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Correspondence

Contribution to the fauna of bat-parasitizing ticks in Bosnia and Herzegovina

Lejla Ušanović[1*], Lejla Lasić[1], Amina Agić[2], Jasna Hanjalić Kurtović[1], Sanja Ćakić[3], Merima Miralem[1] and Belma Kalamujić Stroil[1]

1. *University of Sarajevo – Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina; E-mails:usanovic@ingeb.unsa.ba, lejla.lasic@ingeb.unsa.ba, jasna.hanjalic@ingeb.unsa.ba, merima.miralem@ingeb.unsa.ba, belma.kalamujic@ingeb.unsa.ba*
2. *Center for Karst and Speleology, Sarajevo, Bosnia and Herzegovina; E-mail: amina.agic88@gmail.com*
3. *University of Belgrade, Faculty of Biology, Belgrade, Serbia; E-mail: sanja.cakic@imi.bg.ac.rs*

[*] Corresponding author

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Ticks, as an important group of ectoparasites and vectors of different tick-borne diseases, constitute a growing threat to European public health. Information about the tick population dynamics and their vector potential is crucial for developing preventive measures against the pathogens they transmit. Some European countries have national long-term research projects to monitor the distribution of ticks, both on companion animals (Kooyman *et al*. 2022) and from vegetation (Sormunen *et al*. 2020). In Bosnia and Herzegovina (B & H), studies on tick distribution are sporadic (Omeragić *et al*. 2022) and focused on ticks sampled from domestic animals and vegetation. However, ticks parasitizing on hard-to-catch hosts, such as bats, are poorly investigated in B & H.

Zahn and Rupp (2004) and Hornok *et al*. (2017) reported three hard tick species, *Ixodes ariadnae* (Hornok, 2014), *I. simplex* (Neumann, 1906), and *I. vespertilionis* (Koch, 1844) and at least two soft tick species, *Argas transgariepinus* (White, 1846) and *A. vespertilionis* (Latreille, 1796) as parasites of European bats collected in several countries including southern Bavaria, Portugal, Hungary, Romania, Italy, Vietnam, Kenya, South Africa, and Mexico. Of these, only *I. vespertilionis* was detected in B & H. Between 1908 and 1921, *I. vespertilionis* was recorded in several localities, including northwestern Bosnia (Glibaja Cave, Vrhpolje), central, southern and eastern Bosnia (Han Bulog, Govednica, Kiselica near Trnovo, Dobrun Ruine), Herzegovina (Dolnja near Jablanica, Dragan selo near Konjic, Ilija's Cave), and southern Herzegovina (Grabovica, Slivnica) (Willman 1941). Burazerović *et al.* (2015) found no ticks on bats from three localities in B & H. Hornok *et al.* (2015) detected a nymph of *I. vespertilionis* (GenBank accession number KR902763) removed from the bat *Rhinolophus ferrumequinum* (Schreber, 1774) in Šipovo. To contribute to the scarce data on tick fauna associated with bats in Bosnia and Herzegovina, we analyzed six tick samples collected at the Dardagani quarry in Karakaj, Zvornik, northeastern Bosnia (coordinates: 44.4313603, 19.0883753). Sampling was carried out by researchers from the Center for Karst and Speleology as part of their field surveys on August 25, 2023. Bat species were identified onsite using the

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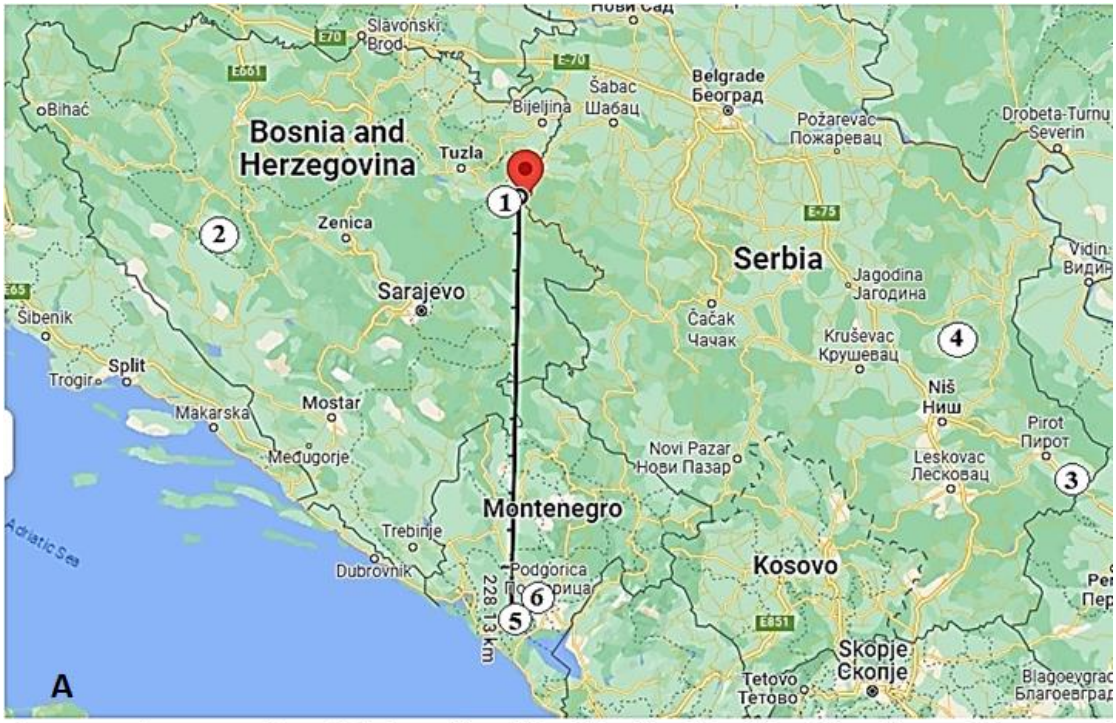
identification key for European bat species (Dietz and Kiefer 2016). The only present species was Schreiber's bent-winged bat, *Miniopterus schreibersii* (Kuhl, 1817). Two ticks (Fig. 1) were removed from the bats and four were collected from the stone walls and stored in 96% ethanol. In the laboratory, all tick samples were washed in 70% ethanol and distilled water, air-dried, and stored at 20 °C until further analysis. Ticks were identified to species level using a morphological key (Arthur 1956) followed by a determination of developmental stage and gender. We performed molecular-genetic analysis to confirm morphological identification, which was based on the rather vague and incomplete available taxonomic key. DNA was extracted from the six specimens using Extract me DNA tissue Kit® (Blirt, Poland). A 710 bp fragment of cytochrome oxidase subunit I gene (*COI*) was amplified using universal primers according to Folmer *et al.* (1994). Sequencing was done on 3500 Genetic Analyzer (Applied Biosystems, HITACHI) in the University of Sarajevo-Institute for Genetic Engineering and Biotechnology, using the same primers as in PCR as one directional. Obtained sequences were edited and analyzed in BioEdit (Hall 1999), identified using the BLASTn tool on the National Center for Biotechnology Information platform (NCBI) and deposited in GenBank.



