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ORIGINAL ARTICLE

Molecular Survey of Head and Body Lice, *Pediculus humanus*, in France

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Abstract

Human lice, *Pediculus humanus,* are obligate blood-sucking parasites. Phylogenetically, they belong to several mitochondrial clades exhibiting some geographic differences. Currently, the body louse is the only recognized disease vector, with the head louse being proposed as an additional vector. In this article, we study the genetic diversity of head and body lice collected from Bobigny, a town located close to Paris (France), and look for louse-borne pathogens. By amplifying and sequencing the *cytb* gene, we confirmed the presence of clades A and B in France. Besides, by amplifying and sequencing both *cytb* and *cox1* gene, we reported, for the first time, the presence of clade E, which has thus far only been found in lice from West Africa. DNA from *Bartonella quintana* was detected in 16.7% of body lice from homeless individuals, but in none of the head lice collected from 47 families. *Acinetobacter* DNA was detected in 11.5% of head lice belonging to all three clades and 29.1% of body lice. Six species of *Acinetobacter* were identified, including two potential new ones. *Acinetobacter baumannii* was the most prevalent, followed by *Candidatus* Acinetobacter Bobigny-1, *Acinetobacter calcoaceticus, Acinetobacter nosocomialis*, *Acinetobacter junii*, and *Candidatus* Acinetobacter Bobigny-2. Body lice were found to be infected only with *A. baumannii*. These findings show for the first time, the presence of clade E head lice in France. This study is also the first to report the presence of DNAs of several species of *Acinetobacter* in human head lice in France.

Keywords: *Acinetobacter* spp., *Bartonella quintana,* clade E, France, *Pediculus humanus*

Introduction

Sucking lice (*Phthiraptera: Anoplura*) are obligate blood-feeding ectoparasites of placental mammals, including humans (Chosidow 2000, Light et al. 2008b). Two recognized genera parasitize humans: *Pthirus* and *Pediculus*. Each genus includes one species affecting humans, *Pthirus pubis* (the crab louse) and *Pediculus humanus* (Chosidow 2000, Veracx and Raoult 2012). The latter is of great concern to public health and includes two ecotypes: head lice and body lice. Both ecotypes have the same life cycle and need to take regular blood meals (approximately five times per day), on human skin to survive a life span of about 4–12 weeks (Veracx and Raoult 2012). However, they occupy distinct ecological niches. Head lice live in the scalp region of humans, where the females lay eggs at the base of hair shafts (Light et al. 2008b). They have a global distribution and affect individuals of broad economic and social status, particularly school-aged children, regardless of living conditions (Chosidow 2000, Izri et al. 2010, Brouqui 2011). Due to reaction to the bites, they can cause an intense pruritus that may lead to irritation and infection (Veracx and Raoult 2012). In contrast, body lice live in clothing and multiply when cold, promiscuity, and lack of hygiene are present (Badiaga and Brouqui 2012, Veracx and Raoult 2012). They are often found in jails and in unstable countries, but are also currently reemerging among homeless populations in developed countries (Raoult et al. 1997, Brouqui 2011, Badiaga and Brouqui 2012, Veracx and Raoult 2012).

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Mitochondrial genes (*cytb* and *COI*) appear to separate lice into five divergent mitochondrial clades (A, B, C, D, and E) exhibiting some geographic differences (Ashfaq et al. 2015, Drali et al. 2015, Amanzougaghene et al. 2016b). Head lice encompass all clades, while body lice belong only to clades A and D (Ashfaq et al. 2015, Drali et al. 2015, Amanzougaghene et al. 2016b). The clade A is the most common and is found around the world, while clade D is only found in the Republic of Congo and Congo Brazaville (Light et al. 2008a, Ashfaq et al. 2015, Drali et al. 2015, Amanzougaghene et al. 2016a). Clade B is found in America, Europe, Australia, and North and South Africa, and was most recently reported in Israel, on head lice remains dated ~2000 years old (Light et al. 2008a, Amanzougaghene et al. 2016b). Clade C has been found in Ethiopia, the Republic of Congo, and in Asia (Amanzougaghene et al. 2016a). Last, clade E consists of head lice from West Africa (Senegal and Mali) (Amanzougaghene et al. 2016b).

Body lice are potentially more harmful than head lice, because of their role as a vector of at least three pathogenic bacteria that have killed millions of people: *Rickettsia prowazekii* (the causative agent of epidemic typhus), *Bartonella quintana* (trench fever), and *Borrelia recurrentis* (relapsing fever) (Raoult and Roux 1999, Veracx and Raoult 2012). Natural and experimental observations have been made that body lice can also transmit *Yersinia pestis*, the causative agent of plague, and that they may be the pandemic vectors of this agent (Blanc and Baltazard 1942, Houhamdi et al. 2006). Some other widespread pathogenic bacteria, such as *Serratia marcescens*, *Acinetobacter baumannii*, and *Acinetobacter lwoffii*, have been detected in human body lice, with the assumption that lice may probably also transmit these agents to humans (La Scola et al. 2001, Houhamdi and Raoult 2006).

