

Early evidence of establishment of the tropical bedbug (*Cimex hemipterus*) in Central Europe

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Abstract. In recent decades, the world has witnessed a remarkable resurgence of bedbugs (Hemiptera: Cimicidae). Although populations of the common bedbug, *Cimex lectularius* L., expanded in temperate regions of its original distribution, the tropical bedbug, *C. hemipterus* (F.), increased its abundance in warmer regions, where it also had been historically distributed. However, *C. hemipterus* has recently been observed to be expanding to other areas, e.g. North Australia, Middle East, the United States and Russia. In other parts of Europe, few sporadic and ephemeral introductions of *C. hemipterus* were recorded until recently. We conducted an extensive sampling of European bedbug populations starting in 2002 and found that *C. hemipterus* has recently become locally established. Among 566 examined infestations, nearly all of which involved *C. lectularius*, *C. hemipterus* occurred in six infestations collected since 2019. In at least three cases, the social background of inhabitants of the infested properties indicated that tropical bedbugs likely spread within local communities. Using cytochrome oxidase subunit I, we linked five of the infestations to the most common haplotype found globally, and one to an African haplotype. In all infestations, we observed two *kdr*-associated mutations in the sodium channel gene, which are also commonly found across the world.

Key words. Cytochrome oxidase subunit I, human ectoparasite, insecticide resistance, invasion, *kdr*, mitochondrial network, pest control.

Introduction

After several decades of being almost forgotten, bedbugs have recently experienced a remarkable resurgence around the world. Until recently, pest management companies (PMCs) across Europe have had to deal with only a single human associated species—the common bedbug, *Cimex lectularius*. However, the last few years have witnessed an increase in reports of a second species, the tropical bedbug *C. hemipterus*, which may create new challenges in pest detection and control.

Bedbugs have evolved a very specific strategy of host exploitation. Unlike most other blood-feeding ectoparasites, they do not live on the host body, but rather reside in the shelters of their hosts, visiting the host body only for blood meals (Reinhardt & Siva-Jothy, 2007). Although widely known as human specialists, the common and tropical bedbugs are two species ancestrally associated with bats (Usinger, 1966). In human dwellings, bedbugs are cryptic, remaining hidden and often going unnoticed, until severe infestations develop. Due to this behaviour, monitoring their presence is difficult. Due to the occurrence of

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multiple insecticide resistance mechanisms (Dang *et al.*, 2017; Romero, 2018) combined with a limited range of effective insecticides, control becomes challenging, especially in countries with strict regulations on insecticidal compounds (Kilpinen *et al.*, 2011).

Although bedbug spread has been facilitated by urbanization, distribution of the two species appears to be largely mutually exclusive (Zorrilla-Vaca *et al.*, 2015). Historically, the tropical bedbug was distributed in warm regions within the 30° north and south latitudes. It has been the primary bedbug species found in SE Asia or inland India, whereas in other tropical regions both species may occur (Usinger, 1966). The well-documented recent resurgence of bedbugs has comprised both species. Population expansions of *C. hemipterus* first occurred in regions where it prevailed in the past (How & Lee, 2010; Lee, 2013). However, soon after, it became established in other regions, including the Middle East (Ramachandran, 2012; Balfour, 2013; Balvín *et al.*, 2013), North Australia (Doggett *et al.*, 2003; Doggett & Russell, 2008), Florida and Hawaii (Campbell *et al.*, 2016; Lewis *et al.*, 2020).

