

Molecular Characterization and Genetic Diversity of Clade E-Human Head Lice from Guinea

Alissa Hammoud¹, Meriem Louni^{2,3*}, Mamadou Cellou Baldé⁴, Abdoul Habib Beavogui^{5,6}, Philippe Gautret², Didier Raoult¹, Florence Fenollar², Dorothée Misse⁷, Oleg Mediannikov^{1*}

¹Aix Marseille Univ, IRD, APHM, MEPHI, IHU-Méditerranée Infection, Marseille, France.

²Aix Marseille Univ, IRD, APHM, VITROME, IHU-Méditerranée Infection, Marseille, France.

³Faculté des Sciences, Université M'Hamed Bougara Boumerdes, Boumerdès, Algérie.

⁴Institut de Recherche en Biologie Appliquée de Guinée (IRBAG), Kindia, Guinée

⁵Centre de Formation et de Recherche en Santé Rurale de Mafèrinyah B.P. 2649, Conakry, Guinée.

⁶Faculté de Médecine, Pharmacie et Odontostomatologie, Université Gamal Abdel Nasser de Conakry, Guinée.

⁷Maladies Infectieuses Et Vecteurs : Écologie, Génétique, Evolution Et Contrôle, MIVEGEC UMR 224, Univ Montpellier, IRD, CNRS, Montpellier, France

*Correspondence: louni_meriem@yahoo.fr and oleguss1@gmail.com

Abstract

Pediculus humanus capitis, the head louse, is an obligate blood-sucking ectoparasite that occurs in six divergent mitochondrial haplogroups (A, D, B, F, C and E), each exhibiting a particular geographic distribution. A few years ago, several studies reported the presence of different pathogenic agents in head lice specimens from different clades collected worldwide. These findings suggest that head louse could be a vector for dangerous diseases and therefore a serious public health problem. Herein, we aimed to study the mitochondrial genetic diversity, the PHUM540560 gene polymorphisms profile of head lice collected in Guinea, as well as to screen for the pathogens present in these lice.

In 2018, a total of 155 head lice were collected from 49 individuals at the Medical Centers of rural (Maférinyah village) and urban (Kindia city) areas, in Guinea. All head lice were subjected to genetic analysis and screened for the presence of several pathogens using molecular tools. The results showed that all head lice belonged to the haplogroups C/E using the duplex qPCR which detects both clades. Standard PCR and sequencing revealed that all specimens belonged to the haplogroup E, including 8 haplotypes, whither 6 new identified for the first time in this study. The study of the PHUM540560 gene polymorphisms in our Guinean head lice revealed that 7/40 (17.5%) of our tested samples exhibit three different polymorphism profiles compared to the clade A-head lice PHUM540560 gene profile, while the remaining specimens 33/40 (82,5%) showed the same PHUM540560 gene polymorphism profile as the previously reported clade A-body lice. Molecular investigations of the targeted pathogens revealed only the presence of *Acinetobacter* species in 9% of our samples using real time PCR. Sequencing results identified highlighted the presence of several *Acinetobacter* species, including *Acinetobacter baumannii* (14.3%), *Acinetobacter nosocomialis* (14.3%), *Acinetobacter variabilis* (14.3%), *Acinetobacter*

haemolyticus (7.2%), *Acinetobacter towneri* (7.2%). Furthermore, a candidate new species of *Acinetobacter* sp. (7.2%) was detected. Positive specimens were collected from 24,5% individuals in Maférinyah. We also investigated in our study the carbapenem's-resistant profile of *A. baumannii*, none of our specimens were positive for the following resistance genes $\text{bla}_{\text{OXA-21}}$, $\text{bla}_{\text{OXA-24}}$ and $\text{bla}_{\text{OXA-58}}$.

To the best of our knowledge, our study is the first to report the existence of the Guinean haplogroup E, the PHUM540560 gene polymorphism profile as well as the presence of *Acinetobacter* species in head lice collected from Guinea.

Keywords: Head lice, haplogroup E, PHUM540560 gene, *Acinetobacter haemolyticus*, *Acinetobacter* spp., Guinea.

1. Introduction

Sucking lice (*Phthiraptera: Anoplura*) are obligate ectoparasites that have co-specified with their hosts for at least 77 million years [1]. Humans are colonized by two genera of lice, *Pediculus* and *Phthirus*, that have been strictly feeding on human blood for nearly 5.6 million years [2]. *Pediculus* genus includes: human (*Homo sapiens*) lice, *Pediculus humanus*; chimpanzees (*Pan troglodytes*) lice *P. schaeffi*; and New World monkeys (*Platyrrhini*) lice, *P. mjobergi* [3]. While *Phthirus* genus includes *Phthirus pubis*, parasite of humans and *Pt. gorilla*, parasite of gorillas (*Gorilla*) [2,4]. The *Pediculus humanus* includes two ecotypes and is a great public health concern: the *Pediculus humanus humanus* (body louse), which lives in clothing and infests people living in poverty and lack of hygiene. *Pediculus humanus capitis* (head louse), lives in the scalp area, with a worldwide distribution regardless of hygienic conditions [5]. Aside from their role as pests, human lice can be a significant health hazard [3]. Indeed, body lice are the main vector of various serious human pathogens, including *Rickettsia prowazekii*, *Bartonella quintana*, *Borrelia recurrentis* and probably *Yersinia pestis*. These agents respectively cause epidemic typhus, trench fever, relapsing fever and plague [3,6]. Although it is assumed that body lice are a more potent vector for pathogens, the role of head lice as pathogens-vector is still debated and misunderstood [7]. This can be explained by the fact that head lice proceed to a rapid elimination of ingested bacteria, caused by a stronger immune response and therefore a weaker vectorial capacity compared to body lice [8, 9]. However, under experimental conditions, the acquisition and the maintenance of *R. prowazekii* and *B. quintana* are reported in head lice [7,10]. Moreover, epidemiological studies have strongly implicated head lice as a vector of infectious pathogens under favorable epidemiological conditions [11]. Various studies have reported that head lice collected worldwide show the presence of DNA from several pathogenic

bacteria including : *B. quintana*, *B. recurrentis*, *Borrelia theileri*, *Y. pestis*, *Coxiella burnetii*, *Rickettsia aeschlimannii*, *Acinetobacter* spp., as well as potential new species of the genera *Moraxella*, *Psychrobacter*, *Ehrlichia* and *Anaplasma* [12–30]. These data highlight the fact that head lice can harbor pathogenic bacteria and probably even transmit them to their human hosts. It is therefore obvious that it is important to prevent potential epidemics related to these ectoparasites.

Robust phylogenetic studies of human lice based on mitochondrial DNA, mainly *cytochrome b* [*cytb*] and *cytochrome oxidase subunit 1* [*cox1*] genes, have inferred *Pediculus humanus* into six divergent mitochondrial clades (haplogroups): A, D, C, E, B and F, each with distinct geographical distribution [5,14,31–33]. Human lice also present an intra-clade diversity in addition to their inter-clade diversity, which is illustrated by several distinct haplotypes for each haplogroup [21,31,34]. Unlike body lice, which only belong to clades A and D, head lice encompass all the genetic diverse clades [18]. Clade A has a global continental distribution and it is the most prevalent [31,34], while clade D is restricted to sub-Saharan African countries, and has so far been reported in the Democratic Republic of Congo (DRC), the Republic of Congo (Congo-Brazzaville), Ethiopia and Zimbabwe [14,21,31]. Clade C has been identified mainly in African and Asian countries, including Ethiopia, Republic of Congo, Nepal, Pakistan and Thailand [18,21,24]. The sister group of clade C, the clade E, has specific distribution to West Africa where it has been reported with high prevalence of lice in Senegal, Mali [18], Nigerian migrants' refugees' communities in Algeria [26] and from migrant communities living in Bobigny, France [25]. Recently, two studies reported for the first time the presence of clade E in Central Africa. A new haplotype that has never been described in clade E has been isolated in Congo. While the Malian clade E haplotype, was highly detected in head lice from Gabon [28,

29]. Clade B is found in a high diversification in America, it has been reported in Western Europe, Australia, North Algeria, South Africa, Saudi Arabia, and is also present among the remains of head lice from the Roman period dating back to about 2000 years [31,34–37]. Recently, a novel clade F, the sister group of clade B, was described in French Guiana, in head lice recovered from the Wayampi community living in a remote Trois-Sauts village. This clade was also found in Argentina and Mexico [33]. All these data confirm important facts about the evolutionary history of lice, as well as the ancestors of their human hosts since their migration out of Africa [38].

Despite their clade diversification and ecological niches, The *Pediculus humanus* ecotypes are morphologically and biologically almost similar [3,5]. Previous genetic studies targeting intergenic spacers, using a highly polymorphic markers were not able to differentiate between body and head lice [38, 39]. Moreover, a study based on the comparison of head and body lice transcriptomes, reported that the two ecotypes had a single 752-base pair (bp) difference in the Phum_PHUM540560 gene, with differential expression that encodes a hypothetical 69-amino acids protein of unknown function [40]. The PHUM540560 gene and 13 others were thought to be missing in head louse. However, a study conducted by Drali and collaborators, showed that the head louse also harbors this gene, but with a rearranged sequence compared to body louse. The variation of the Phum_PHUM540560 gene within the two ecotypes allowed the design and development of a novel molecular tool based on multiplex real-time PCR assays, in order to differentiate the Clade A body and head lice [41].

In Guinea, West Africa, human lice infestation is very frequent but never investigated. In this study, we aimed to identify for the first time in Guinea, the genetic diversity status of head lice collected from two sites: rural (Maférinyah village) and urban (Kindia city), the

Phum_PHUM540560 gene polymorphisms, as well as to assess the occurrence of bacterial pathogens in these lice.

2. Materials and Methods

2.1. *Lice collection and DNA extraction*

In December 2018, head lice collection was carried out at Medical Centers in two areas: Maférinyah (9.5466° N, 13.2866° W) and Kindia (10.0407° N, 12.8630° W) from Guinea in West Africa (Fig.1). All individuals in medical centers with a head lice infestation were asked to perform a complete self-examination for the presence of head and body lice. The medical center personnel obtained verbal consent from the participants and authorization from the head of the medical center to supervise the collection process.

