PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Body lice of homeless people reveal the presence of several emerging bacterial pathogens in northern Algeria

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Abstract

Background

Human lice, *Pediculus humanus*, are obligate blood-sucking parasites. Body lice, *Pediculus h. humanus*, occur in two divergent mitochondrial clades (A and D) each exhibiting a particular geographic distribution. Currently, the body louse is recognized as the only vector for louse-borne diseases. In this study, we aimed to study the genetic diversity of body lice collected from homeless populations in three localities of northern Algeria, and to investigate louse-borne pathogens in these lice.

Methodology/Principal findings

In this study, 524 body lice specimens were collected from 44 homeless people in three localities: Algiers, Tizi Ouzou and Boumerdès located in northern Algeria. Duplex clade specific real-time PCRs (qPCR) and *Cytochrome b* (*cytb*) mitochondrial DNA (mtDNA) analysis were performed in order to identify the mitochondrial clade. Screening of louse-borne pathogens bacteria was based on targeting specific genes for each pathogen using qPCR supplemented by sequencing. All body lice belong to clade A. Through amplification and sequencing of the *cytb* gene we confirmed the presence of three haplotypes: A5, A9 and A63, which is novel. The molecular investigation of the 524 body lice samples revealed the presence of four human pathogens: *Bartonella quintana* (13.35%), *Coxiella burnetii* (10.52%), *Anaplasma phagocytophilum* (0.76%) and *Acinetobacter* species (*A. baumannii*, *A. johnsonii*, *A. berezeniae*, *A. nosocomialis* and *A. variabilis*, in total 46.94%).

Conclusions/Significance

To the best of our knowledge, our study is the first to show the genetic diversity and presence of several emerging pathogenic bacteria in homeless' body lice from Algeria. We also

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report for the first time, the presence of several species of *Acinetobacter* in human body lice. Our results highlight the fact that body lice may be suspected as being a much broader vector of several pathogenic agents than previously thought. Nevertheless, other studies are needed to encourage epidemiological investigations and surveys of louse-associated infections.

Author summary

Head lice, *Pediculus h. capitis*, and body lice, *Pediculus h. humanus*, are obligatory blood-sucking ectoparasites. The body lice occur in two divergent mitochondrial clades (A and D) each exhibiting a particular geographic distribution. Currently, the body louse is the only recognized vector for louse-borne diseases. In this work, we aimed to study the genetic diversity of body lice collected from homeless individuals in Algeria and to investigate louse-borne pathogens in these lice. To the best of our knowledge, our study is the first to show the presence of *Bartonella quintana*, *Coxiella burnetii*, *Anaplasma phagocytophilum* and several species of *Acinetobacter* in human body lice from Algeria. These findings should strongly encourage further epidemiological investigations and surveys of louse-associated infections, and better understanding of the role of body lice as a broader vector of several bacterial pathogens in humans than previously reported in the literature.

Introduction

Two genera are recognized within the human sucking lice order (Phthiraptera: Anoplura): *Pthirus* and *Pediculus* [1,2]. Each genus is presented by one species: *Pthirus pubis* and *Pediculus humanus*, respectively [3,4]. Both louse species are an obligate blood-feeding parasites that thrived exclusively on human blood for thousands years [1,2]. They are probably of the oldest and most intimate human parasites [5,6]. *Pediculus humanus* is of great concern to public health and includes two ecotypes: the head louse, *Pediculus humanus capitis*, which lives and lays its eggs on the human scalp, and the body louse, *Pediculus humanus humanus*, which lives and multiplies in clothing in poor and unhygienic conditions [7,8]. In contrast to the head louse, that preferentially infests schoolchildren throughout the world regardless of their social class or level of hygiene, the body louse is mostly prevalent in people living in precarious conditions [9,10]. Practically, outside of their biotopes, the two ecotypes are morphologically indistinguishable [11]. Indeed, in a study conducted to compare the transcriptional profile of head and body lice, Olds *et al*. argued that the two types of lice had a single 752-base pair (bp) difference in the Phum_PHUM540560 gene, which encodes a hypothetical 69-amino acid protein of unknown function, and that this gene was present and transcribed in body lice, but absent in head lice [12]. More recently, a multiplex real-time PCR assay was conducted, based on the alignment of two portions of the head and body lice Phum_PHUM540560 gene sequences to efficiently distinguish the two ecotypes [11].

Phylogenetic studies, based on mitochondrial genes, widely used to study the genetic diversity of human lice, have revealed the presence of five divergent mitochondrial clades (A, D, B, C, and E). Each clade exhibits a particular geographical distribution [13,14]. Body lice belong only to clades A and D, while head lice encompass all the diversity of clades [13,15]. Haplogroup A is the most common and is worldwide distributed, while haplogroup D is only found in central Africa, specifically in Ethiopia and the Democratic Republic of the Congo

[14–16]. Clade B is confined to the New World (Europe and Australia), and has recently been reported in northern and south Africa [2,15,17,18]. Clade C has been found in Ethiopia, the Democratic Republic of the Congo, Nepal and Thailand [2,16,19,20]. A novel clade, clade E, was described in West Africa [13], and then reported for the first time in head lice in Bobigny, France [21]

Until recently, only the body louse was recognized as a vector of at least three serious human diseases that have killed millions of people, namely epidemic typhus, trench fever, and relapsing fever, caused by *Rickettsia prowazekii*, *Bartonella quintana* and *Borrelia recurrentis*, respectively. Body louse-borne infections are amongst the epidemic diseases described during wars and famine periods in the history [22]. Natural and experimental observations have shown that body lice can also be able to host and possibly transmit *Yersinia pestis*, the causative agent of plague during plague pandemics [23,24]. Subsequently, other widespread pathogenic bacteria, including *Acinetobacter baumannii*, *A. lwoffii* and *Serratia marcescens*, have been detected in human body lice assuming the probability that lice can also transmit these agents [25–28]. Under experimental conditions, infected body lice are also capable of transmitting to rabbits *R. typhi*, *R. rickettsii* and *R. conorii* the causative agents of murine typhus, Rocky Mountain spotted fever and Mediterranean spotted fever, respectively [29,30].

