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Original article

Comparison and complete mitogenomes of two morphologically similar but ecologically different tick species, *Ixodes arboricola* and *Ixodes lividus* (subgenus *Pholeoixodes*)

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ABSTRACT

Ixodes arboricola and *Ixodes lividus* are ornithophilic tick species. The former is typically associated with tree-hole dwelling birds, while the latter is a host-specific parasite of sand martins (*Riparia riparia*). These two tick species share important morphological characters that make them difficult to identify when they are collected from atypical hosts, such as birds of prey. Despite this, high resolution digital pictures have not been reported to compare *I. arboricola* and *I. lividus*, nor was their complete mitogenome reported. The aim of this study was to compensate for this lack of illustrations and sequence data. Nymphs and females of *I. arboricola* and *I. lividus* were used for morphological comparison, and one specimen of each species to generate mitogenome sequences.

The results showed that females of these two species are different in the shape of their scutum, porose areas, the length of basis capituli, palps, coxae, genital pore, anal groove and tarsus I. On the other hand, nymphs of *I. arboricola* and *I. lividus* can be distinguished according to their cervical grooves, cornuae, auricular ridges and spiracular plates. The mitochondrial genome size was 14,539 and 14,536 bp, for *I. arboricola* and *I. lividus*, respectively. The mitogenome sequences of *I. arboricola* and *I. lividus* were 91.1% identical to each other. Phylogenetic analysis of *Ixodes* species showed that *I. arboricola* and *I. lividus* are sister species, and cluster together with *Ixodes crenulatus/canisuga* under strong support.

In conclusion, results of this study confirmed that the front of the basis capituli is crucial in distinguishing *Ixodes* species, especially in the subgenus *Pholeoixodes* where these two species are phylogenetically closest related to *I. canisuga*. Another phylogenetically relevant morphological character is the scutal surface which is wrinkled (rugose) as a common feature of *Pholeoixodes* species in the clade of *I. arboricola* and *I. lividus* (including *I. canisuga, I. rugicollis* and *I. ariadnae*). Although the host ranges of *I. arboricola* and *I. lividus* do not substantially overlap, they may transmit some of the shared pathogens. Relevant data indicate that the eco-epidemiological significance of the two ornithophilic tick species studied here may in part be similar and they may play a role in the transmission of rickettsiae, borreliae and viruses of which birds act as reservoirs.

1. Introduction

Birds are well known as hosts of argasid and ixodid ticks (Ixodida: Argasidae, Ixodidae). Particularly in the context of the latter group, dispersal capacity of birds is considered as having the highest epidemiological impact of all flying and non-flying vertebrates, as tick-

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infested birds might carry these ectoparasites over thousands of kilometers during seasonal migrations (de la Fuente et al., 2015). While generalist ixodid species opportunistically infest birds, avian hosts also have ornithophilic tick parasites that almost exclusively develop on birds and may harbor important animal and human pathogens (Heylen et al., 2014a). The ornithophilic behavior of these tick species do not necessarily prevent them from general epidemiological significance, as bird-associated ticks tend to be nidicolous and thus feed while aggregated (Van Oosten et al., 2016), which may promote occasions of co-feeding and pathogen transmission to other, more generalist tick species, even towards mammals (Heylen et al., 2017).

In general, tick-borne pathogens have specific transmitters, i.e. certain tick species with which they associate as their vector. This implies that tick species have to be identified accurately in order to assess and to take into account the pathogens they may carry. Among the three widespread ornithophilic ixodid tick species in Europe that have passerine birds as typical hosts (i.e., discounting sea bird-associated species), *Ixodes arboricola* and *Ixodes lividus* can be regarded as strictly bird specialists (Keve et al., 2022), while a third species, *Ixodes frontalis* is more frequently reported from mammals including humans (Hillyard, 1996; Gilot et al., 1997). The latter, exophilic tick species is relatively easy to identify on a morphological basis (Reynolds et al., 2022), the other two being endophilic (subgenus *Pholeoixodes*) with completely different niches and host preferences. With this in mind, *I. arboricola* is most frequently identified according to its presence in tree holes and on tree hole-associated passerines, whereas *I. lividus* is a specific parasite of sand martins (*Riparia riparia*) and can be found in its burrows (Keve et al., 2022).

In this context, while the morphological comparison of *I. arboricola* with *I. ricinus* and *I. frontalis* has been reported (Heylen et al., 2014a), these three tick species represent different subgenera (i.e., *Pholeoixodes, Ixodes* and *Trichotoixodes*), thus we can expect them to be relatively easy to differentiate. However, the differential diagnosis of *I. arboricola* and *I. lividus* (both members of subgenus *Pholeoixodes*) was never addressed in detail, and to our knowledge only one study included their high-resolution images simultaneously but only the gnathosoma of females, in pictures made with scanning electron microscope (Cordas et al., 1993). Nevertheless, from time to time, birds of prey (mostly from orders Accipitriformes, Falconiformes) were or have been reported to harbor either *I. arboricola* without illustration of tick morphology (Schilling et al., 1981), or ticks identified as *I. lividus* with pictures that show morphological characters of *I. arboricola* (Ganbold et al., 2024). Therefore, to clarify any potential misidentification and to promote future accuracy in this respect, this study was initiated. It was also within the scope of this investigation to sequence and to analyze phylogenetically, the complete mitogenome of both species.

2. Materials and methods

2.1. Collection of samples and morphological identification of species

Specimens used in this study are shown in Table 1. These were collected during bird ringing, under a license provided by the National Inspectorate for Environment and Nature, Hungary (TMF/1034/2016). Tick species were identified by standard taxonomic keys (*I. arboricola*: Heylen et al., 2014a; *I. lividus*: Estrada-Peña et al., 2017). Pictures were taken with a VHX-5000 digital microscope (Keyence Co., Osaka, Japan).

2.2. Molecular identification of species based on short mitochondrial fragments

The species of morphologically analyzed ticks were confirmed molecularly. For this, DNA was extracted from two legs of adults and nymphs or the whole body of larva with the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) as described (Hornok et al., 2023). This was followed by PCR amplification of short fragments: part of the cytochrome *c* oxidase subunit I (*cox*1) and 16S rRNA genes (Hornok et al., 2023), in order to compare sequences to the complete mitogenome obtained as described below. Purification and sequencing of the short PCR products were done by Eurofins Biomi Ltd. (Gödöllő, Hungary). Quality control and trimming of sequences were performed with the BioEdit program. Obtained sequences were compared to GenBank data by the nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). New sequences were submitted to GenBank under the following accession numbers (*cox*1 gene: PV707163-PV707168, 16S rRNA gene: PV707169-PV707175).