A total of 155 head lice were obtained from 49 individuals (Mean age: 11 [2, 62], 96% female): 130 head lice specimens were collected from 38 individuals in Maférinyah (Mean age: 10 [2, 35], 100% female), and 25 head lice samples were collected from 11 individuals (Mean age: 16,2 [6, 62]; 81.9% female) in Kindia. No body lice were found during the examination. The collected head lice were stored in dry sterile conditions at room temperature and then transported to the laboratory of IHU- Méditerranée Infection, Marseille, France and stored at -20°C until molecular study.

In order to decontaminate the external surface and avoid bacterial contamination, each louse specimen was washed and decontaminated as previously described [42]. Dried louse specimen was cut in half lengthwise, the first half was frozen at -20 °C for subsequent studies. Total DNA was extracted from the remaining half using a DNA extraction kit, QIAamp Tissue Kit (Qiagen, Courtaboeuf, France) in the EZ1 apparatus following the manufacturer's instructions.

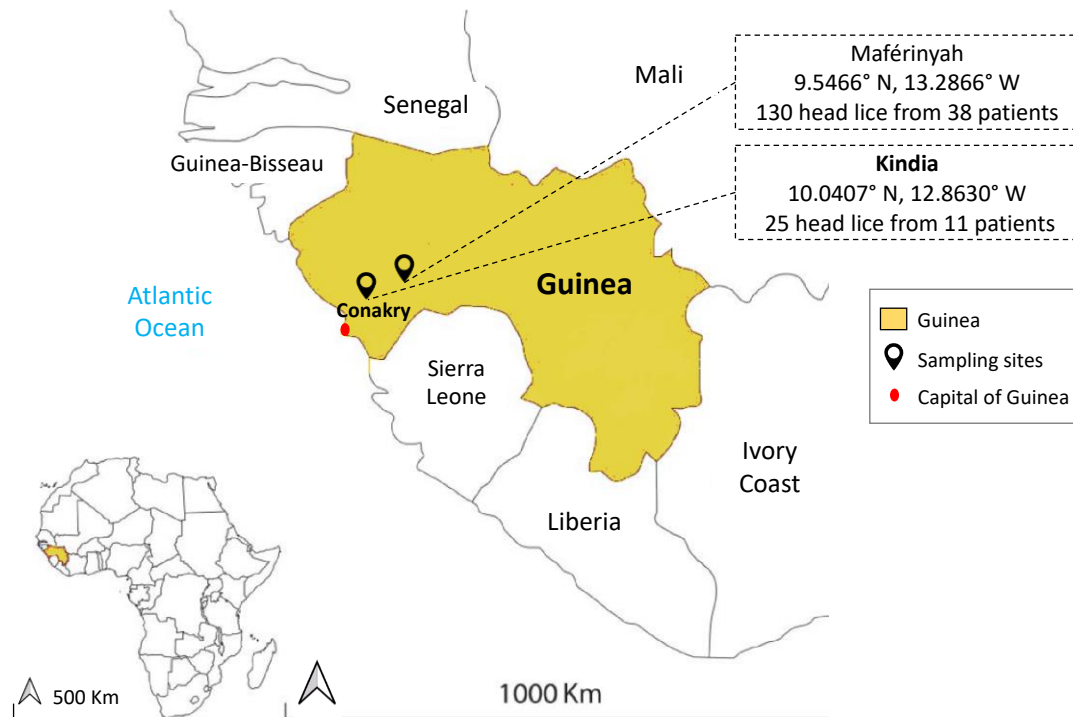


Figure 1. Map of head lice collection sites from infested individuals in two localities in Guinea.

2.2. Genotypic status of lice

2.2.1. Identification of louse mitochondrial haplogroup by qPCR assays

In order to identify the mitochondrial clades of the lice collected in this study, all DNA samples were analyzed by clade-specific quantitative duplex real time PCR (qPCR) assays targeting a portion of the *cytb* gene. Each duplex is specific to clades A-D and B-C as previously described, noting that the B-C duplex amplifies specimens belonging to clade E, classified as a sub-clade within clade C [18, 21]. Clades PCR identification was carried out using a CFX96 Real-Time system (Bio-Rad Laboratories, Foster City, CA, USA). The amplification of DNA was performed using the following parameters: one step of incubation at 50 °C for 2 min (for UDG activation), one step of 95 °C for 5 min for initial denaturation and 45 cycles of 5 seconds

at 95 °C and 30 seconds at 60 °C. The final reaction volume of 20 µl contained 10 µl of Eurogentec™ Probe PCR Master Mix (Eurogentec, Liege, Belgium), 0.5 µM of each primer, probe and water. To validate the qPCR run, lice with known clades were used as positive controls and master mixtures as negative controls for each test.

2.2.2. Identification of louse haplotype by conventional PCR assays and sequencing

In order to perform a phylogenetic study, all head lice specimens were subjected to standard PCR targeting a 347-bp fragment of the *cytb* gene as previously described [38]. PCR amplification was performed in a Peltier PTC- 200 model thermal cycler (MJ Research Inc, Watertown, MA, USA). The final volume PCR consisted of a 25 µl, including 12.5 µl Amplitaq gold master mixes, 0,5 µM of each primer, 5 µl DNA template, and water. The thermal cycling profile comprised an incubation step at 95 °C for 15 min, 40 cycles of 1 min each at 95 °C, 30 s at 56 °C and 1 min at 72 °C, followed by a final extension step of 5 min at 72 °C. The results of the amplification were then confirmed by electrophoresis in agarose gel. To proceed with the purification of the PCR products, a NucleoFast 96 PCR plates (Macherey-Nagel EURL, Hoerd, France) were used according to the manufacturer's instructions. The amplicons were then 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 then assembled and edited using the ChromasPro software (ChromasPro 1.7, Technelysium Pty Ltd., Tewantin, Australia) and compared with those available in GenBank database by NCBI BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

2.2.3. Sequences and phylogenetic diversity analysis

In order to assess clade diversity in human lice, the total Guinean head lice-*cytb* sequences obtained in this study were combined and compared with the worldwide *P. humanus-cytb* mitochondrial sequences previously reported (Supplementary data: Table S1). ClustalW alignments were performed using MEGA software version 6.06. Thereafter, the Maximum-likelihood (ML) analysis was also performed in MEGA6 using the Kimura 2-parameter model for nucleotide sequences under 1000 bootstrap replicates [43]. The *cytb* from *P. schaeffi* (AY696067) was used as an outgroup. A median-joining (MJ) network was also constructed in order to investigate the possible relationships between the haplotypes, using the method of Bandelt performed with NETWORK10.0.0 program (www.fluxus-engineering.com/sharenet.htm) [44].

2.3. Molecular investigation of lice ecotype

2.3.1. Louse ecotype investigation by multiplex qPCR assays

In this study, all lice specimens were collected from the scalp region of each individual; however, we were curious to further study our lice ecotypes. Therefore, all lice samples were analyzed by multiplex real-time PCR, targeting a portion of the PHUM540560 gene. This assay was developed to discriminate between body lice and head lice belonging to clade A [41], which has always been known as a worldwide clade that encompasses the two ecotypes [31,34]. This assay had never been used to study lice belonging to other clades. We used a clade A head and body louse as positive controls.

2.3.2. Louse ecotype investigation by conventional PCR assays and sequencing

To analyze the sequences of the PHUM540560 gene of the Guinean human lice, 40 specimens were randomly selected. Additionally, clade A-human lice were randomly selected from our lice collection, including 7 specimens of Orlando strain from our rabbit rearing colony

and 11 Algerian body lice collected from homeless people [45]. These samples were then subjected to standard PCR and sequencing of the target PHUM540560 gene. The sequences obtained were aligned with the specific PHUM540560 sequences of the clade A-body and head lice, described thorough the previous study [41]. Alignment was performed using the BioEdit v 7.0.5.3 software (available online: <http://en.bio-soft.net/format/BioEdit.html>), in order to reveal the rearranged sequences of the head lice PHUM540560 gene collected in Guinea compared with those of the clade A-human lice.

2.4. Screening for the presence of pathogen's DNA

2.4.1. Identification of pathogen's DNA by qPCR assays

In order to screen louse-borne pathogens, a qPCR was performed for all head lice samples targeting the presence of various pathogenic bacteria: *Rickettsia* spp., *Borrelia* spp., *B. quintana*, *Y. pestis*, *C. burnetii*, *Anaplasma* spp. and *Acinetobacter* spp., using specific primers and probes, as previously reported (Table 1).

Initially, we pooled our DNA's template into 8 samples of 10µl/specimen for each pool; each sample was in a proportion of 1 Log of its initial concentration. Modifications of the qPCR cycles threshold (Ct) from 40 Ct to 45 Ct were performed to ensure that no potentially positive samples were missing from each pool. Thereafter, each louse from each positive pool with a Ct ≤ 38, was tested individually. The qPCR analysis was performed as described above for the *cytb*, a positive control of the targeted DNA and a negative control of master mixtures were included in each PCR run. Samples positive for *Acinetobacter* spp. were subjected to qPCR specific for *Acinetobacter baumannii*, targeting the *OmpA/MotB* gene, as previously reported (Table 1).

2.4.2. Identification of pathogen's DNA by conventional PCR and sequencing

In order to identify the species of *Acinetobacter*, positive samples from qPCR were subjected to a standard PCR targeting a portion of the *rpoB* gene (zone1) using the primers and conditions previously described (Table 1). Successful amplification was confirmed by electrophoresis via agarose gel, amplicons were prepared and sequenced using similar methods as described above. Due to a low specificity of above-mentioned primers, a new specific PCR system was designed in this study in order to amplify only and specifically the DNA of *Acinetobacter* spp. targeting a different portion of the same *rpoB* gene. The Fasta-file was constructed from the sequences available in the GenBank database for all *Acinetobacter* species as well as for *Moraxella* spp. and *Psychrobacter* spp. Sequences were aligned using BioEdit v 7.0.5.3 software (available online: <http://en.bio-soft.net/format/BioEdit.html>) to reveal conserved areas as target regions for *Acinetobacter* spp. specific primers. This region was submitted in Primer3 software v. 0.4.0 (available online: <http://primer3.ut.ee/>) to determine candidate primers based on the criteria for the primer design. PCR primers settings were in accordance with the guidelines as previously described and as recommended by Invitrogen™ (available online: <https://www.thermofisher.com/fr/fr/home/brands/invitrogen.html>) and Applied Biosystems™ (available online: <https://www.thermofisher.com/fr/fr/home/brands/applied-biosystems.html>). Then, the melting temperatures of the primers were tested using the free online software Oligo Analyzer 3.1 (available online: <https://eu.idtdna.com/calc/analyzer>). Primers were then synthesized by Eurogentec (Liège, Belgium). PCR systems and their target gene are described in Table 1.

