THE VIPERS (REPTILIA: VIPERIDAE) FROM EASTERN ROMANIA: ECOLOGY, GENETIC DIVERSITY, AND CONSERVATION.

-Summary of PhD Thesis-

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Introduction

The majority of snake species present a series of traits that increase their pronenes to extinction (e.g. Seigel et al. 1987). Additionally, in numerous regions of the world, the probability of extinction of certain snake species has been dramatically increased by various anthropic factors: habitat destruction and deterioration, introduction of invasive species, environmental pollution, overcollecting for commercial purposes and direct persecution out of fear or superstition (E.g. Gibbons et al. 1999). Most species suffer from habitat fragmentation and declining population sizes, resulting in a loss of genetic diversity. This can lead to an increase the degree of inbreeding and a decrease in the adaptive potential (Frankham 2005, Ursenbacher et al. 2009). Although aspects regarding the genetic diversity in snakes, some researchers have discovered a very low genetic diversity in several species, in isolated populations (e.g. Madsen et al. 2004, Ursenbacher et al. 2009).

Objectives

The current thesis presents the results of investiongations upon the ecology, evolution and the effects of envenomation of vipers from eastern Romania, having certain implications for their conservation.

In the first part of the thesis, I present a statistical analysis of the scientific literature dealing with the Romanian herpetofauna, with the aim of describing the dinamics of research trends as well as for identifying less investigated topics. Subsequently, a characterization of the family Viperidae, implicitally the vipers of Romania, is made, thus presenting the current state of knoweledge on the topic.

In the second part of the thesis, the personal research results are presented, having the following main objectives:

1. Study of the phylogeography of eastern Romanian vipers with the aim of clarifying certain taxonomical and historical biogeograpy problems, and for identifying evolutionary management units for conservation.
2. Study of the current situation and distribution of the Moldavian meadow viper (*Vipera ursinii moldavica*), the most threatened viper taxon from eastern Romania.
3. Study of altitudinal variations of habitat characteristics of the adder (*Vipera berus berus*), the most widespread viper taxon in Romania
4. Long-term study of population ecology (population structure, activity patterns, and reproductive ecology) of some viper populations from eastern Romania.
5. Study of local and systemic effects of adder (*Vipera berus berus*) envenomations.

3. Molecular phylogeography of eastern Romanian vipers: implcations for systematics and conservation.

The present study aims to:

(i) Analyze the genetic variability of the *Vipera ursinii* complex in Europe with the aim of establishing the phylogeographic structure of the populations, the phylogenetic relationships between them in order to answer several systematical and conservational problems.

(ii) Contribute to the phylogeography of the nose-horned viper (*Vipera ammodytes*) by inlcuding the first sequences of *V. a. montandoni* from the subspecies' *terra typica* in the phylogenetic analysis.

(iii) To investigate the phylogeraphic position within the *Vipera berus* complex of the populations from the Central Moldavian Plateau, populations identified morphologically as *Vipera (berus) nikolskii*.

Material and methods

For the *Vipera ursinii* complex, the NADH dehydrogenase subunit 4 (ND4) was amplified and sequenced. For the *Vipera berus* complex and for *Vipera ammodytes,* the cytochrome *b* was also amplified.

Results and discussion

The *Vipera ursinii* complex

Following the alignment and analysis of sequences, we extracted a total number of 10 haplotypes from 27 individuals belonging to the *V. ursinii* complex. Withing the complex, there are two large phlogenetic clades, one comprising of *Vipera renardi* (Ukraine and Russia) and one of all *V. ursinii* s.s.

(Romania, Hungary, Montenegro, Albania and Italy). Populations of *V. u. Moldavica* appear to have a very low genetic diversity.

The *Vipera berus* complex

The phylogenetic reconstructions confirms the determination of the vipers from the Central Moldavian Plateau as *Vipera (berus) nikolskii.* Furthermore, the study confirms the validity of this taxon as a distinct evolutionary lineage.

Vipera ammodytes

The haplotype extracted from Romanian Dobrudja is identical to one previously identified from Bulgaria (*V. a. montandoni*), being grouped in the same clade as the west-Bulgarian and north-Greek haplotypes.

4. Situation and distribution of *Vipera ursinii moldavica* in Romania: the present and future of a critically endangered taxon.

Through the present study, we aimed to:

(i) Model the bioclimatic niceh of the Moldavian meadow viper (*V. u. moldavica*) utilizing the MaxEnt software, with the aim of identifying new potential populations.

(ii) Model the hypothethical bioclimatic niche of the Moldavian meadow viper (*V. u. moldavica*) according to two climate change scenarios, in order to investigate the effectiveness of Natura 2000 network in preserving the taxon.

(iii) To conduct field surveys in order to investigate the distribution and current situation of the Moldavian meadow viper (*V. u. moldavica)* populations, especially from the view points of habitat size and anthropic impact.