2.3. DNA extraction for mitogenome analysis

Two morphologically identified ticks were ground in lysis buffer (ATL buffer) and Proteinase K with a micro-pestle, followed by an overnight incubation at 56°C. Genomic DNA (gDNA) was extracted with the DNeasy Blood & Tissue kit (Qiagen, Germany) following manufacturer's instructions. gDNA was eluted in 75 µl elution buffer (EB). DNA extracts were quality assessed by fluorescence on a Qubit 4.0 fluorometer (Thermo Fisher Scientific, MA, USA) and by a gDNA ScreenTape on a TapeStation 4200 system (Agilent Technologies, CA, USA) prior to library preparation.

2.4. Illumina sequencing

The "Illumina DNA Prep, (M) Tagmentation" kit (Illumina, CA, USA) was used according to the manufacturer's specifications to generate 150PE Illumina-ready libraries. Illumina Unique Dual Indexes were used for indexing. A total of at least five to at most twelve amplification cycles were performed during the library generation step, depending on available input DNA mass. Library yields were quality assessed by fluorescence on a Qubit 4.0 fluorometer (Thermo Fisher Scientific), and

Table 1
Collection data and GenBank accession numbers of specimens with molecularly confirmed species identity. Complete mitogenome accession numbers are shown in bold fonts, marked with asterisk (*).

Species	Stage/sex	Avian host	Date	Place in Hungary (code)	GenBank accession number	
					*Cox*1 gene	16S rRNA gene
Ixodes arboricola	larva	*Parus major*	December 3, 2022	Halászi (PA238)	PV999241*	
	female	*Parus major*	December 3, 2022	Halászi (PA238)	PV707167	PV707173
	nymph	*Parus major*	February 7, 2025	Velence (FL331)	PV707168	PV707174
	nymph	*Parus major*	February 7, 2025	Velence (FL331)	-	PV707175
Ixodes lividus	female	*Riparia riparia*	June 19, 2017	Ócsa (FB1103)	PV999240*	
	female	*Riparia riparia*	June 27, 2024	Aba-Belső-Báránd (FL433)	PV707163	PV707169
	female	*Riparia riparia*	June 27, 2024	Aba-Belső-Báránd (FL436)	PV707164	PV707170
	female	*Riparia riparia*	June 27, 2024	Aba-Belső-Báránd (FL437)	PV707165	PV707171
	nymph	*Riparia riparia*	July 11, 2024	Dinnyés (FL473)	PV707166	PV707172

correct insert sizes were verified using D1000 ScreenTape on a TapeStation 4200 system (Agilent Technologies). Whole Genome Sequencing (WGS) libraries were equimolarly pooled over one/two/three individual sequencing runs, as indicated by the prefixes of the dataset IDs. 150PE sequencing was performed using 300-cycle P1 cartridge and flow cell combination on a NextSeq 2000 system (Illumina). Datasets yielded at least 2 gigabases per sample.

2.5. Mitogenome assembly

An automated bio-informatic pipeline was used for the assembly and annotation of the mitogenomes (van de Vossenberg et al. 2023). In short, Illumina reads were uploaded to CLC genomics workbench v25.0.1 (Qiagen, Germany) and quality trimmed (quality trim: 0.05; ambiguous limit: 2) prior *de novo* assembly (length fraction = 0.8, similarity fraction

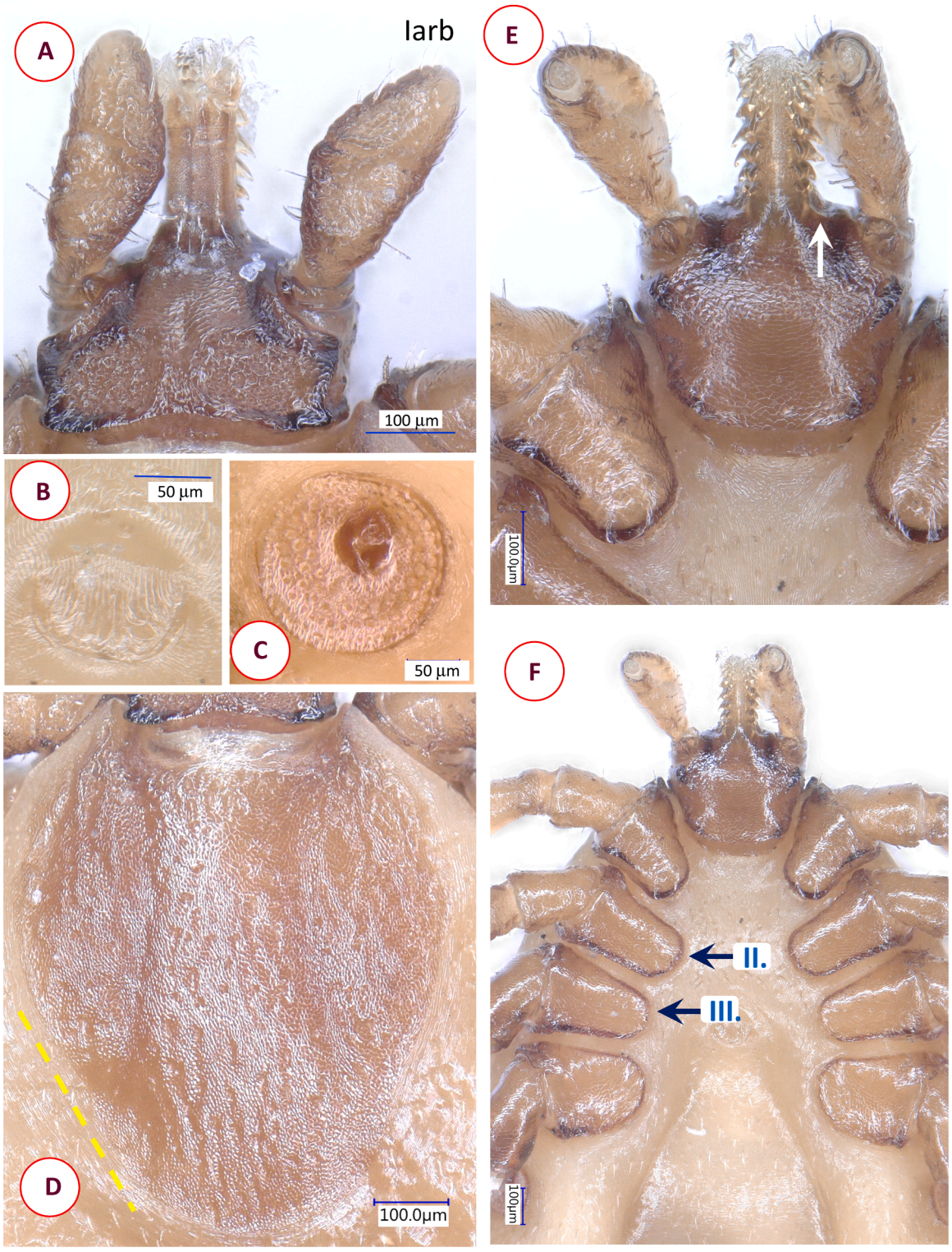


Fig. 1. Morphology of *Ixodes arboricola* female: (A) gnathosoma (dorsal), (B) genital pore, (C) spiracular plate, (D) scutum (dashed line: length of caudolateral straight margin), (E) gnathosoma (ventral) (arrow: lateral plateau), (F) coxae.

