BIHAREAN BIOLOGIST 12 (2): 92-96
Article No.: e181301

http://biozoojournals.ro/bihbiol/index.html

A non-traumatic multi-operational method for individual documentation and identification of nose-horned vipers (*Vipera ammodytes* (Linnaeus, 1758) (Squamata, Viperidae)) allows reliable recognition of recaptured specimens

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Received: 11. November 2017 / Accepted: 26. February 2018 / Available online: 26. February 2017 / Printed: December 2018

Abstract. We developed a combined method for marking and identification of specimens of the nose-horned viper (*Vipera ammodytes*). The method operates on three levels and is very reliable. The first recognition level is based on relatively large numbers, painted on the side of the snakes. We discovered that the markings last for up to five months and even shaded skins can be identified, which was very useful in our field surveys. The second level of identification was based on the morphology of the horn scales of every single snake. We discovered that the horn scale arrangement is constant for every specimen and can be easily recognized on photographs. Furthermore, we found that the horns in *V. ammodytes* are rarely damaged, thus the second level of identification is rather functional. In cases the side marks were lost and the horn was damaged, we were able to identify the specimens based on photographs of the head pholidosis and defined color patterns of the head and the body. This third stage was a kind of safety back up procedure. The identification method proposed in the present study will be useful in the investigation of other snakes with more hidden way of life, as well as for other horned species.

Key words: ecology, Viperidae, snake, individual marking.

Introduction

Marking animals is a key factor for clarification of many ecological aspects of the species, such as population density, life histories, home range, etc. There are numerous methods for marking snakes, e.g. external and PIT tags, tattooing (Spellerberg & Prestt 1978, Fitch 1987, Keck 1994, Jemison et al. 1995, Gibbons & Andrews 2004, Dorcas & Willson 2009), or branding and color marking (Spellerberg & Prestt 1978, Fitch 1987, Henderson & Winstel 1995, Winne et al. 2006, Dorcas & Willson 2009). However, the most widely used method is scale-clipping (Weary 1969, Brown & Parker 1976, Fitch 1987, Dorcas & Willson 2009). Actually, all of these methods have their flaws. External and PIT tags could be lost (Germano & Williams 1993, Roark & Dorcas 2000, Dorcas & Willson 2009). Tattoos, brands and clipped scales are often traumatic and with the time can become obscure (Fitch 1987), but see (Keck 1994, Burger & Zappalorti 2011, Fauvel et al. 2012). Color marking is a non-traumatic method, but it can be used only for short-term personal identification (Henderson & Winstel 1995). Radio-telemetry is very effective for individual identification, home range studies, behavioral studies and even locomotion performance, but this is a method requiring rather expensive gear and is more suitable for a limited number of specimens for tracking (Reinert & Cundall 1982, Reinert 1992, Újvári & Korsós 2000). A very effective method for individual identification for many snake species is the recording of natural markings, such as color pattern or pholidosis (arrangement of head scales) (Fitch 1987, Shine et al. 1988, Sheldon & Bradley 1989, Benson 1999). This method allows the involvement of many scientist and volunteers and is not related to big expenses.

In the present study we describe the implementation of a combination of non-traumatic methods for individual identification of nose-horned vipers (*Vipera ammodytes*). It is based on the documentation, comparison and analysis of combined matrices including color marking, the natural markings of the snakes (color pattern), as well as specifics on pholidosis.

Material and Methods

In Bulgaria, the nose-horned viper (*V. ammodytes*) is found throughout the country and is represented by two subspecies - *V. ammodytes ammodytes* (inhabiting northwestern Bulgaria and the northern part of the Krayshte Region) and *V. ammodytes montandoni* Boulenger, 1904 (inhabiting the rest of the country) (Tomović, 2006; Stojanov et al. 2011). Recent molecular and phylogeographic studies show, that in addition to these two genetic groups, in Bulgaria probably this species is represented by a third group (see Ursenbacher et al. 2008).

For our research, five study sites were chosen along the north-south gradient of the species distribution in Bulgaria. The sites are situated near the villages of Karlukovo (43°10.757'N, 24°3.651'E, subspecies *V. a. montandonii*), Lakatnik (43°5.317'N, 23°22.905E, subspecies *V. a. ammodytes*), Balsha (42°51.410'N, 23°15.139'E, subspecies *V. a. ammodytes*), Bosnek (42°29.860'N, 23°11.830'E, subspecies *V. a. montandonii* or *V. a. meridionalis*) and in the Kresna Gorge (41°45.916'N; 23°9.158'E, subspecies *V. a. montandonii* or *V. a. meridionalis*) (Tomović, 2006; Stojanov et al. 2011, but see also Ursenbacher et al. 2008). Our investigations were performed monthly between 2013 and the spring of 2017, during the active period of the animals, as a part of an ongoing ecological study of the species. The sites were visited once a month (for one day) during 2014 and twice a month (for one day) from 2015 to the spring of 2017. In 2013 the study sites were visited sporadically.

