Amazonian Head Lice-Specific Genotypes Are Putatively Pre-Columbian

Amina Boutellis, Aurélie Veracx, Jônatas Abrahão, and Didier Raoult*

Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes: URMITE, Aix Marseille Université, Faculté de Médecine, Marseille, France; Laboratório de Vírus, Departamento de Microbiologia, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

Abstract. Head and body lice are strict obligate human ectoparasites with three mitochondrial phylotypes (A, B, and C). Using molecular methods for genotyping lice (Cytochrome *b* and multi-spacer typing), and comparing our results with all the sequences of human lice that were genotyped previously, we assessed the presence of a specific American genotype that most likely predates the Columbian era in head lice collected from Amazonia.

INTRODUCTION

Three louse species specifically associated with humans have been described, including the pubic louse (*Phthirus pubis* Linnaeus) and two species belonging to the Pediculidae family.¹ The head louse (*Pediculus humanus capitis* de Geer) is prevalent in all countries, it lives and lays eggs in the hair on the head, and the body louse (*Pediculus humanus humanus* Linnaeus), which lives and lays its eggs in clothing and multiplies when such conditions as cold weather, lack of hygiene, or war are present.² It is responsible for the deaths of millions as a result of vectoring several deadly bacterial pathogens.³ Several phenotypic and genotypic studies have been conducted to assess whether these two species are distinct. The first genetic study was based on the 18S rRNA gene, and EF1 α discriminated lice into two subgroups: lice from sub-Saharan Africa and lice from other places worldwide. In each subgroup, the head lice were genetically different from the body lice.^{4,5} Furthermore, the analysis of mitochondrial DNA, partial cytochrome oxidase subunit 1 (*COI*) gene, and *Cytb* gene sequence (the best template for species identification; high inter-species variability, and low intra-species variation)⁶ classified the lice into three phylotypes of human head lice (clades A, B, and C) and a single phylotype (clade A) of body lice (Figure 1). Each phylotype has a unique geographical distribution: Clade A can be found worldwide, clade B can be found in North America, Central America, Europe, and Australia,⁷ and clade C can be found in Nepal,⁸ Ethiopia,⁹ and Senegal.¹⁰ Li and colleagues⁴ introduced a new genotyping technique involving the sequencing of four variable intergenic spacers for clade A and found a relatively specific geographic distribution for each genotype. This method identified two clusters in France, one cluster in Central Africa, and one cluster in Russia. Veracx and others¹¹ confirmed the presence of an African cluster through a phylogenetic analysis of the spacer PM2 in lice from several different regions. Here, we report the first study of the genotypic distribution of Amazonian head lice.

MATERIALS AND METHODS

This study was approved by the Ethic Committee of the Institute Fédératif de Recherche 48, Marseille, France (Agreement

no. 12-017) and informed verbal consent was obtained from all of the participants involved here. We collected 30 head lice samples from three different states in Brazil. A total of 26 lice were collected from Amazonas State (3°6' S and 60°00' N), two were collected from Pará State (1°52'30"S, 48°07'30"W), and two were collected from Amapá State (3°52'30"N, 51°52'30"W) (Figure 2). All of the lice were preserved dry under sterile conditions and transported to our laboratory in August 2011. All of the samples were photographed with a camera (Olympus DP71, Rungis, France). Before the DNA isolation, each louse was rinsed twice in sterile water, and the total genomic DNA was extracted using a QIAamp Tissue kit (QIAGEN, Hilden, Germany), as described by the manufacturer.

The quantitative real-time polymerase chain reaction (PCR) primers and probes targeting a portion of the *Bartonella* 16S–23S intergenic spacer region⁹ and one *Acinetobacter* sp. specific gene (*rpoB*) were performed as previously described.¹² Negative and positive controls were included in each assay. The mitochondrial gene *Cytb* and each intergenic spacer were amplified as described previously.^{4,11} Each DNA sequence was aligned using the multisequence alignment software (CLUSTALX, version 2.0.11) with previous data performed in 2010 and 2012, which included lice from France, Portugal, Mexico, Russia, Burundi, Rwanda, Senegal, Ethiopia, United States, and United Kingdom.¹¹ The sequence similarities were determined using MEGA 5, and phylogenetic trees were obtained using the maximum likelihood method with 100 bootstrap replicates.¹³

RESULTS

All of the Amazonian head lice are brown, distinct from French lice (gray) and African lice (black) (Figure 2). The quantitative PCR assay showed that all of the samples were negative for *Bartonella* sp. and *Acinetobacter* sp., whereas the positive controls amplified normally.

