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What's in a name: The taxonomic status of human head and body lice

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ABSTRACT

Human head lice (Anoplura: Pediculidae: *Pediculus*) are pandemic, parasitizing countless school children worldwide due to the evolution of insecticide resistance, and human body (clothing) lice are responsible for the deaths of millions as a result of vectoring several deadly bacterial pathogens. Despite the obvious impact these lice have had on their human hosts, it is unclear whether head and body lice represent two morphological forms of a single species or two distinct species. To assess the taxonomic status of head and body lice, we provide a synthesis of publicly available molecular data in GenBank, and we compare phylogenetic and population genetic methods using the most diverse geographic and molecular sampling presently available. Our analyses find reticulated networks, gene flow, and a lack of reciprocal monophyly, all of which indicate that head and body lice do not represent genetically distinct evolutionary units. Based on these findings, as well as inconsistencies of morphological, behavioral, and ecological variability between head and body lice, we contend that no known species concept would recognize these louse morphotypes as separate species. We recommend recognizing head and body lice as morphotypes of a single species, *Pediculus humanus*, until compelling new data and analyses (preferably analyses of fast evolving nuclear markers in a coalescent framework) indicate otherwise.

1. Introduction

Head and body (clothing) lice (*Pediculus humanus capitis* de Geer and *Pediculus humanus humanus* Linnaeus, respectively) have been parasites of humans for thousands of years (Mumcuoglu and Zias, 1988; Araújo et al., 2000; Mumcuoglu et al., 2004). As their names imply, these sucking lice are spatially segregated on their human hosts. Head lice are found on the head and attach their eggs to the base of hair shafts, whereas body lice are found on the body and in clothing and prefer to attach their eggs to clothing rather than body hair (Buxton, 1946). Body lice are believed to have evolved from head lice, invading the body region only recently with the advent of clothing use in modern humans (Burgess, 1995; Kittler et al., 2003, 2004). The ecological separation between head and body lice also is accompanied by several biological and morphological differences (Burgess, 1995). Head lice are common worldwide infesting millions of school children every year. Body lice are less prevalent parasites, associated mainly with those living in poor conditions, but are potentially more harmful because they are known vectors of at least three bacterial pathogens in humans: *Rickettsia prowazekii* (epidemic or louse-borne typhus; but see Robinson et al., 2003), *Borrelia recurrentis* (louse-borne relapsing fever) and *Bartonella quintana* (trench fever; Buxton, 1946; but see Sasaki et al., 2006a, b). Body lice (and their eggs) are generally larger than head lice, most notably in the length of the tibia on the second pair of legs (Schöll, 1955; Busvine, 1978; Reed et al., 2004). These morphological differences are small, and were apparent to Reed et al. (2004) only when assessed with discriminant function analysis. Head lice tend to require more frequent blood meals than do body lice (Alpatov and Nastjukova, 1955). Head and body lice tend to return to their preferred ecological habitat when displaced, and body lice are known to be especially attracted to areas that are occupied by other body lice or body louse feces (Wigglesworth, 1941; Mumcuoglu et al., 1986). These morphological and behavioral differences, however, are not always consistent. For example, louse integument is elastic making identifications based on overall size unreliable (Busvine, 1978). Head and body lice also are known to wander over their hosts' body and may not directly return to their preferred habitat (Nuttall, 1917 and references therein; Keilen and Nuttall, 1919; Busvine, 1944, 1978; Fournier et al., 2002; Brouqui et al., 2005).

Even though the biological differences between head and body lice are not always consistent, it is believed that the physical separation between these lice may facilitate specific differentiation (Schaefer, 1978; Burgess, 1995). Under natural conditions, head and body lice are not known to interbreed (Busvine, 1978), but under experimental conditions they can interbreed and are known to produce viable and fertile offspring (Bacot, 1917). Although some morphological abnormalities have been found in the progeny of laboratory crosses between head and body lice (Buxton, 1946), these same abnormalities are also found in natural infestations of

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either head or body lice (Keilen and Nuttall, 1919; Buxton, 1946). Furthermore, when reared in the laboratory under conditions resembling those of body lice, morphological and behavioral differences disappear with head lice changing into the body louse morphotype within a few generations (Bacot, 1917; Howlett, 1917; Alpatov and Nastjukova, 1955; Levene and Dobzhansky, 1959; see Busvine, 1948 for a single known exception). Alpatov and Nastjukova (1955) also noted high mortality among the progeny of head lice during the early generations of their experiment. Levene and Dobzhansky (1959) described a hypothetical situation in which this high mortality could be the result of natural selection acting to remove lice homozygous for the head louse form, retaining body louse homozygotes and head louse heterozygotes (which, given subsequent generations, will transform into the body louse phenotype). Retention of heterozygotes, and the resulting genetic variability, may be highly adaptive and allow lice to survive when provided with a new habitat. Levene and Dobzhansky (1959) proposed that the reverse also should be true, that body louse heterozygotes would be retained and would transform into the head louse phenotype, if relocated to the head or reared under head louse conditions. Although there have been many studies rearing both head and body lice under body louse conditions (primarily in vivo), there has only been one study that has attempted to rear lice (in this case, only head lice) under head louse conditions (in vitro conditions; Takano-Lee et al., 2003). It is currently unknown if body lice will transform into head lice if raised under head louse conditions. Regardless, most systematists would not consider these traits (e.g., interbreeding, production of viable and fertile offspring, and morphological transformation toward the body louse form) sufficient to describe two distinct species for head and body lice (de Queiroz, 1998; Coyne and Orr, 2004).

The species status of human *Pediculus* has been a topic for debate for over a century. Ferris (1951) and Burgess (1995) provide detailed accounts of this taxonomic confusion, which we briefly summarize here. Nuttall (1917, 1919a,b, 1920) and Ferris (1935) strongly argued that head and body lice represented, at most, subspecies, and that the morphological, behavioral, and ecological differences between these two louse groups represented natural intraspecific variation. Buxton (1946) presented an argument that head and body lice are probably too similar to be considered distinct species, but may represent "species in the making." Fahrenholz (1912, 1915, 1916), Busvine (1944, 1978), and Schaefer (1978), on the other hand, argued that the differences observed between head and body lice were more than sufficient to recognize these taxa as distinct species. This taxonomic confusion regarding the species status of head and body lice continues to be debated today, primarily because of the ecological differences between the louse morphotypes.

In recent years, the specific status of head and body lice has also been addressed in studies examining louse isozymes (Amevigbe et al., 2000), louse primary endosymbionts (Sasaki-Fukatsu et al., 2006; Allen et al., 2007; Perotti et al., 2007) and louse bacterial pathogens (Sasaki et al., 2006a, b; but see Parola et al., 2006). While the results of the endosymbiont and bacteria work indicate that head and body lice are conspecific, the results of the isozyme work indicate that genetic differentiation may exist between these louse forms. There also has been a series of DNA-based molecular studies that have directly addressed the specific status of head and body lice (Leo et al., 2002; Kittler et al., 2003; Yong et al., 2003; Reed et al., 2004; Leo and Barker, 2005; Table 1). The majority of these molecular-based studies have concluded that head and body lice are not distinct species because of the lack of reciprocal monophyly between these two louse morphotypes (Table 1). In the study by Yong et al. (2003), however, several monophyletic groups of head and body lice were found using the nuclear elongation factor-1 alpha (EF-1α) gene, and Yong et al. (2003) hypothesized that body lice diverged from head lice multiple times. BLAST (Basic Local Alignment Search Tool) searches of this EF-1α data revealed that some sequences (GenBank Accession Nos. AY239271–AY239279) are actually fungi (top BLAST result *Escovopsis* sp. DQ848183) and not louse sequences. In light of the fungal contamination, all EF-1α data obtained by Yong et al. (2003) should be either verified or excluded from future louse studies. Additional studies using the EF-1α gene (Leo and Barker, 2005; this study) have not supported Yong et al.'s (2003) findings of multiple monophyletic lineages of head and body lice. Therefore, Yong et al.'s (2003) conclusions were almost certainly the result of misleading signal from the contaminated sequences.

Reed et al. (2004) also had unique findings from their analyses of head and body lice. These authors found three deeply divergent mitochondrial clades of lice all of which contained head lice, whereas only one of these three clades contained body lice (Fig. 1). Furthermore, all three clades had unique geographic distributions: (1) one clade was comprised of a worldwide distribution of both head and body lice, (2) one clade consisting only of head lice was from North America, Central America, and Europe, and (3) the second clade containing only head lice was from Africa and Nepal (further sampling, however, is necessary to determine the geographic range of lice belonging to this third mitochondrial lineage). For convenience, throughout the manuscript we will refer to these mitochondrial clades as Clades A, B, and C, respectively (Raoult et al., 2008). These mitochondrial results, the presence of three deeply divergent clades and the finding that body lice arose from only a subset of head lice (the head lice found in Clade A), were novel compared to previous studies based on both mitochondrial and nuclear data (Leo and Barker, 2002; Kittler et al., 2003; Yong et al., 2003) and resulted in several follow-up studies examining these results (Leo and Barker, 2005; this study).