Although body lice, rather than head lice, are assumed to be potential vectors of pathogens, the epidemiological status of the head louse as a vector of louse-borne diseases is still debated [16]. Studies have demonstrated that the immune reactions of the body louse to different pathogens are weaker than those of head louse, which may allow it to carry a large spectrum of pathogens [31,32]. However, recently, head lice belonging to different mitochondrial clades were found to carry the DNA of several bacterial body louse-borne pathogens, such as *B. quintana*, *B. recurrentis*, *Acinetobacter* species and *Y. pestis* in natural settings [14,16,20,33–38]. Experimental studies have also demonstrated that head lice may also act as a vector of louse-borne diseases [39,40]. Recently, in East Africa, Giroud *et al.* showed in field studies that human lice collected from people living in formerly epidemic areas of Q fever could be infected with *Coxiella burnetii*. The bacterial strains from the infected lice was isolated from guinea pigs [41]. Latterly, a study reported for the first time, the presence of DNA of *C. burnetii* in human head lice collected from two rural villages in Mali, as well as the DNA of *R. aeschlimannii* and two potential new species from the *Anaplasma* and *Ehrlichia* genera of unknown pathogenicity [42].

In northern Africa, notably in Algeria, studies on body lice and the occurrence of their associated emerging pathogens bacteria has never been reported, particularly those involving marginalized people living in precarious sanitary and degraded socio-economic conditions as well as refugees from civil wars, jail population and homeless. People living in these conditions represent an explosive risk factor for outbreaks of arthropod-borne diseases [10]. Several reports have demonstrated that the study of lice-associated pathogens can be used to detect infected patients and therefore estimate the risk of outbreaks of epidemics and assume the control measures to prevent the spread of infection [22,43].

The aim of this work is to investigate louse-borne pathogens of body lice collected from homeless populations in three localities in northern Algeria, and to study the genetic diversity of these lice. An assessment of the frequency of body lice infestation has never been reported previously in this country.

Materials and methods

Ethics statement and louse sampling

This study was approved by the Centre d'Accueil pour Personnes sans Domicile Fixe and the Social SAMU (Service d'Aide Médicale Urgente), Algeria. Body lice were collected from

clothes of homeless individuals during a registered epidemiological study in northern Algeria, with the verbal consent of the infested individuals. Written consent was not obtainable because most of the subjects involved in the study were illiterate. However, the local health center representatives were present during collection. The anonymity of the individuals providing the lice used in the present study was preserved.

An epidemiological investigation was conducted between September 2014 and June 2016, when a massive lice infestation was reported among homeless people attending the Centre d'Accueil pour Personnes sans Domicile Fixe in Algeria. A total of 534 body lice samples were collected from 44 homeless individuals. The collection was conducted in three different localities in northern Algeria: i) Algiers, where 235 lice were isolated from 19 homeless people (17 men and 2 women), ii) Tizi Ouzou, 184 lice isolated from 16 homeless people (12 men and 4 women), and iii) Boumerdès, 115 lice isolated from nine homeless people (7men and 2 women) (Fig 1). All individuals were examined for the presence of both body and head lice, however, no head lice were found during the examination. Visible body lice were removed from the clothing using clamps, live lice were immediately frozen at -20˚C and then transported to (URMITE), Marseille. All body lice collected were then processed for molecular study.

Body lice DNA analysis

DNA extraction. Before DNA isolation, each louse's surface was decontaminated to avoid external contamination, as previously described [40], and each louse specimen was then cut in half length-ways. DNA was then extracted from one-half and the remaining halves of the lice were frozen at -20˚C for subsequent studies. DNA was extracted using the QIAamp DNA tissue extraction kit (Qiagen, Hilden, Germany) on the BioRobot EZ1 (Qiagen, Courtaboeuf, France) following the manufacturer's instructions. DNA was eluted in 100 μl of TE buffer and stored at -20˚C until the next stage of investigation.

Genotypic status of lice

Determination of louse mitochondrial clade by qPCR assays. In order to determine the mitochondrial clades of lice collected in this study, all DNA samples were analyzed by clade-specific quantitative real time PCR (qPCR) assays that targeted a portion of the *cytb* gene specific to clades A, D, B, and C [16]. PCR amplification was carried out using a CFX96 Real-Time system (Bio-Rad Laboratories, Foster City, CA, USA) as previously described [16]. We used lice with known clades as positive controls and master mixtures as a negative control for each test. Sequences of primers and probes are shown in (Table 1).

Cytochrome b amplification and haplotype determination

For phylogenetic study, DNA samples of twenty body lice of the total number of lice collected in each locality were randomly selected to ensure equal distribution of the included lice collected from the three localities. These were then subjected to standard PCR targeting a 347-bp fragment of the *cytb* gene, as previously described [44].

PCRs consisted of 50 μl volume, including 25 μl Amplitaq gold master mix, 1 μl of each primer, 5 μl of DNA template, and water. The thermal cycling profile was one incubation step at 95˚C for 15 minutes, 40 cycles of one minute at 95˚C, 30 seconds at 56˚C and one minute at 72˚C followed by a final extension for five minutes at 72˚C. PCR amplification was performed in a Peltier PTC-200 model thermal cycler (MJ Research Inc., Watertown, MA, USA). The success of amplification was confirmed by electrophoresis on 1.5% agarose gel. Purification of PCR products was performed using NucleoFast 96 PCR plates (Macherey Nagel EURL,

