

## FIRST RECORD OF *IXODES ARIADNAE* IN GERMANY – SHORT COMMUNICATION

Sándor HORNOK<sup>1\*</sup>, Nóra TAKÁCS<sup>1</sup>, Krisztina SZÖKE<sup>1</sup> and Bernd KUNZ<sup>2</sup>

<sup>1</sup>Department of Parasitology and Zoology, Faculty of Veterinary Science,  
Szent István University, István u. 2, H-1078 Budapest, Hungary;

<sup>2</sup>Hauptstraße 111, D-74595 Langenburg, Germany

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A long-legged tick was collected from a hibernating greater mouse-eared bat (*Myotis myotis*) in Baden-Württemberg, Germany. Based on morphological characteristics as well as on partial COI and 16S rDNA gene sequences the tick was identified as an engorged female of *Ixodes ariadnae*. The greater mouse-eared bat is a new host record for this tick species. Taking into account the geographical position of the collection site and the known migration distance of the greater mouse-eared bat, the present data suggest the autochthonous occurrence of *I. ariadnae* in Germany. This is the first record of *I. ariadnae* in Germany, and in any country other than Hungary, where this species has been recently discovered.

**Key words:** Bat, tick, *Ixodes*, *Myotis*

Bats are increasingly recognised as eco-epidemiologically important mammals, in part because many species live in human settlements, even in buildings. In such places humans may be exposed to bites of bat ectoparasites, most importantly of ticks (Jaenson et al., 1994; Estrada-Pena and Jongejan, 1999; Piksa et al., 2013). Bat ticks were shown to carry zoonotic pathogens, including rickettsiae, borreliae, ehrlichiae (Socolovschi et al., 2012) and bartonellae (Hornok et al., 2012).

For more than a century, only two species of ixodid ticks were known to infest bats in Europe, the long-legged bat tick (*Ixodes vespertilionis*) and *I. simplex* (Nosek and Sixl, 1972). However, recently a new long-legged species, *I. ariadnae* has been discovered (Hornok et al., 2014a). The three ixodid bat tick species appear to differ in their host preferences, i.e. *I. vespertilionis* occurs predominantly on *Rhinolophus* spp. (Piksa et al., 2014), *I. simplex* on *Miniopterus schreibersii* (Arthur, 1956), whereas *I. ariadnae* was found on *Plecotus auritus* and *Myotis* spp. (Hornok et al., 2014a). Thus, the various habitat preferences and hibernating behaviour of bat species will significantly influence the geographical distribution of bat ticks.

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\*Corresponding author; E-mail: Hornok.Sandor@aotk.szie.hu; Phone: 0036 (1) 478-4187, Fax: 0036 (1) 478-4193

Despite the fact that *I. vespertilionis* was originally described in Germany (Koch, 1844), it is relatively seldom found on bats (Kulzer and Müller, 2003). One reason for this may be that *Rhinolophus* spp. became nearly extinct in the country, and their habitats (caves) are restricted. According to literature data *I. vespertilionis* occurs in several locations in Germany, but *I. simplex* was recorded only from one place (Petney et al., 2012). Here we report the morphological and molecular identification of a long-legged bat tick collected in Germany.

On 6 March 2015 a long-legged tick was removed from a greater mouse-eared bat (*Myotis myotis*), hibernating in a natural cave at the north rim of the river Bühler valley (49.1550° N and 9.9272° E, ca. 400 m above sea level, northwest of the village Hopfach, Baden-Württemberg, Germany). The collection site is shown in Fig. 1. The authorisation number for bat handling is 55-8850.68/SHA (issued by the Regierungspräsidium Stuttgart Abteilung Umwelt).



Fig. 1. Map of Europe showing the collection site of *Ixodes ariadnae* in Germany (white dot) and its formerly reported occurrence in Hungary (black dots)

The tick was stored in 96% ethanol. Morphological identification was done with a stereomicroscope (SMZ-2T, Nikon Instruments, Japan, illuminated with model 5000-1, Intralux, Switzerland). Molecular analysis was also performed with PCR and sequencing, in order to compare the tick with other tick

isolates of which relevant data are available in the GenBank. The DNA was extracted from one hind leg of the tick as described (Hornok et al., 2014b).