Across Europe, where *C. lectularius* is widespread, most of the available records of *C. hemipterus* are related to sporadic, ephemeral infestations, largely connected with international travel. An early case in Brno, the Czech Republic, had a clear origin in Nepal (V. Kubáň, 1991, personal communication). Two cases in Northern Italy were reported to originate from travels abroad (Masetti & Bruschi, 2007). Another case from Italy was connected with a visit to Brazil (Masini *et al.*, 2020) and one record was from a plane in Switzerland (M. Schmidt, 2018, personal communication). One of the two cases in Southern France was reported from an apartment occupied by students (Bérenger & Pluot-Sigwalt, 2017); the background of the other case is unknown; similarly, for a case from the United Kingdom (Burgess, 2003). Five cases were reported from Sweden, one from 1981, the rest from 2014 to 2016 (Vinnersten, 2017), with no details on the social background or the travel history of the infested inhabitants given. Nevertheless, particularly frequent travels of the Swedes to Thailand are mentioned, and, at the same time, the author speculates that these cases may indicate a permanent establishment of *C. hemipterus* in Sweden. *Cimex hemipterus*, however, appears to have established in Russia, and likely replaced *C. lectularius* in both Moscow and St. Petersburg (Gapon, 2016). Recent data suggest it has spread further in the European part of Russia (Golub *et al.*, 2020).

The control of bedbugs is particularly problematic not only due to their cryptic behaviour but also due to the evolution of multiple mechanisms that confer resistance to insecticides. In *C. hemipterus*, resistance has been reported multiple times, starting in the 1950s (reviewed in Doggett *et al.*, 2018). The mechanisms of resistance have been examined only for mutations in the sodium channel gene, responsible for the knock-down type of resistance (*kdr*) to pyrethrin, pyrethroids, and some organochlorines. Amino acid changes in the sodium channel gene, both in *C. hemipterus* and *C. lectularius*, are considered a major cause of the bedbug resurgence. In *C. lectularius*, despite some considerable world-wide sampling (Zhu *et al.*, 2010, 2013; Booth *et al.*, 2015; Palenchar *et al.*, 2015; Dang *et al.*, 2015a; Balvín & Booth, 2018; Holleman *et al.*, 2019), only three *kdr*-associated

mutations are known, showing unequal distributions at the continent level (Balvín & Booth, 2018; Holleman *et al.*, 2019). In contrast, a total of nine *kdr*-associated mutations were recorded in *C. hemipterus*. Four (L899V, M918I, D953G and L1014F) were found to be unequally distributed across populations in Australia (Dang *et al.*, 2015b) and the remaining five (Y/L995H, V1010L, I1011F, V1016E and L1017F/S) were reported from Sri Lanka (Punchihewa *et al.*, 2019). In the sample representing the invasion of *C. hemipterus* to Hawaii, bedbugs collected in 2009 exhibited three of these mutations, whereas bedbugs found in 2019 possessed four (Lewis *et al.*, 2020).

Specimens of bedbugs are rarely identified to the species level by PMCs in their practice. The specialists in fact have had no reason to do so as only *C. lectularius* has been traditionally known from Europe. It is possible that the identification of *C. hemipterus* in the reported cases was carried out because the tropical bedbug was suspected in connection with trans-continental travel. Also because the two species are difficult to discriminate (Fig. 1), established populations of the tropical bedbug may have been overlooked since the start of the bedbug resurgence or even longer.

Since the resistance levels of *C. hemipterus* compared to *C. lectularius* have not been assessed, the relevance of species identification for the choice of control measures is not currently understood. However, the occurrence of *C. hemipterus* is highly relevant for the consideration of bedbug detection methods. For example, *C. hemipterus* has been shown to have better climbing abilities when compared to *C. lectularius*, due to the presence of a greater number of tenant hairs on its tibial pads (Kim *et al.*, 2017). As such, the higher vertical friction force that these impart enables adults to easily escape the monitors traditionally used for bedbug detection. Knowledge of the species identity of local populations is therefore important.

This study reports on the detection of multiple, likely established populations of *C. hemipterus* in Europe, focusing primarily on Central Europe. Species identification was made through morphological and genetic methods, including both mtDNA and *kdr*-associated mutation presence. Examining the sequences of the cytochrome oxidase subunit I gene of the current samples and using those available in GenBank, we compared the European populations to those sampled in the original areas of distribution. The presence of *kdr*-associated mutations is also reported.

Materials and methods

Records of *C. hemipterus* were made during thorough monitoring of European bedbug populations starting in 2002, with the vast majority of collections derived from two time periods: 2009–2013 and 2019–2020 (Table S1, Fig. S1). The material was gathered with the assistance of multiple PMCs who also provided a brief record of the social background of the affected inhabitants.

