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VARIABLE MICROSATELLITE LOCI FOR POPULATION GENETIC ANALYSIS OF OLD WORLD MONKEY LICE (*PEDICINUS* SP.)

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ABSTRACT: Parasitic lice have been valuable informants of their host's evolutionary history because they complete their entire life cycle on the host and move between hosts primarily through direct host-to-host contact. Therefore, lice are confined to their hosts both in ecological and evolutionary time. Lice on great apes have been studied to examine details of their host's evolutionary history; however, species of *Pedicinus*, which parasitize the Old World monkeys, are less well known. We sampled lice from 2 groups of red colobus (*Procolobus* spp.) in Kibale National Park in Uganda and from red colobus and black and white colobus (*Procolobus polycomos*) in Taï National Park in Côte d'Ivoire. We used next-generation sequencing data analysis and the human body louse (*Pediculus humanus humanus*) genome to search for microsatellites for population genetic studies of *Pedicinus* lice. The 96 primer sets for microsatellite loci designed from the human body louse genome failed to amplify microsatellites in *Pedicinus* sp., perhaps due to the fast rate of evolution in parasitic lice. Of 63 microsatellites identified by next-generation sequencing data analysis of *Pedicinus* sp., 12 were variable among populations and 9 were variable within a single population. Our results suggest that these loci will be useful across the genus *Pedicinus*. We found that the lice in Uganda are not structured according to their hosts' social group; rather, 2 non-interbreeding populations of lice were found on both groups of red colobus. Because direct host-to-host contact is usually required for lice to move among hosts, these lice could be useful for identification and study of behavioral interactions between primate species.

Parasites can be used in the study of the evolutionary history and behavior of their hosts (Whiteman and Parker, 2005; Niedberg and Olivieri, 2007). Parasitic lice have low vagility and mostly move between hosts through direct host-to-host contact. Because of this, lice are confined to their host both in ecological and evolutionary time, and their DNA has recorded their shared evolutionary history. Therefore, lice are useful to understand both long-term evolutionary patterns of their hosts and short-term events such as recent, and past, host behavior. For example, lice have been used to investigate many aspects of human behavior, such as the temporal origin of clothing (Kittler et al., 2003, 2004; Toups et al., 2010) and whether there was contact between modern and archaic humans (Reed et al., 2004).

Mammal sucking lice (Phthiraptera: Anoplura) are blood-feeding obligate ectoparasites that spend their entire life cycle on their mammal hosts. It is thought that approximately one fifth (18%) of all mammal species are parasitized by sucking lice (Durden and Musser, 1994; Light et al., 2010). Although some species of lice are found on more than 1 mammal species, others are more host specific and can only survive on 1 host species (Reed and Hafner, 1997; Reed et al., 2000; Johnson et al., 2002).

There have been many studies of lice that parasitize humans and other great apes; however, much less is known about lice of Old World monkeys, i.e., those in the genus *Pedicinus*. The 14 named species in this genus parasitize 41 species of Old World monkeys in family Cercopithecidae and subfamily Colobinae, which includes red colobus (*Procolobus* spp.; Durden and Musser, 1994).

Red colobus are found along equatorial Africa in rain forest habitats (Struhsaker, 1975, 2010) and are listed as endangered by the International Union for Conservation of Nature because of habitat destruction as well as predation by humans and chimpanzees (Struhsaker, 2010). The red colobus found in Kibale National Park (KNP) in western Uganda, *Procolobus* (*Piliocolobus*) *rufomitratus*, are the only large, unthreatened population remaining (Struhsaker, 1975, 2010). Greater knowledge of the behavior of this endangered primate is critical for understanding group dynamics so that informed conservation plans can be constructed. Observational and genetic studies of red colobus have found that although separate groups behave as unique entities, individuals often move between groups (C. Chapman, unpubl. obs.). Interestingly, parasite genetic data may be structured according to host behavior and therefore may be another avenue for gathering information on host group dynamics.