Although all of these recent molecular-based studies have used a lack of reciprocal monophyly to conclude that head and body lice are not distinct species (Table 1), monophyly may be a poor guide for species status, especially if taxa have recently speciated (Avise,

Table 1
Data and findings from previous studies examining the taxonomic status of head and body lice

Publication	Molecular marker	Analyses performed	Taxonomic conclusion
Leo et al. (2002)	Mitochondrial COI gene	Phylogenetic analyses Population summary statistics	Conspecific
Kittler et al. (2003)	Mitochondrial cytb and ND4 genes Nuclear EF-1α and RPII genes	Phylogenetic analyses Population summary statistics	Conspecific
Yong et al. (2003)	Mitochondrial COI gene Nuclear 18S and EF-1α genes	Percent similarity Phylogenetic analyses	More than two species[a]
Reed et al. (2004)	Mitochondrial COI and cytb genes	Phylogenetic analyses Population summary statistics	Conspecific
Leo and Barker (2005)	Nuclear 18S gene Previously published nuclear data	Phylogenetic analyses	Conspecific
Leo et al. (2005)	Nuclear Microsatellite Loci	Population genetic analyses	Two distinct species

[a] These conclusions, however, are based on contaminated data and subsequent studies (including the study herein) do not support this finding.

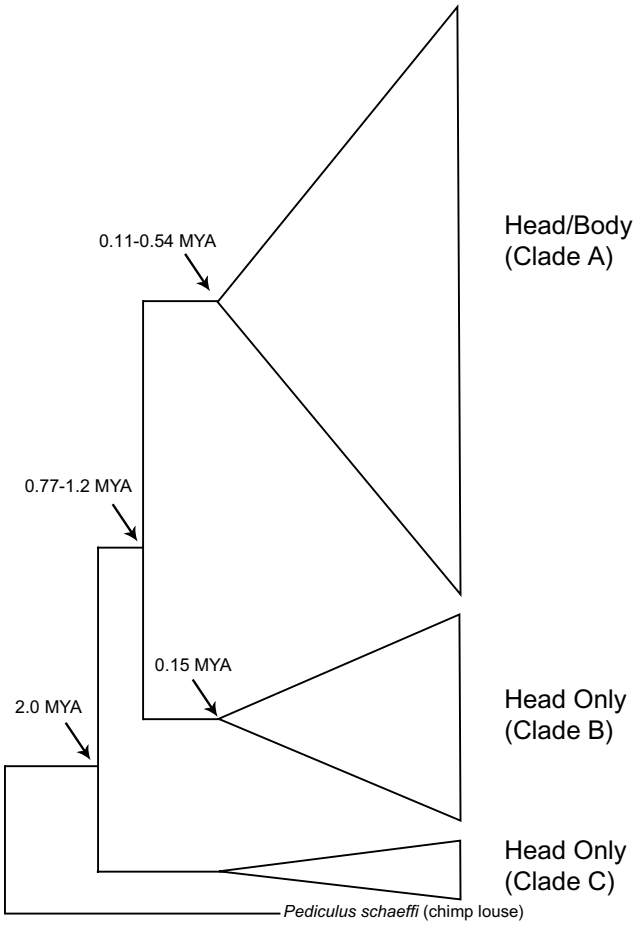

Fig. 1. Phylogeny of louse relationships and timing of divergence events (in millions of years; MYA) based only on the mitochondrial COI and cytb genes analyzed in Kittler et al. (2003) and Reed et al. (2004). Height of the triangles represents the number of specimens in each clade. Head and body lice belonging to Clade A are distributed worldwide, head lice belonging to Clade B are restricted to North and Central America, Europe, and Australia, and head lice belonging to Clade C are restricted to Ethiopia and Nepal. The age of *Pediculus humanus* is approximately 1–2 million years, an order of magnitude older than their modern human hosts. Body lice (Clade A), and the first use of clothing, originated at least 107,000 years ago (Kittler et al., 2004).

2000; Kittler et al., 2003; Leo et al., 2005; Knowles and Carstens, 2007; Shaffer and Thomson, 2007). Within-species (intraspecific) data sets are often very large, and exhibit low divergence, persistent ancestral haplotypes, multifurcations, and reticulated evolution, all of which inhibit traditional phylogenetic analyses (Clement et al., 2000; Posada and Crandall, 2001). Because intraspecific evolution does not always occur in a bifurcating manner, much of the current literature recommends network and population genetic (summary statistic and coalescent) approaches to examine genetic variation within recently speciated taxa (Posada and Crandall, 2001; Excoffier and Heckel, 2006). In fact, the relatively low morphologic and genetic diversity between head and body lice suggests that population genetic approaches may be appropriate to address this taxonomic issue (Demboski and Sullivan, 2003).

Likely realizing that a phylogenetic approach may be inappropriate in studies of head and body lice, Leo et al. (2005) used a population genetics approach to examine nuclear microsatellite allele frequencies (for five loci) of lice from hosts doubly infested with both morphotypes. Leo et al. (2005) examined a total of 11 human hosts from China and Nepal and examined genetic differentiation, migration, and accuracy of morphotype assignment of head and body lice. Although three of the five microsatellite loci showed genetic differentiation between head and body lice, migrants were present and less than half of the lice were assigned to the correct morphotype in some of the comparisons. Despite these contradictory findings, Leo et al. (2005) concluded that head and body lice were genetically isolated on the same host individual and that gene flow (migration) was occurring only among head lice or among body lice from different host individuals. Given these results, as well as the occurrence of morphological abnormalities in the progeny of head and body louse laboratory crosses (but see Keilen and Nuttall, 1919; the same abnormalities are also found in single infestations of either head or body lice), Leo et al. (2005) concluded that head and body lice are genetically distinct species kept separate by ecological and/or behavioral factors.

Although Leo et al. (2005) were the first to publish louse microsatellite data, which are certainly appropriate to address the issue of species status within *P. humanus*, their conclusions must be carefully considered. For one, the use of doubly infested human hosts is not without risk. Because head and body lice can interbreed in the laboratory and because morphological differences do not always hold true, the tendency of lice to move about the host's body (Keilen and Nuttall, 1919; Busvine, 1944, 1978; Fournier et al., 2002; Brouqui et al., 2005) can make it extremely difficult to identify lice from double infestations as having either the head or body louse morphotype. Researchers are left to classify lice based on the habitat in which they were collected (scalp or clothing), which may or may not be the historical habitat of that particular individual louse. Busvine (1978) was the first to utilize doubly infested people to assess the level of gene flow between louse morphotypes, and similar to Leo et al. (2005), Busvine (1978) found no morphological integration and concluded that head and body lice represent two distinct species. Although double infestations may represent an ideal natural environment for gene flow, testing for gene flow (i.e., interbreeding) in nature is not necessarily sufficient to determine whether two populations constitute distinct species (Mayr, 1995).

Sample sizes and geographic sampling also must be carefully considered when interpreting the results presented in Leo et al. (2005). Most population studies employ more loci than the five used by Leo et al. (2005) to make well-supported population level inferences. Sample sizes also were extremely small and the geographic sampling limited, which potentially violates several analytical assumptions (Pritchard et al., 2000; Bergl and Vigilant, 2007). Furthermore, although Leo et al. (2005) show that in close quarters gene flow between head and body lice can be quite low, they did not address the issue of historical gene flow in a worldwide sample of lice, which would be required to determine whether head and body lice should be recognized as distinct species.

The numerous examinations of relationships between head and body lice over the last five years (Leo et al., 2002; Kittler et al., 2003; Yong et al., 2003; Reed et al., 2004; Leo and Barker, 2005; Table 1) have produced a wealth of molecular data from *P. humanus*. Herein, we provide a synthesis of these data and we compare phylogenetic and population genetic methods using the most diverse geographic and molecular sampling presently available. We then provide data and methodological recommendations for future studies of *P. humanus* and offer new information and insights regarding the taxonomic status of human head and body lice.

2. Materials and methods

2.1. Molecular data

All available DNA sequence data from the following genes were downloaded from GenBank (Appendix A): the mitochondrial genes COI (166 sequences), cytb (97 sequences), and ND4 (40 sequences),

and the nuclear genes 18S rRNA (22 sequences), EF-1α (40 sequences; not including any of the sequences from Yong et al., 2003), and RPII (53 sequences). Genetic data from outgroup taxa also were downloaded from GenBank: *Pediculus schaeffi* (AY695999 for COI, AY696067 for cytb, AY316849 for ND4, AY589943 for 18S rRNA, AY316834 for EF-1α, and AY316912 for RPII), *Pthirus pubis* (human crab louse; AY696000 for COI and AY077776 for 18S rRNA), *Pedicinus hamadryas* (Old World monkey louse; AY696007 for COI), and *Fahrenholzia pinnata* (rodent louse outgroup; AY69008 for COI). All mitochondrial and EF-1α and RPII sequences were aligned by eye using MacClade (Maddison and Maddison, 2005) and Se-Al v2.01a11 (Rambaut, 1996). Louse 18S rRNA sequences were aligned manually in reference to secondary structure (Gillespie, 2004; Gillespie et al., 2005; alignment available at the jRNA web site http://hymenoptera.tamu.edu/rna/models/arth/data/alignment/18S_arthropods.00.04.Nex) and ambiguously aligned sites were removed before analysis. Both sequences for specimens heterozygous for the nuclear EF-1α and RPII genes were included in phylogenetic, network, and population genetic analyses.