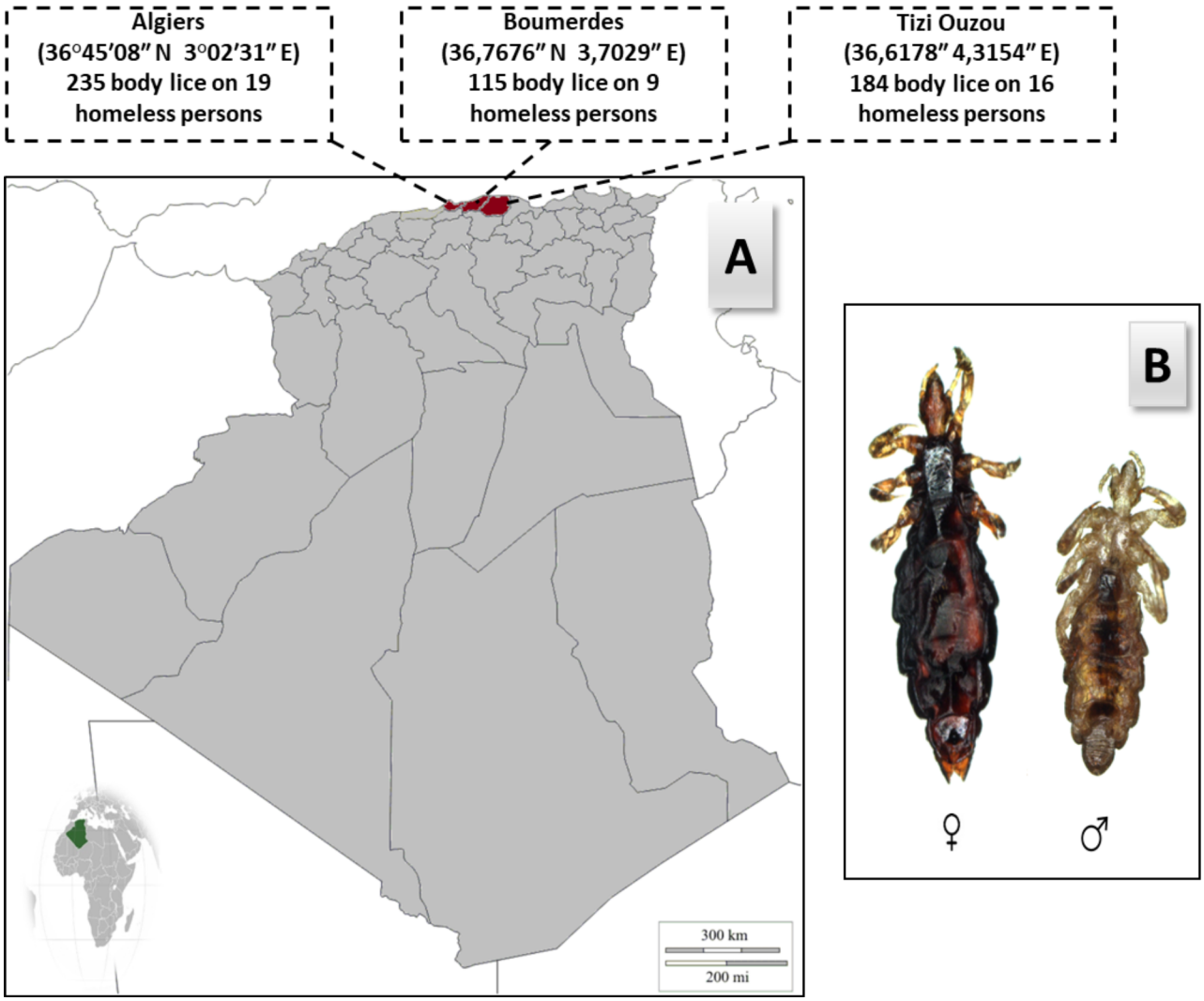

Fig 1. (A) Map of body lice collection from homeless populations in three localities in northern Algeria. Red pins indicate sampling sites. (B): Human body lice collected from the homeless population.

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Hoerdt, France) as per the manufacturer's instructions. The amplicons were sequenced using the Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems, Foster City, CA) with an ABI automated sequencer (Applied Biosystems). The electropherograms obtained were assembled and edited using ChromasPro software (ChromasPro 1.7, Technelysium Pty Ltd., Tewantin, Australia) and compared with those available in the GenBank database by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Molecular confirmation of lice ecotype

All thirty samples belonging to haplotype A5 (a haplotype comprising both head and body lice) were analyzed by multiplex real-time PCR, targeting a portion of the Phum PHUM540560 gene. This assay allowed for the discrimination of body lice from head lice, as described previously [11]. As a positive control, we used a head louse and body louse with genotypic statuses that were detected beforehand. (Multiplex real time PCRs were performed using a CFX96 Real-Time system (Bio-Rad, Marnes-la-Coquette, France).

Table 1. Primers and probes used for real time PCRs and conventional PCRs in this study.

