



Bat ectoparasites: chigger mites (Trombiculidae), ticks (Ixodidae and Argasidae), and bugs (Cimicidae) in the Eastern Palaeartic

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Abstract

Nine species-level taxa of bat ectoparasites, three chigger mites (Trombiculidae), three hard (Ixodidae), and one soft tick (Argasidae) species, as well as two bug (Cimicidae) species from nine bat species hosts were detected in the Eastern Palaeartic. Trombiculid larvae of *Leptotrombidium schlugerae*, *Leptotrombidium album*, and *Ascoschoengastia latyshevi* were first recorded on bats in the temperate zone of eastern Russia. *L. schlugerae* was more abundant than *A. latyshevi* in the same study sites in Eastern Siberia, and the main hosts of both chigger species were *Plecotus ognevi* and *Eptesicus nilssonii*. Ixodid ticks *Dermacentor marginatus*, *Ixodes simplex*, and *Ixodes* sp. were sampled from bats in Kazakhstan, the Far East, and Eastern Siberia, respectively. Phylogenetic analysis based on *Cox1*, 16S rDNA, and ITS2 sequences of *I. simplex* showed that the specimens from the Far East grouped into a clade distributed in the Eastern Palaeartic and India. In turn, the specimen of *Ixodes* sp. from Eastern Siberia was most closely related to *Ixodes soricis* and *Ixodes angustus* with *p*-distance of 9.8–10.7% (*Cox1*), suggesting that this tick probably belongs to a new species. *Argas vespertilionis* larvae were collected from three widespread bat species in Kazakhstan. Two bug species, *Cimex pipistrelli* and *Cimex* aff. *lectularius*, were recorded in the Far East and Eastern Siberia, respectively. Specimens from Transbaikalia were morphologically identified as *Cimex lectularius*. However, they differed from the latter by 12.5–12.9% of *Cox1* sequences, indicating that *C. aff. lectularius* may be a new species.

Keywords Bat ectoparasites · Trombiculidae · *Ixodes* · *Argas* · *Cimex* · Eastern Palaeartic

Introduction

Bat ectoparasites are diverse and are represented in the world by different taxa. For example, the most common and specialized taxa include gamasid (e.g., Macronyssidae and Spinturnicidae), chigger (Trombiculidae) and myobiid

(Myobiidae) mites, hard (Ixodidae) and soft ticks (Argasidae), flies (Nycteribiidae and Streblidae), fleas (Ischnopsyllidae), and bugs (Cimicidae) (Beron 2020; Zajkowska et al. 2018; Bochkov 2009; Farafonova and Gornostaev 2018), and rarer taxa belong to other nine mite families.

More than 397 species of chigger mites (Acari: Trombidiformes, Trombiculidae) included in 18 genera are known to be associated with the bats worldwide (Zajkowska et al.

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2018). Of them, 71 (18%) species of 12 genera are distributed in the Eastern Palearctic area. Half of the trombiculid species (29 species) of the Eastern Palearctic originate from Central Asia, and the other half (42 species) from the Far East — China, Korea, and Japan. There are a few records of chigger mites on bats in the temperate zone of eastern Russia — *Oudemansidium subakamushi* Schluger, 1948 and *Leptotrombidium pavlovskiyi* Schluger, 1948 (Shluger 1948; Zhovty et al. 1962; Emel'yanova and Vysokovskiy 1962).

Four hard tick species (Acari: Ixodidae), *Ixodes vespertilionis* Koch, 1844, *Ixodes simplex* Newmann, 1906, *Ixodes kopsteini* Oudemans, 1926, and *Ixodes ariadnae* Hornok, 2014, are known to infest bats in Eurasia (Robbins and Bangs 2004; Hornok et al. 2014). *Ixodes redikorzevi* Olenov 1927, *Haemaphysalis* spp. and *Amblyomma testudinarium* Koch, 1844 have been found on bats at least in Japan and Iran (Filippova et al. 1976; Yamauchi and Funakoshi 2000; Yamauchi et al. 2021). Bat ixodid ticks from Europe were positive for *Anaplasma phagocytophilum* Foggie 1949, *Bartonella* spp., and *Mycoplasma* sp. (Hornok et al. 2019).

In Palearctic, bat-specialist soft ticks (Acari: Argasidae) are *Argas (Carios) vespertilionis* Latreille, 1802, *Chiropterargas boueti* Roubaud & Colas-Belcour, 1933, *Chiropterargas confusus* Hoogstraal, 1955, *Reticulinasus salahi* Hoogstraal, 1953, and *Secretargas transgaripepinus* White, 1846 (Sándor et al. 2021).

The *Cimex* genus (Insecta, Heteroptera: Cimicidae) has a worldwide distribution and includes 23 valid species. Twenty *Cimex* species exhibit a trophic preference for bats (Akhoundi et al. 2020) and eight *Cimex* species are known in the Eastern Palearctic.

Bats and ectoparasites are well-known natural hosts and vectors for zoonotic infections. For example, at least 88 *Bartonella* species have been found worldwide (McKee et al. 2019). The trombiculid larvae are well-recognized vectors of *Orientia tsutsugamushi* Hayashi, 1920 — the agent of scrub typhus (tsutsugamushi fever) (Blake et al. 1945; Urakami et al. 1999; Frances et al. 2000; Elliott et al. 2019). In total, more than 30 bacterial species from families Rickettsiaceae, Borreliaceae, and Bartonellaceae can infect trombiculids (reviewed in Moniuszko et al. 2022). Soft ticks may be the vectors of pathogenic protozoa (Hornok et al. 2017a, b; Lv et al. 2018), bacteria (Socolovschi et al. 2012; Hornok et al. 2019), and viruses (Varma and Converse 1976; Alkhovsky et al. 2013; Oba et al. 2016). Bat bugs are the vectors of *Bartonella* spp. (McKee et al. 2019; Kejíková et al. 2022). Such a variety of carried pathogens makes the study of the diversity and distribution of ectoparasites of bats important for public health.

The purpose of this research is to identify species composition, distribution, host-parasite, and phylogenetic relationships of temporary bat ectoparasites, chigger mites

(Trombiculidae), hard (Ixodidae), and soft ticks (Argasidae), as well as bugs (Cimicidae), in the Eastern Palearctic.

Material and methods

Sampling sites

The study sites were located in eight ecoregions and five terrestrial biomes (Olson et al. 2001) of the Eastern Palearctic (seven ecoregions in Russia and one ecoregion in Kazakhstan) (Fig. 1). The bat habitats in these ecoregions are defined by different landforms/landscapes such as river valleys, mountain ranges, or sea coasts:

- Sayan alpine meadows/tundra and Sayan montane conifer forests (sites 5, 6, 7) — the Eastern Sayan Mountains and the Irkut River valley;
- East Siberian taiga (sites 8, 9, 10, 14) — the Primorsky Range, Baikal Lake, and the Vitim Plateau;
- Trans-Baikal conifer forests and Okhotsk-Manchurian taiga (sites 11, 12, 13, and 15) — the Chikoy and the Amur River valleys, respectively;
- Ussuri broadleaf and mixed forests, Manchurian mixed forests, and Hokkaido deciduous forests (sites 16, 17, 18) — the Sea of Japan and the Sea of Okhotsk coasts, respectively;
- Tian Shan foothill arid steppe (sites 1, 2, 3, 4) — SW ranges of the Dzungarian Alatau Mountains and the Ili River valley.

Bat surveys and collection of ectoparasites

A total of 359 ectoparasite specimens, 265 chigger mite, 11 hard and 78 soft tick, and six bug specimens, were collected from 46 bat individuals in 18 localities. Bats were caught using mist nets (6/7/9 m × 2.5 m; *Ecotone*, Poland) in foraging sites (over the rivers) from June to August, swarming sites (at the cave entrances) in April to May/August, and found in roosts (city or village buildings) in August and October. Bats were identified by species (Tiunov 1997; Gromov et al. 1963; Corbet 1978) and sex; adults were distinguished from juveniles by the ossification degree of the epiphyseal joints of the finger bones (Racey 1974; Anthony 1988). Afterwards, the bats were ringed with aluminum rings (measuring 2.9 and 3.5 mm) and released at their capture site. Feeding chigger mites, ticks, and bugs were collected using a sterile mounting needle, fixed in 96% ethanol and stored at −20 °C until DNA extraction and identification. All applicable international, national, and institutional ethics statements when using animals in research have been followed.