= 0.9). Putative mtDNA consensus sequences were blast-based identified and annotated with Mitos v1 (Bernt et al. 2013). These consensus sequences were visually assessed on the read mapping. The annotations were adjusted based on the nucleic acid translation (Genetic code: Invertebrate mitochondrial, Translation table 5). GenBank accession numbers of complete mitogenome sequences are PV999240 (*I. lividus*) and PV999241 (*I. arboricola*). Raw sequencing data were also uploaded to the Sequence Read Archive under submission number SUB15754256, and the BioProject accession is PRJNA1358267.

2.6. Phylogenetic analysis of complete mitogenome

ClustalW alignments were computed using Geneious 11.1.4 software (Kearse et al., 2012; Thompson et al., 2002). Phylogenetic inference was

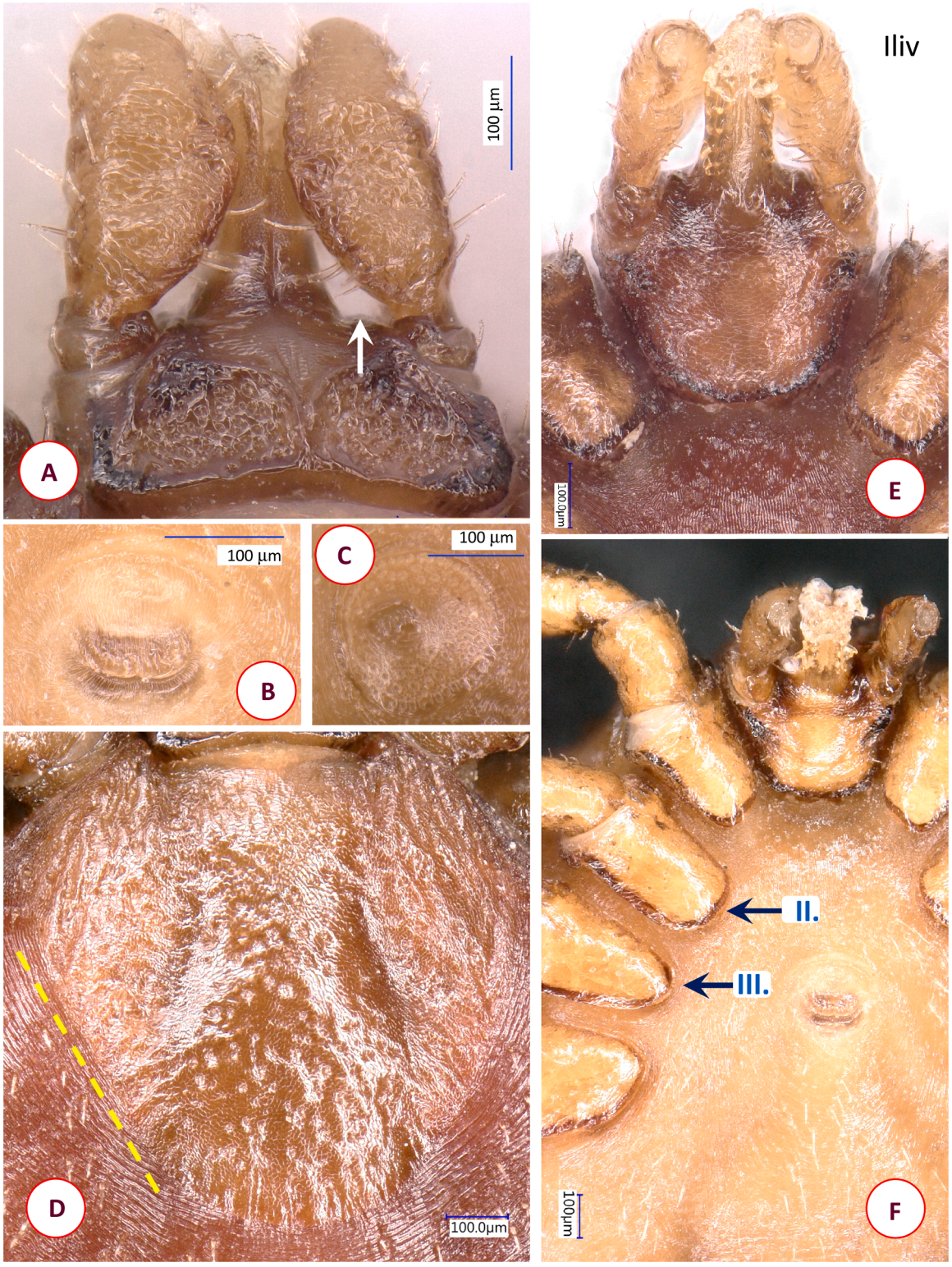


Fig. 2. Morphology of *Ixodes lividus* female: (A) gnathosoma (dorsal) (arrow: lateral plateau), (B) genital pore, (C) spiracular plate, (D) scutum (dashed line: length of caudolateral straight margin), (E) gnathosoma (ventral), (F) coxae.

performed using IQ-TREE 2.1.3 (Minh et al., 2020), and the best-fit evolutionary model was selected based on the Bayesian Information Criterion (BIC) calculated by the integrated ModelFinder (Kalyaanamoorthy et al., 2017), using the options: ModelFinder + tree reconstruction + ultrafast bootstrap (1000 replicates). Branch support was assessed using the ultrafast bootstrap (UFBoot) approximation (Hoang et al., 2018) and the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT) (Guindon et al., 2010). Three sequences of *Hyalomma* spp. were included as an outgroup. The tree was visualized and edited using FigTree (v1.4.1) and Inkscape (v0.91) (Bah, 2011).