2.2. Phylogenetic analyses

No individual louse specimen available on GenBank was sampled for all six genes, therefore each gene was analyzed separately. To assess monophyly of *Pediculus humanus*, a distance-based neighbor-joining (NJ) analysis was performed using PAUP* 4.0b10 (Swofford, 2003). Nodal support was assessed using nonparametric bootstrap analyses (1000 NJ pseudoreplicates; Felsenstein, 1985). Genetic divergence (uncorrected *p* distance) for each gene was assessed across all samples as well as among major (well-supported) clades identified by NJ analyses to determine rate of nucleotide substitution within *P. humanus*.

Phylogenetic analyses also were performed using maximum parsimony (MP), maximum-likelihood (ML) and Bayesian approaches. Each data set was reduced to non-redundant taxa (unique haplotypes) to lessen computational demands. Equally weighted MP searches were performed with 10 random addition replicates and tree bisection-reconnection branch swapping using PAUP* 4.0b10 (Swofford, 2003). To assess nodal support, nonparametric bootstrap analyses were performed (500 pseudoreplicates and 10 random sequence additions; Felsenstein, 1985). To generate the best ML and Bayesian trees, Modeltest (Version 3.7; Posada and Crandall, 1998) was used to examine 56 models of nucleotide substitution to choose a best-fit model of sequence evolution. Models of evolution providing the best approximation of the data using the fewest parameters were chosen for subsequent analyses according to the Akaike Information Criterion (Huelsenbeck and Rannala, 1997; Posada and Buckley, 2004). Full heuristic ML and bootstrap (200 pseudoreplicates) searches were conducted using the best-fit model in PAUP* 4.0b10 (Swofford, 2003). Alternatively, GARLI (v. 0.951; Zwickl, 2006) was used to perform full heuristic ML and bootstrap (100 pseudoreplicates) for larger data sets (COI, cytb, and 18S rRNA). Bayesian phylogenetic analyses were performed using the best-fit model in MrBayes 3.12 (Huelsenbeck and Ronquist, 2001). Model parameters were treated as unknown variables with uniform priors and were estimated as part of the analysis. Bayesian analyses were initiated from random starting trees, run for 10 million generations with four incrementally heated chains (Metropolis-coupled Markov chain Monte Carlo; Huelsenbeck and Ronquist, 2001), and sampled at intervals of 1000 generations. Two independent Bayesian analyses were run to avoid entrapment on local optima, and log-likelihood scores were compared for convergence (Huelsenbeck and Bollback, 2001; Leaché and Reeder, 2002) plotting the log-likelihood scores of sample points against generation time so that burn-in generations (the first 2500 trees) could be discarded. The retained equilibrium samples were used to generate a 50% majority rule consensus tree with the percentage of samples recovering any particular clade representing that clade's posterior probability (Huelsenbeck and Ronquist, 2001).

Alternative phylogenetic hypotheses were compared statistically using the Kishino–Hasegawa (KH) and the Shimodaira–Hasegawa tests (SH) as implemented in PAUP* 4.0b10 (MP and ML analyses using RELL optimization and 1000 bootstrap replicates; Kishino and Hasegawa, 1989; Shimodaira and Hasegawa, 1999; Goldman et al., 2000). Suboptimal trees from the Bayesian analyses also were examined to assess alternative phylogenetic hypotheses. The probability of trees agreeing with alternative hypotheses was calculated by applying constraint-based filter trees implemented in PAUP* 4.0b10 (Ihlen and Ekman, 2002). All executable files for all taxa as well as unique haplotypes are available on TreeBase (http://www.treebase.org; Study Accession Number S2030).

2.3. Network and population genetic analyses

Haplotype networks were assembled to reconstruct historical relationships among haplotypes (i.e., genealogies) for each mitochondrial and nuclear marker. A statistical parsimony analysis (Templeton et al., 1992), implemented in TCS (v. 1.6, Clement et al., 2000) was used to assemble the most parsimonious haplotype tree, with linkages between taxa representing mutational events, and estimate a 95% confidence limit of the reliability of those linkages.

Population summary statistics were performed on each gene for all *Pediculus humanus* specimens. Previous population level studies of lice (Nadler and Hafner, 1989; Nadler et al., 1990; Barker et al., 1991a,b; Johnson et al., 2002) have incorporated louse natural history, host relationships, and (perhaps most importantly) geography into their analyses to provide thorough assessments of louse population structure and genetic differentiation. Humans and their parasitic head and body lice, however, are distributed worldwide and based on the data available in GenBank there are no logical geographic subdivisions with large enough sample sizes that can be applied to the data *a priori* when examining genetic differentiation. We therefore subdivided *P. humanus* samples by morphotype (head or body). Comparisons were made between all head and body lice (each gene), and between head and body lice found only within Clade A for the mitochondrial COI gene. The following measures of DNA sequence variation were calculated using DnaSP software (v. 4.10.9 software; Rozas et al., 2003): number of segregating sites (S), average number of differences (k), number of haplotypes (h), haplotype diversity (Hd; Nei, 1987), nucleotide diversity (π and πJC; Tajima, 1983), gene flow (F_{ST}), effective number of migrants ($M = N_{ef}m$), theta (Θ), and effective population size (N_{ef}).

Coalescent methods provide an alternative to traditional phylogenetic and population genetic analyses by providing estimates of gene flow among populations while taking into account demographic processes and population structure (Posada and Crandall, 2001; Bowie et al., 2006). The same comparisons among populations outlined above also were made using mitochondrial genes in the software package MDIV (Nielson, 2002; Nielson and Wakeley, 2001) which simultaneously estimates theta ($\Theta = 2\ N_{ef}\mu$), scaled migration rate per generation ($M = N_{ef}m$), population divergence time ($T_{pop} = t/N_{ef}$), and a point estimate of the time to most recent common ancestor (TMRCA) where N_{ef} is the effective population size, t is generation time, and μ is the per locus mutation rate. The same parameters were estimated for the nuclear genes using the equalities: $\Theta = 4\ N_{ef}\mu$, $M = 2\ N_{ef}m$, and $T_{pop} = t/2N_{ef}$. Each of the data matrices was run for 5 million generations with 500,000 generations discarded as burn-in. Each analysis was run five times

beginning at random starting points. The prior distributions of M and T_{pop} were set to 0–10 and 0–25, respectively.

In order to calculate effective population size and other measures for DnaSP and MDIV analyses, an estimate of the per locus mutation rate (μ) was required. We took a conservative approach by estimating μ based on the expected number of substitutions under a best-fit model of nucleotide selection between *Pediculus humanus* and *P. schaeffi*, whose lineages were estimated to have diverged from a common ancestor approximately 6 million years ago (Reed et al., 2004, 2007). Substitution rates were calculated once for mitochondrial genes, and once each for the 18S rRNA, EF-1α, and RPII nuclear genes. We converted per generation parameter estimates into per year estimates using the generation time of 18 generations per year (approximately 21 days per generation for *P. humanus*; Nuttall, 1917).

3. Results

3.1. Phylogenetic analyses

In total, six genes were individually examined in this study, totaling 4961 base pairs (bp). Nucleotide and potentially parsimony informative sites (in parentheses and for ingroup taxa only) are as follows: 879 (92) bp for COI, 707 (68) bp for cytb, 579 (59) bp for ND4, 1658 (17) bp for 18S rRNA, 536 (4) bp for EF-1α, and 602 (21) bp for RPII. Genetic divergences (uncorrected p distances) and best-fit models of sequence evolution (from Modeltest; Posada and Crandall, 1998) are shown in Tables 2 and 3, respectively. All phylogenetic analyses resulted in a monophyletic *Pediculus humanus* and a lack of reciprocal monophyly of head and body lice (Figs. 2–5). NJ analyses including all louse samples for the 3 mitochondrial genes resulted in three well-supported clades corresponding to Clades A, B, and C (Fig. 1), however relationships among the 3 clades were undetermined (Fig. 2). Results were similar for MP, ML, and Bayesian analyses of non-redundant taxa (Fig. 3), however nodal support was lacking for Clades A and B. There was little to no nodal support using NJ, MP, ML, and Bayesian analyses of the nuclear genes (Figs. 4 and 5), with the exception of an African clade for the 18S rRNA gene. NJ, ML, and Bayesian analyses each produced one best tree with two exceptions. ML analysis for the mitochondrial cytb and nuclear EF-1α genes resulted in 55 and 2 equally likely trees, respectively, due to minor rearrangements of terminal taxa (1 most likely tree is shown in Figs. 3 and 5). MP analyses resulted in very large numbers of equally parsimonious solutions and heuristic searches failed to reach completion for a majority of the six genes without setting a limit on the number of trees saved. Maxtrees were set to 500 and 10,000, with no overall change in topology or support, which was similar to the ML and Bayesian trees (Figs. 3 and 5). ML bootstrap support values are not shown for COI, cytb, and 18S rRNA (Figs. 3 and 5) because the bootstrap analyses failed to complete even a few replicates on large computer clusters.