The snakes were captured by hand, using leather gloves. All captured animals were measured and weighted. Photographs of the dorsal and ventral sides of the body and the tail, as well as all sides of the head (including the anterior side of the horn) were taken. The sex of the snakes was determined, by inspecting the color and pattern of the body and the tail morphology (the length and the width of the tail, as well as the ratio between the snout-vent length and tail length). The animals were marked with a non-toxic, alcohol-free red color pen (Faber-Castell Multimark 1525 permanent). As a mark was used a number painted onto the mid-dorsal section of the body with size 2.5-3 cm for adults and subadults and 1-1.5 cm for juveniles (Fig.

Figure 1. Color mark on an adult *V. ammodytes* male (L.tot = 51.9 cm) 11 days after its application.

1). We did not check whether each captured individual is already been registered in the database, because this procedure is time consuming and it is performed in the laboratory. If a viper, that already has been registered was marked with a new number, this duplication was corrected in the database after the method of long term identification was applied in the lab (see below). After all of the procedures were carried out, the snakes were released. Gravid females from the beginning of August to the second half of September were brought to the laboratory until parturition. Neonates were measured, weighted, photographed, and their sex was determined. Then both the juveniles, as well as the adult females were released on the site of capture of the females. Neonates weren't marked with a color pen, because shedding was still in process.

For long term identification, we used the number, shape and arrangement of the scales on the front of the viper's horn (including the suprarostral) (Fig. 2). The rostral was not included in the count. We first grouped the vipers by sex and size, and then compared the pictures by the number of the horn-scales. However, due to injuries, the horn can be damaged. Also, snakes with similar horn morphology can be misidentified. Therefore, for additional certainty, we also compared the pholidosis and the color pattern of the head, as well as the color pattern of the body to confirm the positive identification, made by the horn structure (Fig. 3). This third stage was a kind of safety back up procedure. To obtain information whether the number of the horn scales remains the same throughout life, we compared the pictures of the captured viper not only with those animals with the same number of scales on the horn, but also with those having one and two scales more (for potential polymerization, e.g. increasing of the number of the scales by sub-division of scales) and one and two scales less (for potential oligomerization, e.g. decreasing of the number of the scales by adhesion of scales). In these cases we used the pholidosis and the color pattern of the head, as well as the color pattern of the body for identification. For example, in case we caught a female with seven scales on the horn, and total length (L.tot) was 55 cm, we compared the shape and arrangement of the scales of the horn of that animal with the photos of all previously

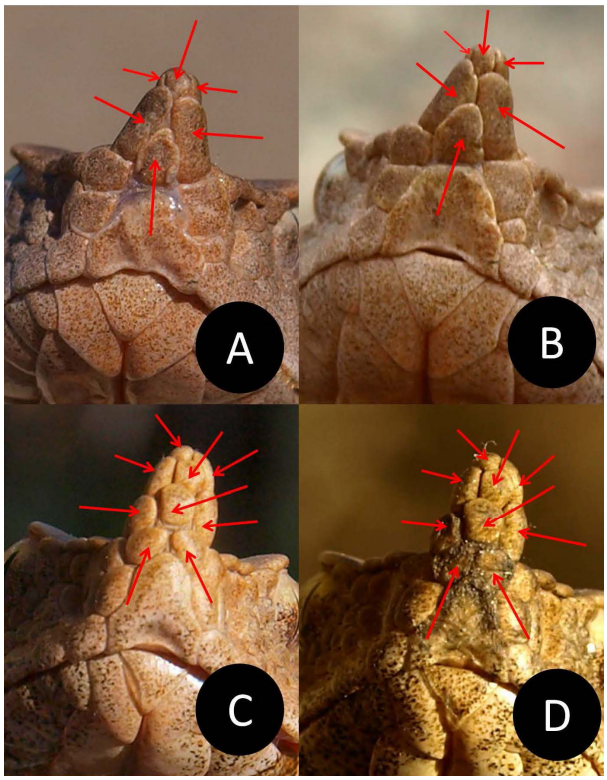

Figure 2. Two different *V. ammodytes* females, identified by their horn-morphology. Specimen represented on 2a was photographed on 08.07.2015, the same specimen represented on 2b was recaptured and photographed on 25.08.2016; Specimen represented on 2c was photographed on 11.04.2015, the same specimen represented on 2d was recaptured and photographed on 11.11.2015; arrows mark the scales used for identification.

