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A review and molecular study of bugs of the genus *Cimex* in Russia with an emphasis on the findings on bats

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Abstract

New geographical and host records of bed bug species of the genus *Cimex* L. (Hemiptera Cimicidae) are present in the article, and we also document and curate previously known data. In Russia, six species of the genus *Cimex* (*C. dissimilis*, *C. hemipterus*, *C. hirundinis*, *C. lectularius*, *C. pipistrelli*, *C. sibiricus*) are confirmed. *Cimex pipistrelli* are newly recorded for northern bat *Eptesicus nilssonii* and eastern water bat *Myotis petax*. *C. lectularius* was registered on greater noctule bat *Nyctalus lasiopterus*, Nathusius' pipistrelle *Pipistrellus nathusii* and Turanian serotine *Eptesicus serotinus turcomanus* for the first time. Our molecular data support the earlier assumption about a low level of genetic differences between *C. pipistrelli* and *C. japonicus*, which puts taxonomic status of *C. japonicus* into question.

Key words: *Cimex pipistrelli*, *Cimex lectularius*, bat bugs, Russia.

Introduction

Cimicid bugs (Hemiptera Cimicidae) include approximately 110 described species, which are obligate, haematophagous ectoparasites of birds and mammals (Balvín *et al.*, 2012a). Bats are regarded as the primary (ancestral) hosts of bugs in the Cimicidae, with subsequent switches to other hosts, including birds and humans (Balvín *et al.*, 2012a). The majority (approximately two thirds) of cimicid species are bat-associated (Usinger, 1966; Balvín *et al.*, 2012a), and the taxonomic status, distribution and host preferences of some species remains largely unknown.

According to Usinger (1966), the genus *Cimex* L. consists of the four groups discussed in a molecular study by Balvin *et al.* (2015).

1. *C. lectularius* group (*C. lectularius*, *C. pulveratus*, *C. emarginatus*, may be *C. columbarius*);
2. *C. hemipterus*, only one within a group;
3. *C. pipistrelli* group;
4. *C. pilosellus* group.

The group of *C. pipistrelli* consists of 11 described species, including *C. burmanus* Usinger, *C. cavernicola* Usinger, *C. dissimilis* (Horvath), *C. emarginatus* Simov, Ivanova et Schunger, *C. flavifuscus* (Wendt), *C. japonicus* Usinger, *C. limai* Pinto, *C. hirudinis* (Lamarck), and *C. pipistrelli* Jenyns, which are dispersed throughout the Palaearctic region (Akhoundi *et al.*, 2020).

The group of *C. pilosellus* consists of *C. adjunctus* Barber, *C. antennatus* Usinger et Ueshima, *C. brevis* Usinger et Ueshima, *C. incrassatus* Usinger et Ueshima, *C. latipennis* Usinger et Ueshima, and *C. pilosellus* (Horvath), and inhabits the Nearctic region (North America).

In Palaearctic, there are currently more than 10 species of genus *Cimex*. Despite the large number of articles devoted to the comprehensive study of species of the genus *Cimex* in European territory, Russia is still a "blind-spot", where information about this genus is reduced to scattered publications of different years (Dubinin, 1947; Kirichenko, 1951; Kerzhner, 1988; Vinokurov and Kanyukova, 1995). Dubinin (1947) mentioned only two species of genus *Cimex* for Eastern Siberia (*C. lectularius* and *C. pipistrelli*), in Kirichenko's monography (1951) there were also two species of the genus for Russia. Both in Dubinin's and Kirichenko's studies swallow bug *C. hirundinis* was attributed to genus *Oeciacus* Stal. In "Key to insects of the Far East of Russia" (Kerzhner, 1988) the same species was recorded for the Far East, but swallow bug was not mentioned. Vinokurov and Kanyukova (1995) described a new species of the genus *Cimex* in Southern Siberia.

