SHORT COMMUNICATION

# Evidence from Mitochondrial DNA That Head Lice and Body Lice of Humans (Phthiraptera: Pediculidae) are Conspecific

N. P. LEO,[1,2] N.J.H. CAMPBELL,[2] X. YANG,[3] K. MUMCUOGLU,[4] AND S. C. BARKER[2]

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**ABSTRACT** The specific status of the head and body lice of humans has been debated for more than 200 yr. To clarify the specific status of head and body lice, we sequenced 524 base pairs (bp) of the cytochrome oxidase I (COI) gene of 28 head and 28 body lice from nine countries. Ten haplotypes that differed by 1–5 bp at 11 nucleotide positions were identified. A phylogeny of these sequences indicates that these head and body lice are not from reciprocally monophyletic lineages. Indeed, head and body lice share three of the 10 haplotypes we found. $F_{ST}$ values and exact tests of haplotype frequencies showed significant differences between head and body lice. However, the same tests also showed significant differences among lice from different countries. Indeed, more of the variation in haplotype frequencies was explained by differences among lice from different countries than by differences between head and body lice. Our results indicate the following: (1) head and body lice do not represent reciprocally monophyletic lineages and are conspecific; (2) gene flow among populations of lice from different countries is limited; and (3) frequencies of COI haplotypes can be used to study maternal gene flow among populations of head and body lice and thus transmission of lice among their human hosts.

**KEY WORDS** *Pediculus humanus*, *Pediculus capitis*, lice, Phthiraptera, Pediculidae, cytochrome oxidase I

ALTHOUGH THE HEAD and body lice of humans are very similar morphologically, investigators have reported consistent morphological differences between the two types (Busvine 1978, Schaefer 1978, Tarasevich et al. 1988). Head and body lice interbreed readily in the laboratory, yet one study of their morphology suggested they do not interbreed in nature (Busvine 1978). However, whether or not these lice are conspecific remains controversial (Durden and Musser 1994, Burgess 1995, Khudobin 1995). We studied a fragment of the cytochrome oxidase I (COI) gene of mitochondrial DNA (mtDNA) from lice collected from nine countries. Our aim was to collect evidence that would clarify the specific status of head and body lice. If they are different species, we would expect head and body lice to be reciprocally monophyletic lineages; whereas if they are conspecific, we would expect that these two types of lice would not be in reciprocally monophyletic lineages (clades). We also examined genetic differentiation among different populations of these lice using $F_{ST}$ values and exact tests of haplotype frequency differences.

## Materials and Methods

Lice from nine countries were studied: Australia, China, Hungary, Israel, Japan, Kenya, New Zealand, Papua New Guinea, and the United States (28 head and 28 body lice, Table 1). These included four lice from Inner Mongolia Province in China that were from two hosts infested with both head lice and body lice. We sampled one head louse and one body louse from each of these two hosts.

DNA was extracted from lice by either a phenol-chloroform method (Sambrook et al. 1989) or with chelex beads (Bio-Rad, Hercules, CA). The latter involved crushing a louse with a micropestle in a tissue grinding tube with liquid nitrogen. One milliliter of boiling 5% chelex beads in 1 × TE buffer with RNaseA (1 μl of 25 mg/ml RNaseA for every 100 ml of chelex in 1 × TE buffer) was added to the tube and then put in boiling water for 15 min. Tubes were cooled for 10 min at −20°C and then spun in a microcentrifuge at 12,000 × *g* for 10 min. Three microliters of the top layer was used in each 25 μl polymerase chain reaction (PCR). The insect-specific primer C1-J-1718 (forward: 5′-GGAGGATTTGGAAATTGATTAGTTCC-3′) (Simon et al. 1994) and a primer specific to *Pediculus humanus* (designed by N.L.) C1-N-2191

[1] E-mail address: n.leo@imb.uq.edu.au.
[2] Department of Microbiology & Parasitology, and Institute for Molecular Biosciences and ARC Special Research Centre for Functional and Applied Genomics, University of Queensland, Brisbane, Queensland, 4072, Australia.
[3] Animal Medical Department, Inner Mongolia Agricultural University, Huhehot, 010018, China.
[4] Department of Parasitology, Hebrew University, Hadassah Medical School (P.O. Box 12272), and the Department of Dermatology, Hadassah University Hospital (P.O. Box 12000), 91120 Jerusalem, Israel.