2.5. *Acinetobacter* resistance to carbapenem

To eventually investigate this paradigm in our study, all positive *A. baumannii*-Guinean head lice were subjected to qPCR analyzes targeted three carbapenem resistant encoding genes including bla_{OXA-23}, bla_{OXA-24} and bla_{OXA-58}, as previously described [51] (Table 1). Results were considered positive when the cycle threshold value of real-time PCR is ≤ 35 .

1

Table 1. Real time PCR and conventional PCR primers and probes used in this study.

Target	Name	Primers and probes (5'-3')	Source	
<i>Pediculus humanus</i>	Cytb. Duplex A/D	FAM-CATTCTTGTCTACGTTTCATATTTGG-TAMRA	[18]	
		VIC-TATTCTTGTCTACGTTTCATGTTTGA-TAMRA		
		F_ GATGTAAATAGAGGGTGGTT		
		R_ GAAATTCCTGAAAATCAAAC		
	Cytb. Duplex B/C-E	FAM-GAGCTGGATAGTGATAAGGTTTAT- TAMRA		
		VIC-CTTGCCGTTTATTTTGTGGGGTTT-TAMRA		
		F_ TTAGAGCGMTTRTTTACCC		
		R_ AYAAACACACAAAAMCTCCT		
	Cytb	F_ GAGCGACTGTAATTACTAATC		[38]
		R_ CAACAAAATTATCCGGGTCC		
	Phum54 0560	FAM-CGATCACTCGAGTGAATTGCCA-TAMRA		[41]
		VIC-CTCTTGAATCGACGACCATTGCT-TAMRA		
GTCACGTTTCGACAAATGTT				
<i>Rickettsia</i> spp. <i>citrate</i> <i>synthase (gltA)</i>	RKND03	TTTCTATAACCACGACACGATAAAT	[46]	
		FAM-CTATTATGCTTGCGGCTGTCGGTTC-TAMRA		
		F_GTGAATGAAAGATTACACTATTTAT		
<i>Borrelia</i> spp. <i>16S</i> <i>ribosomal RNA</i>	Bor16S	R_GTATCTTAGCAATCATTCTAATAGC	[47]	
		FAM-CCGGCCTGAGAGGGTGAACGG-TAMRA		
		F_AGCCTTTAAAGCTTCGCTTGTAG		
<i>Bartonella quintana</i>	yopP - Hypothetical	R_GCCTCCCGTAGGAGTCTGG	[48]	
		FAM-GCGCGCGCTTGATAAGCGTG-TAMRA		
		F_GATGCCGGGGAAGGTTTTTC		

	intracellular effector	R_GCCTGGGAGGACTTGAACCT		
<i>Yersinia pestis</i>	PLA	FAM-TCCCGAAAGGAGTGCGGGTAATAGG-TAMRA	[13]	
		F_ATG GAG CTT ATA CCG GAA AC		
		R_GCG ATA CTG GCC TGC AAG		
<i>Coxiella burnetii</i>	IS1111	FAM-CCGAGTTCGAAACAATGAGGGCTG-TAMRA	[49]	
		F_CGCTGACCTACAGAAATATGTCC		
		R_GGGGTAAGTAAATAATACCTTCTGG		
<i>Anaplasma</i> spp. 23S ribosomal RNA	TiAna	FAM-GGATTAGACCCGAAACCAAG-TAMRA	[50]	
		F_TGACAGCGTACCTTTTGCAT		
		R_TGGAGGACCGAACCTGTTAC		
<i>Acinetobacter</i> spp. RNA polymerase β subunit gene	rpoB	FAM-CGCGAAGATATCGGTCTSCAAGC-TAMR	[51]	
		F_TACTCATATACCGAAAAGAAACGG		
		R_GGYTTACCAAGRCTATACTCAAC		
	(zone1)	rpoB	F_TAYCGYAAAGAYTTGAAAGAAG	[52]
			R_CMACACCYTTGTTMCCRTGA	
			F_TACAARATCTTYGAAGAAGC	
(zone1)	rpoB	R_CCACAACADAGDTTGTARRA	This study	
<i>Acinetobacter baumannii</i> . Type VI secretion system <i>OmpA/MotB</i>	<i>OmpA/MotB</i>	FAM_AAGTCGCCAAGAAACCTTGA_TAMRA	[53]	
		F_TCAACATCACAATCTTTAGTAGCTGA		
		R_CGCTCTTGCCAGCATAAAGA		
Carbapenems genes	OXA-23	6-FAM-CCAGTCTATCAGGAAGTTGCGCGA-BHQ_1	[56]	
		F_GACACTAGGAGAAGCCATGAAG		
		R_CAGCATTACCGAAACCAATACG		
		TET-AGTAACACCCATTCCCCATCCACTTTT-IABkFQ		

