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First official report of bed bug (Hemiptera, Cimicidae) infestations in Algeria

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ABSTRACT

Background: Bed bugs are hematophagous insects with a long history of presence in human communities. Over the last three decades, infestations by bed bugs in human dwellings have drastically increased, leading to a rise in bed bug concerns. Nevertheless, very little is known about the bed bug species and their population diversity in Algeria.
Method: A pilot entomological inventory was performed in May 2019 in Tizi Ouzou, in northern Algeria. The gathered bed bug specimens were identified by morphological and molecular approaches, followed by neighbor-joining and network phylogenetic analyses.
Results: A total of seven out of 12 requested locations were allowed to inspect for bed bug infestation. Of these, three locations were found with active bed bug infestations. A total of 145 specimens belonging to different life stages [egg (21), nymph (74), adult male (17), and female (33)] were collected and analyzed using morphological and molecular approaches. The adult specimens were identified as *Cimex lectularius* according to specific morphological criteria, most importantly the pronotum laterally expanded with more flattened extreme margins. Morphological identification of the adults was confirmed further by conventional PCR targeting 450 bp fragment of the COI gene. All the nymphs and eggs were also molecularly identified as *C. lectularius*. Neighbor-Joining phylogenetic tree reconstructed with the collected specimens provides clues on the presence of two closely phylogenetic groups. The first one gathers our samples of Algeria with previously reported COI haplotype sequences from Asian, European, and North American countries. The second group encompasses a lesser-documented haplotype reported in Europe and Central America. These findings were further confirmed by network analysis.

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Conclusions: These results provide evidence of established *C. lectularius* infestation in Algeria and its potential dispersal capacity by travelers or immigrants and will help future management of these ectoparasites.

1. Introduction

Bed bugs (Hemiptera, Cimicidae) are obligate ectoparasitic insects of humans with a strictly hematophagous diet (Reinhardt and Siva-Jothy, 2007). The two cosmopolitan species, *Cimex lectularius,* and *C. hemipterus*, feed almost exclusively on humans and are responsible for significant infestation outbreaks. Although no proven evidence has been demonstrated the vectorial role of these insects, they have been the main issue of public health and responsible for several clinical, psychological, and economic disorders (Goddard and deShazo, 2009; Benkacimi et al., 2020; Akhoundi et al., 2023). In addition to medical issues, removing the bed bugs from an infested area is a costly mission. Therefore, bed bug infestation control remains a significant challenge in public health (Akhoundi et al., 2023). Since the 1990s, bed bugs (*C. lectularius* and *C. hemipterus*) have undergone a significant resurgence worldwide. Algeria is the largest country in Africa continent in which the presence of bed bugs was documented in ancient references (e.g. books, dissertations) but very little is known in the recently released literature about the bed bugs' identity, species composition, and infestation rate across the country as well as in North Africa. In this study, we provided one of the first evidences about the species composition and epidemiology of bed bug infestations in a district in the north of Algeria using morphological and molecular approaches.

2. Materials and methods

2.1. Bed bugs sampling

The bed bug specimens of various life stages were collected within private houses and apartments in the Tizi Ouzou region in the north of Algeria. Sampling was carried out with entomological forceps (Insecta-Pro®) and the collected specimens were placed in 40 mL sterile mini-glass vials including a piece of folded bound paper as an artificial shelter to avoid excessive mortality of bed bugs. They were brought to the laboratory and identified morphologically under a stereomicroscope (WF10X/21 OPTIKA, Japan), according to identification keys published by Usinger (1966) and Walpole (1987). All the specimens were labeled individually and kept at −20 °C for further molecular analysis.

2.2. DNA extraction and PCR amplification

For further identification, the DNA extraction of bed bug specimens was performed using Chelex 10% (Bio-Rad, France), and its concentration was then quantified by Qubit (Thermoscientific, USA). The extracted DNA was subjected to conventional PCR performed in a final volume of 25 μL, with 12.5 μL master mixture, 8 μL distilled water, 1 μM of each of the forward (COIF: 5′-GCATTYCCACGAATAAATAAYATAAG-3′) and reverse (COIR: 5′-TAAACTTCTGGATGTCCAAAAATCA-3′) primers and 2.5 μL extracted DNA (Seri Masran and Majid, 2017). The amplification was performed under the following conditions: initial denaturation for 2 min at 95 °C, followed by 5 cycles of 40 s at 94 °C, 40 s at 45 °C, 1 min at 72 °C; and then 35 cycles of 40 s at 94 °C, 40 s at 51 °C, 1 min at 72 °C, and 5 min at 72 °C. A couple of negative (distilled sterile water) and positive (laboratory-reared bed bug specimen's DNA previously positive in PCR examination) controls were used for each PCR batch. The amplicons were analyzed using electrophoresis on 1.5% agarose gel containing ethidium bromide.