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**Table 1. Location and hosts of the lice used in this study**

| Country | Locality | Type | Haplotype (s) | Host |
|---|---|---|---|---|
| Australia | Townsville, Queensland | Head | 2 | 1 louse from 1 host |
| | Brisbane, Queensland | Head | 4 | 1 louse from 1 host |
| China | Cele, Cele County, Xinjiang Province | Body | 2, 2, 2 | 3 lice from 1 host |
| | | Body | 3, 3, 3 | 3 lice from 1 host |
| | | Body | 3, 3, 10 | 3 lice from 1 host |
| | | Head | 2, 2 | 2 lice from 1 host |
| | | Head | 2, 2 | 2 lice from 1 host |
| | | Head | 2, 2, 3 | 3 head lice from 3 separate hosts |
| | Yiliqi, Hotan County, Xinjiang Province | Body | 1, 3 | 2 lice from 1 host |
| | | Body | 3, 3, 3, | 3 body lice from 3 separate hosts |
| | Huhehot, Inner Mongolia Province | Head | 2 | 1 louse from 1 host |
| | | Body | 6 | 1 louse from 1 host |
| | Mountain village, Inner Mongolia Province | Body, Head | 6, 7 | 2 lice from a double infestation |
| | | Body, Head | 6, 6 | 2 lice from a double infestation |
| | | Head | 7 | 1 louse from 1 host |
| | Longxi County, Ganshu Province | Head | 8 | 1 louse from 1 host |
| Hungary | Budapest | Head | 2 | 1 louse from 1 host |
| Israel | Bet-Shemesh | Head | 2, 4, 9 | 3 lice from 3 separate hosts |
| Japan | Sapporo | Body | 5, 5, 5 | 3 lice from a laboratory colony |
| | Tokyo | Body | 2, 2 | 2 lice from 1 host |
| Kenya | Nairobi | Head | 2, 2, 2 | 3 lice pooled from 3 hosts |
| | | Body | 2, 2, 2 | 3 lice pooled from 3 hosts |
| New Zealand | Auckland | Head | 4, 4, 4 | 3 lice pooled from 5 hosts |
| Papua New Guinea | Askaroff, Lake Murray | Head | 2, 2, 4 | 3 lice from 3 separate hosts |
| | Hole in the wall village, Madang | Head | 2 | 1 louse from 1 host |
| United States of America | Orlando, Florida & Washington DC | Body | 2, 2, 2 | 3 lice from a laboratory colony |

(reverse: 5′-CCAGGAAGAATAAGAATATAAACTTC-3′) were used to amplify 524 bp of the mitochondrial COI gene. Primer names refer to where they anneal by gene, strand and 3′ base position relative to the *Drosophila yakuba* sequence (after Simon et al. 1994). PCR reactions contained 1–3 μl of DNA template, 2.5 μl of Reaction Buffer IV at 10× concentration (AB Gene, Epsom, UK), 2.25 μl of $MgCl_2$ (25 mM), 1.1 μl of dNTPs (5 mM), 0.3 μl of each primer (10 μM), 0.2 μl of Red Hot *Taq* polymerase (AB Gene), and MilliQ water to a final volume of 25 μl. The cycling conditions were 94°C for 1 min; 35 cycles of 30 s at 94°C, 30 s at 55°C, and 40 s at 68°C; and a final extension time of 5 min at 68°C. PCR products were visualized under UV light after electrophoresis in an ethidium bromide-stained agarose gel. If insufficient DNA was amplified for sequencing, hot-start PCR was attempted with a fresh sample of DNA from that louse. The conditions for hot-start PCR were 5 min at 94°C, polymerase added, 2 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 55°C and 40 s at 68°C; and then 5 min at 68°C. PCR products were purified with Qiaquick columns (QIAGEN, Venlo, The Netherlands) and sequenced directly (DyeDeoxy Terminator; PE Applied Biosystems, Foster City, CA) with the PCR primers (above), by an ABI 377 gene sequencer. The first 24 lice were sequenced with both forward and reverse primers. This revealed haplotypes 1–5. The rest of the lice were sequenced with the forward primer only. If the sequence data indicated a new haplotype, or if the identity of a nucleotide was ambiguous, the gene was then sequenced with the reverse primer.