The lice (*Pedicinus* sp.) for this project were collected from KNP red colobus (*Procolobus* [*Piliocolobus*] *rufomitratus*). We searched for variable microsatellites from these lice that could be used to determine the level of parasite gene flow between host groups in KNP, and we looked for genetic structure in the parasite populations. To determine whether these microsatellites could be useful across this louse genus, lice were also collected from primates in Taï National Park (TNP), Côte d'Ivoire (Leendertz et al., 2010), from red colobus (*Procolobus* [*Piliocolobus*] *badius*), and black and white colobus (*Procolobus polycomos*).

We used 2 methods to find microsatellites for the study of population genetics in these lice. First, we scanned the major contigs from the recently published human body louse (*Pediculus humanus humanus*) genome (Kirkness et al., 2010); and second, we searched DNA sequence data for *Pedicinus badii* acquired through next-generation sequencing (NGS).

MATERIALS AND METHODS

Collection

Individuals from two groups of red colobus were captured as described in Goldberg et al. (2009) to evaluate health issues and to attach collars that permitted the recognition of individual monkeys (Tombak et al., 2011). From each of these individually marked monkeys, lice were collected by combing through the hair of the host with a Nit Free Terminator louse comb (Natural Ginesis, Franklin, Tennessee). Overall, 31 red colobus were sampled from 2 groups, SC and LM. Three of the 31 individuals had no

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TABLE I. Locus name, forward primer, reverse primer, repeat motif, size, and melting temperature for the 12 variable microsatellite loci in an Old World monkey louse genus *Pedicinus*. The size range is reported from the full data set of 32 individuals.

Locus	Left primer (forward)	Right primer (reverse)	Repeat	Size range	Temp (C)
PbTri1	TGTCAATGGTAATAAAACAGAATCAA	CAGGCTCAAGTGCGGAATAC	(AAT)11	152–170	52
PbTri2	GCTTTCTTTTTCAAAGCAATTT	CGTACTCGACCCGGAGTAAA	(AAT)15	202–223	55
PbTri3	AATCGAAGCCGTTAATTTGC	TTGGATAAGGTCAATTGATTCG	(ATC)13	143–164	52
PbTri4	AGCGTGAAGCCATACAACTTT	TCGAATTAATTGATCGATAGGTTG	(ATC)12	155–167	52
PbTri5	GGGTGAGAGAGAAAGAAAATGC	TCAAGCATAACACCATCACTAACA	(GT)12	177–192	50
PbTri6	TGAATTTTTCCGCTTTAGTTGTG	GAAAACAAATTTTTGACAGCTTTT	(AAT)11	169–189	52
PbTri7	CCCTACTTCCCCTTAAAGTTTCTT	CATAATAAACGTACAATTTAAAAACGA	(ATT)16	156–202	55
PbTri8	TTGAGCCCACAAAGTTATTTCA	CGTGGGTTTCCAGTTCATCT	(ACT)11	104–165	52
PbDi1	AATTCACACTACGGCTCACG	TTGGTTTTCCACCTTTCACC	(GA)15	345–377	55
PbDi2	AAATGAAAAACTTTGCCAGGAA	GTAACTGGGAAAACGCAAGG	(GA)15	161–183	55
PbDi3	TTTGTGCCACATTGAAAATGA	TGCACAATAGTGGTGGGAGA	(GA)10	226–232	52
PbDi4	TGTGAGTGAATTCAAAAACAAAG	ACAACCCAAAGGAATTCTTTAAT	(GA)10	200–240	52

lice. From the remaining 28 monkeys, a single louse was selected (SC = 11 individuals, LM = 17 individuals). These lice were preliminarily identified as *P. badii*. From TNP, 9 red colobus and 3 black and white colobus were sampled. Seven red colobus and 2 black and white colobus were found to have lice. These lice were preliminarily identified with louse keys (Ferris, 1934; Kuhn and Ludwig, 1964) as *P. badii* and *Pedicinus pictus*. Lice were sampled from hosts that had more than a single louse. Three red colobus lice were selected (2 from 1 host representing both putative species) and 1 louse selected from a black and white colobus host.