2.3. Phylogenetic reconstruction and species assignation

PCR products were subjected to bidirectional DNA sequencing using the same primer pairs used for amplification. The acquired sequences were identified at the species level using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST). All nucleotide sequences were deposited in the GenBank database. Sequence alignment was performed with the BioEdit v7.0.0 software, and the phylogenetic analysis was carried out using MEGA v.6 software. The inferred phylogenetic tree of *Cimex* species (identified in this study) and homonym sequences from GenBank were constructed using the neighbor-joining (NJ) method and bootstrap values, determined by 1000 replicates. To display the genetic relationships within *C. lectularius* populations, the median-joining algorithms were implemented using network v. 5 software.

3. Results

A total of 12 human dwellings including nine apartments and three private houses in the Tizi Ouzou region were requested to be visited that among them, seven locations were allowed to be examined for the presence of bed bugs. Based on visual inspection, three sites were found to be infested, while no bed bug was collected in the remaining visited locations. A total of 145 specimens belonging to different life stages [egg (21), nymph (74), adult male (17) and female (33)] were collected (Fig. 1, Table 1). All adult specimens were identified as *C. lectularius* according to specific morphological criteria most importantly the pronotum laterally expanded with more flattened extreme margins. Morphological identification of the adults was confirmed further by molecular approach. Furthermore, all

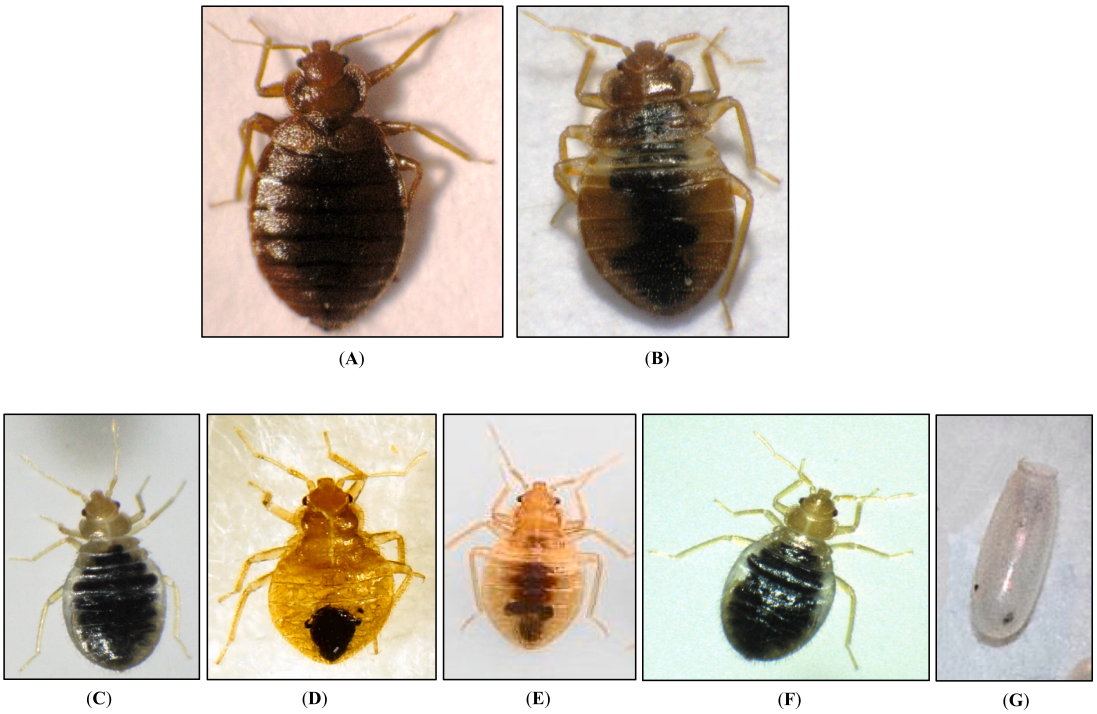

Fig. 1. Various life stages of bed bug specimens caught in Tizi-Ouzu, northern Algeria: Male (A), female (B), immature nymphal stages (C to F) & egg (G).

Table 1
Detailed information of the bed bug specimens processed in the present study.

