Medical and Veterinary Entomology

Medical and Veterinary Entomology (2015) **29**, 94–98 doi: 10.1111/mve.12058

SHORT COMMUNICATION

Evidence that clade A and clade B head lice live in sympatry and recombine in Algeria

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Abstract. *Pediculus humanus* L. (Psocodea: Pediculidae) can be characterized into three deeply divergent lineages (clades) based on mitochondrial DNA. Clade A consists of both head lice and clothing lice and is distributed worldwide. Clade B consists of head lice only and is mainly found in North and Central America, and in western Europe and Australia. Clade C, which consists only of head lice, is found in Ethiopia, Nepal and Senegal. Twenty-six head lice collected from pupils at different elementary schools in two localities in Algiers (Algeria) were analysed using molecular methods for genotyping lice (*cytochrome b* and the multi-spacer typing (MST) method. For the first time, we found clade B head lice in Africa living in sympatry with clade A head lice. The phylogenetic analysis of the concatenated sequences of these populations of head lice showed that clade A and clade B head lice had recombined, suggesting that interbreeding occurs when lice live in sympatry.

Key words. *Pediculus humanus capitis*, clade A, clade B, genotype, recombination, sympatry.

There are three recognized taxa of lice that feed on humans: the pubic louse *Pthirus pubis* (Phthiraptera: Pthiridae), the clothing louse *Pediculus humanus humanus*, and the head louse *Pediculus humanus capitis* (Reed *et al.*, 2004). Each type has its own distinct ecology and morphology (Veracx *et al.*, 2012a). Most of the genetic studies on human lice have been performed using cytochrome oxidase subunit 1, COXI, and cytochrome b, suggesting that the divergence of the three mitochondrial clades (A, B and C) occurred approximately 2 m.y.a. (Reed *et al.*, 2004; Ascunce *et al.*, 2013). Each clade has a unique geographical distribution (Raoult *et al.*, 2008). The most common mtDNA haplogroup is found in both head and clothing lice (type A) and is distributed worldwide (Raoult *et al.*, 2008). The second mtDNA group (type B) occurs only in head lice and has been found in the New World, including in America and Australia, as well as in western Europe, but not in Africa or Asia (Leo & Barker, 2005; Raoult *et al.*, 2008). The third type (type C) has been found only among head lice from Nepal (Reed *et al.*, 2004), Ethiopia (Angelakis *et al.*, 2011) and Senegal (Boutellis *et al.*, 2012).

Recently, two phylogenetic studies of clade A head and clothing lice based on four intergenic spacers have been reported. The first study evaluated the genotypic distribution of 207 human lice and identified two clusters in France, one cluster in central Africa and one cluster in Russia (Li *et al.*, 2010). A second study identified two clusters of lice: worldwide lice (A1), and sub-Saharan African lice (A2) (Veracx *et al.*, 2012b). However, no genetic study has been performed in North African lice. In the present study, *cytb* and multi-spacer typing (MST) were used to elucidate the genotypic distribution of head lice collected from two different locations in Algiers in Algeria.

An epidemiological investigation was conducted by the Service d'Ecologie des Systèmes Vectoriels in Algiers in September 2011 in Algiers elementary schools to estimate the prevalence of

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Table 1. The name, size and primer sequences used for the amplification of the four intergenic spacers and the cytochrome b gene.

Name	Forward primer (5′–3′)	Reverse primer (59–39)	Size, bp
S2	ATGATGTGCATTGCGAGTGT	AAACTTAACCCGGGCCCTAT	488
S5	TCCAAATGAAACCCACACTTT	TGGCAGACACTGCTTCCTTA	492
PM1	GAAATAATATCCAACCTCGTTCA	CATTCTTCCTCATCAAGCTGC	217
PM2	CCGAAGGAGCTGATTCTTTT	CCACAACGAGTGATGTGAGC	437
Cytb	GAGCGACTGTAATTACTAATC	CAACAAAATTATCCGGGTCC	360

