FINAL REPORT

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Nose-horned viper in Serbia-continuation of monitoring population status and conservation efforts

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Project objectives

Nose-horned viper is listed in the IUCN Red List in the Least Concern category, Appendix II of the Bern Convention, Annex IV of the EU Habitats Directive, and, according to Serbian national law, it is in category of protected species. In the first project financed by the Rufford Foundation we collected basic population data on, mapped habitat of, and recognized main threats to nose-horned vipers (*Vipera ammodytes*) in Serbia. We began population monitoring at several locations. Main findings of our first project included confirmation of illegal harvesting for the venom supply in the surroundings of Krupanj, pollution with human waste, habitat fragmentation (by roads), and deliberate killing at every locality.

Main objectives of this project were:

* Continuation of population monitoring to draw population trends and assess the status of this species in Serbia (including collection of buccal swabs for parasite analyses, collection of DNA samples and installation of camera traps)
* Investigation of venom markets
* Continuation of creating better understanding of nose-horned vipers and snakes in general and help in changing the social perception of this rarely aggressive species through education activities.

Results

I MONITORING POPULATION STATUS

1.DEMOGRAPHIC DATA

We have continued population monitoring on locations from previous project (Đerdap National Park, Jelašnička Gorge Special Nature Reserve, Krupanj surroundings, Svilajnac surrundings and Ibar River Valey) and added two additional populations (Lazarev Canyon and Kragujevac surroundings) (fig.1). We have selected these populations to get good comparation between populations which were/are harvested (red populations on the map), populations which inhabit protected areas (blue populations on the map) and "neutral" populations e.g. those not harvested in the past and which inhabit unprotected area (yellow populations on the map).

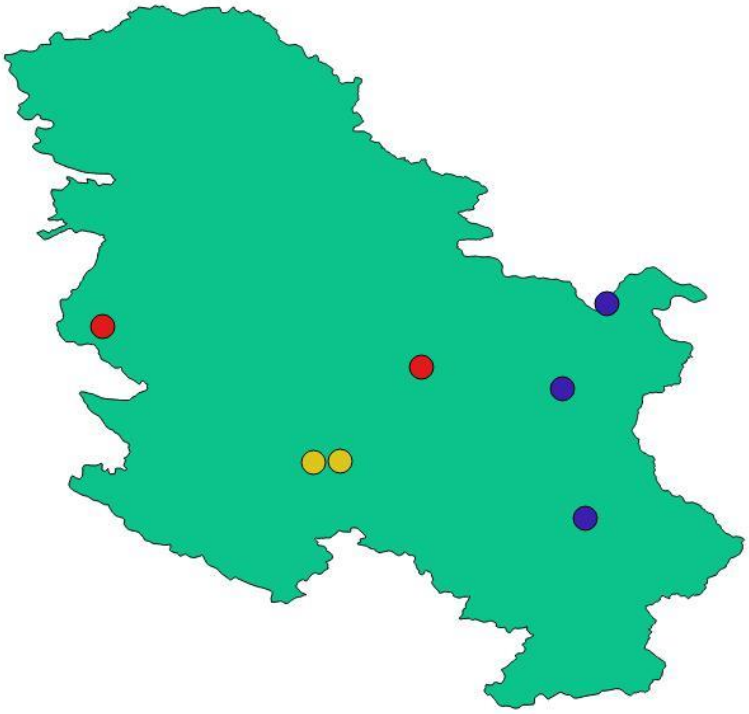


Fig.1. Map of our monitored populations

Preliminary analysis indicated that the biggest population occurs at Svilajnac surroundings while the smallest one is in Đerdap National Park. Main characteristics of individuals from these respective populations are given in Table 1. As every ectotherm organism, the nose-horned viper has considerable fluctuations in it's detectability. Therefore, it is very important to take different detectability of sex and age classes per year into consideration. In the figures 2-8 I showed differentiation in detectability between sexes and between adults and juveniles for all years of research (during my first and second Rufford projects. The graphs also show population trend from 2016. to 2018. I have classified individuals with total length above 30 cm as adults and smaller from 30 cm as juveniles.