Residence type	Inspection	Bed bug species	Number of collected specimens	Life stage
Apartment 1	X	–	–	–
Apartment 2	X	–	–	–
Apartment 3	√	*C. lectularius*	67	9♂, 17♀, 33 nymphs, 8 eggs
Private house 1	√	–	–	–
Private house 2	√	–	–	–
Apartment 4	X	–	–	–
Apartment 5	X	–	–	–
Private house 3	√	*C. lectularius*	41	3♂, 9♀, 18 nymphs, 11 eggs
Apartment 6	√	–	–	–
Apartment 7	√	–	–	–
Apartment 8	√	*C. lectularius*	37	5♂, 6♀, 23 nymphs, 3 eggs
Apartment 9	X	–	–	–

the nymphs and eggs were identified molecularly as *C. lectularius*. BLAST analysis of COI gene sequences revealed ≥99% identity with counterpart sequences of *C. lectularius* (e.g., GU985525) in GenBank. The obtained sequences were deposited in GenBank with assigned numbers of MK3456812 to MK3456957. A Neighbor-Joining phylogenetic tree constructed based on COI sequences of *C. lectularius* specimens processed in this study compared to homologous sequences from GenBank revealed the presence of two well-differentiated and supported clades. First big clad consists of 40 specimens sampled from Europe [Republic Czech (GU985524, GU985524, MF680529, KJ937990), Finland (MK141705, MK141706)], America [USA (AY253011, KR002572, KR002575, JQ782821, JQ782823, MG596836)] and Asia [Thailand (JX826481)], while the second consists of 4 specimens found in Hungary (MF161522, MF161527), Finland (MK141700) and Costa Rica (MN271345) (Fig. 2A). Furthermore, the clustering displayed by the median-joining network agrees well with the topology of the phylogenetic tree generated by the neighbor-joining analysis (Fig. 2B).

4. Discussion

Cimex lectularius is a hematophagous species commonly found in temperate regions of the Nearctic and Palearctic areas (Asia, Australia, Africa, and South America) (Zorrilla-Vaca et al., 2015; Akhoundi et al., 2020). It has recently been observed to expand its range to a number of tropical locations. International travel, immigration, and secondhand business (primarily within cities) are such factors that contribute to its widespread distribution across the world (Suwannayod et al., 2010; Doggett et al., 2017).