After sequence correction and assembly of the partial *Cytb* gene sequence, all of the lice were found to belong to clade A (GenBank accession nos. JX178718–JX178747) and very little variation was observed among the collected specimens (Figure 1).

The intergenic spacer S2 was analyzed in 14 of the Amazonian head lice. These samples exhibited six different genotypes, three of which (genotype 117, 118, and 119) are novel (GenBank accession nos. JX178748–JX178750) (Table 1). Four of the six genotypes are specific to the Amazonian lice and were found in (71%) samples, and the other genotypes (39 and 40) are common with the other lice from America, Africa, and Asia (Li and others⁴) (Figure 3).

*Address correspondence to Didier Raoult, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes: URMITE, Aix Marseille Université, UMR CNRS 7278, IRD 198, INSERM 1095, Faculté de Médecine, 27 Bd Jean Moulin, 13005, Marseille, France. E-mail: Didier.raoult@gmail.com

FIGURE 1. Phylogenetic distribution of lice based on *Cytb*.

Spacer S5 was analyzed in 26 of the Amazonian lice, and four different genotypes were found. Two of these genotypes (68 and 69) (GenBank accession nos. JX178751–JX178752) are specific to the Amazonian head lice and were found in (38%) of the samples. We found two lice specimens (1 and 22)

that were heterozygous for genotype 23 and genotype 67 (Table 1). Genotype 23 was found in (42%) Amazonian lice and was also found in Europe and America.⁴

The analysis of spacer PM1 showed that 72% of the 25 Amazonian lice had genotype 13; this genotype is prevalent

FIGURE 2. Photographs of the Amazonian brown head louse (A), French gray head louse (B), and Senegalese black head louse (C), showing the three different phenotypes.

FIGURE 3. The phylogenetic organization of Amazonian lice (green), American lice (blue), European lice (red), Asian lice (yellow), and African lice (black), constructed using the maximum likelihood method with each spacer region independently. (A) Spacer S2; (B) spacer S5; (C) spacer PM1; (D) spacer PM2.

in Europe and is also found in America and Africa.¹¹ A new genotype (36) (GenBank accession no. JX178753) was found to be specific to the Amazonian head lice and was present in 28% of the tested samples (Table 1).

All of the Amazonian head lice presented genotype 47 for spacer PM2 but four samples were not properly amplified. For the phylogenetic tree constructed using the PM2 spacer, we included sequences from lice found in the United States and United Kingdom that were previously genotyped only for PM1 and PM2 (Figure 3). These lice exhibited genotype 47 for spacer PM2 and belonged to clade B based on *Cytb* analysis.⁴

Two clusters are shown: one cluster containing all of the African lice and another containing all of the non-African lice. Among the non-African lice, the Amazonian lice cluster

separately with some Mexican, British, and American lice (Figure 3).

This clustering is correlated with the two bases specific to the genotype 47. Another tree was constructed using the concatenated sequences of the four intergenic spacers S2, S5, PM1, and PM2. The dendrogram showed the same topology as the PM2 tree.

Among the non-African lice, the Amazonian lice are clustered together with four Mexican lice (Figure 4). This tree supports the hypothesis that the head lice in Amazonia represent an independent cluster.

DISCUSSION

In this study, we confirmed that the sequencing of the partial mitochondrial *Cytb* gene and multi-spacer typing

FIGURE 4. The phylogenetic organization of Amazonian lice (green), American lice (blue), European lice (red), Asian lice (yellow), and African lice (in black), determined using the maximum likelihood method. The tree was constructed based on the concatenated sequences of the four nuclear intergenic spacer regions, S2, S5, PM1, and PM2.

