Mitochondrial diversity and phylogeographic analysis of Pediculus humanus reveals a new Amazonian clade "F"

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Abstract

Pediculus humanus is an obligate and highly intimate bloodsucking insect parasite of humans that has two ecotypes, head louse and body louse. This study analyzed genetic diversity at three mitochondrial genes (*cytochrome b* [*cytb*], *cytochrome oxidase subunit 1* [*cox1*] and 12S ribosomal RNA [12S]) in 98 head lice collected from an isolated Native American population from the Wayampi community in Trois-Sauts, French Guiana. These results are integrated with all prior data of P. humanus (1402 cytb, 743 cox1 and 344 12S) from other parts of the world. The phylogenetic analysis revealed six highly divergent and well-supported monophyletic clades. Five clades corresponded to the previously recognized mitochondrial clades A, D, B, C and E, while the sixth (clade F) was novel, as it exhibited 5.4%, 3.7% and 3.6% divergence at *cytb*, *cox1* and 12S, respectively, from its nearest neighbor clade B. Interestingly, the clade F has only been recovered in a few lice sequences from Mexico and Argentina, while it was the most common lineage in the Amazonian lice, which hints its association with the Native American region. Furthermore, *Pediculus mjobergi*, a New World monkeys' louse, which is thought to be transmitted to monkeys from the first humans that had reached the American continent thousands of years ago, also belonged to this clade, suggesting that this louse may not be a separate species but an evolutionary lineage of *P. humanus*.

The discovery of new Amazonian clade F with the recovery of additional haplotypes within each of the five clades demonstrates that the levels of genetic diversity in *P. humanus* are higher than previously thought.

Keywords: *Pediculus humanus*; phylogeography; genetic diversity; Clade F; Amazonia

1. Introduction

Sucking lice (Phthiraptera: Anoplura) are obligate blood-feeding ectoparasites of placental mammals that have coevolved with their hosts for more than 65 million years (Durden and Musser, 1994; Light et al., 2010). Humans are parasitized by two species of sucking lice, the pubic louse (*Pthirus pubis*) and head/body lice (*Pediculus humanus*) (Reed et al., 2004). The association between the *P. humanus* and its human host can be traced back to at least 6 million years ago (MYa) to a common ancestor of humans and chimpanzees (Reed et al., 2004). *P. humanus* includes two ecotypes, head lice (*P. h. capitis*) and body lice (*P. h. humanus*), that are morphologically and biologically almost similar but ecologically distinct (Reed et al., 2004; Veracx and Raoult, 2012). Head lice are confined to the scalp and feed on human blood every 4–6 hours (Veracx and Raoult, 2012). Body lice live in clothing and feed less frequently but take larger blood meals than head lice (Veracx and Raoult, 2012). Aside from their role as pests (Chosidow, 2000), body lice are the main vectors of serious human pathogens; *Rickettsia prowazekii* (the causative agent of epidemic typhus), *Bartonella quintana* (trench fever), *Borrelia recurrentis* (relapsing fever) and probably *Yersinia pestis* (plague) (Raoult, 2016; Raoult and Roux, 1999). It was once believed that only body lice could transmit disease. However, recently several combined epidemiological and laboratory studies have strongly implicated head lice as a vector of infectious agents, although its vectorial capacity is lower as compared to body lice (Amanzougaghene et al., 2017, 2016a; Angelakis et al., 2011; Diatta et al., 2014; Goldberger and Anderson, 1912; Kim et al., 2017; Murray and Torrey, 1975; Sangaré et al., 2014).

The genetic diversity of human lice has been extensively studied using mitochondrial genes (mainly *cytochrome b* [*cytb*] and *cytochrome oxidase subunit 1* [*cox1*] genes) revealing the presence of five highly divergent mitochondrial clades (A, D, B, C and E) (Amanzougaghene et al., 2016b; Ascunce et al., 2013; Ashfaq et al., 2015; Drali et al., 2015;

Kittler et al., 2003; Reed et al., 2004). In addition to this inter-clade diversity, human lice also present intra-clade diversity, illustrated by several distinct haplotypes for each clade (Amanzougaghene et al., 2016a; Ascunce et al., 2013; Light et al., 2008). Body lice belong to clades A and D, while head lice encompass the full genetic diversity of clades (Amanzougaghene et al., 2016a; Drali et al., 2016; Light et al., 2008). Clade A is the most common and widely distributed across all continents, whereas the other clades are geographically restricted (Amanzougaghene et al., 2016b; Ascunce et al., 2013; Light et al., 2008; Raoult et al., 2008). Clade D is restricted to Africa and is found in the Democratic Republic of Congo (DR Congo), the Republic of Congo (Congo-Brazzaville), Ethiopia and Zimbabwe (Amanzougaghene et al., 2016a; Drali et al., 2015). Clade B is found in America, Europe, Australia, Algeria, South Africa, Saudi Arabia and has recently been found among the remains of Israeli head lice, which date back about 2,000 years (Al-Shahrani et al., 2017; Amanzougaghene et al., 2016b; Ascunce et al., 2013; Ashfaq et al., 2015; Boutellis et al., 2015; Light et al., 2008; Raoult et al., 2008). Clade C has been found in some African and Asian countries including Ethiopia, Congo-Brazzaville, Nepal, Pakistan and Thailand (Amanzougaghene et al., 2016a; Ashfaq et al., 2015; Kittler et al., 2003; Raoult et al., 2008; Sunantaraporn et al., 2015). Finally, clade E appears to be specific to West African lice, including Senegal and Mali, where it is highly prevalent, and has recently been identified in the head lice from Nigerian refugees arriving in Algeria and from migrant communities living in Bobigny, France (Amanzougaghene et al., 2017; Candy et al., 2018; Louni et al., 2018).

Lice are highly host specific and fast-evolving parasites that have evolved in tandem with their primate hosts for thousands of years (Light et al., 2008; Reed et al., 2007). Previous studies have shown that the time of divergence among lice clades (around 2 MYa) was far anterior to the time of modern human divergence, about 200,000 years ago (Light et al., 2008; Tishkoff et al., 2009). Previous reports have also suggested that different lice clades

have evolved on other archaic hominids, likely those known from 2.3 to 0.03 MYa (such as *Homo erectus*, Neandertal and Denisovan), and have only switched to modern humans during the recent period of overlaps (Ashfaq et al., 2015; Reed et al., 2004). Moreover, the presence of highly divergent clades and their geographical isolation can yield important information regarding the evolutionary history of the lice as well as their human hosts (Ascunce et al., 2013; Light et al., 2008). Therefore, a more detailed analysis of genetic diversity in *P. humanus* and current distributions of its major clades will provide a more detailed picture of evolutionary pattern of this parasite and will clarify additional events in human evolution.

