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FIRST RECORD OF *IXODES ARIADNAE* IN WESTERN EUROPE, BELGIUM – SHORT COMMUNICATION

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Fourteen long-legged ixodid ticks (6 nymphs and 8 larvae) were collected from Bechstein's bat *(Myotis bechsteinii)* in Rochefort, Belgium. All ticks were morphologically identified as *Ixodes ariadnae*, based on their long legs (Haller's organ longer than maximum diameter of tarsus I), broad palps and posteriorly reverse bell-shaped scutum with wavy surface. The DNA was extracted from these ticks, followed by PCR amplification of part of their cytochrome oxidase subunit I (COI) gene. All obtained sequences were 100% identical with each other, and with the COI sequence of *I. ariadnae* reported previously from Hungary and Germany. Taking into account that the collection site in the present study is close to the French border of Belgium, and migration of Bechstein's bat is known between Belgium and France, it is reasonable to suppose that *I. ariadnae* also occurs in France. This is the first record of *I. ariadnae* in Western Europe, outside its formerly known geographical range (Central Europe).

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

Bats form the second largest order (Chiroptera) of mammals. Their epidemiological significance has become increasingly recognised, owing to their flight ability, migratory habit and presence in the urban environment, where they may use man-made buildings (houses, cellars, churches, stables) for roosting (Hutterer et al., 2005). In addition, numerous bat species and their blood-sucking ectoparasites have been identified as carriers of important pathogens to which humans and/or domestic animals are susceptible (Socolovschi et al., 2012). Feeding of bat ectoparasites (particularly of ticks) on humans (Jaenson et al., 1994; Piksa et al., 2013) may represent an opportunity for zoonotic pathogen transmission.

Ixodes ariadnae is a recently described hard tick species of bats (Hornok et al., 2014). Hitherto it has been reported from two Central European countries, Hungary and Germany (Hornok et al., 2015*a*). However, long-legged bat ticks

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morphologically and genetically most similar to *I. ariadnae* are geographically more widespread, and have already been reported from southern and eastern Asia (Hornok et al., 2015*b*).

The preferred hosts of *I. ariadnae* are *Myotis* spp. and *Plecotus auritus* (Chiroptera: Vespertilionidae) (Hornok et al., 2014, 2015*a,b*). The genus *Myotis* is represented by the highest number of (i.e. sixteen) bat species in Europe, and at least half of them occur in the western part of our continent (Hutterer et al., 2005). Taking also into account that some *Myotis* spp. are able to migrate (relatively) long distances, it is justifiable to suppose that *I. ariadnae* should be present (but was hitherto unknown to occur) in Western Europe. Here we report the morphological and molecular identification of long-legged bat ticks collected in Belgium.

In September of 2013 and 2015, fourteen long-legged ixodid ticks (6 nymphs and 8 larvae) were collected from Bechstein's bat *(M. bechsteinii)* in Grotte de Lorette (50.155N, 5.2281E), Namur Province, Rochefort, Belgium. This natural cave is a very important site for bats in the region, during both hibernation and swarming, with at least eleven bat species (Nyssen et al., 2015). The collection site is shown in Fig. 1. The authorisation number for bat handling was SPWDNF-DGO3 (2013/RS/n°15 and 2014/RS/n°12, issued in Belgium).

Fig. 1. Map of Europe showing the collection site of *I. ariadnae* in Belgium in the present study (red dot) and previously known occurrence of this tick species in Germany and Hungary (black dots). The shaded area marks the distribution range of *Myotis bechsteinii*

The ticks were stored in 96% ethanol. Morphological identification was based on the description of *I. ariadnae* (Hornok et al., 2014, 2016). Pictures of representative specimens were taken with a digital microscope (Zeiss Stereo Discovery microscope LZO 0191 with AxioCam ICc5 camera and ZEN software). Consequently, DNA was extracted from the ticks individually using the Qiagen DNeasy Blood & Tissue Kit according to the manufacturer's protocol for the purification of total DNA from ticks (Qiagen, Venlo, The Netherlands).

The cytochrome oxidase subunit I (COI) gene was chosen for confirmation and genetic comparison of the species, because this gene is regarded as the most suitable marker for molecular identification (barcoding) of tick species (Lv et al., 2014). The PCR was modified from Folmer et al. (1994) and amplifies an approx. 710-bp-long fragment of the gene. The PCR was performed with the HotStarTaq master mix (Qiagen, Venlo, The Netherlands) and 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'). For amplification, an initial denaturation step at 95 °C for 15 min was followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 40 s and extension at 72 °C for 50 s. Final extension was performed at 72 °C for 10 min.

PCR products were visualised in a 1.5% agarose gel. Sequencing was performed with Sanger method BaseClear (BV BaseClear, Leiden, The Netherlands) and sequences were compared by nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). One representative sequence was submitted to GenBank (accession number: KX375410).

