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Mitochondrial Diversity in Human Head Louse Populations Across the Americas

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ABSTRACT Anthropological studies suggest that the genetic makeup of human populations in the Americas is the result of diverse processes including the initial colonization of the continent by the first people plus post-1492 European migrations. Because of the recent nature of some of these events, understanding the geographical origin of American human diversity is challenging. However, human parasites have faster evolutionary rates and larger population sizes allowing them to maintain greater levels of genetic diversity than their hosts. Thus, we can use human parasites to provide insights into some aspects of human evolution that may be unclear from direct evidence. In this study, we analyzed mitochondrial DNA (mtDNA) sequences from 450 head lice in the Americas. Haplotypes clustered into two well-supported haplogroups, known as A and B. Haplogroup frequencies differ significantly among North, Central and South America. Within each haplogroup, we found evidence of demographic expansions around 16,000 and 20,000 years ago, which correspond broadly with those estimated for Native Americans. The parallel timing of demographic expansions of human lice and Native Americans plus the contrasting pattern between the distribution of haplogroups A and B through the Americas suggests that human lice can provide additional evidence about the human colonization of the New World. Am J Phys Anthropol 152:118-129, 2013. © 2013 Wiley Periodicals, Inc.

The genetic makeup of modern human populations in the Americas is expected to primarily reflect the two major settlements of the New World: the first peopling of Americas and the European colonization after Columbus. Although the timing, geographic routes, and number of initial colonizations into the Americas are still under debate, there is a general agreement that Native American populations originated in Asia (Wallace et al., 1985; Torroni et al., 1993; Forster et al., 1996; Kolman et al., 1996; Dillehay, 1999; Kaufman and Golla, 2000; Schurr and Sherry, 2004; Tamm et al., 2007; Goebel et al., 2008; Gilbert et al., 2008; O'Rourke and Raff, 2010). However, after five centuries of admixture between Native Americans, Europeans, Africans, and modern Asians, current populations in the Americas contain great genetic heterogeneity. Because of the recent fast-paced demographic processes that shape the current genetic makeup of human populations in the Americas, we propose to use a host-specific parasite to reveal information about contemporary human population structure. Parasite features differ from that of their hosts in that parasites generally have faster evolutionary rates and larger population sizes, which allow them to maintain greater levels of genetic diversity than their hosts. These attributes also offer greater resolution for the estimation of population genetic parameters such as effective population size (N_e) and coalescent times. Previously, several human parasites have provided insight into aspects of human evolution that were less clear from studies of direct human evidence such as fossil or molecular data. For example, the evolutionary history of the bacterial parasite, *Helicobacter pylori*, which infects the stomach of most humans, reflected major events in human migrations such as the out of Africa expansion (Falush et al., 2003; Moodley et al., 2012), the colonization of Austronesian speaking region (Moodley et al., 2009), and the settlement of the Americas by Asians across the Bering Strait (Ghose et al., 2002). However, microorganisms such as bacteria and viruses often reproduce clonally, increasing the probability of observing a selective sweep such that the parasite phylogeny is likely to reflect the selection history of some genes rather than of the host tree. Conversely, macroparasites (such as

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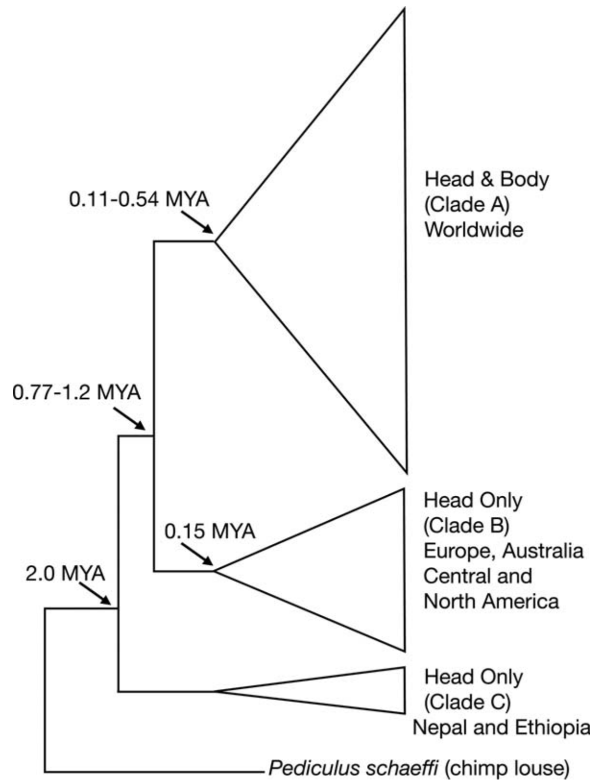

Fig. 1. Phylogenetic relationships, timing of divergence events (in millions of years; MYA), and geographic distribution among human lice based on the mitochondrial COX1 gene from Light et al. (2008). Height of the triangles represents the number of specimens in each clade.

parasitic arthropods) often reproduce sexually and may track host evolutionary history more accurately because selective sweeps affect only closely linked loci in their genomes (Grenfell et al., 2004). In cases where parasites coevolve with their hosts, parasite phylogenies will, to some extent, reflect host evolutionary history (Hafner and Nadler, 1988; Hafner et al., 1994). For this reason, the use of macroparasites such as pinworms (Araújo et al., 2008) and lice (Reed et al., 2004; Raoult et al., 2008) has become increasingly common in the study of human evolution. In fact, the human louse, *Pediculus humanus*, is one of the most well studied human parasites.

Human lice are blood-sucking, wingless, host-specific ectoparasites of humans that are both obligate (cannot live off the host) and permanent (complete their life cycle on a single host species). This parasite combines several features that make it an ideal candidate for coevolutionary studies. Studies have shown that primate lice synchronously speciated with their primate hosts (cospeciation) during the last 25 million years (Reed et al., 2004, 2007). Lice from humans and chimpanzees diverged about 5.6 million year ago (MYA), which is strikingly similar to the estimated dates for the divergence of humans and chimpanzees themselves (Reed et al., 2004; Light and Reed, 2009). The human louse genealogy shows three deeply divergent mitochondrial (mtDNA) lineages referred to as clades or haplogroups: A, B, and C (Light et al., 2008). Louse lineages coalesce to a common ancestor around 2 MYA ago (Reed et al., 2004; Light and Reed, 2009) (Fig. 1). Haplogroup A has a worldwide distribution, is the most common of the human louse haplogroups, and shows the signs of a recent demographic expansion about 100,000 years ago (Reed et al., 2004). This date coincides with the out-of-Africa expansion of anatomically modern humans (AMHs) (Reed et al., 2004), thus reflecting a codemographic pattern between lice and humans. Haplogroup B diverged from haplogroup A between 0.7 and 1.2 MYA (Light and Reed, 2009), and perhaps evolved on Neanderthals living in Europe and Asia only switching to modern humans recently during periods of overlap (Reed et al., 2004). Haplogroup C has only been found in Nepal and Ethiopia and given its early divergence in the *Pediculus* tree (ca. 2 MYA), may have evolved on archaic hominids in Asia or Africa (Reed et al., 2004). A key feature of lice is that we can use lice to track host ecological interactions including those that are nonsexual because lice can be transmitted horizontally through host-to-host physical contact or even by sharing of sleeping sites.

