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First Report on a Trichromatic Lowland *Vipera berus bosniensis* Population in Serbia

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***Abstract*.—During a capture-mark-recapture (CMR) study in the lowland *Vipera berus bosniensis* population inhabiting an agricultural landscape, we found that the snakes co-occur in three color morphs and that individual body coloration in adults changes in time. Further investigations should reveal if different morphs offer some survival/selective advantages. Although this population is isolated from conspecifics and exposed to numerous other anthropogenic pressures, snakes are relatively abundant hence the population is of considerable conservation significance. Continuation of our CMR surveys should reveal its trends and prospects.**

Key Words.—Balkan Adder; isolated population; rural landscape; dorsal pattern variation; conservation

Introduction

The Common Adder, *Vipera berus*, is the most widely distributed venomous terrestrial snake, the only serpent inhabiting areas north of the Arctic Circle (Strugariu and Zamfirescu 2011; Lourdais et al. 2013). In fact, it has a very wide distribution among wild terrestrial vertebrates in general (Bates 1990). Because it is predominately a boreal specialist (Lourdais et al. 2013), its current distribution is disjunct and often restricted to refugial mountainous habitats. However, since the Pleistocene glaciations, adder populations have persisted in lowlands as well (Ursenbacher et al. 2006). Records exist of their earlier wider distribution in central European plains (Fejérváry 1923), especially along large rivers including Danube and Sava, also in Croatia and Serbia, where the Balkan or Bosnian subspecies, *V. b. bosniensis* is present (Jelić et al. 2013). Knowledge about the current distribution, biology, and status of lowland adder populations is meager (e.g., Újvári et al. 2001), and recent long-term population studies are, to our knowledge, nonexistent.

The International Union for the Conservation of Nature (IUCN) regards *V. berus* as of Least Concern (globally), but no information on the Balkan subspecies is available (Crnobrnja Isailović et al. 2009). Like other viperids worldwide, *V. berus* is at high risk compared to other snake groups/species due to its life-history characteristics (Tomović et al. 2015; Maritz et al. 2016). In Serbia, it is strictly protected by law and regarded as Vulnerable (Anonymous 2011; Ajtić and Tomović 2015). Lowland adder populations are at especially high risk due to the high anthropogenic presence and intense activities, compared to highland areas, where human presence is often merely sporadic or seasonal.

Northern distribution limits of *V. b. bosniensis* lie below 190 m above sea level (asl) in south-western Hungary (Malina et al. 2011). In lowlands and hills of northern Serbia (southern portion of the Pannonian Plain), the subspecies persists in the Fruška gora and Vršačke planine mountains (up to 539 and 641 m asl, respectively), and around wetlands along the Sava River (Ajtić and Tomović 2015). Dorsal skin coloration pattern typical for *V. b. bosniensis* is a broken zigzag line (Jelić et al. 2013). Descriptions and explanations of the high variability of this pattern, and the presence of other morphs in *Vipera* sp. exist in the scientific literature for over a century, at least from Leighton (1901) to Dubey et al. (2015). It has been long known that in adder populations, dark and melanistic individuals are fairly common (Fejérváry 1923; Malina et al. 2011), even abundant (40–50%) in high-altitude and closed and/or humid forested habitats, but also in lowlands in Hungary (Monney et al. 1996; Újvári et al. 2001; Ducrest et al. 2014). These two morphs are present in most populations throughout the species range (Luiselli et al. 1994). Usually, the zigzag pattern is found in neonates and snakes may become melanistic in their second or third year of life (Forsman 1995a). Újvári et al. (2001) also noted that coloration alters significantly during ontogeny. However, cases are documented of zigzag females giving birth to melanistic neonates (Strugariu and Zamfirescu 2009). In a close relative, *Vipera aspis*, an additional unpatterned variant was recorded in high percentages in certain populations: light-brown dorsum without a zigzag line or dark blotches (Mebert et al. 2011). Its persistence in populations was explained, *inter alia*, through ecological interactions: such individuals are inconspicuous in open alpine habitats (Dubey et al. 2015). Recently, a flavistic, bright