Medical impact

Previously, it was believed that bed bugs and bat bugs have no epidemiological significance. Over the past two decades, there has been a widespread annual increase in the number of these insects, which has now become a worldwide problem. Therefore, increased attention is now being paid to elucidating the epidemiological status of these insects.

According to available data, at present almost 80% of people have various allergic reactions to bed bug bites. Bed bug saliva secreted during bites can cause various allergic manifestations in humans, such as itching, burning, redness of the skin, formation of papules, dermatitis, impetigo, ecthyma, lymphangitis, folliculitis, diarrhoea. Bed bug saliva secreted during bites can cause various allergic manifestations in lethargy up to an anaphylactic shock and asthmatic crisis (Center of Disease Control and Preventions: Bed Bugs FAQs - https://www.cdc.gov/parasites/bedbugs/faqs.html).

With intensive scratching of bite sites, there may appear pustules, the gateway to a secondary infection. Bed bugs are also a serious disturbing factor that deprives people of normal sleep and rest.

With a high number of bed bugs, their bites often cause iron deficiency anaemia (Pritchard and Hwang, 2009), especially in children, and also a decrease in immunity.

In the body of bed bugs can live pathogens of various infectious and parasitic diseases, theoretically capable of being transmitted through saliva during blood-sucking and through excrements. When bloodsucking, a pathogen immediately enters the bloodstream.

Basing on available information on the role of bed bugs as reservoirs and carriers of pathogens, the susceptibility of bed bugs from various natural populations to 45 human pathogens (viruses, rickettsia, bacteria, fungi, protozoa, and helminths, including *Trypanosoma cruzi*, *Bacillus anthracis*, *Mycobacterium leprae*, *Francisella tularensis*, *Brucella melitensis*, hepatitis B virus, smallpox virus, yellow fever virus, *Brugia malayi* and *Wuchereria bancrofti*) to date (Zorrilla-Vaca *et al*., 2015). In most cases, significant additional research is required to confirm the role of bed bugs and bat bugs in the transmission of a given pathogen. Nevertheless, the data above should be kept in mind when hordes of bed bugs spread into homes (Delaunay *et al*., 2011).

However, it should be noted, there are no definitive reports of pathogens transmitted by Cimicidae to human so far. There are several studies that show the possibility of their future role in the field of vector-borne diseases (Cockburn *et al*., 2013; Meriweather *et al.,* 2013; Barbarin *et al*., 2014) but without enough evidence of diseases arising from that transmission.

Materials and methods

Field investigations

Bat trappings were carried out in the summer, mainly in June-July from 1997 to 2017, almost throughout the entire territory of Russia (from the European part to Eastern Siberia), mainly in the forest zone, with the exception of the Daurskiy Reserve, Bolotovsk town and Klyuchkovka Village (steppe zone). Bats (Chiroptera Vespertilionidae) were captured using mist nets and were identified using morphological measurements following Dietz *et al.* (2009). The pelage, ears, propatagium, plagiopatagium, dactylopatagium, and uropatagium of every captured bat were carefully searched using an LED headlamp. Bugs from each bat were individually collected using forceps and placed into a single vial filled with 95% ethanol in the field. They were transferred into a new vial containing 96% ethanol in the laboratory for identification and long-term preservation. All captured bats were released at the study sites after identification and collection of ectoparasites.

The collected bugs were identified by the first and the third authors (MO, DS), following Usinger (1966).

Scanning electron microscopy

A high-performance scanning electron microscope JEOL JSM-6510 LV, Japan was used in accordance with the manufacturer's instructions (University of Pittsburgh, “Gertrude E. and John M. Petersen” institute of NanoScience and Engineering).

Molecular analysis

Specimens used in molecular analysis are given in table 1.

Collected specimens were stored in an ethanol (96%) at −75 °C. The ethanol (95%) used to preserve the bed bugs was first removed. We obtained tissue for DNA extraction from the thorax of each specimen. Extractions were performed using the HiPure Universal DNA Kit (Magen, China) according to the standard protocols of the manufacturer. The extracted DNA was stored at −20 °C.