The Americas were the last continental landmasses colonized by humans (with the exception of Antarctica), thus understanding the genetic diversity of humans and their parasites in the Americas could reveal the extent of ancient human demographic events that occurred before the first people arrived to the continent. We examined 450 lice collected from 15 localities throughout North, Central, and South America and analyzed their mitochondrial genetic diversity to evaluate the geographic distribution of the haplogroups. Based on previous human louse studies (Reed et al., 2004; Light et al., 2008), and what is known about human migrations, our predictions are that (1) if haplogroup A lice evolved on AMHs and lice reflect human out-of-Africa expansion ~100,000 years ago, we will find a low number of A-haplotypes as a result of recent demographic expansion and (2) if haplogroup B lice evolved on Neanderthals, who expanded in both Europe and Asia, we will expect high within-haplogroup differentiation among B-haplotypes, plus a secondary contact in the Americas between B-Asian haplotypes brought by the first people coming from Asia and B-European haplotypes brought by European colonizers in the last 500 years. A better understanding of the gene pool of American lice can provide insights into the population dynamics of this parasite, the history of human colonization of the Americas through time, and how ancient pre-New World events impacted American human louse diversity.

MATERIALS AND METHODS

Sampling

Head lice were collected mostly from school-age children. Each child's head was visually inspected, and head lice were removed using a fine-toothed anti-louse metal comb. Then lice were picked up with forceps and placed in individual vials containing 95% ethanol. In the laboratory, vials were stored at −20°C. A total of 380 human head lice were collected throughout the Americas (Table 1, Fig. 2). We retrieved 70 sequences belonging to head lice from the Americas from GenBank generating a final dataset of 450 sequences (Supporting Information Table S1). In addition, 41 human louse COX1 sequences from around the world were included in the analysis (Supporting Information Table S1).

TABLE 1. Geographic distribution of mitochondrial DNA haplogroups in human head lice from the Americas based on the COX1 gene

		Haplogroups	
		A	B
North America	USA		
	California	13	0
	Utah	13	15
	Washington	51	8
	Florida	8	15
	Georgia	0	2
	New York	9	5
	Tennessee	0	4
	Texas	2	20
	Subtotal	96	69
Meso and Central America	Mexico	4	10
	Honduras	4	25
	Panama	0	2
	Subtotal	8	37
South America	Argentina	224	11
	Colombia	2	0
	Ecuador	2	0
	Peru	1	0
	Subtotal	229	11
	Total	333	117

DNA extraction

Lice were cut in half, placed into 1.5 ml Eppendorf tubes containing a cell lysis and proteinase K solution, and ground using a pestle. DNA was extracted from each individual louse using the Puregene Core Kit A (QIAGEN, Valencia, CA) following manufacturer protocols with modifications. We used a NanoDrop 1000 spectrophotometer (Thermo Scientific) to determine the amount of DNA in each sample and then diluted them from their original concentrations to approximately 5–10 ng/μl.

PCR amplification and sequencing

A polymerase chain reaction was performed to amplify the mitochondrial gene cytochrome c oxidase 1 (COX1) using the primers H7005 and L6625 (Hafner et al., 1994) as described in Reed et al. (2004). PCRs consisted of 25 μl total volume including 10 μl of MasterMix (5 PRIME), 1 μl of each primer, 2–4 μl of total genomic DNA, and water. The thermal cycling profile began with an initial denaturation at 94°C (10 min) followed by 10 cycles of 94°C (1 min), 48°C (1 min), and 65°C (2 min) (decreased by 0.5°C per cycle). This was followed by 35 cycles of 94°C (1 min), 52°C (1 min), and 65°C (2 min) and then a final extension of 65°C (10 min). Amplified

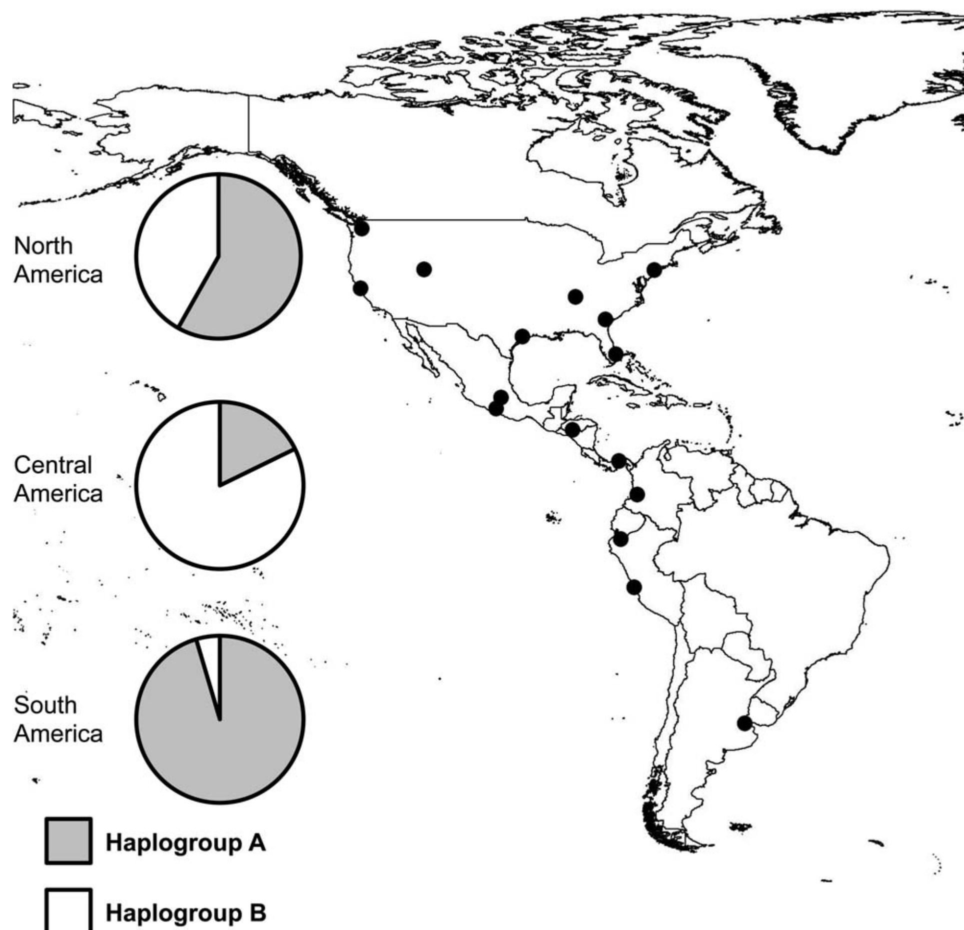

Fig. 2. Collecting sites of head lice (black dots) and haplogroup composition throughout the Americas (North, Central, and South) indicated by the colored pies: haplogroups A (light gray) and B (white).