**Table 2. Variable sites in the 524 bp region of the cytochrome oxidase I (COI) gene of *Pediculus humanus*, and the haplotypes found in each country**

| Base position | | | | | | | | | | | Haplotype | No. of lice from each haplotype from each locality | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 91 | 106 | 116 | 120 | 150 | 255 | 264 | 291 | 345 | 357 | 453 | | AUS | CHI | | HUN | ISR | JAP | KEN | | NZ | PNG | USA | Total |
| | | | | | | | | | | | | H | H | B | H | H | B | H | B | H | H | B | |
| T | T | G | C | C | A | T | G | A | A | T | 1 | | | 1 | | | | | | | | | 1 |
| A | . | . | . | . | . | . | . | . | . | . | 2 | 1 | 7 | 3 | 1 | 1 | 2 | 3 | 3 | | 3 | 3 | 27 |
| A | . | . | . | T | . | . | . | . | G | C | 3 | | 1 | 9 | | | | | | | | | 10 |
| A | . | . | . | . | G | . | . | . | . | . | 4 | 1 | | | | 1 | | | | 3 | 1 | | 6 |
| A | . | . | . | T | . | C | . | T | G | . | 5 | | | | | | 3 | | | | | | 3 |
| A | . | . | . | T | . | . | . | . | G | . | 6 | | 1 | 3 | | | | | | | | | 4 |
| A | . | . | T | . | . | . | . | . | . | . | 7 | | 2 | | | | | | | | | | 2 |
| A | . | . | . | T | . | . | A | . | G | . | 8 | | 1 | | | | | | | | | | 1 |
| A | . | A | . | . | . | . | . | . | . | . | 9 | | | | | 1 | | | | | | | 1 |
| A | A | . | . | . | . | . | . | . | . | . | 10 | | | 1 | | | | | | | | | 1 |
| | | | | | | | | | | | | 2 | 29 | | 1 | 3 | 5 | 6 | | 3 | 4 | 3 | 56 |

Sites are numbered according to the alignment of sequences. A dot indicates identity with the sequence of haplotype 1. AUS, Australia; CHI, China; HUN, Hungary; ISR, Israel; JAP, colony originally from Japan; KEN, Kenya; NZ, New Zealand; PNG, Papua New Guinea; USA, colony originally from the United States of America; H, head lice; B, body lice.