Table 1. Population characteristics

Population	age and sex	snouth to vent length(cm) -mean-	tail length (cm) -mean-	Mass (g)
Đerdap National Park	males (>30 cm)	44.1	6.25	61.5
	females (>30cm)	51.45	6.7	156.25
Jelasnicka Gorge Special Nature Reserve				

	males (>30 cm)	45.9	7	76
	females (>30cm)	55.28	6.62	181.6
	juveniles (<30cm)	24.32	3.72	58
Lazarev kanjon Nature Monument				
	males (>30 cm)	46.14	6.88	70
	females (>30cm)	53.38	5.57	121.42
	juveniles (<30cm)	21.77	2.82	11.25
Kragujevac				
	females (>30cm)	29.9	4	25
Ibar river Valey				
	males (>30 cm)	45.1	6.15	77.43
	females (>30cm)	42.93	4.95	79.43
	juveniles (<30cm)	23	3	20
Krupanj surroundings				
	males (>30 cm)	58.82	7.67	187.5
	females (>30cm)	56.68	6.35	186.87
	juveniles (<30cm)	21.06	2.76	16.66
Svilajnac surroundings				
	males (>30 cm)	54.99	7.88	119.36
	females (>30cm)	47.92	6.13	114
	juveniles (<30cm)	20.9	2.77	22