fragments were purified using ExoSAP-IT (USB Corporation, Cleveland, OH). Sequencing was performed at the University of Florida DNA Sequencing Core Laboratory (ICBR, Gainesville, FL) using standard fluorescent cycle-sequencing PCR reactions (ABI Prism Big Dye terminator chemistry, Applied Biosystems).

Sequence editing, alignment, and haplotype reconstruction

The forward and reverse sequences were edited and aligned using Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, MI) with base calling confirmed by eye. Consensus sequences were generated for each sample using both forward and reverse sequences. One hundred eleven additional head louse COX1 sequences previously obtained by our group (Supporting Information Table S1) were aligned to the sequences obtained in this study, leading to a 379 bp alignment for 491 human head louse COX1 sequences. For the sequences obtained in this study, unique haplotypes were defined using DnaSP v5.10 (Librado and Rozas, 2009) and these haplotype sequences have been deposited in GenBank (KF250510-KF250546). Basic haplotypic diversity measurements including haplotype diversity (Hd) (Saitou and Nei, 1987) and nucleotide diversity (π) (Tajima, 1983) were also estimated using the program DnaSP v5.10 (Librado and Rozas, 2009).

Genetic distance analyses

A matrix of pairwise differences using uncorrected *p*-distances (proportion of nucleotide sites at which two sequences being compared are different) among COX1 unique haplotypes was calculated with MEGA 4 (Tamura et al., 2007). The genetic relationships were estimated by constructing neighbor-joining (NJ) trees (Saitou and Nei, 1987) using PAUP* 4.0b10 (Swofford, 2003). Bootstrapping was performed using 100 pseudoreplications of the dataset. This analysis provides unambiguous assignment of clade membership: A, B, and C to each sequence. Two outgroup COX1 sequences from chimpanzee lice (*Pediculus schaeffi*) (GenBank Accession numbers AY695999 and EF152553) were aligned to the human louse dataset. The genealogical relationships among haplotypes within each human louse mitochondrial clade were analyzed using the program TCS version 1.13 (Clement et al., 2000).

Statistical analysis

Haplotype frequencies for the Americas were determined and graphed using Microsoft Excel. For the comparison in the Americas (North, Central, and South America), haplotype frequency was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Calculated means were compared and separated by the Tukey test at $P < 0.05$ (StatSoft, 2001). The observed vs. expected frequency X^2 test to evaluate the fit of the data (frequencies) to any arbitrary set of expected frequencies was made among North, Central, and South American regions. All statistical tests were performed with $\alpha = 0.05$ for significance of statistical tests.

Demographic analysis

To investigate the demographic history of human lice in the Americas, we used two different statistics implemented in the program Arlequin v3.5 (Excoffier and Lischer, 2010). First, we compared the mismatch distributions of pairwise nucleotide differences among all the COX1 sequences, and then also for each clade. As population size changes are expected to leave detectable patterns in the distribution of genetic differences (Slatkin and Hudson, 1991; Rogers and Harpending, 1992; Harpending, 1994), the smoothness of the mismatch distribution was measured using the raggedness index *rg* (Harpending, 1994). The second statistic used was Fu's statistic (F_S) (Fu, 1997), which considers the haplotype distribution. Significance of these tests was evaluated with a parametric bootstrap of 1,000 replicates assuming a neutral infinite-sites model and constant population size (Hudson, 1990) The simulations estimate the probability of obtaining values of the statistics (*rg* and F_S) equal to or lower than the observed using empirical sample sizes and $\theta = 2N_e\mu$, which is estimated from the data. With this program, measures of mitochondrial haplotype diversity (Hd) (Nei, 1987), nucleotide diversity (π) (Nei, 1987), the number of segregating sites (S), the mean number of pairwise differences (Π), and estimator of the mutation time (τ) were also estimated. The latter was used to calculate the time of population expansion in human lice from the Americas based on the method devised by Rogers and Harpending (1992) and Rogers et al. (1995). The time of possible expansion in generations (t) was calculated using the formula $\tau = 2\mu t$, where τ is an estimate of the mutation time and μ is the mutation rate for the whole sequence. The time at which the expansion event took place was dated following the expression, $t = \tau/2\mu k$, where τ is the estimated number of generations since the expansion, μ is the mutation rate per site per generation, and k is the sequence length. The formula was applied using a mutation rate of 6.28×10^{-8} substitutions per site per year previously used in studies of human lice (Toups et al., 2011).

Coalescent analysis

The Bayesian Markov Chain Monte Carlo method implemented in BEAST software v1.7.2 (Drummond et al., 2012) was used to estimate the times to most recent common ancestor (TMRCA) for each mitochondrial clade. To avoid over-parameterization—considering that the sequences were short—we used a strict clock with the same mutation rate as described above. Best fitting substitution models for each dataset were determined based on Bayesian information criterion (Schwarz, 1978) using a maximum likelihood (ML) model test implemented in MEGA 5 (Tamura et al., 2011). The whole dataset was tested using coalescence priors under two demographic models (constant size and exponential growth) with strict clock conditions. We compared these two models by calculating Bayes Factors, which is the difference in log marginal likelihoods between two model combinations (Kass and Raftery, 1995). We calculated approximate marginal likelihoods for each model via importance sampling using the harmonic mean (HME) of the sampled likelihoods (with the posterior as the importance distribution) (Newton and Raftery, 1994). In addition, we used a new marginal likelihood estimator: path sampling as it is suggested to substantially outperform HME (Baele et al., 2012) (Supporting Information Table S2). Bayesian Skyline Plots (BSP), which use the coalescent properties of gene trees to plot population size changes over time, were

constructed for each mtDNA haplogroup using BEAST (Supporting Information Fig. S3). All Markov chains were run for 100,000,000 generations with samples taken every 1,000 generations and the first 1,000,000 generations were discarded as burn-in. Convergence of parameters was assessed by calculating the effective sample size (ESS) using Tracer v1.5 (Rambaut and Drummond, 2007). All parameter estimates for each run showed ESS values >100. Three independent runs were combined using LogCombiner v.1.7.1 (Drummond and Rambaut, 2007). Tracer v1.5 (Rambaut and Drummond, 2007) was also used to calculate the mean and upper and lower bounds of the 95% highest posterior density interval (95% HPD) for divergence times. Tree topologies were assessed using TreeAnnotator v.1.7.1 (Drummond and Rambaut, 2007).