For species identity, we examined between 1 and 10 individuals per infestation. Specimens from 2002 to 2018 were identified based on morphological characters (Usinger, 1966).



Fig. 1. Adult females of *Cimex lectularius* (left) and *C. hemipterus* (right). *Cimex lectularius* has a wider body, and broader pronotum relative to head width. The lateral lobes of the pronotum are broad in *C. lectularius*, broadening forwards and rounded in the front, whereas they are narrow, parallel and frontally rather pointed in *C. hemipterus*. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 1. Details of *Cimex hemipterus* records reported in the study, collected from Central European homes in 2019–2020.

Country	Locality and social context	Date	No. of individuals	COI GenBank accession no.
Czech Republic	Prague; hostel for foreign workers	02 Dec 2019	2	MW449538
Slovakia	Bratislava; apartment for socially disadvantaged	19 Jul 2019	2	MW449539
Slovakia	Bratislava; private apartment, seniors	09 Jul 2019	2	MW449540
Slovakia	Bratislava; hostel for socially disadvantaged	28 Nov 2019	1	MW449542
Slovakia	Bratislava; private apartment, young family	03 Oct 2019	3	MW449541
Switzerland	Genève; hotel	05 Jan 2020	3	MW449543

Additionally, for 169 infestations out of 267, the species was confirmed through sequencing of a fragment of the cytochrome oxidase subunit I gene (COI), following the methods outlined in Balvín *et al.* (2012). Samples collected between 2019 and 2020 (299 locations) were identified based on morphology, and those believed to be *C. hemipterus* were confirmed by sequencing the COI. Sequences of *C. hemipterus* were compared to those publicly available in GenBank with the length of 530 bp and more (Table S2). Sequences were shortened for sites with undefined states to 513 bp and an intraspecific mitochondrial network was constructed using the TCS Statistical Parsimony method (Clement *et al.*, 2000) implemented in PopART v.1.7.2 (Bandelt *et al.*, 1999, <http://popart.otago.ac.nz>).

Specimens identified as *C. hemipterus* were then sequenced for two fragments of the para-type sodium channel gene, in an effort to identify mutations previously connected with *kdr* in *C. hemipterus* (Dang *et al.*, 2015b; Punehiwa *et al.*, 2019). Primer combinations used were BBParaF1/BBParaR1 and BBParaF3/BBParaR3, following Zhu *et al.* (2010). Chromatograms were visualized using CodonCode Aligner version 9.0.1 (CodonCode Corporation, Dedham, MA, USA) and manually inspected.

Results

A total of 566 infestations were analysed, from the years 2002–2020, collected from 15 European countries (Table S1). Of these, 284 samples came from the Czech Republic and Slovakia. Between the years 2002 and 2018, 267 infestations from 15 countries were analysed. Among these, only *C. lectularius* was identified. Since 2019, specimens from 299 sample locations spanning seven countries were analysed. This latter sampling yielded *C. hemipterus* in six infestations from three countries (Table 1). Four cases were found in Bratislava, Slovakia, one each in an apartment and a hostel both rented to socially disadvantaged people, one in an apartment inhabited by seniors, and the final in an apartment occupied by a young family. In Prague, Czech Republic, one case was recorded in a hostel for workers, mostly of Ukrainian origin. In Geneva, Switzerland, *C. hemipterus* was found in a hotel.

Thirteen specimens morphologically identified as *C. hemipterus* were confirmed also by COI sequencing. A dataset of 79 sequences (66 downloaded from GenBank) was collapsed into a network consisting of 19 unique haplotypes (Fig. 2). The resulting network contained one central haplotype