OXA-24

F_GATGACCTTGCACATAACCG

R_ CAGTCAACCAACCTACCTGTG

OXA-58

Cy5-TGGACCAATACGACGTGCCAATTCT-IAbRQSp

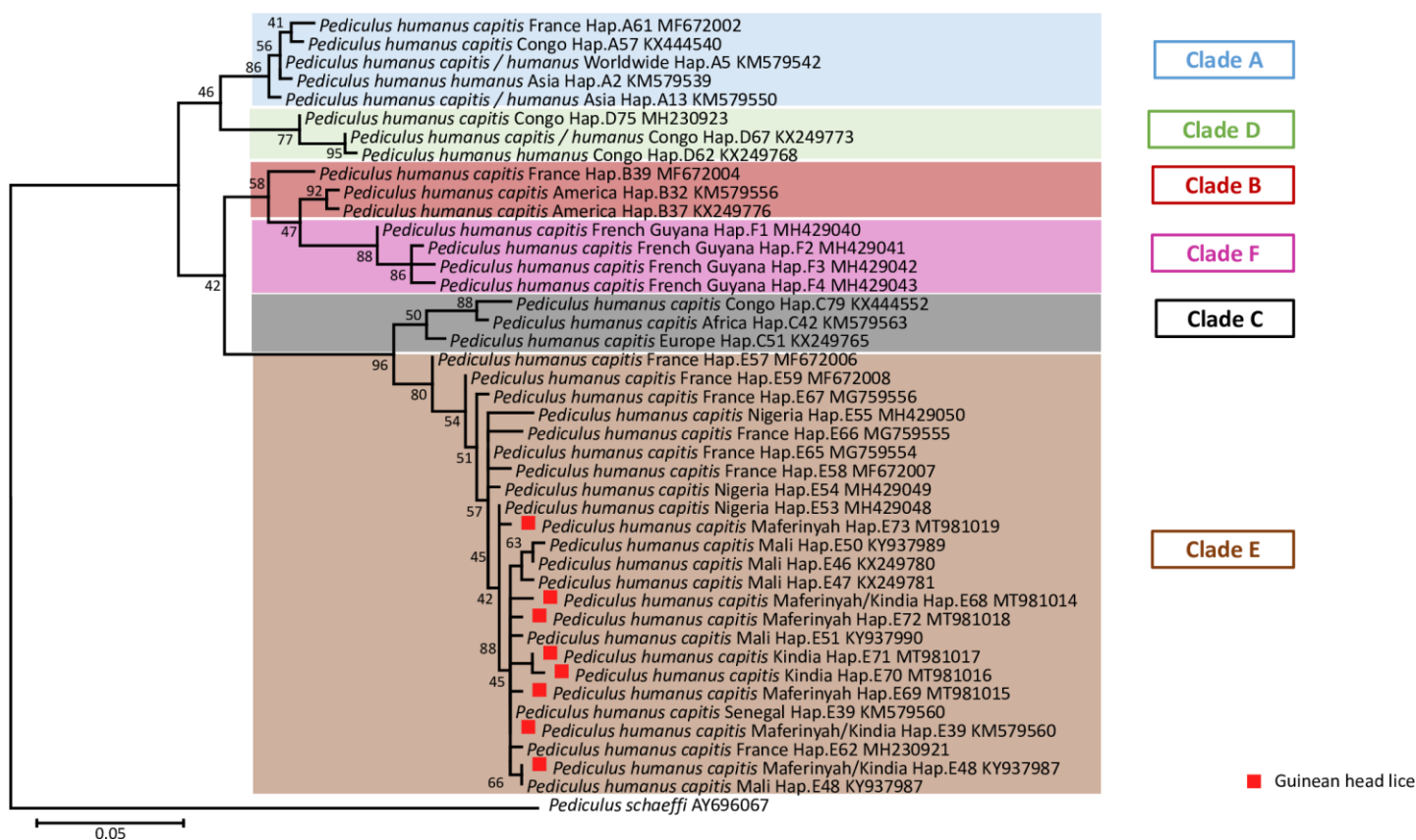
AAGATTTTACTTTGGGCGAAGC

CAACTTCCGTGCCTATTTGC

3. Results

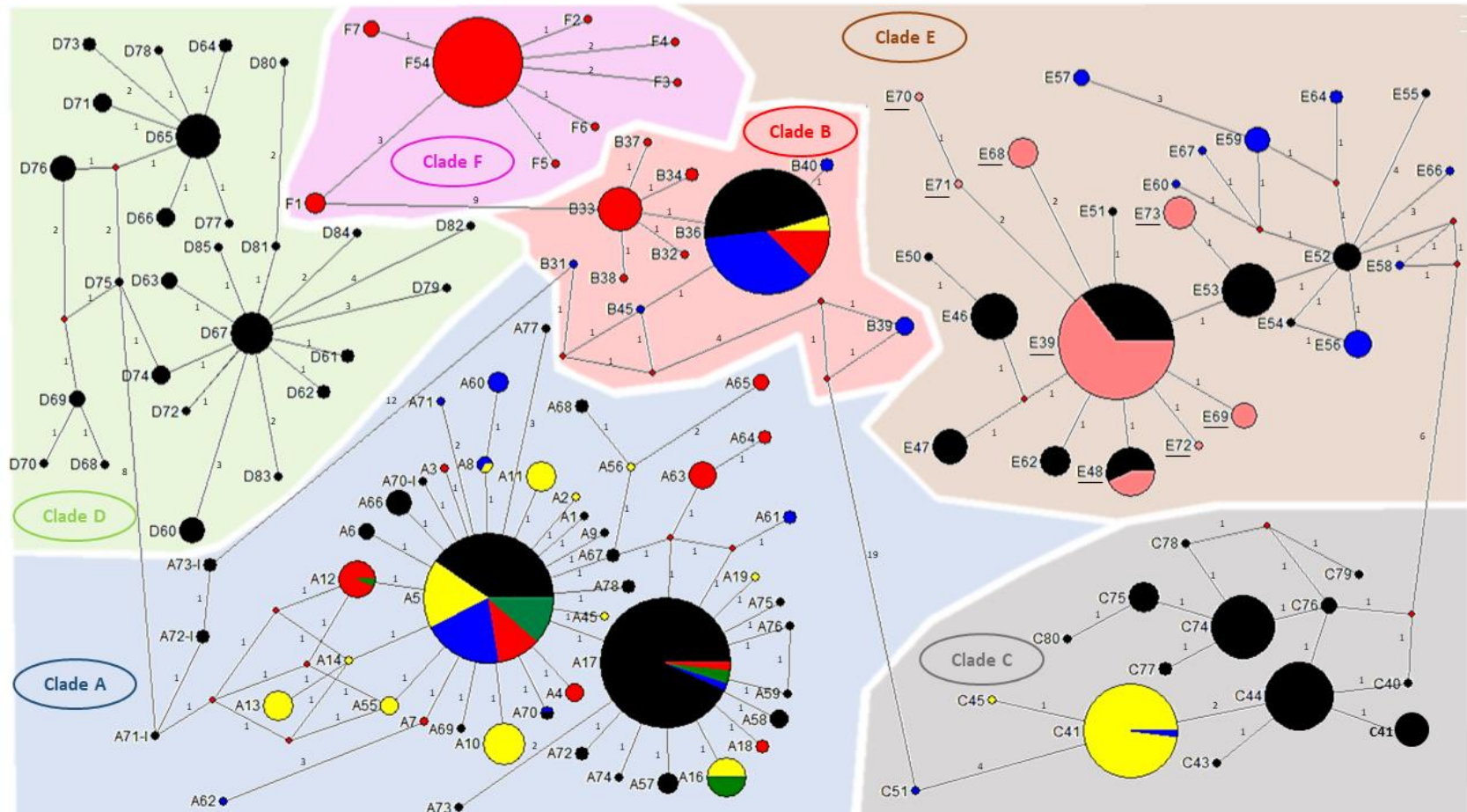
This study included 155 head lice specimens collected from 49 individuals, (96% female and 4% male), from two regions of Guinea, Maférinyah and Kindia. First, All *P. h. capitis* specimens were subjected to a duplex qPCR to determine their clade. The results of the amplification curve revealed that our samples were positive for the clade C-E. Standard PCR and sequencing showed that all our samples belonged to the haplogroup E. For the phylogenetic analysis, we were able to generate 141 sequences from the 155 samples analyzed, due to the low DNA concentration. The generated Guinean sequences were then aligned and combined with all sequences available for *cytb* haplotypes and were then used to construct a maximum-likelihood (ML) tree (Fig. 2) and a median-joining (MJ) network (Figs. 3). The results revealed the existence of 8 haplotypes, including 6 new haplotypes referred here as E68, E69, E70, E71, E72 and E73 with the attributed GenBank accession numbers MT981014-MT981019 respectively. In addition, E39 and E48, the two most prevalent haplotypes present in clade E, accounted for the majority of our lice samples. Indeed, most of our head lice specimens, 96 (68.1%), belonged to the haplotype E39, 12 (8.5%) to the haplotype E48 and 33 (23.4%) to the six novel haplotypes, with 10 (30.3%) to E68, 8 (24.3%) to E69, 1 (3%) to E70, 1(3%) to E71, 1(3%) to E72 and 12 (36.4%) to E73.

20



21

22 **Figure 2.** Maximum-likelihood (ML) phylogenetic tree of the mitochondrial *cytb* gene showing the relationship of haplotypes
 23 identified in this study with other *P. humanus* haplotypes reported in the literature. Phylogenetic inference was conducted in MEGA 7
 24 using the maximum likelihood method under the Kimura 2-parameter with 1000 bootstrap replicates. There were a total of 141
 25 positions in the final dataset.



26

27

Figure 3. *Cytb* haplotype networks of human body and head lice including our samples. Each circle indicates a unique

28

haplotype, and variations in circle size are proportional to haplotype frequencies. Pie colors and sizes in circles represent the

29

continents and the number of their sequence for a haplotype. The length of the links between nodes is proportional to the number of

30

mutations. The types of haplotypes identified in this study are underlined.

31 Besides, all *Pediculus humanus* lice were tested by multiplex qPCR targeting the
32 PHUM540560 gene to investigate their ecotype; this method was used previously to distinguish
33 between head and body lice belonging to clade A. All our specimens were collected by the
34 patients from their scalp hair, and belong to clade E. Using this method, all the 155 Guinean head
35 lice specimens exhibited a real-time PCR FAM-labeled probe amplification specific to the body
36 lice profile, as demonstrated previously [41]. These results confirm the fact that the
37 PHUM540560- multiplex qPCR is a restricted molecular tool to differentiate only between clade
38 A-human lice. Based on these data, we proceeded with the analysis of the PHUM540560 gene
39 sequences of our samples and those belonging to clade A reported in the literature [41]. For this
40 purpose, diverse human lice specimens encompassing both ecotypes were randomly selected
41 from our lice collection. These samples were then subjected to standard PCR and sequencing of
42 the targeted PHUM540560 gene. The obtained sequences were aligned with the specific
43 PHUM540560 sequences of body and head lice. Alignment was conducted using the BioEdit v
44 7.0.5.3 software (available online: [http://en.bio-soft.net/ format/BioEdit.html](http://en.bio-soft.net/format/BioEdit.html)), in order to reveal
45 the rearranged sequences of the Guinean-head lice PHUM540560 gene and to compare the
46 polymorphisms obtained with those of the clade A body and head lice.

47 The comparison of the PHUM540560 gene of Clade A-head lice and clade A-body lice
48 revealed the presence of 22 single nucleotides polymorphisms (SNPs) in the head lice-
49 PHUM540560 gene. The first two point mutations situated on the first exon and the remainder of
50 the polymorphisms were spread throughout the first intron [41]. Interestingly, none of our clade
51 E-head lice showed the clade A head lice-PHUM540560 gene profile. Indeed, 33/40 (82.5%) of
52 Guinean head lice are characterized by the absence of all the above-mentioned SNPs present in
53 clade A-Amazonian head lice, thus exhibiting a clade A-body lice profile, while 7/40 (17.5%)

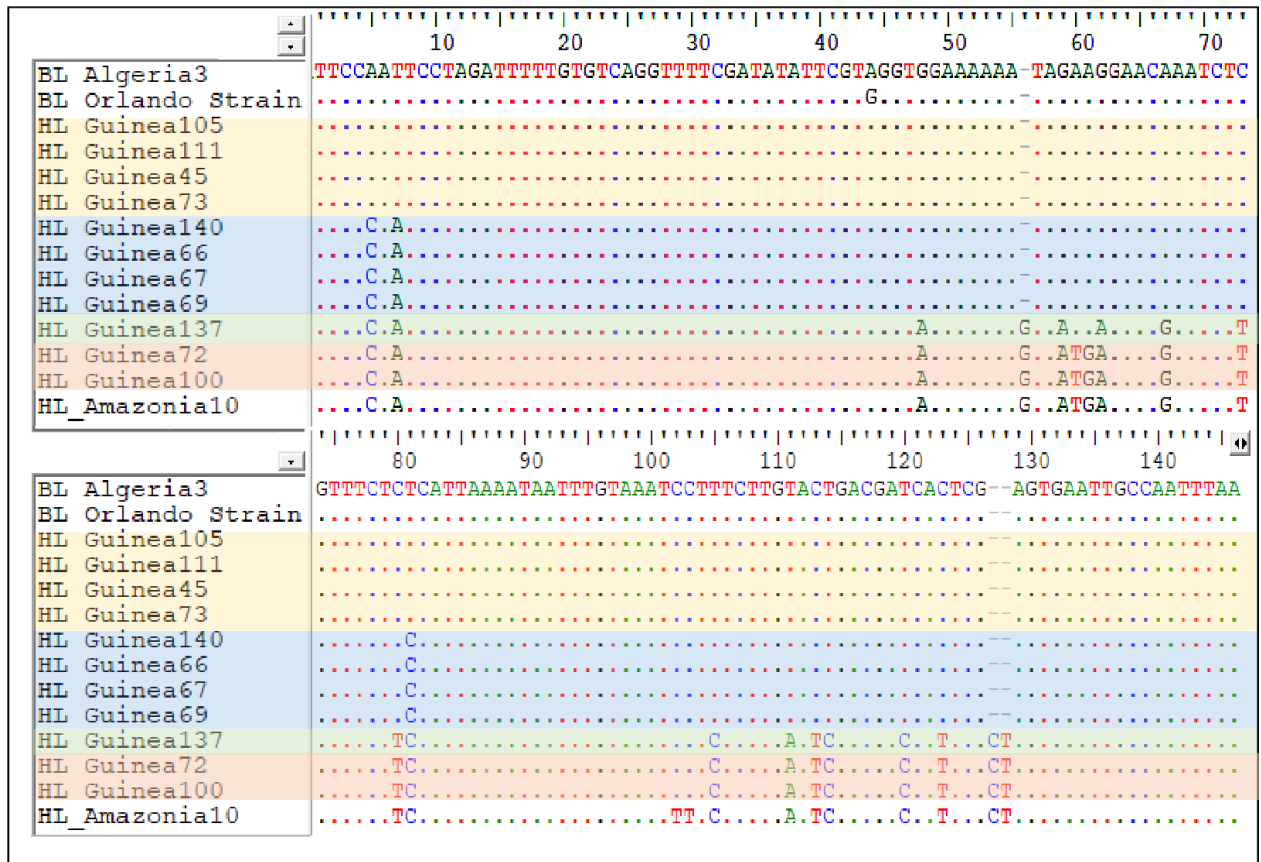
54 samples displayed different kinds of SNPs: 4/7 (57.1%) revealed the existence of 3 SNPs, 1/7
 55 (14.3%) the existence of 18 SNPs and 1/7 (14.3%) the existence of 20 SNPs. Regarding our body
 56 lice tested, all (18 samples) had the same PHUM540560 gene profile of body lice reported by
 57 Drali *et al.* [41]. Details of head lice numbers, codes and PHUM540560 gene SNPs profiles are
 58 listed in Table 2. Details of PHUM540560 sequences alignments are listed in Figure 2.