RESULTS

COX1 sequence variation and phylogenetic analysis

COX1 sequence data from 380 head lice were collected and combined with 70 sequences retrieved from GenBank generating a dataset with a total of 450 sequences spanning 15 geographic locations throughout the Americas (Table 1). Variable nucleotide positions accounted for 12.4% of the sites. A NJ tree supported the presence of the two major haplogroups of head lice (100% bootstrap support): A and B (Fig. 3). Within the A and the B haplogroups, average *p*-distances were 0.674% and 1.652%, respectively, whereas between-haplogroup average *p*-distance value was of 8.143%.

Lice from the three geographical regions (North, Central, and South America) were found in each of the two clades, although with different frequencies (see Statistical Analysis below). Thirty-seven unique COX1 haplotypes were identified, 12 haplotypes belonging to haplogroup A and 25 haplotypes from haplogroup B (Table 2). Haplotypic diversity (Hd) and nucleotide diversity (π) were 0.6050 and 0.001723, respectively, for haplogroup A, and 0.7946 and 0.00831, respectively, for haplogroup B. The most common haplotypes were found in more than one location. From haplogroup A, the two most common haplotypes were: A-H002 ($N = 191$, in five locations), followed by A-H003 ($N = 83$, in nine locations). Within clade B, B-H001 ($N = 43$, in eight locations) was the most common and also found throughout the Americas. The second most common B-haplotype, B-H002 ($N = 30$, in four locations) was found in North and Central America, but not South America.

We constructed haplotype networks using the statistical parsimony method implemented in the program TCS to further understand the relationships among the COX1 haplotypes within each major haplogroup. In addition, we conducted the same analysis using available data generated in our group with known geographic location (Supporting Information Table S1). Missing and ambiguous characters were collapsed resulting in a slight reduction of the number of haplotypes. In the haplogroup A network, A-H002, the most common haplotype occupied a central position, with six haplotypes derived by single mutational steps, and the remaining two A-haplotypes connected by only two mutational steps (Fig. 4A). Within haplogroup B, the most common haplotype was B-H001 and was only found in North and Central America (Fig. 4B). Haplotypes B-H010 and B-H018 were merged with the larger haplogroup B-H001 due to ambiguous bases. The second most common B-haplotype was B-H002, which contained the merged haplotypes B-H006, B-H021, and B-H022, and was also only found in North and Central America. B-H002 was the central B-haplotype in the network with seven derived haplotypes connected by a single mutational step (Fig. 4B). There are three groups of highly derived haplotypes that are separated from the main group by more than seven mutational steps. The first of these groups included B-H003 and B-H004, neither of which is found in North America. The other two groups are composed of (1) a highly differentiated haplotype B-H020 from Honduras and (2) haplotypes B-H024 and B-H025 from Mexico, singleton B-H023 from Texas, and singleton B-H016 from Utah.

Larger sets of networks were constructed by adding COX1 sequences from two countries in Europe: Norway and the UK. All Norway and some UK sequences belonged to haplogroup A (Supporting Information Table S1). Again for the haplogroup A network, there was a lack of geographic structure (Supporting Information Fig. S1). In the haplogroup-B network, most of the UK sequences belong to the same B-haplotype, which is the most common within the B-haplogroup. Moreover, this haplotype has been found throughout USA sites with high prevalence in Texas, and also in Mexico. Thus, this could be a common and widely distributed B-haplotype. For example, if this haplotype was originally present in archaic populations of the Middle East, when AMHs arrived, lice could have switched onto AMHs and migrated with them through the world along with lice of haplogroup-A. Alternatively, Europeans could have brought this haplotype to the New World. The current sampling, however, cannot differentiate between the two hypotheses.

Statistical analyses

Haplotype frequencies differ geographically (Fig. 2). Haplogroups A and B were both relatively common in North America, with 58% haplogroup A and 42% haplogroup B. In Central America and Mexico, haplogroup A was much less common than haplogroup B (18% and 82%, respectively). In contrast, 95% of the head lice from South America belonged to haplogroup A with only 5% of the haplotypes from haplogroup B. The haplotype frequency through the Americas (North, Central, and South America) was compared using an ANOVA. These results show that there is a significant difference in haplotype distribution (A vs. B) in the Americas ($P = 0.007$).

Demographic analysis

The distribution of pairwise differences displayed a bimodal shape consistent with the presence of two divergent haplogroups (Fig. 5A). The left peak corresponded to comparisons within haplogroups, and the right peak to between-haplogroup comparisons (Fig. 5A). The average numbers of pairwise differences between haplogroups was 11.04 while within each haplogroup were 0.653 and 2.860 for clades A and B, respectively (Table 3). The multiple peaks observed in the mismatch distribution within B (Fig. 5B) are consistent with the divergent haplotypes observed in the network analysis (Fig. 4B). This pattern could be the result of either population differentiation, which corresponds to demographic growth and a subsequent bottleneck, or migration that resulted in secondary contact among previously