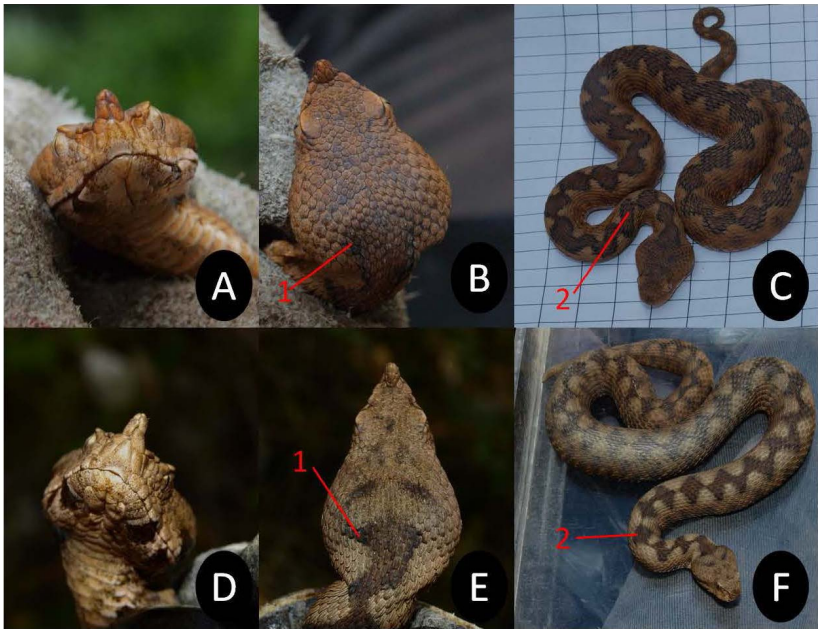

Figure 3. Two different *V. ammodytes* females with very similar horn morphology (3a and 3d). 3b and 3e shows the difference in the head pattern between the two vipers (1 red); 3c and 3f shows the difference in the body pattern between them- the shape of the dorsal zig-zag stripe (2 red).

caught females with 5, 6, 7, 8 and 9 horn-scales and L.tot to about 60 cm. In few cases, for reliable identification of specimens the shape of Gulare, Preanale and Anale, as well as other easily recognizable ventralia were also used.

The procedure could be made more efficient with the use of a formula for scale arrangement. For example, the formula for the specimen showed on Fig. 2a is 1+2+3 and for specimen showed on Fig. 2c is 2+3+4, where the first number is the number of the scales in direct contact with rostrale, the second number- the row of scales above the first, and the third- the rest of the scales. However, we did not use this formula, because we tested this trait for variability.

Results

During the field surveys, a total of 335 vipers were captured. Sixty-one (18.21%) of them were identified at least once (33 female and 28 male). Some of the snakes were recaptured more than once. The maximum number of recaptures for an individual was six times. During the analysis of the recaptured specimens, we successfully identified snakes 88 times, excluding daily resightings. One of the neonates, born in the laboratory, was captured and identified on the following year.

28.41% of all identifications of snakes were made by the color marks (25 identifications of 22 specimens). The other 71.59% of the identifications (63 identifications of 49 specimens) were made using the morphology of the horn scales, and were confirmed by the comparison of the pholidosis and the color pattern of the head and the body. In the cases when the identification was made by the color mark, the scale morphology and the color pattern were also compared for possible variations.

The color marks lasted up to two months during the active period (mean: 23.64 days, range: 5−55, SD = 14.3; n = 22), and up to five months, during the hibernation of the animals (mean: 147.33 days, range: 118−168, SD = 26.1; n = 3). Normally, the mark was clearly visible for 3-5 weeks. In the cases that it lasted two months, it was either very pale or only sections of the marker were visible. This was also the case with two of the marks that remained during the hibernation. In the third case the mark was starting to fade away, but was still easily recognizable (Fig. 4). In many of the cases, detection of the mark was very hard or not possible without capturing the viper, either because the snake was coiled, or because it was within tall vegetation. In one case the exuvia with retained color mark was found next to the viper.

The 61 positively identified snakes, belonging to all age groups (juvenile, subadult and adult), showed no variation of the number and shape of the scales of the horn. No signs of postnatal instability in this trait were found. The mean number of days from the first capture to the last recapture and identification was 314.49 days (range: 5−981, SD = 253.23; n = 61) (Fig. 5). Only one animal with the damaged horn was found during the study period.