Target	Name	Primers (5'-3') and probes	Reference
***Rickettsia* spp.** *citrate synthase* (*gltA*)	**RKNDO3**	**F_GTGAATGAAAGATTACACTATTTAT**	[78]
		R_GTATCTTAGCAATCATTCTAATAGC	
		FAM-CTATTATGCTTGCGGCTGTCGGTTC-TAMRA	
Borrelia spp. *16S ribosomal RNA*	**Bor16S**	**F_AGCCTTTAAAGCTTCGCTTGTAG**	[79]
		R_GCCTCCCGTAGGAGTCTGG	
		FAM-CCGGCCTGAGAGGGTGAACGG-TAMRA	
Bartonella quintana	***yopP*** Hypothetical intracellular effector	**F_GATGCCGGGGAAGGTTTTC**	[80]
		R_GCCTGGGAGGACTTGAACCT	
		FAM-GCGCGCGCTTGATAAGCGTG-TAMRA	
	fabF3 3-oxoacyl-synthase gene	**F_GCGGCCTTGCTCTTGATGA**	
		R_GCTACTCTGCGTGCCTTGGA	
		FAM-TGCAGCAGGTGGAGAGAACGTG-TAMRA	
Yersinia pestis	***PLA***	**F_ATG GAG CTT ATA CCG GAA AC**	[81]
		R_GCG ATA CTG GCC TGC AAG	
		FAM-TCCCGAAAGGAGTGCGGGTAATAGG-TAMRA	
***Acinetobacter* spp.** *RNA polymerase β subunit gene*	***rpoB***	**F_TACTCATATACCGAAAAGAAACGG**	[33]
		R_GGYTTACCAAGRCTATACTCAAC	
		FAM-CGCGAAGATATCGGTCTSCAAGC-TAMR	
	rpoB (zone1)	**F_TAYCGYAAAGAYTTGAAAGAAG**	[45]
		R_CMACACCYTTGTTMCCRTGA	
Coxiella burnetiid Spacers	***IS1111***	**F_CAAGAAACGTATCGCTGTGGC**	[63]
		R_ CACAGAGCCACCGTATGAATC	
		FAM- CCGAGTTCGAAACAATGAGGGCTG-TAMRA	
	IS30A	**F_ CGCTGACCTACAGAAATATGTCC**	
		R_ GGGGTAAGTAAATAATACCTTCTGG	
		FAM-CATGAAGCGATTTATCAATACGTGTATGC-TAMRA	
	Cox2	**F_CAACCCTGAATACCCAAGGA**	[47]
		R_GAAGCTTCTGATAGGCGGGA	
	Cox5	**F_CAGGAGCAAGCTTGAATGCG** **R_TGGTATGACAACCCGTCATG**	
	Cox18	**F_CGCAGACGAATTAGCCAATC**	
		R_TTCGATGATCCGATGGCCTT	
	Cox22	**F_GGGAATAAGAGAGTTAGCTCA**	
		R_CGCAAATTTCGGCACAGACC	
***Anaplasma* spp.** 23S ribosomal RNA	**TtAna**	**F_TGACAGCGTACCTTTTGCAT**	[46]
		R_TGGAGGACCGAACCTGTTAC	
		FAM-GGATTAGACCCGAAACCAAG-TAMRA	
	Ana23S	**F_ATAAGCTGCGGGGAGTTGTC**	
		R_ TGCAAAAGGTACGCTGTCAC	
Anaplasma phagocytophilum	***apaG***	**F_TAAGCGCAGTTGGAAGATCA**	[50]
		R_CGGCACATCCACATAAAACA	
		FAM-TGATGAACGGCTGGTATCAG-TAMRA	

(*Continued*)

Table 1. (Continued)

Target	Name	Primers (5'-3') and probes	Reference
Cytochrome b	**Duplex A-D**	**F_ GATGTAAATAGAGGGTGGTT**	[16]
		R_ GAAATTCCTGAAAATCAAAC	
		FAM-CATTCTTGTCTACGTTCATATTTGG-TAMRA	
		VIC-TATTCTTGTCTACGTTCATGTTTGA-TAMRA	
	Duplex B-C	**F_ TTAGAGCGMTTRTTTACCC**	
		R_ AYAAACACACAAAAMCTCCT	
		FAM-GAGCTGGATAGTGATAAGGTTTAT-MGB	
		VIC-CTTGCCGTTTATTTTGTTGGGGTTT-TAMRA	
	Cytb	**F_ GAGCGACTGTAATTACTAATC**	[44]
		R_ CAACAAAATTATCCGGGTCC	
PHUM	**Phum540560**	**GTCACGTTCGACAAATGTT**	[11]
		TTTCTATAACCACGACACGATAAAT	
		FAM-CGATCACTCGAGTGAATTGCCA-TAMRA	
		VIC-CTCTTGAATCGACGACCATTCGCT-TAMRA	
Universal vertebrate (vCOI) Cytochrome C, Oxidase I gene	**(vCOI)**	**F_AAGAATCAGAATARGTGTTG, R_ AACCACAAAGACATTGGCAC**	[49]

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Molecular screening for the presence of pathogen DNA

The qPCRs were performed to screen all body lice samples using previously reported primers and probes for *Rickettsia* spp., *Borrelia* spp., *B. quintana*, *Y. pestis*, *Acinetobacter* spp., *C. burnetii* and *Anaplasma* spp. All the sequences of primers and probes as well as their respective sources used in this study are presented in Table 1. All qPCRs were performed using a CFX96 Real-Time system (Bio-Rad, Marnes-la-Coquette, France) and the Eurogentec Master Mix Probe PCR kit (Eurogentec, Liège, Belgium). We included the DNA of the target bacteria as positive control and master mixtures as a negative control for each test. Samples were considered positive when the cycle threshold (Ct) was lower than 35 Ct. All *B. quintana* and *C. burnetii* positives samples were confirmed by a second specific qPCR targeting the *fabF3* gene and the IS30A spacer, respectively (Table 1).

To identify the species of bacteria, all positive samples from qPCRs for *Acinetobacter* spp. and *Anaplasma* spp. were further subjected to standard PCR, targeting a 350-bps fragment of the *rpoB* gene (zone1) and the 525-bps fragment of the *rpoB* gene, for each genus respectively [45,46]. In order to perform genotyping of *C. burnetii*, all positive lice were also subjected to PCR amplification and sequencing targeting four spacers (Cox2, Cox5, Cox18 and Cox22). Primers and all conditions used for the investigation were as described previously [47]. Successful amplification was confirmed via gel electrophoresis and amplicons were prepared and sequenced using similar methods as described for *cytb* gene above.

Data analysis

For comparison, the body lice nucleotide sequences obtained in this study were combined with the *cytb* database which comprised haplotypes spanning different geographic location in the five continents, as reported by Amanzougaghene *et al.* [13], in order to investigate the possible relationships between the haplotypes. MEGA 6.06 was used for the phylogenetic analyses under the Kimura 2-parameter model with 500 replicates as described previously [16,48].

All obtained sequences of *Acinetobacter* species were analyzed using BLAST and compared with sequences in the GenBank database. A maximum-likelihood method was also used to infer the phylogenetic analyses, as described for the analyses above [48].

Body lice' blood-feeding source identification

In order to identify the blood meal source, 30 body lice specimens with positive bacterial-DNA results were tested using conventional PCR targeting the vertebrate universal specific primers cytochrome c oxidase I gene (vCOI) fragment, as previously described [49] (Table 1). Successful amplifications have been treated using similar methods as described above for *Cytb* and bacteria.