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Figure 1. The habitat where we found the Balkan Adder, *Vipera berus bosniensis*, north of the Sava River, Srem region, Vojvodina Province, Serbia. (A) Young forest stand with clearings; (B) One of the corridors that was (inexistent in March 2017) a stretch of scrub between two fields (the main forest stand is visible in the back). (Photographed by Sonja Nikolić).

yellow-orange *V. b. bosniensis* individual was reported from Vitosha Mountains in Bulgaria (Stojanov 2014). Leucism and albinism also occur in *Vipera* sp., but rarely (Krecsák 2008). Such pronounced variability in coloration strongly correlates with the adaptability of the species to various habitat types and ecological niches (including predator avoidance), and contributes to their wide distribution, implying their old age and survival capabilities (Pizzato and Dubey 2012; Ducrest et al. 2014; Dubey et al. 2015).

Although usually explained as a result of (sex-specific) trade-offs between metabolic/thermoregulation needs and crypsis, recent considerations suggest that melanism alone is not necessarily adaptive, or clearly beneficial, in terms of heat gaining (Forsman 1995a; Strugariu and Zamfirescu 2011; Santos et al. 2014; Azócar et al. 2016). A single, general explanation for its persistence has not yet been offered, especially bearing in mind interactions between various environmental factors (e.g., Forsman 1993) and opposite trends concerning cryptic vs. melanistic individuals in closely related species (Monney et al. 1996). Also, the possible genetic basis of coloration variability was found (Ducrest et al. 2014), which implies the need for further studies concerning color polymorphism in ectotherms.

Lack of information regarding the distribution, population trends, and extinction risks of reptiles is common. According to Tingley et al. (2016), only 45% of described species have been assessed by the IUCN, of which 19% are Data Deficient. Bland and Böhm (2016) also found that 52% of Data Deficient reptiles lack information on population status and trends. The sole IUCN assessment process has been being criticized lately, and the need for improvements is recognized (Frankham et al. 2014, Collen et al. 2016). Additional and alternative evaluation criteria have been suggested (Filippi and Luiselli 2000; Tomović et al. 2015).

Given the above facts, we believe it is important that all types of data regarding all lowland adder populations be published rapidly so they become available to other researchers and conservationists. The locality we report on is one of only two where CMR studies of lowland *Vipera berus* in Serbia are being conducted. Our site is the first from where we recorded the presence of all possible color morphs in a single, restricted population.

Materials and Methods

Study site.—In March 2014 we started a capture-mark-recapture study in the population of *Vipera berus bosniensis* inhabiting a lowland area (70–90 m asl) north of the Sava River (Srem region, Vojvodina province, Serbia). Our study population inhabits a young oak forest plantation (Fig. 1A) surrounded by crop fields. Several irrigation canals crisscross the terrain; their edges are comparatively bare in the forest, but overgrown with small trees and scrub in open fields. Dry shrub corridors also exist between adjacent fields (Fig. 1B). We intensively searched an area of about 20 ha total surface. We usually found adders on forest, road, and canal edges (i.e., in a small percentage of entire habitat). For substantial periods of time, the ground was covered with fallen leaves and dry grass (Fig. 1A and 2F). The dry bare soil was grayish-brown (see Fig. 2C). In times of high precipitation, the waterlogged soil is dark for prolonged periods (Fig. 1B).

Field procedure and statistical analysis.—We captured adders by hand (with leather gloves on), during random walks along selected parts of the habitat. Two to five people participated in surveys. We marked the exact location of each snake with noticeable satin bows. We kept snakes in calico bags until processing. We recorded the morphometric and meristic traits of snakes,

Figure 2. Three co-occurring Balkan Adder (*Vipera berus bosniensis*) morphs. (A) predominant, typical, zigzag coloration pattern; (B) fully melanistic adult; (C) an almost concolor sub-adult female; (D) male number 25 in 2014; (E) male number 25 in 2015; and (F) melanistic individual on the early spring background, March 2014. (Photographed by Aleksandar Simović and Sonja Nikolić).