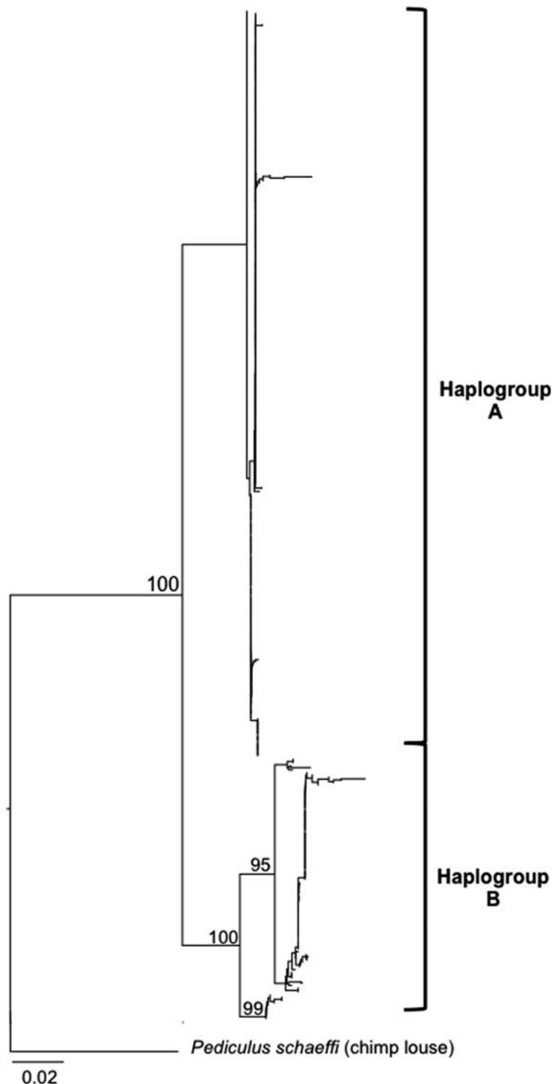

Fig. 3. Neighbor-joining tree constructed from COX1 sequences data from 450 human lice. Bootstrap values greater than 90% (based on 100 NJ bootstrap replicates) are located above main nodes. Mitochondrial clade membership is indicated to the right of the tree.

differentiated lineages. Thus, in addition, within haplogroup B we created a dataset including only the large group of haplotypes connected for less than eight mutational steps, and designated this group as subhaplogroup B1 (Fig. 4B). Using this dataset, we also performed the mismatch distribution analysis (Fig. 5B), and subhaplogroup B1 had an average pairwise difference of 0.848 as well as low *rg* and negative F_S values (Table 3). In contrast, the observed mismatch distribution for haplogroup B did not differ significantly from the expectations under conditions of constant growth ($rg = 0.0708$, $P = 0.5600$). However, F_S, which considers the number of different haplotypes, was significant ($F_S = -11.1627$, $P = 0.0010$) reflecting an excess of recent mutations over neutral predictions (Table 3). For haplogroup A and subhaplogroup B1, the estimation of the time of the population expansion in human lice in the Americas was estimated from the mismatch distribution based on the method devised by Rogers and Harpending (1992); using the formula $\tau = 2\mu t$ (described in Materials and Methods). When the mutation rate was applied to the formula, and the average τ value was used, dates of population expansion were estimated as 16,000 years ago (range = 11,154–29,221) for haplogroup A and 20,000 years ago (range = 9,894–34,788) for subhaplogroup B1, respectively. The BSP showed evidence of a subtle but persistent population growth for A and B1 starting around 1,000 years ago (Supporting Information Fig. S2). It is probable that the BSP analysis was limited by low within-haplogroup diversity. The lack of bottleneck signal in the BSP plot for the clade B also suggests that the bimodal distribution within clade B is more likely due to secondary contact among previously differentiated lineages.

Coalescent times

The ML model test implemented in MEGA 5 (Tamura et al., 2011) indicated the best-fit models for each set of data. For all head louse sequences, BEAST analysis was performed with a T92+I nucleotide substitution model, for haplogroup A and for haplogroup B1, a T92 substitution model were used. BEAST analyses under a strict molecular clock yielded median estimates of the time to the most recent common ancestor (TMRCA) of 49,000 years ago (95% HPD: 88,890–16,814) for haplogroup A, 300,000 years ago (95% HPD: 453,970–156,930) for haplogroup B, and 147,000 years ago (95% HPD: 253,200–51,677) for subhaplogroup B1. The TMRCA of A+B from the Americas was estimated at approximately 707,000 (95% HPD: 1.0215 MYA–402,600), which differs from the 1.28 MYA (95% HPD: 2.03–0.72 MYA) estimate derived from worldwide samples and using mitochondrial and nuclear genes with relaxed molecular clocks (Light and Reed, 2009). It is to be noticed that in Light and Reed (2004) besides using sequences from different areas of the world, divergence times were estimated using Bayesian approaches implemented in multidivtime (Thorne et al., 1998; Kishino et al., 2001) and in a previous version of BEAST (v1.46) (Drummond and Rambaut, 2007) under a Yule model of speciation. Both methods rely on a phylogenetic framework, where our current Beast analyses are based on the coalescent, which is more appropriate due to the population based questions of our study. It is expected that the rate of coalescence within species is dramatically faster than the rate of cladogenesis.

DISCUSSION

Insights into the peopling of the Americas using louse data

To our knowledge, this is the most geographically widespread study to evaluate the genetic diversity in a human eukaryotic parasite in the Americas. Mitochondrial genetic data of 450 human head lice from 15 localities throughout the Americas showed the presence of two major mitochondrial haplogroups: A and B with frequencies of each haplogroup differing geographically. Our estimates for the coalescent time for haplogroup A $\approx$ 49,000 years ago (95% HPD: 88,890–16,814) and for haplogroup B $\approx$ 300,000 years ago (95% HPD: 453,970–156,930) predate the mitochondrial coalescent time of Native Americans (e.g., Horai et al., 1993; Achilli et al., 2008; Kumar et al., 2011). Much like with mtDNA in Native Americans, the TMRCA dates for human lice in

TABLE 2. Haplotype frequency of human head lice per site in the Americas

	CA	UT	WA	FL	GA	NY	TN	TX	Mexico	Honduras	Panama	Argentina	Colombia	Ecuador	Peru	Total	Acc.#
A_H001	–	–	–	–	–	–	–	–	–	–	–	18	–	–	–	18	KF250510
A_H002	–	2	–	–	–	–	–	–	–	3	–	182	2	2	–	191	KF250511
A_H003	13	9	34	8	–	7	–	1	4	–	–	6	–	–	1	83	KF250512
A_H004	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	2	KF250513
A_H005	–	–	–	–	–	–	–	–	–	–	–	8	–	–	–	8	KF250514
A_H006	–	1	7	–	–	2	–	1	–	–	–	–	–	–	–	11	KF250515
A_H007	–	1	–	–	–	–	–	–	–	–	–	5	–	–	–	6	KF250516
A_H008	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	2	KF250517
A_H009	–	–	9	–	–	–	–	–	–	–	–	–	–	–	–	9	KF250518
A_H010	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1	KF250519
A_H011	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	KF250520
A_H012	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	KF250521
B_H001	–	7	1	6	–	5	1	17	5	–	–	1	–	–	–	43	KF250522
B_H002	–	5	–	5	–	–	–	2	–	18	–	–	–	–	–	30	KF250523
B_H003	–	–	–	–	–	–	–	–	2	–	–	7	–	–	–	9	KF250524
B_H004	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	3	KF250525
B_H005	–	–	–	2	–	–	1	–	–	–	–	–	–	–	–	3	KF250526
B_H006	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	2	KF250527
B_H007	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	3	KF250528
B_H008	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	4	KF250529
B_H009	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	1	KF250530
B_H010	–	–	–	–	1	–	2	–	–	–	–	–	–	–	–	3	KF250531
B_H011	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	KF250532
B_H012	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	KF250533
B_H013	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1	KF250534
B_H014	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	1	KF250535
B_H015	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	1	KF250536
B_H016	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	KF250537
B_H017	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	KF250538
B_H018	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1	KF250539
B_H019	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	KF250540
B_H020	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	KF250541
B_H021	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	KF250542
B_H022	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	KF250543
B_H023	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1	KF250544
B_H024	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	2	KF250545
B_H025	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1	KF250546
	13	28	59	23	2	14	4	22	14	29	2	235	2	2	1	450	