Results

Population description and genetic status of lice

Of the 44 homeless individuals infested by body lice, majority were male (sex ratio M/F = 4.5) and were aged between 30 and 63 years. In total, 524 body lice were collected from 44 homeless people from three different localities in northern Algeria, and all collected lice were analyzed by two duplex qPCRs to determine their clade. The result showed that all body lice were clade A. Phylogenetic analysis of the 60 *cytb* sequences of randomly selected lice yielded to define 3 different haplotypes. The first haplotype (30 sequences) belonged to the worldwide haplotype A5 comprising both head and body lice within Clade A. The second haplotype (14 sequences) belonged to haplotype A9. While the remaining 16 sequences belonged to the third haplotype which was novel and named here A63. These haplotypes, together with references from all the body lice and haplogroups, were used to construct a maximum-likelihood (ML) phylogenetic tree (Fig 2).

Unexpectedly, the results showed that 5 five of the 30 (16.66%) body lice exhibited a Phum540560 profile typical for head lice. These lice belonged to haplotype A5 and were collected from the same patient in Algiers.

Molecular detection of bacterial pathogens

In this study, we did not detect the DNA of *Rickettsia* spp., *Borrelia* spp. and *Y. pestis* in any of the 524 body lice specimens studied. The DNA of *B. quintana* was detected in 70/524 (13.35%) of the body lice collected from 30/44 (68.18%) individuals, targeting two specific genes. *Bartonella quintana*-positive lice were haplotype A5, A9 and A63 clade A (Fig 2) and found in two localities: 48 (68.57%) of these infected lice were from Algiers and 22 (31.43%) from Tizi Ouzou (Table 2).

Coxiella burnetii DNA was found in 10 of the 524 body lice collected (1.90%) from 2/19 (10.52%) of the homeless individuals in Algiers (Table 2) and belonged to the A5 worldwide haplotype (Fig 2). These results were also confirmed by qPCR targeting two specific genes for *C. burnetii*, supplemented by amplification and sequencing of one spacer for genotyping *C. burnetii*. We only succeeded in obtaining sequences for the Cox22 spacer, probably due to the low concentration of *C. burnetii* DNA in these body lice samples.

The DNA of *Anaplasma* spp. was found in 22/524 (4.19%) body lice collected from three homeless individuals using qPCR targeting the TtAna (23S ribosomal RNA) specific gene. Conventional PCR and sequencing targeting a 525-bps fragment of the *rpoB* specific gene succeeded in only 4 of the 22 samples that were positive in qPCR. This could be due to the lower sensitivity of standard PCR compared to qPCR. The portion of the *rpoB* gene amplified was of poor quality, probably due to existence of several genotypes, but when BLASTed, it matched with *Anaplasma phagocytophilum*. We therefore tested these samples by qPCR specific to *A. phagocytophilum* targeting *apaG* gene as described previously [50]. Four samples were found to be positive for *A. phagocytophilum*, all positive lice were collected from the same homeless person from Algiers, and all belonged to the worldwide A5 haplotype.