59

60 **Table 2.** PHUM540560 gene SNPs profile of Guinean head lice compared to clade A-
 61 human lice.

N. of HL	Code Sample	N. of SNPs	Type of SNPs
1	S137	18	All except 13, 14, 6 and 7
2	S72, S100	20	All except 13 and 14
4	S66, S67, S69, S140	3	1, 2 and 12
33	Remaining tested samples (45, 73, 105 and 111 in Fig. 2)	0	0

62 **N:** Number; **HL:** head lice; **S:** sample, **SNPs:** Single Nucleotide Polymorphisms



63

64 **Figure 4. Alignments of a portion of Guinean head lice-Phum_PHUM540560 gene**65 **sequences with those from body and head lice reported in this study and previously in the**66 **literature. HL_Amazonia10 [40] represent the 22 SNPs that are specific to clade A-head lice.**

67 HL_Guinea group contains 7 specimens: yellow block nucleotides represent 22 SNPs that are

68 specific to body lice; blue block nucleotides represent 3 SNPs that are specific to body lice;

69 green block nucleotides represent 18 SNPs that are specific to body lice and orange block

70 nucleotides represents 20 SNPs that are specific to *P.h. humanus*.71 **BL:** body louse; **HL:** head louse; *P.h. humanus* strain USDA 1103172108290 Phum_PHUM540560 (gene

72 sequence available in GenBank accession N. NW_002987859.1).

73

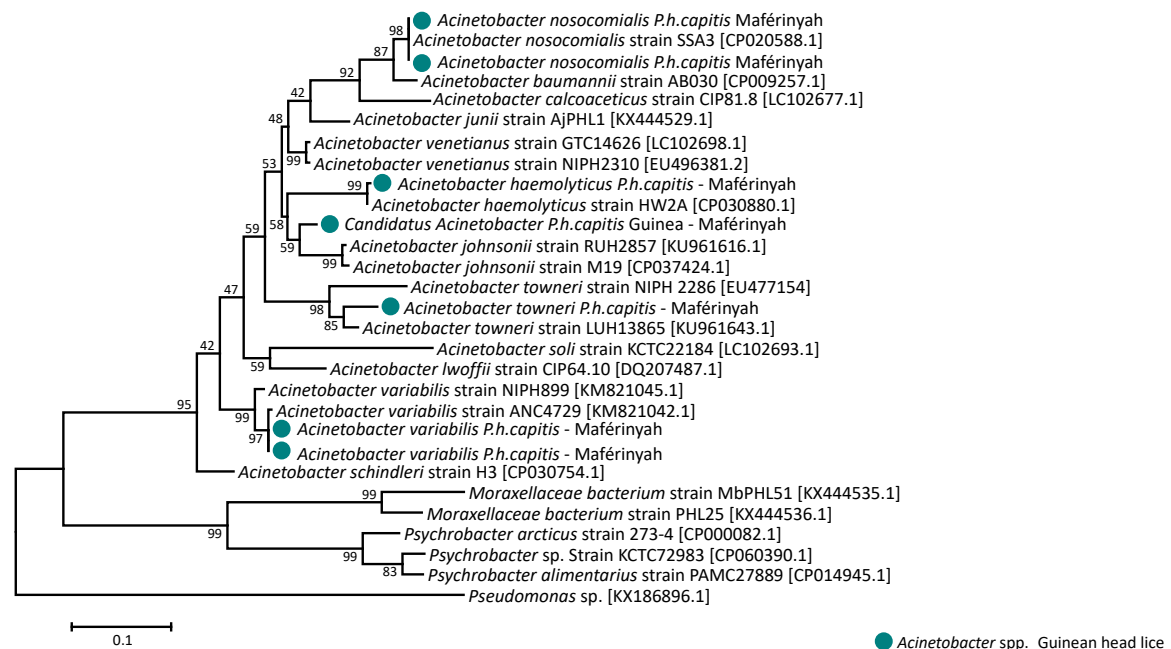
74

75

76 In addition, none of the head lice samples revealed the presence of *Rickettsia* spp., *B.*
77 *quintana*, *Borrelia* spp., *Y. pestis*, *C. burnetii* and *Anaplasma* spp. However, the DNA of
78 *Acinetobacter* spp. was found in 14/155 (9%) samples collected from 11/46 (24.5%) individuals.
79 All positive samples were collected in 14/130 (10.8%) Maférinyah. Positive *Acinetobacter*
80 specimens were then tested for a specific *A. baumannii*-qPCR, revealing that 2 of the 14 (14.3%)
81 samples were positive, these samples belonged to the haplotype E39 and were collected from 2
82 different patients in Maférinyah. The first amplification of *Acinetobacter* DNA in positive head
83 lice samples showed the presence of the DNA from *Moraxella bacterium* and *Psychrobacter*
84 spp., which explains the low specificity of the target gene-amplification by these primers. We
85 designed a more specific system targeting the *rpoB* gene of this bacterium and, due to the low
86 concentration of *Acinetobacter* DNA in our positive head lice, we succeeded in amplifying the
87 DNA of the pathogen in only 7/14 positive samples. Sequencing and blast analysis of 350-bps
88 fragment *rpoB* gene revealed that 6/7 (85.7%) of our sequences shared 99–100% identity with 4
89 *Acinetobacter* species including: 2/7 (28.6%) E69 and E39 head lice matching with
90 *Acinetobacter nosocomialis*, 2/7 (28.6%) E39 and E48 head lice with *Acinetobacter variabilis*,
91 1/7 (14.2%) E69 head louse with *Acinetobacter towneri* and finally, 1/7 (14.2%) E48 specimen
92 with *Acinetobacter haemolyticus*. The remaining generated sequence 1/7 (14.3%), shared a lower
93 similarity (<94%, coverage 100%) with two *Acinetobacter* species: *A. johnsonii* and *A.*
94 *venetianus*, suggesting that this *Acinetobacter* is a potential new specie, named here “*Candidatus*
95 *Acinetobacter P.h capitis* Guinea”. This positive sample belonged to haplotype E39. All
96 positives head lice for *Acinetobacter* species were collected at Maférinyah, and none at Kindia.
97 Interestingly, one of the patients included in this study was infested with four head lice, two of
98 them were infected with *A. nosocomialis* and *A. variabilis*; belonging respectively to haplotypes

99 E48 and E39. The remaining seven of the 14 sequences (50%) also had similarities with
 100 *Acinetobacter* spp. However, the sequences were of poor quality, which is assumed to be due to
 101 the co-infection of several *Acinetobacter* species. The phylogenetic tree of all *Acinetobacter*
 102 species identified in this study is presented in Fig.4.

103 In our study, none of the DNA *A. baumannii* samples tested positive for carbapenem's-
 104 resistant encoding genes (bla_{OXA-21} , bla_{OXA-24} , bla_{OXA-58}).



105
 106 **Figure 5.** Phylogenetic tree highlighting the position of the *Acinetobacter* species
 107 identified in the head lice collected from Guinea compared to *Acinetobacter* spp. available in the
 108 GenBank database. Phylogenetic inferences were conducted in MEGA 7 using the maximum
 109 likelihood method based on the Kimura 2-parameter model for nucleotide sequences. Statistical
 110 support for internal branches of the tree was evaluated by bootstrapping with 1000 replicates.
 111 There was a total of 7 positions in the final dataset.