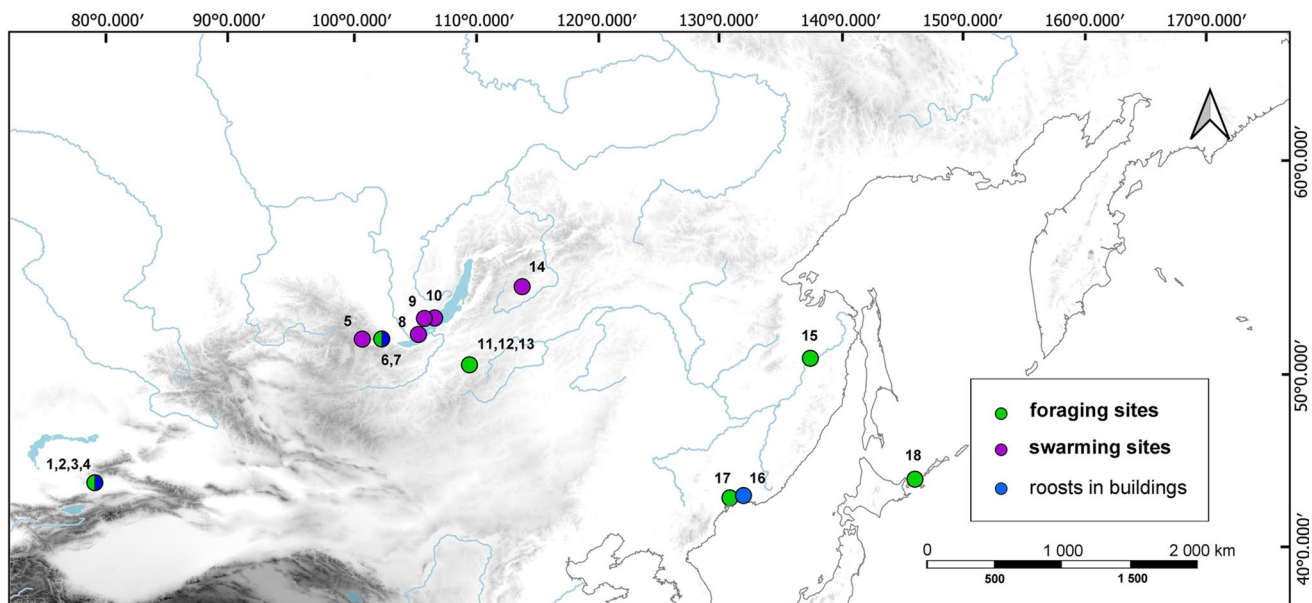


Fig. 1 Map showing bat capture and ectoparasite collection sites in the Eastern Palaearctic. Kazakhstan: 1, 2, 3, 4 — Almaty Region, Basshi village, Shygan, Malyj Kalkan, and Kosbastau cordons. Russia: Buryatia Republic: 5 — Belyj Irkut River; 6, 7 — Kharagun River and Badary tract; 14 — Dolganskaya Yama Cave; Irkutsk Region: 8 — Okhotnichya Cave; 9 — Chekanovskogo Cave; 10

— Mechta Cave; Transbaikal Territory: 11, 12, 13 — 11 km S of Khilogoson village, Arey River; Khabarovsk Territory: 15 — Gorin River, near the mouth of Malaya Talandinka River; Primorsky Territory: 16 — Vladivostok; 17 — Ovchinnikovo village; Sakhalin Region: 18 — Kunashir Island, Ozernaya River

Identification of mites, ticks, and bugs

Trombiculid larvae ($n = 265$) were mounted on microscopic slides using Faure–Berlese fluid as a clearing agent. Slides were examined under a Micromed-3 Professional microscope (Ningbo Sheng Heng Optics & Electronics, China) supplied with phase contrast. Photos were made using a ToupCam camera (ToupTek Photonics, China). Measurements were taken from photos by the calibrated software ToupView (ToupTek Photonics, China). The morphological terminology follows Goff et al. (1982) and Stekolnikov (2013): AW — distance between anterolateral scutal setae; PW — distance between posterolateral scutal setae; SB — distance between bases of sensillae; ASB — distance from the bases of sensillae to extreme anterior margin of the scutum; PSB — distance from the bases of sensillae to extreme posterior margin of scutum; SD — the length of the scutum (ASB + PSB); P–PL — distance from the level of posterolateral scutal setae to extreme posterior margin of the scutum; AP — distance between bases of antero- and posterolateral scutal seta on one side; AM — the length of anteromedian scutal seta; AL — the length of anterolateral scutal setae; PL — the length of posterolateral scutal setae; H — the length of humeral setae; Dmin — the length of the shortest dorsal idiosomal seta; Dmax — the length of the longest dorsal idiosomal seta; Vmin — the length of the shortest ventral

idiosomal seta; Vmax — the length of the longest ventral idiosomal seta; pa — the length of leg I (excluding claws and including coxa); pm — the length of leg II (excluding claws and including coxa); pp — the length of leg III (excluding claws and including coxa); Ip — the sum of legs lengths (pa + pm + pp); DS — the number of dorsal idiosomal setae (excluding scutal and including humeral); NDV — the number of idiosomal setae (DS + VS). The identification of the trombiculid larvae was made with the help of Vercammen-Grandjean and Langston (1976), Kudryashova (1998), and Stekolnikov (2013). The specimens examined are deposited at the Department of Entomology, Faculty of Biology, Lomonosov Moscow State University (Moscow, Russia).

Hard (nine larvae and two nymphs) and soft (78 larvae) ticks were removed from a storage buffer, washed in 70% alcohol and sterile phosphate buffered saline (PBS, pH = 7.4), and transferred on microscopic slides. Slides were examined under an MBS-1 stereomicroscope. The identification of developmental stage and tick species was carried out using key guides to the tick fauna of Russia and adjacent countries (Filippova 1977; 1997). The bug specimens were examined under a stereoscopic microscope (Olympus SZX9) and identified according to Usinger (1966) and Péricart (1972) by Dr. Dmitry A. Gapon (Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia).

DNA extraction, amplification, and sequencing

In total, single larva of Trombiculidae, seven larvae and one nymph of Ixodidae and single larva of Argasidae ticks were subjected to molecular analysis as well as six specimens of Cimicidae. Genomic DNA was isolated from individual mite specimens and bug legs using the QIAamp DNA Micro Kit (Qiagen, Germany) following the manufacturer's protocol with modifications (Matthews et al. 2018) or the RealBest Extraction100 Kit (VektorBest, Russia) according to the manufacturer's protocol. A fragment of the *Cox1* barcoding gene (658 bp) was amplified as in Young et al. (2019). We used LepF1-LepR1 and LCO-HCO primers (Hebert et al. 2004; Folmer et al. 1994) or LepF1-LepR1 primers only for chigger mites/ticks, and LepF-LepR primers modified for bugs (Balvín et al. 2013). For the PCR, the 20 µl PCR mix contained 4.0 µl of 5.0×PCR buffer (1.0×, 3.5 mM MgSO₄), 0.4 µl of dNTPs (10 mM each; 0.2 mM), 0.8 µl of each 10 µM forward and reverse primers, 0.3 µl of 50×Encyclo polymerase Mix (0.75), and 0.5 µl of DNA template per sample. Milli-Q water was used to make up the remaining volume. The complete internal transcribed spacer 2 (ITS2) and a fragment of the mitochondrial 16S rRNA gene (16S rDNA) for ixodid ticks were amplified as in Black and Piesman (1994) and Fukunaga et al. (2000), respectively.

All final PCR products were visualized on 1.0% agarose gels, purified using the *Cleanup S-Cap* Kit (Evrogen JSC, Russia) or QIAquick Gel Extraction Kit (Qiagen, Germany), and sequenced in both directions using the same primers on an ABI 3500 Genetic Analyzer (Applied Biosystems) or NanoFor-05 Genetic Analyzer (Syntol, Russia). Sequences are deposited in the GenBank database under accession no. OQ658533-40, OQ661875-80 (*Cox1*), OQ685053-55 (16S rDNA), and OQ685074-80 (ITS2).

Phylogenetic and population genetic analyses

The original sequences were aligned with published sequences taken from the GenBank database (Online Resource 1) using the CLUSTALW algorithm (Sievers et al. 2011). The original sequences were compared to those in public databases using the Nucleotide Basic Local Alignment Search Tool (BLASTn) on the NCBI website (<https://blast.ncbi.nlm.nih.gov/>). Maximum likelihood reconstructions were conducted in the IQTREE v. 1.6.12 software (Nguyen et al. 2015) with 1000 bootstrap replicates to test topology stability. The ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the optimum partitioning scheme and the best-fit substitution model under BIC criterion. Median-joining networks of *Cox1* haplotypes were constructed using NETWORK v. 10.2 (<http://www.fluxus-engineering.com/>).

Genetic distances were estimated using the MEGA v. 11 (Tamura et al. 2021).

Results

Acari.

Trombidiformes.

Family Trombiculidae.

Leptotrombidium schlugerae (Emeljanova et Gorbacheva, 1960)

Material. Russia, Irkutsk Region, collected by Denis V. Kazakov: 28 LL on adult males of *Plecotus ognevi* Kishida, 1927 (No. PO19006 / 010–11 / 045 / 056 / 080 / 119), 13 LL on adult male of *Eptesicus nilssonii* Keyserling et Blasius, 1839 (No. EN19017), Okhotnichya Cave, 52.13 N 105.45 E, 29–30-Apr-2019; 13 LL on adult male of *P. ognevi* (No. PO19121), Chekanovskogo Cave, 53.057 N, 106.0161 E, 6-May-2019.