Trees constraining head and body lice to be monophyletic were significantly worse than the best ML trees (KH and SH tests $p < 0.05$) only for the cytb and 18S rRNA data sets (non-redundant taxa only). However, all trees constraining head and body lice to be monophyletic were rejected using Bayesian tree sampling (in fact, none of the Bayesian trees were consistent with the topological constraint; $p < 0.001$). Because the KH and SH tests can be biased (Shimodaira, 2002), we place more weight on our analyses using Bayesian sampling.

Table 2
Mean uncorrected p distances (in percentage) between *Pediculus humanus* and *Pediculus schaeffi* (the chimp louse) and within and among *P. humanus* Clades A, B, and C (Figs. 1 and 2)

Taxonomic comparison	Gene					
	COI	cytb	ND4	18S rRNA	EF-1α[b]	RPII[b]
P. humanus vs *P. schaeffi*	16.90	22.68	35.03[a]	1.91	6.15	4.88
Within *P. humanus*	1.18	1.55	1.46	0.49	0.27	0.77
Within Clade A	0.32	0.37	0.42			
Within Clade B	0.34	0.34	0.00			
Within Clade C	1.01	0.30	0.65			
Clade A vs Clade B	5.95	6.04	4.95			
Clade A vs Clade C	7.78	9.59	6.86			
Clade B vs Clade C	7.52	9.02	6.74			

Each gene was assessed separately and all raw data were obtained from GenBank (see text).

[a] This genetic divergence is excessively high, possibly the result of contamination (see also publications discussing the outgroup sequence for cytb; Reed et al., 2004; Kittler et al., 2004). This high divergence does not affect the results of the current manuscript.

[b] For EF-1α and RPII, the genetic divergences may actually be inflated because both alleles from heterozygous individuals were included in the analyses.

Table 3
Best-fit nucleotide substitution models for data sets including all taxa and non-redundant taxa using Modeltest (Version 3.7; Posada and Crandall, 1998)

Gene	All taxa	Non-redundant taxa
COI	GTR + I + G	K81uf + I + G
cytb	GTR + I	GTR + 1
ND4	TrN + I	TrN + 1
18S rRNA	TrN + I	—
EF-1α	TIM + I + G	TIM + I + G
RPII	HKY + I + G	K81uf + I + G

All taxa in the 18S rRNA data set were non-redundant.

3.2. Network and population genetic analyses

Statistical parsimony analyses of the mitochondrial COI gene resulted in 3 unconnected subnetworks (Fig. 6a–c), corresponding to Clades A, B, and C resolved in the NJ analyses above (Fig. 2). The most common and geographically widespread clade (Clade A) was highly reticulated with all haplotypes parsimoniously connected to each other by up to three mutational steps (Fig. 6a). Several haplotypes were shared by both head and body lice (in gray circles; Fig. 6a) and geographic substructuring was minimal (data available upon request). Statistical parsimony analyses of cytb and ND4 had similar results (data available upon request), with one additional unconnected subnetwork each consisting of the same louse from Ethiopia (AY316774 and AY316872, respectively; Kittler et al., 2003). Analysis of the 18S rRNA nuclear gene resulted in a highly reticulated network, shared haplotypes between head and body lice, and an absence of geographic structure (Fig. 6d; results for EF-1α and RPII were similar and are available upon request). Haplotype networks were similar for all genes using several additional network programs (data available upon request).

Using the best-fit model of nucleotide substitution (Table 3) and a divergence date of approximately 6 million years ago (Reed et al., 2004, 2007), the substitution rate for the mitochondrial genes was estimated to be 2.28×10^{-8} substitutions per site per year (subs/site/yr). Estimates for the other genes were 1.94×10^{-9} subs/site/yr, 1.14×10^{-8} subs/site/yr, and 2.38×10^{-8} subs/site/yr for 18S rRNA, EF-1α, and RPII, respectively. Substitution rates were converted to substitutions per locus per generation as required by MDIV using 18 louse generations per year. Estimates for Θ, N_{ef}, F_{ST} and other measures from DnaSP are presented in Table 4. For the mitochondrial genes, estimates of N_{ef} ranged from 6.67 million to 10.97 million individuals between all head and body lice with a slightly lower estimate (2.34 million individuals) between head and body lice from Clade A of the COI gene. With the exception

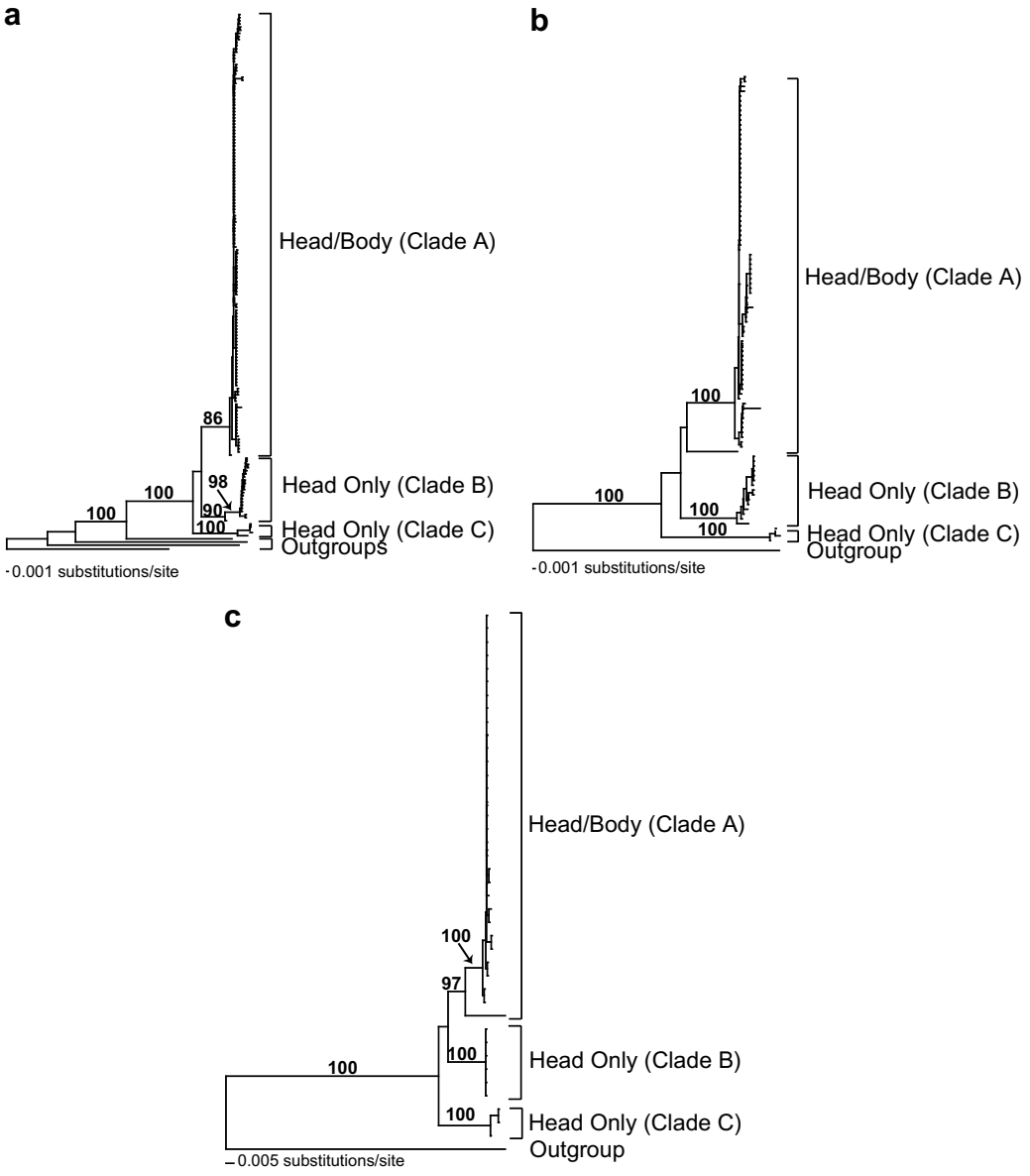

Fig. 2. Neighbor-joining (NJ) phylograms resulting from analysis of all mitochondrial data available on GenBank: (a) COI, (b) cytb, and (c) ND4. Bootstrap support values greater than 75 (based on 1000 NJ bootstrap replicates) are located above the nodes. Mitochondrial clade memberships (Fig. 1) are indicated to the right of each tree.

of 18S rRNA, estimates of N_{ef} were much lower for the nuclear markers. The number of effective migrants per generation ($M = N_{ef}m$) between head and body lice ranged from 1.83 to 2.63 with a slightly higher estimate between head and body lice from Clade A of the COI gene. Estimates of M for the nuclear genes were similar, although slightly lower. For all genes, F_{ST} values ranged from 0.14 to 0.21 between all head and body lice, and 0.11 between head and body lice from Clade A (Table 4).