3. Results

3.1. Morphological comparison of females

Females of *I. arboricola* and *I. lividus* are similar to each other in most

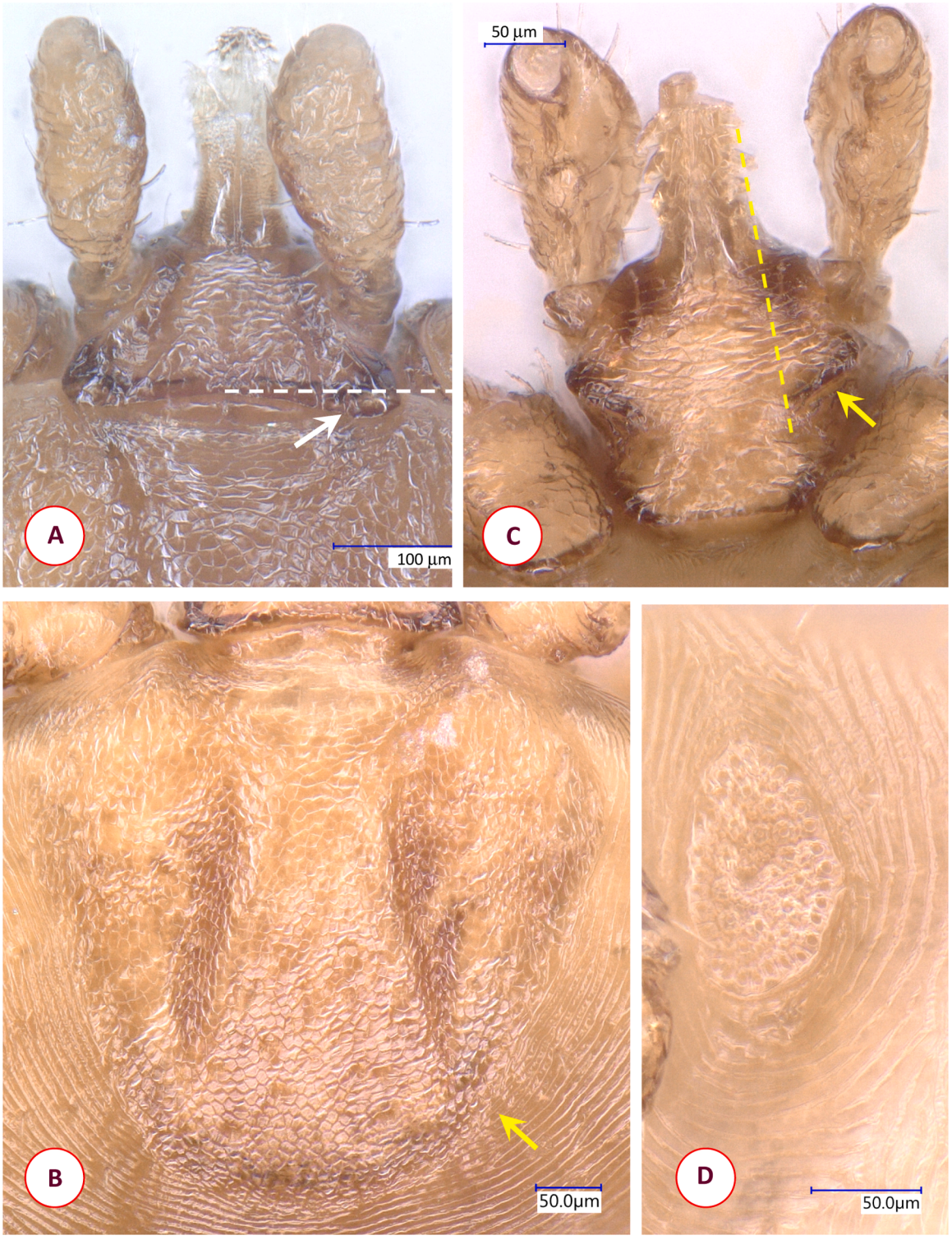


Fig. 3. Morphology of *Ixodes arboricola* nymph: (A) gnathosoma (dorsal) (dashed line: caudal margin, arrow: cornua), (B) scutum (arrow: end of cervical groove), (C) gnathosoma (ventral) (dashed line: prolongation of hypostome margin, arrow: auricular ridge), (D) spiracular plate.

of their characters, including (1) the heart-shape of the scutum with the broadest part at the anterior third of its length; (2) wrinkling of the scutal surface laterally and caudally; (3) broad, shallow and curved cervical grooves (Fig. 1D, 2D); (4) anterior part (front, tectum) of basis flattened laterally, perpendicular to hypostome axis (Fig. 1E, 2A); (5) some of the pores fused to form larger units in porose areas; (6) medially curved and laterally straight palps with similar number of 0.02–0.05 mm long hairs (Fig. 1A, 2A); (7) ventrally on the basis low and short auricular ridges; (8) inapparent, blunt internal spur on coxa I (Fig. 1E, 2E); (9) genital grooves diverging caudally from coxa IV (Supplementary Figure 1.A-B); (10) subcircular spiracles, with 3 to 6 rows of sparsely distributed aeropyles, opening eccentric (Fig. 1C, 2C).

However, some characters allow to distinguish these two species. The scutal index (length-to-width ratio) is approx. 1.1–1.2 in *I. arboricola* vs it is around 1 in *I. lividus*. The number of pores in the central region of the scutum of *I. lividus* exceeds that in case of *I. arboricola* (Fig. 1D, 2D), but this is difficult to assess due to the wrinkled surface. The caudolateral straight edge of the scutum is shorter, only in the caudal half of