Russia, Buryatia Republic, collected by Denis V. Kazakov: 55 LL on five adult males and three adult females of *P. ognevi* (No. DO18003 / 007 / 011 / 018 / 022 / 023 / 045 / 049), Dolganskaya Yama Cave, 54.44 N 113.78 E, 1-May-2018; 28 LL on adult male and adult female of *P. ognevi* (No. DO18570 / 636) and 17 LL on adult females of *Myotis petax* (No. DO18541-542), Dolganskaya Yama Cave, 54.44 N 113.78 E, 23-Aug-2018; 1 LL on adult male of *P. ognevi* (No. ES19153/01) and 2 LL on adult male of *E. nilssonii* (No. ES19155/03), Belyj Irkut River, 51.77 N 100.70 E, 7-Aug-2019; 4 LL on lactating female of *P. ognevi* (No. ES19157/05), Kharagun River, 51.60 N 102.36 E, 11-Aug-2019; 2 LL on adult male of *P. ognevi* (No. ES19162/10), Badary tract, 51.75 N 102.24 E, 17-Aug-2019.

Russia, Primorsky Territory, collected by Uliana V. Gorobeyko: 3 LL (No. PR22506.Ch1-3) on adult male of *Vespertilio murinus* (No. PR22506), Vladivostok, 43.12 N 131.92 E, 8-Oct-2022.

***Cox1* sequence** for No. PR22506.Ch1: OQ658540.

Localization: inside the ears in *P. ognevi* (Fig. 2) and *E. nilssonii*; near the eye, the dorsal side of the plagiopathagium and the body on the border with the uropatagium in *V. murinus*.

Other bat hosts: E. Palaearctic — unknown; the *L. schlugerae* record on bats in Kazakhstan (Dzhanokmen 1969) needs revision according to Kudryashova (1998).

Remarks. 4.78% OQ658540 vs *Leptotrombidium* sp. (Japan) AB300494.

The specimens examined were highly variable in the number of dorsal setae: fD = 2H–10(9–13)–8(8–11)–8(7–9)–2(2–8)–6(2–6)–4(2–4)–2(0–2). The measurements were overlapping *Leptotrombidium myotis* Ewing, 1929 (Table 1).



Fig. 2 *Leptotrombidium schlugerae* on *Plecotus ognevi* from Buryatia Republic, Russia. Photo by Denis V. Kazakov

***Leptotrombidium album* (Kamo et al. 1957)**

Material. Russia, Sakhalin Region, collected by Denis V. Kazakov and Uliana V. Gorobeyko: 8 LL (KU20138.Ch1-8) on adult male of *Barbastella pacifica* Kruskop, Kawai &

Tiunov, 2019 (No. KU20138), Kunashir Island, Ozernaya River, 43.87 N 145.48 E, 25-Jul-2020.

Localization: along the outer ear edges.

Other bat hosts: E. Palearctic — *Rhinolophus nippon* as “*Rhinolophus ferrumequinum*,” *Rh. cornutus*, Japan (Kamo et al. 1957).

Leptotrombidium sp

Material. Russia, Irkutsk Region, collected by Denis V. Kazakov:

52 LL on adult males of *P. ognevi* (No. PO19010-11 / 045 / 073 / 080 / 087–88 / 119) and 3 LL on adult male of *E. nilssonii* (No. EN19017), Okhotnichya Cave, 52.13 N 105.45 E, 29–30-Apr-2019; 14 LL on adult male of *P. ognevi* (No. PO19120), Chekanovskogo Cave, 53.057 N 106.0161 E, 6-May-2019.

Localization: inside the ears.

Remarks. Identification to species-level was impossible due to poor quality of the slides.

Table 1 Standard measurements (in micrometer) of *Leptotrombidium schlugerae* from this study, and *L. schlugerae* and *L. myotis* from previously published data

	<i>L. schlugerae</i> (n = 18) Okhotnichya and Chekanovskogo Caves, Russia		<i>L. schlugerae</i> (Kudryashova 1998) Transbaikalia, Russia	<i>L. myotis</i> (Vercammen-Grandjean and Langston 1976) USA
	Range	Median	Range	Range
AW	60–69	66	63	62–70
PW	72–79	76	77	72–78
SB	29–33	31	31	27–32
ASB	22–28	25	27	24–27
PSB	14–18	16	16	13–16
SD	39–46	42	43	38–42
P-PL	12–19	16	16	–
AP	21–27	25	23	23–25
AM	38–49	45	45–58	38–48
AL	28–39	31	36–40	30–40
PL	49–56	53	54–59	44–54
H	48–54	50	58	44–53
Dmin	34–39	37	36	36–47
Dmax	46–54	50	49	41–52
Vmin	23–27	25	27	29–32
Vmax	34–43	39	40	38–47
pa	256–301	278	275	249–278
pm	225–258	237	247	220–243
pp	253–298	277	277	262–275
Ip	748–833	803	799	738–790
DS	38–46	42	37–48	42
VS	33–43	38	32–46	36
NDV	73–88	82	94	78

Ascoschoengastia latyshevi (Schluger 1955)

Material. Russia, Irkutsk Region, collected by Denis V. Kazakov: 11 LL on adult males of *P. ognevi* (No. PO19056, PO19073), Okhotnichya Cave, 52.13 N 105.45 E, 29–30-Apr-2019.

Russia, Buryatia Republic, collected by Denis V. Kazakov: 6 LL on adult male of *P. ognevi* (No. DO18570), Dolganskaya Yama Cave, 54.44 N 113.78 E, 23-Aug-2018 and 5 LL on adult male of *P. ognevi* (No. ES19154/02), Belyj Irkut River, 51.77 N 100.70 E, 7-Aug-2019. The measurements are shown in Table 2.

Localization: inside the ears.

Other bat hosts: E. Palaearctic — unknown. W. Palaearctic — *Nyctalus lasiopterus*, Spain (Stekolnikov et al. 2022).

Ixodida.

Family Ixodidae.

Dermacentor marginatus (Sulzer, 1776)

Material. Kazakhstan, Almaty Region, collected by Denis V. Kazakov: 2 LL (No. KZ22014.1–2) on adult male of *Pipistrellus pipistrellus* (No. KZ22014), Shygan cordon, 44.11 N 78.71 E, 24-Jun-2022.

Table 2 Standard measurements (in micrometer) of *Ascoschoengastia latyshevi* ($n=6$) from this study

	Range	Median
AW	50–56	53
PW	60–70	66
SB	24–27	26
ASB	20–25	25
PSB	20–25	22
SD	42–50	45
P-PL	11–15	12
AP	20–22	21
AM	29–33	30
AL	24–31	28
PL	37–41	39
H	37–44	41
Dmin	20–23	21
Dmax	28–34	31
Vmin	16–19	18
Vmax	26–28	27
pa	195–221	212
pm	163–188	177
pp	182–209	202
Ip	546–618	593
DS	52–61	58
VS	43–48	46
NDV	100–106	103

Cox1 sequence for No. KZ22014.1: OQ658539. **16S rDNA sequence:** OQ685055.

Localization: on the outer side of the ear, on the wing membrane near the forearm.

Other bat hosts: E. Palaearctic — *P. pipistrellus*, Iran (Filippova et al. 1976).

Remarks. *Cox1*: 0–3.58% OQ658539 vs *D. marginatus*/as “*D. niveus*” (China) KU364300, MK213075, MT890492, KU880561–62 and etc.

16S rDNA: 0% OQ685055 vs *D. marginatus*/as “*D. niveus*” (China) KF547986, KU880610, NC_062070, MN860525.

The sequences of *D. marginatus* from Kazakhstan were clustered with *D. marginatus* from Western China both in *Cox1* and 16S rDNA analyses (Fig. 3). The host *P. pipistrellus* was caught near buildings that housed horses and dogs.

Ixodes simplex Neumann, 1906

Material. Russia, Primorsky Territory, collected by Denis V. Kazakov and Uliana V. Gorobeyko: 1 nymph and 7 LL (No. KH21121.1–8) on adult male of *Myotis macrodactylus* (No. KH21121) (Fig. 4 a, b), Ovchinnikovo village, 43.23 N 131.35 E, 28-Jul-2021.

Cox1 sequences: OQ658533–36. **16S rDNA sequence:** OQ685053. **ITS2 sequences:** OQ685074–79.

Localization: inside the ears and near the eyes.

Other bat hosts: E. Palaearctic — *Miniopterus fuliginosus*, *M. macrodactylus*, Japan (Yamauchi and Funakoshi 2000). W. Palaearctic — *Mi. schreibersii*, Caucasus, Russia (Filippova 1977); *Mi. schreibersii*, Europe; Oriental *Mi. magnater*, India (Hornok et al. 2015).

Remarks. *Cox1* (645–651 nt): 0.157% OQ658533–36 vs *I. simplex* (Japan) LC055099, LC651625.