MDIV estimates for Θ, N_{ef}, scaled migration rate per generation (M), time since population divergence (T_{pop}), and TMRCA are given in Table 5. For the mitochondrial genes, N_{ef} estimates ranged from 2.71 million to 4.46 million individuals (2.02 within Clade A), slightly lower than the estimates from DnaSP (Table 4). Similar to the DnaSP results, N_{ef} estimates for 18S rRNA were comparable to the mitochondrial estimates, but much lower for EF-1α. For all genes, migration between head and body lice ranged from 0.50 to 2.00 effective migrants per generation, similar to those estimated in DnaSP (Table 4). MDIV produced point estimates for the expected TMRCA for the mitochondrial and 18S rRNA genes around 1.50 million years, with much younger estimates for EF-1α and RPII.

4. Discussion

Assessing the taxonomic status of human head and body lice has traditionally been, and remains, a difficult endeavor. Although these louse morphotypes exhibit morphological, behavioral, and ecological differences, analyses using molecular data thus far have been unable to differentiate head from body lice (Leo et al., 2002; Kittler et al., 2003; Yong et al., 2003; Reed et al., 2004; Leo and Barker, 2005). The discordance between biological (morphology, behavior, and ecology) and genetic data from head and body lice may be the result of both the data and methodologies used to examine this difficult taxonomic question (Hypsa, 2006). By using a variety of approaches (phylogenetic, network, and population genetic) to analyze the most diverse sample of head and body lice presently available, we feel we have significantly updated the current knowledge regarding the taxonomic status of head and body lice.

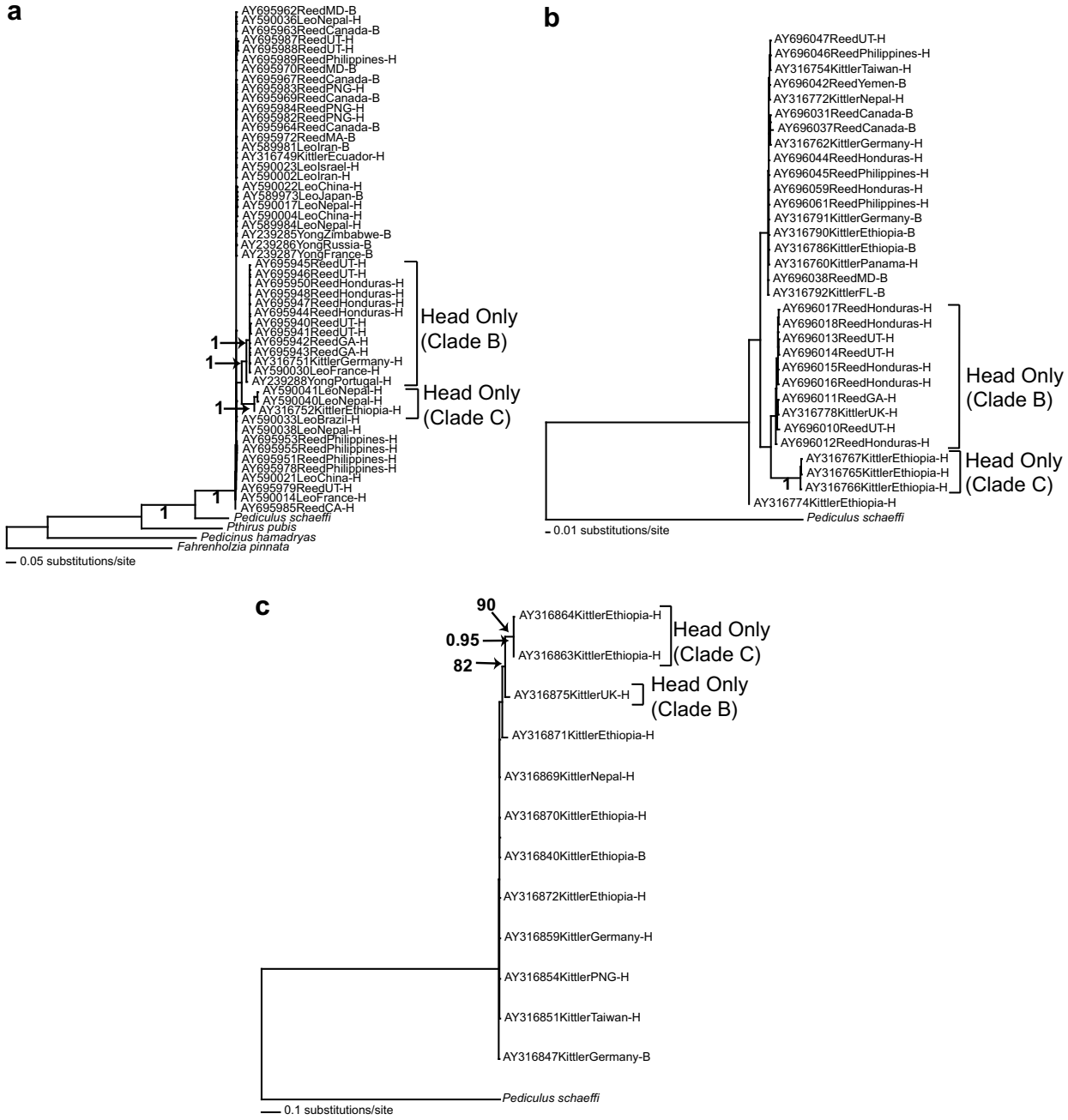

Fig. 3. Maximum-likelihood (ML) phylograms resulting from analysis of non-redundant taxa (see text) for (a) COI, (b) cytb, and (c) ND4. Maximum-likelihood bootstrap support values (greater than 75) and Bayesian posterior probabilities (greater than 0.95) are located above and below the nodes, respectively. Maximum parsimony support values were similar and are available upon request. Mitochondrial clade memberships (Fig. 1) are indicated to the right of each tree. GenBank accession numbers, manuscript lead author, locality, and louse type [head (H) or body (B)] are indicated for each louse specimen. Localities are abbreviated as follows: California (CA), Florida (FL), Georgia (GA), Maryland (MD), Massachusetts (MA), Papua New Guinea (PNG), United Kingdom (UK), and Utah (UT).

4.1. Phylogenetic analyses

In agreement with previous research, the phylogenetic analyses performed here (utilizing both mitochondrial and nuclear data) did not result in reciprocally monophyletic clades of head and body lice (Figs. 2–5). Likelihood scores from trees constraining the monophyly of the louse morphotypes were significantly worse than best tress, thus reciprocal monophyly of head and body lice is not accepted for any of the genes sampled. Head and body lice appeared scattered throughout the phylogenetic trees (Figs. 2–5), with no apparent consistencies in placement except among the mitochondrial genes (although support was often lacking). Given the potential for discordance between gene and species trees, this variable phylogenetic placement (or clade membership) of head and body lice among genes is not surprising. In a recent study, Leo and Barker (2005) identified a head only and a head plus body clade based on 18S rRNA data and concluded that these clades were equivalent to the mitochondrial clades identified by Reed et al., (2004; their Fig. 2). Leo and Barker (2005), however, did not actually test clade membership using both molecular markers from the same louse individuals and it is unclear if these nuclear and mitochondrial clades are homologous.