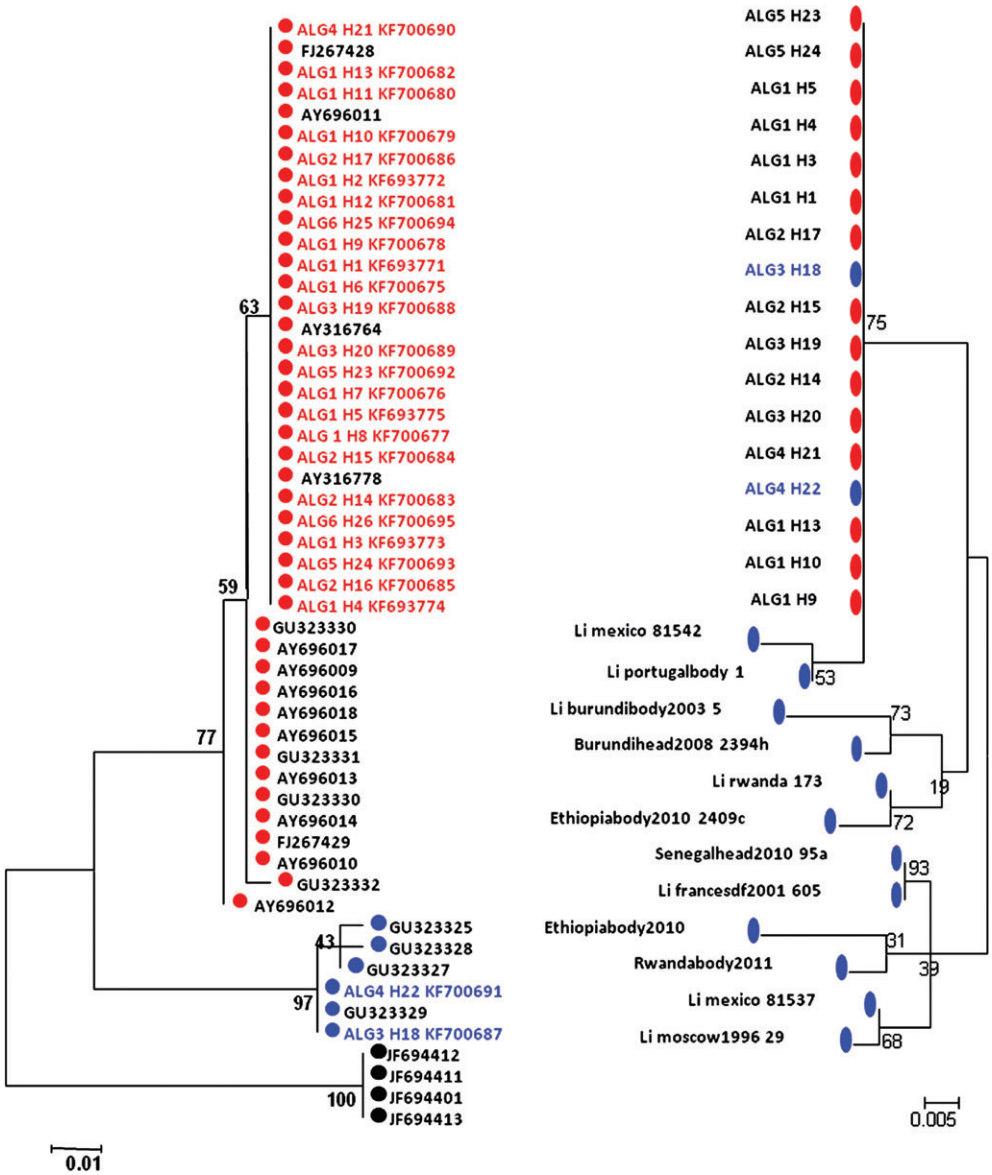

Fig. 1. A comparison of the topologies of the *cytb* and multi-spacer typing (MST) concatenated sequence trees. To facilitate the comparison of the two trees, we used only clade A lice for which both the MST and cytochrome b sequences were available. The *cytb* phylogenetic tree (left) and the MST concatenation tree (right) show recombination events between clade A (blue) and clade B (red) Algerian head lice.

Table 2. List of GenBank accession numbers for each sequence from each host.

