not possible, based on our data, to assess its width and extent.

All the populations from the forest-steppe zone of Romania and Republic of Moldova demonstrate the whole list of diagnostic features of *V. b. nikolskii*: greater numbers of scales in almost all characters (table 5), colorless venom and the dorsal coloration pattern similar to that of the *V. b. nikolskii* newborns in the Kharkiv region. Proteolythic activity and composition of venom from populations near Znamenka (CLIS) and Kodry (KOD) were found to be typical for *V. b. nikolskii* in Southern Russia and Eastern Ukraine (Malenyov et al., 2007; Bakiev et al., 2008). Thus, all localities of *V. berus* sensu lato of the southern part of the forest-steppe between Kaniv and the Central Moldavian plateau (fig. 1) should be referred to as *V. b. nikolskii*. Within the outlined area, it is also known from the Northern part of Odessa region (the Savran' and the Balta rivers basins, the Southern Bug tributaries – Kotenko, 1992; Tabachishin and Zavialov, 2003). It is still unclear how far *V. b. nikolskii* could intrude into the steppe zone in Ukraine: it is reported that there was a finding of *V. b. nikolskii* 20 km to the North-West from Mykolayiv, well inside the steppe zone in the Southern Bug river valley (Franzen and Heckes, 2000), but this location is questionable because of trade origin of snakes (M. Schweiger, pers. comm.). In Russia *V. b. nikolskii* was also found in the floodplain forests of the rivers Medveditsa, Don and Khopior in the steppe zone (Tabachishin, Tabachishina and Zavjalov, 2003; Milto and Zinenko, 2005).

The extent of the range of *V. b. nikolskii* to the west, up to the eastern slopes of Carpathians is reasonable. The typical habitats for *V. b. nikolskii* are continuously present, without significant gaps, from the known populations in Kaniv and Znamenka vicinities in Central

Ukraine (Milto and Zinenko, 2005) up to the Central Moldavian plateau. As in the eastern part of its range, the distribution of *V. b. nikolskii* to the West of the Dnieper is connected with moderate heights (Central Moldavian plateau, Kodry, Dniester plateau, southern part of Podolian heights) and regions of refugia of forest vegetation and fauna during glaciation (Blagovolin et al., 1982; Markova, Simakova and Puzachenko, 2002). The list of sympatric species remains the same, except for the frequent replacement of *Lacerta agilis* L., 1758 with *Lacerta viridis* Laurenti, 1768 (Zinenko, 2006a; Strugariu et al., 2008). The connection between the distribution of *V. b. nikolskii* and oak forests, slopes, ecotones with bushes along the forest edges, etc., found in Ukraine and Russia (Zinenko, 2006a) also persists.

Very few specimens from the southern Carpathians have been available for analyses. The fact that these specimens were from distant and isolated localities precludes us to analyze them as a pooled sample. Two specimens from the southwestern Carpathian localities Gheboaia (Dîmboviţa County) and Brezoi (Vîlcea County) were classified as *V. b. nikolskii* (table 1). However, we cannot exclude the possibility of extreme manifestation of variation of *V. b. berus* (because the precise determination is reliable only for samples, not single specimens) or that of a mistake in labeling. Specimens from the inner part of the Carpathian basin (Cluj and Maramureş counties) have high probabilities of classification and pronounced morphology of *V. b. berus* (table 1).

Additional specimens from the eastern slopes of the Central Carpathians, the Trotuş river valley (Neamţ and Suceava counties), have an uncertain discrimination, with bias to *V. b. berus* (table 1, fig. 1) as do other specimens from the vicinal BAC group. Our data from Ceahlău (Neamţ) supported more certain discrimination of snakes from this region as *V. b. berus* (table 1). The single specimen from Liteni, lowland part of Suceava County was discriminated as *V. b. nikolskii*. This raises a question of the

existence of populations of *V. b. nikolskii* to the North of the Iaşi region.

The territory of Western Ukraine remains poorly sampled. Samples and sole specimens allow us to draw the northern border of the *V. b. nikolskii* range along the East and South from the Dniester basin in Ukraine (Ivano-Frankivs'k region) and Khotin height (Chernivtsi region), where vipers have rather intermediate morphology, more similar to *V. b. berus* (table 1, fig. 1). Specimens from "Medobory" Natural Reserve (Ternopil' region) were discriminated with high probability as *V. b. berus*. The absence of typical *V. b. nikolskii* habitats in Vinnitsa and Khmelnitsky vicinities (pers. obs.) gives us grounds to consider that only the southern part of Podolian heights is inhabited by *V. b. nikolskii*.

