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Description of the male and the larva of *Ixodes ariadnae* Hornok, 2014

Sándor Hornok[a,*], Dávid Kováts[b], Dorottya Angyal[c], László Dányi[c], Richárd Kovács[d], Jenő Kontschán[e]

[a] Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary
[b] Department of Evolutionary Zoology and Human Biology, University of Debrecen, Debrecen, Hungary
[c] Department of Zoology, Hungarian Natural History Museum, Budapest, Hungary
[d] Ariadne Caving Group, Pilis, Hungary
[e] Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

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ABSTRACT

Ixodes ariadnae is a tick species of bats so far reported only in Central Europe, with its description based on the female and nymph. This study describes the male and larva in order to complete the description of the species.

Male ticks collected from cave walls in Hungary showed a different morphology from those of *I. vespertilionis* and *I. simplex*. Molecular analysis of the cytochrome oxidase subunit I (COI) gene of these ticks verified them as conspecific to *I. ariadnae*. In addition, a larva of *I. ariadnae* was removed from a Daubenton's bat (*Myotis daubentonii* Kuhl, 1817). The male of *I. ariadnae* is characterized by long legs (7–8 mm; *I. vespertilionis*: 8–10 mm; *I. simplex*: 2–2.2 mm), relatively short palpal setae (30–100 μm; *I. vespertilionis*: 100–200 μm; *I. simplex*: 20–50 μm) and straight lateral edge of palps, the genital aperture (enclosed by a line of sclerotization) situated anteriorly to second intercoxal space and rounded coxae. The larva of *I. ariadnae* has long legs (2–2.2 mm; *I. vespertilionis*: 1.6–1.8 mm; *I. simplex*: 1 mm), broad palps (length × width: 200 × 90 μm; *I. vespertilionis*: 200 × 70 μm; *I. simplex*: 140 × 60 μm), pentagonal and posteriorly reverse bell-shaped scutum. These features allow to distinguish the male and the larva of *I. ariadnae* from those of *I. vespertilionis* (of which the male has longer palpal setae and curved lateral edge of palps, the genital aperture is situated posterior to the second intercoxal space, and the second coxae are squared; the larva of *I. vespertilionis* has narrower palps and posteriorly triangular scutum) and *I. simplex* (of which the male and the larva have considerably shorter legs, palps).

1. Introduction

Ixodes ariadnae Hornok is an ixodid tick species infesting bats (Hornok et al., 2014), which has been so far reported from two countries in Central Europe, i.e. Hungary and Germany (Hornok et al., 2015a). According to a recent survey (Hornok et al., 2015b), *I. ariadnae* appears to be geographically more widespread than previously thought, because ticks morphologically similar to and phylogenetically clustering with *I. ariadnae* also occur in more distant regions of Eurasia (Vietnam, Japan). Hitherto only the female and the nymph of *I. ariadnae* have been described (Hornok et al., 2014). This study provides the description of the male and the larva of the species.

2. Materials and methods

2.1. Sample collection

Bat ticks were collected from cave walls in Hungary, including formerly unknown locations (specimens No. 4-6 below) of *I. ariadnae*. Six males showed morphology different from that of *I. vespertilionis*. In the present study these six specimens were used for morphological comparison:

(1) Male from Legény Cave (coordinates: 47.41 N, 18.50 E), part of the Ariadne Cave System, Pilis Mountains (collected by D. Kováts, in December 2015; accession number UNIVET-PAR-HS101);

(2)–(3) Two males from Szopláki-ördöglyuk Cave (coordinates: 47.42 N, 18.52 E), Pilis Mountains (collected by D. Kováts, on February 6, 2015; accession numbers UNIVET-PAR-HS102-3);

(4) Male from Csodabogyós Cave (coordinates: 46.47 N, 17.21 E), Keszthelyi Mountains (collected by D. Angyal and L. Dányi on December 14, 2014; accession number UNIVET-PAR-HS104);

* Corresponding author.
E-mail address: hornok.sandor@univet.hu (S. Hornok).

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(5) Male from Pilis Cave (coordinates: 47.42 N, 18.52 E), Pilis Mountains (collected by D. Kováts, on August 3, 2015; accession number UNIVET-PAR-HS105);

(6) Male from Pálvölgyi Cave (coordinates: 47.31 N, 19.09 E), part of the Pálvölgyi Cave System, Budapest (conditions of collection unknown; accession number UNIVET-PAR-HS106);

(7) Larva from *Myotis daubentonii* Kuhl, 1817 (caught with mist-net for ringing/monitoring purpose) at Szopláki-ördöglyuk Cave (coordinates: 47.42 N, 18.52 E), Pilis Mountains (collected by D. Kováts, on August 26, 2015; accession number UNIVET-PAR-HS107);

Ticks were immediately put into 96% ethanol. The above specimens are deposited in the tick collection of the Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary.