TABLE 1
Results of the multi-spacer typing of Amazonian head lice*

Locality	Subject	Age	Louse ID	S2 type	S5 type	PM1 type	PM2 type
Amazonas	F	12	1	NA	23/67	13	47
Amazonas	F	11	2	NA	NA	13	47
Amazonas	M	15	3	43	<u>69</u>	NA	47
Amazonas	F	17	4	NA	NA	NA	47
Amazonas	F	19	5	NA	<u>69</u>	13	47
Amazonas	F	11	6	43	<u>69</u>	13	47
Amazonas	F	13	7	NA	<u>69</u>	13	47
Amazonas	F	14	8	NA	<u>69</u>	13	47
Amazonas	F	10	9	NA	23	13	(-)PCR
Amazonas	M	9	10	43	<u>69</u>	13	(-)PCR
Amazonas	M	5	11	<u>117</u>	<u>69</u>	13	(-)PCR
Pará	F	6	12	NA	67	13	(-)PCR
Pará	M	13	13	43	<u>69</u>	13	47
Amapá	F	12	14	NA	<u>69</u>	13	47
Amapá	F	12	15	39	67	13	47
Amazonas	F	6	16	NA	23	NA	47
Amazonas	F	6	17	43	23	<u>36</u>	47
Amazonas	F	30	18	NA	67	<u>36</u>	47
Amazonas	F	8	19	40	67	<u>36</u>	NA
Amazonas	F	30	20	NA	67	<u>36</u>	47
Amazonas	F	3	21	<u>118</u>	23	13	47
Amazonas	F	6	22	<u>119</u>	23/ 67	NA	47
Amazonas	F	3	23	NA	NA	13	47
Amazonas	F	3	24	40	<u>68</u>	13	47
Amazonas	F	3	25	40	NA	13	47
Amazonas	F	6	26	NA	23	<u>36</u>	47
Amazonas	F	6	27	NA	23	<u>36</u>	47
Amazonas	F	8	28	43	23	<u>36</u>	47
Amazonas	F	8	29	43	23	NA	NA
Amazonas	F	3	30	NA	23	13	47

* Underlined and bold genotypes are new.

NA = not available; (-) = negative; PCR = polymerase chain reaction.

(MST) are sensitive methods for evaluating the genotypic distribution of human lice. The genotyping of the *Cytb* gene showed that all of the Amazonian head lice belonged to clade A. The MST results indicate that genotype 47 of the PM2 spacer is found among all of the clade A Amazonian lice and is associated with the clade B American and British lice.⁴ The phylogenetic tree based on the PM2 spacer and the concatenated sequences show a net separation of the Amazonian lice.

Clade A includes three subtypes of lice. The first subtype, A1, is the ubiquitous gray louse,⁷ the second subtype, A2, includes black African lice from Rwanda and Burundi,¹¹ and the third subtype, A3, is the American subtype that grouped the Amazonian brown head lice with the lice from Mexico and the United States.⁴

The biodiversity of body lice is lower compared with head lice (three clades for head lice versus only one clade for body lice). However, the head louse has proliferated in all countries with an uncontrolled expansion caused by globalization. The A3 subtype may date from the pre-Columbian era. Our previous study of the head lice from Peruvian mummies confirmed that the clade A lice were prevalent in America before the arrival of Columbus.⁷ These lice may date from the Clovis culture, which is presumed to be responsible for populating America¹⁴ or may provide from the third member of the *Pediculidae* family “*Pediculus humanus mjobergi* Ferris,” which is found on certain American monkeys of the family *Cebidae*.¹⁵ It seems that when Clovis penetrated the New World across the Bering Straits, these New World monkeys were present.¹⁶ Nevertheless, Maunders (1983) thinks that these monkeys, until then possessing no lice of their own, were able to acquire the human head louse, which developed into *P. h. mjobergi*. Amazonia is one of the few places in the world that has not been strongly affected by globalization, and these lice may be the descendants of a pre-Columbian population.

In conclusion, we confirmed the presence of a specific, likely pre-Columbian American louse genotype. Although it was interesting to test the lice from ancient mummies, the DNA extracted was insufficient for phylogenetic analysis. Collecting more louse samples from a wide variety of origins, particularly lice from the New World monkeys and ancient American mummies, such as the Andean mummy of Arica in Chile,¹⁷ for MST and *Cytb* analyses will help us to clarify the origin and the distribution of human lice in the Americas.

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Authors' addresses: Amina Boutellis, Aurélie Veracx, and Didier Raoult, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes: URMITE, Aix Marseille Université, Faculté de Médecine, Marseille, France, E-mails: amina.boutell@yahoo.fr,

veracxaurelie@hotmail.com, and didier.raoult@gmail.com. Jônatas Abrahão, Laboratório de Vírus, Departamento de Microbiologia, Universidade Federal de Minas Gerais, Minas Gerais, Brazil, E-mail: jonatas.abrahao@gmail.com.

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