112 4. Discussion

113 To the best of our knowledge, the present study is the first to investigate both the
114 phylogeny and associated pathogens of head lice collected in Guinea. A total of 155 head lice
115 were collected from 47/49 (95.9) females and 2/49 (4.1%) males living in two different rural and
116 urban areas, Maférinyah, a village, and the city of Kindia, Guinea, West Africa. A genetic study
117 using the qPCR duplex first showed that all the lice samples analyzed belonged to the
118 mitochondrial clade C or E. Standard PCR and sequencing revealed that all the head lice
119 belonged to the haplogroup E. The qPCR duplex method is not discriminative enough for the
120 screening of African human lice, which mainly belong to haplogroups C and E. In the future, this
121 method should be optimized by a design that also includes a specific clade E monoplex, to obtain
122 a better identification of African human lice, as well as to establish an optimal qPCR duplex for
123 the discrimination of all human lice belonging to all existing haplogroups, including the recently
124 described clade F. The phylogenetic study showed the existence of 8 haplotypes including 6
125 novels described for the first time in this study. The presence of clade E in both rural and urban
126 communities in Guinean lice is not surprising, as it confirms the high prevalence of the “African
127 endemic” clade E, as previously reported so far [18, 26, 28, 29]. The most prevalent haplotype
128 reported in our head lice is the E39 obtained with 68.1%, followed by the haplotype E48 with
129 8.5% of the total prevalence. In addition, six new haplotypes were identified in Guinean head
130 lice, including E68 (7.1%), E69 (5.6%), E70 (0.71%), E71 (0.71%), E72 (0.71%) and E73
131 (8.5%). So far, haplogroup E has only been found in head lice collected in West African
132 countries, including Senegal and Mali [18], in head lice samples collected from Nigerians
133 refugees in Algeria [26] and in *P.h. capitis* collected from migrant communities living in
134 Bobigny, France [25]. However, a recent study showed for the first time the presence of clade E,

135 more specifically the E62 haplotype in Central Africa, in Congo, suggesting that Congonians are
136 in direct contact with West African populations or travelers arriving from West African countries
137 [28]. More recently, this clade has also been found in head lice collected from individuals in
138 Gabon, belonging to haplotype E46 [29], already reported among lice collected in Mali [18].
139 These results suggest that the significant migratory exchange between Gabon and the Republic
140 of the Congo can be the source for the clade E expansion [29]. In addition, among the 141 head
141 lice *cytb* sequences analyzed, 4 lice with two different haplotypes, E39 and E70, were collected
142 from the same 34-year-old woman. In addition, four haplotypes were also identified within the
143 same person infested with 11 lice, in Kindia, belonging to haplotypes E39, E48, E71 and E73.
144 Co-infestations by different mitochondrial DNA clades of human head lice within the same
145 person were reported previously, showing that human lice belonging to different clades can live
146 in sympatry and interbreed [36, 39], including the association of clades A and B [36], clade A
147 and D [14,29], clade A and C [39]. All these results underline the fact that more phylogenetic
148 studies on human lice-mitochondrial clades, from larger sampling zones, with different
149 geographical areas, and more human lice samples, should be carried out to broaden our
150 knowledge of the inter and intra-haplogroup diversity.

151 In this study, we investigated the Phum540560 gene polymorphisms of Guinean head lice
152 belonging to clade E. We could not confirm the ecotype of our head lice samples using the
153 Phum540560-multiplex qPCR, previously used to discriminate between *P. humanus* ecotypes
154 belonging to clade A, therefore, our results are consistent with those reported previously [41], the
155 clade E head lice ecotype cannot be identified by this molecular tool. However, in our study, we
156 were interested in analyzing the Phum_PHUM540560 gene sequences from Guinean head lice to
157 better understand their polymorphism profile. Interestingly, none of our clade E-head lice

158 showed the clade A head lice-PHUM540560 gene profile. Indeed, 33/40 (82.5%) of Guinean
159 head lice were characterized by the absence of all the above-mentioned SNPs present in clade A-
160 Amazonian head lice, consequently exhibiting a clade A-body lice profile, while 7/40 (17.5%)
161 samples showed different kinds of SNPs: 4/7 (57.1%) revealed the existence of 3 SNPs, 1/7
162 (14.3%) the existence of 18 SNPs and 1/7 (14.3%) the existence of 20 SNPs. These results
163 highlight the fact that the majority of the Clade E-Guinean head lice exhibit the profile of the
164 clade A-body lice, noting that the clade A lice is the origin of clades B and C, where clade B
165 head lice were reported to diverge from clade A between 0.7 and 1.2 Mya, whereas clade C is
166 even older (ca. 2 Mya) [11]. These results should encourage further study of the polymorphism
167 profile of the PHUM540560 gene from *Pediculus humanus* belonging to the six divergent
168 mitochondrial clades reported so far. In addition, further studies, with a wider sampling, are
169 necessary to study a larger portion of the Phum_PHUM540560 gene. These investigations will
170 allow a better understanding and will probably lead us to design a more efficient molecular tool,
171 which will be able to discriminate between the two ecotypes. At this point, we can affirm the fact
172 that the morphological, biological and genetic characteristics of *P. humanus* species are almost
173 similar and remain obscure. However, body and head lice are extremely different in their
174 ecological niches, which remain, until now, the main criterion for distinguishing between these
175 two ecotypes.

176 In recent decades, the paradigm that *P.h.humanus* was the only vector of dangerous
177 diseases has been challenged [8]. Indeed, many studies have reported the presence of several
178 pathogenic agents in head lice specimens collected worldwide [12–30]; thus, demonstrating that
179 the potential pathogen-vector-competence of the head louse is not yet understood [8]. In this
180 study, among all the bacteria examined, we found the presence of *Acinetobacter spp.* in only 14

181 head lice infesting 11 individuals. Findings from previous studies reported a worldwide spread
182 of several *Acinetobacter* species, including *A. baumannii*, *A. junii*, *A. ursingii*, *A. johnsonii*, *A.*
183 *schindleri*, *A. lwoffii*, *A. nosocomialis*, *A. townneri*, *A. variabilis*, *A. radioresistens*, *A.*
184 *calcoaceticus*, *A. soli*, *A. pittii* and potential new species in head lice collected from different
185 population categories, including elementary school children in Algeria [26,27], France [22],
186 Thailand and Georgia-USA [19,24], of the Pygmy population in the Republic of Congo [21],
187 Nigerian refugee children in Algeria [26] and, more recently, in head lice collected from healthy
188 women in Gabon [29], and even in ancient Roman-era head lice remains [32]. The diversification
189 of *Acinetobacter* species was reported from head lice belonging to the majority of the existing
190 head lice-haplogroups A, D, C, E and B, and in different haplotypes of each haplogroup [11].
191 Among the 14 *Acinetobacter* positives *P.h.capitis* samples, we identified the existence of *A.*
192 *baumannii*, *A. nosocomialis*, *A. variabilis* *A. townneri* and for the first time in head lice *A.*
193 *haemolyticus* as well as one potential new specie named here “*Candidatus Acinetobacter P.h*
194 *capitis* Guinea”. Based on these results, we confirmed the diversity of the *Acinetobacter* species
195 in head lice. Our study is the first to describe the presence of *A. haemolyticus* in human head lice.
196 *A. haemolyticus* is a pathogenic bacterium widely distributed in nature and commonly found in
197 soil, water and hospitals. Like *A. baumannii*, *A. johnsonii* and *A. junii*, *A. haemolyticus* is an
198 important clinical microorganism responsible for nosocomial infections and associated with
199 endocarditis, bacteremia and others types of infections in hospitals [58].

200 Body lice can also be infected by a diverse *Acinetobacter* species, including *A. baumannii*,
201 *A. johnsonii*, *A. berezeniae*, *A. nosocomialis* and *A. variabilis* [23, 45]. Indeed, *A. baumannii* was
202 isolated for the first time from body lice collected on homeless people in France and,
203 subsequently, the bacterium was detected in body lice collected worldwide [55]. Most of the

204 *Acinetobacter* species are pathogenic bacteria that can survive for a long period in the
205 environment and have been associated with carbapenem resistance, especially *A. baumannii* and
206 *A. haemolyticus*, because of their multiple resistance to many common antibiotics [58].

207 In recent decades, *Acinetobacter* bacteria have shown high ability to develop resistance to
208 almost all major classes of antibiotics. So far, the incidence of carbapenem resistance in *A.*
209 *baumannii* has continued to increase worldwide [54]. In human lice, *A. baumannii* isolates were
210 remarkably susceptible to carbapenems [55]. Indeed, a study performed on head lice collected in
211 Senegal reported that 21.4% of the positive *A. baumannii*- head lice harbored a bla_{OXA-23}
212 carbapenem resistant encoding gene [56]. A precedent study reported for the first time the
213 presence of bla_{OXA-23} gene in positive *A. baumannii* human head lice in Senegal [54]. None of
214 our positive-*A. baumannii* head lice were positive to the carbapenem's-resistant encoding genes
215 (bla_{OXA-21}, bla_{OXA-24}, bla_{OXA-58}). Further studies are needed to investigate the association between
216 *Acinetobacter* infections and human lice, the compatibility of *Acinetobacter* strains present in
217 lice and those responsible for human infections, as well as investigate the carbapenem's-resistant
218 in *A. baumannii* strain present in human lice.

219 5. Conclusion

220 Herein, to the best of our knowledge, we report here the first molecular data on the genetic
221 diversity and associated pathogens of head lice collected in Guinea. Overall, polygenetic analysis
222 reveal that all our specimens belonged to haplogroup E within 8 haplotypes, with 6 novels
223 described for the first time in this study. Genetic study of the PHUM540560 Gene
224 polymorphisms profile revealed that the majority of our Guinean head lice exhibit a clade A-
225 body lice PHUM540560 gene polymorphism profile, showing the importance of conducting a
226 more in-depth genetic study of the PHUM540560 gene, targeting human lice belonging to the six

227 divergent mitochondrial clades to better understand this paradigm. None of the pathogenic
228 bacteria tested were detected in our *P. h. capitis* samples, except for *Acinetobacter spp.* for
229 which we were able to identify several species, including *A. baumannii*, *A. nosocomialis*, *A.*
230 *variabilis*, *A. towneri*, a potential new specie “*Candidatus Acinetobacter P.h capitis* Guinea”
231 and, for the first time in head lice, *A. haemolyticus*. Further studies are needed to study the
232 genetic diversity of Guinean head lice and to evaluate their role as a potential vector for their
233 associated bacteria.

234 **Supplementary Materials**

235 **Table S1.** Geographical and frequencies occurrences of *cytb* haplotypes of human head
236 and body lice worldwide.

237 **Author contributions:** M.L., F.F. and O.M. designed the study. CB and HB supervised the
238 collection of the samples. A.H. and M.L performed the lab work. A.H., M.L. and O.M. carried out
239 the data analysis. A.H. and M.L. wrote the manuscript. A.H, M.L, CB, HB, F.F, D.R, D.M, P.G
240 and O.M revised the manuscript. All authors read and approved the final manuscript.

241 **Funding:** This study was supported by the Institut Hospitalo-Universitaire (IHU)
242 Méditerranée Infection, the National Research Agency under the program «Investissements
243 d’avenir», reference ANR-10-IAHU-03, the Région Provence-Alpes-Côte d’Azur and European
244 funding FEDER PRIMI.

245 **Acknowledgments:** We are grateful for the assistant’s member who participated in the
246 supervision of the data and samples collection.