	L	C	GenBank	44	146	254	283	305	473	476	504	References
1	Zvornik	BH	OR726849	C	G	A	C	T	A	G	C	This research
2	Šipovo	BH	KR902763.1	A	A	G	*	C	*	*	*	
3	Dimitrov grad	SR	KR902764.1	A	A	*	*	C	G	A	*	
4	Zlot	SR	KR902765.1	A	A	*	*	C	G	A	*	Hornok et al., 2015
5	Rijeka Crnojevića	MN	KR902766.1	*	*	*	*	*	*	*	*	
6	Jama Šutonjića cave	MN	KM062043.1	*	*	*	*	*	*	*	*	Burazerović et al., 2015

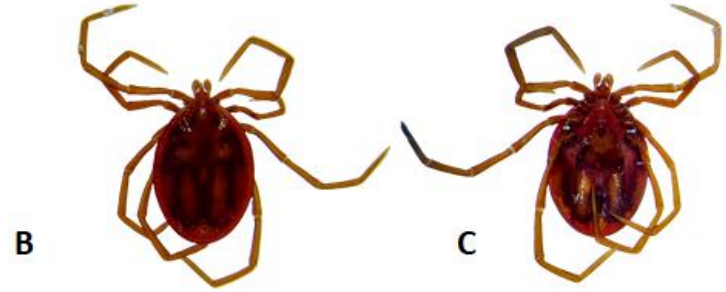


Figure 1. **A.** Distribution of haplotypes obtained in this research and earlier studies. L and C markings represent the location and country, respectively. The locations are marked with numbers from 1 to 6, whereby (1) denotes Zvornik, (2) Šipovo, (3) Dimitrov grad, (4) Zlot, (5) Rijeka Crnojevića, and (6) Jama Šutonjića cave; **B.** Dorsal surface of tick sample; **C.** Ventral surface of tick sample.

The results of both approaches were congruent in identifying all samples as *I. vespertilionis*, being its first molecular record in this part of B & H. All specimens were adult males and displayed the same *COI* haplotype (GB acc. No: OR726849). Hornok *et al*. (2014) observed identical haplotypes in caves 20 km apart, but different haplotypes were found in more distant caves. The authors suggest that the allopatric distribution of *I. vespertilionis COI* genotypes may be influenced by the geographical distance between surveyed caves and bats' preference for the same hibernation spot. However, the haplotype detected in the present study was identical to that found in ticks collected in Montenegro despite the considerable geographic distance between these two locations (Fig. 1). Such a finding could be explained by the migratory behavior of *Miniopterus schreibersii*. Rodrigues and Palmeirim (2007) recorded the migration distance between a nursery and another roost up to 260 km in the Iberian peninsula, while the longest detected movement between a hibernaculum and a spring roost was 306 km. Literature shows that this species can travel far greater distances, with a record of 833 km in Spain (Hutterer *et al*. 2005). Our hypothesis is corroborated by Pejić and Budinski (2021), who studied the diversity of bat flies in *M. schreibersii* from Serbia, and Bosnia and Herzegovina, including the location from the present study. They found identical *COI* haplotypes previously detected in Romania and Hungary, proving the migratory potential of Schreiber's bent-winged bat. *Ixodes vespertilionis* is a highly relevant species due to its broad host range and worldwide distribution. It has been found to carry Bartonellae (Hornok *et al*. 2012) and Borrelia (Michalik *et al*. 2020). Although *I. vespertilionis* preferentially infests *Rhinolophus* spp., tick samples collected in this research were parasitizing on *M. schreibersii*, which is predominantly a host for *I. simplex* (Hornok *et al*. 2015). Even though we did not find *I. simplex* on the investigated site, nor there is any data of its existence in B & H, it is expected that this tick species might already be present in B & H bat populations, given its record in Serbia (Burazerović *et al*. 2015) and proven regional bat migrations (Pejić and Budinski 2021). Since pathogens transmitted by ticks can also migrate alongside their bat hosts, it is imperative to conduct comprehensive research on bat species composition and migratory patterns in B & H as well as monitoring of tick species presence to better understand the implications of host-parasite dynamics for public health and wildlife ecology.

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