Although body lice are currently assumed to be more potent vectors of pathogens, the vector potential of head lice is not yet fully understood. Studies have demonstrated that the immune reactions of head lice to different pathogens are stronger than those of body lice, which obviously may carry a broad spectrum of pathogens (Previte et al. 2014, Kim et al. 2017). In laboratory-reared lice, it has been demonstrated that head lice can support a persistent load of *B. quintana* for several days following acquisition in a bloodmeal (Previte et al. 2014). An experimental infection with *R. prowazekii* has also shown that head lice can be readily infected and disseminate these pathogens in their feces, showing that these lice might be a vector of pathogens under optimal epidemiologic conditions (Robinson et al. 2003). Indeed, a substantial number of studies have reported body louse-borne pathogens on head lice collected from different parts of the world. This is the case of *B. quintana, B. recurrentis*, and *Y. pestis* DNA, found in head lice belonging to different mitochondrial clades (Angelakis et al. 2011a, 2011b, Boutellis et al. 2012, 2013, Drali et al. 2015, Amanzougaghene et al. 2016a). Several *Acinetobacter* species have also been detected in human head lice (Sunantaraporn et al. 2015, Amanzougaghene et al. 2016a).

In this study, we examined the genetic diversity of head and body lice collected from Bobigny, a town located 3 km north of Paris (France) (48°54′38″N 2°26′23″E), to look for louse-borne pathogens in these lice.

Materials and Methods

Ethical clearance

The protocol was reviewed and approved by the *Comité de Protection des Personnes* (institutional review board) of the CPP-Ile-de-France X (2017-02) Ethics Committee. Informed consent was obtained from all patients.

Study area and lice sampling

Between September 2015 and December 2016, head lice were collected from patients attending the Avicenne Hospital in Bobigny. One hundred forty-one patients belonging to 47 families were enrolled in this study, and 5 head lice were randomly selected per parasitized family (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/vbz). Body lice were obtained from two homeless individuals hospitalized in the same facility. In all, 16 and 8 body lice were collected, from patients 1 and 2, respectively. After collection, lice were immediately frozen at −80°C and were then transported to the laboratory of Marseille. In total, 235 head lice and 24 body lice were processed for molecular study.

DNA extraction

Before DNA isolation and to avoid external contamination, the surface of each louse was decontaminated as described previously (La Scola et al. 2001). DNA was extracted using the QIAamp DNA tissue extraction kit (Qiagen, Hilden, Germany) in an EZ1 apparatus following the manufacturer's instructions.

Genotypic status of lice

Identification of louse mitochondrial clade by qPCR assays. To identify the mitochondrial clades of the lice, all DNA samples were analyzed using clade-specific quantitative real-time PCR (qPCR) assays that targeted a portion of *cytochrome b* (*cytb*) gene specific to clades A, D, B, and C, as previously described (Amanzougaghene et al. 2016a). It is important to note that, when the design of the qPCR specific to clade C was performed, clade E was classified as subclade within clade C; therefore, this qPCR detects both clades C and E (Amanzougaghene et al. 2016a). To discriminate between them, we performed another qPCR essay, specific only to clade E (Amanzougaghene et al. 2017). We used lice with known clades as positive controls and master mixtures as a negative control for each test.

PCR amplifications and sequencing. For phylogenetic study, 79 (79/235) head lice and 5 (5/24) body lice were randomly selected and subjected to standard PCR, targeting a 347-bp fragment of the cytb gene as previously described (Li et al. 2010). To confirm the presence of clade E, sixteen lice already identified as clade E by the previous PCR were subjected to another standard PCR, targeting another mitochondrial gene, cytochrome oxidase subunit 1 (cox1), as previously described (Drali et al. 2016).

PCR amplification was performed in a Peltier PTC-200 model thermal cycler (MJ Research, Inc., Watertown, USA). The reactions were carried out using the Hotstar Taq

polymerase (Qiagen), in accordance with the manufacturer's instructions. Purification of PCR products was performed using NucleoFast 96 PCR plates (Macherey-Nagel EURL, 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 were assembled and edited using ChromasPro software (ChromasPro 1.7; Technelysium Pty Ltd., Tewantin, Australia).

Table 1. Oligonucleotide Sequences of Primers and Probes Used for Real-Time PCRs and Conventional PCRs in This Study

