Ticks (Acari: Ixodidae) on Nose-horned Vipers, *Vipera ammodytes* (Linnaeus, 1758), at the Danube's Iron Gates, Serbia, with a brief review of tick infestations on European vipers

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Parasitiform mites (Acari) are frequent ectoparasites of ecto- and endothermic terrestrial vertebrates. At least 242 species of Acari have been recorded as permanent parasites of reptiles (Fajfer, 2012), with hematophagic ticks (Ixodida) particularly common. Reptiles are even important reservoirs for tick-borne human pathogens, such as the spirochaete Borrelia burgdorferi, the cause of Lyme borreliosis (Mendoza-Roldan and Colella, 2019; Mendoza-Roldan et al., 2020), and hematophagic ticks are frequently found parasitizing lizards. This becomes particularly evident when considering that mite pockets, small skin invaginations along the gular, axillar, inguinal, and postfemoral regions that should prevent ectoparasites from attaching more widely and causing more damage, have evolved in at least five different lizard families (Arnold, 1986).

Snakes, on the other hand, lack mite pockets or equivalent structures and are also less often parasitized by hematophagic ticks (Bury et al., 2020). Even though ixodid ticks have been recorded from snakes in the wild both in the tropics (e.g., Pandit et al., 2011; Oda et al., 2022), where ecological correlates of tick parasitism have been demonstrated (e.g., Natusch et al., 2018), and in temperate zones (e.g., Takahashi et al., 2012; Tsapko, 2017), where legless squamates show an extremely low prevalence of ticks in contrast to lacertids, birds, or smaller mammals (Bury et al., 2020). Thus appears to suggest that snakes have a strong capacity for avoiding tick infestation, but to date there has not been any research to investigate this. With records of tick infestations still scarce for many European snake species (Bury et al., 2020), we here report records of hematophagic ticks (Acari: Ixodidae) on two individuals of *Vipera ammodytes* at the Danube's Iron Gates in Serbia, and briefly review other known cases of ticks infesting European viperid snakes.

During a field trip as part of the 21st Congress of the Societas Europaea Herpetologica in Belgrade, we visited the right side of the Iron Gates, the narrowest part of the Danube River (44.6062°N, 22.2822°E, elevation 320 m) on 4 September 2022. This area is an important one for amphibian and reptile diversity in Serbia and was proclaimed as Đerdap National Park in 1974 (Crnobrnja-Isailović et al., 2015). During a hike to the Ploče Viewpoint guided by rangers of the national park, we uncovered two juvenile *Vipera ammodytes* (estimated total length 40 cm each) along the path in the debris of a small rockslide at ca. 15:30 h (Fig. 1). We photographed both individuals, which were released at the site of the encounter.

The head region of both vipers was found to have ticks embedded in the skin. In one individual, the engorged tick was positioned below and slightly in front of the eye, above the fourth supralabial scale (Fig. 2A). In the second individual at least eight ticks, apparently of different life stages (nymphs, adults) based on their sizes, were attached laterally in the posterior neck region (Fig. 2B).

The ticks infesting these vipers could be identified as members of the genus *Ixodes* due to the absence of festoons (in contrast to *Haemaphysalis*; Estrada-Peña et al., 2018). Ixodid ticks have been reported for *V. ammodytes* before, with *Haemaphysalis sulcata* (listed as *H. cholodkovskyi*¹) and *H. punctata* (as *H. cinnabarina punctata*¹) from former Yugoslavia (Oswald, 1939,

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¹ *Haemaphysalis cholodkovskyi* is considered a synonym of *H. sulcata* (Abbassian-Lintzen, 1960; Guglielmone et al., 2014), while *H. cinnabarina* is a neotropical tick species found only in Brazil (Barros-Battesti et al., 2008).



Figure 1. A small rockslide close to a hiking path in the Iron Gates area of Đerdap National Park, Serbia, where two juvenile *Vipera ammodytes*, infested with ixodid ticks, were found. The red arrow points to the same juvenile individual depicted in Fig. 2A. Photo by Thore Koppetsch.