In the present study, we obtained and analyzed head lice collected from an isolated Native American population from the Wayampi community in Trois-Sauts, French Guiana. These results are integrated with all prior mitochondrial data from over the world to expand perspectives on the number, distributions and diversification rates of clades of *Pediculus* lice.

2. Materials and methods

2. 1 Ethical approval

The Amazonian head lice were collected from infested Amerindians after obtaining their verbal consent or that of their legal representatives in the case of children, because most subjects were illiterate. Local authorities approved and were present at the lice collection. The study was validated by the ethics committee of the Institut Fédératif de Recherche 48, Faculty of Medicine, Marseille, France, under agreement12-017.

2. 2 Louse samples

The Amazonian head louse specimens were recovered in 2013 from Amerindians of the Wayampi community living in Trois-Sauts (2°15'0" N and 52°52'60" W, 122 m), a remote and isolated village in French Guyana. A total of 98 head lice were recovered from 22 individuals. No body lice were found during the examination. Collected lice were then preserved in 70% ethanol before being sent to our laboratory in Marseille (France). All the

126 samples were photographed with a camera (Olympus DP71, Rungis, France) prior molecular
127 analysis. In addition, a total of 327 louse specimens were also included in this study,
128 corresponding to body and head lice collected from several countries. These samples were
129 obtained from the private frozen collection of world lice belonging to our laboratory.
130 Additional supporting information including, collection locations and numbers of lice tested
131 are described in Table S1.

132 Moreover, because there were no available 12S sequences of New World monkey louse
133 *Pediculus mjobergi* in the GenBank database, we have also amplified the 12S gene for this
134 louse. In total three *P. mjobergi* lice were amplified in this study targeting 12S gene. These
135 samples were obtained from the same *P. mjobergi* collection, from our laboratory, that was
136 previously used by Drali et al. (Drali et al., 2016). More specifically, the louse specimens
137 were collected from a wild howler monkey, *Alouatta caraya*, (#B2188) from the Iguazú
138 National Park, Misiones Province (Drali et al., 2016).

139 **2. 3 DNA extraction, PCR amplification and sequencing**

140 Genomic DNA was isolated from louse specimens using the DNeasy tissue kit (Qiagen,
141 Courtaboeuf, France) as described previously (Amanzougaghene et al., 2017). Three
142 mitochondrial genes *cytb, cox1* and 12S ribosomal RNA were targeted for sequencing. PCR
143 amplifications were conducted in a Peltier PTC-200 thermal cycler (MJ Research Inc.,
144 Watertown, MA, USA) with the Hotstart Taq-polymerase (Qiagen) in accordance with the
145 manufacturer's instructions. The success of PCR amplification was then verified by
146 electrophoresis of the PCR product on a 1.5% agarose gels. All primers used for these
147 experiments and PCR conditions are described in Table 1. All PCR products were purified
148 using the PCR filter plate Millipore NucleoFast 96 PCR kit (Macherey-Nagel EURL, Hoerdt,
149 France) following the manufacturer's recommendations. The sequence reaction was carried
150 out using the Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems,

151 Foster City, CA) as per the manufacturer's instructions. Sequencing was performed with an
152 ABI Prism 3130xl Genetic Analyzer capillary sequencer (Applied Biosystems). Finally, all
153 the generated sequences were assembled and corrected using ChromasPro 1.7 software
154 (Technelysium Pty Ltd., Tewantin, Australia).

155 **2. 4 Sequence analysis.**

156 All the 98 Amazonian head louse sequences for the three mitochondrial genes (cytb,
157 cox1 and 12S) obtained in this study were combined with all the available mitochondrial
158 sequence data of *P. humanus* (1402 *cytb*, 743 *cox1* and 344 12S) from other parts of the
159 world to generate a global dataset to examine clade diversity in *P. humanus*. In addition, the
160 newly amplified 12S sequence of *P. mjobergi* in this study, as well as, seven *P. mjobergi*
161 sequences (six *cytb* and one *cox1* sequences) reported by Drali et al. (Drali et al., 2016), were
162 also included in the analysis (Table S2).

163 The DNA sequences obtained from the literature varied in length, so sequences were
164 trimmed to produce a dataset that maximized the number of sequences incorporated. The
165 sequences between nucleotide positions 433–705 of *cytb* (272-bps, according to Genbank
166 Accession KC685778), 748–1031 of *cox1* (283-bps, according to Genbank Accession
167 KC685838) and 109-666 of 12S (557-bps, according to Genbank Accession KC685877) were
168 used for analysis. ClustalW alignments were performed in MEGA6 (Tamura et al., 2013).
169 Haplotypes were identified using DnaSP v5.10 software (Librado and Rozas, 2009). Finally,
170 we created three datasets for which a total of 1500, 841 and 442 sequences were included,
171 respectively, for *cytb*, *cox1* and 12S (Tables S3-S5).

172 **2. 5 Phylogenetic analysis**

173 Neighbor-joining (NJ) analysis was performed in MEGA6 using the K2P model with
174 pairwise-deletion and 500 bootstrap replicates. The Maximum-likelihood (ML) analysis was
175 also performed in MEGA6 using the Kimura 2-parameter model for nucleotide sequences

under 500 bootstrap replicates. The subtree for each clade of lice was collapsed with the "compress/expand subtree" function. *Cytb, cox1* and 12S sequences from *P. schaeffi* (AY695999, KC241887, AY696067, KC241883 and KR706169) were employed as outgroups.

2. 6 Genetic diversity and haplotype analysis

For each dataset, population genetic indices including number of sequences (n), number of polymorphic sites (S), average number of pairwise nucleotide differences (k), nucleotide diversity (π), number of haplotypes (H) and haplotype diversity (Hd) were calculated using DNASP v5.10 software (Librado and Rozas, 2009). Kimura-2-parameter (K2P) pairwise distances among the *cytb*, *Cox1* and 12S haplotypes were calculated using MEGA6 with pairwise deletion of gaps and missing data (Tamura et al., 2013). Neutrality tests (Fu & Li's D and Tajima's D) were calculated with DNASP v5.10 (Librado and Rozas, 2009). In order to investigate the possible relationships between the haplotypes, networks haplotypes for each of the three genes were constructed with the median joining method of Bandelt available in NETWORK5.0 (www.fluxus-engineering.com/sharenet.htm) using equal weights for all mutations (Bandelt et al., 1999).