247 **Conflicts of Interest:** The authors declare that they have no competing interests. The funders
248 had no role in the design of the study; in the collection, analyses, or interpretation of the data; in
249 the writing of the manuscript, or in the decision to publish the results.

References

1. Light, J.E.; Smith, V.S.; Allen, J.M.; Durden, L.A.; Reed, D.L. Evolutionary history of mammalian sucking lice (*Phthiraptera: Anoplura*). *BMC Evol. Biol.* **2010**, *10*, 292. doi:10.1186/1471-2148-10-292.
2. Reed, D.L.; Smith, V.S.; Hammond, S.L.; Rogers, A.R.; Clayton, D.H. Genetic analysis of lice supports direct contact between modern and archaic humans. *PLoS Biol.* **2004**, *2*, e340. doi: 10.1371/journal.pbio.0020340.
3. Raoult, D.; Roux, V. The body louse as a vector of re-emerging human diseases. *Clin. Infect. Dis.* **1999**, *29*, 888–911, doi:10.1086/520454.
4. Durden, L.A.; Musser, G.G. The sucking lice (*Insecta, Anoplura*) of the world: a taxonomic checklist with records of mammalian hosts and geographical distributions. *Bull. AMNH* no. 218,” in *Sucking Lice and Hosts*. **1994**. <http://digitallibrary.amnh.org/handle/2246/825> (accessed April 15, 2018).
5. Light, J. E.; Toups, M.A.; Reed, D.L. What’s in a name: the taxonomic status of human head and body lice. *Mol. Phylogenet. Evol.* **2008**, *47*, 1203–1216. doi: 10.1016/j.ympev.2008.03.014.
6. Raoult, D. A personal view of how paleomicrobiology aids our understanding of the role of lice in plague pandemics. *Microbiol. Spectr.* **2016**, *4* : PoH-0001-2014. doi: 10.1128/microbiolspec. PoH-0001-2014
7. Robinson, D.; Leo, N.; Prociw, P.; Barker, S.C. Potential role of head lice, *Pediculus humanus capitis*, as vectors of *Rickettsia prowazekii*. *Parasitol. Res.* **2003**, *90*, 209–211. doi:10.1007/s00436-003-0842-5.
8. Kim, J. H.; Min, J.S.; Kang, J.S.; Kwon, D.H.; Yoon, K.S.; Strycharz, J. et al. Comparison of the humoral and cellular immune responses between body and head lice following bacterial challenge. *Insect Biochem. Mol Biol.* **2011**, *41*, 332–339.
9. Kim, J.H.; Yoon, K.S., Previte, D.J.; Pittendrigh, B.R.; Clark, J.M.; Lee, S. H. Comparison of the immune response in alimentary tract tissues from body versus head lice following *Escherichia coli* oral infection. *J. Asia Pac. Entomol.* **2012**, *15*, 409–412. doi: 10.1016/j.aspen.2012.05.010
10. Kim, J.H.; Previte, D.J.; Yoon, K.S.; Murenzi, E.; Koehler, J.E.; Pittendrigh, B.R.; Lee, S.H.; Clark, J.M. Comparison of the proliferation and excretion of *Bartonella quintana* between body and head lice following oral challenge. *Insect Mol. Biol.* **2017**, *26*, 266–276, doi:10.1111/imb.12292.

11. Amanzougaghene, N.; Fenollar, F.; Raoult, D.; Mediannikov, O. Where are we with human lice? A review of the current state of knowledge. *Front. Cell. Infect. Microbiol.* **2020**, *9*, 474, doi:10.3389/fcimb.2019.00474.
12. Sasaki, T.; Poudel, S.K.S.; Isawa, H.; Hayashi, T.; Seki, N.; Tomita, T.; et al. First molecular evidence of *Bartonella quintana* in *Pediculus humanus capitis* (Phthiraptera: Pediculidae), collected from Nepalese children. *J. Med. Entomol.* **2006**, 110–112. doi: 10.1603/0022-2585(2006)043[0110:fmeobq]2.0.co;2.
13. Piarroux, R.; Abedi, A. A., Shako; J.-C., Kebela; B., Karhemere; S., Diatta; G., et al. Plague epidemics and lice, Democratic Republic of the Congo. *Emerg Infect Dis*, **2013**, *19*, 505–506. doi: 10.3201/eid1903.121542.
14. Drali, R.; Shako, J.-C.; Davoust, B.; Diatta, G.; and Raoult, D. A new clade of African body and head lice infected by *Bartonella quintana* and *Yersinia pestis*-Democratic Republic of the Congo. *Am. J. Trop. Med. Hyg.* **2015**, 990–993. doi: 10.4269/ajtmh.14-0686.
15. Boutellis, A.; Mediannikov, O.; Bilcha, K. D.; Ali, J.; Campelo, D.; Barker, S. C. et al. *Borrelia recurrentis* in head lice, Ethiopia. *Emerg. Infect. Dis.* **2013**, 796–798. doi: 10.3201/eid1905.121480.
16. Angelakis, E.; Diatta, G.; Abdissa, A.; Trape, J.-F.; Mediannikov, O.; Richet, H. et al. Altitude-dependent *Bartonella quintana* genotype C in head lice, Ethiopia. *Emerg. Infect. Dis.* **2011**, 2357–2359. doi: 10.1111/j.1574-695X.2011.00804.x .
17. Sangaré, A.K.; Boutellis, A.; Drali, R.; Socolovschi, C.; Barker, S. C.; Diatta, G. et al. Detection of *Bartonella quintana* in African body and head lice. *Am. J. Trop. Med. Hyg.* **2014**, 294–301. doi: 10.1016/j.ijantimicag.2016.01.001.
18. Amanzougaghene, N.; Fenollar, F.; Sangaré, A. K.; Sissoko, M. S.; Doumbo, O. K.; Raoult, D. Detection of bacterial pathogens including potential new species in human head lice from Mali. *PLoS ONE.* **2017**, e0184621. doi: 10.1371/journal.pone.0184621.
19. Eremeeva, M.E.; Capps, D.; Winful, E.B.; Warang, S.S.; Braswell, S.E., Tokarevich, N.K., et al. Molecular markers of pesticide resistance and pathogens in human head lice (*Phthiraptera: Pediculidae*) from rural Georgia, USA. *J. Med. Entomol.* **2017**, 1067–1072. doi: 10.1093/jme/tjx039.
20. Boutellis, A.; Veracx, A.; Angelakis, E.; Diatta, G.; Mediannikov, O.; Trape, J.-F. et al. *Bartonella quintana* in head lice from Sénégal. *Vector Borne Zoonotic Dis.* **2012**, 564–567. doi: 10.1089/vbz.2011.0845.

21. Amanzougaghene, N.; Akiana, J.; Mongo Ndombe, G.; Davoust, B.; Nsana, N.S.; Parra, H.-J.; Fenollar, F.; Raoult, D.; Mediannikov, O. Head Lice of Pygmies Reveal the Presence of Relapsing Fever *Borreliae* in the Republic of Congo. *PLoS Negl.Trop.Dis.* **2016**, *10*, e0005142. doi: 10.1371/journal.pntd.0005142.
22. Bouvresse, S.; Socolovshi, C.; Berdjane, Z.; Durand, R.; Izri, A.; Raoult, D. et al. No evidence of *Bartonella quintana* but detection of *Acinetobacter baumannii* in head lice from elementary schoolchildren in Paris. *34*, 475–477. *Comp. Immunol. Microbiol. Infect. Dis.* **2011**, *34*.doi: 10.1016/j.cimid.2011.08.007.
23. Kempf, M.; Abdissa, A.; Diatta, G.; Trape, J.-F.; Angelakis, E., Mediannikov, O., et al. Detection of *Acinetobacter baumannii* in human head and body lice from Ethiopia and identification of new genotypes. *Int. J. Infect. Dis.* **2012**, e680–683. doi: 10.1016/j.ijid.2012.05.1024
24. Sunantaraporn, S.; Sanprasert, V.; Pengsakul, T.; Phumee, A.; Boonserm, R.; Tawatsin, A.; Thavara, U.; Siriyasatien, P. Molecular survey of the head louse *Pediculus humanus capitis* in Thailand and its potential role for transmitting *Acinetobacter* spp. *Parasit. Vectors.* **2015**, *8*, 127, doi:10.1186/s13071-015-0742-4.
25. Candy, K., Amanzougaghene, N., Izri, A., Brun, S., Durand, R., Louni, M., et al. Molecular survey of head and body lice, *Pediculus humanus*, in France. *Vector Borne Zoonotic Dis.* **2018**, 243–251. doi: 10.1089/vbz.20.
26. Louni, M.; Amanzougaghene, N.; Mana, N.; Fenollar, F.; Raoult, D.; Bitam, I.; Mediannikov, O. Detection of bacterial pathogens in clade E head lice collected from Niger’s refugees in Algeria. *Parasit. Vectors.* **2018**, *11*, 348, doi:10.1186/s13071-018-2930-5.
27. Mana, N.; Louni, M.; Parola, P.; Bitam, I. Human head lice and pubic lice reveal the presence of several *Acinetobacter* species in Algiers, Algeria. *Comp. Immunol. Microbiol. Infect. Dis.* **2017**, 33–39. doi: 10.1016/j.cimid.2017.06.003
28. Boumbanda Koyo, C.S.; Amanzougaghene, N.; Davoust, B.; Tshilolo, L.; Lekana-Douki, J.B.; Raoult, D.; Mediannikov, O.; Fenollar, F. Genetic diversity of human head lice and molecular detection of associated bacterial pathogens in Democratic Republic of Congo. *Parasit. Vectors.* **2019**, *12*, 290, doi:10.1186/s13071-019-3540-6.
29. Boumbanda-Koyo, C.S.; Mediannikov O., Amanzougaghene N., Oyegue-Liabagui S.L.; Imboumi-Limoukou, R.K.; Raoult, D. ; Lekana-Douki, J.B. Fenollar, F. Molecular identification of head lice collected in Franceville (Gabon) and their associated bacteria. *Parasites & Vectors*, **2020**. *13*, 410. doi: 10.1186/s13071-020-04293-x.