DNA extraction

High-resolution digital images were taken of each individual louse before DNA extraction because whole lice were crushed and macerated such that morphological examination would be impossible after molecular work. Crushed lice were extracted using a QiAmp DNA micro kit (QIAGEN, Venlo, The Netherlands) according to the manufacturer's protocol. The GenomiPhi V2 rolling cycle amplification does not alter microsatellite size (Holbrook et al., 2005) and was therefore used to obtain enough DNA for Next Generation Sequencing (NGS). Five reactions were conducted using GenomiPhi V2 DNA kit (GE Healthcare, Piscataway, New Jersey) from a single louse DNA extract. This amplified total genomic DNA to levels suitable for NGS. The 5 GenomiPhi products were then cleaned using an ethanol precipitation and combined, yielding ~17 μg of DNA (quantified using a NanoDrop fluorometer; NanoDrop Wilmington, Delaware). Finally, this product was run on a 454 Genome Sequencer FLX standard series system (Roche, Indianapolis, Indiana) using one eight of a plate.

Genome mining

MSATCOMMANDER (Faircloth, 2008) was used to search for tetranucleotide microsatellites in the major contigs of the human body louse genome (Kirkness et al., 2010), and di- and trinucleotide microsatellites were chosen from the NGS data from *Pedicinus* sp. After identifying microsatellites, the program Primer3 (Rozen and Skaletsky, 2000) was used to design primers for each locus (Tables I, II).

Phylogenetics and mitochondrial cytochrome c oxidase subunit I (COI) gene

The mitochondrial COI gene was amplified by polymerase chain reaction (PCR) from all 32 DNA extracts. The reaction mix consisted of 11 μl of water, 10 μl of HotMasterMix (5 PRIME, Gaithersburg, Maryland), 2 μl of DNA template (10 ng/μl), and 1 μl each of forward and reverse primers (10 μM; H7005 from Hafner et al. [1994]; LC01718 from Reed et al. [2004]), for a total reaction volume of 25 μl. The temperature protocol was 94 C for 10 min, followed by 4 cycles of 94 C for 1 min, 48 C for 1 min, 65 C for 2 min, and then 29 cycles of 94 C for 1 min, 52 C for 1 min, 65 C for 2 min, followed by a final extension step at 65 C for 10 min. The products were purified with ExoSAP-IT (GE Healthcare) and sequenced using an ABI 3130 MegaBACE 4500 sequencer (Applied Biosystems, Foster City, California). The sequences were edited in Sequencher version 4.5 (GeneCodes, Ann Arbor, Michigan) and combined with outgroup sequences from human head lice (GenBank accessions EU493446 and EU493447), chimpanzee louse (*Pediculus schaeffi*; AY695999), human pubic louse (*Pthirus pubis*; EF152554), gorilla louse (*Pthirus gorillae*; EF152555), and baboon louse (*Pedicinus hamadryas*; AY696007.1). The sequences were aligned with SeaView (Galtier et al., 1996) using MUSCLE (Edgar, 2004) and were checked by eye.

To determine the best model of molecular evolution, jModelTest (Guindon and Gascuel, 2003; Posada, 2008) was used with the Akaike Information Criterion (Akaike, 1974). A TPM1uf+I+G nucleotide substitution model was selected and was used in PAUP* version 4.0b (Swofford, 2003) to obtain a maximum likelihood tree by using a heuristic search and tree bisection and reconnection branch swapping. RAxML (Stamatakis, 2006) was used to create 200 rapid bootstraps and a best maximum likelihood tree by using a GTR+G model (Tavaré, 1986; Yang, 1996) of molecular evolution. To compare the results from a maximum likelihood run with a Bayesian analysis, Mr. Bayes (Hulsenbeck and Ronquist, 2001; Ronquist and Hulsenbeck, 2003) was run for 2×10^6 generations with 10% burnin excluded from the posterior distribution of trees, sampling every 1,000 generations. This was performed twice, first using a GTR+I+G model, and then using a model more similar to that selected with jModeltest, HKY+I+G (Hasegawa et al., 1985). Two independent runs were conducted and combined to create a posterior distribution of trees that included 3,602 trees. A 50% majority rule

TABLE II. Locus name, number of alleles, and observed and expected heterozygosities for the 12 variable microsatellites. These microsatellites were found to be variable in the initial 8 lice tested, which included both populations. Results here are shown for only 1 population, which is the large COI clade from Figure 1, and some loci were monomorphic for this population. Tests for Hardy–Weinberg equilibrium were conducted using Genepop.