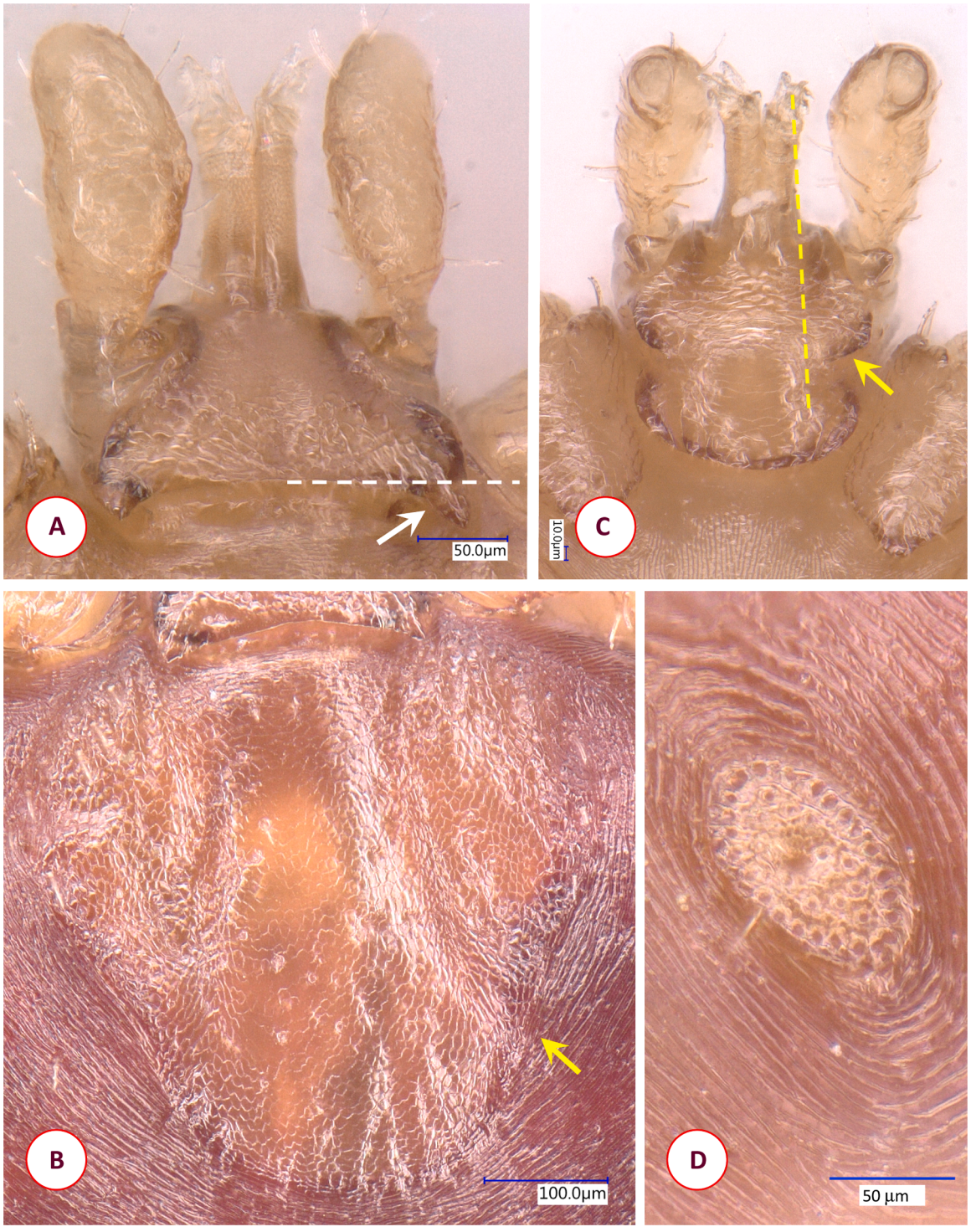


Fig. 4. Morphology of *Ixodes lividus* nymph: (A) gnathosoma (dorsal) (dashed line: caudal margin, arrow: cornua), (B) scutum (arrow: end of cervical groove), (C) gnathosoma (ventral) (dashed line: prolongation of hypostome margin, arrow: auricular ridge), (D) spiracular plate.

the scutum in *I. arboricola*, but it is longer, extending into the anterior half of the scutum in *I. lividus* (Fig. 1D, 2D). On the gnathosoma, the interval between porose areas of *I. arboricola* is usually broader (0.05 mm) than in case of *I. lividus* (varying between 0.01–0.05 mm, Fig. 1A, 2A). Only in *I. lividus*, some of the posterior pores are on the caudal margin of basis, while the porose areas of *I. arboricola* are surrounded by a rim. The anteriolateral broadening of the basis dorsally is more evident in *I. arboricola* than in *I. lividus* (Fig. 1A, 2A). The basis capituli is more than twice as long as the length of porose areas in *I. arboricola*, whereas it is less than twice as long as the length of porose areas in *I. lividus*. In other words, the hypostome is shorter than the basis in *I. arboricola*, whereas it is longer than the basis in *I. lividus* (Fig. 1A, 2A). The separation of palpal segments II and III is more evident (as broad and deep groove) in *I. arboricola* (Fig. 1A) than in *I. lividus* (Fig. 2A). Ventrally, three differences are evident between the two species. First, the medial edge of all coxae of *I. arboricola* are rounded, the third in particular only slightly narrower than the second (Fig. 1F); by contrast, in *I. lividus*, the second coxa has rounded square-shaped medial edge, and compared to it the third coxa is much narrower, rounded, almost peaked (Fig. 2F). Second, the genital pore of *I. arboricola* is narrow U-shaped (twice broad than long) (Fig. 1B), whereas this is broad U-shaped in *I. lividus* (approx. four times broader than long) (Fig. 2B). Third, sides of the anal groove of *I. arboricola* diverge backwards, whereas these are nearly parallel in *I. lividus* (Supplementary Figure 1.A-B). Finally, tarsus I has a less elevated, but distally steeper hump in *I. lividus* compared to *I. arboricola* (Supplementary Figure 1.C-D).

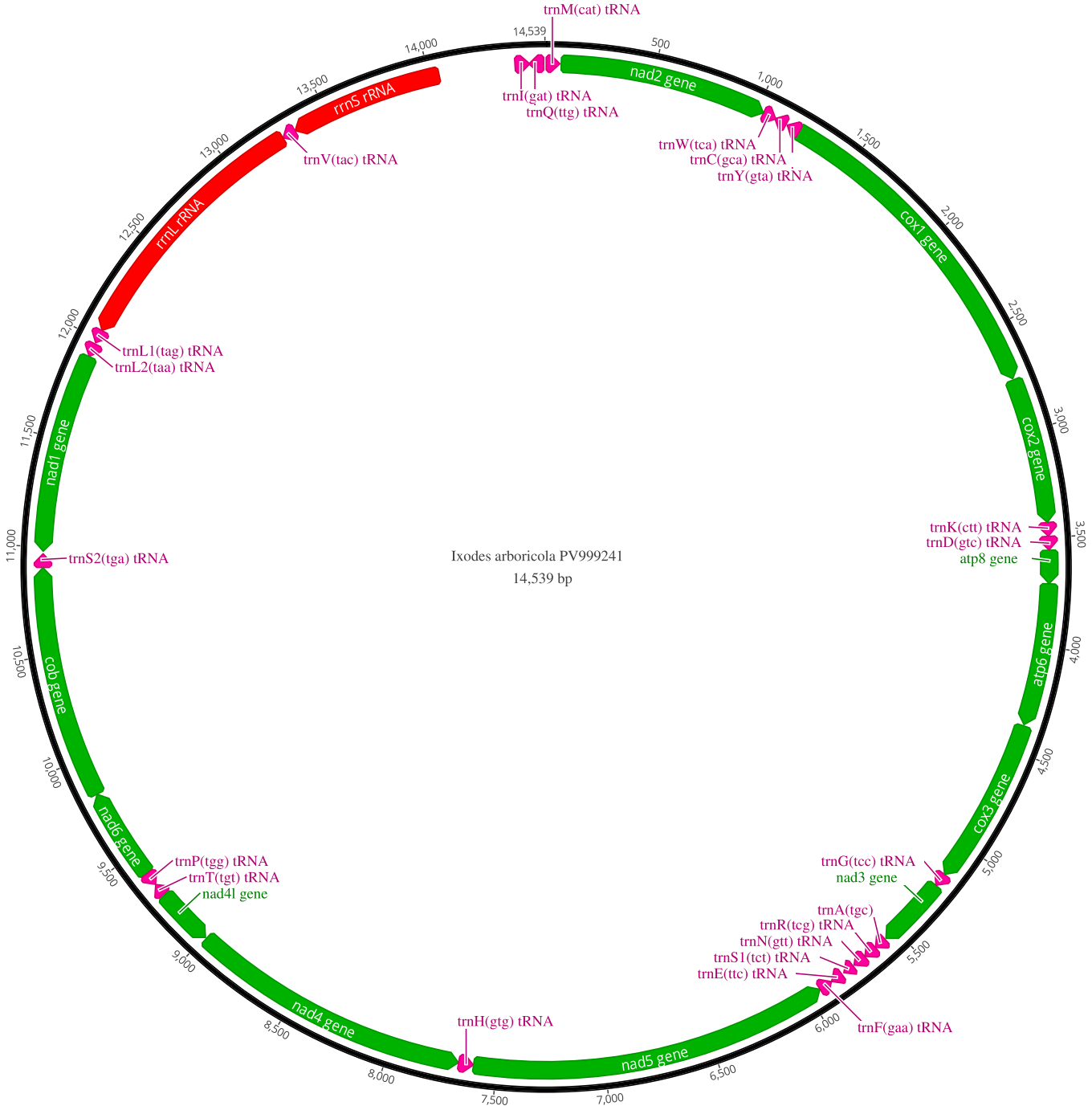


Fig. 5. Arrangement of the complete mitogenome of *Ixodes arboricola*. Protein-coding genes (abbreviated) are indicated with green, and tRNAs with red color.