30. Amanzougaghene, N.; Mediannikov O.; Anh Ly T.D.; Gautret P.; Davoust B.; Fenollar F.; Izri A. Molecular investigation and genetic diversity of *Pediculus* and *Phthirus* lice in France. *Parasites & Vectors*, **2020**, 13, 177. doi: 10.1186/s13071-020-04036-y.
31. Ashfaq, M.; Prosser, S.; Nasir, S.; Masood, M.; Ratnasingham, S.; Hebert, P.D.N. High diversity and rapid diversification in the head louse, *Pediculus humanus* (*Pediculidae: Phthiraptera*). *Sci. Rep.* **2015**, 5, 14188. doi:10.1038/srep14188.
32. Amanzougaghene, N.; Mumcuoglu, K.Y.; Fenollar, F.; Alfi, S.; Yesilyurt, G.; Raoult, D.; Mediannikov, O. High Ancient Genetic Diversity of Human Lice, *Pediculus humanus*, from Israel Reveals New Insights into the Origin of Clade B Lice. *PLOS ONE*. **2016**, 11, e0164659, doi: 10.1371/journal.pone.0164659.
33. Amanzougaghene, N.; Fenollar, F.; Davoust, B.; Djossou, F.; Ashfaq, M.; Bitam, I.; et al. Mitochondrial diversity and phylogeographic analysis of *Pediculus humanus* reveals a new Amazonian clade “F.” *Infect. Genet. Evol.* **2019**, 1–8. doi: 10.1016/j.meegid.2019.02.006.
34. Ascunce, M. S.; Fane, J., Kassu; G., Toloza; A. C., Picollo, M. I.; González-Oliver, A. et al. Mitochondrial diversity in human head louse populations across the Americas. *Am. J. Phys. Anthropol.* **2013**, 118–129. doi: 10.1002/ajpa.22336.
35. Raoult, D.; Reed, D.L.; Dittmar, K.; Kirchman, J.J.; Rolain, J.; Guillen, S.; Light, J.E. Molecular Identification of Lice from Pre-Columbian Mummies. *J. Infect. Dis.* **2008**, 197, 535–543. doi:10.1086/526520.
36. Boutellis, A.; Bitam, I.; Fekir, K.; Mana, N.; Raoult, D. Evidence that clade A and clade B head lice live in sympatry and recombine in Algeria. *Med. Vet. Entomol.* **2015**, 94–98. doi: 10.1111/mve.12058.
37. Al-Shahrani, S.A.; Alajmi, R.A.; Ayaad, T.H.; Al-Shahrani, M.A.; Shaurub, E.-S.H. Genetic diversity of the human head lice, *Pediculus humanus capitis*, among primary school girls in Saudi Arabia, with reference to their prevalence. *Parasitol. Res.* **2017**, 2637–2643. doi: 10.1007/s00436-017-5570-3
38. Li, W.; Ortiz, G.; Fournier, P-E.; Gimenez, G.; Reed, D.L.; Raoult, D. Genotyping of Human Lice Suggests Multiple Emergences of Body Lice from Local Head Louse Populations. *PLoS Negl. Trop. Dis.* **2010**, 4, 10. doi: e641. doi: 10.1371/journal.pntd.0000641.

39. Veracx, A., Boutellis, A., Merhej, V., Diatta, G., Raoult, D. Evidence for an African Cluster of Human Head and Body Lice with Variable Colors and Interbreeding of Lice between Continents. *PLoS One*. **2012**, 7: e37804. doi:10.1371/journal.pone.0037804.
40. Olds, B.P.; Coates, B.S.; Steele, L.D.; Sun, W.; Agunbiade, T.A. et al. Comparison of the transcriptional profiles of head and body lice. *Insect Mol Biol*, **2012**, 21: 257–268. doi: 10.1111/j.1365-2583.2012.01132.x.
41. Drali, R.; Boutellis, A.; Raoult, D.; Rolain, J.M.; Brouqui, P. Distinguishing Body Lice from Head Lice by Multiplex Real-Time PCR Analysis of the Phum_PHUM540560 Gene. *PLoS ONE*. **2013**, 8, e58088. doi: 10.1371/journal.pone.0058088.
42. La Scola, B.; Fournier, P.-E.; Brouqui, P.; Raoult, D. Detection and Culture of *Bartonella quintana*, *Serratia marcescens*, and *Acinetobacter* spp. from Decontaminated Human Body Lice. *J. Clin. Microbiol.* **2001**, 39, 1707–1709. doi:10.1128/JCM.39.5.1707-1709.2001.
43. Tamura K.; Stecher G.; Peterson D.; Filipski A.; Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol.* **2013**, 30: 2725–2729. <https://doi.org/10.1093/molbev/mst197> PMID: 24132122
44. Bandelt, H.J.; Forster P.; Rohl A.; Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol.* **1999**, 37–48. doi: pmid:10331250.
45. Louni, M.; Mana, N.; Bitam, I.; Dahmani, M.; Parola, P.; Fenollar, F.; Raoult, D.; Mediannikov, O. Body lice of homeless people reveal the presence of several emerging bacterial pathogens in northern Algeria. *PLOS Neglected Tropical Diseases*. **2018**. 12, 4, e0006397. doi: org/10.1371/journal.pntd.000639
46. Rolain, J.-M.; Stuhl, L.; Maurin, M.; Raoult, D. Evaluation of Antibiotic Susceptibilities of Three *Rickettsial* Species Including *Rickettsia felis* by a Quantitative PCR DNA Assay. *Antimicrob. Agents Chemother.* **2002**, 46, 2747–2751. doi:10.1128/AAC.46.9.2747-2751.2002.
47. Parola, P.; Diatta, G.; Socolovschi, C.; Mediannikov, O.; Tall, A.; Bassene, H.; Trape, J.F.; Raoult, D. Tick-Borne Relapsing Fever Borreliosis, Rural Senegal. *Emerg. Infect. Dis.* **2011**, 17, 883–885. doi:10.3201/eid1705.100573.

48. Raoult, D.; Angelakis, E.; Socolovschi, C. *Bartonella quintana* in *Cimex hemipterus*, Rwanda. *Am. J. Trop. Med. Hyg.* **2013**, *89*, 986–987. doi:10.4269/ajtmh.13-0182.
49. Mediannikov, O.; Fenollar, F.; Socolovschi, C.; Diatta, G.; Bassene, H.; Molez, J.-F.; Sokhna, C.; Trape, J.-F.; Raoult, D. *Coxiella burnetii* in Humans and Ticks in Rural Senegal. *PLoS Negl. Trop. Dis.* **2010**, *4*, e654. doi:10.1371/journal.pntd.0000654.
50. Dahmani, M.; Davoust, B.; Rousseau, F.; Raoult, D.; Fenollar, F.; Mediannikov, O.; Natural *Anaplasmatocae* infection in *Rhipicephalus bursa* ticks collected from sheep in the French Basque Country. *Ticks Tick Borne Dis. Elsevier GmbH.* **2017**, *18–24*. doi.org/10.1016/j.ttbdis.2016.09.009.
51. Bouvresse, S.; Socolovschi, C.; Berdjane, Z.; Durand, R.; Izri, A.; Raoult, D. et al. No evidence of *Bartonella quintana* but detection of *Acinetobacter baumannii* in head lice from elementary schoolchildren in Paris. *Comp Immunol Microbiol Infect Dis. Elsevier Ltd.* **2011**; *34*: 475–477. doi: .org/10.1016/j.cimid. 2011.08.007.
52. La Scola, B.; Gundi, V.A.K.B.; Khamis, A.; Raoult, D. Sequencing of the *rpoB* Gene and Flanking Spacers for Molecular Identification of *Acinetobacter Species*. *J. Clin. Microbiol.* **2006**, *44*, 827–832, doi:10.1128/JCM.44.3.827-832.2006.
53. Ly, T.D.A.; Kerbaj, J.; Edouard, S.; Hoang, V.T.; Louni, M.; Dao, T.L.; Benkouiten, S.; Badiaga, S.; Tissot-Dupont, H.; Raoult, D.; et al. The Presence of *Acinetobacter baumannii* DNA on the skin of homeless people and its relationship with body lice infestation. Preliminary Results. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 86, doi:10.3389/fcimb.2019.00086.
54. Kempf, M.; Rolain, J.-M.; Diatta, G.; Azza, S.; Samb, B.; Mediannikov, O.; Sow, G.A.; Diene, S.M.; Fenollar, F.; Raoult, D. Carbapenem Resistance and *Acinetobacter baumannii* in Senegal: The Paradigm of a Common Phenomenon in Natural Reservoirs. *PLoS ONE.* **2012**. *7*, *6*, e39495. doi: id=10.1371/journal.pone.0039495
55. La Scola, B.; Raoult, D. *Acinetobacter baumannii* in Human Body Louse. *Emerg. Infect. Dis.* **2004**, *10*, 1671–1673, doi:10.3201/eid1009.040242.
56. Huang, X.-Z.; Cash, D.M.; Chahine, M.A.; Nikolich, M.P.; Craft, D.W. Development and validation of a multiplex TaqMan real-time PCR for rapid detection of genes encoding four types of class D carbapenemase in *Acinetobacter baumannii*. *J. Med. Microbiol.* **2012**, *61*, 1532–1537, doi:10.1099/jmm.0.045823-0.
57. Xiong, H.; Campelo, D.; Boutellis, A.; Raoult, D.; Alem, M.; Ali, J.; et al. SNPs in entire mitochondrial genome sequences (≈ 15.4 kb) and *coxI* sequences (≈ 486 bp)

resolve body and head lice from doubly infected people from Ethiopia, China, Nepal, and Iran but not France. *J Med Entomol.* **2014**, 51:1199–207. doi: 10.1603/ME14001

58. Liang, B.; ShaoCun, Z.; Yong, D.; ChengCheng, S., GuangBo, K.; Yan, D.; Yue, W.; Feng, G.; He, H.; Comparative genomics analysis of *Acinetobacter* haemolyticus isolates from sputum samples of respiratory patients. *Genomics.* **2020**, 112, 4, 2784-2793. doi : pii/S0888754319308699.