Discussion

The durability of the color mark may depend on the humidity of the habitat and the activity of the snakes. In our similar study on the Balkan adder (*Vipera berus bosniensis* Boettger, 1889) (unpublished data), the mark very rarely lasted for more than 20 days. However, the Balkan adder lives in habitats with higher humidity compared to *V. ammodytes* (see Stojanov et al. 2011), and the increased humidity may cause the fast removal of the marks from the skins. In their study of *Corallus grenadensis* Barbour, 1914 (referred in the paper as *Corallus enydris*), Henderson & Winstel (1995) stated that the paint mark flaked off within about two weeks - this boinae species inhabits rather humid localities (see Henderson & Winstel 1995). Having in mind that *V. ammodytes* lives in dryer habitats (Stojanov et al. 2011), color marking might be a very effective method for specimen identification in short-term surveys.

The activity of the horned vipers probably also impact the durability of the markers - in more active snakes the mark is exposed to higher abrasion. During hibernation the marks were retained for a long time due to the low activity of the snakes. Of course, the mark is lost with the shedding of the snakes, however, exuviae with color marks can also be used for identification and may provide valuable ecological information.

An important question is, whether the color mark makes the vipers more detectable to predators, thus increasing depredation of the marked specimens. In our opinion this is not

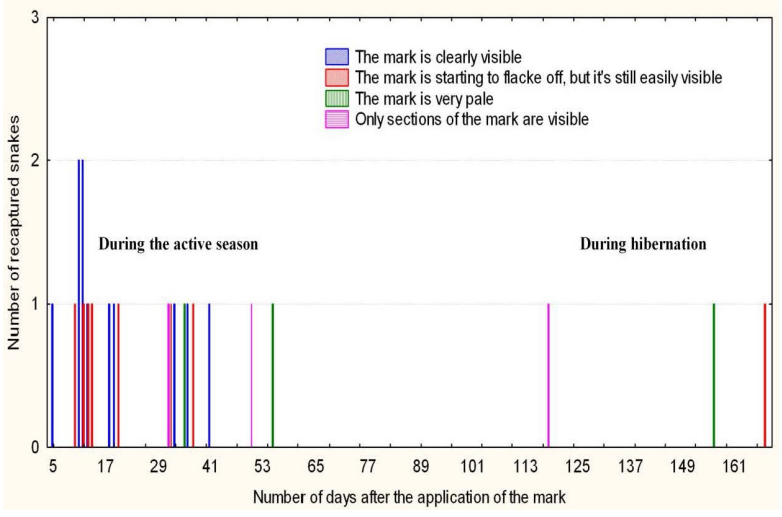

Figure 4. Retention rate and condition of the color mark during the active period and during hibernation based on 25 identifications of 22 marked and recaptured specimens *V. ammodytes*.

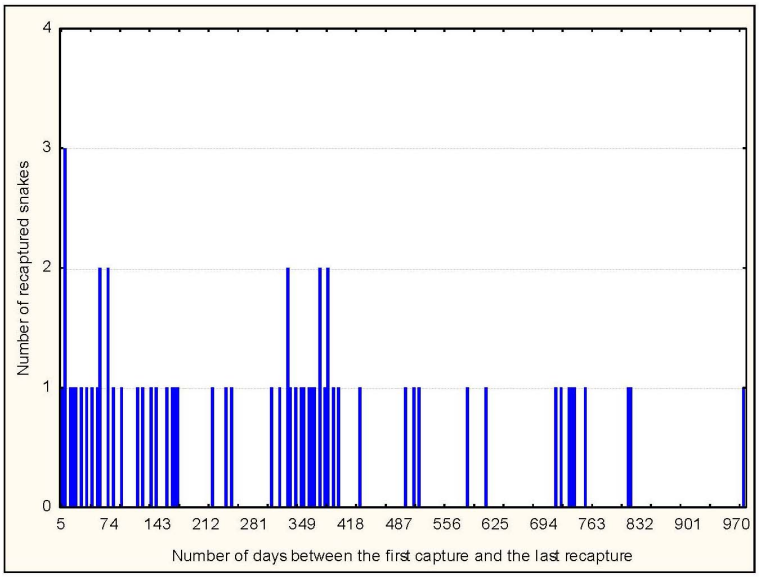

Figure 5. Time interval between the first capture and the last recapture and identification, made by the morphology of the horn scales of 61 specimens *V. ammodytes*.

the case. None of the recaptured marked animals had injuries or fresh scars from predators (although a small number of vipers can survive the predator's attack). As mentioned above, often the detection of the mark was very hard or not possible prior to the capture of the viper. It could be argued that the relatively low recapture rate is due to increased predation of the marked individuals. In our opinion the lower recapture rate is due to the fact that the sampling effort was concentrated on five different populations, which resulted in lower sampling periods for each population.