Table 1. The findings of bat bug *C. pipistrelli* in Russia used in molecular analysis.

N. in figure 3 and GenBank	Locality	Host species	Bug sex	Date	Collector
249 OQ747991	Southern Russia: Astrakhan’ Province, Nature Reserve “Astrakhanskiy” 46°18'N 48°58'E	*Nyctalus noctula*	♂	28.V.2018	D.G. Smirnov
261 OQ747992	Nature Reserve “Astrakhanskiy”	*N. noctula*	♂	28.V.2018	D.G. Smirnov
290 OQ747997	Nature Reserve “Astrakhanskiy”	*N. noctula*	♂	28.V.2018	D.G. Smirnov
340 OQ747993	European Part: Samara Province, Nature Reserve “Samarskaya Luka” 53°26'N 49°55'E	*N. noctula*	♀	13.VII.2018	D.G. Smirnov
794 OQ747994	Nature Reserve “Samarskaya Luka”	*N. noctula*	♂	15.VII.2019	D.G. Smirnov
876 OQ747998	Nature Reserve “Samarskaya Luka”	*Vespertilio murinus*	♂	25.VII.2019	D.G. Smirnov
1113 OQ747995	Southern Russia: Dagestan Republic National Park “Samurskiy” 41°52'N 48°32'E	*N. noctula*	♀	15.VII.2022	D.G. Smirnov
1362 OQ747996	European Part: Penza Province Biological station of Penza State University 52°57'N 45°21'E	*Pipistrellus nathusii*	♀	22.VI.2022	D.G. Smirnov

Figure 1. Bat bug *C. pipistrelli* (♀) dorsal view.

Amplification of cytochrome oxidase subunit I (COI) was performed with PCR using the primers: Lep1F (5′-ATT CAA CCA ATC ATA AAG ATA TTG G-3′), Lep1Fdeg (5′-ATT CAA CCA ATC ATA AAG ATA TNG G-3′), and Lep3R (5′-TAT ACT TCA GGG TGT CCG AAA AAT CA-3′) (Hajibabaei *et al*., 2006) as reported (Balvín *et al*., 2015).

These primers used in a reaction volume of 25 µl, containing 12.5 µl Biomaster HS-Taq PCR-Color (2×) (includes highly processive recombinant HS-Taq DNA polymerase, deoxy nucleoside triphosphate mixture, 2× PCR buffer, Mg2+ and marker dyes), 0.25 µl (0.5 µM final concentration) of each Lep1F and Lep1Fdeg primers and 0.5 µl (1 µM final concentration) of Lep3R primer, 6.5 µl ddH2O and 5 µl template DNA.

Reactions were run using T100 Thermal Cycler, Bio-Rad (USA) thermocycler following cycling the procedures from 95 °C for 5 minutes was followed by 40 cycles of denaturation at 94 °C for 40 seconds, annealing at 53 °C for 1 minute, and extension at 72 °C for 1 minute. Final extension was performed at 72 °C for 10 minutes.

Statistical analysis and visualization

Alignment was conducted using the MUSCLE algorithm implemented in MEGA 11 (Tamura *et al*., 2021). The evolutionary history was inferred by using the Maximum Likelihood method using Hasegawa-Kishino-Yano model (Hasegawa *et al*., 1985). Bootstrap value was obtained using 1000 iterations (Felsenstein, 1985). Initial tree for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites - 5 categories (+G, parameter = 0.1944). This analysis involved 65 nucleotide sequences. There was a total of 658 nucleotide positions in the final dataset.

Sequences were deposited in GenBank NCBI under the numbers OQ747991 - OQ747998.

Results

List of Russian bat bug species

Cimex pipistrelli Jenyns

Material: beside the specimens used in molecular analysis seven individuals of *C. pipistrelli* (figure 1) have been found in several years.