All ticks were morphologically identified as *I. ariadnae*. This diagnosis is based on the presence of long legs (i.e. Haller's organ elongated, longer than the maximum diameter of tarsus I), like in the case of *I. vespertilionis*. However, specimens investigated in the present study had laterally straight and medially curved palps (Fig. 2), broader than those of *I. vespertilionis*. In addition, the scutum was posteriorly reverse bell-shaped, with wavy surface (Fig. 2), unlike in the case of *I. vespertilionis* (Arthur, 1956).

All obtained COI sequences were 100% identical with each other and with the COI sequence of *I. ariadnae* reported formerly from Hungary (KJ490306) and Germany (KR093169). Accordingly, while *I. vespertilionis* was shown to have different COI genotypes within Hungary (most likely due to the sedentary habit and limited seasonal movements of its main host, *Rhinolophus hipposideros*: Hutterer et al., 2005), *I. ariadnae* appears to show highly conserved COI sequences over large geographical distances, as demonstrated here. This can be explained by longer distance movements of *Myotis* spp. (the preferred hosts of *I. ariadnae*) in Europe (Hutterer et al., 2005). In particular, *M. bechsteinii*, from which all specimens of this study were collected (i.e. it appears to be an important host of *I. ariadnae*), is known to have a social system and roosting behaviour

allowing transmission of ectoparasites and their genetic exchange between different bat colonies (van Schaik et al., 2015).

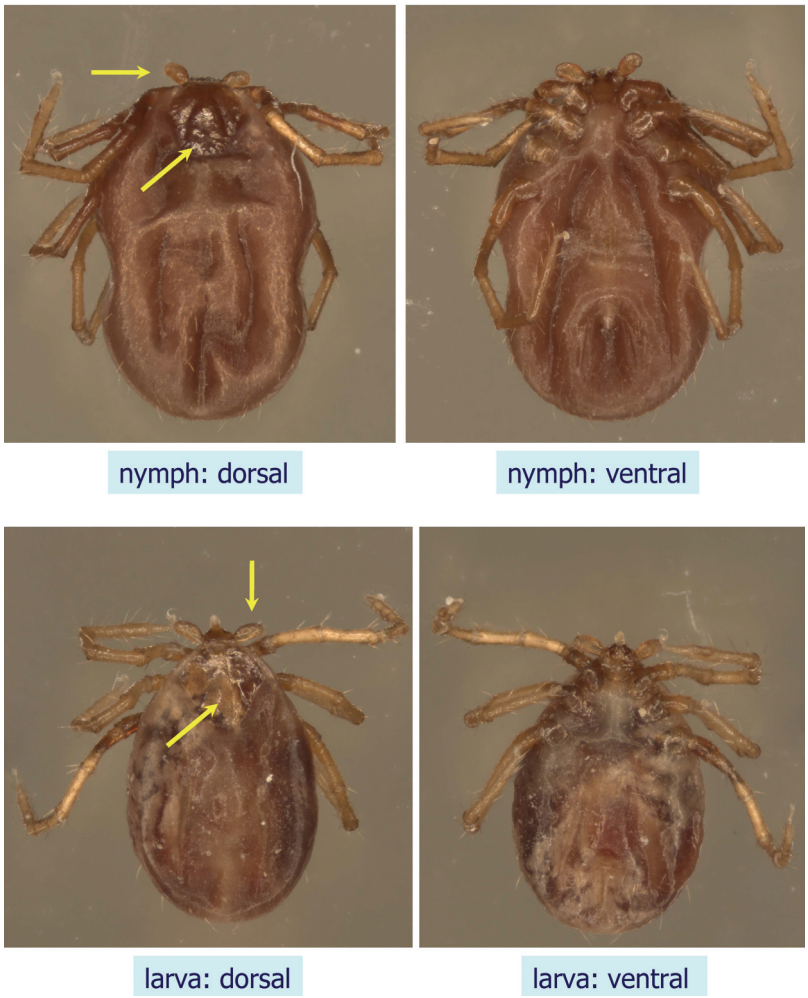

Fig. 2. Dorsal and ventral views of *I. ariadnae* nymph and larva collected in the present study. Arrows indicate species characteristics (broad palps curved medially but straight laterally; wavy surface of scutum in its reverse bell-shaped, posterior part)

This is the first record of *I. ariadnae* in Western Europe, outside its formerly known geographical range (Central Europe). Taking into account that the collection site in the present study is close to the French border of Belgium, and migration of Bechstein's bat is known between Belgium and France (Hutterer et al., 2005), it is reasonable to suppose that *I. ariadnae* also occurs (at least on this bat species) in France. Therefore, it is suggested to extend tick collections from

Myotis spp. to other countries in Western Europe, in order to uncover the full geographical range of *I. ariadnae* in the region.

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