The cytochrome oxidase subunit I (COI) gene was chosen as the first target for molecular analysis, on account of its suitability as a DNA-barcode sequence for tick species identification. The PCR was modified from Folmer et al. (1994) and amplifies an approx. 710 bp long fragment of the gene. The primers HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') were used in a reaction volume of 25 µl, containing 1 U (0.2 µl) HotStarTaq Plus DNA polymerase, 2.5 µl 10× CoralLoad Reaction buffer (including 15 mM MgCl<sub>2</sub>), 0.5 µl PCR nucleotide Mix (0.2 mM each), 0.5 µl (1 µM final concentration) of each primer, 15.8 µl ddH<sub>2</sub>O and 5 µl template DNA. For amplification, an initial denaturation step at 95 °C for 5 min was followed by 40 cycles of denaturation at 94 °C for 40 s, annealing at 48 °C for 1 min and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 10 min.

Another PCR was used to amplify an approx. 460 bp fragment of the 16S rDNA gene of Ixodidae (Black and Piesman, 1994), with the primers 16S+1 (5'-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3') and 16S-1 (5'-CCG GTC TGA ACT CAG ATC AAG T-3'). Other reaction components, as well as cycling conditions were the same as above, except for annealing at 51 °C.

PCR products were electrophoresed in a 1.5% agarose gel (100 V, 60 min), stained with ethidium bromide and visualised under ultraviolet light. Purification and sequencing was done by Biomi Inc. (Gödöllő, Hungary). The sequences were submitted to the GenBank (accession numbers KR093169 and KR093170, respectively).

Based on morphological characteristics, i.e. 5 mm size, long legs, short palps, broad and posteriorly rounded scutum (Fig. 2) and sparse covering with setae, the tick was identified as an engorged female of *I. ariadnae*. The greater mouse-eared bat (*M. myotis*), from which the specimen was collected, is a new host record for this tick species.

The partial COI sequence of the tick (KR093169) showed 100% homology with *I. ariadnae* (KJ490306). The partial 16S rDNA gene sequence of the tick (KR093170) was identical with genotype F among Hungarian isolates, collected at the main habitat of *I. ariadnae* in Hungary (Ariadne cave system, Pilis Mountains: Hornok et al., 2015).

The collection site of *I. ariadnae* in Germany is at least 250 km from the southeastern country borders, and approx. 650 km from the known habitats of this tick species in Hungary. Taking into account that the tick in the present study was removed from a bat species with migration ranges of approx. 100–250 km in Germany and Hungary (Griffin, 1970), these data suggest the autochthonous occurrence of *I. ariadnae* in Germany.

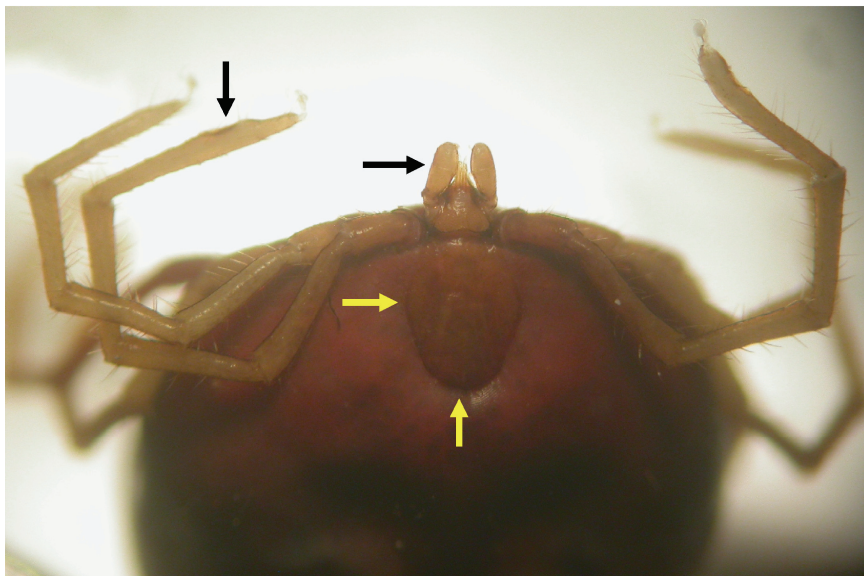


Fig. 2. Anteriodorsal view of the bat tick collected in the present study. Arrows indicate the characteristics of *Ixodes ariadnae* mentioned in the text

This is the first record of *I. ariadnae* in Germany, and in any country other than Hungary, where this species has been recently discovered (Hornok et al., 2014a). The tick will be deposited in the Museum für Naturkunde Berlin. In order to ascertain if the present finding represents a unique case, or *I. ariadnae* has a more widespread occurrence in Germany or the surrounding countries, further bat tick monitoring is recommended.

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