16S rDNA (420 nt): 0.74–1.23% OQ685053 vs *I. simplex* (Japan, China, India) AB901140, NC_062060, KR902774, MW132810. *ITS2* (522 nt): 15.89% OQ685074–79 vs *I. simplex* (South Africa) KY457498. *p*-distances based on *Cox1* sequences between *I. simplex* from Primorye and other regions are shown in Table 3.

Sequences from Primorye, Japan, and China formed a single haplogroup (Fig. 4c) with mean within-group *p*-distance of 0.315%, and a single clade with high support (99–100) in *Cox1* and 16S rDNA (+ India) analyses (Fig. 3). Phylogenetic analysis based on *ITS2* sequences showed a sister position of *I. simplex* from Primorye and South Africa (Fig. 5).

Ixodes sp

Material. Russia, Irkutsk Region, collected by Denis V. Kazakov: 1 LL on adult male of *E. nilssonii* (No. ME21158), Mechta Cave, 52.95 N 106.78 E, 12-Aug-2021.

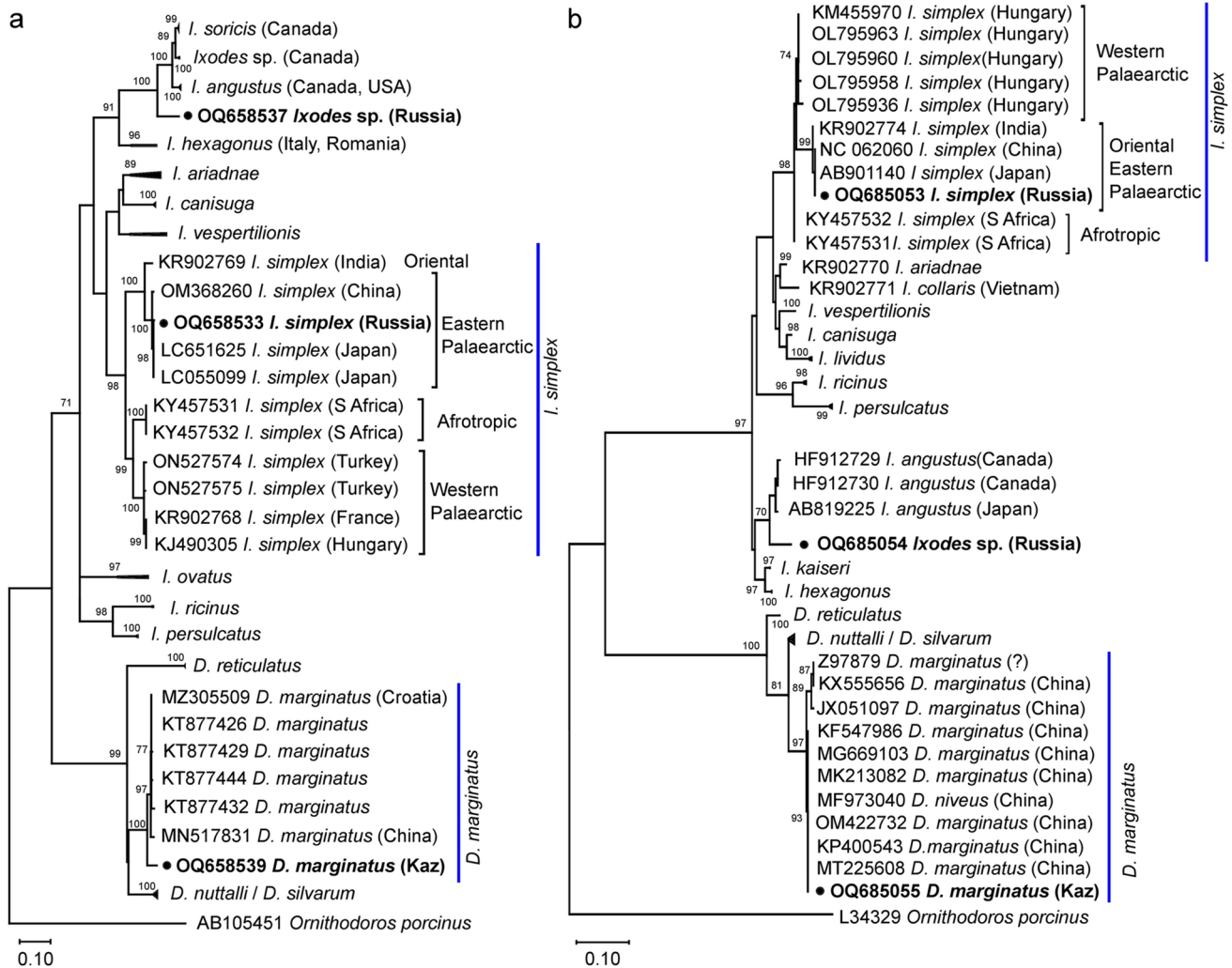


Fig. 3 Maximum likelihood (ML) trees based on *Cox1* (a) and 16S rDNA (b) sequences of *Ixodes* spp., *Dermacentor* spp., and outgroup. Nodes are labeled with the bootstrap support values. The GenBank

accession nos. and references for the sequences used in the phylogenetic analyses are listed in the Supplementary Information

***Cox1* sequence:** OQ658537. **16S rDNA sequence:** OQ685054. **ITS2 sequence:** OQ685080.

Localization: inside the ear.

Other bat hosts: unknown.

Remarks. *Cox1* (618 nt): 9.76–10.41% OQ658537 vs *I. soricis* (Canada) KX360373, HM398837, MN354578, MN350728, MN349810, MN352634, MN352350, MN350446, MG412230; 10.08–10.73% OQ658537 vs *I. angustus* (USA) NC_067905, (Canada) KX360397, KX360406, KX360411.

16S rDNA (378 nt): 6.07–7.14% OQ685054 vs *I. angustus* (USA, Japan) HF912729, AB819225, NC_067905, and etc. *ITS2* (779 nt): 19.67–20.50% OQ685080 vs *I. hexagonus* (Germany, Poland) GU001679, GQ924083, MG962875, and etc.

The *Cox1*-barcoding system was unable to match any species in *Cox1* species database. The phylogenetic analysis of *Cox1* gene fragment suggests grouping of *Ixodes* sp.

from Eastern Siberia to the clade of *I. soricis*, *Ixodes* sp., and *I. angustus* (Fig. 3a) those were recently described as parasites of small mammals in Canada. The closes sister branch was formed by the well-studied *I. hexagonus* tick. The phylogenetic analysis based on 16S rDNA sequences also showed the grouping of *Ixodes* sp. from Eastern Siberia with *I. angustus* (Fig. 3b), and ITS2 analysis — the grouping with *I. hexagonus* with no closest relatives (Fig. 5).

Family Argasidae.

***Argas vespertilionis* Latreille, 1802 (syn. *Carios vespertilionis* Latreille, 1796)**

Material. Kazakhstan, Almaty Region, collected by Denis V. Kazakov: 7 LL on adult male of *Eptesicus serotinus* (No. KZ22003), Basshi village, 44.16 N 78.75 E, 21-Jun-2022;