2.2. Sample analyses

Molecular genetic analysis of three additional males from Ajándék Cave (coordinates: 47.70 N, 18.84 E; close to Ariadne Cave System, Pilis Mountains, collected by R. Kovács in March 2014), which showed the same morphology as specimens No. 1-6 above, was performed as reported (Hornok et al., 2014). The relevant PCR amplifies an approx. 710 bp long fragment of the cytochrome oxidase subunit I (COI) gene, with 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′). PCR products were electrophoresed in a 1.5% agarose gel for visualisation. Purification and sequencing was done by Biomi Inc. (Gödöllő, Hungary). The sequences were compared to those already deposited in GenBank by nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). Obtained sequences were not submitted to GenBank, because they were 100% identical to each other and to already submitted sequences of conspecific females (KR093169, KJ490306).

Pictures of males No. 2-3. were made with a VHX-5000 (Keyence Co., Osaka, Japan) digital microscope.

3. Results

Based on the amplified fragment of their COI gene, all three molecularly evaluated males were identified as *I. ariadnae*, having 100% sequence identity to formerly reported isolates of this tick species (KR093169, KJ490306: the latter from the same cave system as male No. 1).

The sizes in the descriptions below are provided in millimeters, for males including the range and the means (in parentheses) of all evaluated specimens.

3.1. Ixodes ariadnae *Hornok, male*

Length of idiosoma (from half point between scapular apices to posterior margin) 3.01–3.59 (3.35), breadth 2.2–2.6 (2.44), ratio of idiosomal length/breadth 1.36–1.39 (1.37). Conscutum elongated, elliptical, broadest behind its mid-length (Fig. 1b). Length of scutum 2.95–3.53 (3.3), breadth 1.7–2.06 (1.9), ratio length/breadth 1.71–1.74 (1.72). On posterior half of conscutum shallow paramedian grooves and scattered, large punctuations. Lateral punctuations outlined by ivory coloration, making them more conspicuous. Idiosoma with sparse setae covering ventral surface (Fig. 2d). Genital aperture enclosed by a line of sclerotization, slightly anterior to second intercoxal space (Fig. 2b). Spiracular plates slightly oval, with excentric opening. Anal groove posteriorly convergent (Fig. 2d).

Gnathosoma: length from palpal apices to posterior margin of basis 0.53–0.635 (0.59), width of basis dorsally 0.34–0.41 (0.38), ratio of length to width 1.55–1.57 (1.56). Basis capituli dorsally broadest at base of palps, posteriorly narrow, with a broad U-shaped anterior elevation behind anterior margin of gnathosoma (Fig. 1d); posterior margin straight. Ventrally basis capituli trapezoidal, posteriorly tapering (Fig. 2b). Palps short, length 0.33–0.39 (0.37), breadth 0.14–0.165 (0.155), ratio length/breadth 2.35–2.37 (2.36). Palpal segment I. 0.04, II–III [separation not visible] 0.31, segment IV. 0.04. Segments II–III medially curved and broad, laterally straight. Palpal setae anteriorly (on segment IV) short (0.03), longest (0.1) around mid-length laterally, none of them exceeding width of palps (Fig. 1d). Hypostome slightly lanceolate, length 0.24–0.26 (0.25), breadth 0.08–0.1 (0.09), ratio length/breadth 2.4–2.6 (2.5). Teeth underdeveloped (ill-defined), dental formula 3/3.

Legs long, 7–8 (7.5). Coxae medially rounded, without spines or spurs, but with few setae (0.07-0.12) (Fig. 2b). Tarsus I 1.7–1.9 (1.83). Haller's organ open, with a prominent sensillum (0.07) laterally to middle group of anterior pit sensillae.

3.2. Ixodes ariadnae *Hornok, larva*

Length of idiosoma 1.1, breadth 0.92, ratio idiosomal length/breadth 1.2. Scutum pentagonal, posteriorly reverse bell-shaped, broadest slightly anteriorly to its mid-length (Fig. 3b). Length of scutum 0.43, breadth 0.48, ratio length/breadth 0.9. Scutum with uneven (wavy) surface, especially posterolaterally. Cervical grooves long, reaching posterolateral margin of scutum behind deepest point of concavity of reverse bell-shaped part (Fig. 3b). Scutal setae short (0.02) anteriorly, longer (0.03) laterally, close to point of maximum breadth. Alloscutal setae progress in size backwards (from 0.08 to 0.15), longest caudally, protruding well behind the body (Fig. 3b). Ventrally, sternal setae shorter anteriorly than posteriorly (0.07 vs. 0.12); pre-anal setae 0.12; premarginal and marginal ventral setae slightly shorter anteriorly (0.12) than posteriorly (0.14) (Fig. 3d).