1. 1♀ ex *Pipistrellus pygmaeus* (Leach) from Solodcha settlement (European Part, Volgograd Province) 49°39'N 44°18'E, 14.VI.1996, leg. D.G. Smirnov.
2. 1♀ ex *Vespertilio murinus* L. from Pervouralsk town (Urals, Sverdlovsk Province) 56°55'N 59°57'E, 20.VII.2015, leg. O.L. Orlov.
3. 2♀ ex *Eptesicus nilssonii* (Keyserling et Blasius) (figure 2) from Bolshaya rechka settlement (Western Siberia, Krasnoyarsk territory, Ermakov district) 53°02'N 92°25'E, 2.VII.2011, leg. A.V. Zhigalin.
4. 1♀ ex *Myotis petax* Hollister from Rechka Vydrino settlement (Eastern Siberia, Buryatia Republic, Kaban district) 51°29'N 104°50'E, 18.VII.2014, leg. D.V. Kazakov.
5. 1♀ ex *M. petax* from Baikalskiy National Nature Reserve (Eastern Siberia, Buryatia Republic, Kaban district) 51°20'N 105°09'E, 23.VII.2014, leg. D.V. Kazakov.
6. 1♀ ex *E. nilssonii* from Daurskiy Reserve (Eastern Siberia, Zabaykalie territory, Onon district, Nizhniy Tsasuchey settlement) 50°30'N 115°08'E, 18.VII.2015, leg. D.V. Kazakov.

Distribution in Russia: Moscow (Povolný, 1957), Kursk (Povolný, 1957), Volgograd Province (this study, new record), Urals (Balvín *et al.*, 2012b; this study), Western Siberia (Péricart, 1972; this study), Eastern Siberia (Dubinin, 1947; this study), Primorsky territory (Kerzhner, 1988).

Distribution outside Russia: Belarus, Ukraine, Sweden, and the United Kingdom (Povolný, 1957), Czech Republic, Slovak Republic and Germany (Roer, 1969), the Netherlands, Hungary, Romania, Greece and Kazakhstan (Péricart, 1972), Tadjikistan (Delaunay *et al*., 2011).

Chiropteran hosts (all known bat hosts of *C. pipistrelli* belong to Vespertilionidae): *Myotis myotis* (Aukema and Rieger, 1996), *M. blythii* (Roer, 1969), *M. bechsteinii* (Balvín *et al*., 2015), *M. dasycneme* (Morkel, 1999), *M. nattereri* (Roer, 1969), *M. daubentonii* (Balvín *et al*., 2012b), *M. brandtii* (Roer, 1969), *M. emarginatus* (Usinger, 1966), *M. petax* (this study, new record), *Nyctalus leisleri* (Heise, 1988), *N. noctula* (Heise, 1988; this study), *N. lasiopterus* (Balvín *et al*., 2012b), *Pipistrellus kuhlii* (Krištofík and Kaňuch, 2006), *P. nathusii* (Roer, 1969; this study), *P. pipistrellus* (Usinger, 1966), *P. pygmaeus* (Bartonička, 2007; this study), *Eptesicus nilssonii*

Figure 2. Bat bug *C. pipistrelli* on *E. nilssonii* (Photo by A. Zhigalin).

(this study, new record), *E. serotinus* (Bartonička and Gaisler, 2007), *Vespertilio murinus* (Dubinin, 1947; this study).

Non-chiropteran host: humans (Kejíková *et al.*, 2022).

Cimex lectularius L.

Material:

1. 1♀ ex *Eptesicus serotinus turcomanus* (Eversmann) from Kletskaya stanitsa (European Part, Volgograd Province) 49°39'N 44°18'E, 16.VI.1996, leg. D.G. Smirnov.
2. 1♀ ex *V. murinus* from Bolotovsk town (Southern Russia: Orenburg Province) 52°14'N 59°57'E, 18.VI.2002, leg. D.G. Smirnov.
3. 1♀ ex *Nyctalus noctula* Schreber from Vorona River (European Part: Penza Province) 52°54'N 42°57'E, 4.VIII.2002, leg. D.G. Smirnov.
4. 1♂ ex *N. noctula* from Klyuchkovka Village (Southern Russia: Rostov Province) 49°08'N 40°39'E, 8.VI.2006, leg. D.G. Smirnov.
5. 1♀ ex *Nyctalus lasiopterus* Schreber from Nature Reserve "Samarskaya Luka", 9.VII.2007, leg. D.G. Smirnov.
6. 1♀ and 1♂ ex *N. noctula* from Nature Reserve "Samarskaya Luka", 15-27.VII.2007, leg. D.G. Smirnov.