the Americas do not reflect when the lice arrived with the first people of the Americas, but rather reflect the level of genetic diversity present in the parasite founding populations. Another potential explanation for the deeper coalescent times for lice could relate to the larger effective population sizes (N_e) of parasites compared to their hosts.

Demographic expansions

BSP analyses were probably limited by the low within-haplogroup diversity and only showed a subtle but persistent population growth for A and B1 starting around 1,000 years ago (Supporting Information Fig. S1). Conversely, in addition to the unimodal mismatch distribution observed for haplogroup A and subhaplogroup B1, the two statistics used to investigate departures from constant population size also supported the idea of demographic expansions in each of the haplogroups. Specifically, these analyses showed low raggedness (*rg*) and negative Fu's statistic (F_S). The mean date of expansions varied from 16,000 years ago for haplogroup A (CI: 11,154–29,221) and 20,000 years ago for subhaplogroup B1 (CI: 9,894–34,788), which are contemporaneous with estimates of demographic expansions in human populations in the Americas (Tamm et al., 2007; Goebel et al., 2008; Kitchen et al., 2008; Fagundes et al., 2008; Mulligan et al., 2008; Reich et al., 2012). Thus, demographic expansions in human lice seem to resemble those in Native Americans. This pattern was also found in other parasites. Particularly, a recent study in a human parasite endemic to the Americas, *Trypanosoma cruzi* (the agent of Chagas disease), revealed that the domestic TcI genotype underwent a sharp reduction in effective population size approximately 27,000 years ago followed by a gradual expansion from 23,000 years ago until the present (Ramirez et al., 2012).

Different genetic, anthropological and paleoenvironmental evidence support the presence of humans in the greater Beringia area (particularly the Yana River site) as early as 30,000 years ago (Pitulko et al., 2004; Goebel, 2007). Archeological evidence also supports an ancient presence of human lice from sites throughout the Americas. In North America, head lice and their eggs have been found on mummified remains of prehistoric Indians from the American Southwest (Ewing, 1924; Graham, 1965; Horne and Kawasaki, 1984; Cockburn, 1998). Lice have been found in hunter-gatherer and agricultural sites in the Unites States (the Great Basin of Utah and surrounding states, and the Colorado Plateau) and in central Mexico (El-Najjar and Mulinski, 1980). In South America, mummies of Inca sacrifices carried lice (Horne and Kawasaki, 1984), and louse eggs were recovered from human hair found in Brazil and were carbon dated

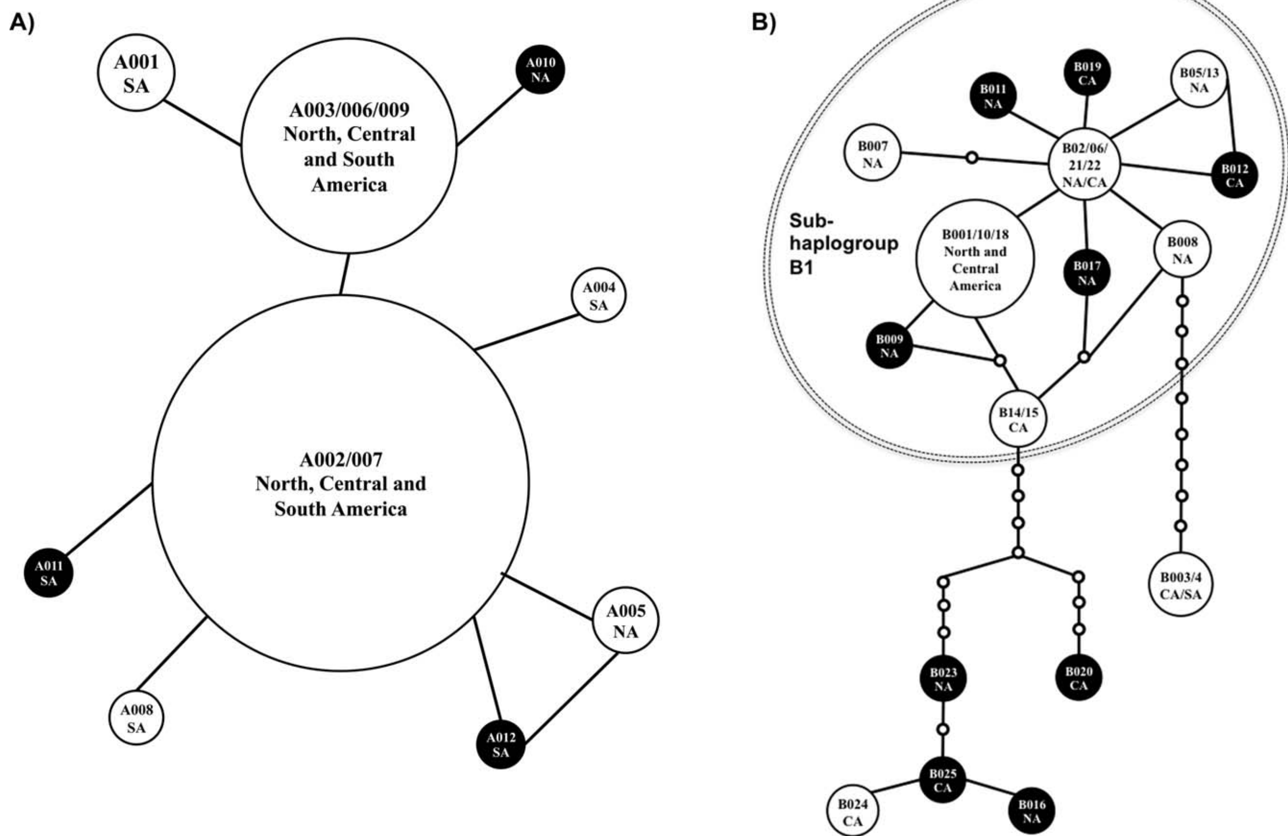

Fig. 4. Statistical parsimony networks for the COX1 haplotypes found in haplogroup A (**A**) and in haplogroup B (**B**). Each connecting branch represents a single mutational step, and inferred missing intermediate haplotypes are represented by open circles. Sizes are scaled and represent relative frequencies (see Table 2 for absolute frequencies). Circles with black background and white letters are singletons (unique haplotypes found in a single location). For each haplotype, the geographic distribution as North (NA), Central (CA), and South America (SA) is indicated. Mexico was included with the Central America countries due to its different colonization history than the rest of North America.