3. Results

A total of 98 head lice collected from 22 Amazonian individuals were analyzed, targeting three mitochondrial genes (*cytb*, *cox1* and 12S). We obtained 11 haplotypes of *cytb*, 8 haplotypes of *cox1* and 13 of 12S that were defined by 31, 25 and 41 polymorphic sites, respectively (Fig. S1-S4). The distribution of the head lice haplotypes identified in this study, according to mitochondrial genes, among the 22 infested Amazonian individuals are presented in Table S6. The generated Amazonian sequences (98 sequences for each gene) were then combined with all available sequences for *cytb*, *cox1* and 12S. The number of haplotypes in each dataset within each clade and their distributions were determined for each

gene. The details of the identified haplotypes, their GenBank accession numbers and geographic locations are described in Tables S3-S5.

In addition, six *cytb* and one *cox1 P. mjobergi* sequences reported by Drali et al. (Drali et al., 2016), as well as, the three *P. mjobergi* 12S sequences amplified in this study were also included in the analysis (Table S2).

For the *cytb* dataset, 1506 sequences were included (1500 sequences of *P. humanus* and 6 sequences of *P. mjobergi*) from which 105 haplotypes, including two haplotypes from *P. mjobergi*, were identified from 45 countries on five continents. For the *cox1* dataset, 842 sequences were included (841 sequences of *P. humanus* and one sequence of *P. mjobergi*) from which 57 haplotypes, including one haplotype from *P. mjobergi*, were identified from 27 countries on five continents. For the 12S dataset, 445 sequences were included (442 sequences of *P. humanus* and 3 sequences of *P. mjobergi*) from which 49 haplotypes, including one haplotype from *P. mjobergi*, were identified from 18 countries on five continents.

Neighbor-joining (NJ) and Maximum-likelihood (ML) analyses, including all haplotypes, were performed for each of the three mtDNA genes, consistently recovered six highly divergent and well-supported monophyletic clades (Fig. 2 and Fig. S5). Five clades corresponded to the mitochondrial clades previously recognized A, D, B, C and E, while the sixth was novel, here named “clade F”.

This novel clade consists mainly of Amazonian head lice (a total of 84 out of 98 [85.7%] Amazonian lice sequences belonged to this clade) as well as one haplotype from the New World monkey louse *P. mjobergi*, while the remaining 14 of the 98 (14.3%) Amazonian lice sequences belonged to clade A.

More precisely, for the 12S gene, the clade F consisted of nine haplotypes; eight haplotypes were for Amazonian lice (referred to here as F19-F26) whose F9 haplotype was

the most common (83.3% of 84 sequences), while the ninth haplotype was from *P. mjobergi*. For the *cox1* gene, 8 haplotypes were identified, six haplotypes were from Amazonian lice (referred to here as F29-F34), one haplotype named F18 consisted of sequences reported by Ascunce et *al.* (Ascunce et al., 2013) from Argentina (10 *cox1* sequences) and Mexico (2 *cox1* sequences), the eighth haplotype was *P. mjobergi.* Lastly, for the *cytb* gene, the clade F also included nine haplotypes; eight haplotypes were from Amazonian lice (named here F54 and F1-F7) of which haplotype F54 was the most widespread (84.3% of 89 sequences were from Amazonian head lice sequenced in this study, while 15.7% of 89 sequences also from Amazonian lice and were recovered from Genbank database, the remaining haplotype was from *P. mjobergi*. It is important to note that the second *cytb* haplotype of *P. mjobergi* has the *P. humanus'* haplotype A5 within clade A (see Drali et al., 2016).

The median-joining networks for all *cytb*, *cox1* and 12S haplotypes corroborated the neighbor joining and Maximum-likelihood phylogenetic reconstructions, with all the recovered haplogroups forming separate clusters represented by six connected subnetworks corresponding to clades A, D, B, C, E and "F" (Fig. 3-5).

The maximum intra-clades distances at *cytb* were 1.2%, 1.9%, 1.4%, 1.4%, 1.5% and 1.1% for clades A (n= 34 haplotypes), D (n= 17), B (n= 9), C (n= 13), E (n= 23) and "F" (n= 8), respectively. The maximum intra-clades distances at *cox1* were 1.2%, 1.0%, 1.3%, 1.3%, 1.3% and 1.1% for clades A (n= 17), D (n= 7), B (n= 12), C (n= 6), E (n= 7) and "F" (n= 7), respectively. The maximum intra-clades distances at 12S were 0.9%, 1.6%, 0.4%, 1.4%, 0.3% and 0.5% for clades A (n= 15), D (n= 8), B (n= 3), C (n= 10), E (n= 4) and "F" (n= 8), respectively. The nearest neighbors (NN) distances between clades and the nodal supports are presented in Fig. 2A (*cytb*), Fig. 2B (*cox1*) and Fig. 2C (12S). The new clade "F" shows divergence from its NN clade B of 5.4%, 3.7% and 3.6%, respectively, at *cytb, cox1* and 12S.

Estimates of genetic diversity indices and the results of neutrality tests for *cytb*, *cox1* and 12S are presented in Table 2. The average number of nucleotide diversity (π), pairwise nucleotide differences (k) and haplotype diversity (Hd) varied among the clades of three genes. The highest haplotype diversity was found within clade D in both *cytb* and 12S genes (Hd= 0.831 and 0.899, respectively, in cytb and 12S), while in *cox1* gene, the highest haplotype diversity was found within clade B (Hd = 0.899). Overall, both (k) and (π) were similar in *cytb, cox1* and 12S.

4. Discussion

In this study, we analyzed the genetic diversity of head lice collected from Amazonian individuals of the Wayampi community living in Trois-Sauts, a remote and isolated village. In total, three mitochondrial genes were targeted (*cytb*, *cox1* and 12S). By coupling these results with all available mitochondrial data of *P. humanus* from 45 countries, the present study has expanded understanding of its levels, sequence divergence pattern and revealed higher levels of mtDNA diversity in *P. humanus* corroborating results reported by others (Ashfaq et al., 2015). To the best of our knowledge, this is the most geographically widespread study to evaluate the mitochondrial genetic diversity in human lice based on three mtDNA genes. Previous studies on *P. humanus* showed that the maximum distances within clades were 1.4% at *cytb* and 1.9% at *cox1*, while the NN distances at these genes were 4.6% and 2.3%, respectively (Ashfaq et al., 2015). In the present study, the maximum distances within clades were almost similar (*cytb* 1.9%, *cox1* 1.3% and 1.6% 12S), while NN distances were higher (*cytb* 5.6% and *cox1* 6.5%). These results reflect the extended geographic coverage and the large sample sizes, which allowed the recovery of additional clades and haplotypes within each clade.

Three phylogenetic methods (NN, ML and MJ) at three mtDNA genes, have consistently recovered six highly divergent and well-supported monophyletic clades. Five

clades corresponded to the previously recognized mitochondrial clades A, D, B, C and E (Amanzougaghene et al., 2016b; Ashfaq et al., 2015), while the sixth (clade F) was novel, consisting mainly of head lice from Amazonian individuals analyzed in this study and 12 sequences of head lice from Argentina and Mexico reported by Ascunce et *al*. (Ascunce et al., 2013). In that study, the authors found these sequences to be highly derived B-haplotypes that are separated from the main group by more than seven mutational steps (Ascunce et al., 2013).