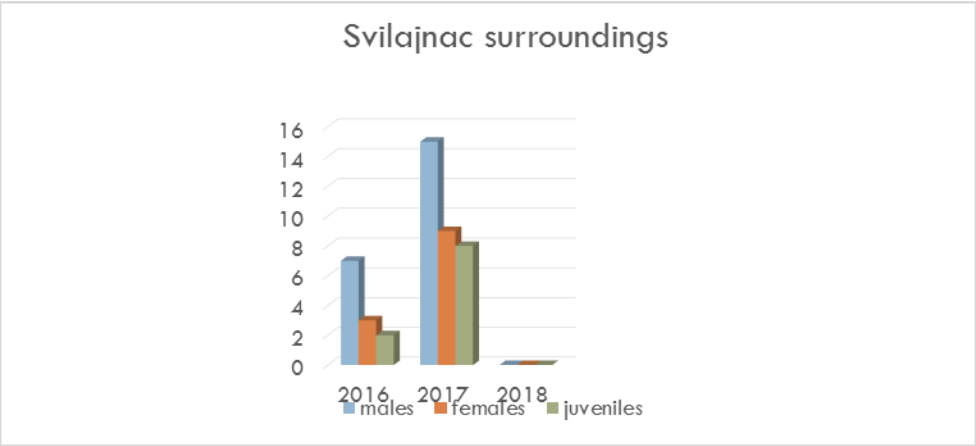


Fig. 2 Sex and age detectability

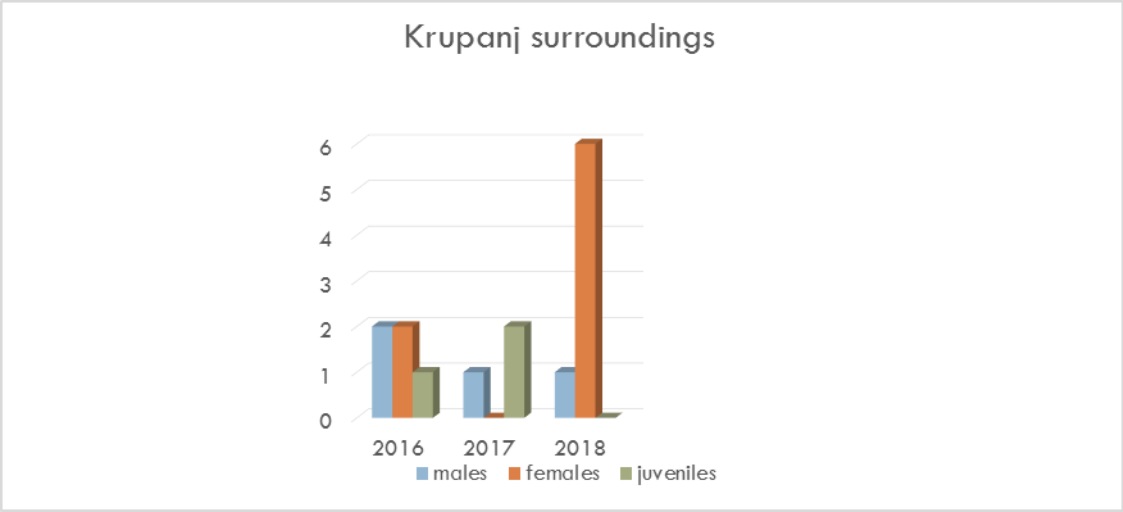


Fig. 3. Sex and age detectability

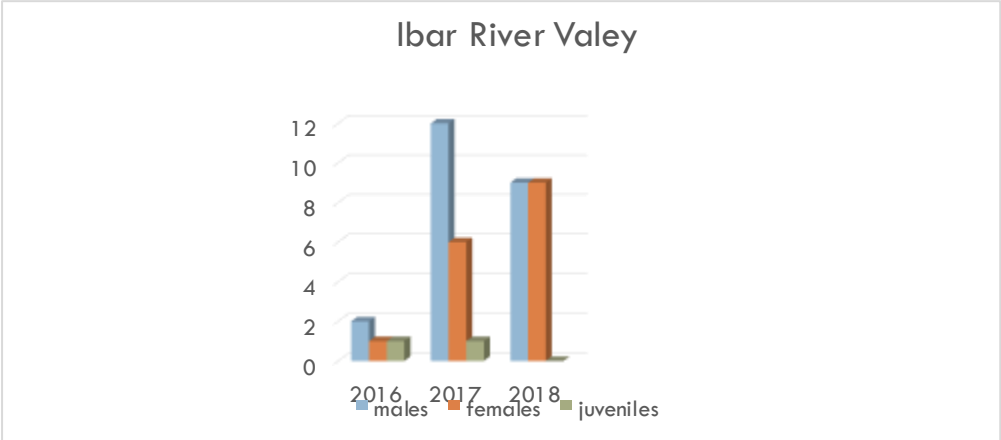


Fig.4. Sex and age detectability

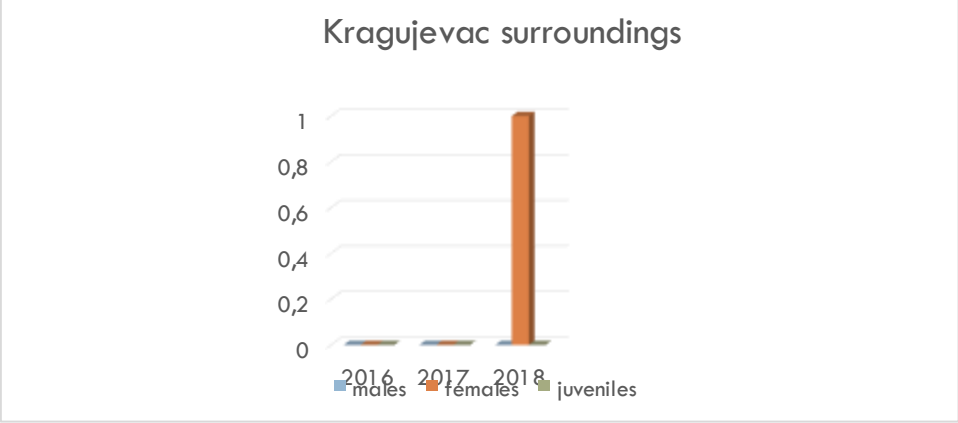


Fig.5 Sex and age detectability

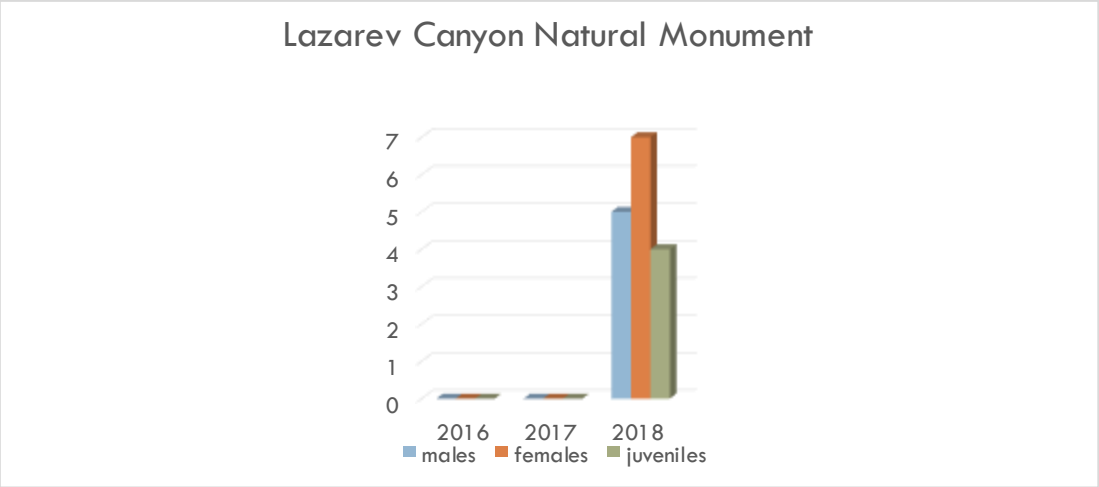


Fig.6. Sex and age detectability

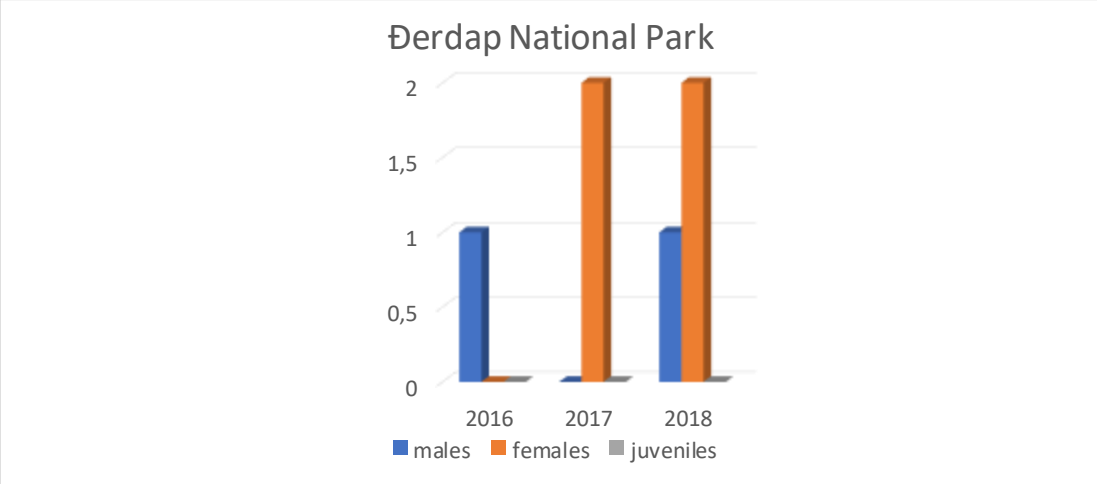


Fig.7. Sex and age detectability

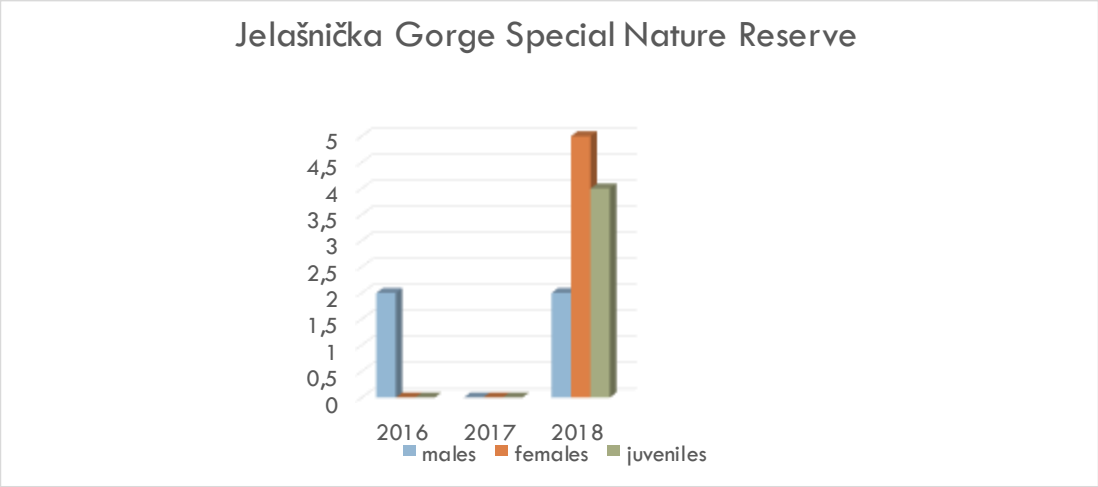


Fig.8. Sex and age detectability

I have calculated body condition index as body condition is important measure of animal fitness and overall health. I have used residual index which is calculated as the residual score from the general linear regression of log transformed mass against log transformed snout to vent length. I have calculated residual index separately for sexes and age groups. Main assumption in this method is that animal who weighs less than predicted mass for her size, will have negative body condition index and it is than considered that that animal is in poor condition. In our sample, 50.68% of measured animals had negative body condition index. For the populations where I had statistically big enough sample, I performed ANOVA analyses to check is there any interpopulation difference in body condition and I did not find any ($p>0.05$) (Fig.9).