GenBank accession no.	Head/body	*Cytb*
GU323325	Body louse	Clade A
GU323327	Body louse	Clade A
GU323328	Body louse	Clade A
GU323329	Body louse	Clade A
FJ267428	Head louse	Clade B
AY316764	Head louse	Clade B
AY316778	Head louse	Clade B
GU323330	Head louse	Clade B
AY696017	Head louse	Clade B
AY696009	Head louse	Clade B
AY696016	Head louse	Clade B
AY696018	Head louse	Clade B
AY696015	Head louse	Clade B
GU323331	Head louse	Clade B
AY696013	Head louse	Clade B
GU323330	Head louse	Clade B
AY696014	Head louse	Clade B
FJ267429	Head louse	Clade B
AY696010	Head louse	Clade B
GU323332	Head louse	Clade B
AY696012	Head louse	Clade B
JF694412	Head louse	Clade C
JF694411	Head louse	Clade C
JF694401	Head louse	Clade C
JF694413	Head louse	Clade C

head lice infestation. After informed consent was obtained from the parents of students, 26 head lice were collected by visual screening of the scalp and hair of schoolchildren at schools in two different locations in Algiers.

In the locality of El Madania, we collected 22 head lice from four different subjects attending three different schools. In the locality of Tessala El Merdja, we collected four head lice from two subjects (two head lice per person) at two different schools. The lice were preserved dry in Eppendorf tubes and sent to the laboratory in Marseilles. Before DNA isolation, each louse was rinsed twice in sterile water for 15 min and then dried on sterile filter paper. Each specimen was longitudinally cut into two equal halves. Then, total genomic DNA was extracted with a QIAamp Tissue Kit (Qiagen SAS, Courtaboeuf, France) with the EZ1 apparatus, as described by the manufacturer. Extracted genomic DNA was stored at $-20\,^{\circ}C$ under sterile conditions to avoid cross-contamination until further processing.

The mitochondrial gene *cytb* (270 bp) and each intergenic spacer (S2, S5, PM1 and PM2) were amplified from louse samples with a PTC-200 automated thermal cycler (MJ Research, Inc., Waltham, MA, U.S.A.), as described previously (Li *et al.*, 2010). The success of the polymerase chain reaction (PCR) amplification was verified by the migration of the product in 2% agarose gel. The products were then purified using a NucleoFast 96 PCR plate (Macherey-Nagel EURL, Hoerdt, France), as recommended by the manufacturer. Purified PCR products were sequenced using the primers used for the PCR (Li *et al.*, 2010) (Table 1) using the BigDye Terminator Version 1.1 Cycle Sequencing Ready Reaction Mix (Applied Biosystems, Inc., Foster City, CA, U.S.A.) with an ABI 31000 automated sequencer (Applied Biosystems, Inc.). The sequences were assembled and analysed with ChromasPro Version 1.34 (Technelysium PTY, Australia) and BLAST to identify the genotype. When results showed homology of less than 100%, a new genotype was recorded and a new number attributed to the sequence.

Unrooted phylogenetic trees were obtained from DNA sequences using the maximum likelihood (ML) method. The trees were constructed with 100 bootstrap replicates. The sequences of the selected intergenic spacers were concatenated and all of them were represented in a phylogenetic tree. All the obtained sequences were deposited on the GenBank (Table 2).

Overall, 26 head lice obtained from six schoolchildren studying in five different schools in two localities in Algiers were tested. After correction and assembly of the *cytb* sequences obtained from these head lice, we were surprised to observe that the phylogenetic tree classed most head lice collected in Algiers among clade B. This is the first evidence of the presence of clade B in Africa. Two lice (ALG3 H18 and ALG4 H22) were from clade A1 and were sampled from two children co-infested with clade B lice (Fig. 1) (GenBank accession nos KF700687 and KF700691). This is the first report of a finding of head lice from both clades A and B on a single head in Africa. One base at position 2 distinguished Algerian clade B head lice from other sequences found in GenBank: this base is an 'A' in Algerian lice and a 'G' in the others (Fig. S1, online). Additionally, the sixth base was able to distinguish between three different groups of the 24 clade B Algerian head lice. The first group contained 13 lice (54%) and had an 'A' at this position, similarly to the other GenBank sequences. The second group contained seven lice (29%) and had a 'T' at this position, and the third group contained four lice (17%) and had a 'C' at this position (Fig. S1). The two sequences of Algerian lice belonging to clade A1 differed at positions 13 and 14; louse ALG3 H18 had 'GG', and louse ALG4 H22 had 'TT'.

