First report on the group mating of *Vipera ammodytes* (Linnaeus, 1758)

Many snake species exhibit courtship behavior and ritualized combats between males prior to mating (Vitt & Caldwell 2014). During reproduction, many species engage in aggregation behavior during mating, forming a mating ball (e.g., *Thamnophis sirtalis* (Linnaeus, 1758), *Natrix* spp. (Whittier et al. 1985, Luiselli 1996). Nevertheless, to the best of our knowledge, social aggregations during mating were not described in the genus *Vipera*. In this genus, females usually mate with one to two males at the same time. Prior to courtship, males usually engage in male-to-male combats, and the winner of the battle gets to court and mate with the female. Often the males display female guarding behavior between the copulation events (Andrén 1986, Madsen et al. 1993, 1996).

Vipera ammodytes (Linnaeus, 1758) is a viviparous venomous snake that ranges from southern Austria and northern Italy through the Balkan Peninsula to Asia Minor (Crnobrnja-Isailović & Haxhiu 1997). The species has a distinct sexual dimorphism most prominent in the tail length (males have a longer and wider tail), the number of subcaudal scales (males have a higher number) and the ventral scale width (females have wider scales) (Tomović et al. 2002). There are very few studies regarding aspects of the nose-horned viper's reproductive biology (Beshkov 1977, Luiselli & Zuffi 2002, Strugariu et al. 2011, Dyugmedzhiev et al. 2018, Anđelković et al. 2021, Tomović et al. 2022). Mating usually occurs during April and May (Speybroeck et al. 2016). It was also suggested that this species displays a biennial reproductive cycle (Luiselli & Zuffi 2002).

Here we report the first-ever observation (to the best of our knowledge) of the group mating behavior of *V. ammodytes*.

We observed a group mating of the nose-horned vipers on the 16th of May 2022 at 13:40h (local time) near Velika Morava River in Central Serbia during our regular monitoring, which has been conducted since 2016. The habitat consisted of the edge of an oak forest, some shrubs, and a non-paved village road. We measured the captured vipers' length, mass, and cloacal temperature. Further, we measured substrate, air temperature, and humidity at the mating place. Cloacal temperature, environmental temperatures, and humidity were measured (environmental temperatures were compared between different devices to avoid error) with a digital cloacal thermometer (Dostmann digital Einstich-Thermometer with an accuracy of ±0.1 °C), a data logger (Voltcraft multi data logger with an accuracy of ±2 °C for temperature, ±5 rF% for humidity) and non-contact infrared thermometer (ExoTerra). Environmental parameters were measured at the exact spot where the animals were found (the cloacal thermometer was used in the shade).

We found five males and one female mating (Fig. 1. and Fig. 2.) on the leaf debris near the edge of the oak forest. The vipers were partially hidden in the shade. The males were lying on top of each other, and the female was positioned on the bottom (Fig.1). We took a photograph, then walked away from the vipers, and continued to observe them at a distance of 5.10 m (measured with laser meter) from the snakes. During the observation, we took some photographs from a distance (Fig. 2.). When copulation was completed, approximately at 14:00h (local time), and the vipers started departing, we managed to catch four males while one male and the female escaped. Data from snakes' measurements are presented in Table 1. The recorded temperatures were 46.5 °C, 46.3 °C, and 44.9 °C for the substrate, the air at 5 cm above the ground, and the air at 60cm; humidity was 43.8%.

Table 1. Body size measurements and cloacal temperatures of the captured and measured male *Vipera ammodytes*

Individual	No.1	No.2	No.3	No.4
Total length (cm)	70.5	59.1	68.2	62.5
Tail length (cm)	8.5	7.2	8.5	7
Mass (g)	120	75	135	125
Cloacal temperature ◦C	26.6	32.8	30.1	30.7

Figure 1. Group mating between five males and one female *Vipera ammodytes* observed on 16 May 2022.

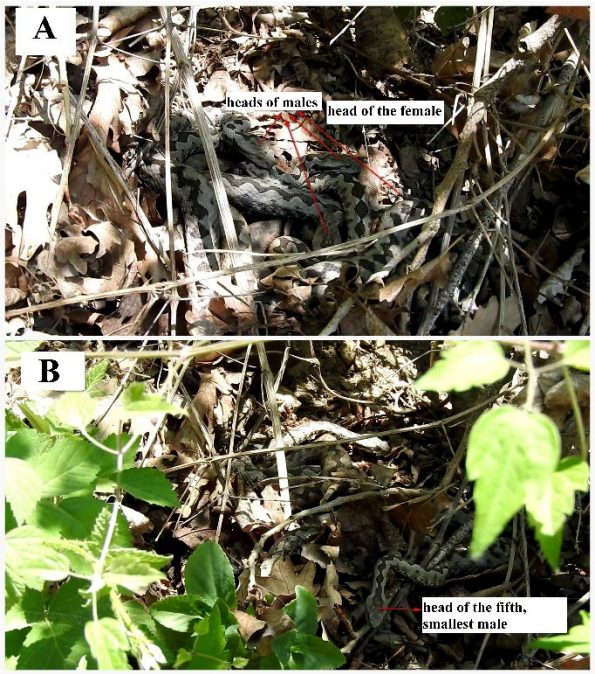

Figure 2. Nose-horned Vipers departing after copulation. In 2A, the heads of the four bigger males and the female are indicated with red and white arrows, respectively. On 2B, the head of the smallest male is indicated with a red arrow.