The data from Kittler et al. (2003) offer an ideal situation to track louse phylogenetic placement because both nuclear (EF-1α

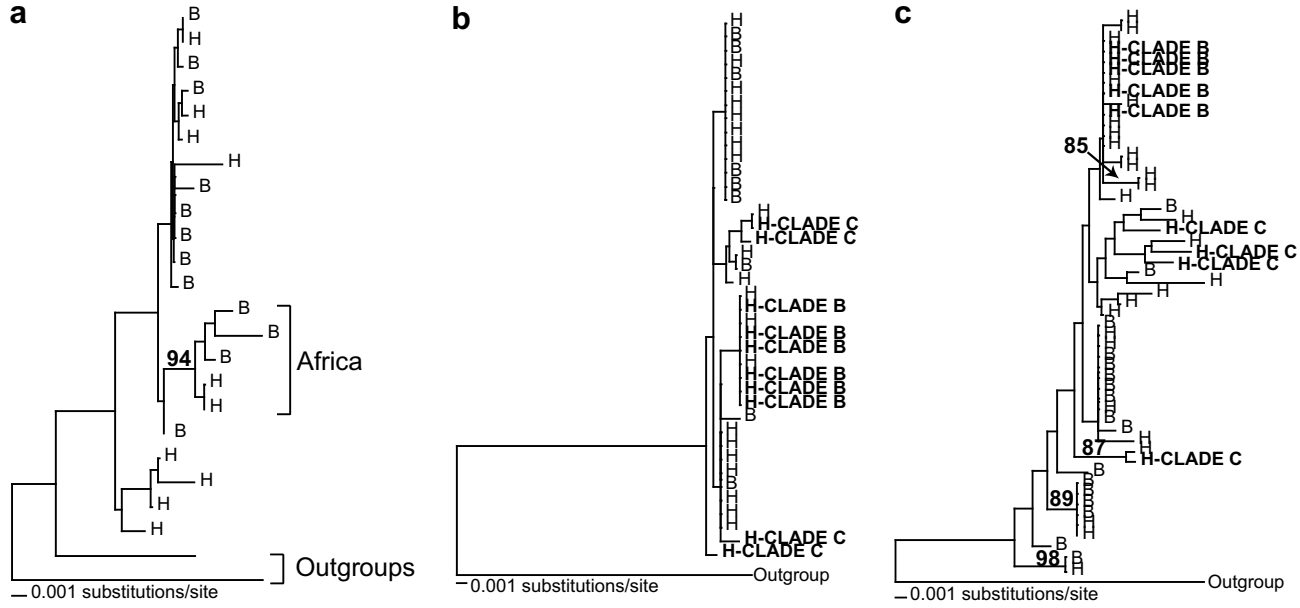

Fig. 4. Neighbor-joining (NJ) phylograms resulting from analysis of nuclear data available on GenBank: (a) 18S rRNA, (b) EF-1 α, and (c) RPII. Bootstrap support values greater than 75 (based on 1000 NJ bootstrap replicates) are located above the nodes. Mitochondrial clade memberships (Fig. 1) are indicated for EF-1 α and RPII (Clades B and C only). Louse specimens are identified as either head (H) or body (B) lice and a well-supported African clade is indicated for the 18S rRNA gene (a). Taxa in bold (EF-1α and RPII only) correspond to taxa from which mitochondrial data were also available (Kittler et al., 2003).

and RPII) and mitochondrial (COI, cytb, and ND4) data were available for several individual lice, specifically louse isolates 4, 18, and 33 from Africa (all members of Clade C; Fig. 1) and isolates 1, 37, 38, 41, 58, and 59 from Europe (all members of Clade B; Fig. 1). Although Clades A, B, and C could be easily identified using NJ analysis of mitochondrial data (Fig. 2), these same three clades were not recovered using nuclear data (Figs. 4 and 5). Rather, members of mitochondrial Clade C were dispersed throughout the trees and there was little support for a monophyletic Clade B (Figs. 4 and 5, taxa in bold). Given the phylogenetic placement of the Kittler et al. (2003) specimens and different genealogical histories of mitochondrial versus nuclear genes (see below), it is clear that analyses of these genes do not necessarily produce identical results with respect to individual lice.

The nuclear 18S rRNA, EF-1α, and RPII markers traditionally have been useful in resolving higher-level taxonomic questions (e.g., Barker et al., 2002; Whiting, 2002; Regier et al., 2004; Danforth et al., 2006). For addressing the taxonomic status of head and body lice, however, these genes are phylogenetically uninformative because of a lack of significant genetic divergences (uncorrected *p* distances; Table 2). In contrast, mitochondrial markers show more promise for phylogenetic studies because they are fast evolving markers that have been useful in many other louse studies (e.g., Hafner et al., 1994; Johnson et al., 2002; Weckstein, 2004; Whiteman et al., 2004; Light and Hafner, 2007a,b; Reed et al., 2007). Although these markers are deeply divergent among *P. humanus* Clades A, B, and C (6–9% uncorrected *p* distance; Table 2; Fig. 2), they are unable to phylogenetically differentiate head and body lice. Rather, phylogenetic analyses of the mitochondrial markers indicate a lack of both reciprocal monophyly and genetic differentiation between the two morphotypes.

4.2. Network and population genetic analyses

Phylogenetic methods applied to nuclear and mitochondrial markers may be insufficient to properly assess the taxonomic status of head and body lice. Stochastic variation within genealogical histories and recent divergences can cause monophyly to be a poor indicator of species status (Avise, 2000; Hudson and Coyne, 2002; Knowles and Carstens, 2007; Shaffer and Thomson, 2007). In this case, intraspecific (network and population genetic) methodologies are preferable because when taxa are young, very closely related, and exhibit only a small number of substitutions that differentiate unique haplotypes, phylogenetic approaches often provide limited resolution and result in multiple equally probable solutions (Figs. 3 and 5; Crandall, 1996; Crandall and Templeton, 1996; Demboski and Sullivan, 2003; Cassens et al., 2003). Previous intraspecific studies examining population structure of hosts and their parasites have found equal or higher structure in the parasites compared to the hosts (Criscione et al., 2006). This is a promising finding for future human-parasite work, where parasites such as *P. humanus* could potentially be used to uncover events during the evolutionary history of humans that currently cannot be observed using human genetic or fossil data (Ashford, 2000). Thus, properly assessing relationships within *P. humanus* using appropriate methodologies could possibly enhance studies regarding human evolutionary history.

Network approaches are a logical first step to examine population structure within *P. humanus* because these analyses take into account several features that are normally associated with intraspecific gene evolution (e.g., persistence of ancestral haplotypes, presence of multiple descendent haplotypes, and low levels of sequence variation; Chen et al., 2006). Network analyses of the mitochondrial genes resulted in shared haplotypes between head and body, several unconnected networks (Fig. 6a–c for COI), and extensive reticulation within each subnetwork (Fig. 6). Although these networks are concordant with results from the NJ analyses, it is unclear if multiple subnetworks are the result of large genetic divergences among clades (Table 2) or analytical failure (Morrison, 2005). Additionally, coalescent theory predicts that the ancestral haplotype is present in data at the highest frequency (Donnelly and Tavaré, 1986), but we cannot say with certainty that the ancestral louse type is a member of Clade A (Fig. 6a) because of the disconnected networks as well as the extremely small sample sizes of Clades B and C. Disconnected networks, reticulation, and shared haplotypes make interpretation difficult and, at most, we could