Gnathosoma: length from palpal apices to posterior margin of basis 0.28, width of basis capituli dorsally 0.3, ratio of length to width 0.93. Basis capituli dorsally triangular, with straight posterior margin. Ventral basis with two pairs of short (0.015) posthypostomal setae. Posterior margin of ventral basis caudolaterally rounded (Fig. 3d). Palps short and broad, laterally straight: length 0.2, breadth 0.09, ratio length/breadth 2.2. Palpal setae dorsally of medium length (0.05), ventrally short (0.01–0.02) or long (0.07–0.08). Hypostome conical, short (0.1), with dental formula 2/2.

Legs long (2–2.2: Fig. 3a and c). Coxae medially tapering and rounded, without spurs. Single long (0.1) seta on coxa II, one short (0.025) and one long (0.1) setae on coxae I and III. Tarsus I length 0.7.

3.3. Differential diagnosis

Based on the redescriptions of *I. vespertilionis* by Arthur (1956) and Feider (1965), males of *I. ariadnae* show the following distinctive characteristics from males of *I. vespertilionis*: the size is smaller (3.4 mm vs. 4.7 mm); the lateral ivory colouration shows different pattern (i.e. along the sides posteriorly tapering vs. posteriorly broadening in *I. vespertilionis*: Fig. 1a and b); genital opening enclosed by a line of sclerotization and anterior to second intercoxal space (vs. posterior in *I. vespertilionis*: Fig. 2a and b); the palpal setae (especially apically) are short (30–100 μm vs. 100–200 μm in *I. vespertilionis*), not exceeding palpal breadth as contrasted to *I. vespertilionis* (Fig. 1c and d); lateral edge of palps straight (but curved in *I. vespertilionis*: Fig. 1c and d); coxa II rounded (vs. squared in *I. vespertilionis*: Fig. 2a and b). In comparison with the male of *I. simplex* (Filippova, 1977), the size of *I. ariadnae* male is larger (3.4 mm vs. 2.4 mm), the palps are laterally straight (vs. bent in an angle in

Fig. 1. Dorsal view of *Ixodes vespertilionis* male (a—habitus, c—gnathosoma) and *I. ariadnae* male (b—habitus, d—gnathosoma).

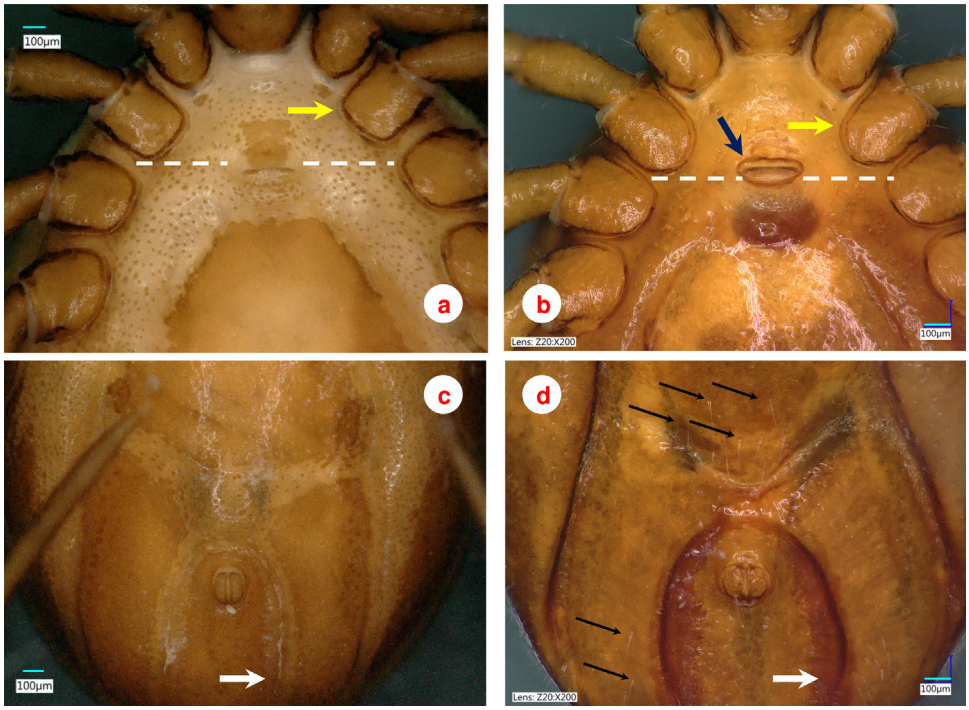

Fig. 2. Ventral view of *Ixodes vespertilionis* male (a—anterior, c—posterior part of the idiosoma) and *I. ariadnae* male (b—anterior, d—posterior part of the idiosoma). The yellow arrows mark the second coxae, the white dashed line indicates the second intercoxal space. The thick black arrow shows the marginated genital aperture, and the thin black arrows point at sparse setae of *I. ariadnae*.