We performed the necessary measurements on the vipers approximately 70 meters away from the place of their capture, in a completely shaded place, to avoid exposing the animals to high temperature and strong direct sunlight while exposing them already to the stress of handling, measuring, and taking swabs. After the measurement procedure on the captured males was completed and we returned to the place of capture and started releasing them (appr. 45 minutes after capture), we noticed that the female started mating with the smaller male who had previously escaped. When we released the measured males, the female started retreating with the small male still attached to her cloaca. To not further disturb the copulation, we departed the spot of this finding.

To the best of our knowledge, this is the first published report on group mating of *V. ammodytes*. In our field experience of the last 30 years, we have not encountered group mating in this species. The reported behavior could be due to several different causes and/or combinations of them:

(a) This behavior could be more common but remained unnoticed/unpublished in the literature. Reproduction in snakes comes with costs such as death, disease transmission, etc.; therefore, they need to alter their reproductive behavior due to different ecological factors. Those alterations include variations in seasonal timing of reproduction, allocation of total energy to reproduction, variation in the frequency of reproduction, variation in offspring size and quality, number of mating events, mate quality, and, for males, also sperm competition (Shine 2003 and references therein). Considering the general plasticity that snakes may exhibit (Krohmer & Jurkovic 2020 and references therein), specific abiotic or biotic factors could contribute to the vipers' shifting reproductive behavior. Those factors could include skewed sexual ratio and/or females being difficult to find, predator influence, anthropogenic pressure, and thermal conditions (especially if we consider the reported environmental temperatures in this study).

(b) Although *V. ammodytes* displays male combat, it was not observed in this particular case. The possible causes could include the similar size, and thus supposed similar strengths, of the four captured males; this might prolong the time involved in the combat, and make it too risky, particularly under the observed combination of different factors (high environmental temperatures observed, the continuous exposure to predation risk and the risk of road killing due to the vicinity of the local road). The costs of the male combat would outweigh the benefits from the lesser sperm competition if combat lasted too long. For example, it was shown in captive *Montivipera raddei* that the two males engaged in a very long-lasting combat were of similar size, and there was no obvious winner at the end (Ettling & Marfisi 2002).

(c) Further, some males in this study could display female mimicry, as the males who display this behavior benefit from being warmed from other males; the "she-males" are in this way also protected from predators (Shine et al. 2000, 2001). To answer this question, further analysis of chemical and pheromone sexual communication is needed.

(d) Males of several snake species usually mate with fatter rather than thin females, probably due to the correlation of female's size and body shape to the size and quality of the clutch (Shine 2003 and references therein). Unfortunately, we did not manage to measure the female, but it was obvious to the naked eye that this was a large individual and, therefore, might be preferred by the males.

Of course, the involvement of several males in the mating event does not necessarily imply insemination of the female by all of them, as larger males can push aside the tails of smaller ones and, in that way, allow more space for themselves for the mating (Shine 2003 and references therein). Regarding the continued mating of the female with the smaller male, this kind of behavior was previously noted in *Vipera berus,* where small males wait nearby for the departure of the larger male (Shine 2003 and references therein).

The substrate and ambient temperatures at the time of the observation were much higher than the species is known to be active at (Dyugmedzhiev et al. 2021 found that *V. ammodytes* was active only up to 35 °C). This may signal an urgency for the vipers to mate at this particular habitat, as they are ready to risk mating at such high temperatures. This urgency might be due to various abiotic factors (such as generally warm weather) or biotic ones, such as a skewed sex ratio in the population.

In conclusion, further studies are needed to determine the cause, frequency of occurrence, and possible consequences of group mating in *V. ammodytes*.

Acknowledgments

TČ is grateful to Dr. Angel Dyugmedzhiev for his comments and suggestions regarding the manuscript. The fieldwork was kindly funded by The Rufford Foundation (grants no: 19578-1 and 23392-2) for TČ. TČ and JCI were also supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia – contract no. 451-03-68/2022-14/200124 (2022). JCI was additionally funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia – contract no. 451-03-68/2022-14/200007 (2022).

References

Andrén, C. (1986): Courtship, mating and agonistic behavior in a free-living population of adders, *Vipera berus* (L.). Amphibia-Reptilia 7(4): 353-383.

Anđelković, M., Nikolić, S., Tomović, Lj. (2021): Reproductive characteristics, diet composition and fat reserves of nose-horned vipers (*Vipera ammodytes*). Herpetological Journal 31: 151-161.

Beshkov, V. (1977): A study of biology and ecology of the snakes in Maleshevska Planina Mts. (Southwestern Bulgaria). III. On the food and reproduction of the Nose-horned viper (*Vipera ammodytes meridionalis* Boulenger). Ecologia, Sofia 4: 3–12 (in Bulgarian).