Target	*Name*	*Primers (5′-3′) and probes*	*Source*
Pediculus humanus *Cytochrome b*	Duplex A–D	F_GATGTAAATAGAGGGTGGTT R_GAAATTCCTGAAAATCAAAC FAM-CATTCTTGTCTACGTTCATA TTTGG-TAMRA VIC-TATTCTTGTCTACGTTCATGTTT GA-TAMRA	Amanzougaghene et al. (2016a)
	Duplex B–C/E	F_TTAGAGCGMTTRTTTACCC R_AYAAACACACAAAAMCTCCT FAM-GAGCTGGATAGTGATAAGGTTT AT-MGB VIC-CTTGCCGTTTATTTTGTTGGGGTT T-TAMRA	
	Monoplex E	GGTTGGAATTGGATAGTGAT GGGTCCATAAAGAAATCC G FAM- TAGGAGGCTTTGTGTGTCTATC CT-TAMRA	Amanzougaghene et al. (2017)
	Cytb	F_GAGCGACTGTAATTACTAATC R_CAACAAAATTATCCGGGTCC	Li et al. (2010)
Cytochrome oxidase subunit I	*Cox1*	F_TTAGGGGGTGGTGATCCTGT R_TGAGAGTGCATTCTTGCTGGT	Drali et al. (2016)
Acinetobacter spp. RNA polymerase β subunit gene	*rpoB*	F_TACTCATATACCGAAAAGAAACGG R_GGYTTACCAAGRCTATACTCAAC FAM-CGCGAAGATATCGGTCTSCAAG C-TAMRA	Bouvresse et al. (2011)
	rpoB (zone1)	F_TAYCGYAAAGAYTTGAAAGAAG R_CMACACCYTTGTTMCCRTGA	La Scola et al. (2006)
Rickettsia prowazekii *rOmpB* gene	*ompB*	F_AATGCTCTTGCAGCTGGTTCT R_TCGAGTGCTAATATTTTTGAAGCA FAM-CGGTGGTGTTAATGCTGCGTTA CAACA-TAMRA	Nguyen-Hieu et al. (2010)
Yersinia pestis Plasminogen activator gene	PLA	F_ATGGAGCTTATACCGGAAAC R_GCGATACTGGCCTGCAAG FAM-TCCCGAAAGGAGTGCGGGTAA TAGG-TAMRA	Nguyen-Hieu et al. (2010)
Borrelia spp. *16S ribosomal RNA*	Bor16S	F_AGCCTTTAAAGCTTCGCTTGTAG R_GCCTCCCGTAGGAGTCTGG FAM-CCGGCCTGAGAGGGTGAACG G-TAMRA	Parola et al. (2011)
Bartonella quintana Hypothetical intracellular effector	yopP	F_TAAACCTCGGGGGAAGCAGA R_TTTCGTCCTCAACCCCATCA FAM-CGTTGCCGACAAGACGTCCTT G-TAMRA	Angelakis et al. (2011a)
3-oxoacyl-synthase gene	fabF3	F_GCGGCCTTGCTCTTGATGA R_GCTACTCTGCGTGCCTTGGA FAM-TGCAGCAGGTGGAGAGAACG TG-TAMRA	
Anaplasma spp. 23S ribosomal RNA	TtAna	F_TGACAGCGTACCTTTTGCAT R_TGGAGGACCGAACCTGTTAC FAM-GGATTAGACCCGAAACCAA G-TAMRA	Dahmani et al. (2017)
Coxiella burnetii Spacers IS1111	IS1111	F_CAAGAAACGTATCGCTGTGGC R_CACAGAGCCACCGTATGAATC FAM-CCGAGTTCGAAACAATGAGGG CTG-TAMRA	Mediannikov et al. (2010)

Molecular screening for the presence of pathogen DNA

The qPCRs were performed to screen all lice samples, using previously reported primers and probes, for *Acinetobacter* spp., *Borrelia* spp., *B. quintana*, *Acinetobacter* spp., *R. prowazekii*, *Y. pestis*, *Coxiella burnetii*, and *Anaplasma* spp. (Mediannikov et al. 2010, Nguyen-Hieu et al. 2010, Angelakis et al. 2011a, Bouvresse et al. 2011, Parola et al. 2011, Dahmani et al. 2017). All *B. quintana*-positive samples were confirmed by a second specific qPCR targeting the *fabF3* gene (Angelakis et al. 2011a). All sequences of primers and probes used for qPCRs and conventional PCRs in this study are shown 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 controls and master mixtures as negative control for each test.

To identify the species of *Acinetobacter* spp., all positive samples from qPCRs were subjected to standard PCR, targeting a portion of the *rpoB* gene as described previously (La Scola et al. 2006). Amplicons were prepared and sequenced using similar methods as described for the *cytb* gene for lice above.

Data analysis

For the head and body lice *cytb* and *-cox1* sequences, unique haplotypes were defined using DnaSPv5.10 and compared with the *cytb* and *cox1* haplotypes as described previously (Amanzougaghene et al. 2016a, Drali et al. 2016). All sequences of *Acinetobacter* species were analyzed using BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) and compared against sequences in the GenBank database. A maximum-likelihood method was used to infer the phylogenetic analyses, and tree reconstruction was performed using MEGA software version 6.06 (Tamura et al. 2013).

Results

Lice clade and phylogenetic analysis

Of the 141 patients (47 families) infested by head lice, the majority were female (sex ratio M/F = 0.1) aged between 2 and 53 years. No body lice were found.