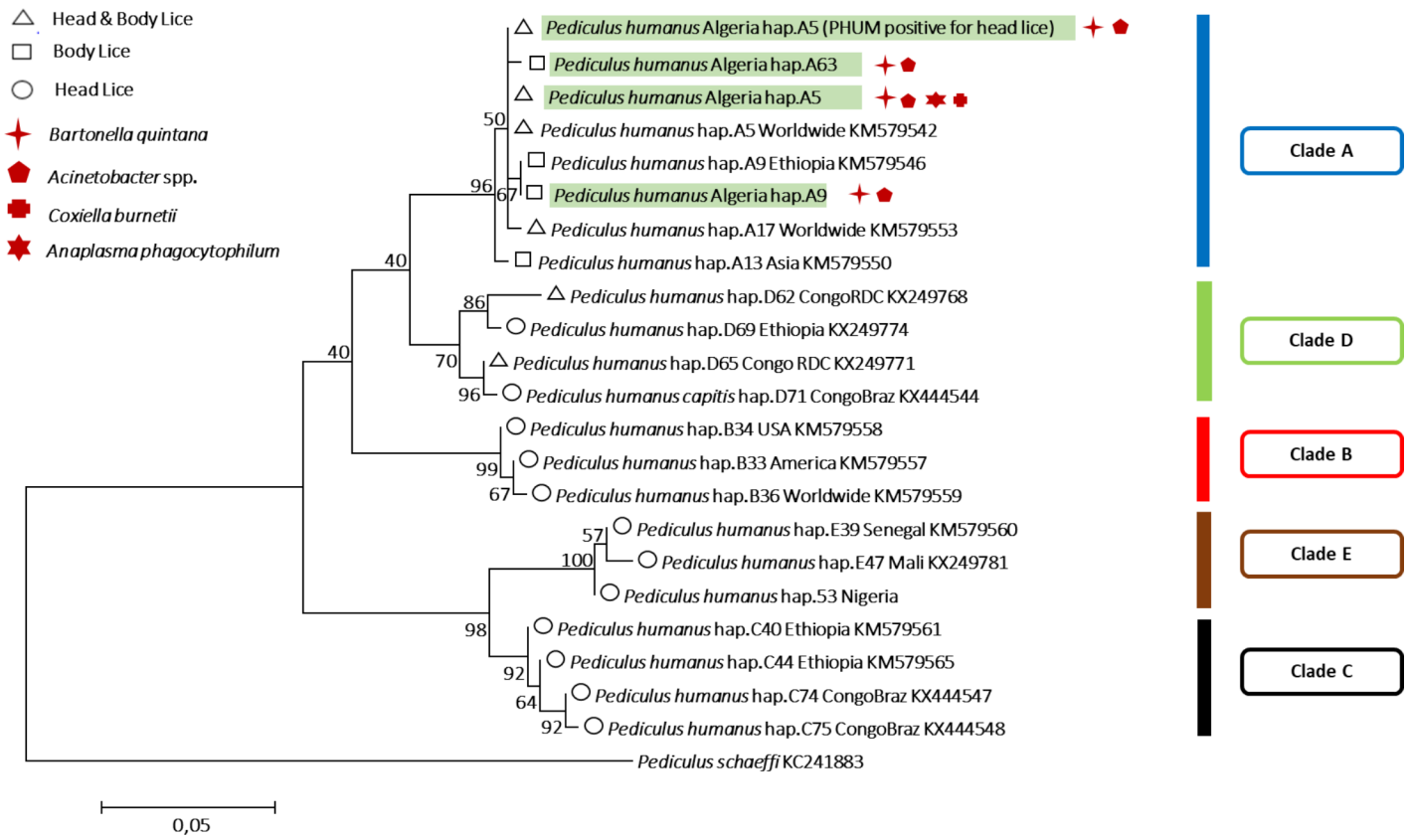

Fig 2. Phylogenetic tree showing the relationship of haplotypes identified in this study with other *Pediculus humanus* haplotypes. Phylogenetic inferences were conducted in MEGA 6 using the maximum likelihood method based on the Kimura 2-parameter. The GenBank accession numbers are indicated at the end. The mitochondrial clade memberships are indicated to the right of each tree. Specimens analyzed in this study are in green. **A)** Bacterial DNAs detected in body lice reported in this study. **B)** Lice samples positive for *B. quintana*, *C. burnetii*, *A. phagocytophilum* and *Acinetobacter* spp. and there are specified genotypes.

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The *Acinetobacter* spp. DNA was detected in 246/524 (46.94%) body lice collected from 25/44 (56.81%) homeless people. These positive lice included the 60 lice selected for phylogenetic analysis and which belonged to the three haplotypes found in the study. One hundred and two of these infected lice were from Algiers, 96 from Tizi Ouzou, and 48 from Boumerdes (Table 2). For molecular identification of the *Acinetobacter* species, we succeeded in amplifying a 350-bps fragment of the *rpoB* gene only in 190 of the 246 that were positive in qPCR for *Acinetobacter* spp. Based on a BLAST search, comparison of the nucleotide sequences with the GenBank database sequences revealed the existence of five species of *Acinetobacter* sharing 99–100% identity with their corresponding references. The *Acinetobacter* species identified were *A. baumannii* (83/190; 43.68%), *A. johnsonii* (46/190; 24.21%), *A. berezeniae* (27/190; 14.21%), *A. nosocomialis* and *A. variabilis* (18/190; 9.40% for both) (Fig 3).

The DNA of none of the pathogens tested, except *A. baumannii*, was identified from the five lice with the head louse genotype based on PHUM540560 gene analysis.

Body lice blood-meal analysis

The bacteria found in this study (*C. burnetii*, *A. phagocytophilum* and *Acinetobacter* spp.) are usually not associated with human body lice, so we used additional tools to confirm that the amplified microorganisms were really associated with engorged human lice.

Table 2. Pathogenic agents detected from infested homeless population in three localities, northern Algeria.

Bacterial pathogen	Algiers		Tizi Ouzou		Boumerdès		Total*	
	Persons N = 19	Body lice N = 235	Persons N = 16	Body lice N = 184	Persons N = 9	Body Lice N = 115	Persons N = 44	Body Lice N = 524
Bartonella quintana	17	48	13	22	0	0	30 (68.18%)	70 (13.35%)
Coxiella burnetii	2	10	0	0	0	0	2 (10.52%)	10 (1.90%)
***Acinetobacter* spp.**	12	102	7	96	6	48	25 (56.81%)	246 (46.94%)
Anaplasma phagocytophilum	1	4	0	0	0	0	1 (2.27%)	4 (0.76%)
***Rickettsia* spp.**	-	-	-	-	-	-	-	-
***Borrelia* spp.**	-	-	-	-	-	-	-	-
Yersinia pestis	-	-	-	-	-	-	-	-
Total*	17/19 (89.47)	122/235 (51.91%)	13/16 (81.47%)	74/184 (40.21%)	6/9 (66.66%)	48/115 (41.73%)	42 (95.45%)	317 (60.49%)

(-): no presence of pathogen, **N**: number

*: Total persons infested with infected lice, and total of infected lice

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Blood-meal sources were successfully identified by DNA sequencing based on the vertebrate vCO1 gene from 30 of the body lice specimens analyzed which were positive for at least one pathogen tested. Thus, the 30 obtained sequences were compared with homologous sequences deposited in the GenBank using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and, as expected, all specimens showed 100% identity with the vCO1of *Homo sapiens*.

Discussion

In this study, we report the first molecular data on human body lice, *P. h. humanus*, infesting the homeless population in Algeria, northern Africa.

The 524 body lice samples collected were analyzed using clade-specific qPCR, which showed that all the samples belong to clade A. Genotyping of 60 body lice reveals the presence of three haplotypes belonging to clade A: haplotype A5 was the most prevalent (56%) followed by haplotype A69 (26.66%), which is a novel haplotype characterized in this study and, finally, haplotype A9 (23.33%). A research study conducted on Algerian body lice has reported that they belong to sub-clade A2, which is the main clade in sub-Saharan Africa [37]. As expected, our results confirm that clade A has worldwide distribution, as reported by previous studies [11,18,19,51], and indicate a low mtDNA diversity among the body lice studied, unlike head lice which have been identified as having a high mtDNA diversification [16].

Five of the 30 (16.66%) lice tested showed a head lice-specific profile in the PHUM540560 gene. These lice were collected from the same patient in Algiers and belonged to the A5 haplotype, proving that, in conditions of massive infestation, head lice can change ecotype and migrate from the scalp to colonize clothing. A study has shown that the opposite is true, whereby body lice can migrate and colonize the hair [11].