The four nuclear intergenic spacers S2, S5, PM1 and PM2 were phylogenetically analysed and compared with those in lice from other geographical areas (Li *et al.*, 2010). For spacer PM1, all clade A and B lice were of genotype 13, which is predominant in Europe and the U.S.A. For spacer PM2, all clade A and B lice were of genotype 47. This genotype is found in Mexico, the U.S.A. and the U.K. For spacer S2, we found two genotypes: genotype 39 was found in 10 clade B head lice and the two clade A head lice. The new genotype 120 was found in 14 clade B lice and differed from genotype 39 by a deletion of three bases (AGT) at position 72 of the sequence (GenBank accession no. JX848552).

With reference to spacer S5, all of the head lice were of genotype 23, which has been found previously in Portugal and Mexico (Table 3). The discrepancy in the phylogenetic organization obtained from the concatenated sequences of the four intergenic spacers S2, S5, PM1 and PM2 and the *cytb* sequence suggests that the histories of mitochondrial genes and chromosomal genes differ in lice. The lice obtained from subjects 3 and 4 demonstrated the same chromosomal genotype but different mitochondrial genotypes (A and B). This result suggests that head lice from different clades can recombine (Fig. 1).

Table 3. Results of the genotyping of head lice from Algeria.

Area	School	Subject	Lice ID	*Cytb*	Genotype			
					S2	S5	PM1	PM2
El Madania	1	S1	ALG 1	Clade B	120	23	13	47
			ALG 2	Clade B	NA	NA	NA	NA
			ALG 3	Clade B	120	23	13	47
			ALG 4	Clade B	120	23	13	47
			ALG 5	Clade B	120	23	13	47
			ALG 6	Clade B	NA	NA	13	47
			ALG 7	Clade B	NA	23	13	47
			ALG 8	Clade B	NA	23	13	47
			ALG 9	Clade B	39	23	13	47
			ALG 10	Clade B	39	23	13	47
			ALG 11	Clade B	39	NA	13	47
			ALG 12	Clade B	NA	23	13	47
			ALG 13	Clade B	39	23	13	47
	2	S2	ALG 14	Clade B	39	23	13	47
			ALG 15	Clade B	39	23	13	47
			ALG 16	Clade B	NA	23	13	47
	3	S3	ALG 17	Clade B	39	23	13	47
			ALG 18	Clade A	39	23	13	47
			ALG 19	Clade B	39	23	13	47
		S4	ALG 20	Clade B	39	23	13	47
			ALG 21	Clade B	39	23	13	47
			ALG 22	Clade A	39	23	13	47
Tessala El Merdja	4	S5	ALG 23	Clade B	120	23	13	47
			ALG 24	Clade B	120	23	13	47
	5	S6	ALG 25	Clade B	NA	23	13	47
			ALG 26	Clade B	NA	23	13	47

NA, not available.