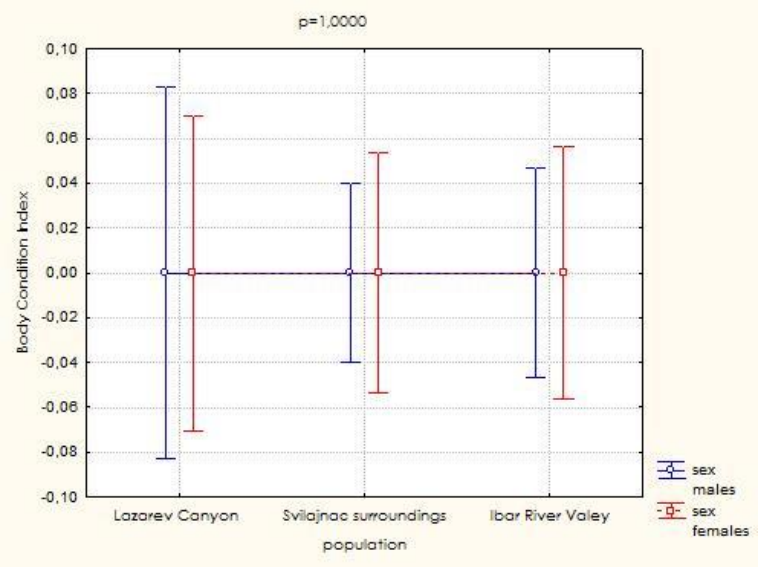


Fig. 9. Interpopulation difference in body condition index

Fig.10 Field work

2.GENETIC DATA

We investigated phylogenetic relations among vipers whose samples were collected by us and by our colleagues, and have also included available sequences from GenBank. We used mtDNK sequence of the 16S rRNA gene collected from 25 (47 with sequences from GenBank) individuals at 15 localities. Results showed that one individual from the locality where vipers were harvested and released during nineties, clustered with north-western clade, while the remaining 5 individuals from same locality clustered with north-eastern clade (for the description of the main genetic clades in this species see Ursenbacher et al., 2018). Also, one individual from another locality clustered with north-western clade. Based on the Ursenbacher et al (2018), the north-western clade does not inhabit Serbian territory but exact boundaries of the distribution of clades are not defined due to the lack of samples. Our findings could imply that presence of this clade in Serbia could be due to the natural contact zone between two clades or those individuals originated from other countries and occurre here due to previous release out of the place of origin. In conclusion, it is essential to continue our work because, even if this is natural contact zone and not result of the anthropogenic misconduct, it still further underlines importance of conservation measures. Namely, it is critical to maintain genetic diversity within this species as we have confirmed illegal harvesting, intentional killing, habitat fragmentation and pollution in Serbia and different responses on these threats in different populations often have a genetic component. Understanding genetic component with demographic data will enable us to have insight in population differences and prioritize actions in conservation. We wrote a manuscript about these findings which hopefully soon will be published.

3. Collection of buccal swabs and camera traps

We have collected buccal swabs for parasite analyses and we will analyze them soon. Our camera trap has already given some interesting data about this species thermoregulation and habitat use but we need more data to publish it. We have used some of this footage in our amateur educational video.

4.THREATS

Unfortunately, we have confirmed the same threats as in our previous projects with one additional:

1. Deliberate killing. We documented deliberate killing of nose-horned viper in Jelašnička Gorge and in surroundings of Svilajnac.
2. Habitat fragmentation and degradation. All habitats were polluted with human waste. Also, most habitats were fragmented with roads.
3. Harvesting. As we stated in our last project, in Krupanj we located population were this viper is harvested for venom supply.
4. Keeping nose-horned viper as pet. In Kragujevac and Kraljevo surroundings we have spoken with two men who keep wild nose-horned vipers as pets.

Fig.11 Polution with pesticide bottles

II Investigation of the venom markets

Nose-horned viper is regularly used for production of anti-venom for administrating in case of snake bite on the whole European territory and for all European species. Even though, according to literature review by Lamb et al. (2017) there are very limited pre-clinical comparative data and no randomised controlled trials assessing effectiveness of the

antivenoms against different viper species, it is still widely and effectively used. For this reason, this viper was heavily, uncontrollably harvested during the 80s and 90s in Serbia and surrounding countries. In Serbia, along 16 consecutive years, 9800 nose-horned vipers were sold to the Institute for Immunology and Virusology "Torlak" in Belgrade where they later all died due to improper care (Ajtic, 2009).