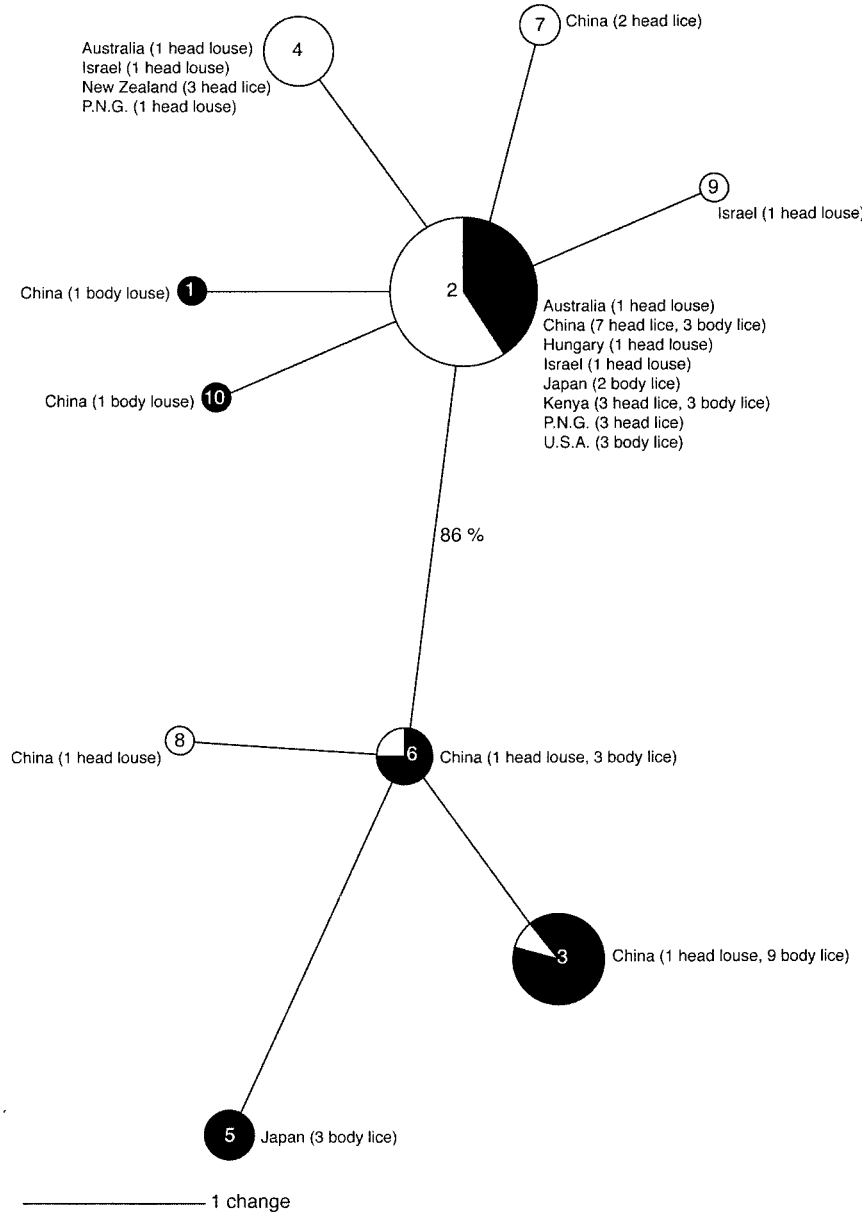


**Fig. 1.** The single most parsimonious (shortest) unrooted tree (11 steps) of the 10 haplotypes of head and body lice from a branch and bound search in PAUP. There was only one internal branch in this tree; it had a bootstrap support of 86%. Numbers in circles show the haplotype identification number (refer to Table 2). The size of the circles indicates the frequency of the haplotypes. Shaded areas indicate the proportion of body lice; nonshaded areas indicate the proportion of head lice.

Sequences were aligned by eye in Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, MI), then compared with COI sequences of other insects in GenBank to check that the COI had been amplified. We executed a branch and bound search in PAUP 4.0b3a (Swofford 1998) to find the maximum parsimony tree(s) for the sequences, and then tested the robustness of these relationships with 1,000 cycles of bootstrap resampling.

Arlequin 2.000 (Schneider et al. 2000) was used to calculate $F_{ST}$ values and to execute an exact test of sample differentiation from haplotype frequencies, for differences between all head and body lice tested, and for differences among lice from different countries.