Interestingly, the *P. mjobergi* 12S and *cox1* haplotypes, as well as, one of the two *P. mjobergi cytb* haplotypes previously classified within clade B by Drali et *al.*, (Drali et al., 2016) belong to this new clade F. This result suggests that *P. mjobergi* may not be a distinct species of *P. humanus*, but probably an evolutionary lineage of *P. humanus* species within clades A and F. A similar suggestion was offered by Drali et *al.* (Drali et al., 2016). Indeed, these authors argued that *P. mjobergi* was originally a human louse and was switched to new world monkeys from the first humans who reached the American continent thousands of years ago (Drali et al., 2016). Further studies are needed to confirm this hypothesis.

Clade "F" shows a divergence from its NN clade B of 5.4%, 3.7% and 3.6%, respectively, at c*ytb*, *cox1* and 12S. Clade B was first described in contemporary lice in the American continent, where it was highly prevalent and diversified (Ascunce et al., 2013; Light et al., 2008; Reed et al., 2004). This discovery and its identification in the lice of pre-Columbian mummies led researchers to deduce initially an American origin for this clade (Boutellis et al., 2013). However, its recent discovery among head lice remains from Israel, dating back about 2,000 years, has challenged this hypothesis (Amanzougaghene et al., 2016b). In that study, the authors strongly argued in favor of an Asian origin of clade B, that resulted probably from a recent host switch from Neanderthals or Denisovans to modern humans which was followed by its introduction into the New World with the migration of

early peoples (Amanzougaghene et al., 2016b; Ascunce et al., 2013; Light et al., 2008; Reed et al., 2004). Because clade F was found only in head lice from Mexico and Argentina and it was the dominant lineage found in the Amazonian lice, knowing that Amazonia is one of the few places in the world that has not been strongly affected by globalization. This clade F may be the descendants of a pre-Columbian population and was derived from clade B brought by the first humans who had reached the American continent via the Bering straits thousands of years ago, thus representing Native America louse mitochondrial diversity. Interestingly, Ascunce et *al*. (Ascunce et al., 2013) have also proposed a similar suggestion for their sequences from Mexico and Argentina that we have reclassified here as members of clade F.

Previous studies reported that clade A is the most common and has a global distribution (Amanzougaghene et al., 2017; Ascunce et al., 2013; Ashfaq et al., 2015), results supported by its detection in approximately 46% of the analyzed lice from 49 countries on the five continents. Furthermore, the clade A subnetworks at the three analyzed mitochondrial genes (Fig. 3-5) were star-like in structure, combined with its significant negative Tajima's D value (-2.322, -1.866 and -2.055, respectively at *cytb*, *cox1* and 12S; $P<0.05$), indicating the signature of population expansion for this clade (Aris-Brosou and Excoffier, 1996), and corroborating the results reported by others (Ascunce et al., 2013; Reed et al., 2004). In addition, Reed et al. (Reed et al., 2004) estimated that the demographic expansion of this clade occurred about 100,000 years ago, coinciding with the out-of-Africa expansion of *Homo sapiens*, thus reflecting a codemographic pattern between lice and humans (Ascunce et al., 2013; Reed et al., 2004). Clade D (referred as clade E in Ashfaq et al. 2015) diverged from clade A between 0.37 and 0.54 MYa (Ashfaq et al., 2015) and is restricted to Central Africa including DR Congo and Congo-Brazzaville, where it was detected mainly among indigenous Pygmy populations (Amanzougaghene et al., 2016a; Drali et al., 2015). This clade

has also been reported in lice in Ethiopia (Amanzougaghene et al., 2016a) and we identified in this study its occurrence, for the first time, in body lice from Zimbabwe.

Our sampling did not encounter any new specimens of clade C, so it remains restricted to Africa and Asia (Amanzougaghene et al., 2016a; Ascunce et al., 2013; Light et al., 2008; Sunantaraporn et al., 2015). Given its early divergence in the *Pediculus* tree around 2 MYa, this clade may have evolved on archaic hominids in Asia or Africa such as *H. erectus* (Light et al., 2008; Reed et al., 2004). Lastly, clade E (referred as clade D in Ashfaq et al, 2015) diverged from the MRCA of clade C between 0.28 and 0.42 MYa (Ashfaq et al., 2015). This clade consists of head lice from West Africa including Senegal and Mali where it was highly prevalent (Amanzougaghene et al., 2017). Its recent detection in the head lice of Nigerian refugees arriving in Algeria and migrant communities living in Bobigny (France) is probably the result of a recent migratory flow from West African countries (Candy et al., 2018; Louni et al., 2018).

5. Conclusion

Our study underlines the importance of the use of mitochondrial genes in the analysis of phylogeographic patterns and genetic diversity of *P. humanus*. Six highly divergent and well-supported monophyletic clades were identified. Five clades corresponded to the previously recognized mitochondrial clades A, D, B, C and E, while the sixth "clade F'' was novel. The new clade F was found mainly in Amazonia, where it is also shared with the monkey louse *P. mjobergi* and could therefore represent Native American louse mitochondrial diversity.

The recovery of additional haplotypes within each of the five clades (A, D, C, E and B) along with the discovery of new clade F, demonstrate that levels of genetic diversity in *P.*

humanus is higher than previously thought, reinforcing the importance of continuing to survey and phylogeographically characterize human lice.

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Figure captions

Figure 1. Map showing the head lice collection site from the Amerindians of the Wayampi community living in Trois-Sauts. A) Geographical localization of louse sampling. B) Amerindian children infested with head lice. C) Human head lice from Trois-Sauts. ♀ female; ♂ male; nymphs and nits.

Figure 2. Neighbor-joining cluster analysis of *Cytb* (A), *Cox1* (B) and 12S (C) haplotypes of *P. humanus*. Bootstrap values (500 replicates) are shown above the branches. The scale bar indicates K2P distances. The node for each clade with multiple haplotypes is collapsed to a vertical triangle, with the horizontal depth indicating the level of intra-clade divergence. Bracketed numbers next to each clade name indicate the number of haplotypes analyzed and the average intra-clade distance. Analyses were conducted in MEGA6

Figure 3. *Cytb* haplotype networks of human body and head lice. Each circle indicates a unique haplotype and variations in circle size are proportional to haplotype frequencies. Pie colors and sizes in circles represent the continents and the number of their sequences for a haplotype. *P. mjobergi* that belong to the haplotype A5 are included in the portion representing human lice from America.