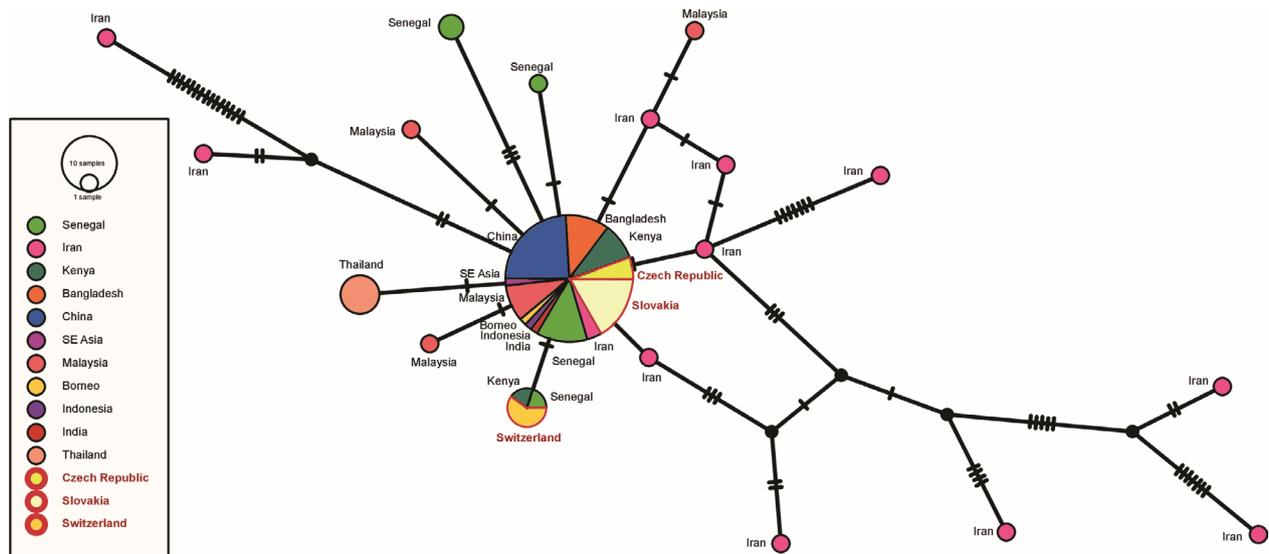


Fig. 2. Mitochondrial network of *Cimex hemipterus* based on cytochrome oxidase subunit I sequences (513 bp) reconstructed using TCS Statistical Parsimony method (Clement *et al.*, 2000). Circle size represents the number of specimens. Crossed lines correspond to the number of nucleotide substitutions. Country names and circles in red represent samples reported in this study. [Colour figure can be viewed at wileyonlinelibrary.com].

comprising samples from geographically distant localities, including all of those sampled from the Czech Republic and Slovakia. The samples from Geneva differed from the central haplotype by 2 bp, sharing a haplotype with sequences previously reported from Senegal and Kenya. Senegalese, Malai and Thai haplotypes were separated from the main haplotype by two or more bp mutations. By contrast, Iranian samples created a complex network with more distant haplotypes.

Two *kdr*-associated mutations (M918I and L1014F) were detected in the sodium channel gene, consistently in a homozygous state across all populations examined.

Discussion

The results presented here reveal convincing evidence of the recent establishment of local *C. hemipterus* populations in Central Europe. Among our records dating back to 2002, only in the collections made since 2019 were *C. hemipterus* detected. This suggests that the invasion was not synchronous with the re-emergence of *C. lectularius* across Europe. It thus started only recently and had not been largely overlooked by PMCs, as hypothesized. The establishment of local populations is supported by the information on the social background of the inhabitants of the infested properties, which indicates that the *C. hemipterus* infestations were likely transferred within cities or communities for at least three cases. This is in contrast to the information known for most previous cases recorded from Europe, which, except for findings in Russia and possibly Sweden, indicated rather sporadic and ephemeral introductions, connected with international travel.

Among the cases reported here, three findings from Bratislava were made among inhabitants who were very unlikely to travel to areas with frequent occurrence of *C. hemipterus*. The case

from Prague was recorded among foreign workers. However, their nationality was European. One case is likely connected with a travel to areas with a regular occurrence of *C. hemipterus* (Geneva, hotel), and the origin of one case is inconclusive, with an introduction resulting from foreign travel or within-city spread equally plausible (Bratislava, young family).