In spite of the general similarity with topotypical populations of *V. b. nikolskii*, the populations from Moldova and Romania differ on account of the presence of non-melanistic adult specimens. Melanistic specimens are even completely absent at times (in NMOL sample). It was previously considered that black coloration of adults is a diagnostic character for *V. b. nikoskii* (Vedmederja, Grubant and Rudajeva, 1986; Milto and Zinenko, 2005) and that the occurrence of non-melanistic specimens could be a consequence of hybridization with *V. b. berus* (Zinenko, 2004). We do not reject this explanation for some populations from contact zones. However, the occurrence of both melanistic and non-melanistic adult *V. b. nikolskii* in Moldova and Romania is more likely to reflect ancestral polymorphism, which has perhaps been lost in Eastern Ukraine and Russia (e.g., due to a more severe selection in more continental climates or via stochastic genetic processes). Thus, the diagnosis of *V. b. nikolskii* should be corrected. Melanism in vipers occurs in most taxa and is widespread in the *V. berus* range (Thiesmeier and Völkl, 2002; Völkl and Thiesmeier, 2002) and in all taxa in the species complex. Plenty of hypotheses explaining color polymorphism and melanism in vipers were suggested. Most of them discuss the adaptive value of col-

oration type. Thus, the importance of coloration type for selection is generally accepted (Andren and Nilson, 1981; Madsen and Stille, 1988; Luiselli, 1992; Monney, Luiselli and Capula, 1995; Thiesmeier and Völkl, 2002; Wüster et al., 2004; Niskanen and Mappes, 2005). Consequently, a character being under selection and arising independently in many different populations or evolutionary lineages has a low value for phylogenetic reconstruction.

The pattern of coloration of non-melanistic specimens from Romania and the Republic of Moldova is typical for *V. b. nikolskii* and differs from the pattern of *V. b. berus*, particularly by the number and the shape of zigzag windings (table 5, fig. 3). Infrequent and deep zigzag dorsal band of *V. b. nikolskii* (both non-melanistic adult specimens from western part of its range and newborns in the East) makes it also similar to *V. barani* and *V. b. bosniensis* (Zinenko and Bondarenko, unpublished data). Snakes from Northern Moldova and non-melanistic specimens from the Central Moldavian plateau (Romania) exhibit a less pronounced sexual dimorphism in coloration than *V. b. berus*: sexes differ only in pattern details and its contrast, like newborns of *V. b. nikolskii* from Ukraine (Milto and Zinenko, 2005), while *V. b. berus* has well expressed dimorphism in coloration: males are usually grayish and females are brownish (e.g., Bruno and Maugeri, 1990; Shine and Madsen, 1994).

The lack of genetic differences in mtDNA sequences between *V. b. berus* and the majority of *V. b. nikolskii* populations is one of the most problematic questions in the systematics of this taxon. The reference samples of *V. b. nikolskii* used in this paper also partly consist of populations where only *V. b. berus* mtDNA cytochrome b haplotypes were found (Kalyabina-Hauf et al., 2004; Joger et al., 2007). At the same time, all *V. b. nikolskii* populations, both with *V. b. berus* and its own specific mtDNA haplotypes (Kalyabina-Hauf et al., 2004; Joger et al., 2007), combine into morphological and ecological unity, showing concor-
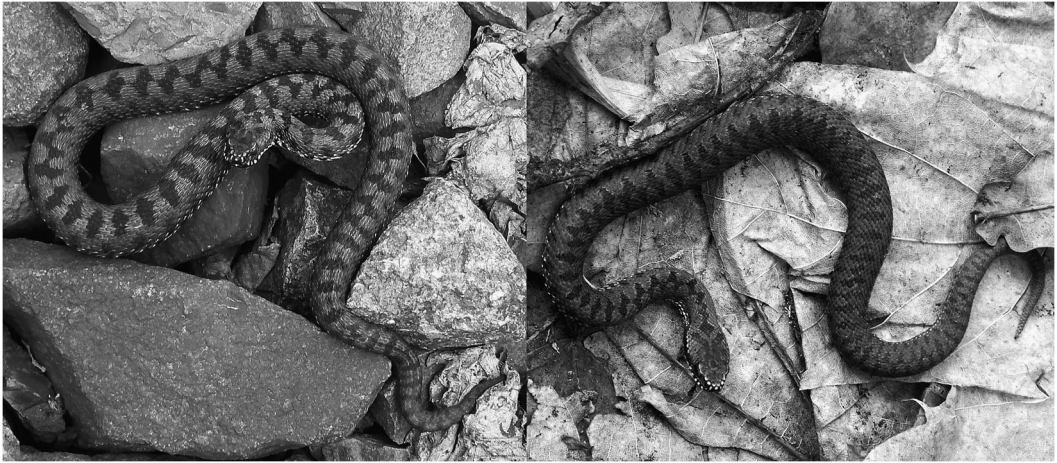
Figure 3. Adult non-melanistic male specimen of *V. b. nikolskii* from RMOL group (left, Bârnova, Iaşi, Romania) and male juvenile *V. b. nikolskii* from Kharkiv vicinities, Ukraine (right).