Crnobrnja-Isailović, J., Haxhiu, I. (1997): *Vipera ammodytes*. pp. 384-385. In: Gasc, J.P., Cabela, A., Crnobrnja-Isailović, J., Dolmen,D., Grossenbacher, K., Lescure, J., Martens,H., Martinez Rica, J.P., Maurin, H., Oliveira, M.E., Sofianidou, S., Veith, M., Zuiderwijk, A. (eds.), Atlas of Amphibians and Reptiles in Europe. Societas Europaea Herpetologica and Museum Nationall d' Histoire Naturelle, Paris.

Dyugmedzhiev, A., Tzankov, N., Naumov, B.Y. (2018): A case of abnormal pregnancy in *Vipera ammodytes* (Linnaeus, 1758) (Reptilia: Viperidae) from Bulgaria. Acta Zoologica Bulgarica 70 (2): 277-282.

Dyugmedzhiev, A., Naumov, B., Tzankov, N. (2021): Thermal ecology of the Nose-Horned Viper (*Vipera ammodytes* (Linnaeus, 1758)) under natural conditions. North-Western Journal of Zoology 17(1): 44-56.

Ettling, J., Marfisi, A. (2002): Male combat in two species of mountain vipers, *Montivipera raddei* and *M. wagneri*. pp.163-166. In: Schuett, G.W., Hoggren, M., Douglas, M.E., Greene, H.W. (eds.), Biology of the vipers. Eagle Mountain Publication, Eagle Mountain, Utah.

Krohmer, R.W., Jurkovic, J. (2020): Neuronal plasticity in the forebrain of the male red-sided garter snake: Effect of season, low temperature dormancy, and hormonal status on dendritic spine density. Physiology & Behavior 215: 112789.

Luiselli, L. (1996): Individual success in mating balls of the grass snake, *Natrix natrix*: size is important. Journal of Zoology 239(4): 731-740.

Luiselli, L, Zuffi, M. (2002): Female life-history traits of the aspic viper (*Vipera aspis*) and sand viper (*V. ammodytes*) from the Mediterranean region. pp. 279–382. In: Schuett, G.W., Hoggren, M., Douglas, M.E., Greene, H.W. (eds.), Biology of the vipers. Eagle Mountain Publication, Eagle Mountain, Utah.

Madsen, T., Shine, R., Loman, J., Håkansson, T. (1993): Determinants of mating success in male adders, *Vipera berus*. Animal Behaviour 45(3): 491-499.

Madsen, T., Stille, B., Shine, R. (1996): Inbreeding depression in an isolated population of adders *Vipera berus*. Biological Conservation 75(2): 113-118.

Shine, R., O'Connor, D., Mason, R.T. (2000): Female mimicry in gartersnakes: behavioural tactics of 'she-males' and the males that court them. Canadian Journal of Zoology 78: 1391-1396.

Shine, R., Phillips, B., Waye, H., LeMaster, M., Mason, R. T. (2001): Advantage of female mimicry to snakes. Nature 414: 267.

Shine, R. (2003): Reproductive strategies in snakes. Proceedings of the Royal Society of London, Series B: Biological Sciences 270: 995-1004.

Speybroeck, J., Beukema, W., Bok, B., Van der Vort, J. (2016): Field guide to the amphibians and Reptiles of Britain and Europe. Bloomsbury Publishing.

Strugariu, A., Zamfirescu, S.R., Gherghel, I., Sahlean, T.C. (2011): Unusual early parturition in temperate region viviparous snakes during the atypically hot summer of 2007. Herpetologica Romanica 5: 43-49.

Tomović, L., Radojičić, J., Dzukić, G., Kalezić, M.L. (2002): Sexual dimorphism of the sand viper (*Vipera ammodytes* L.) from the central part of Balkan Peninsula. Russian Journal of Herpetology 9(1): 69-76.

Tomović, Lj., Anđelković, M., Golubović, A., Arsovski, D., Ajtić, R., Sterijovski, B., Nikolić, S., Crnobrnja-Isailović, J., Lakušić, M., Bonnet, X. (2022): Dwarf vipers on a small island: body size, diet, and fecundity correlates. Biological Journal of the Linnean Society 137: 267-279.

Vitt, L.J., Caldwell, J.P. (2014): Herpetology. An introductory biology of Amphibians and Reptiles. Fourth Edition. Elsevier, Amsterdam.

Whittier, J.M., Mason, R.T., Crews, D. (1985): Mating in the red-sided garter snake, *Thamnophis sirtalis parietalis*: differential effects on male and female sexual behavior. Behavioral Ecology and Sociobiology 16(3): 257-261.

Keywords: Nose-horned Viper, Viperidae, copulation, reproductive behavior, Serbia.

Article No.: e237502
Received: 15 October 2022 / Accepted: 12 December 2022
Available online: December 2022 / Printed: June 2023

Tijana ČUBRIĆ[1]*, Jelka CRNOBRNJA-ISAILOVIĆ [1,2]

1. Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18 000 Niš, Serbia
2. Department of Evolutionary Biology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Despota Stefana 142, 11000 Belgrade, Serbia
* Corresponding author: T. Čubrić, E-mail: tijana.cubric@pmf.edu.rs