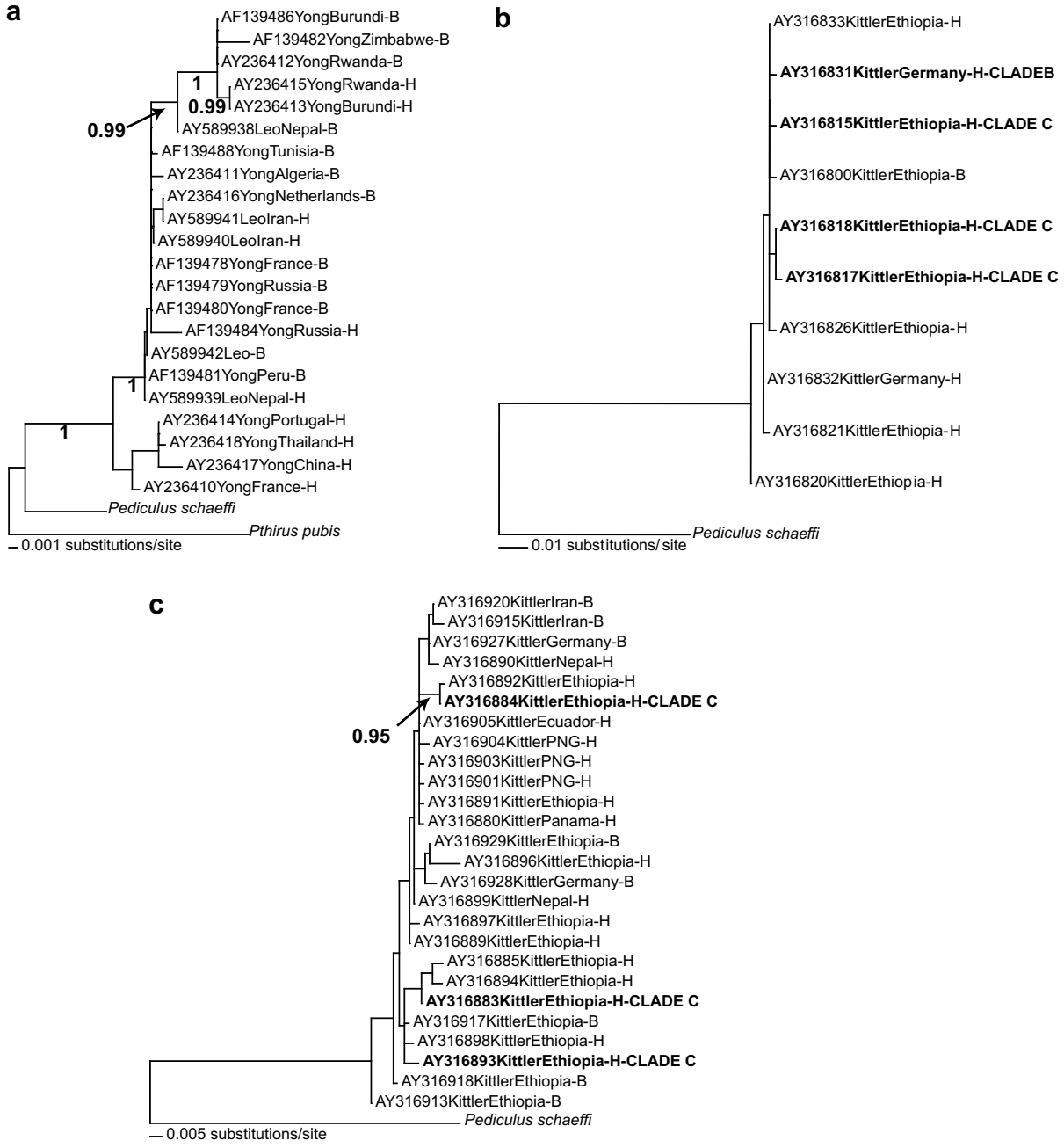

Fig. 5. Maximum-likelihood (ML) phylograms resulting from analysis of non-redundant taxa (see text) for (a) 18S rRNA, (b) EF-1α, (c) RPII. Maximum-likelihood bootstrap support values (greater than 75) and Bayesian posterior probabilities (greater than 0.95) are located above and below the nodes, respectively. Maximum parsimony support values were similar and are available upon request. Mitochondrial clade memberships (Fig. 1) are indicated for EF-1α and RPII (Clades B and C only). GenBank accession numbers, manuscript lead author, locality (if known), and louse type [head (H) or body (B)] are indicated for each louse specimen. Localities are abbreviated as follows: Florida (FL), Georgia (GA), Maryland (MD), Massachusetts (MA), Papua New Guinea (PNG), United Kingdom (UK), and Utah (UT). Taxa in bold (EF-1α and RPII only) correspond to taxa from which mitochondrial data were also available (Kittler et al., 2003).

suggest that head and body lice do not represent distinct species, but additional data and analyses would be necessary to support this statement.

Population genetic analyses (summary statistic and coalescent approaches) offer an advantage over phylogenetic and network analyses because population parameters such as effective population size, gene flow, and migration can be estimated from the data. Estimates of N_{ef}, Θ, F_{ST}, and migration for head and body were similar regardless of which analysis (DnaSP and MDIV) or gene was used (Tables 4 and 5). DnaSP diversity and MDIV dating estimates also were similar across genes (Tables 4 and 5), although some parameters could not be estimated presumably because of the lack of genetic signal. Comparisons made between head and body lice within Clade A of the mitochondrial COI gene resulted in population estimates that were generally lower than estimates resulting from inclusion of all head lice. This finding is not unexpected given the decreased diversity within Clade A when compared to all samples (data not shown). Genetic diversity within head lice also was higher (although not necessarily significantly higher) than body lice for both mitochondrial and nuclear genes. This increased genetic diversity lends support to the ancestral status of head lice where body lice are derived from head lice with the advent of

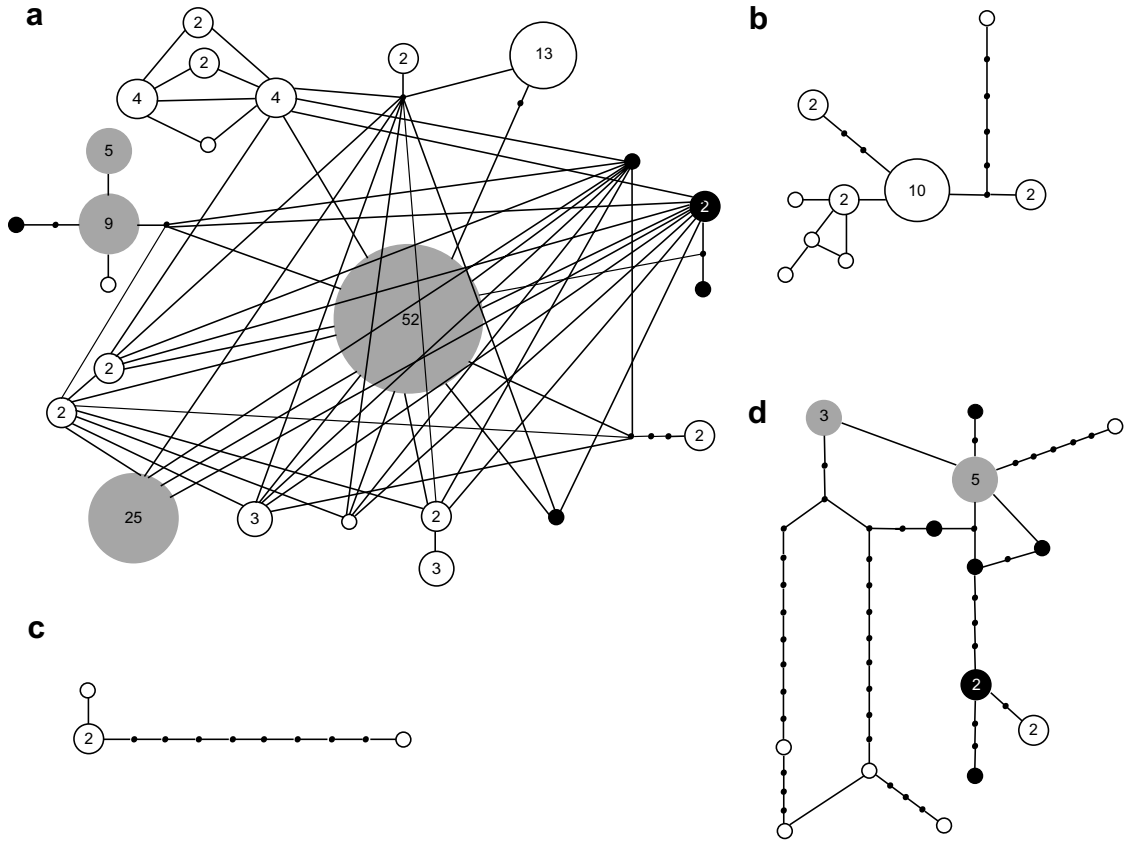

Fig. 6. Statistical parsimony networks for mitochondrial COI Clades (a) A, (b) B, and (c) C, and the nuclear ribosomal 18S rRNA gene (d). Each connection represents a single mutational step with inferred haplotypes represented by small black circles. Observed haplotypes are shown as large circles with haplotype frequency indicated within the circles (no number indicates a single haplotype). White circles indicate haplotypes found only among head lice, black circles indicate haplotypes found only among body lice, and gray circles indicate haplotypes shared between head and body lice.

clothing use in modern humans (Kittler et al., 2003, 2004). Although genetic diversity within head lice is higher than body lice, low F_{ST} values (0.11–0.21; Table 4) and a relatively large number of migrants per generation (>1; Wright, 1978) indicates that there is evidence of recent or ongoing gene flow and little genetic differentiation between these two louse morphotypes.

4.3. Additional findings

Interestingly, NJ and network analyses of the mitochondrial data (Figs. 2 and 6) uncover three mitochondrial clades, in agreement with previous studies (Reed et al., 2004; Fig. 1). Support for relationships among the three clades, however, is lacking (Fig. 3), possibly due to insufficient molecular signal, inappropriate methods (see above), and/or oversampling of ingroup taxa (trimming ingroup taxa to a moderate number of representatives per mitochondrial clade results in high support within and among the clades; Reed et al., 2004; J.E. Light, unpublished data). The presence of these isolated clades within *P. humanus* is fascinating, possibly indicating multiple colonization events of lice on their human hosts (Creer et al., 2001; Reed et al., 2004). Alternatively, the genetic differentiation observed among these three mitochondrial clades may be the result of retained ancestral polymorphism, especially given that recent louse studies using mitochondrial markers have found genetic divergences up to 20% between species (Johnson et al., 2002, 2003; Reed et al., 2004; Light and Hafner, 2007a,b). Future investigation of relationships within and among these three clades is undeniably warranted.

The age of *P. humanus* has also been an issue in previous research and recently, Leo and Barker (2005) expressed concern about discordant age estimates present in the literature. Kittler et al. (2003) and Reed et al. (2004) estimated that *P. humanus* originated 77,000 years ago and 1.18 MYA, respectively. But these estimates are not markedly different, especially when one considers the standard deviation of the Reed et al. (2004) estimate overlaps the Kittler et al. (2003) estimate and that the Kittler et al. (2003) estimates were based on more conserved amino acid data. Coalescent analyses of the mitochondrial and 18S rRNA data estimated that *P. humanus* originated 1.26–1.61 MYA (Table 5) and these dates are similar to phylogenetic estimates (Kittler et al., 2003; Reed et al., 2004, 2007; J.E. Light, unpublished data). Therefore, based on both mitochondrial and nuclear makers, phylogenetic and coalescent-based approaches provide estimates of the age of *P. humanus* between 1 and 2 million years, and further concern may not be necessary.