Figure 4. *Cox1* haplotype networks of human body and head lice. Each circle indicates a unique haplotype and variations in circle size are proportional to haplotype frequencies. Pie

colors and sizes in circles represent the continents and the number of their sequences for a haplotype

Figure 5. 12S haplotype networks of human body and head lice. Each circle indicates a unique haplotype and variations in circle size are proportional to haplotype frequencies. Pie colors and sizes in circles represent the continents and the number of their sequences for a haplotype

Table 1. Primer sequences used in this study

Target gene	Primer name	Primer sequences (5′-3′)	Product size (bp)	Tm	source
Cytochrome b	cytbF	GAGCGACTGTAATTACTAATC	348	56°C	(Li et al., 2010)
	cytbR	CAACAAAATTATCCGGGTCC			
Cytochrome oxidase subunit 1	cox1F	GGAGTGAGTTCGATTTTAG	828	55°C	This study
	cox1R	GTGCTGAGGAAAGAAAGTC			
12S ribosomal RNA	12SF	CAGCACTAGCGGTCATACAT	596	56°C	This study
	12SR	AATGACGGGCGATATGTAC			

Table 2. Analysis of genetic diversity indices and neutrality tests (Fu & Li's D and Tajima's D) on mitochondrial *cytb*, *cox1* and 12S sequences

	n	S	K	π	h	Hd	Fu & Li's D	Tajima's D
Cytb all	**1500**	96	20.341	0.075	**105**	0.999	-2,878 (P <0.05) S*	-0.172 (P> 0.1) NS
Clade A	769	33	3.098	0.011	34	0.750	-3.751 (P <0.02) S**	-2.322 (P < 0.01) S**
Clade D	69	20	5.044	0.018	17	0.831	-1.425 (P> 0.1) NS	-0.583 (P> 0.1) NS
Clade B	200	13	3.694	0.013	9	0.789	-1.633 (P> 0.1) NS	-1.357 (P> 0.1) NS
Clade F	104	11	2.750	0.010	9	0.700	-1.924 (P < 0.05) S*	-1.757 (P < 0.05) S*
Clade C	205	16	3.744	0.014	13	0.792	-1.471 (P> 0.1) NS	-1.151 (P> 0.1) NS
Clade E	153	26	4.075	0.015	23	0.803	-2.747 (P < 0.05) S*	-1.752 (P > 0.05) NS
COI all	**842**	67	17.255	0.061	**57**	0.946	-0.065 (P > 0.1) NS	0.457 (P> 0.1) NS
Clade A	443	21	3.294	0.012	17	0.769	-2.244 (P > 0.05) NS	-1.866 (P < 0.05) S*
Clade D	48	8	2.762	0.010	7	0.739	-0.971 (P > 0.1) NS	-1.318 (P> 0.1) NS
Clade B	157	12	3.697	0.013	12	0.786	-0.564 (P > 0.1) NS	-0.292 (P > 0.1) NS
Clade F	139	8	2.952	0.010	8	0.738	-0.473 (P > 0.1) NS	-0.503 (P > 0.1) NS
Clade C	25	8	3.667	0.012	6	0.782	0.457 (P > 0.1) NS	0.274 (P > 0.1) NS
Clade E	30	8	3.524	0.012	7	0.781	0.321 (P > 0.1) NS	0.415 (P > 0.1) NS
12S all	**442**	110	26.928	0.048	**49**	0.964	-0.332 (P > 0.1) NS	-0.130 (P > 0.1) NS
Clade A	145	30	4,886	0.009	15	0.830	-2.502 (P < 0.05) S*	-2.055 (P < 0.05) S*
Clade D	62	23	8.821	0.016	8	0.899	0.337 (P > 0.1) NS	-0.029 (P > 0.1) NS
Clade B	36	3	0.271	0.001	3	0.679	-0.319 (P > 0.1) NS	-1.399 (P > 0.1) NS
Clade F	87	10	2.893	0.005	9	0.746	-1.612 (P > 0.1) NS	-1.589 (P > 0.05) NS
Clade C	69	24	7.622	0.014	10	0.885	-0.096 (P > 0.1) NS	-0.483 (P > 0.1) NS
Clade E	43	3	1.500	0.003	4	0.606	-0.754 (P > 0.1) NS	-0.754 (P > 0.1) NS

n: number of sequences; S: number of polymorphic sites; k: average number of pairwise nucleotide differences; π: nucleotide diversity; h: number of haplotypes; Hd: haplotype diversity. Tajima's D: A negative Tajima's D signifies an excess of low frequency polymorphisms relative to expectation. A positive Tajima's D signifies low levels of both low and high frequency polymorphisms. Statistical significance: Not significant, $P > 0.10$.

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Supporting Information

Figure S1. (A) Cytb sequences alignment and (B) DNA sequence chromatograms of clade F haplotypes identified in this study showing the polymorphic sites.

Figure S2. (A) Cox1 sequences alignment and (B) DNA sequence chromatograms of clade F haplotypes identified in this study showing the polymorphic sites.

Figure S2. (A) 12S sequences alignment and (B) DNA sequence chromatograms of clade F haplotypes identified in this study showing the polymorphic sites.

Figure S4. (A) 12S sequences alignment of clade F haplotypes and (B) 12S DNA sequence chromatogram of P. mjobergi amplified in this study.

Figure S5. Maximum-likelihood (ML) analysis of *Cytb* (A), *Cox1* (B) and 12S (C) haplotypes of *Pediculus humanus*. Bootstrap values (500 replicates) are shown above the branches. The scale bar shows K2P distances. The node for each clade with multiple haplotypes is collapsed to a vertical triangle, with the horizontal depth indicating the level of intra-clade divergence. Bracketed numbers next to each clade's name indicate the number of haplotypes analyzed and the average intra-clade distance. Analyses were conducted in MEGA6.

Table S1. Additional louse specimens included in this study, obtained from the private frozen collection of world lice belonging to our laboratory.

Table S2. *Pediculus mjobergi* sequences from new world monkey (Alouatta caraya) included in this study. The cox1 and cytb *P. mjobergi* sequences analyzed in this study were those reported by Drali et al. (2016) collected from two monkey individuals B2188 and B1395. The 12S sequences were amplified in this study from three *P. mjobergi* specimens from monkey individual B2188.