In total, 235 head lice collected from the 47 families and 24 body lice collected from 2 homeless persons were analyzed using qPCRs to determine their clade. The result showed that 82 lice (31.8%, [82/258]) belonged to clade A, 42 (16.3%, [42/258]) to clade B, and 134 (51.9%, [134/258]) to clade E. All the body lice were clade A, while the head lice belonged to all three clades (A, B, and E).

The analysis of 84 cytb sequences yielded 44 variable positions defining 18 different haplotypes. Two haplotypes belonged to the worldwide haplotypes, A5 (14 head lice sequences and 5 body lice sequences) and A17 (5 head lice sequences), within clade A. Three haplotypes, all from head lice, also belonging to clade A, were novel and are named here as A60–A62. Within clade B, two haplotypes were found, one belonged to the B36 haplotype, the most widespread and prevalent in the B haplogroup, the second was novel and is referred to here as B39. The remaining 11 haplotypes belonged to clade E, all were novel, and they are named here as E56–E67 (Table 2). The phylogenetic position of these haplotypes is shown in Figure 1.

The analysis of *-cox1* sequences from 16 head lice belonged to clade E, yielded 6 variable positions defining 4 different haplotypes; all were novel named here as E40–E43 (Table 3). Phylogenetic tree (Fig. 2) showed that all these haplotypes clustered with haplotypes from Mali within clade E, confirming the identity of this clade.

Molecular detection of bacterial pathogens

In this study, the qPCR investigation of all lice samples for *Rickettsia* spp., *R. prowazekii*, *Borrelia* spp., *Y. pestis*, *C. burnetii*, and *Anaplasma* spp. produced no positive results. However, we obtained positive results when testing for the presence of *B. quintana* and *Acinetobacter* spp.

B. quintana DNA was found in 4 of 24 (16.66%) body lice collected from the same homeless patient. The patient was a 35-year-old man originally from Pakistan, who was hospitalized with an unexplained fever at the time of the sample collection. No head lice were found to be infected with *B. quintana*.

Acinetobacter DNA was detected by qPCR targeting the *rpoB* gene in 27 of 235 head lice (11.5%) collected from 8 of 47 families and in 7 of 8 body lice collected from the homeless patient 2, which represented a total of 29.1% (7/24) of all body lice tested. Among these 7 body lice *Acinetobacter*-DNA positive, 3 lice were also co-infected with *B. quintana*. Conventional PCR and sequencing targeting a 350-bp fragment of the same gene was successful only in 23 of the 34 samples that were positive in qPCR. This may be due to the lower sensitivity of standard PCR compared to qPCR.

Based on a BLAST search, seven sequences were identified as *A. baumannii*, two sequences as *Acinetobacter nosocomialis*, one sequence as *Acinetobacter junii*, and three

Table 2. Haplotype Frequency of Head and Body Lice Identified in Bobigny, France, Based on *cytb* Gene

Haplotype	*Head lice*	*Body lice*	*Total*	*Acc. no.*
A5	14	5	19	KM579542
A17	5	0	5	KM579553
A60	6	0	6	MF672001
A61	3	0	1	MF672002
A62	1	0	1	MF672003
B36	22	0	22	KM579559
B39	4	0	4	MF672004
E56	9	0	9	MF672005
E57	3	0	3	MF672006
E58	1	0	1	MF672007
E59	7	0	7	MF672008
E60	1	0	1	MF672009
E61	1	0	1	MF672010
E63	4	0	4	MG759552
E64	2	0	2	MG759553
E65	1	0	1	MG759554
E66	1	0	1	MG759555
E67	1	0	1	MG759556
Total	79	5	84	