3.2. Distinguishing characters of nymphs

On the scutum of *I. arboricola* nymphs, the cervical grooves are nearly straight, reaching the caudolateral scutal margin close to the posterior end of its straight part (Fig. 3B). By contrast, the cervical grooves of *I. lividus* nymphs are curved, reaching the caudolateral scutal margin at the deepest point of its concavity (Fig. 4B). Nymphs of *I. arboricola* have inapparent, caudolaterally directed blunt cornuae (Fig. 3A), while *I. lividus* nymphs have prominent, backward-projecting cornuae (Fig. 4A). Ventrally on the basis, the auricular ridges of *I. arboricola* nymphs are relatively long, similar in length to the interval between them, and reaching the prolongation of the hypostome's margin (Fig. 3C). This is unlike in *I. lividus* where the auricular ridges are short, with an interval more than twice their length and not reaching the prolongation of the hypostome's margin (Fig. 4C). The spiracular plates of both species are elongated in the nymphal stage, but in *I. arboricola* these do not have parallel edges (Fig. 3D), but in *I. lividus* they do (Fig. 4D).

3.3. Mitogenome and phylogenetic analyses

From one specimen of each tick species, *I. arboricola* and *I. lividus*, a circular mitochondrial genome was obtained, including 13 protein-encoding genes, 22 transfer RNA genes (tRNAs), and 2 ribosomal RNA genes (rRNAs) (Figs. 5 and 6). *Ixodes lividus* has three alternative stop codons (*cox*2, *cox*3 and *nad*1) and *I. arboricola* has four (*cox*2, *cox*3, *cob*

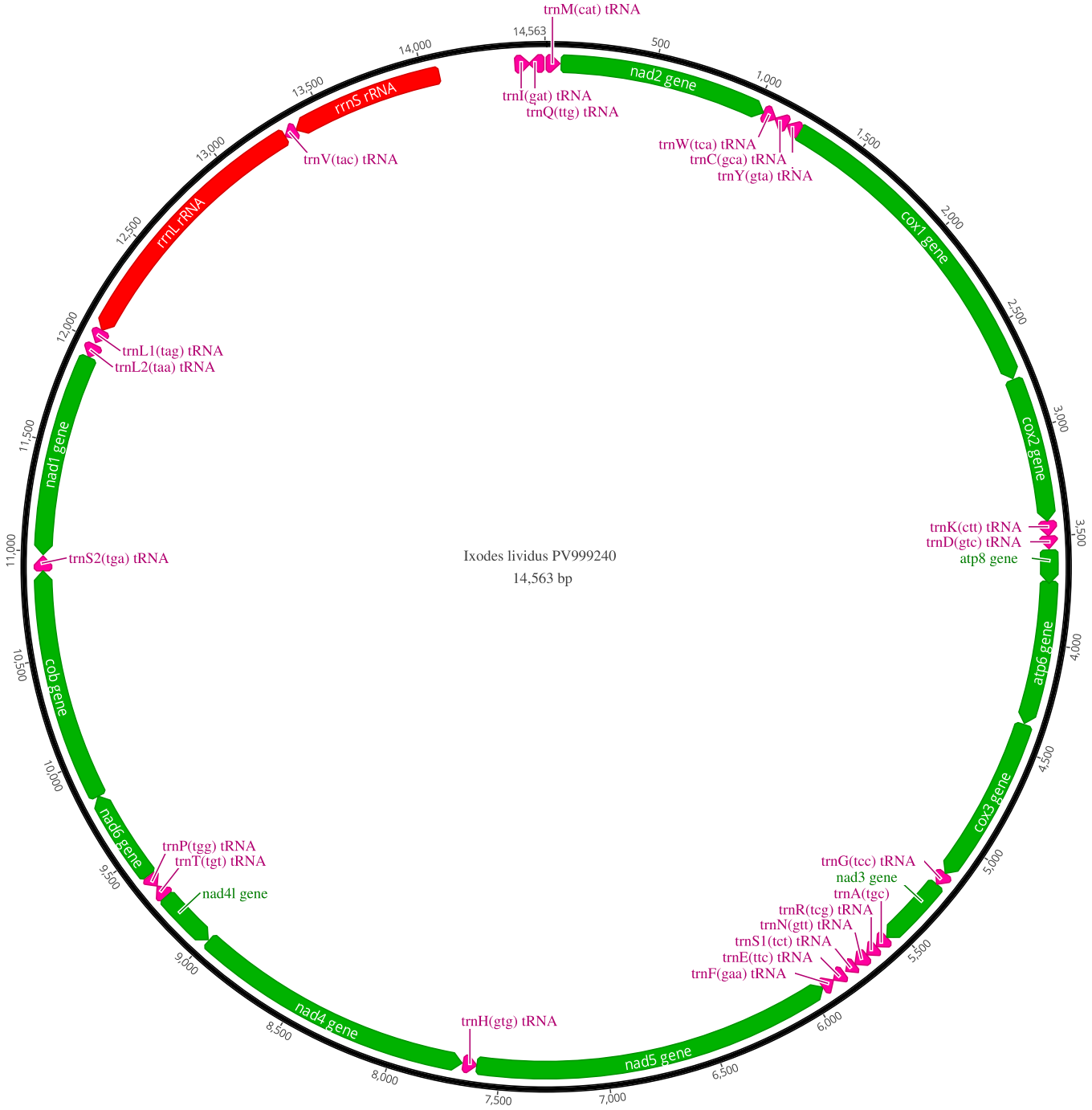


Fig. 6. Arrangement of the complete mitogenome of *Ixodes lividus*. Protein-coding genes (abbreviated) are indicated with green, and tRNAs with red color.

and *nad*1). The average read coverage (ARC) of the contigs was 43x in the case of *I. arboricola*, whereas 342x in the case of *I. lividus*. The mitochondrial genome size was 14,539 and 14,536 bp, for *I. arboricola* and *I. lividus*, respectively. The guanine-cytosine base content (GC) of the mitochondrial genomes was slightly higher for *I. arboricola* (22.4%) in comparison to *I. lividus* (22.0%). The sequences of *I. arboricola* and *I. lividus* were 91.1% identical to each other (Table 2) (based on the ClustalW alignment generated for six complete mitochondrial genomes of *Pholeoixodes* ticks, using Geneious software).