Our reconstruction of the relationship among European samples with those from the rest of the world showed limited mtDNA variability. Excluding samples from Iran, the network showed a starburst-like pattern, likely reflecting a recent population expansion. However, the number of recorded mutations suggests that the expansion occurred deep in the past and is not connected to the recent resurgence. One of the likely scenarios behind this pattern may be the expansion of host species from bats to humans. The distance of the Iranian samples (Samiei *et al.*, 2020) from the rest may suggest a deeper history of association of *C. hemipterus* with humans in the region. However, such diversity is highly unlikely to stay restricted to one country through the history of humans.

The mitochondrial data, in concert with the single *kdr*-associated variant found, provide only limited information on the origin of the European population, when compared with data available in GenBank. The COI haplotype found in infestations sampled in the Czech Republic and Slovakia was previously reported across many countries and appears to be the most common COI variant of *C. hemipterus*, at least in Asia. Similarly, the sodium channel variant, containing M918I and L1014F mutations, has been reported multiple times, from India and Australia (Dang *et al.*, 2015b), and from China (Zhao *et al.*, 2020), but not in Hawaii (Lewis *et al.*, 2020) or Sri Lanka (Punchihewa *et al.*, 2019). Although the country of origin cannot be pinpointed accurately at this time, the genetic uniformity of the Czech and Slovakian infestations may suggest a single introduction followed by spread. However, this is speculative,

as they represent the most common and widespread variants for the examined loci in the original areas of distribution. The Swiss finding is linked by its COI haplotype to two African countries, suggesting possible import from this continent.

The presence of M918I and L1014F mutations suggests that the new European populations of *C. hemipterus* are resistant to pyrethroids, however, the level of resistance has yet to be determined. Nevertheless, the cooperating PMCs reported no problems controlling the sampled infestations using treatments commonly implemented for *C. lectularius* control. Although the authors are not aware of current insecticidal treatments failing to control *C. hemipterus* that would otherwise be effective for the control of *C. lectularius*, insecticide resistance mechanisms are diverse and there is a potential for new, species-specific forms to evolve.

In conclusion, the authors document a relatively recent and, for now, low level invasion of the *C. hemipterus* into Central Europe. Within Europe, the first occupied areas were Russia and possibly Sweden. It is possible that the climate had been originally a reason for different areas of distribution of the two bedbug species. The current situation indicates that climate no longer constrains bedbug distribution and that indoor conditions, including central heating and/or the use of indoor humidifiers, mostly used in bedrooms, may facilitate the spread of both bedbug species, including the presumably tropical *C. hemipterus*. At this moment, no evidence suggests that the European population of *C. hemipterus* is harder to control than *C. lectularius*. However, in an area with limited spectrum of insecticides available on the market, the occurrence of two species may represent a broader genetic pool, upon which evolution may yield further resistance mechanisms. Currently, it is clear that the successful establishment of invasions is in its early stages; however, understanding the occurrence and spread of *C. hemipterus* is important with respect to bedbug detection methods. With regard to that, bedbug-detection dogs trained for *C. lectularius* have been found to be capable of locating other cimicid species, specifically *C. hemipterus* and *C. pipistrelli* (A. Hamker, J. Hofferová, 2021, personal communication). However, bedbug monitoring using conventional climb-up type traps may fail to identify early-stage infestations of *C. hemipterus* due to their greater climbing ability. The reliability of detection is important in evaluating the efficiency of bedbug control measures, but also in the monitoring of eventual further spread of the tropical bedbug. Therefore, we recommend that PMCs make efforts to identify bedbug species at least occasionally, particularly in cases where traditional treatments have failed.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Map of the infestations examined in this study.

Table S1. List of bedbug material examined.

Table S2. List of GenBank accession codes of COI sequences of *Cimex hemipterus* compared with our samples and used in PopArt genealogy reconstruction.

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Author's contributions

M. Sasínková, T. Bubová and O. Balvín organized the sample collection. O. Balvín identified the specimens morphologically. O. Balvín, J. Martinů and M. Sasínková carried out the molecular investigations. All authors took part in interpretation of the molecular data and writing the manuscript. All authors gave final approval for publication.

Data availability statement

All data used in study are enclosed as supplementary files, with nucleotide sequences deposited at GenBank with reference codes in Supplementary Table 2.

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