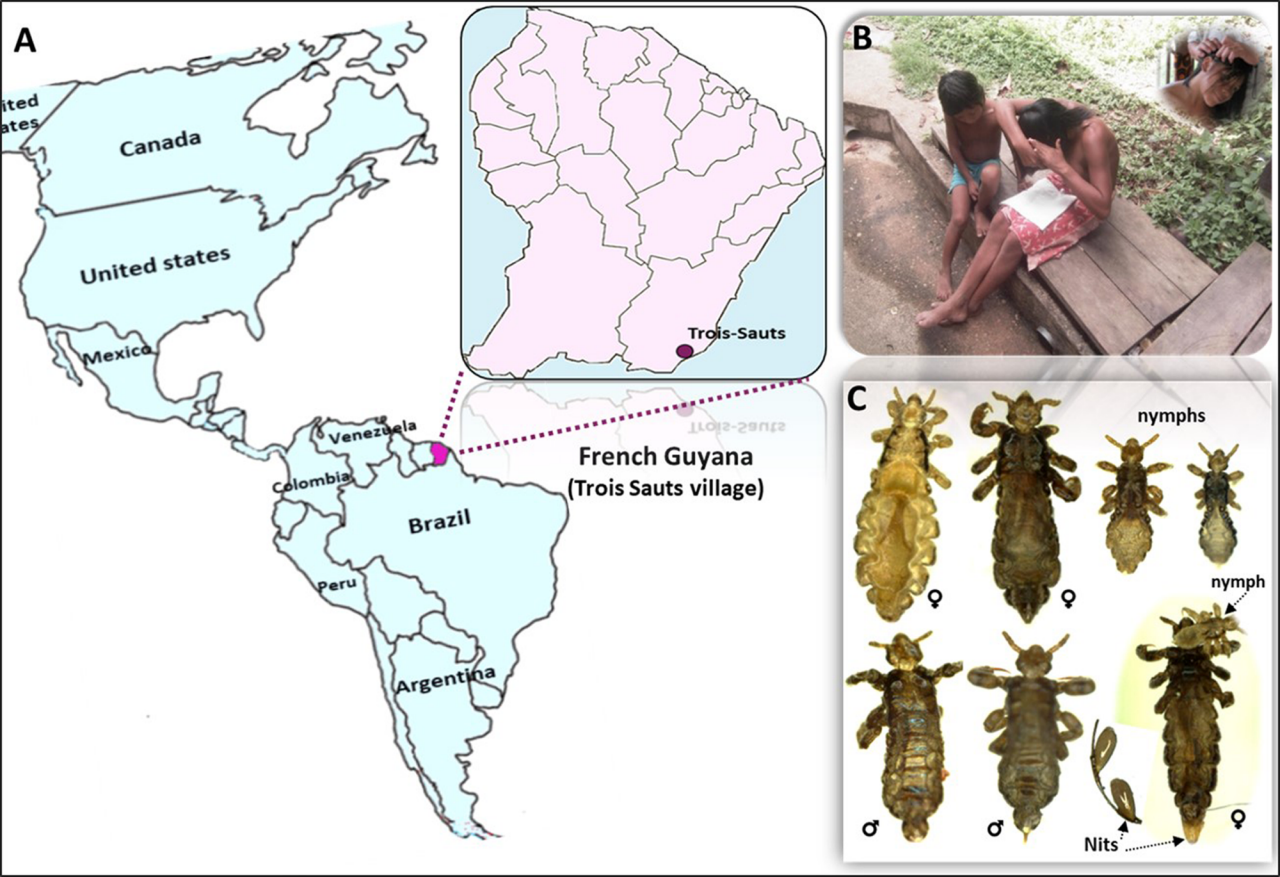
572 **Table S3. Geographic occurrences and frequencies of *cytb* haplotypes of human head**
573 **and body lice.**

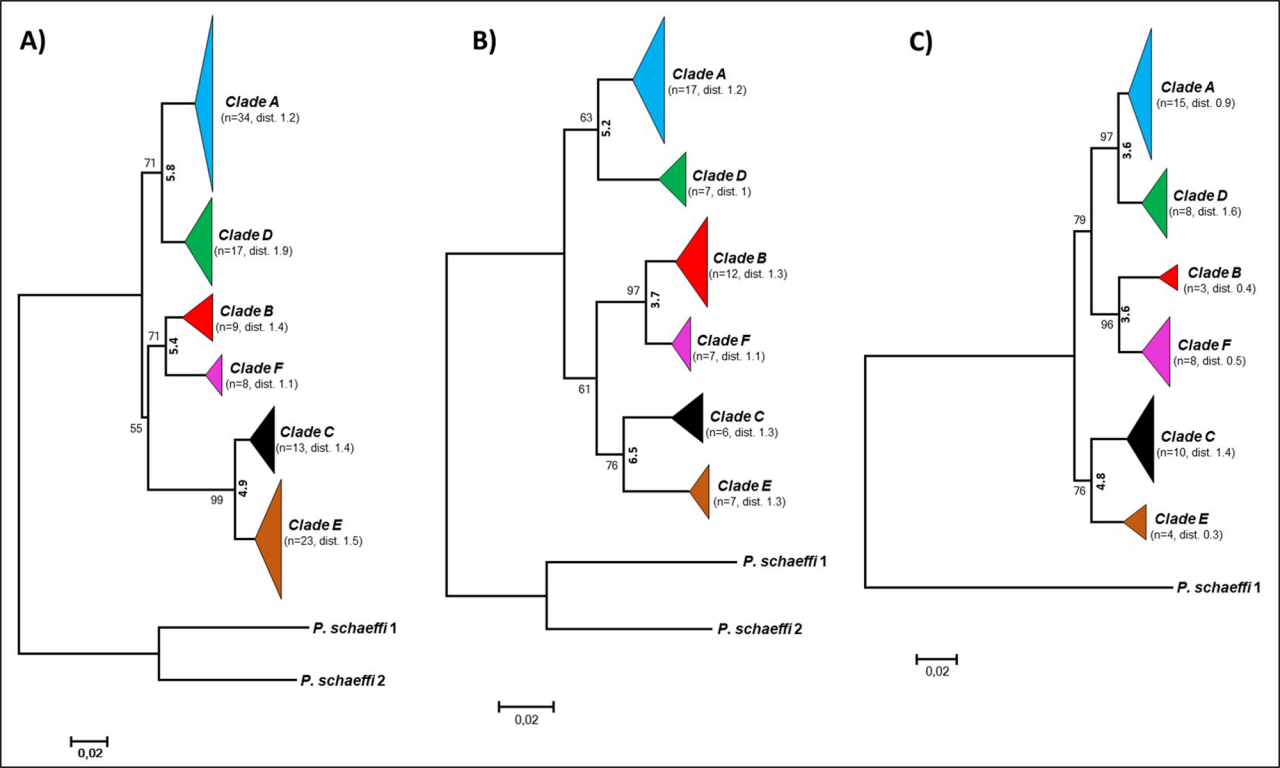
574 **Table S4. Geographic occurrences and frequencies of *cox1* haplotypes of human head**
575 **and body lice.**