Distribution: temperate and subtropical regions worldwide.

Chiropteran hosts: Vespertilionidae: *Myotis myotis* and *M. blythii* (as *M. oxygnathus*) (Usinger, 1966), *M. bechsteinii* (Southwood and Leston, 1959), *M. daubentonii* (Schefler, 2008), *M. mystacinus* (Usinger, 1966), *M. emarginatus* (Wagner, 1967), *Nyctalus leisleri* (Hornok *et al.*, 2017), *N. noctula* (Roer, 1969), *N. lasiopterus* (this study, new record), *Eptesicus nilssonii* (as *Vesperugo borealis*) (Usinger, 1966), *E. serotinus* (Bobkova, 2001), *E. serotinus turcomanus* (this study, new record), *Pipistrellus kuhlii* (Abul-Hab, 1979), *P. nathusii* (this study, new record), *P. pipistrellus* (Baagøe, 2011), *Vespertilio murinus* (Aukema and Rieger, 1996; this study), *Plecotus auritus* (Roer, 1969). Rhinolophidae: *Rhinolophus ferrumequinum* (Aukema and Rieger, 1996).

Non-chiropteran host: humans; wide variety mammals and birds (Kerzhner, 1987).

Note: *C. lectularius* are almost exclusively synanthropic or domestic, and most wild mammals and birds likely serving only as occasional hosts. Probably permanent populations of *C. lectularius* exist only on humans, bats, pigeons and poultry.

Cimex hirundinis (Lamarck)

Distribution in Russia: European part, Eastern Siberia (Aukema and Rieger, 1996).

Distribution outside Russia: Europe; Asia (Kazakhstan); Africa (Algeria, Morocco, Tunisia) (Aukema and Rieger, 1996).

Chiropteran hosts: unknown.

Non-chiropteran hosts: humans; *Glis glis*, *Muscardinus avellanarium*; *Asio otus*, *Merops apiaster*, *Apodemus sylvaticus*, *Galerida cristata*, *Delichon urbicum*, *Hirundo rustica*, *Riparia riparia*, *Pica pica*, *Parus major*, *Turdus merula*, *Turdus philomelos*, *Certhia familiaris*, *Ficedula albicollis*, *Muscicapa striata*, *Motacilla alba*, *Fringilla coelebs*, *Passer domesticus*, *Passer montanus*, *Apus apus* (Országh *et al.*, 1990; Hamlili *et al.*, 2023).