dance between such different characteristics as morphology (both scalation and coloration pattern) and venom polypeptide composition and enzymatic activity (Zinenko, 2006b; Malenyov et al., 2007; Bakiev et al., 2008; Ramazanova et al., 2008), size of newborns (Zinenko et al., 2005) etc. This supports monophily of *V. b. nikolskii* according to the principle of genealogical concordance (Avise, 1994) and the existence of *V. b. nikolskii* as an evolutionary lineage.

To explain this complex situation, when morphologically and ecologically united populations carry diverged mitochondrial haplotypes, one possibly could be of mtDNA intogression (Zinenko, 2006b). Many cases of introgressive hybridization have been observed, with recent examples of *Lissotriton vulgaris* and *Lissotriton montandoni* in Europe (Babik et al., 2005) among others. If this is the case, geographically isolated populations like the ones studied from CLIS and NMOL are candidates to be "pure" *V. b. nikolskii*, free from introgression with *V. b. berus*. However, such assumptions should be tested with genetic methods.

References

Andren, C., Nilson, G. (1981): Reproductive success and risk of predation in normal and melanistic colour morphs of the adder, *Vipera berus*. Biol. J. Linn. Soc. **15**: 235-246.

Arnold, S.J., Peterson, C.R. (2002): A model for optimal reaction norms: the case of the pregnant garter snake and her temperature-sensitive embryos. American Naturalist. **160**: 306-316.

Avise, J.C. (1994): Molecular Markers, Natural History and Evolution. Chapman and Hall, New York.

Babik, W., Branicki, W., Crnobrnja-Isailovic, J., Cogălniceanu, D., Sas, I., Olgun, K., Poyarkov, N.A., Garcia-Paris, M., Arntzen, J.W. (2005): Phylogeography of two European newt species – discordance between mtDNA and morphology. Mol. Ecol. **14**: 2475-2491.

Bačescu, M. (1933): *Vipera berus* L. in Moldova şi Basarabia. Revista Stiintifica "V. Adamachi" **19**: 3-13.

Bakiev, A., Böhme, W., Joger, U. (2005): *Vipera (Pelias)* [*berus*] *nikolskii* Vedmederja, Grubant et Rudaeva, 1986 – Waldsteppenotter. In: Handbuch der Reptilien und Amphibien Europas, Bd. 3/2B. Schlangen (Serpentes) 2, Viperidae, p. 293-309. Joger, U., Stümpel, N., Eds, Aula-Verlag.

Bakiev, A.G., Malenyov, A.L., Chetanov, N.A., Zajtseva, O.V., Peskov, A.N. (2008): Common adder *Vipera berus*

(Reptilia, Viperidae) in Volga bassin: materials on biology, ecology and toxinology. Newsletter of the Samara bend **26**: 759-816.

Blagovolin, N., Leontjev, O., Muratov, V., Ostrovsky, L., Rychagov, G., Serebryanny, L. (1982): Paleogeography of Europe During the Last One Hundred Thousand Years (Atlas-monograph). Nauka, Moscow.

Bruno, S., Maugeri, S. (1990): Serpenti d'Italia e d'Europa. Milano.

Dotsenko, I.B. (2004): About reptiles' collections of A.A. Brauner in zoological museum of NMNH NAS of Ukraine. Actual Problems of Herpetology and Toxinology: Collection of Scientific Papers **7**: 60-70.