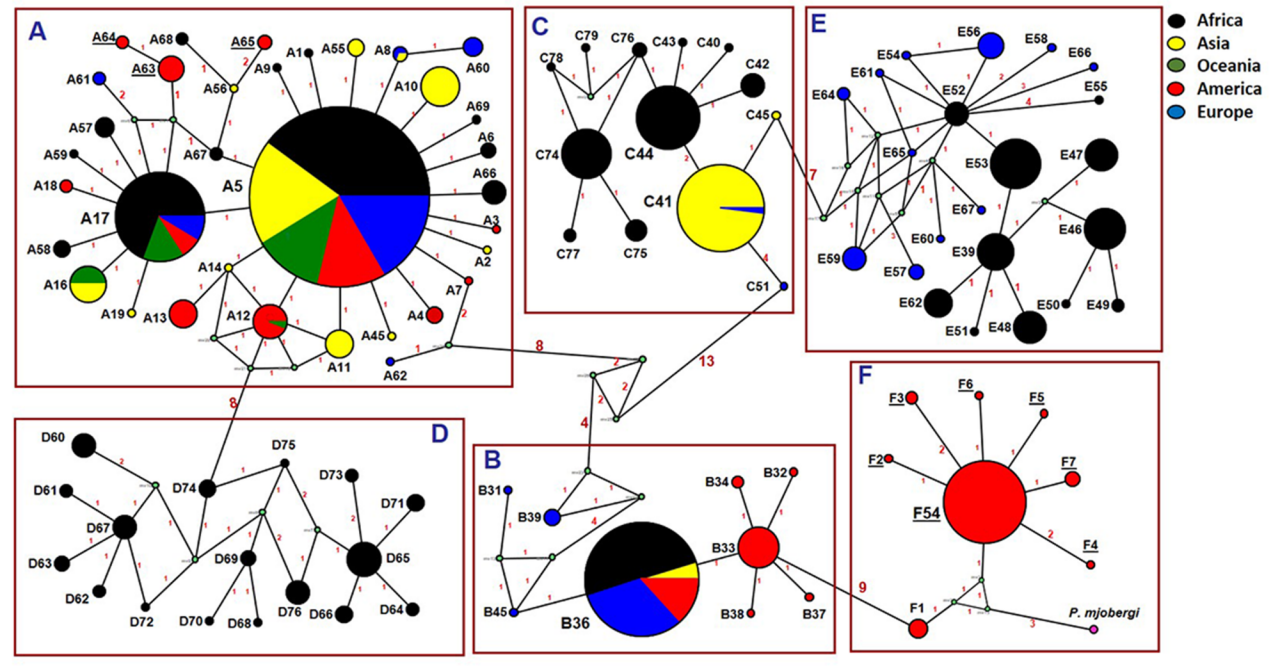
576 **Table S5. Geographic occurrences and frequencies of 12S haplotypes of human head**
577 **and body lice.**

578 **Table S6. Distribution of the head lice haplotypes identified in this study, according to**
579 **mitochondrial genes, among the 22 infested Amazonian individuals.**

580


A
Canada
United states
Mexico
Venezuela
Colombia
Brazil
Peru
Argentina
Trois-Sauts
French Guyana
(Trois Sauts village)
B
C
nymphs
nymph
Nits


A)
Clade A
(n=34, dist. 1.2)
71
5.8
Clade D
(n=17, dist. 1.9)
Clade B
(n=9, dist. 1.4)
71
5.4
Clade F
(n=8, dist. 1.1)
55
Clade C
(n=13, dist. 1.4)
99
4.9
Clade E
(n=23, dist. 1.5)
P. schaeffi 1
P. schaeffi 2
0,02
B)
Clade A
(n=17, dist. 1.2)
63
5.2
Clade D
(n=7, dist. 1)
Clade B
(n=12, dist. 1.3)
97
3.7
Clade F
(n=7, dist. 1.1)
61
Clade C
(n=6, dist. 1.3)
76
6.5
Clade E
(n=7, dist. 1.3)
P. schaeffi 1
P. schaeffi 2
0,02
C)
Clade A
(n=15, dist. 0.9)
97
3.6
Clade D
(n=8, dist. 1.6)
79
Clade B
(n=3, dist. 0.4)
96
3.6
Clade F
(n=8, dist. 0.5)
Clade C
(n=10, dist. 1.4)
76
4.8
Clade E
(n=4, dist. 0.3)
P. schaeffi 1
0,02


A
A64
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A6
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A5
A3
A17
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A2
A14
A16
A7
A19
A13
A12
A4
A45
A11
A62
C
C79
C78
C76
C43
C40
C42
C45
C74
C44
C41
C77
C75
C51
E
E56
E54
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E48
Africa
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Oceania
America
Europe
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D76
D66
D64
B
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B32
B34
B39
B33
B45
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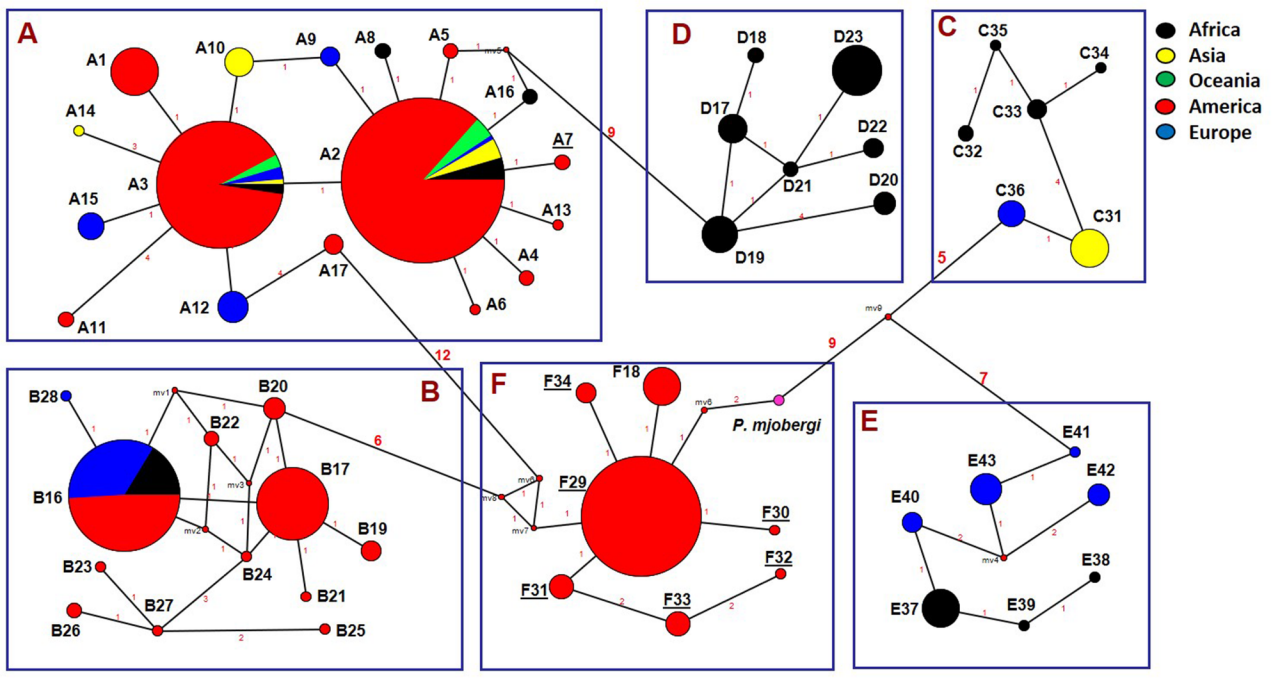
F6
F5
F2
F7
F54
F4
9
F1
P. mjobergi