4.4. Recommendations for future studies and taxonomic conclusions

The lack of reciprocal monophyly in phylogenetic analyses cannot be considered sufficient evidence that head and body lice present only ecological variants of the same species (Knowles and Carstens, 2007; Shaffer and Thomson, 2007). Network and population genetic analyses, especially of mitochondrial data, indicate that head and body lice do not represent distinct species and that there is negligible, if any, population differentiation between these louse morphotypes. Yet, mitochondrial markers represent only a single gene history and recent research has questioned the utility of mitochondrial data to infer population and phylogeographic structure because selection may be acting on these maternally inherited markers (Hurst and Jiggens, 2005; Bazin et al., 2006;

Table 4
Estimates of population diversity, size, and gene flow from the software package DnaSP (v. 4.10.9 software; Rozas et al., 2003) for head and body lice based on the mitochondrial (COI, cytb, and ND4) and nuclear (18S rRNA, EF-1α, and RPII) genes

Estimate	COI, all		COI, Clade A		cytb		ND4		18S rRNA		EF-1 α		RPII	
	Head	Body	Head	Body	Head	Body	Head	Body	Head	Body	Head	Body	Head	Body
Number of samples	108	58	83	58	66	31	26	14	10	12	31	9	36	17
Segregating sites (S)	59	10	15	10	51	3	70	3	19	8	5	3	25	13
Number of haplotypes (h)	19	8	12	8	12	4	10	3	7	5	9	4	21	8
Haplotype diversity (Hd)	0.84	0.71	0.75	0.71	0.73	0.50	0.85	0.38	0.91	0.67	0.82	0.58	0.92	0.80
Average number of differences (K)	11.89	1.40	1.58	1.40	11.11	0.55	19.15	0.67	6.96	2.38	1.48	0.83	4.42	3.75
Nucleotide diversity (π)	0.0260	0.0030	0.0034	0.0030	0.0300	0.0016	0.0030	0.0012	0.0057	0.0020	0.0030	0.0017	0.0074	0.0062
Nucleotide diversity	0.0270	0.0030	0.0034	0.0030	0.0300	0.0017	0.0340	0.0012	0.0057	0.0020	0.0030	0.0017	0.0074	0.0063
Jukes–Cantor (πJC)														
F_{ST}	0.16		0.11		0.21		0.21		0.14		0.16		0.16	
Effective number of migrants (M)	2.63		3.80		1.83		1.87		1.46		1.28		1.24	
Theta per sequence (θ)	10.55		3.62		10.30		16.93		6.31		1.41		5.95	
Effective population size (N_{ef}; in millions)	6.83		2.34		6.67		10.97		8.81		0.30		0.63	

For all genes, populations examined included all head and body lice. For the COI gene, comparisons were also made between head and body lice only from Clade A (see text for descriptions of clades).

Table 5
Estimates of population parameters between head and body lice based on the coalescent for the mitochondrial (COI, cytb, and ND4) and nuclear (18S rRNA, EF-1α, and RPII) genes calculated in the software package MDIV (Nielson, 2002; Nielson and Wakeley, 2001)

Estimate	COI, all	COI, Clade A	cytb	ND4	18S rRNA	EF-1α	RPII
Theta (θ)	5.66	3.12	4.18	6.89	4.46	1.30	10.17
N_{ef} (in millions)	3.67	2.02	2.71	4.46	6.23	0.96	3.20
Scaled migration rate/ generation (M)	1.90	1.48	0.50	3.16	1.00	N/A	2.00
Scaled time since divergence (T)	N/A	N/A	N/A	N/A	0.32	N/A	0.48
Time since divergence (T_{pop})	N/A	N/A	N/A	N/A	0.22	N/A	0.17
TMRCA (in millions of years)	1.38	1.26	1.34	1.48	1.61	0.24	0.38

For all genes, populations examined included all head and body lice. For the COI gene, comparisons were also made between head and body lice only from Clade A (see text for descriptions of clades).

Criscione and Blouin, 2007). In addition to selection, incomplete lineage sorting or introgression can cause mitochondrial data to be misleading. Furthermore, although the mitochondrial markers appear to be fast evolving (Table 1), it is possible that there may not be enough variation in these markers to be informative at the population level and differentiate head and body lice. It will therefore be necessary to verify our results using fast evolving nuclear markers (representing different gene histories) sampled from a worldwide distribution of both head and body lice. Finding fast evolving nuclear markers can be a difficult task (Leo and Barker, 2002; Leo et al., 2005), however with the upcoming release of the body louse genome (Pittendrigh et al., 2006) we are optimistic that microsatellites, SNPs and SNPSTRs can be characterized from *P. humanus*.

Analyses of fast evolving nuclear makers should rely on intraspecific, preferably coalescent, analyses to assess relationships within *P. humanus* (Knowles and Carstens, 2007). Traditional population genetic models frequently rely on unrealistic, and often violated, biological assumptions such as symmetrical rate of gene flow among populations and equal population sizes (Hey and Machado, 2003; Bowie et al., 2006). Coalescent approaches are able to overcome these limitations by taking population structure and demographic processes into account when calculating estimates within and among populations (Beerli and Felsenstein, 1999, 2000; Bowie et al., 2006). Future analyses of fast evolving nuclear markers in a coalescent framework will either support or refute our mitochondrial findings of no population differentiation between head and body lice. If the mitochondrial data are refuted, head and body lice may represent distinct species. Alternatively, depending on the amount of genetic differentiation, head and body lice may be incipient species. Body lice represent a relatively young morphological variant and insufficient time may have elapsed to result in genetic differentiation from head lice (Buxton, 1946). Regardless, the genetic data presented here are in conflict with the morphological, behavioral, and ecological differences between the two louse morphotypes. These biological differences, however, may not be as substantial as previously thought. The morphological differences are primarily quantitative (Schöll, 1955; Busvine, 1978; Reed et al., 2004), louse habitat preferences are not necessarily absolute (Nuttall, 1917 and references therein; Keilen and Nuttall, 1919; Busvine, 1944, 1978; Fournier et al., 2002; Brouqui et al., 2005), and head lice have been shown to host, and possibly vector, bacterial pathogens previously thought to be transmitted only by body lice (Robinson et al., 2003; Sasaki et al., 2006a,b). Although the definition of a "species" is controversial, the overall lack of genetic differentiation

presented here, as well as the small amounts of morphological, behavioral, and ecological variability, is hardly sufficient to characterize head and body lice as distinct species at this time. Not one of the major species concepts employed today (reviewed in de Queiroz, 1998, 2007 and Coyne and Orr, 2004), including the biological species concept (Mayr, 1942, 1995), genotypic cluster species concept (Mallet, 1995), recognition species concept (Paterson, 1985), cohesion species concept (Templeton, 1989), evolutionary species concept (Wiley, 1978), ecological species concept (Van Valen, 1976), or even all variants of the phylogenetic species concept (de Queiroz and Donoghue, 1988; Cracraft, 1989; Baum and Donoghue, 1995), would recognize head and body lice as evolutionary discrete units. Therefore, although these louse morphotypes have often been cited as a classic example of sympatric speciation, it is more likely that head and body lice may only represent weakly isolated ecotypes (Coyne and Orr, 2004).

In any taxonomic study, it is necessary to collect and rigorously analyze appropriate data before making definite taxonomic statements. In the case of economically or medically important species, it is imperative that this new taxonomy is sound because it will often be immediately applied to conservation, control, or eradication efforts. It is helpful to provide to the larger community (beyond taxonomists) a point of reference for taxonomic clarification to prevent the perpetuation of invalid names (e.g., *P. corporis* for body lice), and to provide guidance on the current status of species that are in flux. We therefore recommend retaining the single species *P. humanus*, consisting of two morphotypes (head and body lice), until compelling new data suggest otherwise.

Acknowledgments

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Appendix A

Accession numbers for all louse sequences used in this study

Publication	COI	cytb	ND4	18S rRNA[a]	EF-1α	RPII
Leo et al. (2002)	AF320286[b]					
Kittler et al. (2003)	AY316748–AY316752	AY316753–AY316792	AY316835–AY316875		AY316794–AY316833	AY316876–AY316911, AY316913–AY316929
Yong et al. (2003)	AY239285–AY239288			AY139478–AY139482, AY139484, AY139486, AY139488, AY236410	AY239271 – AY239284	
Reed et al. (2004)	AY695940–AY695999	AY696009–AY696066				
Leo and Barker (2005)				AY589938–AY589942		
Leo and Barker, unpublished	AY589944–AY590041					

Louse sequences are divided by publication.

[a] An additional 18S rRNA sequence (AY077775) was also available on GenBank but was not used because it was not known if this sequence was from a head or body louse.

[b] AF320286 was accidentally omitted from the analyses performed herein. This sample is identical in sequence to many of the other COI sequences and subsequent analyses including AF320286 did not change the results presented in this study (data available upon request).

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