Phylogenetic analysis of *Ixodes* species showed that *I. arboricola* and *I. lividus* are sister species, and cluster together with *Ixodes crenulatus/ canisuga* under strong (100%) support (Fig. 7). These three *Pholeoixodes* species occupied a sister clade to bat-associated *Ixodes* species.

4. Discussion

In this study, high resolution digital photography was used to compare the morphology of two closely related ornithophilic tick species, at the same time obtaining their complete mitochondrial genome sequences. Nevertheless, the sample size was too small to provide quantitative ranges of measurements (mean ± standard deviation), and mitogenome sequences were generated from a single specimen of each species. Therefore, no conclusions could be drawn on intraspecific variation and future studies will be necessary, using more individuals to confirm the observed interspecific genetic distance (91.1 % identity).

Ixodes arboricola is a widespread Palearctic tick species, occurring in most countries of Europe, in North Africa, Transcaucasia, as well as in the eastern part of Asia (Keve et al., 2022; Fedorov and Hornok, 2024). It has a relatively broad range of avian hosts, primarily being a parasite of tree hole dwelling bird species (songbirds: order Passeriformes, owls: order Strigiformes, woodpeckers: order Piciformes) but can also infest relatively frequently diurnal birds of prey associated with cliffs (orders Accipitriformes, Falconiformes) (Keve et al., 2022; Fedorov and Hornok, 2024).

On the other hand, *I. lividus* has a broader, nearly pan-Eurasian geographical distribution (Keve et al., 2022; Fedorov and Hornok, 2024), but a narrower host range than *I. arboricola*. Considering *I. lividus*, it is a strict host-specialist, with most observations linked to the sand martin (*Riparia riparia*), which is a common nest-dweller all over Europe and Asia. It was recorded from eight other bird species, either from other swallows (barn swallow, *Hirundo rustica* and house martin *Delichon urbicum*) which regularly roost together with sand martins while in migration (Kaczmarek 1982, Hind et al. 2022) or other birds, like European bee-eaters (*Merops apiaster*), tree sparrows (*Passer montanus*) or starlings (*Sturnus vulgaris*), which may occupy breeding holes of sand martins in riverbanks or loess walls (Krumpál et al. 1995, Trilar 2004, Rusev 2009). These ecological data suggest that the report of *I. lividus* from diurnal birds of prey (*Falco* sp.: Ganbold et al., 2024) is probably a misidentification and may be instead relating to *I. arboricola*. In that study generated sequence(s) were not deposited in GenBank, and morphology of ticks in the illustrations resemble *I. arboricola* in characters described here (e.g., basis longer than the diameter of porose areas, all coxae medially rounded, anal grooves diverging). This and other possible misidentifications underline the importance of morphological differential diagnosis of these two cavity-dwelling ornithophilic tick species.

Ixodes arboricola and *I. lividus* were exceptionally also recorded to feed on mammals. In particular, both of these tick species were collected from rodents and bats. *Ixodes arboricola* was collected from dormouse (hazel dormouse, *Muscardinus avellanarius*), an arboreal species which regularly uses tree holes for diurnal resting (Krumpál et al. 1995), while *I. lividus* was reported from house mouse (Filippova, 1977). Among bats, *I. arboricola* was reported from two bat species, the common noctule (*Nyctalus noctula*) and the Nathusius' pipistrelle (*Pipistrellus nathusii*) (Sándor et al. 2025). Both bat species are forest specialists and crevice-dwellers, commonly roosting in tree holes and even artificial nest boxes erected for birds (Dietz et al. 2009). Sharing a roost may enhance the transfer of this bird specialist tick to bats, especially because the occurrence of common noctule individuals was recorded even in active nests of common starlings (Myczko et al. 2016), while this latter bird species is known to be a regular host for *I. arboricola. Ixodes lividus* was recorded on common pipistrelles (*Pipistrellus pipistrellus*) in the UK, the Netherlands and Germany (Sándor et al. 2025). Occurrence of bird specialist ticks on bats is a rare phenomenon but becomes more interesting in light of the phylogenetic analysis of this study, because the two *Pholeoixodes* species examined here, occupied (together with *I. canisuga*) a sister clade to bat-associated *Ixodes* species, hinting towards either birds or bats as hosts for their respective common ancestor.