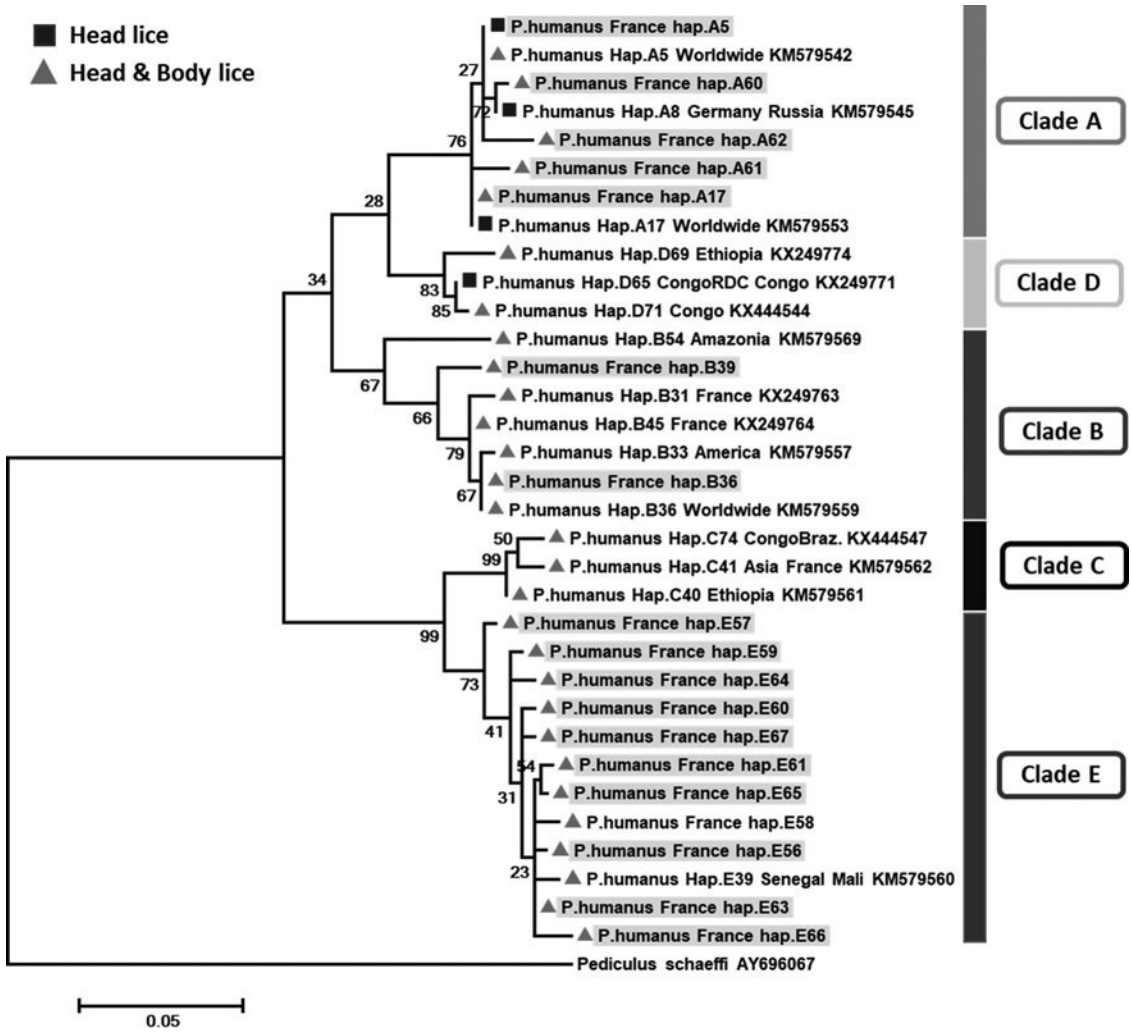

FIG. 1. Phylogenetic tree showing the relationship of haplotypes identified in this study with other *Pediculus humanus* haplotypes. The *cytb* sequences were aligned using CLUSTALW and phylogenetic inferences were conducted in MEGA 6 using the maximum likelihood method, based on the Kimura 2-parameter. Statistical support for internal branches of the trees was evaluated by bootstrapping with 500 iterations. There was a total of 270 positions in the final dataset. The scale bar represents a 5% nucleotide sequence divergence.

sequences as *Acinetobacter calcoaceticus*, sharing 99–100% identity with their corresponding reference *Acinetobacter* species.

For 5 of the 23 sequences, BLAST analysis showed a homology score of under 95%, meaning that these sequences are likely to correspond to new species, provisionally referred to here as *Candidatus* Acinetobacter Bobigny-1 and *Candidatus* Acinetobacter Bobigny-2. The most closely related species are *Acinetobacter johnsonii* (GenBank number CP010350) for *Candidatus* Acinetobacter Bobigny-1 with 94% similarity (314 of 334 base positions in common), and *Acinetobacter venetianus* (GenBank no. LSVC01000004) for Acinetobacter Bobigny-2 with 91.9% similarity (307 of 334 base positions in common). The phylogenetic position of these *Acinetobacter* are shown in Figure 3. Interestingly, *Candidatus* Acinetobacter Bobigny-1 was identified in four clade E head lice collected from the same family, while *Candidatus Acinetobacter* Bobigny-2 was found in one clade B head louse from another family.

Table 3. Haplotype Frequency of Head Lice Clade E Identified in Bobigny, France, Based on *Cox*1 Gene

Haplotype	*Head lice*	*Acc. no.*
E40	3	MG759563
E41	1	MG759564
E42	4	MG759565
E43	8	MG759566
Total	16	

The remaining 5 of 23 (21.7%) sequences, which were also rated, resembled *Acinetobacter*, but were of poor quality, which is assumed to be due to co-infection with several *Acinetobacter* species. The distribution of these species according to lice ecotypes and clades are presented in Table 4. The partial *rpoB* sequences obtained in this study