During our first Rufford project we have confirmed illegal harvesting for the venom supply in the village Sokograd in the Krupanj surroundings. Therefore, in this project we have started venom market investigation. We have:

- talked with the local collector in Krupanj surroundings,
- searched the available literature on anti venoms used in case of a snake bite of European vipers,
- searched the literature data in papers dealing with toxicology of nose-horned vipers venom in order to get information where the researchers obtained the venom.
- Searched on the internet for the available information about manufacturers of anti-venoms and other medical companies which sell raw venom to the manufactures and medical researchers
- -we have also sent e-mails to manufactures and other medical companies and called the manufacturer in Serbia and
- Surveyed the state and private hospitals in Serbia about the names of the manufacturers of the anti-venom they use

Here are the results:

1. Venom buyer in Serbia

We have talked with the local collector in Krupanj suroundings (village Sokograd) and he insisted that he sells the venom to the National Institute for Immunology and Virology ''Torlak' in Belgrade and that he has ''license'' for this. Even though this certainly was the case in the past, this is not true now. Namely, we have called the Institute Torlak and ask them if they purchase animals from Serbia and they said no. They confirmed that they obtain animals from import (mostly from neighbor country Montenegro, where this species is unfortunately unprotected). We have then contacted Institute for Nature Protection and asked them is it legal to purchase snakes in Serbia and they answered that there is a ban for collecting nose-horned vipers for several years now. In Institute for Nature Protection, Rastko Ajtić (the

herpetologist in charge) has tight relationships with the inspectors for nature and environmental protection and they monitor every imported snake. He is collaborating with Institute for Immunology and Virusology ''Torlak'' to possibly establish population in captivity where the specimens will be kept in proper conditions and their venom will be used which will contribute in dismissing the import.

Than during our second field trip in Krupanj, we tried to engage again with the local collector in Krupanj but he did not want to talk.

What is worth reporting, Krupanj is located on the state boundary with Bosnia and Herzegovina, where (according to the interviews in the Bosnian newspapers) the nose-horned vipers are sold for only 40-50 euros per animal to some pharmaceutical companies which produce anti-venom. The Bosnian collector said in the interview that he sells to Torlak in Belgrade (same as collector in Serbia told us) and to some pharmaceutical company in Slovenia (we were not able to find on the internet any information about any medical company in Slovenia which produces anti-venom). Therefore, there is a possibility that local collectors in Serbia and Bosnia sell to the same company and that they both are lying which company it is. Or it could be some sale mediator which does not tell them the truth.

2. Anti-venoms used in a case of the snake-bite available in the literature data and manufacturers of anti-venom and other medical companies which sell raw venom to the manufacturers and medical researchers

In the very comprehensive study by Lamb et al. (2017), authors listed 8 main manufacturers of anti-venom as follows: Biomed from Poland, Bulbio from Bulgaria, Microgen from Russia, Vetal Serum and Biological Products Manufacturing Industry and Commerce from Turkey, Torlak from Serbia, Microfarm from United Kingdom, Sanofi-Pasteur from France and Institute for Immunology from Croatia.

According to our search and sent e-mails to the manufacturers and other medical companies which sell raw venom, we present manufacturers of anti-venoms, their venom resources (where they were willing to answer) and information about their recognition by the World Health Organization as well as the companies who sell raw venom in the following table:

Name of institution	Country	Venom resource	Recognized by WHO	Commentary

Institute for Immunology and Virology ''Torlak''[1]	Serbia	Animals from import (mostly from Montenegro)	Yes	Production of anti-venom
Institute for Immunology[1]	Croatia	from captivity with some of them captured in Croatia	Yes	Production of anti-venom
Bulbio	Bulgaria	Not known	No	Production of anti-venom
Vetal Serum and Biological Products Manufacturing Industry and Commerce	Turkey	Turkey	No	Production of anti-venom
Sanofi-pasteur	France	Not known	Yes	Production of anti-venom
Vascera	Egypt	Not known		
Latoxan Laboratory	France	Italy and most Balkan countries and they also have livestock	/	This is a company which sells raw venom
Sigma-Aldrich Chemie GmbH	Germany and USA	From recently they have discontinued sell of nose-horned viper venom	/	This is a company which sells raw venom
FaviAmmodytes[3]	Romania	Breeding in captivity	/	This is a company which sells raw venom for

				cosmetics companies and they also produce their own cosmetics from this viper's venom
S.C. Viperin Invest S.R.L	Romania	Breeding in captivity	/	They sell raw venom
Vipers Family SRL	Romania	Breeding in captivity	/	They sell raw venom
Snake venom	Austria	Not known	/	They sell raw venom and there e-mail address is unavailable
Biomed[2]	Poland	/	Yes	Production of antivenom Use another viper species, *Vipera berus*
Microphar[2]	United Kingdom	/	Yes	Production of anti-venom Use another viper species, *Vipera berus*