Franzen, M., Heckes, U. (2000): *Vipera barani* Böhme & Joger, 1983 aus dem östlichen Pontus-Gebirge, Türkei: Differentialmerkmale, Verbreitung, Habitate. Spixiana **23**: 61-70.

Fuhn, I., Vancea, S. (1961): Reptilia. In: Fauna Republicii Populare Romine, vol. 14, 2.

Golay, P., Monney, J.-C., Conelli, A., Durand, T., Gilles, T., Zuffi, M., Ursenbacher, S. (2008): Systematics of the Swiss asp vipers: some implications for the European *Vipera aspis* (Linnaeus, 1758) complex (Serpentes: Viperidae) – a tribute to Eugen Kramer. Amphibia-Reptilia **29**: 71-83.

Joger, U., Lenk, P., Baran, I., Böhme, W., Ziegler, T., Heidrich, P., Wink, M. (1997): The phylogenetic position of *Vipera barani* and of *V. nikolskii* within the *Vipera berus* complex. Herpetologia Bonnensis: 185-194.

Joger, U., Fritz, U., Guicking, D., Kalyabina-Hauf, S., Nagye, Z.T., Wink, M. (2007): Phylogeography of western Palaearctic reptiles – Spatial and temporal speciation patterns. Zoologischer Anzeiger **246**: 293-313.

Kalyabina-Hauf, S., Schweiger, S., Joger, U., Mayer, W., Orlov, N., Wink, M. (2004): Phylogeny and systematics of adders (*Vipera berus* complex). Mertensiella. Verbreitung, Okologie und Schutz der Kreuzotter (*Vipera berus* [Linnaeus, 1758]) **15**: 7-16.

Kotenko, T.I. (1992): Protection of amphibians and reptiles. In: Protection of Animal World, p. 36-50. Borejko, V.S., Ed., "Urozhaj", Kyiv.

Lourdais, O., Shine, R., Bonnet, X., Guillon, M., Naulleau, G. (2004): Climate affects offspring phenotypes in a viviparous snake. Oikos **104**: 551-560.

Luiselli, L. (1992): Reproductive success in melanistic adders: a new hypothesis and some considerations on Andren and Nilson's (1981) suggest. Oikos **63**: 601-604.

Madsen, T., Stille, B. (1988): The effect of size dependent mortality on colour morphs in male adders, *Vipera berus*. Oikos **52**: 73-78.

Malenyov, A.L., Bakiev, A.G., Zajtseva, O.V., Shurshina, I.D., Zinenko, A.I. (2007): Proteolitic activity of adders venom in some populations of Russia and Ukraine. News of Samara scientific centre of Russian Academy of Sciences **9**: 1040-1044.

Malhotra, A., Thorpe, R. (1997): Microgeographic variation in scalation of Anolis oculatus (Dominica, West Indies): a multivariate analysis. Herpetologica **53** (1): 49-62.

Markova, A.K., Simakova, A.N., Puzachenko, A.U. (2002): Ecosystems of Eastern Europe during Late Glacial Maximum of Valday Glaciation (24-28 thousand years ago) on floristic and teriological data. Doklady RAN **386**: 681-685.

Milto, K.D., Zinenko, O.I. (2005): Distribution and morphological variability of *Vipera berus* in Eastern Europe. In: Herpetologia Petropolitana, p. 64-73. Ananjeva, N., Tsinenko, O., Eds, St. Petersburg.

Monney, J.-C., Luiselli, L., Capula, M. (1995): Correlates of melanism in a population of adders (*Vipera berus*) from the Swiss Alps and comparisons with other alpine populations. Amphibia-Reptilia **16**: 323-330.

Niskanen, M., Mappes, J. (2005): Significance of the dorsal zigzag pattern of *Vipera latastei gaditiana* against avian predators. Journ. of Anim. Ecol. **74**: 1091-1101.

Osgood, D.W. (1978): Effects of temperature on the development of meristic characters in *Natrix fasciata*. Copeia **1**: 33-47.

Ramazanova, A.S., Zavada, L.L., Starkov, V.G., Kovyazina, I.V., Subbotina, T.F., Kostyukhina, E.E., Dementieva, I.N., Ovchinnikova, T.V., Utkin, Yu.V. (2008): Heterodimeric neurotoxic phospholipases A2 – the first proteins from venom of recently established species *Vipera nikolskii*: implication of venom composition in viper systematics. Toxicon **51**: 524-537.