marked them by scale clipping and branding (Winne et al. 2006), and released them unharmed at exact places where they were caught. We assigned snakes into age/size classes according to Újvári et al. (2001): individuals < 300 mm snout-vent length (SVL) are considered newborns/juveniles, and we regarded snakes > 550 mm SVL as adults. We classified snakes between these sizes as sub-adults. We photographed the head (dorsal view and both sides), venter, and, sometimes, the dorsum of snakes, and we noted all peculiarities and anomalies (unusual coloration, specific markings, scale anomalies, etc.). Regarding their dorsal coloration/pattern, we classified snakes as normal zigzag (in numerous shades and intensities, but patterned, typical cryptic adder coloration), dark zigzag (zigzag pattern clearly visible, but dorsum darker than usual), melanistic (completely black, and those with only faint traces of zigzag on the neck), and concolor (beige background ± pale traces of dark pattern along the back).

Whenever possible, we recorded the cloacal temperature of snakes (T_{cl}), substrate temperature (grass, leaf litter, or soil: T_{sub}), and air temperature (T_{air}). As an estimate of differences in the heat gaining efficacy of the two prevailing morphs, we calculated differences between cloacal temperature and air temperature (T_{cl} minus T_{air}) and substrate temperature (T_{cl} minus T_{sub}). Temperature difference data (ΔT_{cl}-T_{sub}) for the two morphs were normally distributed ($P > 0.050$) and variances were not significantly different ($P = 0.190$). We used a *t*-test to compare ΔT_{cl}-T_{sub} between zigzag and melanistic individuals.

In studies of significance and effects of polychromatism in snakes, the incidence of wounds/scars is often used as a potential indicator of predation risk for melanistic compared to cryptic vipers (e.g., Niskanen and Mappes 2005). For that reason, we also recorded all wounds and scars and their exact locations on the body of the snakes. For future examinations of intra- and inter-population genetic variability, we also collected tissue samples for DNA analyses.

Results

Of all snakes we encountered, we found only two on bare ground: we found all others resting along the forest/bushes edges, on grass / leaf litter or around piles of cut branches. Also, only three individuals were moving (escaping), while the rest were basking when we saw them. Among the 73 adders of both sexes (52 males, 21 females) and all age classes (seven juveniles, 66 sub-adults and adults) we marked, 60 (82.19%) had the typical zigzag dorsal pattern (with variations: Fig. 2A), four (5.48%) were noted as dark zigzag, seven (9.60%) were melanistic (only males: Fig. 2B), and one sub-adult female (1.37%) was light, almost concolor (for one individual we missed to record the pattern). This concolor female had a clear arrowhead-shaped head marking, but only pale rhombi along the body (Fig. 2C). All seven juveniles (9.60%) had the typical, zigzag pattern. In all adders we processed, large white patches were present on supralabial scales (Fig. 2B). Until March 2015 we made 17 recaptures, many of which only a week or a month after the initial capture, and we only noted the encounter, without taking any measurements, notes, or photographs.

At least two (2.74%) individuals we captured as sub-adults and recaptured as adults darkened considerably during the year of study. We captured a sub-adult male with the distinct zigzag pattern 13 April 2014 at 52.8 cm SVL. He was light grey with black zigzag pattern delineated with white contour (which is uncommon), vividly colored, and highly conspicuous (Fig. 2D). We recaptured this animal 19 April 2015 (59.4 cm SVL). The color of this snake was much darker than when we caught it the first time (Fig. 2E). We caught another male also in 2014 (43.2 cm SVL) and it had a normal zigzag pattern. At recapture in 2015 (57.0 cm SVL), we noted it as dark (we did not photograph it again).

Cloacal temperatures of snakes ranged from 18.0° C to 31.7° C (n = 34). The three highest (> 30° C) and lowest (around 18.0° C) cloacal temperatures were for normal zigzag-patterned snakes. Differences between cloacal and air temperatures (T_{cl}-T_{air}, n = 25) ranged from -6.7° C to 9.6° C. We recorded both extremes in dark/melanistic individuals. The three highest temperature differences were 9.6° C (dark zigzag, 15 March 2015), 9.3° C, and 8.7° C (normal zigzag, 29 and 14 March 2014, respectively). The three lowest negative differences (the snakes were colder than the air temperature, all from 21 April 2014) were -6.7° C (melanistic snake), -5.1° C, and -3.9° C (normal zigzag pattern).