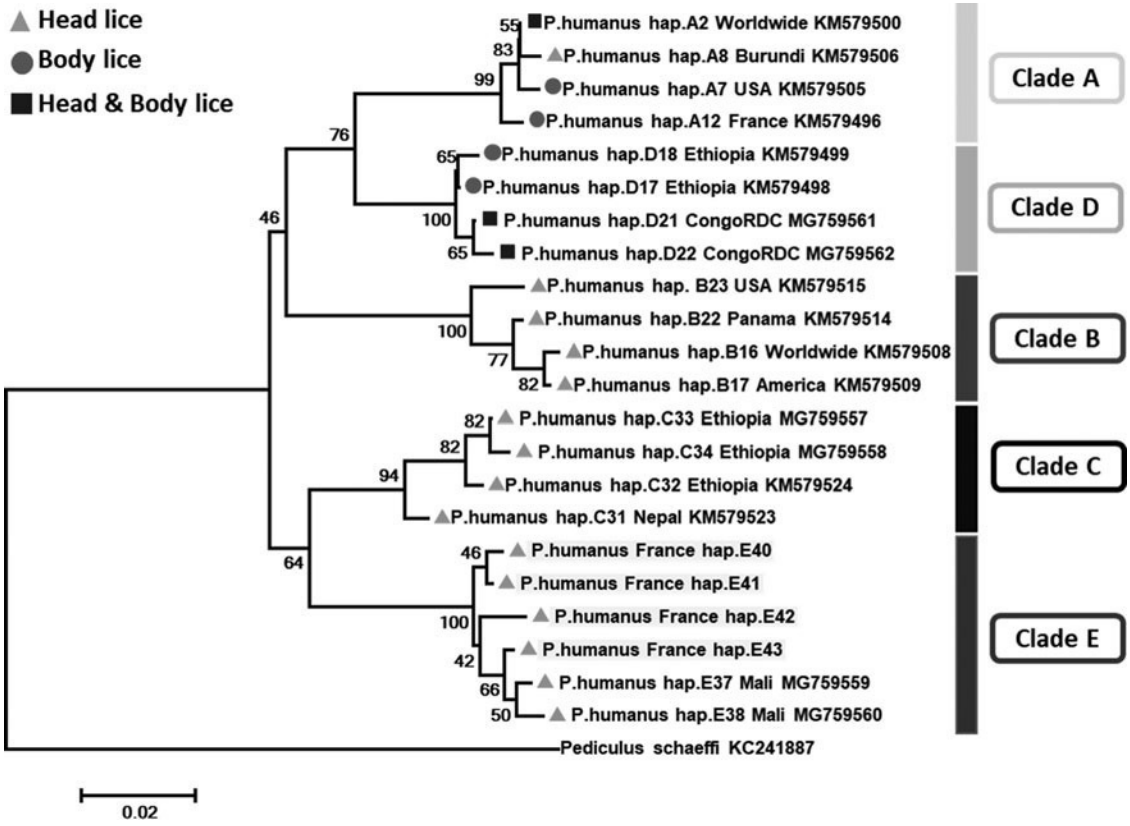

FIG. 2. Phylogenetic tree showing the relationship of haplotypes identified in this study with other *P. humanus* haplotypes. The *Cox1* sequences were aligned using CLUSTALW and phylogenetic inferences were conducted in MEGA 6 using the maximum likelihood method based on the Kimura 2-parameter. Statistical support for internal branches of the trees was evaluated by bootstrapping with 500 iterations. There was a total of 283 positions in the final dataset. The scale bar represents a 2% nucleotide sequence divergence.

were deposited in the GenBank under the accession no.: MF672011-MF672020.

Discussion

Louse infestation remains a social and public health concern around the world in the 21st century (Bonilla et al. 2013). In this study, the patients affected by pediculosis capitis were mainly female (sex ratio M/F = 0.1). During an epidemiological investigation in the same area, 70% (402/574) of infested children were girls and the median age was 8.9 years (Bouvresse et al. 2012).

The mtDNA analysis of the 235 head and 24 body lice showed the presence of three major haplogroups: A, B, and E. Haplogroup E was the most prevalent (51.9%, [134/258]), followed by haplogroups A (31.8%, [82/258]) and B (16.3%, [42/258]). All the body lice belonged to clade A. These data confirm the existence of clade A and B in France, as reported by others (Light et al. 2008a, Drali et al. 2015, Amanzougaghene et al. 2016b). Previous studies reported that clade E was limited to West African countries, namely Senegal and Mali (Ashfaq et al. 2015, Amanzougaghene et al. 2016b, 2017). This is the first report of clade E found in head lice in France, which may be explained by the intercontinental migration flow. Furthermore, clade C lice that were previously only observed in Africa and Asia have also recently been found in head lice collected in Paris (Drali et al., unpublished data). Our sampling failed to find this clade among the head lice tested, possibly due to the limited size and region of louse sampling.

B. quintana infection is the most common reemerging louse-borne disease in homeless populations living in poor hygiene conditions in developed countries, where it is responsible for a range of clinical manifestations in humans, including asymptomatic chronic bacteremia, endocarditis, and bacillary angiomatosis (Raoult and Roux 1999, Brouqui 2011, Badiaga and Brouqui 2012). In this study, we found *B. quintana* DNA in four of 24 body lice collected from one of the hospitalized homeless patients, consistent with the standard view that body lice are the major natural vector for this pathogen, which is among the most prevalent parasitic infestations in the homeless population (Raoult and Roux 1999, Badiaga et al. 2008). Epidemiologic studies of homeless populations have reported the prevalence of 7–22% for body louse infestations and 2–30% for *B. quintana* infections (Badiaga et al. 2008).