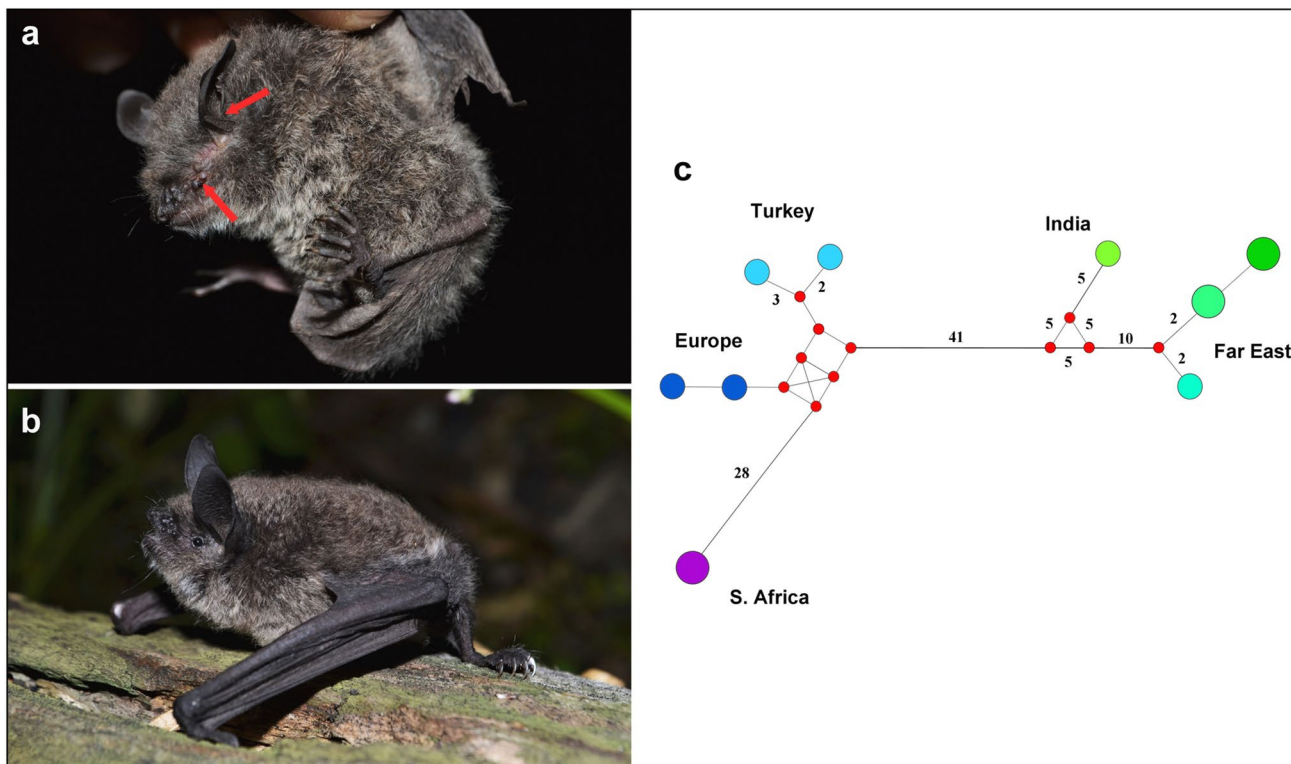


Fig. 4 *Ixodes simplex* on *Myotis macrodactylus* (No. KH21121) (a), and *M. macrodactylus* (b) from Primorsky Territory, Russia. Photo by Denis V. Kazakov. Median-joining network of *Cox1* haplotypes of

I. simplex (c), color-coded based on their geographical areas. Circle size corresponds to the number of samples, and numbers represent connections separated by more than one mutation

Table 3 Within-species *Cox1* *p*-distances (%) of *I. simplex*

Region	Japan	Primorye	China	India	Europe	Turkey	S. Africa
Japan (<i>n</i> = 2)	0.00						
Primorye (<i>n</i> = 4)	0.157	0.00					
China (<i>n</i> = 1)	0.667	0.833	n/c				
India (<i>n</i> = 1)	3.667	3.833	3.667	n/c			
Europe (<i>n</i> = 2)	9.917	10.083	10.083	9.083	0.17		
Turkey (<i>n</i> = 2)	9.917	10.083	10.083	9.250	1.167	0.83	
S. Africa (<i>n</i> = 2)	9.167	9.333	9.500	9.167	5.083	5.167	0.00

Data in bold font indicate the samples obtained in this study, values in bold indicate closest *p*-distance

3 LL on lactating female of *P. pipistrellus* (No. KZ22013), Shygan cordon, 44.11 N 78.71 E, 24-Jun-2022; 2 LL (KZ22064.1–2) on adult male of *Nyctalus noctula* (No. KZ22064) and 52 LL on juvenile male of *E. serotinus* (No. KZ22070), Malyj Kalkan cordon, 43.878 N 78.394 E, 2-Jul-2022; 6 LL on lactating female of *P. pipistrellus* (No. KZ22083) and 13 LL on adult male of *E. serotinus* (No. KZ22091), Kosbastau cordon, 43.92 N 78.79 E, 4–5-Jul-2022.

Cox1 sequence for No. KZ22064.1: OQ658538

Localization: on the lips, on the outside of the ear base, mostly on the body in hair.

Other bat hosts: E. Palearctic — *P. pipistrellus*, XUAR China (Sheng et al. 2019); *V. murinus*, XUAR China (Hornok et al. 2017a, b); *P. abramus*, *Mi. fuliginosus*, Japan (Yamauchi and Funakoshi 2000); *V. sinensis*, Japan (Fukui 2015); *M. petax*, Primorye, Russia (Medvedev et al. 1991); *Scotophilus kuhlii*, Pakistan (Ullah et al. 2019).

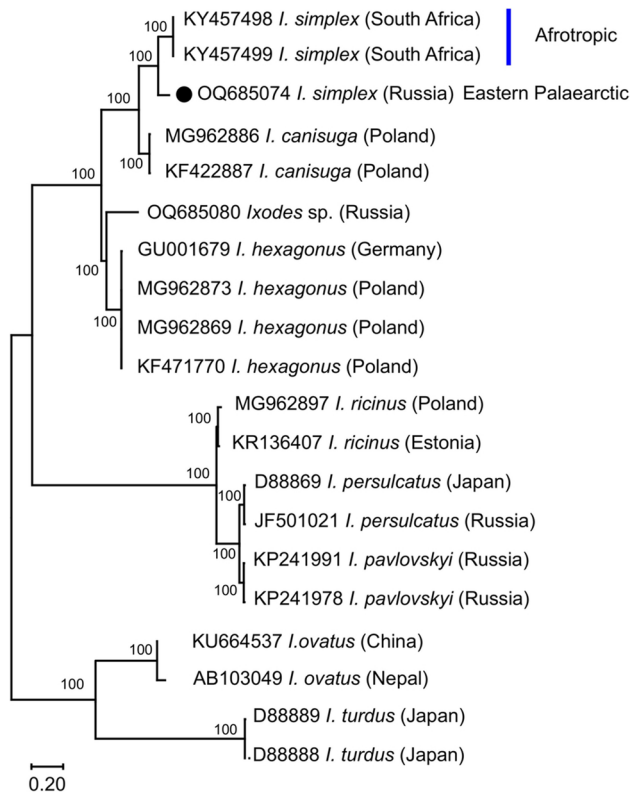


Fig. 5 Maximum likelihood (ML) tree based on ITS2 sequences of *Ixodes* spp. Nodes are labeled with the bootstrap support values. The GenBank accession nos. and references for the sequences used in the phylogenetic analysis are listed in the Supplementary Information

Remarks. 2.03% OQ658538 vs *A. vespertilionis* (China, Japan) MT762370, OM368318. Sequences from Kazakhstan, China and Japan formed a single haplogroup (Fig. 6) with mean within-group *p*-distance of 1.13%. *p*-distances based on *Cox1* sequences between *A. vespertilionis* from Kazakhstan and other regions are shown in Table 4.

Insecta.

Heteroptera.

Family Cimicidae.

Cimex pipistrelli Jenyns, 1839

Material. Russia, Khabarovsk Territory, collected by Denis V. Kazakov and Uliana V. Gorobeyko: 1 m and 1 f (No. KK18019b, KK18030b) on juvenile males of *V. murinus* (No. KK18019, KK18030) (Fig. 7 a, b), Gorin River, near the mouth of Malaya Talandinka River, 50.83 N 137.48 E, 7–8-Aug-2018.

Cox1 sequences: OQ661875-76

Other bat hosts: E. Palaearctic — “unknown bat host,” Mongolia (Balvín et al. 2018); *M. davidii* as “*Myotis aurascens*?” *V. murinus*, Mongolia (Scheffler et al. 2012); *M.*

petax, “*Myotis mystacinus*” Mongolia (Scheffler et al. 2010). W. Palaearctic — *M. dasynceme*, *V. murinus*, Russia, Urals; *M. daubentonii*, *M. myotis*, *M. nattereri*, *M. brandtii*, *M. dasynceme*, *M. blythii*, *N. noctula*, *N. lasiopterus*, *N. leisleri*, *P. pygmaeus*, Europe (Balvín et al. 2013).

Remarks. 1.04–1.10% OQ661875 (No. KK18019) vs *C. pipistrelli* (UK) KY561683, KC503539, GU985534; 1.32–1.42% OQ661876 (No. KK18030) vs *C. pipistrelli* (Netherlands, Russia: Urals) MK140096, KC503537 (Table 5). *p*-distances based on *Cox1* sequences between *C. pipistrelli* from the Lower Amur and other regions are shown in Table 5. Haplotypes from the Lower Amur occupied an intermediate position between haplogroups “Urals, Mongolia” and “*C. pipistrelli* haplogroup 1” (Fig. 7c). The sequences of *C. pipistrelli* from the Lower Amur were clustered with *C. pipistrelli* from other regions and *C. japonicus* from Japan in *Cox1* analysis with high bootstrap support (Fig. 8).

Cimex aff. lectularius Linnaeus, 1758

Material. Russia, Transbaikalia Territory, collected by Denis V. Kazakov and Maxim A. Khasnatinov: 2 f (No. ZA21062b, ZA21064b) on adult females of *P. ognevi* (No. ZA21062, ZA21064) (Fig. 9), 5 km SW of Fomichevo village, 50.48 N 109.19 E, 6-Jul-2021; 1 m (No. ZA21083b) on adult male of *M. petax* (No. ZA21083), Steklozavod village, mouth of Bobrovka River, 50.58 N 110.22 E, 9-Jul-2021; 1 m (No. ZA21084b) on adult male of *M. petax* (No. ZA21084), 11 km S of Khilogon village, Arey River, 51.05 N 110.62 E, 10-Jul-2021.

Cox1 sequences: OQ661877-80

Other bat hosts: As *C. lectularius*: E. Palaearctic — *M. petax*, *M. sibiricus* as “*M. gracilis*,” *E. nilssonii*, Mongolia (Scheffler et al. 2012, 2016).