Boulenger (1903), Biella (1983), Biella & Blattler (1989) and Tomović & Džukić (2003), point out on the diversity of the horn-type in *V. ammodytes* as a taxonomic trait. This trait however, has never been used for individual identification in the species. According to our results, using the horn-morphology for individual recognition in horned vipers may be very useful tool and will ease the identification. Shine et al. (2005) suggest that discrete values of particular scalation characters are set at birth and cannot change thereafter. According to Maderson (1965) the scales and scale hinges are differentiated and their topography is established during embryogenesis. Post-natally changes in scalation may arise only after injuries which could cause alterations in the size, shape, distribution or number of the scales (see Maderson et al. 1978). Tomović et al. (2008) stated that scale variation in individual's life could occur in viperid snakes in the form of oligomerization, polymerization and shape changes. The authors stated that considerable changes in head scalation appeared in 52.2% of the recaptured specimens of *Vipera ursinii macrops* Méhely 1911. Üveges et al. (2012) contradicted these findings, as the authors found no evidence for post-natal changes in *Vipera ursinii racosiensis* Méhely 1893. Hodges & Seabrook (2014) stated that new head-scales can be created quickly in *Vipera berus*, but this occurs rarely and it is unlikely to result in significant inaccuracy in the identification, especially when other indicators (such as color pattern) had also been used. Our results showed no signs of ontogenetic changes in the horn structure of the vipers. The degree of variation that we used (± two scales) may not be enough to detect all probable polymerizations or oligomerizations, but in our opinion it should have detected at least some of them, if they occurred. It could be argued that the relatively low recapture rate is due to changes in the horn scalation, leading to unsuccessful identification of those specimens. Changes in the horn morphology may occur as a result of traumas. However, our results show that traumas of the horn occur very rarely in *V. ammodytes* and even in cases of injury, other traits such as pholidosis and the color pattern of the head and the body can be used. We recommend however multi-variance traits documentation to be performed and used together with the horn-morphology (at least for detection of potential variability of the horn, or for preventing misidentification of animals with very similar horn-morphology.

The advantages of the multi-operational documentation method described in the present article are related its low costs, its reliability (for both short-term, as well as long-termed studies) and the possibility to be performed by various experts trained in handling venomous snakes. Additionally, the method is non-traumatic for the animals. The process of analysis of a large number of pictures, that must be compared to the positive identification of the animals, may be crucially optimized. By grouping the animals by sex and size, the time for picture comparisons can be significantly reduced. The described method has some limitations. First, it is a time-required process. With marking by scale clipping + branding of ventral and dorsal scales or using PIT-tags, it is easier to determine whether the individual has already been recorded in the database. There are several potential sources of error associated with the use of natural markings to identify individuals. For example, image quality influences error. Also it is possible that two or more individuals in a population will have such similar natural markings that they cannot be distinguished from one another (Pennycuick 1978). When more people are involved then there is a greater chance of error in identifying individuals This method is not practical for other species with a higher density of the population

In conclusion, horned-viper specimens can be reliably identified based on the morphology of their horn scales. These scales do not change their form and number during the ontogenesis and represent a useful tool for recognition of individuals in long-term studies. The documentation of ad-

ditional patterns such as head pholidosis and body coloration will ensure the correct identification of the animals even in case the horn is damaged.

The color marking with numbers of the side of the vipers proofed to be a rather useful tool for studies with duration from two to five months. This method allows relatively limited potential for recognition of the animals from distance, but is rather useful for fast and exact identification of the horned vipers.

Acknowledgements. All regulations under the Biodiversity law of the Republic of Bulgaria were abide during this study. For the purpose of the study a special permit by the Ministry of Environment and Water of the Republic of Bulgaria was granted (Permit № 656/08.12.2015). This study was funded by the Bulgarian Academy of Sciences Program to help young scientist of BAS, project № DFNP- 38/20.04.2016 "Study of some biological and ecological features of the nose-horned viper (*Vipera ammodytes* Linnaeus, 1758) and the Balkan adder (*Vipera berus bosniensis* Boettger, 1889), clarification and prevention of the man-snake conflict". We would like to thank our colleague and friend Andrei Stojanov (who sadly passed away in June 2016) for the valuable advises and recommendations during this study. We would also like to thank Nikolay Todorov for designing a special box, which facilitated the safe photographing of the vipers.

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