I. simplex), the legs are considerably longer (7–8 mm vs. 2–2.2 mm in *I. simplex*), and the row of 12 setae situated posteriorly on coxa IV of *I. simplex* male is missing in *I. ariadnae*.

Concerning the larva of *I. ariadnae* relative to the larva of *I. vespertilionis*, its scutum is broadest anteriorly to mid-length (*I. vespertilionis*: at mid-length). The cervical grooves of the larva of *I. ariadnae* reach the posterolateral margin of the scutum behind the deepest point of concavity, as contrasted to *I. vespertilionis* larva, which has cervical grooves reaching the posterolateral margins exactly at the deepest point of concavity (Arthur, 1956). The larva of *I. ariadnae* has broad palps (length × width: 200 × 90 µm), whereas the larva of *I. vespertilionis* has forward broadening, club shaped and narrower palps (200 × 70 µm: Arthur, 1956; Feider, 1965). In comparison with the larva of *I. simplex* (Feider, 1965), the larva of

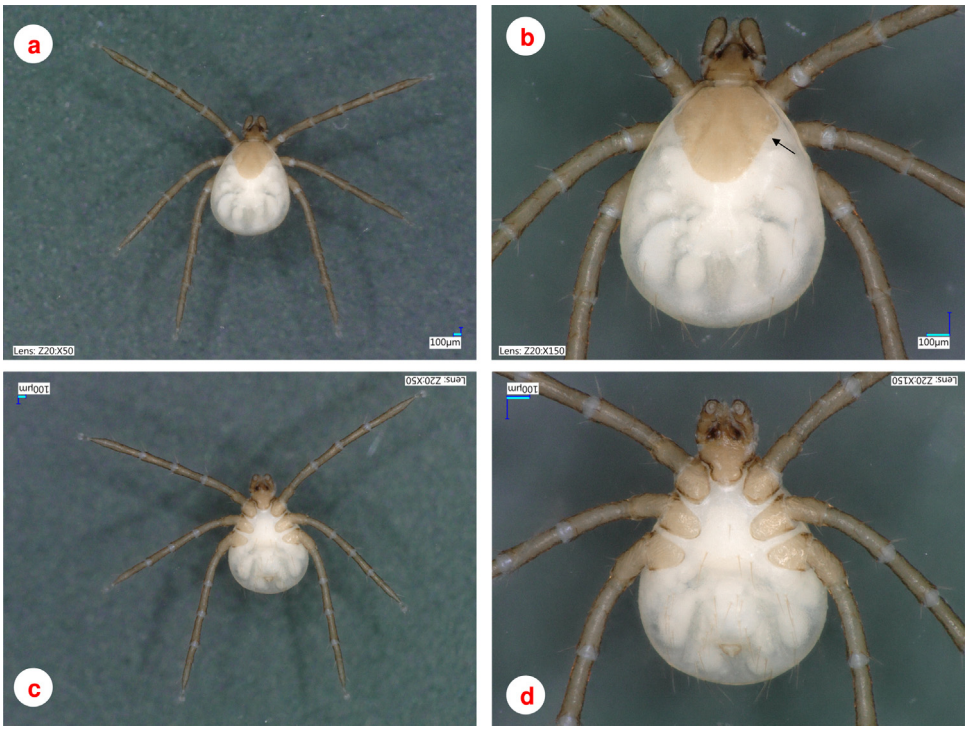

Fig. 3. Dorsal and ventral views of *I. ariadnae* larva with two magnifications. The arrow marks the deepest point of concavity in the reverse bell-shaped posterior part of the scutum.

I. ariadnae has different shape of palps (laterally straight, medially curved vs. slightly curved both laterally and medially in *I. simplex*), longer palpal setae (10–80 μm vs. 10–30 in *I. simplex*) and longer legs (2–2.2 mm vs. 1 mm in *I. simplex*).

4. Discussion

With the morphological traits (high resolution pictures) provided in the present study, the description of all stages of *I. ariadnae* is now complete. Adding to the genetic differences between *I. ariadnae*, *I. vespertilionis* and *I. simplex* as already reported (Hornok et al., 2014, 2015c), these ixodid bat tick species are morphologically distinguishable in all stages, as shown formerly for females and nymphs (Hornok et al., 2014, 2015c) and here for males and larvae.

Ethical approval

Authorization for bat capture was provided by the National Inspectorate for Environment, Nature and Water (No. 14/2138-7/2011). Bat banding licence number is TMF-14/ 4 32/2010 (DK).

Acknowledgement

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