Locus	No. alleles	H_o	H_e	Probability test
PbTri1	2	0.23	0.21	1.00
PbTri2	4	0.42	0.43	0.85
PbTri3	2	0.00	0.08	0.02
PbTri4	1	0.00	0.00	NA*
PbTri5	2	0.58	0.49	0.44
PbTri6	1	0.00	0.00	NA
PbTri7	6	0.67	0.63	0.15
PbTri8	2	0.00	0.33	0.00
PbDi1	5	0.67	0.65	0.86
PbDi2	3	0.12	0.18	0.08
PbDi3	1	0.00	0.00	NA
PbDi4	4	0.04	0.41	0.00

* NA = not applicable.

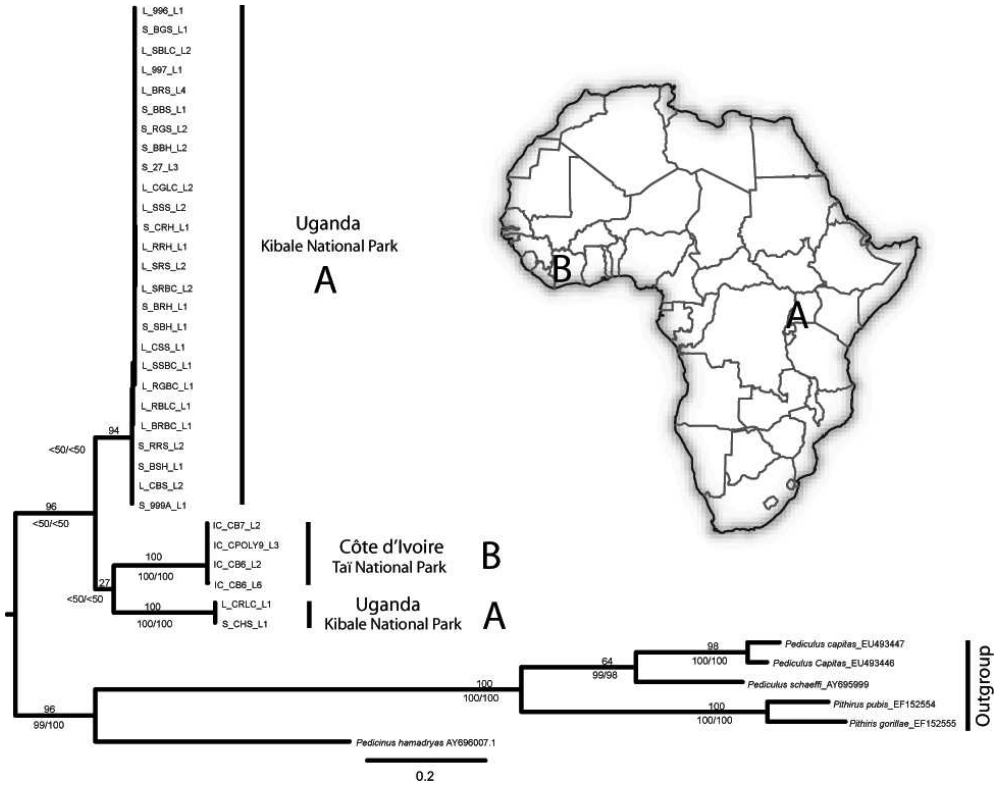

FIGURE 1. RaxML maximum likelihood tree of the mitochondrial COI gene. The lice are identified as follows: host group_host identification_louse number. Host groups are either SC (S), LM (L), or IC (IC). Bootstrap values are listed above the branches, and posterior probabilities for the GTR+I+G/HKY+I+G Bayesian consensus tress are listed below branches. The 2 sampling localities are indicated on the map of Africa, KNP (A) and TNP (B). Map was obtained through the creative commons license and was originally created by Eric Gaba.

consensus tree was computed using PAUP* for both runs to calculate posterior probabilities.