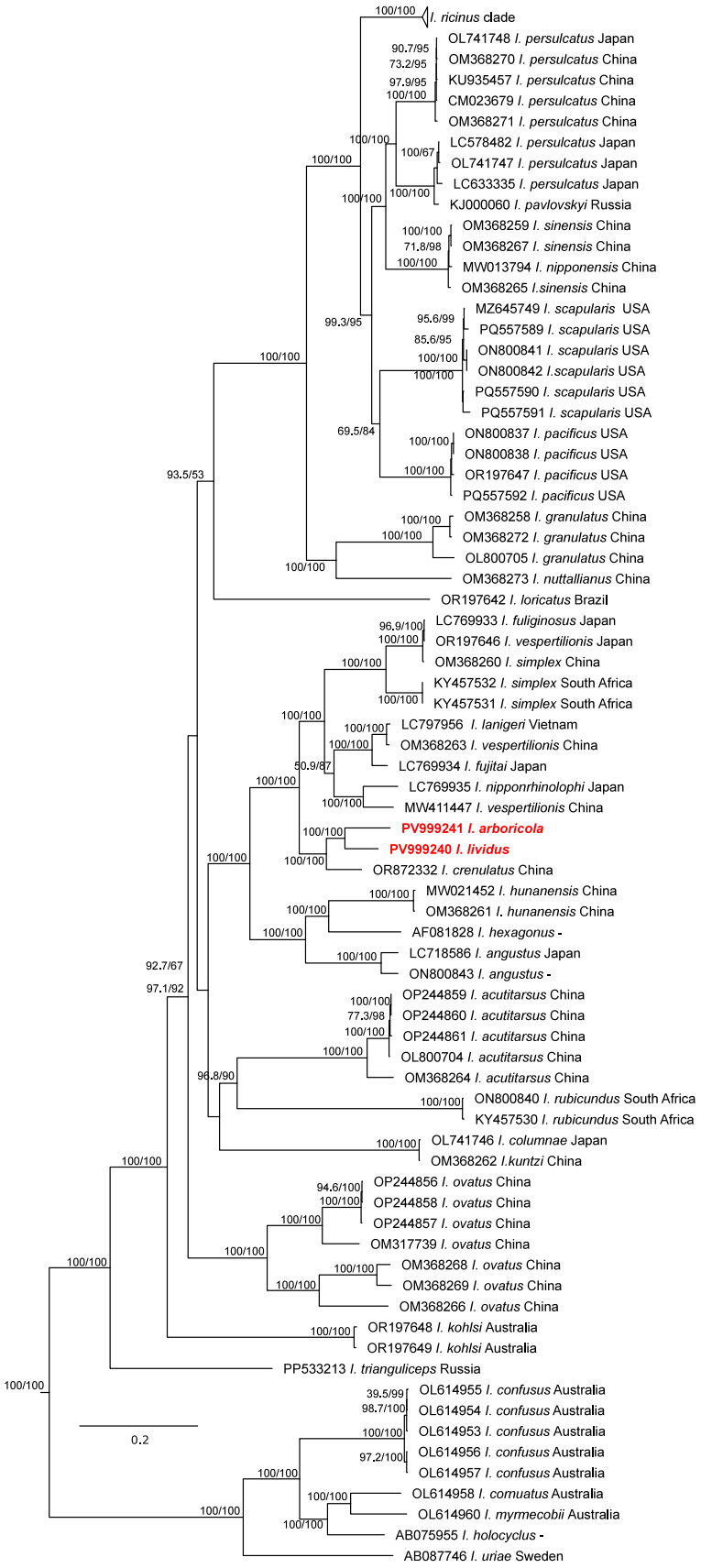
Results of this study confirmed what was already postulated (Arthur, 1953) that the front of the basis capituli (tectum) is crucial in distinguishing *Ixodes* species, especially in subgenus *Pholeoixodes* (Hornok et al., 2017) where both *I. arboricola* and *I. lividus* belong. In particular, as it was shown here based on their complete mitogenome, these two species are phylogenetically closest related to *I. canisuga* (Hornok et al., 2023), and all these three species share the character of a flattened part (plateau) at the hypostome basis. However, for *I. canisuga* females and nymphs this plateau encircles the base of hypostome and is also visible anteriorly (Hornok et al., 2017; Hornok et al., 2021), unlike shown here for *I. arboricola* and *I. lividus*. Another phylogenetically relevant morphological character is the scutal surface which is wrinkled (rugose) as a common feature of *Pholeoixodes* species in the clade of *I. arboricola* and *I. lividus* (including *I. canisuga*: Hornok et al., 2017; *I. rugicollis*: Hornok et al., 2023; and *I. ariadnae*: Hornok et al., 2014).

These structures appear to be phylogenetically and taxonomically more relevant to define closely related species within the subgenus *Pholeoixodes*, than for instance the posterior edge of basis capituli in nymphs which is provided with prominent cornuae both in the case of closely related *Pholeoixodes* species (e.g., *I. lividus* and *Ixodes kandingensis*: Guo et al., 2017) and in a phylogenetically more distant one (e.g., *I. kaiseri*: Hornok et al., 2021).

While the host ranges of *I. arboricola* and *I. lividus* do not significantly overlap, they may share pathogens, as exemplified by *Rickettsia vini* (Nováková et al., 2018). *Borrelia burgdorferi* sensu lato and *Borrelia garinii* were also demonstrated in *I. arboricola* and *I. lividus*, respectively, and the first was even transstadially inherited (Movila et al., 2008; Heylen et al., 2014b). Several viruses were identified in bird specialist *Pholeoixodes* ticks. Both *I. arboricola*, as well as *I. lividus* are suspected to have a vectorial role for tick-borne encephalitis virus (Lichard & Kožuch 1967, Nosek & Blaškovič 1973, Efremova & Gembitsky 1998), while a specific flavivirus (Kama virus) is transmitted by *I. lividus* (L'vov et al.

Table 2
The matrix of genetic identity (%) among six complete mitochondraial genomes belonging to *Pholeoixodes*.

AF081828 *I. hexagonus*						
PV999241 *I. arboricola*	80.09					
PV999240 *I. lividus*	80.39	91.09				
MW021452 *Pholeoixodes* sp.	83.91	79.75	80.11			
OM368261 *Pholeoixodes* sp.	83.89	79.74	80.11	99.52		
OR872332 *I. crenulatus*	80.5	89.52	90.35	80.03	80.04	
	AF081828 *I. hexagonus*	PV999241 *I. arboricola*	PV999240 *I. lividus*	MW021452 *Pholeoixodes* sp.	OM368261 *Pholeoixodes* sp.	OR872332 *I. crenulatus*



(caption on next page)

Fig. 7. Schematic representation of the maximum likelihood phylogenetic tree based on the complete mitogenomes of 114 sequences of *Ixodes* spp. The final length of the alignment was 15,784 bp and the tree was constructed using the evolution model GTR+F+I+G4. The highlighted clade represents the newly obtained mitogenome of *I. arboricola* and *I. lividus* generated in this study; bootstrap values (SH-aLRT/UFB) above the 60/85 threshold are displayed; all sequences included in the analysis are listed in Supplementary Table 1. The outgroup is not displayed. Sequences acquired from the GenBank database and sequences obtained in this study (highlighted in red) are presented by their accession number, host and country of origin. The scale bar indicates the number of nucleotide substitutions per site. The clade representing the *I. ricinus* complex is shown collapsed.

1998). These data indicate that the eco-epidemiological significance of the two ornithophilic tick species studied here may in part be similar and they may play a role in the transmission of rickettsiae, borreliae and viruses for which birds act as reservoirs (Spitalská et al., 2011). On the other hand, their non-overlapping host ranges and niches might limit pathogen exchange in nature, even if both are competent vectors of the same viruses, bacteria or protozoa under experimental conditions.

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Data availability

All three sequences obtained during this study were identical, deposited in GenBank under the following accession numbers: PV707163-PV707168 (*cox*1 gene), PV707169-PV707175 (16S rRNA gene). GenBank accession numbers of complete mitogenome sequences are PV999240 (*I. lividus*) and PV999241 (*I. arboricola*). Raw sequencing data were also uploaded to the Sequence Read Archive under submission number SUB15754256, and the BioProject accession is PRJNA1358267. All other relevant data are included in the manuscript and the supplementary material or are available upon request by the corresponding author.

Supplementary Material

Supplementary Figure 1. Additional morphologic characters of females. Ventral habitus of (A) *Ixodes arboricola*, (B) *Ixodes lividus*. Tarsus I of (A) *Ixodes arboricola*, (B) *Ixodes lividus*.

CRediT authorship contribution statement

Sándor Hornok: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Paulina Lesiczka:** Methodology, Investigation, Data curation. **Tim Warbroek:** Methodology, Investigation, Data curation. **Tijs J.M. van den Bosch:** Methodology, Investigation, Data curation. **Andor Pitó:** Methodology, Investigation. **Gergő Keve:** Methodology, Investigation. **Nóra Takács:** Methodology, Investigation, Data curation. **Jenő Kontschán:** Investigation, Data curation. **Attila D. Sándor:** Writing – review & editing, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2025.102589.

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