Remarks. 12.50% OQ661877-80 vs *C. lectularius* (Hungary) MF161521. Within-group *Cox1 p*-distance was 0.35%. *p*-distances based on *Cox1* sequences between *C. aff. lectularius* and other *Cimex* species are shown in Table 6. The sequences of *C. aff. lectularius* from Transbaikalia formed a separate clade within the *C. lectularius* group (Fig. 8). Morphologically, they belonged to *C. lectularius*, so the ratio of the head width to the third antennal segment was 1.36–1.43.

Discussion

In this study the diversity of bat ectoparasites is described in poorly characterized regions of north-eastern Palaearctic. The sampling sites covered most of land biomes in North Asia across approximately 6000 km in latitude direction. This provided the representative and diverse collection of bats and associated ectoparasites. Besides often described

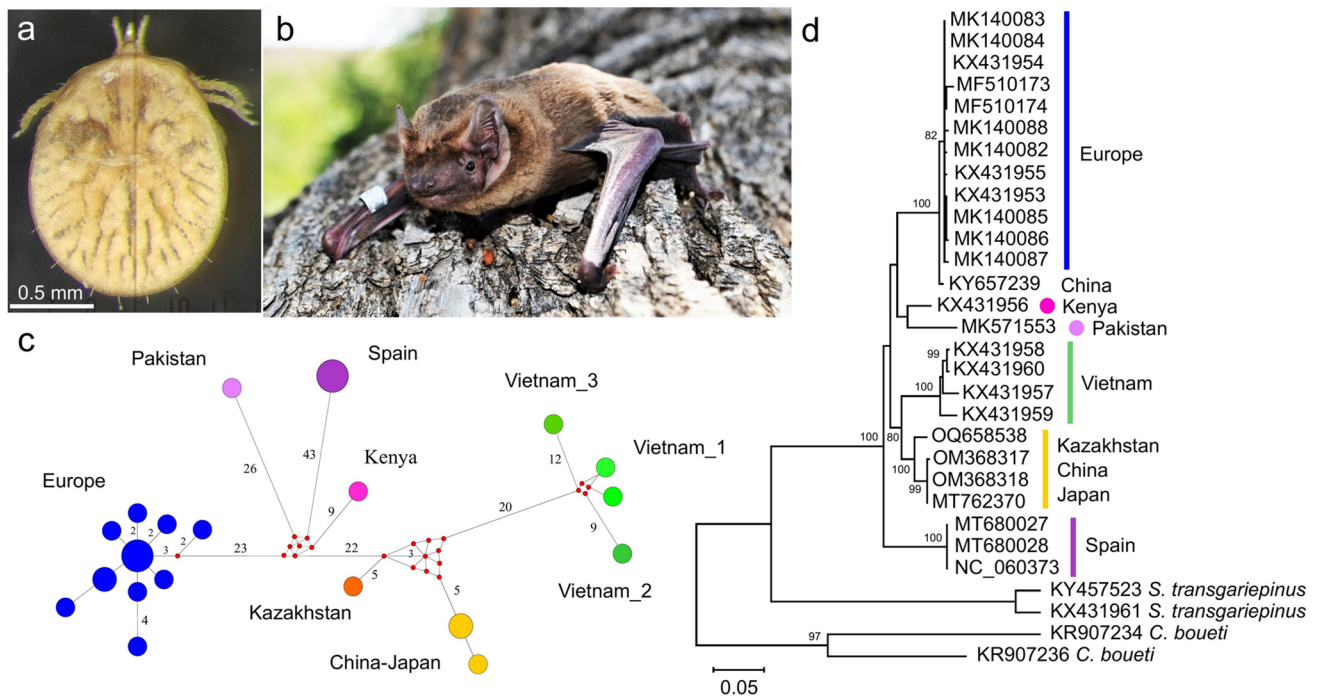


Fig. 6 *Argas vespertilionis*, No. KZ22070.1 (a), and one of hosts, *Nyctalus noctula*, No. KZ22064 (b). Photo by Denis V. Kazakov. Median-joining network of *Cox1* haplotypes of *A. vespertilionis* (c), color-coded based on their geographical areas. Circle size corresponds to the number of samples, and numbers represent connections

separated by more than one mutation. The maximum likelihood (ML) tree based on *Cox1* sequences of *A. vespertilionis* and outgroups (d). Nodes are labeled with the bootstrap support values. The GenBank accession nos. and references for the sequences used in the phylogenetic analysis are listed in the Supplementary Information

Table 4 Within-species *Cox1* *p*-distances (%) of *A. vespertilionis*

Region	Eu	Ken	Pak	Viet_2	Viet_1	Viet_3	Spain	China-Japan	Kaz
Europe (<i>n</i> = 13)	1.00								
Kenya (<i>n</i> = 1)	5.83	n/c							
Pakistan (<i>n</i> = 1)	8.24	5.99	n/c						
Vietnam_2 (<i>n</i> = 1)	8.03	7.28	8.25	n/c					
Vietnam_1 (<i>n</i> = 2)	7.57	6.96	8.01	1.86	0.00				
Vietnam_3 (<i>n</i> = 1)	7.59	7.44	8.90	2.43	2.27	n/c			
Spain (<i>n</i> = 3)	8.94	7.77	9.71	9.39	9.06	8.90	0.00		
China-Japan (<i>n</i> = 3)	6.58	6.09	7.71	6.04	4.99	5.88	7.98	0.00	
Kazakhstan (<i>n</i> = 1)	6.44	5.83	6.96	5.99	4.94	5.99	7.61	2.16	n/c

Data in bold font indicate the samples obtained in this study, values in bold indicate closest *p*-distance

from bat invertebrates such as Trombiculidae, Argasidae, and Cimicidae our observations revealed the bat infestation with relatively rare Ixodidae ticks from the *Ixodes* and *Dermacentor* genera.

Both *L. schlugerae* and *A. latyshevi* are wide-spread bat ectoparasites in Siberia. However, 170 specimens of *L. schlugerae* were found on 25 bats in six geographic localities, whereas only 22 mites of *A. latyshevi* were collected from 4 infested bats in three geographic localities. These differences may indicate that geographic range,

occurrence, and the abundance of *L. schlugerae* are notably higher than *A. latyshevi*. Joint parasitism of *L. schlugerae* and *A. latyshevi* on *P. ognevi* was detected at the swarming site in August. It is believed that the main hosts of *L. schlugerae* are rodents (e.g., *Ochotona* spp., *Cricetulus* spp., *Alticola* spp., and *Microtus* spp.). The broadly overlapping body measurements for *L. schlugerae* and *L. myotis* (Table 1) suggest that these two species are synonyms. Several bat species were recorded as specific hosts for *L. myotis* including *Myotis* sp. in South Korea