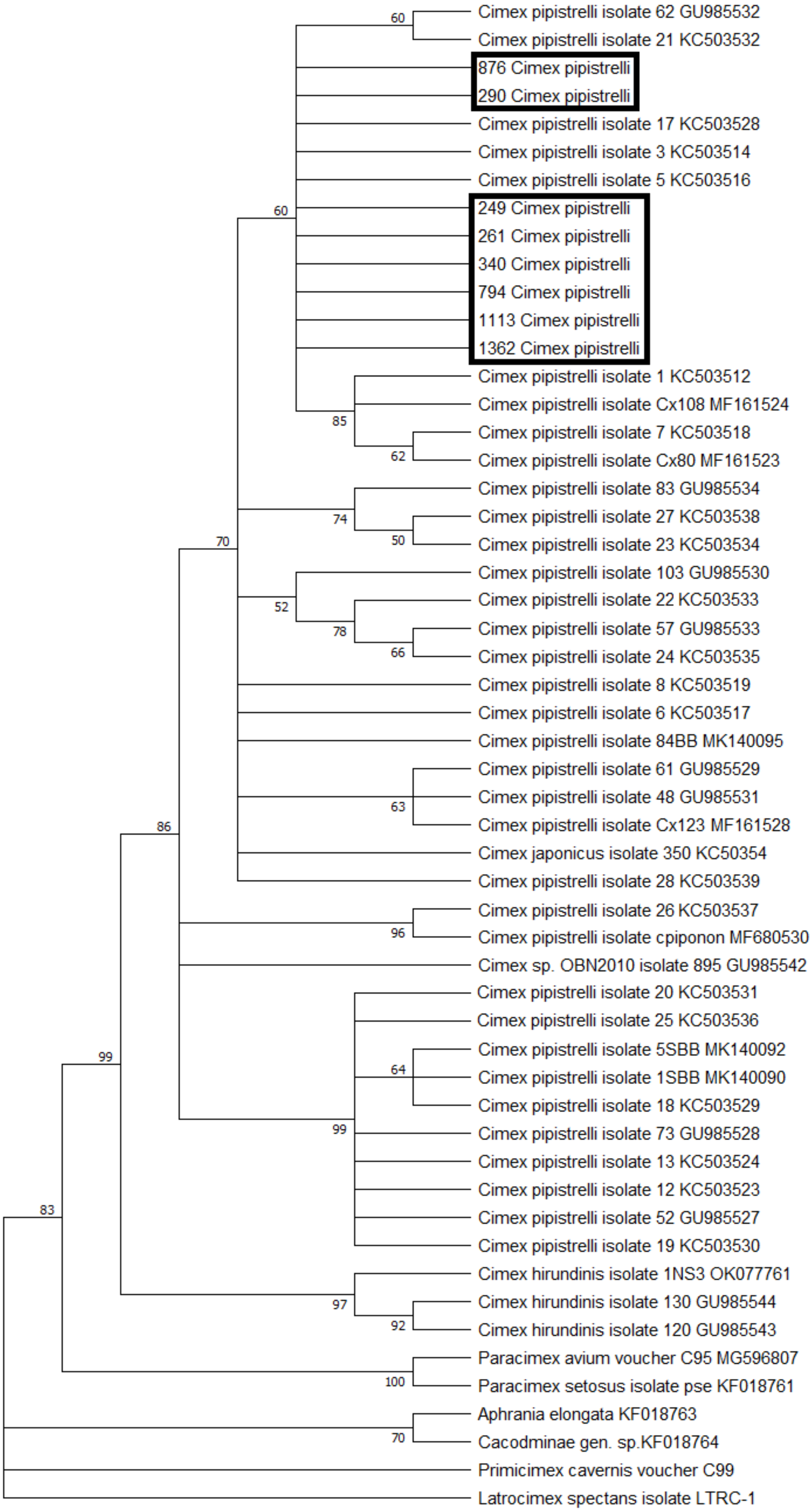

Figure 3. Phylogenetic relationships between species of the cimicid bugs genera based on cytochrome-c-oxidase first subunit (COI). The Maximum Likelihood method conducted using the best nucleotide substitution model (Hasegawa-Kishino-Yano) selected by MEGA 11. The tree with the highest log likelihood (−3887.30) is shown. The percentage of trees in which the associated taxa clustered together is shown below the branches (support values less than 70 were not shown in the figure). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st + 2nd + 3rd + Noncoding. This analysis involved 54 nucleotide sequences.

C i m e x d i s s i m i l i s (H o r v a t h)

Distribution in Russia: European part (Aukema and Rieger, 1996).

Distribution outside Russia: Great Britain, Germany, Hungary, Czech Republic [as Bohemia], and Netherlands (Usinger, 1966), Belarus (Larchenko and Lukashuk, 2021), Denmark, Sweden, Poland, Slovakia, France, Switzerland, Kazakhstan, Tadjikistan, and Uzbekistan (Aukema and Rieger, 1996), Bulgaria and Greece (Simov *et al*., 2006), Italy (Lanza, 1999).