to approximately 10,000 years old (Araújo et al., 2000). Moreover, the lice collected in 1,000-year-old Peruvian mummies showed the presence of haplogroup A lice in the Americas before the time of Columbus (Raoult et al., 2008). Even though the lice used in this study were collected in general mixed populations, they seem to retain a substantial fraction of Native American louse mtDNA, reflecting a similar demographic history as human mixed populations despite the extensive genetic input from Old World populations (mainly from Europe and Africa) (Perego et al., 2010).

Finally, louse nuclear genetic diversity based on microsatellite data of a small sampling from four main geographic regions (North America, Central America, Asia, and Europe) showed that the head louse populations were strongly structured geographically, where one cluster included head lice from North America and Europe, a second cluster encompassed only head lice from Central America, with a third Asian cluster (Ascunce et al., 2013). Principal coordinate and gene flow analyses indicated the closer relationship between Central America and Asia clusters suggesting that the Central America cluster is probably from Native American origin since Asia has been suggested as a potential source population for the first people of the Americas (Torroni et al., 1993; Kolman et al., 1996). Thus, both mitochondrial and microsatellite data support the idea that current populations of human lice in the Americas retain human louse genetic diversity brought by the first people. In the microsatellite study, most of the head lice from North America were collected in the New York area, suggesting that in this particular region, head lice might have retained European louse diversity. A larger sampling and combination of both nuclear and mitochondrial data are needed to fully understand the evolutionary history of head lice in the Americas.

Variation in haplogroup frequencies among North, Central, and South America

There are strong differences in the geographic distribution of louse haplogroups. Haplogroups A and B were both relatively common in North America, with 58% haplogroup A and 42% haplogroup B. In Central America, haplogroup A was much less common than haplogroup B (18% and 82%, respectively). In contrast, 95% of the head lice from South America belonged to Clade A with only 5% of the haplotypes from Clade B. By comparing the haplotypic distribution among the continents, some haplotypes from both clades have a wide distribution, suggesting that they are common worldwide haplotypes, or were brought with the first peoples of the

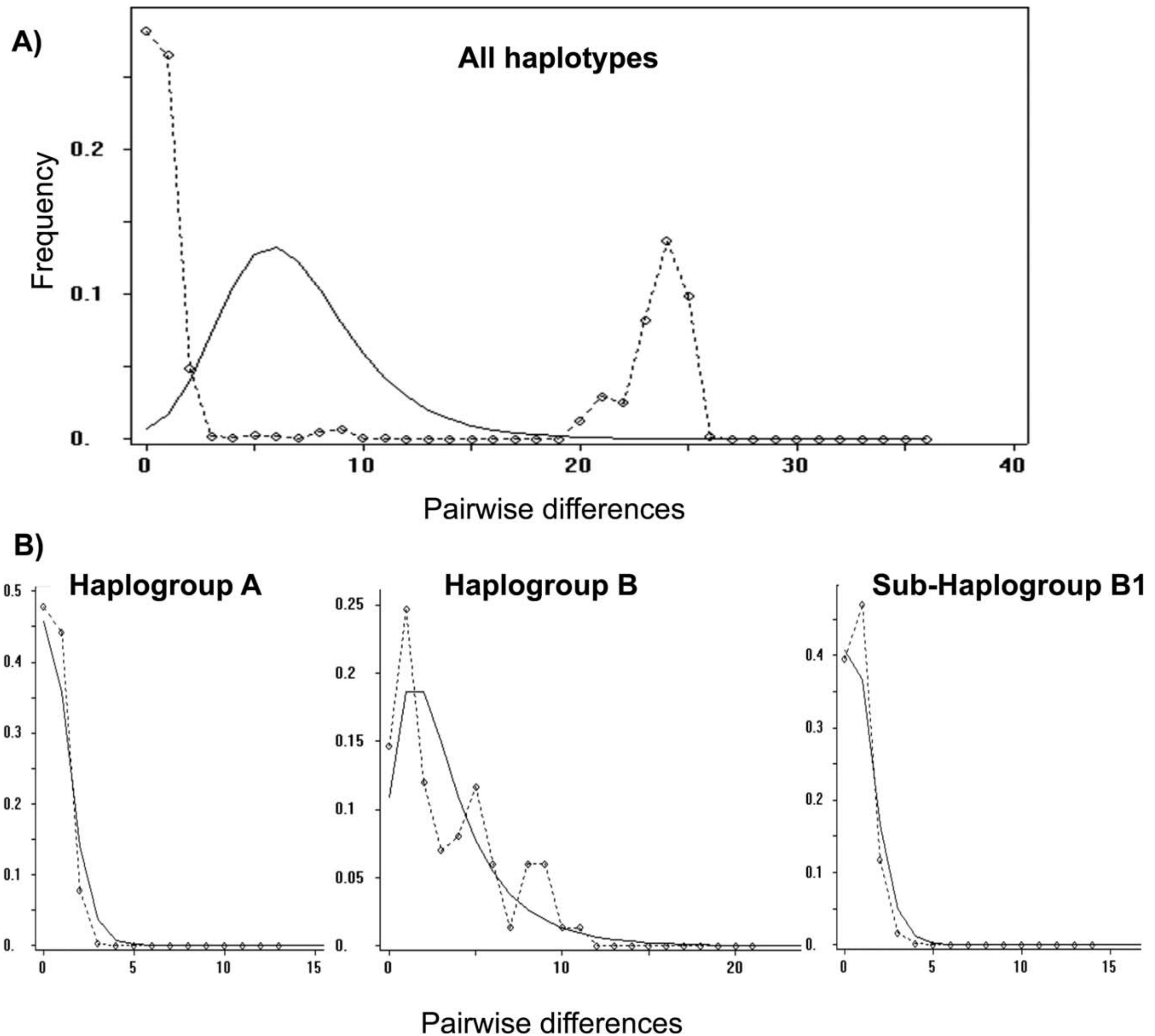

Fig. 5. Mismatch distributions between and within clades using COX1 sequence data. The horizontal axis represents site differences and the vertical axis relative frequencies of mismatches. Solid line represents the expected distribution of an expanding population and dashed line is the observed distribution. Analysis of all haplotypes revealed a bimodal distribution, which is consistent with the presence of two divergent haplogroups (**A**). Haplogroups A, B, and B1 were analyzed separately (**B**).