Bartonella quintana is the most common re-emerging louse-borne pathogen associated with humans dating back over 4,000 years [52]. It is the causative agent of trench fever, an infection that was common in France during Napoleon's Russian war but also during World Wars I and II [53]. In addition to trench fever, this bacterium is responsible for a range of clinical manifestations in humans, including asymptomatic chronic bacteremia, endocarditis, and bacillary angiomatosis [10,22]. For a long time, body lice were considered as the principal natural vector for the transmission of *B. quintana* in humans [22,43]. However, in recent years, *B.*

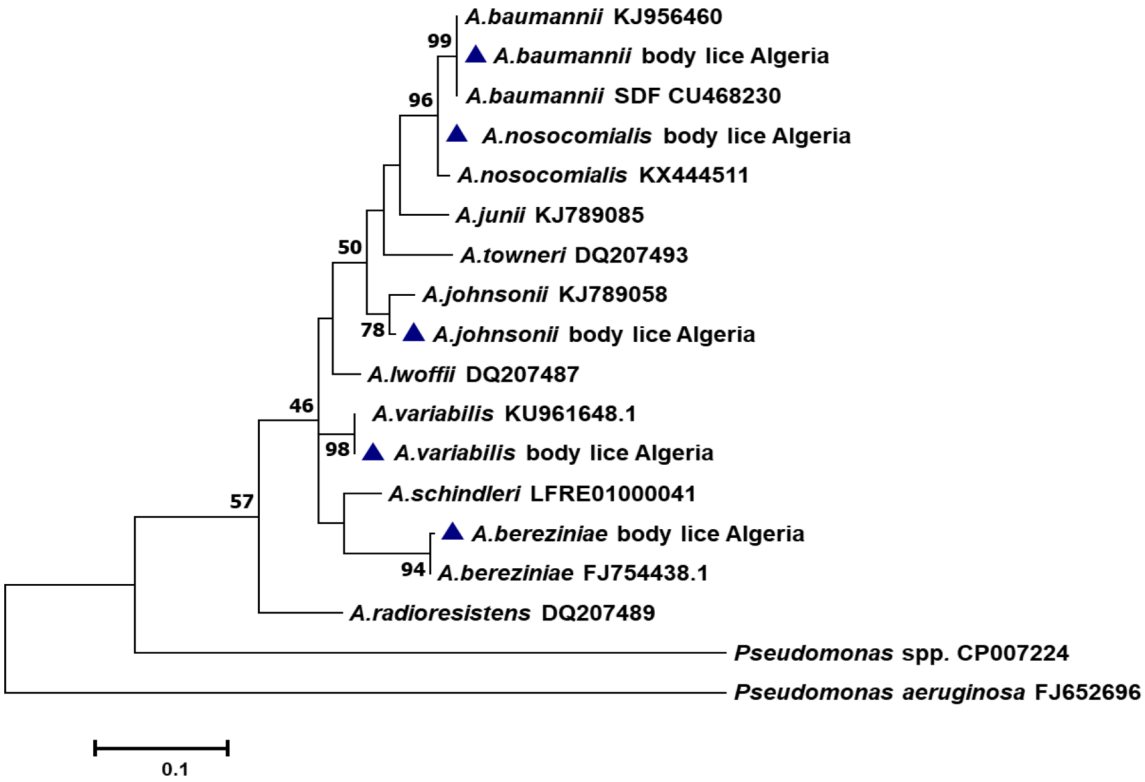

Fig 3. Phylogenetic tree highlighting the position of the *Acinetobacter* species identified in body lice compared to another Acinetobacter available in the GenBank database. Phylogenetic inferences were conducted in MEGA 7 using the maximum likelihood method based on the Kimura 3-parameter model for nucleotide sequences. GenBank accession numbers are indicated at the end. Statistical support for internal branches of the tree was evaluated by bootstrapping with 1,000 iterations.

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quintana-DNA has been detected in head lice worldwide, usually in people infested with both head and body lice [25,54,55], as well as those with head lice and no body lice infestation [56–58].

Bartonella quintana is regarded as a re-emerging pathogen in poor countries, as well as in the homeless population living in precarious and overcrowding conditions from the United States, France, the Netherlands, Russia, Japan, Ethiopia, and Mexico [25]. The prevalence of body lice infestation is 7%-22% of the homeless population worldwide, with 2%-30% for *B. quintana* infection [59,60]. In north Africa, notably in Algeria, it is reported that the bacterium is the principal common cause of infective endocarditis, in addition to *Brucella melitensis*, and *C. burnetii* [60,61]. Two studies conducted by Sangaré *et al*. and Fournier *et al*. failed to detect this bacterium in human lice collected in Algeria [37,62]. In this study, we report the presence of *B. quintana* in 70 of 524 (13.35%) body lice analyzed. All the positive lice were collected from homeless people living in the two localities, Algiers and Tizi Ouzou (Table 2). No positive samples were found in Boumerdès. This finding suggests a local occurrence for each of these pathogens. All *B. quintana* positive body lice belong to all clade A-haplotypes found in this study (Fig 2).

Coxiella burnetii is the causative agent of Q fever, a highly infectious zoonotic intracellular bacterium. It is found worldwide and has a diverse multi-host range: mammals, birds, reptiles, and arthropods, mainly ticks [63]. In humans, the infection is usually contracted through aerosol inhalation and can be acute or chronic exhibiting a wide range of clinical manifestations

[63,64]. Q fever has been reported throughout the African continent as a significant public health threat with a higher prevalence in western Africa, principally in Senegal [63].

In East Africa, although human lice are not a known to be a vector of *C. burnetii*, studies showed that lice collected from individuals living in formerly epidemic areas of Q fever can be infected with this bacteria [41]. Most recently, a research study showed for the first time that 1% of 600 clade E head lice infesting 5% of 117 individuals from Mali were positive *for C. burnetii* [42]. In contrast, in Ethiopia, a molecular study conducted on head and body lice showed no evidence of *C. burnetii* in all 98 louse pools tested [65].