Material and Methods

For modeling the bioclimatich nich of the species in Romania, we used all viable records for the species indicated by Krecsak & Zamfirescu (2008). For georeferencing and

realizing the model, we used the softwares ArcGIS 9.3, MaxEnt 3.3.3 and the WorldClim Database, version 1.4.

During field research, we conducted exhaustive and intensive herpetofaunal surveys in numerous regions of eastern Romania in the 2002-2014 periods.

Results and Discussion

The strongest model generated by MaxEnt indicated a strong correlation between the potential distribution of the meadow viper in Romania and three climatic variables. Except for the areas where populations have already been identified (the Danube Delta, the Moldavian Plain and the Cluj County area), the generated model identified two other potential areas of occurence: Vaslui County with the Bârlad Valley and the plains and coridors along the Prut river.

In Iaşi County, we confirmed the presence of the two previously know stable populations. Furthermore, we reconfirmed the presence of the population from the Ciritei Valley – from which the species was only known from a single dead specimen collected in 2001 (Krecsak et al. 2003). Aditionally, we identified four new habitats of *V. u. moldavica*, in the relative vicinity of the known ones.

The only populations of *V. u. moldavica* that we identified in Dobrudja, were recorded in the Danube Delta, on the levees of Letea, Câşla Vădanei (Sfântu Gheorghe) and Perişor-Periteasca.

6. Altitudinal variations of the characteristics of adder (*Vipera berus berus)* habitats in the Northern Eastern Carpathians

The present study aimed to answer the following questions:

i. Are there important altitude-associated differences between the habitats that *Vipera berus* inhabits in the Northern Eastern Carpathians?

ii. What characteristics are associated with which altitude groups?
iii. Is the complexity of the habitats influenced by habitat?

Material and Methods

We studied 25 sites from the northern Eastern Carpathians, sites from which *Vipera berus* had been recently recorded from (Covaciu-Marcov et al. 2008). Habitat characteristics of each site were studied, being recorded a series of abiotical and biotical variables. The similarity between the sites was studied based on all recorded characteristics and via a multivariate hierarchal clustering analysis.

Results and Discussion

The results of the multivariate analysis of similary have split the habitats in two groups, correspondinc perfectly to altitude differences. One is formed by the only lowland site while the other is formed by all other montane habitats. The results of the current study indicate that, despite *Vipera berus* is a highly spread species that inhabits diverse habitat types, it is not a true generalist. The adder selects different habitats, regardlesss of altitude, that offer a relatively narrow spectrum of temperature and humitidy/ or precipitations.

7. The adaptive value of chromatic polymorphism in a population of *Vipera berus berus* from the Northern Eastern Carpathians

The present study aimed to investigate characteristics of a color polymorphic population of *Vipera berus* from the Northern Eastern Carpathians, in order to test a series of hypotheses regarding the maintanance of color polymorphism.

Material and methods

Field investigations for the current study were conducted during 2002-2014, in site A, in each year from March to October. Since 2004, we measured the total length of individuals with using measuring tape. The statistical significance of the differences betweenthe length of the color mophs were tested using two-way ANOVA, followed by multiples Tukey post-hoc comparisons.

Results and discussion

Melanistic individuals were relatively rare, representing only 15.58% of the total number of vipers. Melanism was more frequently encountered in males but this association was not significant. There were significant differences between the size of males and females but there were no inter-morph differences within sexes. The structure of the population is atypical compared to literature data. We consider that color polymorphism is maitained through nonadaptive mechanisms in the studied population.

8. Activity patterns and habitat selection in vipers from eastern Romania

The present study aimed to investigate inter-sexual, ontogenetic and seasonal shifts in patterns of activity and habitat selection in populations belonging to all viper taxa from eastern Romania: (*Vipera berus berus, V. (b.) nikolskii, V. ursinii moldavica* şi *V. ammodytes montandoni*).

Material and methods

The study areas for all populations are described in chapter 3. For each viper individual observed, we recorded a series of biologial and ecological traits, the frequency of values being subsequently analyzied via Simple and Multiple Correspondence Analysis.

Results and discussion

Vipera berus berus: During spring, all sex-age category ov vipers were associated with a wide range of variable states. During summer, there is a clear separation between the activity patterns of adult males and females.

Vipera (berus) nikolskii: Multivariate correspondance analysis has shown that, during spring, males are associated with a wide range of character states while adult females and immature individuals are associated with a narrower range of variable states. During summer, there are clear sex-age related differences.