1941), Ixodes sp. from Greece (Pantazis, 1947; Doss et al., 1974), and H. marginatum from Romania (Mihalca et al., 2012). Within the genus Ixodes, the castor bean tick (I. ricinus) is common across Eurasia, and its larvae and nymphs have been recorded on tortoises, lacertid lizards, grass snakes, and at least three viperid snakes, Vipera berus, V. renardi, and V. ursinii (Lewin and Grabda-Kazubska, 1997; Lenders, 2013; Tsapko, 2017; Orlova et al., 2022). Three other tick species (H. inermis, H. otophila, I. ricinus) have been observed on V. ursinii in the former USSR (Serdyukova, 1956; Doss et al., 1974), with H. inermis also reported for V. renardi (Tsapko, 2017). Haemaphysalis parva was reported to occur on V. renardi by Tsapko (2017), and H. punctata was also recorded on V. aspis by Neumann (1901, 1911, cited in Doss et al., 1974) and Nuttall and Warburton (1915; cited in Tovornik and Brelih, 1980). It is a testament to the relative rarity of tick infestations on vipers that Tovornik and Brelih (1980: Table 1) listed

V. ammodytes, *V. berus*, and *V. ursinii* among the reptiles they personally studied in the former Yugoslavia that they never saw infected with ticks.

The low prevalence of hematophagic ticks in temperate zones, in contrast to tropical regions, is not thought to be associated with climatic factors (Pandit et al., 2011; Natusch et al., 2018). The reason why snakes or legless lizards are infested less frequently than fourlegged lizards might be the lack of easily penetrable sites or specialized skin invaginations, like mite pockets (Bury et al., 2020), and because the movement of a smooth, near-cylindrical surface against a rough substrate can make successful questing² tricky and more easily dislodge ectoparasites before they can firmly embed. In contrast, since snakes possess flexible skin in which each scale is surrounded by depressed areas of elastic epidermis, once a tick has successfully attached it might be found in body regions all over the host, and mite pockets would not be a focal point for the presence of ectoparasites (Arnold, 1986). Our observations on V. ammodytes show that ticks penetrated particularly the region around the eye and neck (Fig. 2), both body parts that are slightly less exposed to routine slithering movements. The causes of the observed differences in ectoparasite infestation between quadruped lizards and legless squamates can only be revealed by comparative experimental studies in which potential reptile hosts are exposed to questing tick larvae. We expect that several additional species of hematophagic ticks will be recorded as permanent or temporary ectoparasites of snakes in the future

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² Questing is the technical term for the process by which tick larvae seek and attach to a host (Leal et al., 2020).

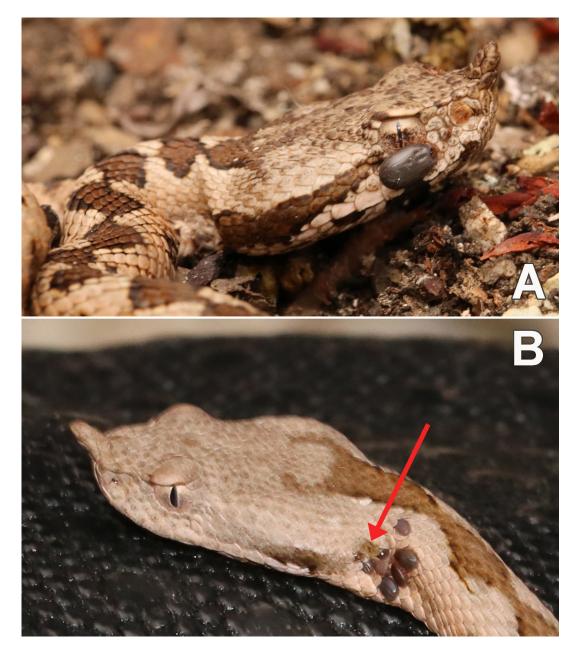


Figure 2. Two juvenile *Vipera ammodytes* from the Iron Gates area of Derdap National Park, Serbia, with attached ixodid ticks (*Ixodes* sp.). (A) A juvenile with a tick below its eye. (B) Another juvenile with ticks of different sizes and at different stages of engorgement attached to the lateral neck region. The red arrow points to a discoloured area where a scar is visible and where a tick had likely been attached. Photo by Thore Koppetsch.

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