Americas (e.g. A-H001 and B-H001). However, other haplotypes, particularly from haplogroup B, are more geographically structured. The haplotypes closest to B-H002 are only found in North and Central America, which may suggest a common Native American origin (or ancestral Asian populations) for both the B-H002 haplotype as well as the derived ones. The first of the groups of highly derived B-haplotypes was distributed in North and Central America (Utah, Texas, and Mexico). The connection between Southwestern North America and Mesoamerica Native American human populations has been well supported from different evidence (Smith et al., 2000; Malhi et al., 2003; Kemp et al., 2010). The B-003 and B-004 haplotypes on the other hand are distributed in both Mexico and Argentina, which may represent lice genetic diversity brought by the Europeans, or still Native America louse mitochondrial diversity.

It is striking the near lack of haplogroup B in South America despite haplogroup B being so abundant in Central America. Although sampling is comparatively less in Central America (N = 45) than in South America (N = 240) and a larger and geographical spread sampling is needed, the dichotomy in the distribution between haplogroups may reflect human colonization events. The number of migration waves of humans from Asia to the New World is still a topic of debate. Initially, four major mitochondrial haplogroups were identified in Native Americans and were named A, B, C, and D, but are now A2, B2, C1, and D1. The original interpretation was that these four haplogroups indicated more than one migratory wave during the initial colonization of the Americas (e.g., Horai et al., 1993; Torroni et al., 1993). Some studies have suggested that the vast majority of Native Americans descend from a homogeneous

TABLE 3. Measurements of mitochondrial diversity and historical demographic analyses in human head louse populations from the Americas

		Haplogroup	Haplogroup B	
	Total	A	B	B1
Number of lice	450	333	117	99
Number of haplotypes	37	12	25	15
Hd: Haplotype diversity	0.7706	0.6050	0.7946	0.7137
π: nucleotide diversity	0.0299	0.00172	0.0083	0.0024
S: Segregating sites	49	8	23	6
Π: mean number of pairwise differences	11.0369	0.6529	2.8607	0.848
τ: estimator of the mutation time	0.2090	0.7810	0.4394	0.9710
Confidence interval	[0.000–4.711]	[0.531–1.391]	[0.000–4.189]	[0.471–1.656]
rg: raggedness statistic	0.0594	0.1388	0.0708	0.1074
P^1	0.6900	*0.0000*	0.5600	*0.0200*
F_S: Fu's statistic	0.3142	−7.1127	−11.1627	−12.4153
P^a	0.6240	*0.0150*	*0.0010*	*0.0000*

[a] P: The probability of obtaining rg or F_S values, respectively, equal to or lower than the observed.

Amerindian ancestral population, which presumably crossed the Bering Strait more than 15,000 years ago and from at least two additional streams of Asian gene flow into America (e.g., Greenberg et al., 1986; Bortolini et al., 2003; Volodko et al., 2008; Reich et al., 2012). In human lice, do the Central and South America populations reflect different waves of human migrations to the New World with a different louse haplotype composition in each wave? Other human parasites have supported a multiwave colonization of the New World. Particularly, archaeoparasitological studies of human intestinal parasites suggested that due to temperature survival potential, some human parasites may have arrived with humans using alternative routes to Beringia, such as trans-oceanic or costal routes (Araújo et al., 2008).

Alternatively, some studies have proposed that Native Americans descended from a single migration wave crossed the Bering Strait (Tamm et al., 2007; Fagundes et al., 2008; Kitchen et al., 2008; Mulligan et al., 2008). The southward migration of humans left a footprint in the human mitochondrial genetic diversity, which is represented as a reduction of the mt diversity in Central America and South America compared with North America. This pattern was interpreted (Kolman et al., 1996) as reflecting a population bottleneck among human populations that settled in Central America. Under this scenario, could it be possible that lice carried by Paleoindians underwent a bottleneck in the migration into South America and "lost" lice from haplogroup B? In humans, a large number of studies have shown that Native American populations have the greatest differentiation among most human populations with a significant decrease of genetic diversity as a function of geographic distance from the Bering Strait (Cavalli-Sforza et al., 1994; Wang et al., 2007). Thus, the significant difference in haplogroup frequencies among the Americas could be the result of the strong genetic drift experienced by humans during the colonization of the continent. Alternatively, Hunley and Healy (2011) examined not only how founder effects and gene flow have molded patterns of Native American genetic diversity but also the effect of European admixture. They found that previous evidence for serial founder effects in the Americas might also have be driven in part by high levels of European admixture in northern North America, intermediate levels in Central America, and low levels in eastern South America (Hunley and Healy, 2011). Our group recently developed a pannel of microsatellite loci for human lice (Ascunce et al., 2013) that we will use with the samples presented in this study to further evaluate the impact of serial founder effect, gene flow and European admixture in lice of the Americas. These future results could help clarify human evolutionary processes in the Americas. In addition, genetic analysis from lice recovered from Native American mummies could be used to provide new insights into human precontact migration routes.

Final remarks

This work is part of the growing number of studies looking into human parasites as probes for human migration around the world. Particularly, the human louse is one of our oldest parasites as indicated by archeological remains, the worldwide codistribution of both humans and lice, and coevolutionary studies. This ancient human-louse association allows us to investigate parasite genetic diversity to determine whether it accurately reflects host migrations and host–host interactions. Our study represents the most geographically widespread work in a human ectoparasite in the Americas. We show that there is a parallel timing of demographic expansions of human lice and Native Americans. These results suggest that human lice can provide additional evidence about the human colonization of the New World. Moreover, human louse genetic diversity seems to reflect ancient human demographic events that predate the colonization of the New World. The deep genetic differentiation found in haplogroup B is consistent with the hypothesis that haplogroup B evolved in a different hominid other than AMHs such as Neanderthals. The ability to detect the impact of those ancient host interactions in the current genetic diversity of a human parasite suggest the existence of rich and dynamic interactions among different hominids during the migration of AMHs around the world. Further studies in human lice including larger number of genetic markers and more samples would provide better resolution to these questions.

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