[1]Both Insitutes for Immunology in Serbia and Croatia are currently in bad financial situation. Insitute for Immunology from Croatia lost license for selling the anti-venom at 2013. which is concerning as this Institution is one of the rare institutions that actually has established nose-horned viper population in captivity (in proper conditions) and most probably does not often purchase animals or venom from natural populations in comparison to the company Torlak in Serbia that haven't yet established population in captivity (in proper conditions) and does purchase the snakes.

[2]According to information on the internet, main manufacturers on the market for anti-venom are in United Kingdom and Poland, but their anti-venom is based on venom of different species of snake which is adder (*Vipera berus)* and not on the nose-horned viper and it is probably well controlled by the government.

[3]Besides this company, there are (according to the interview with the director of this company) 70 other companies (certified and uncertified) in Romania which sell raw venom to the cosmetic companies to very high price of: '' 800 Euros per gram…as this venom is used in anti-rheumatic ointments and facial wrinkle creams as it is well known that nose-horned viper's venom which is included in the facial creams acts deep within the tissues of the face's skin, thus eliminating wrinkles and oxygenating cells, and it represents a 100% natural non-invasive alternative to Botox''.

Based on our research the price for *Vipera ammodytes* venom goes from 100 euros to 800 euros per gram.

According to Beshkov (1993), in Bulgaria there are 40 nose-horned viper farms regulated by law and 20-25 illegal ones.

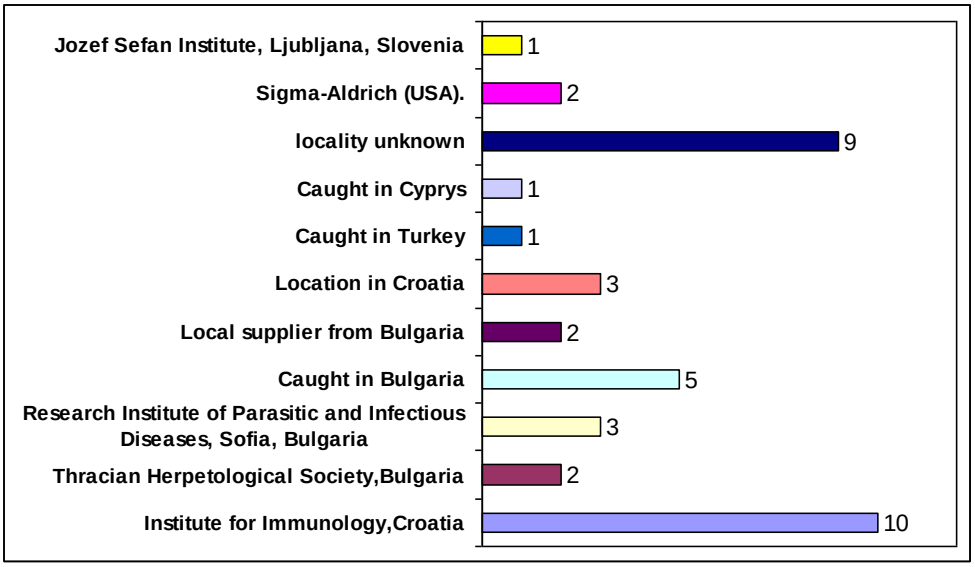
To the best of our knowledge there are no such farms in Serbia. There could be uncertified ones which are not known in the public, but the probability of their occurrence is low.

3. Manufacturers of anti-venoms used in hospitals in Serbia

Hospitals which were willing to answer us, named Institute for Immunology and Virology .,Torlak''as the manufacturer of the venom they use.

4. Sources of venoms in the scientific papers dealing with venom toxicology

We have analyzed available literature published from 1980. to 2018. and the following graph shows our results.



There were no vipers or venom from vipers from Serbia, but in 9 papers localities of used vipers/viper venoms are unknown.