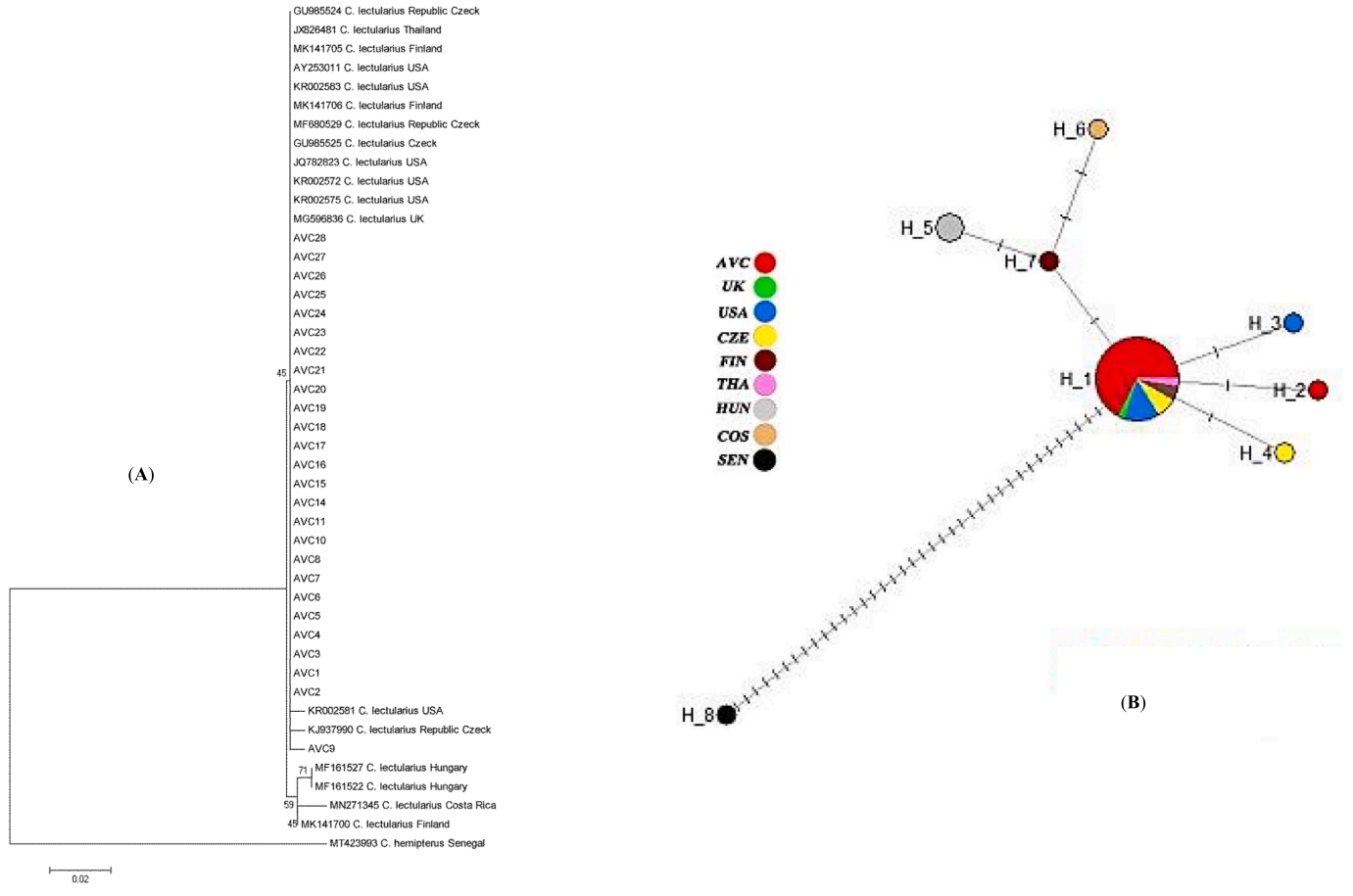

Fig. 2. Neighbor-joining (NJ) tree reconstructed from COI sequences of bed bug specimens collected in the present study (beginning with AVC) and sequences collected from GenBank (A); Median-joining network for COI sequences of Algerian *C. lectularius* specimens processed in the present study (B).

In Africa, bed bug infestations have been poorly documented over the past two decades. *Cimex hemipterus* was considered the most prevalent species, while *C. lectularius* was restricted to some countries in central and southern Africa such as Nigeria, Sierra Leone, Tanzania, and Madagascar (Jupp et al., 1978, Newberry and Jansen, 1986, Gbakima et al., 2002, Emmanuel et al., 2014, Akhoundi et al., 2022). Furthermore, the accurate diagnosis of these species is crucial for designing effective control programs involving insecticide selection and the type of monitoring system used.

In Algeria, bed bug infestations seem to be an underreported nuisance. Although bed bugs were documented in the ancient references (Buquet, 1832; Notice sur l'Institut Pasteur d'Algérie, 1934), there is a serious lack of knowledge about the identity and distribution of bed bug species in the released literature. To fill out partially this gap of knowledge on the bed bug infestation, we conducted the current study which highlights the bed bug infestations in the Tizi Ouzou region. This is the first official evidence of *C. lectularius* infestation in Algeria and North Africa. The record of *C. lectularius* in the present study is in accordance with those reports documented from other African countries such as Sierra Leone and Nigeria (Gbakima et al., 2002; Emmanuel et al., 2014). Although it is impossible to track the origin of infestation by *C. lectularius*, regarding the information gathered from the residents of infested locations, no history of national/international travels or accommodation of foreign guests was recorded indicating the presence of *C. lectularius* as an established species in this country. Besides, it seems that one of the reasons why the bed bug issue in Algeria is ignored might be due to a kind of embarrassment that people have from reporting their homes infested with bed bugs, which has led to resistance to visiting or restricting access to infected places. In this study, 12 houses were requested to be checked for bed bug presence, of which only seven residents were allowed to visit the house.

Phylogenetic analysis depicted the occurrence of two *C. lectularius* populations in which our specimens were clustered in a major clade together with homologous sequences from Europe, America, and Asia (Fig. 2A,B). No hybrid or genetic combination notion was observed among our specimens compared with those coming from other countries. These findings were consistent with a previous study by Chebbah et al. (2021) certifying heterogeneity among processed bed bug specimens in Europe. It is worth mentioning that three Genbank sequences originating from Finland (MK141700, MK141705, and MK141706) were clustered in two independent clades demonstrating the presence of heterogeneity among the mentioned sequences.

5. Conclusion

This pilot study provides the first evidence of the presence of *C. lectularius* in the Tizi Ouzou district in northern Algeria as well as in North Africa, which can serve as baseline information for further epidemiological and public health studies and for Algerian health authorities to apply the appropriate control management strategies against these ectoparasites.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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