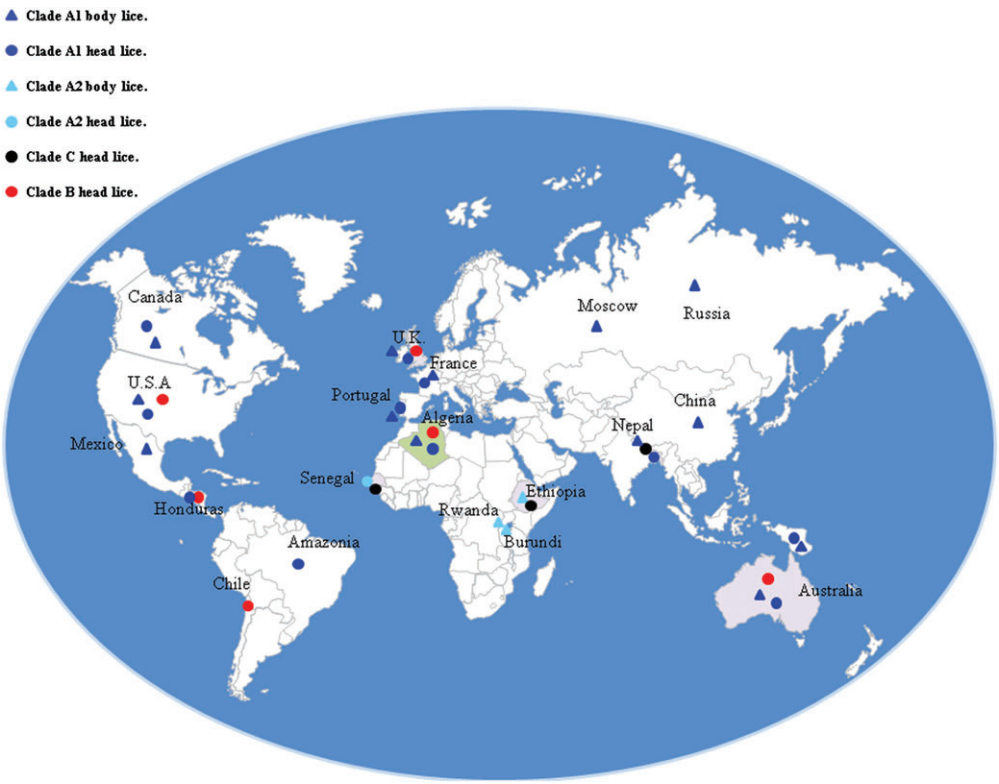

Fig. 2. The current repartition of lice haplogroups is indicated in light blue (type A2), dark blue (type A1), red (type B), and black (type C). Head lice are represented as circles and clothing lice as triangles.

In this study, clade B head lice in Africa were identified for the first time. These lice are likely to have been imported through international travel between America and the Old World during early globalization (Ascunce *et al.*, 2013) (Fig. 2). Lice are insects without spermatheca, which allows them to mate more frequently, and the possibility of their mating is increased by their ability to live together reflected in the observation of gene exchanges and recombination (Veracx *et al.*, 2013).

In this study, the classification of lice into clades A and B based on *cytb* mainly reflected mitochondrial cladistics; however, these two clades were indistinguishable based on intergenic spacers, which are more distinctive (Fig. 1). This result suggests that head lice can live in sympatry, as has been shown previously in lice of clades A and C in Africa (Veracx *et al.*, 2013).

Pediculus humanus DNA shows greater diversity among African lice than among non-African lice, suggesting an African origin of human clade A and clade C lice (Veracx *et al.*, 2013). However, the exact source of clade B head lice before their diversification remains unknown. It has been suggested that lice from Central America may have descended from lice imported by the first people to immigrate from Asia (Ascunce *et al.*, 2013). The U.S.A. (50.8%) and the U.K. (40.2%) have the highest prevalences of clade B head lice (Light *et al.*, 2008). Moreover, lice from Honduras were mostly of clade B (Ascunce *et al.*, 2013). Lice from clade B are also present in several other European countries, which suggests a European origin of clade B head lice; however, clade B head lice have recently been confirmed to have an American origin based on the analysis of nits belonging to a pre-Columbian Chilean mummy from Camarones (Boutellis *et al.*, 2013). Moreover, it appears that louse classification based on mitochondrial genes does not identify species but rather ecotypes and that globalization allows for a sympatric lifestyle in head lice. Finally, given the wide genomic plasticity of louse mitochondria, which is divided among 20 minicircular chromosomes, this may not be surprising and may depend on the exchange of a single plasmid (Georgiades & Raoult, 2011) (Fig. 1).

In conclusion, this report presents the first evidence of the presence in North Africa of clade B head lice, which are most likely to have been imported from Europe to Africa subsequent to globalization, and shows that lice from different clades interbreed, as has been previously suggested based on nuclear microsatellite data (Ascunce *et al.*, 2013). Clade B head lice have never been found in Asia or in any other region known to have contributed to the peopling of the Americas; therefore, further sampling from different regions will be necessary to determine the origin and distribution of clade B head lice.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/mve.12058

Figure S1. Alignment of a partial sequence of the *cytb* clade B sequences (Algerian lice and other clade B lice present in GenBank), showing the differences between Algerian lice and other clade B head lice.

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Accepted 18 October 2013
First published online 25 October 2014