Vipera ammodytes as pet

As this species is considered as an attractive pet we have conducted research about this problem too. And the results are devastating. Namely, this species is not very hard to catch as it is very lethargic. Also, this snake is very beautiful especially colorful morphs such are red, yellow, with wide pattern etc. Therefore, demand for specific morphs and supply for specific morphs is huge. The price of this viper goes from 70 euros for the babies to 800 euros to even 3000 euros for specific morphs. This species is even heavily displayed on the famous animal exhibition and fair TerraPlaza in Budapest, Hungary.

In Serbia, there are two people who hold or held these animals as pets, according to our research. One is from Kragujevac and other is from Kraljevo. They both claim that they hold them (usually one or two of them) for a certain period of time and then they release them. It is worth reporting that psychology of these people (they are extremely shallow minded) harden us to persuade them to not collect them. As this is a deadly viper, it boosts their ego to have specimens at home and ''show it off'' and they think that they know the best (even though they are not biologists) and they do not appreciate our advices. Law force in Serbia is not very focused on saving the endangered animals (only if there is a extremely strong lobi or financial gain) so that further hardens up our efforts, but we are getting some results and support from people surrounding these kind of people.

On the forums and Facebook pages we have found hundreds of pictures of nose-horned vipers held in captivity. Most of the vipers are from Montenegro, Croatia, Bosnia and Serbia. Pet owners are from different European countries: Germany, Switzerland, Netherlands. Some of them are kept in good conditions and some of them in bad. I have tried logging into forums about snake keeping and soon as I was start asking questions, admins banned me. I have also tried to send some interviews to the pet owners on FB page and most of them did not want to answer me or they told me that they breed vipers themselves or buy them from breeding places which is probably the truth for some of them but not for the majority as they post publicly on their respective pages where their vipers are from.

Main conclusion

Besides further investigations in other parts of Serbia in order to find other local collectors (if they exist) willing to talk and sending the report of our findings to the Institute for Nature Protection in Serbia, the only efficient way to find out who is buying the venom could be transboundary collaboration between scientists and government bodies in their respective countries, where we could collect the samples of vipers used for venom production and do genetic analysis in order to find their country of origin and try to react accordingly.

III EDUCATION ACTIVITIES

We have held several lectures in primary and high schools and to the mountaineer societies. There was radio show about our project activities on radio station ,,Zlatousti''.The children in schools and members of mountaineer societies were very interested to hear about snakes. We have filmed our amateur educational video where we speak about proper procedure in case of encounter with nose-horned viper and in the case of the snakebite, about different venomous and non-venomous snake species which inhabit Serbia and about importance of the snakes in general. We have uploaded it on You Tube: https://youtu.be/VHzXTJVq4QU and we have also put English translation for people from other countries within this viper's range. Based on our interviews with the local farmers we saw that some of them are slowly changing their opinion about this viper. We have concluded that repeated conservation actions and engagement with them can drastically change their behaviour. We also have nice following and engagement on our Facebook page (https://www.facebook.com/sacuvajmoposkoka).

We have also formed a blog where we write some interesting facts about snakes (https://snakecorner.wordpress.com/). The main threat to this viper is human (throw killing and harvesting). So, having people know the right information and having them understand the importance of preserving this species (and their impact of members of their own community) is a powerful tool in conservation.

Fig.12 Lecture at primary school

Fig.13 Lecture at Institute for Biological Research ,,Sinisa Stankovic'' in Belgrade

Important future steps

- It is from immense importance to continue our field research to obtain sample size large enough to satisfy statistical requirements to do and publish demographic studies. We have already collected considerable amount, but we lack more findings for our smaller populations. This is important for two reasons. First, our government bodies will have, for the first time, published population data for this species and will be able to react and even to monitor the populations which are in the protected areas. Second, we are using methods which are easily repeated on any other populations of nose-horned vipers so the colleagues from surrounding countries can use them in their respective countries and therefore our results will be comparable.
- Regarding our previous step, it is also important to widen our research area to as many locations (populations) as possible to get the wider picture and more informations about populations, their habitats and threats.
- Large scale genetic analyses are very important at this point as they can answer us quicker on important questions than monitoring.

- Further, radiotelemetric studies would be very useful to give us information about habitat use, reproduction patterns, growth rate, data about natality and mortality. This is non-invasive method which produces important data which are used in any conservation planning.
- Education must be continued. It would be very useful that besides our lectures and our documentary, we do series of other documentaries about snakes in Serbia and photograph exhibit with symbolic prizes for participants.