Chiropteran hosts: Vespertilionidae: *Nyctalus noctula* (Usinger, 1966), *Myotis myotis* (Usinger, 1966), *M. daubentonii*, *Pipistrellus nathusii*, and *P. pygmaeus* (Larchenko and Lukashuk, 2021).

Non-chiropteran hosts: unknown.

C i m e x h e m i p t e r u s (F .)

Distribution in Russia: Moscow Province, Smolensk, Saransk (Mordovia Republic) and Saint-Petersburg (Gapon, 2016), Belgorod (Prisniy, 2020), Voronezh (Golub *et al*., 2020).

Distribution outside Russia: oriental region and all tropical countries of the Old and New World; Norway (Hage *et al*., 2022), Sweden (Vinnersten, 2018), France (Chebbah *et al.*, 2021), the Czech Republic, Slovakia, and Switzerland (Balvín *et al.*, 2021).

Chiropteran hosts: Vespertilionidae: *Scotophylus kuhlii* (also as *Scotophylus temminckii*) (Usinger, 1966).

Non-chiropteran host: humans (Usinger, 1966).

Note: most of the European records of *C. hemipterus* are only occasional introductions, likely without permanent settlement (except for Bratislava).

C i m e x s i b i r i c u s V i n o k u r o v

Distribution in Russia: type locality - the Torgalyg settlement (Southern Siberia: Tuva Republic) (Vinokurov and Kanyukova, 1995).

Chiropteran hosts: bats (without specification) (Vinokurov and Kanyukova, 1995).

Non-chiropteran hosts: *Sitta europaea* (nest) (Vinokurov and Kanyukova, 1995).

Cimicid bug species possibly inhabiting Russia

C i m e x j a p o n i c u s U s i n g e r

Distribution: Japan (Delaunay *et al*., 2011; Yamauchi *et al.*, 2021).

Chiropteran hosts: Vespertilionidae: *Nyctalus aviator* (Usinger, 1966; Yamauchi *et al.*, 2021), *Myotis frater*, *M. petax*, *M. sibiricus* (as *M. gracilis*), *Plecotus sacrimontis*, and *Vespertilio sinensis* (Yamauchi *et al.*, 2021).

Non-chiropteran hosts: unknown.

Cimicid bug species erroneously attributed to Russia

C i m e x c a v e r n i c o l a U s i n g e r

It has been described in Turkmenistan and erroneously mentioned by Akhoundi *et al*. (2020) for Russia.

Discussion

The two groups observed in *C. pipistrelli* (figure 3) have a level in 3% of differences between nucleotide consequences in the first subunit of cytochrome-c-oxidase (COI) (Balvín *et al*., 2013). Such a level of differences does not indicate the existence of separate species; however, they show a high genetic variability within the species examined. The level of genetic differences between *C. japonicus* and *C. pipistrelli* is also comparatively low and *C. japonicus* should probably be considered as a synonym of *C. pipistrelli*. Our sequences are placed into the clade A of *C. pipistrelli* found in various localities across Europe and identical to haplotype 5 from the Czech Republic, Bulgaria and Russia (Balvín *et al*., 2013). Bugs from the clade A are distributed in 14 European countries, while haplotypes from the clade B found in Switzerland, Poland, Greece, Bulgaria, and the Czech Republic (Balvín *et al*., 2013).

Conclusions

We have been recorded for Russia six species of *Cimex* genus (*C. pipistrelli*, *C. lectularius*, *C. hirundinis*, *C. hemipterus*, *C. sibiricus*, *C. dissimilis*). For the first time *C. pipistrelli* have been found on *E. nilssonii*. Finding *C. pipistrelli* in Volgograd Province is the first record for the territory. For the first time *C. lectularius* have been recorded on *N. lasiopterus*, *P. nathusii* and *E. serotinus turcomanus* hosts. Further researches in the territory of Russia are needed to identify possible new species of the genus *Cimex* and to confirm the status of previously described species.

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