Differences between body and substrate temperatures were more pronounced than with air temperature (-6.9° C to 20.5° C, n = 26). The three highest differences we recorded were on 15 March 2014 in two zigzag-patterned snakes (15.2° C and 20.5° C) and one dark/melanistic individual (11.0° C). The lowest negative differences were found on 21 April 2014 (-3.8° C and -5.1° C in zigzag-patterned snakes, and -6.9° C in a melanistic individual). The ΔT_{cl}-T_{sub} between zigzag and melanistic individuals were not significantly different (t = 0.720, df = 24, P = 0.480). We did not test differences between cloacal and air temperatures because we had only two records of T_{air} for melanistic individuals. We found possible indications of predator attacks in only seven adult snakes (9.60%: two females, one normal another dark, and five males, of which two melanistic), which were either fresh wounds or scars. Of these seven, four snakes had wounds or scars on the head, two on the neck and mid-body, and one on a ventral scale.

Discussion

Although known for other viper species and only preliminary for our study population, the findings we present are interesting and open new questions. This is the first population in Serbia with documented all possible color patterns and color variants: typical cryptic/aposematic zigzag, melanistic, and light, beige unpatterned (concolor). To the best of our knowledge, the light unpatterned morph of *Vipera berus* has not been reported from any lowland population before. In another Serbian adder population where the CMR was started (Vršačke Planine Mountains) a single sub-adult concolor individual has been found so far (Milivoj Krstić, pers. comm). This means that light unpatterned *V. berus bosniensis* are probably more frequent than previously thought. The variety we report of occurred in a very small area, which is surrounded by uniformly looking arable land. As noted before, in different periods of the year and in different parts of our study area, the (back)ground coloration varies from the very dark wet soil, through green vegetation and dry grass and leaves, to light dry soil in summer. Therefore, each adder pattern type can cryptically match a habitat subtype (Luiselli et al. 1994). Causes of such polymorphism and mechanisms of its maintenance in our study population remain to be revealed. Previous studies usually investigated the interplay between crypsis, thermoregulation, growth, and mortality rates and reproductive success (including different effects on fitness in males and females) in cryptic and melanistic snakes (Madsen and Stille 1988; Forsman 1995b). Despite its persistence in scientific literature, the hypothesis of higher growth rates in melanistic individuals is not always supported by field data (Forsman and Ås 1987). Abiotic factors also have to be considered (Capula and Luiselli 1995; Broennimann et al. 2014).

Many of the dark or melanistic individuals in our sample still possessed faint traces of a zigzag pattern along the neck and anterior portions of the dorsum. During the 2015 field surveys, we found several such individuals. This supports the change in coloration with time, even seasonally (Madsen and Shine 1992; Forsman 1995a; Újvári et al. 2001). To further confirm this assumption and understand its causes, we must continue our study, and, if possible, monitor adders from birth until adulthood, taking records of changes in weather, vegetation cover, as well as of composition and abundance of prey and predators of the snake.

Dark body coloration may be positively related to increased humidity and warmth. Such a relationship, explained *inter alia* as a mode of camouflage in darker surroundings, has been found in several groups of endotherms (but in some plants as well), and is referred to as Gloger's Rule (e.g., Roulin and Randin 2015; Lev-Yadun 2016). Another explanation, related to birds, is the higher resistance of dark (melanin-rich) feathers to certain bacteria (Gunderson et al. 2008). We were not able to find a reference for such a phenomenon in ectotherms, but both options sound plausible for this group as well because feathers developed from ancestral reptilian scales (Di-Poï and Milinkovitch 2016). This appears possible especially for *V. berus*, which often occupies comparatively moist habitats (Zinenko 2006). The fact that prominent color change in one male happened in a single year might be related to high humidity during that period. The year 2014 was extremely wet, with severe floods along the entire Sava and other river courses. In Serbia, 2014 was the rainiest and the second warmest year in the period from 1951 to 2014 (Anonymous. 2015. Republički hidrometeorološki zavod: Godišnji bilten za Srbiju 2014. godina [Republic Hydrometeorological Service: Annual bulletin for Serbia, year 2014]. Available at www.hidmet.gov.rs/podaci/meteorologija/latin/2014.pdf. [Accessed 17 October 2015]).