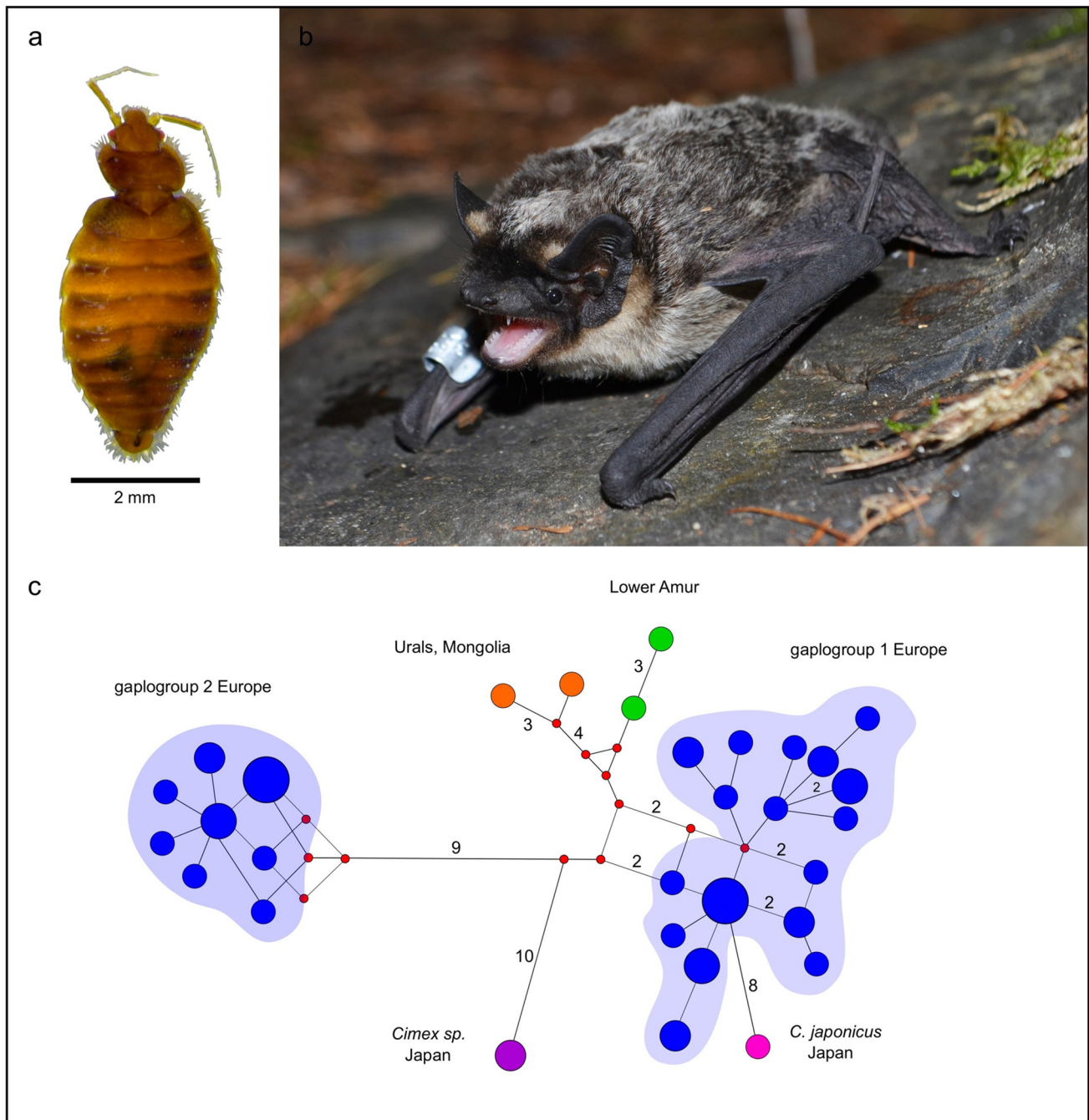


Fig. 7 *Cimex pipistrelli*, m, No. KK18019 (a), and its host *Vespertilio murinus*, No. KK18019 (b) from Khabarovsk Territory, Russia. Photo by Denis V. Kazakov. Median-joining network of *Cox1* haplotypes of

Cimex pipistrelli (c), color-coded based on their geographical areas. Circle size corresponds to number of samples, and numbers represent connections separated by more than one mutation

(Vercammen-Grandjean and Langston 1976), *Nyctalus aviator* in China (Wen 1984) and various bats in USA and Canada (Vercammen-Grandjean and Langston 1976). For *A. latyshevi*, the main hosts are rodents (*Apodemus* spp., *Myodes* spp., and *Sciurus vulgaris*), shrew (*Crocidura suaveolens*), and two bird species (Kudryashova 1998). In addition, *A. latyshevi* was recently recorded on bats

in Spain for the first time (Stekolnikov et al. 2022). The finding of *A. latyshevi* on bats may be associated to a low host specificity of these chigger mites. Previously, *Oudemansidium subakamushi* on *V. murinus* and *V. sinensis* (as “*V. superans*”), and *L. pavlovskiyi* on *P. ognevi* were identified in the temperate zone of eastern Russia (Zhovty et al. 1962; Emel’yanova and Vysokovskiy 1962). In the

Table 5 *Cox1 p*-distances (%) of *C. pipistrelli* and other *Cimex* taxa as in Balvín et al. (2013)

Species/region/group	Haplogroup 1	Haplogroup 2	Urals, Mongolia	<i>C. japonicus</i> Japan	<i>C. sp.</i> Japan	Lower Amur
<i>C. pipistrelli</i> Europe haplogroup 1 (<i>n</i> = 31)	0.74					
<i>C. pipistrelli</i> Europe haplogroup 2 (<i>n</i> = 16)	2.63	0.54				
<i>C. pipistrelli</i> Urals, Mongolia (<i>n</i> = 2)	2.23	3.04	0.67			
<i>C. japonicus</i> (<i>n</i> = 1) Japan	1.76	3.02	2.85	n/c		
<i>C. sp.</i> Japan (<i>n</i> = 2)	2.73	3.55	3.02	3.35	0.00	
<i>C. pipistrelli</i> Lower Amur (<i>n</i> = 2)	1.44	2.46	1.42	2.18	2.26	0.50

Data in bold font indicate the samples obtained in this study, values in bold indicate closest *p*-distance

arid zone of Mongolia, *O. subakamushi* from unknown bat species and *Willmannium cavus mongoliensis* from *Plecotus* sp. (as “*P. austriacus*”) were also known (Kudryashova and Lushchekina 2011). *O. subakamushi* (as “*Chiroptella (Oudemansidium) muscae*”), *L. nyctali*, and *L. myotis* were found on *Nyctalus* spp. in China (Wen 1984). Another 25 chigger species from bats in China were reported in the Wen (1984), but for the most of these chiggers, it is not possible to verify the collection region and host bat species.

Dermacentor marginatus is a three-host tick species, whose larvae prefer such hosts, as rodents, small to medium size insectivores, lagomorphs, and carnivores (Hornok 2017). Previously *D. marginatus* has been previously found on *P. pipistrellus* in Iran (Filippova et al. 1976). The foraging sites and day roosts of *P. pipistrellus* are often associated with urban areas and buildings (Tzortzakaki et al. 2019; Khabilov and Tadzhibaeva 2021).

The phylogenetic analysis of *I. simplex* revealed three main clades of this species. Previously described Asiatic and European clades (Hornok et al. 2015) are comprised by the specimens from India, China, Japan, Hungary, France, and Turkey, respectively. African clade includes specimens from South Africa and had been previously shown to be related to the Australian *I. simplex* according to the 18S rDNA analysis (Mans et al. 2019). In our analysis, this clade is more similar to the European *I. simplex* according to the *Cox1* sequences and branches separately by the 16S rDNA analysis. By both genome fragments, the specimens from Primorye belong to the Asiatic clade with the closest relatives found in Japan and China. In the ITS2 analysis, *I. simplex* is the only representative of the Asiatic clade and forms separate branch from the African clade. In addition to *I. simplex*, a second bat-specialist hard tick, *I. vesper-tilionis* from *M. dasynceme*, was also recorded in the temperate zone of eastern Russia (Khanty-Mansi Autonomous Okrug) (Starikov et al. 2017); however, no representatives of this species were found in our surveys so far. The genetic analysis of *Ixodes* sp. from Eastern Siberia revealed that

differences in the evolution distances reached the species level in all three phylogenetic reconstructions suggesting that the *Ixodes* sp. from Eastern Siberia belongs to a new tick species most closely related to Canadian nidicolous ticks *I. angustus* and *I. soricis*. It is necessary to collect other stages and study the morphology.

A. vesper-tilionis has a wide host range (more than 40 bat species) in the Western Palaearctic. The hosts of *A. vesper-tilionis* from Kazakhstan belong to three bat species and correspond to the main host species in the Western Palaearctic (Sándor et al. 2021). *Cox1*-barcoding of *A. vesper-tilionis* across species' range shows the presence of four lineages with high support (100), with specimens from Pakistan and Kenya in an uncertain position. *P*-distances in 27 of 36 pairwise comparisons (Table 4) exceed the value of 6% (from 6.0% for Kazakhstan vs Vietnam to 9.7% for Pakistan vs Spain) proposed for closely related ixodid tick species (Lv et al. 2014). However, it was shown that morphologically *A. vesper-tilionis* from Europe and Vietnam belong to the same species, while it was proposed to consider this species as a complex (group) of at least two putative cryptic species (Hornok et al. 2017a, b).

In this study, two bug species, *Cimex pipistrelli* and *C. aff. lectularius*, are sampled from bats in eastern Russia. *C. aff. lectularius* from Transbaikalia is identified as *C. lectularius* by morphometric parameters, whereas the phylogenetic analysis shows a sister position to *C. lectularius* with *p*-distance of 12.5–12.9%. The specimens from Transbaikalia differ from *C. columbarius*, another species of the *C. lectularius* group, in the ratio of head width to the third antennal segment, 1.36–1.43 vs 1.78, respectively (Usinger 1966). High genetic differentiation may be associated with introgression during hybridization of *C. lectularius* with one of the other *Cimex* species distributed in Asia. Alternatively, the bug specimens from Transbaikalia represent an unknown cryptic species. Thus, Johnson (1939) and Titschack (1949) showed that *C. lectularius* and *C. columbarius* interbreed freely, but at the same time, the population of hybrids will never stabilize in nature (Usinger 1966).