Vipera ursinii moldavica: In Moldavia, during spring, vipers tend to concentrate around the hibernation habitats, which are represented by small bluffs with sparse vegetation cover. In the Danube Delta, during spring, vipers concentrate on the highest sand dunes, that are covered by *Juncus litoralis* and *Juncus maritimus*. At the start of summer, vipers disperse towards more varied microhabitats, except for the females from Sfantu Gheorghe that continue occupying higher elevations. Females continue feeding throughout pregnancy.

Vipera ammodytes montandoni: During spring, adult snakes no not feed, recent feeding atempt being detectable only in imature females. The Multiple Correspondence Analysis has indicated that adult and immature males are associated with a broader range of variable states. During summer, both males and females were associated with broad ranges of variable states but very different ones.

9. Reproductive ecology of the vipers of eastern Romania

The aim of this study was to investigate reproductive characteristics of female vipers (*Vipera b. berus, V. (b.) nikolskii* and *V. ursinii moldavica)* from eastern Romania.

Material and methods

The reproductive state (gravid or nongravid) of female vieprs was detected dirrectly in the field. With the aim of investigating neonate characteristis and reproductive success, a sample of gravid females was taken into the lab for temporary study and kept in individual terraria until after parturition.

Results and discussion

Vipera berus berus: The female vipers from the studied population present (at least during certain conditions) an annual reproductive cycle. The Spearman nonparametric correlation has indicated no significant relationships between fecundity and maternal body size.

Vipera (berus) nikolskii: Reproductive females from this taxon are considerably larger than those of *V. b. berus.* The reproductive cycle for females is biennial as we have usually observed both gravid and nongravid females during the summer.

Vipera ursinii

The subspecies (*moldavica* and *rakosiensis*) differed significantly regarding litter size (those from Transylvania being superior). There were also significant differences with regards to some characters between the Moldavian and Danube Delta populations of *V. u. moldavica.*

10. Clinical and biological data regarding common adder (*Vipera berus)* bites from north-eastern Romania

The aim of the study was to describe the epidemiological, clinical and biological data of *Vipera berus* bites from north-eastern Romania.

Material and methods

We realized a retrospective study on common adder bites that were treated at the "Unitatea de Primire Urgenţe a

Spitalului Județean de Urgențe ”Sf. Ioan cel Nou” Suceava in the period 2003 – 2011.

Results and discussion

Within the series of patients studied, a very rare manifestation of neurotoxity was reported in a 9 year old girl.

General conclusions of the thesis

1. The molecular phylogepgrahical studies, which included all taxa of the *Vipera ursinii* complex from Europe, concluded that the *Vipera renardi* taxon should be considered a distinct species and that some of the subspecies of *Vipera ursinii* could also merit this status. The genetic study showed that in eastern Romania, the meadow viper has a very low genetic diversity, being especially threatened in the region.
2. The phylogepgraphical studies conducted on *Vipera ammodytes* and on the *Vipera berus* complex have showed that the populations of vipers formerly identified as *Vipera (berus) nikolskii* are, indeed, a separate philogenetic clade, distinct from other forms of the complex, and that the Dobrudjan *V. ammodytes* populations are inclued in the same clade as the ones from Bulgaria and northern Greece.
3. The study of the bioclimati niche and current distribution of the *Vipera ursinii moldavica* populations has indicated that the niche fundamental niche of the taxon will not dicrease according to global climate change scenarios, and that the extant populations are severly threatened, especially by habitat destruction.
4. The analysis of altitudinal variations of habitat characteristics for populations of *Vipera berus* from the Northern Eastern Carpathians represents the first study of this type conducted upon a European viper. It has

indicated a clear separation of three altitudinal groups of habitats that are used by *Vipera berus*. Therefore, despite its wide range, the species is specialized to areas with high precipiations or humitidy values and lower temperatures.

5. The study of color polymorphism in a *Vipera b. berus* population has indicated significant differences between certain ecological characteristics (sex-ratio, body size, melanism) between the studied population and other populations of the species, described in the literature. Thus, the sex-ratio is slightly biased towards the females, adders have a small body size and melanism is most frequent in males. Unlike previous studies which have revealed an adaptive role of melanism, we conclude that in our population, color polymorphism is maintained via nonadaptive mechanisms.
6. The study of activity patterns and habitat selection in several viper populations belonging to all taxa from eastern Romania, has indicated seasonal, inter-generic and ontogenetic differences in microhabitat use. These results have been observed in all studied viper population and represent important information for management and conservation of the populations.
7. The study on the reproductive ecology of eastern Romanian vipers have indicated relativel similar characteristics to those presented by previous studies, conducted on other population. The annual reproductive cycle observed in the *Vipera berus berus* population was the most remarkable observation, being extremely rare in this species.
8. The study of the clinical and biological effects of *Vipera b. berus* bites have indicated that pai and edema are the most frequent local simptoms while hipotension and leucocitosis are the most common systemic simptoms.

We also reported a very rare case of neurotoxicity following enveomation.

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