In recent decades, *B. quintana* DNA has also been detected in head lice collected from poor and homeless persons in the United States, Nepal, Senegal, Ethiopia, the Democratic Republic of the Congo, and France (Sasaki et al. 2006, Bonilla et al. 2009, Angelakis et al. 2011a, 2011b, Boutellis et al. 2012, Cutler et al. 2012, Drali et al. 2014, 2015). Conversely, all attempts to detect *B. quintana* in head lice collected from schoolchildren living in family households have failed (Fournier et al. 2002, Bouvresse et al. 2011, Sunantaraporn et al. 2015). Taken together, these results suggest that head lice

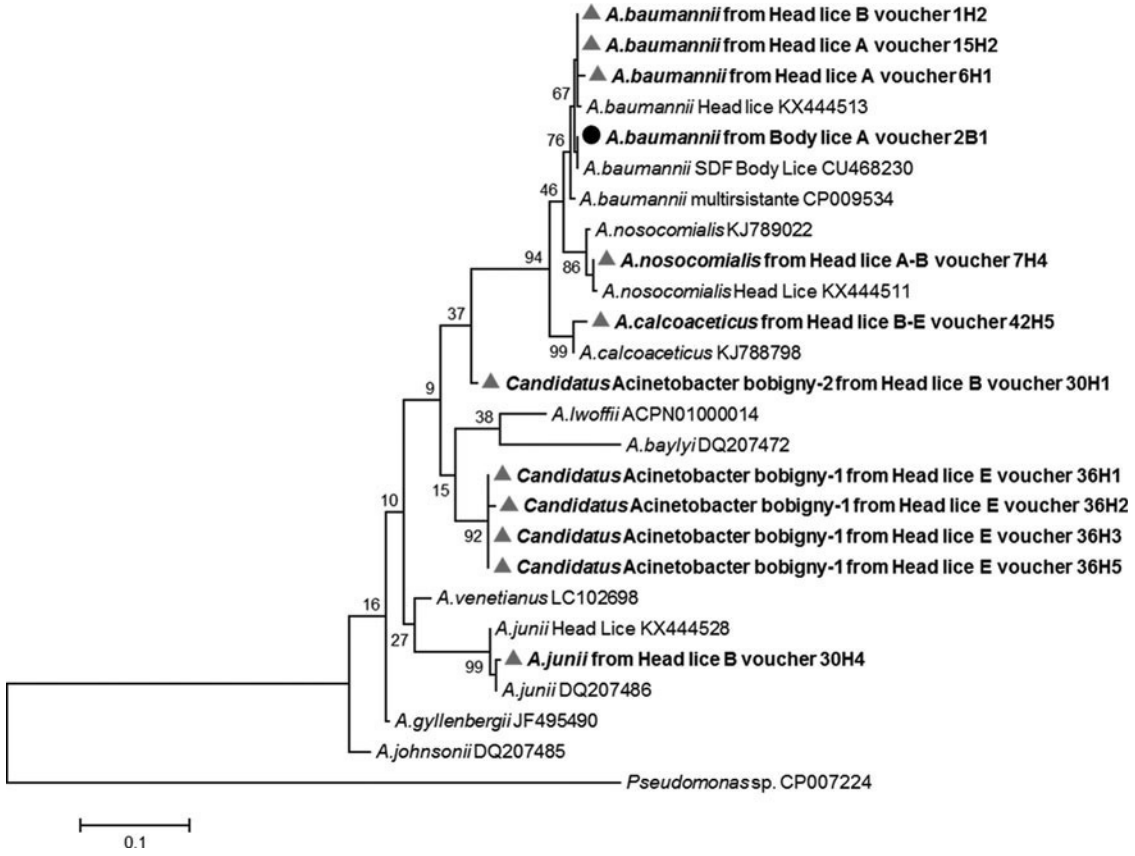

FIG. 3. Phylogenetic tree highlighting the position of *Acinetobacter* species identified in head and body lice compared to another Acinetobacter available in the GenBank database. The *rpoB* sequences were aligned using CLUSTALW, and phylogenetic inferences were conducted in MEGA 6 using the maximum likelihood method based on the Kimura 3-parameter model for nucleotide sequences. The GenBank accession numbers are indicated at the end. Statistical support for internal branches of the trees was evaluated by bootstrapping with 1000 iterations. There was a total of 345 positions in the final dataset. The scale bar represents a 10% nucleotide sequence divergence.