Sanders, K., Malhotra, A., Thorpe, R. (2004): Ecological diversification in a group of Indomalayan pitvipers (Trimeresurus): convergence in taxonomically important traits has implications for species identification. J. Evol. Biol. **17**: 721-731.

Shine, R., Madsen, T. (1994): Sexual dichromatism in snakes of the genus *Vipera*: a review and a new evolutionary hypothesis. J. Herpetol. **28**: 114-117.

Strugariu, A., Zamfirescu, S., Nicoară, A., Gherghel, I., Sas, I., Puşcaşu, C., Bugeac, T. (2008): Preliminary data regarding the distribution and status of the herpetofauna in Iaşi County (Romania) North-West. J. Zool. **4** (Supplement 1): 1-23.

Tabachishin, V.G., Zavialov, E.V. (2003): Forest-steppe Viper distribution in the south of the Podolsk(sic!) height. Povolzhje Ecological Journal **2**: 202-203.

Tabachishin, V.G., Tabachishina, I.E., Zavialov, E.V. (2003): Modern habitats and some ecological aspects of the Forest-steppe Viper in the north of the Lower Volga region. Povolzhje Ecologycal Journal **1**: 82-86.

Thiesmeier, B., Völkl, W. (2002): Zur Verbeitung und Okologie schwarzer Kreuzottern – ein Uberblick. Zeitschrift fur Feldherpetologie **9**: 1-16.

Thorpe, R., Brown, R., Malhotra, A., Wüster, W. (1991): Geographic variation and population systematics: distinguishing between ecogenetics and phylogenetics. Bolletino di Zoologia **58**: 329-335.

Ţurcanu, V. (2006): On the systematic status of common adder *Vipera berus* (Linnaeus, 1758) in Moldova. In: Academician Leo Berg – 130: Collection of Scientific Articles, p. 151-163. Elan Poligraph, Bender.

Vancea, Şt., Fuhn, I.E., Borcea, M. (1982): L'étude morphologique des populations de *Vipera b. berus* du Plateau Central Moldave, des Carpathes Orientales et de Transylvanie. Vertebrata Hungarica **30**: 245-250.

Vedmederja, V.I., Grubant, V.N., Rudaeva, A.V. (1986): On the name of the black adder in woodland steppe zone of European part of USSR. Vestnik Kharkovskogo Universiteta **288**: 83-85 (in Russian).

Völkl, W., Thiesmeier, B. (2002): Die Kreuzotter: ein Leben in festen Bahnen? Laurenti-Verlag, Bielefeld.

Wüster, W., Allum, C.S.E., Bjargardottir, I.B., Bailey, K.L., Dawson, K.J., Guenioui, J., Lewis, J., McGurk, J., Moore, A.G., Niskaneny, M., Pollard, C.P. (2004): Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. Proc. R. Soc. Lond. **271**: 2495-2499.

Zinenko, O.I. (2004): New data about hybridization between *Vipera nikolskii* Vedmederya, Grubant et Rudaeva, 1986 and *Vipera berus berus* (Linnaeus, 1758) and their contact zones in Ukraine. Mertensiella. Verbreitung, Okologie und Schutz der Kreuzotter (*Vipera berus* [Linnaeus, 1758]) **15**: 17-28.

Zinenko, O.I. (2006a): Habitats of *Vipera berus nikolskii* in Ukraine. In: Herpetologia Bonnensis II. Proceedings of the 13th Congress of the Societas Europaea Herpetologica, p. 205-209. Vences, M., Kohler, J., Ziegler, T., Bohme, W., Eds, Bonn.

Zinenko, O.I. (2006b): Reptiles of the forest-steppe zone of Left-bank Ukraine (distribution, morphology, taxonomy, biology, ecology). Ph.D. Dissertation, Institute of Zoology, Kyiv.

Zinenko, O.I., Ruzhilenko, N.S. (2003): On the systematic position of adders inhabit the Kanev Natural Reserve territory. Zapovidna Sprava v Ukraini **9**: 51-55 (in Russian).

Zinenko, O., Bondarenko, Z., Claus, C. (2005): Comparison of size and body proportions of newborns *Vipera berus berus* L., 1758 and *Vipera berus nikolskii* Vedmederja, Grubant et Rudaeva, 1986. Actual Problems of Herpetology and Toxinology **8**: 48-54.