A
A1
A10
A9
A8
A5
mv5
A16
A14
A7
A2
A3
A15
A13
A4
A17
A12
A6
A11
9
D
D18
D23
D17
D22
D21
D20
D19
C
C35
C34
C33
C32
C36
C31
5
Africa
Asia
Oceania
America
Europe
mv0
9
7
12
B
B28
mv1
B20
B22
6
B17
B16
mv3
mv2
B19
B23
B24
B21
B27
B26
B25
F
F34
F18
mv8
P. mjobergi
mv0
F29
mv6
mv7
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F32
F31
F33
E
E41
E43
E42
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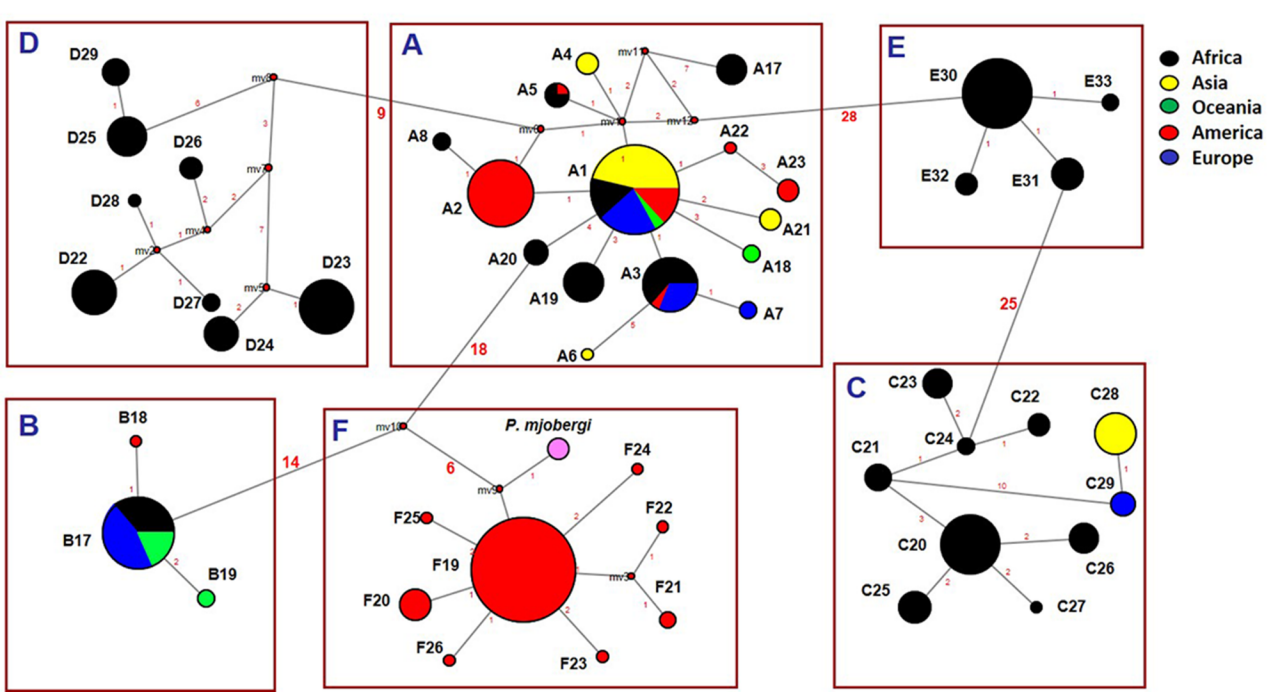
mv4
E38
E37
E39


D
D29
D25
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E
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E32
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Africa
Asia
Oceania
America
Europe
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25
14
B
B18
B17
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F
P. mjobergi
6
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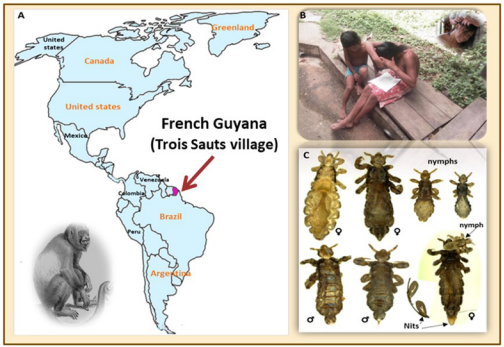
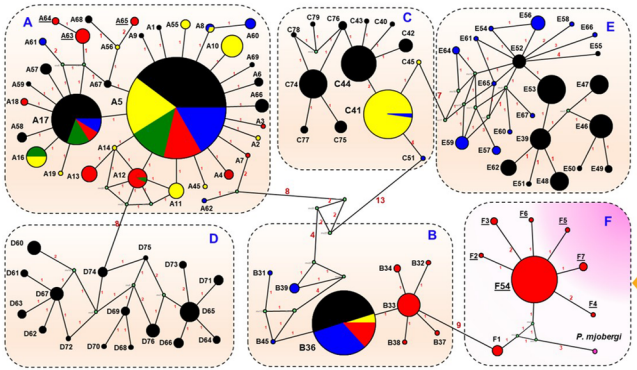
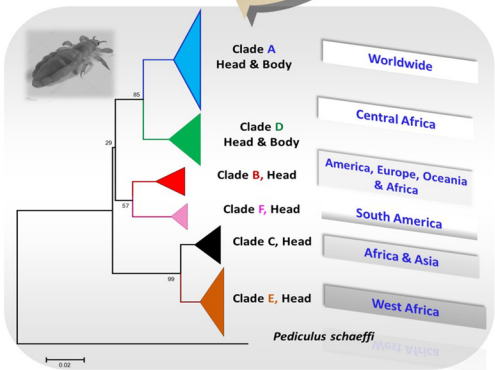


Head lice from an isolated Native American population were analyzed and integrated with all prior data of *P. humanus* from over the world

⇒ New World monkey louse, *P. mjobergi* , was also included in the analysis





Phylogeny based on mitochondrial genes