## Results

There was little nucleotide variation among the lice we studied: we found 10 COI haplotypes in the 56 lice from nine countries. These haplotypes differed from each other by 1–5 base substitutions (0.2–1.1%) at 11 variable sites in the 524 bp fragment (Table 2). Eight of the substitutions were conservative (silent) transitions at the third codon position. The other three substitutions were, relative to the most common haplotype, nonconservative transversions in haplotypes 1 and 10 at the first codon position, and in haplotype 9 at the second codon position (sites 91, 106, and 116, respectively, Table 2). Haplotype 2 was the most common and widespread haplotype overall; it was found in 27 of the 56 lice and in lice from all countries except New Zealand (GenBank accession number AF320286). This haplotype was the most common haplotype in both head lice (16 of 28 lice) and body lice (11 of 28 lice) (Table 2).

The single most parsimonious tree of the COI haplotypes had 11 steps and only one internal branch; this branch had bootstrap support of 86% (Fig. 1). This internal branch divided the lice into two clades: one with both head and body lice from all nine countries (haplotypes 1, 2, 4, 7, 9, and 10) and the other with head and body lice from China and Japan (haplotypes 3, 5, 6, and 8). Haplotypes from both of these clades were found in a head louse (haplotype 7) and a body louse (haplotype 6) from one host in Inner Mongolia Province, in China. Both lice from the other host infested with two types of lice (also from Inner Mongolia Province) had haplotype 6.

Both the $F_{ST}$ values and the exact test of haplotype frequencies showed significant differences between head and body lice ($F_{ST} = 0.09$, $P = 0.00880$; exact $P$ value = 0.00000). However, both tests also showed significant differences among lice from different countries ($F_{ST} = 0.23$, $P = 0.00098$; exact $P$ value = 0.00236).

Some infestations (lice from one host) had more than one haplotype (Table 1). Of nine hosts, from which two to three lice were studied, three hosts had lice with two different haplotypes: (1) a person from Xinjiang Province in China had two body lice with haplotype 3, and one body louse with haplotype 10; (2) another person from Xinjiang Province in China had one body louse with haplotype 1 and another body louse with haplotype 3; and (3) a girl from a remote mountain village in Inner Mongolia Province in China had a body louse with haplotype 6 and a head louse with haplotype 7.

## Discussion

We examined 28 head and 28 body lice from nine countries. Ten COI haplotypes were identified. Phylogenetic analysis revealed a single tree with one internal branch that separated haplotypes 1, 2, 4, 7, 9, and 10 from 3, 5, 6, and 8. Both of these clades contained head and body lice; therefore, the phylogeny of the COI sequences indicates that head and body lice do not come from reciprocally monophyletic lineages. This is evidence that head and body lice are conspecific, however, we note that the phylogeny of a single gene does not necessarily indicate the true phylogeny. But it is noteworthy that head and body lice share three of 10 haplotypes. These shared haplotypes may be ancestral *Pediculus humanus* haplotypes that have been retained by both types of lice after divergence, or the shared haplotypes may be evidence of conspecificity. We propose that haplotype 2 is an ancestral *P. humanus* haplotype, because it is common and widespread. Haplotypes 3 and 6 were common in the two provinces in China, Xinjiang Province and Inner Mongolia Province, respectively, but were not found in any other locations, so they are probably not ancestral haplotypes. Therefore, the simplest explanation for the presence of haplotypes 3 and 6 in both head and body lice is that head and body lice are conspecific.

We looked for differences between head and body lice by comparing haplotype frequencies. The $F_{ST}$ and exact tests showed significant differences between the haplotype frequencies of head and body lice. However, comparison of lice from different countries explained even more of the variation in haplotype frequencies; this is further evidence for conspecificity.

Our results from COI provide evidence that the head and body lice of humans belong to the same species. The COI sequences from the head and body lice that we studied did not come from reciprocally monophyletic lineages. Indeed, the head and body lice shared three of the 10 haplotypes we found, which is evidence for conspecificity. Moreover, analysis of haplotype frequencies showed that although there were significant differences between the head and body lice we studied, more of the variation was explained by differences among lice from different countries than by differences between head and body lice. Further analyses of COI should reveal much about transmission and maternal gene flow among populations of lice on global, local and individual host levels.

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