Regarding the relationship between body temperatures of snakes and temperature of their environment, our findings are inconclusive, for the time being. This relationship was widely studied in *V. berus*, but often the conclusion was that the color itself could not explain different rates of heat gaining and body temperature maintaining (e.g., Forsman 1995a). Ontogenetic integument darkening in ectotherms further complicates the already complex considerations regarding the implications of body coloration for metabolism, growth rates, body condition, and other life-history traits determined by these, such as sexual maturity attainment, sexual dimorphism, success of black vs. zigzag adders in male-male confrontations, and hypothetical larger litters in/by melanistic individuals of both sexes. Further, many animals grow fast before they reach maturity (e.g., Webb et al. 2002) and that is the period when adders are usually cryptically colored (Forsman 1995a).

In this initial phase of research, we could not find any regularity concerning the frequency of wounds as a measure of predator pressure on different adder morphs. Usually, the melanistic adders were presumed or shone to suffer from higher predation (e.g., Andrén and Nilson 1981). However, more recent studies showed that the zigzag pattern typical for European vipers is not only cryptical/disruptive (helps the animals to stay less detectable to predators, and to prey as well), but also serves as a warning (aposematic signal) to potential visually oriented predators (Wüster et al. 2004; Niskanen and Mappes 2005; Valkonen et al. 2011).

As in other parts of the Pannonian basin (Újvári et al. 2001), adder populations in northern Serbia are mutually isolated in small remnants of suitable habitat in agriculturally dominated landscape (Ajtić and Tomović 2015). These lowlands are usually strongly altered by centuries of human presence and activities. There are numerous archaeological sites in the Srem region along the Sava River from at least 6,000 BC (Radmanović et al. 2013). The remnants of wilderness along rivers and irrigation canals and remnants of old forests most probably are the only possible routes of connectivity among wildlife populations (e.g., Balestrieri et al. 2015). Therefore, these corridors must be at least preserved as such, if not improved and formally protected. We made a visit to our study site at the beginning of March 2017 and found that two stretches of shrub, including one corridor between two fields, had been clearcut. Isolation of populations can lead to speciation, but also poses high risk of inbreeding and possibly extinction (Madsen et al. 1996; Ursenbacher et al. 2006, 2009). On the other hand, bearing in mind their pronounced polymorphism, the adders we studied could be genetically diverse (Dubey et al. 2015). Considering all this, lowland adder populations in Serbia should be considered of high conservation concern. Also, the ecology of adders was almost exclusively studied in high-altitude populations; therefore, our future findings would be very valuable in terms of adder biology. In the future, *V. b. bosniensis* ecology and demography should be more thoroughly studied and the species must be properly protected, including protecting their habitat.

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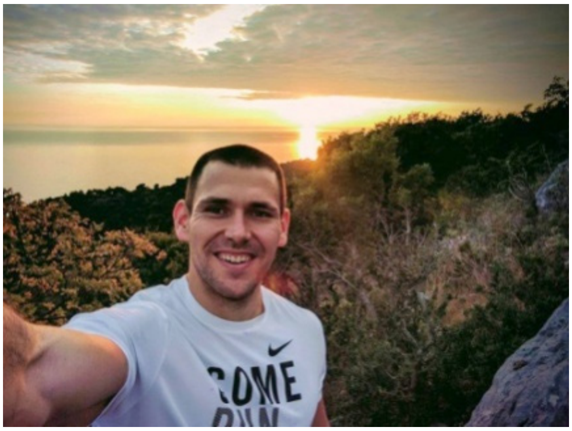

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