Table 4. Summary of the Bacterial Species Detected in Head and Body Lice Collected from Infested Individuals in Bobigny Per Lice Ecotype and Clade

Bacterial species	*Head or body lice H/B (no.)*	*Clade of lice (no.)*	*Total (%)*
Bartonella quintana	B	A	4 (1.5)
Acinetobacter baumannii	B (4) and H (3)	A (6) and B (1)	7 (2.7)
Acinetobacter nosocomialis	H	B (1) and A (1)	2 (0.7)
Acinetobacter junii	H	B	1 (0.4)
Acinetobacter calcoaceticus	H	B (1) and 2 (E)	3 (1.15)
Candidatus Acinetobacter bobigny-1	H	E	4 (1.5)
Candidatus Acinetobacter bobigny-2	H	B	1 (0.4)
Acinetobacter spp.	H	A, B, and E	5 (1.9)

probably can also transmit *B. quintana* if people live close together in poor sanitary conditions and if they lack medical treatment. This view is supported by our finding, as we were unable to detect the bacterium in any of the head lice collected from the 47 middle-class suburban families in the study.

In this study, we also assessed our collected lice for the presence of *Acinetobacter* species. More attention is now paid to extrahospital reservoirs of these ubiquitous opportunistic bacteria and their potential involvement in emerging human community-acquired infections, as pan drug-resistant strains are increasingly being identified worldwide (Eveillard et al. 2013).

Recent works have shown the *Acinetobacter* infection to be highly prevalent among body and head lice (La Scola and Raoult 2004, Bouvresse et al. 2011, Amanzougaghene et al. 2016a). However, it is still unknown how lice acquire these infections. Some authors argue that infections could occur after the ingestion of an infected blood meal from patients with ongoing bacteremia (La Scola and Raoult 2004) or possibly by passage through the human skin while feeding (Kempf et al. 2012). Furthermore, an experimental study showed that the human body louse, feeding on bacteremic rabbits, can acquire and support a persistent life-long infection with *A. baumannii* and *A. lwoffii* (Houhamdi and Raoult 2006). Another study compared two sequenced genomes of *A. baumannii* and showed that the *A. baumannii* homeless strain

from the body louse had several hundred insertion sequence elements, which played a crucial role in its genome reduction compared to the human multidrug-resistant *A. baumannii* AYE strain, and which have also been shown to have low catabolic capacities, suggesting the specific adaptation of this strain to the louse environment (Vallenet et al. 2008).

Our results showed the presence of *Acinetobacter* DNA in 11.5% of head lice and 29.1% of body lice. Six species were identified, including two potential new ones. *A. baumannii* was the most prevalent, followed by *Candidatus* Acinetobacter Bobigny-1, *A. calcoaceticus, A. nosocomialis*, *A. junii*, and *Candidatus* Acinetobacter Bobigny-2. Body lice were found to be infected only by *A. baumannii*, while the head lice belonging to all three clades were found to be infected by at last one of all six species. As a result, it appears that *Acinetobacter* lice infection is not specific to a particular louse clade, but probably to louse ecotypes, as body lice were found to be infected only by *A. baumannii*. This hypothesis is supported by the detection of *A. baumannii* in 21% of body lice collected worldwide (La Scola and Raoult 2004). In contrast, studies performed on head lice collected from elementary school children in Thailand and from pygmy population in the Republic of Congo, showed the presence of the DNA of several *Acinetobacter* species, in 3.62% of head lice belonging to both clade A and C, and 37.3% of head lice belonging to clades A, D, and C (Sunantaraporn et al. 2015, Amanzougaghene et al. 2016a), respectively. In these two studies, the *Acinetobacter* species identified were *A. junii*, *A. ursingii, A. baumannii, A. johnsonii, A. schandleri, A. lwoffii, A. nosocomialis, A. towneri*, and *A. radioresistens* (Sunantaraporn et al. 2015, Amanzougaghene et al. 2016a). When comparing the panel of *Acinetobacter* species found in all these studies with our findings, *A. ursingii, A. johnsonii, A. schandleri, A. lwoffii, A. towneri*, and *A. radioresistens* were not identified in our head lice collection. Conversely, our sampling showed, for the first time, the presence of the DNA of *A. calcoaceticus* and two potentially new species in human head lice.

These studies together demonstrate the widespread infection of human lice with several species of *Acinetobacter,* suggesting that lice could be a preferential host for these bacteria. However, it remains to be determined whether these *Acinetobacter* strains present in lice are the same as those that are responsible for human infections.

In conclusion, we confirmed that head lice from Bobigny (France) belong to haplogroups A and B, and reported, for the first time, the presence of haplogroup E, which is specific to West Africa, reflecting the heterogeneous communities found in the studied area. We detected *B. quintana* only in body lice from homeless individuals, but not from head lice collected from the 47 middle-class suburban families. Several *Acinetobacter* species were also detected, including two potentially new ones, indicating that lice could be a source for *Acinetobacter* spp. infections in humans.

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Authors' Contributions

Conceived and designed the experiments: O.M., F.F., and A.I.. Collected samples: K.C., A.I., and S.B. Conducted the experiments: K.C., N.A., O.M., and F.F. Analyzed the data: K.C., N.A., and O.M., and F.F. Wrote the article: N.A. K.C., O.M., F.F. A.I., R.D., M.L., and D.R.

Author Disclosure Statement

No competing financial interests exist.

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