In Algeria, only two human cases of Q fever have been reported and documented in Oran [66]. Regarding prevalence of *C. burnetii* in animals, a study reported that *C. burnetii* DNA was identified in the spleens of 1/117 (0.85%) dogs and 1/107 (0.93%) cats from Algiers [67]. DNA of this bacterium was also identified in 3/19 (15.8%) *Ixodes vespertilionis* from bats in the north-east of Algeria [68]. Recently, a study revealed a high seroprevalence of *C. burnetii* infection in camel populations in south-eastern Algeria, providing strong evidence that Q fever represents a public health and veterinary concern in Algeria [69].

In our study, the DNA of *C. burnetii* was detected in 10 of the 524 (1.90%) body lice infesting two (4.54%) of 44 homeless individuals. The positive lice were from Algiers. To the best of our knowledge, this is the first molecular evidence of the presence of *C. burnetii* DNA in body lice infesting homeless indigenous populations in Algeria. Under experimental conditions, infection with *C. burnetii* through body lice remains possible [70]. Based on our results from Algeria, combined with data from the literature, the role of human lice in the epidemiology of Q fever should be further investigated.

The family of *Anaplasmataceae* comprises, among others, the genera of *Anaplasma*, *Ehrlichia* and *Neorickettsia*. The *Anaplasma* genus is a worldwide tick-borne pathogen, and several species of vector-borne *Anaplasmataceae* are emerging pathogens associated with human and animal infection [71]. Surprisingly, Amanzougaghene *et al*. have reported, for the first time, the detection of DNA of two potential new species of *Anaplasma* and *Ehrlichia* genera of unknown pathogenicity in head lice collected from two rural villages in Mali. The DNA of a potential new *Anaplasma* species was detected in 1.58% of 600 head lice collected from two persons. BLAST analysis of the *rpoB* gene showed that this *Anaplasma* sp. was significantly different from all previously reported *Anaplasma* species and that the closest related species is *A. phagocytophilum* with 83% similarity [42]. In this work, to the best of our knowledge, we detect for the first time the DNA of *A. phagocytophilum* in four of the 524 (0.76%) body lice collected from one homeless person in Algiers. *A. phagocytophilum* is the agent of an emerging tick (*Ixodes* spp.) transmitted disease, which is the causative agent of human granulocytic anaplasmosis [72]. In Africa, this agent has not been completely studied, notably in Algeria where *A. phagocytophilum* has been reported from cattle [73] and serologically from dogs [74]. *A. phagocytophilum* was detected in *I. persulcatus* ticks collected in neighboring Morocco and Tunisia [75]. However, further field and experimental studies are required to clarify and determine the significance of our findings.

In this study, we also assessed the body lice collected for the presence of *Acinetobacter* species. Findings from several studies on head lice collected from elementary school children in Algeria and Thailand and from pygmyies' population in the Republic of the Congo, has shown a widespread infection of head lice with several species of *Acinetobacter* including *A. baumannii*, *A. junii*, *A. ursingii*, *A. johnsonii*, *A. schindleri*, *A. lwoffii*, *A. nosocomialis*, *A. towneri* and *A. variabilis* [16,20,34]. Recent studies have shown that *A. baumannii* infection can be highly prevalent among body lice [20]. It was firstly isolated from body lice from homeless people in France and, subsequently, the bacterium was detected in 21% of body lice collected worldwide [26]. *A. baumannii* was also detected in 71% of body lice collected from healthy individuals in

Ethiopia [35], however, the acquisition of lice for these infections is still unknown. Studies revealed that the infections occur either after lice-ingestion of an infective blood meal from bacteraemic patients, or from superficial contamination through human skin while feeding [26].

Furthermore, experimental studies have demonstrated that the human body louse is able to acquire and maintain a long-persistent life infection with *A. baumannii* and *A. lwoffii* in experimental conditions on bacteremic rabbits [27]. Further studies comparing two sequenced genomes of *A. baumannii* have shown that the *A. baumannii* homeless strain from the body louse had several hundred insertion sequence elements which have played a major role in its genome reduction, compared to the human multidrug-resistant *A. baumannii* (AYE strain), and also showed that it has a low catabolic capacity, suggesting a specific adaptation of this strain to the louse environment [76].

Our sampling showed, for the first time, the existence of four species of *Acinetobacter* spp. in human body lice. In addition to *A. baumannii*, other species such *A. johnsonii* (24.21%), *A. berezeniae* (14.21%), *A. nosocomialis* and *A. variabilis* (9.40%) were identified. As a result, it appears that the diversity of the *Acinetobacter* species is not specific to the head louse, and that body lice can also be infected by a widespread infection with several species of this genera, suggesting that body lice could be a host for these bacteria. The *Acinetobacter* species are widespread in nature, including in water, the soil, living organisms and the skin of patients and healthy subjects [76]. However, it still not clear whether these *Acinetobacter* strains present in lice are the same as those responsible for human infections [35].

Furthermore, molecular evidence for the presence of DNA of these pathogenic bacteria: *C. burnetii*, *A. phagocytophilum* and several *Acinetobacter* species cannot distinguish between pathogens accidentally acquired from the blood of infected individuals and those established in a competent vector which can maintain and transmit the pathogen. Previous studies showed that the bacteria have the capacity to survive in the midgut of lice [22], or in the phagocytes of body lice [77]. Further field studies as well as experimental studies are required to clarify the role of body lice in harboring or transmitting these pathogens.

The present study provides for the first time the presence of several emerging bacterial pathogens in body lice collected from homeless people in three different localities in northern Algeria. We identified the presence of the dangerous human pathogens *B. quintana* and *C. burnetii*, the causative agents of trench fever and Q fever, respectively. Findings from this study also show, for the first time, the presence of DNA of *A. phagocytophilum* and the widespread infection of body lice with several species of *Acinetobacter* in our samples.

Epidemiological investigations and surveys of louse-associated infections are needed in Algeria to define the public health consequences of these emerging louse-associated pathogens detection.

This finding highlights the fact that the body lice may have the ability and ubiquity to be much broader vectors of several pathogenic agents than previously thought. Further study of louse-borne pathogens would be needed for a better understanding of lice specificity to different pathogenic bacteria.

Author Contributions

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