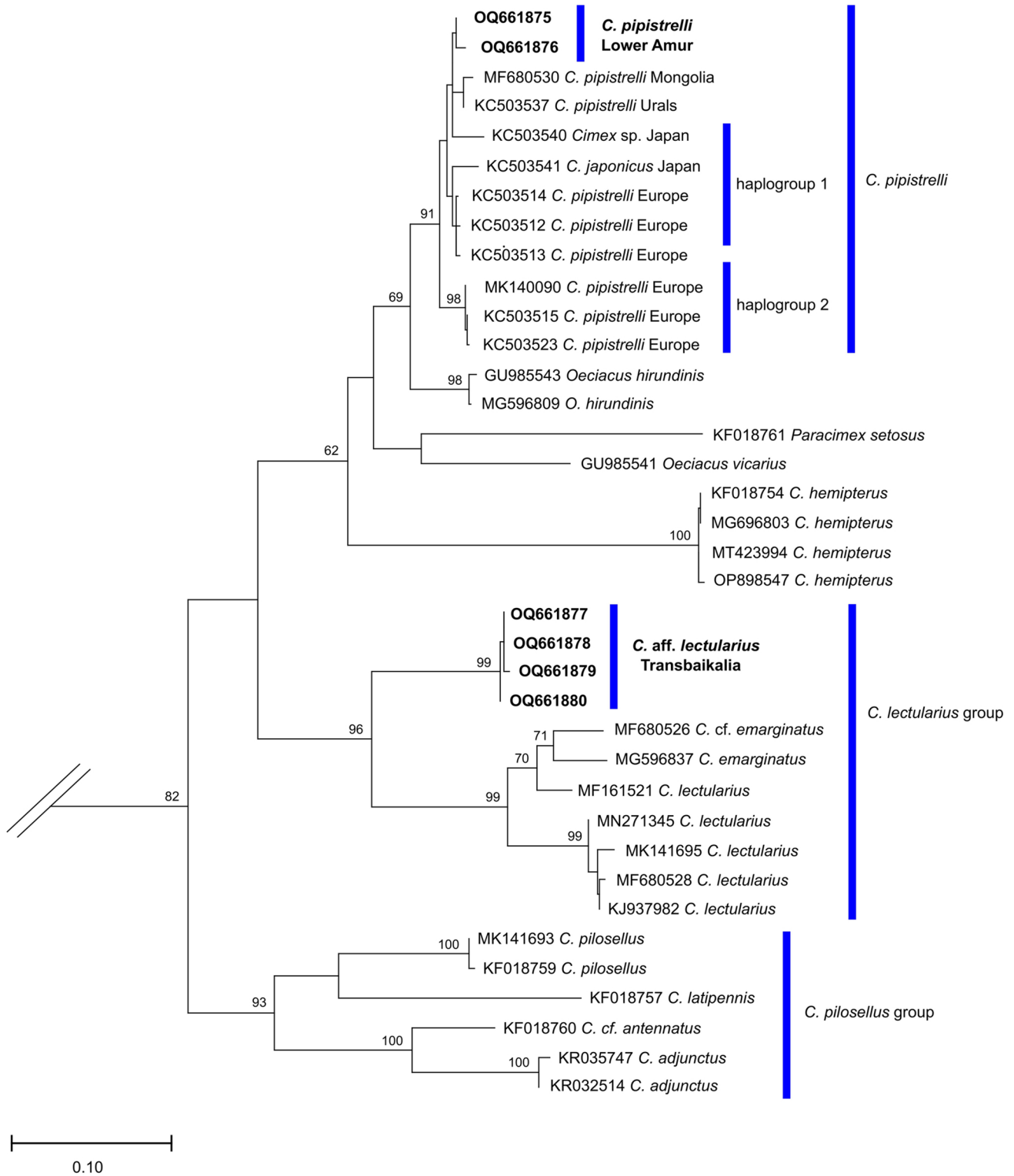


Fig. 8 Maximum likelihood (ML) tree based on *Cox1* sequences of *Cimex* spp. and outgroups (as in Ossa et al. 2019). Nodes are labeled with the bootstrap support values. The GenBank accession nos. and

references for the sequences used in the phylogenetic analyses in the Supplementary Information

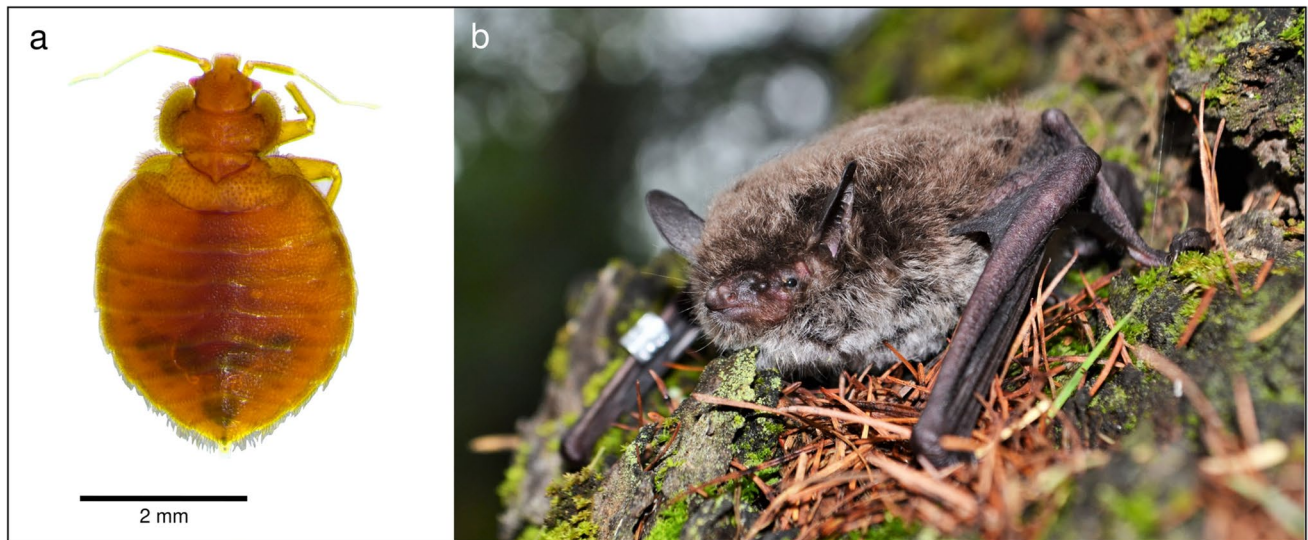


Fig. 9 *Cimex* aff. *lectularius*, f, No. ZA21064 (a), and host *Myotis petax*, No. ZA21084 (b) from Transbaikalia Territory, Russia. Photo by Denis V. Kazakov

Table 6 *Cox1* *p*-distances (%) of *C. aff. lectularius* and other *Cimex* species

Species\group	sp.	sp. OP963073	<i>lec</i>	<i>lec.</i> MF161521	<i>lec.</i> Transb	<i>cf. emar</i>	<i>lat</i>	<i>adjun</i>	<i>anten</i>
<i>Cimex</i> sp. (<i>n</i> =7)	0.51								
<i>Cimex</i> sp. OP963073	16.25	n/c							
<i>C. lectularius</i> (<i>n</i> =16)	18.30	17.31	0.83						
<i>C. lectularius</i> MF161521	18.85	16.69	7.37	n/c					
<i>C. aff. lectularius</i> (<i>n</i> =4) Transbaikalia	16.93	15.76	12.88	12.50	0.35				
<i>C. cf. emarginatus</i> (<i>n</i> =1)	18.94	16.86	8.70	5.51	12.88	n/c			
<i>C. latipennis</i> (<i>n</i> =2)	16.61	7.54	18.74	18.31	16.00	18.48	1.46		
<i>C. adjunctus</i> (<i>n</i> =3)	10.97	17.23	19.46	18.96	17.14	19.18	17.29	0.65	
<i>C. cf. antennatus</i> (<i>n</i> =1)	9.77	15.40	18.31	19.61	17.46	19.45	15.24	9.94	n/c

Values in bold indicate closest *p*-distance

Conclusions

Bat ectoparasites belonging to the families of mites Trombiculidae, ticks Ixodidae and Argasidae, as well as insects Cimicidae have been poorly studied in the northeastern Palaearctic so far. The role and place of chigger mites in the bat ectoparasite communities at the study region were especially underestimated. The new records and molecular analyses have identified probably new, previously unknown species of tick and bug, demonstrating a higher diversity of bat ectoparasite communities than previously thought. More detailed research of ectoparasite fauna associated with bats is necessary to clarify the status of presumed novel species as well as their distribution, prevalence, and abundance.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00436-023-08093-x>.

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Author contribution D.V.K. conceived and designed the study. D.V.K., M.A.Kh., and U.V.G. collected the bat ectoparasites in the field. A.A.A. identified trombiculid mites. M.A.Kh. identified ixodid and argasid ticks. D.V.K. and M.A.Kh. performed molecular and data analyses. D.V.K., M.A.Kh., A.A.A., and U.V.G. prepared the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval All procedures involving animals were in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 18 March 1986), and ethical approval was granted by the University of Tyumen Biomedical Ethic Committee (No. 8, July 2023, Tyumen, Russia). All bat species in this study are listed in *The IUCN Red List of Threatened Species* (Coroiu 2016a, b; Csorba and Hutson 2016; Fukui et al. 2019; Kruskop and Fukui 2019; Fukui and Sano 2020; Godlevska et al. 2020, 2021) as Least Concern.

Consent to participate This is not applicable to the present manuscript.

Consent for publication All authors reviewed and approved the final version of the manuscript.

Conflict of interest The authors declare no competing interests.

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