Microsatellite identification and amplification

To determine whether the microsatellite primers designed from the human louse genome would amplify microsatellites in *Pedicinus* sp., PCRs were performed to amplify the microsatellites for cloning and sequencing. The reaction mix consisted of 11 µl of water, 10 µl of HotMasterMix (5 PRIME), 2 µl of DNA template (10 ng/µl), and 1 µl each of forward and reverse primers (10 µM), for a total reaction volume of 25 µl. The temperature protocol for the PCR had a denaturation step at 95 C for 5 min, followed by 35 cycles of 95 C for 30 sec, 55 C for 1 min 30 sec, and 65 C for 30 sec, with a final extension step at 65 C for 30 min. PCR products were visualized using gel electrophoresis and cloned using TOPO TA cloning kit (Invitrogen, Carlsbad, California). Recombinant clones were then sequenced using the same protocol as described above. After sequencing, we found that none of the PCR products contained a microsatellite; therefore, they were not used for genotyping.

With the primers designed from the 454 data, 8 lice were examined for variability. These 8 lice were representative of both host groups, and both geographic locations. A 3-primer nested PCR approach (Schuelke, 2000) was used to amplify microsatellite loci designed from the 454 data. The mix for these reactions consisted of 5.38 µl of water, 7.5 µl of Type-it microsatellite PCR mix (QIAGEN), 1 µl of DNA template (10 ng/µl), 1 µl of dye (10 µM), and 0.06 µl each of forward and reverse primer (5 µM). The 5′ end of each forward primer was modified to include a tag, either an M13 tag (CACGACGTTGTAAAAC) or a CAG tag (CAGTCGGGGTCA) to incorporate the florescent dye into the product as in Schuelke (2000). The total volume of the reaction mixture was 15 µl. The temperature protocol was initial denaturation at 95 C for 5 min, followed by 10 cycles of 94 C for 30 sec, a locus-specific annealing temperature (Table I) for 45 sec and 72 C for 45 sec, followed by 25 cycles of 94 C for 30 sec, 55 C for 45 sec, and 72 C for 45 sec, with final extension at 72 C for 10 min. Fragment size was determined using an ABI 3730 Automated Sequencer (Applied Biosystems). The microsatellite alleles were scored using GeneMarker version 1.85 (SoftGenetics, State College, PA). To reduce scoring errors, all loci were scored by K.S. and checked by J.M.A. Twelve loci were found to be variable with the initial 8 lice and were then amplified for all specimens (n = 32).

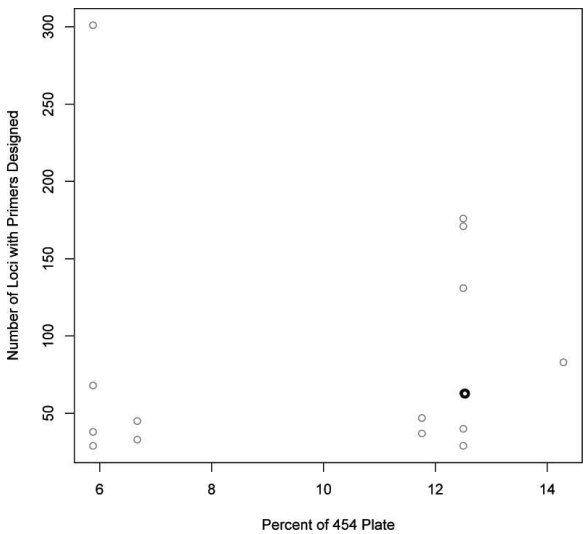

FIGURE 2. Number of loci with good flanking sequences for primer design shown as a function of the percentage of 454 plate used based on invertebrate specimens taken from Gardner et al. (2011). Data for other invertebrates is in light gray, and our *Pedicinus* sample is in black. Our 454 data fall within range found for other invertebrates.

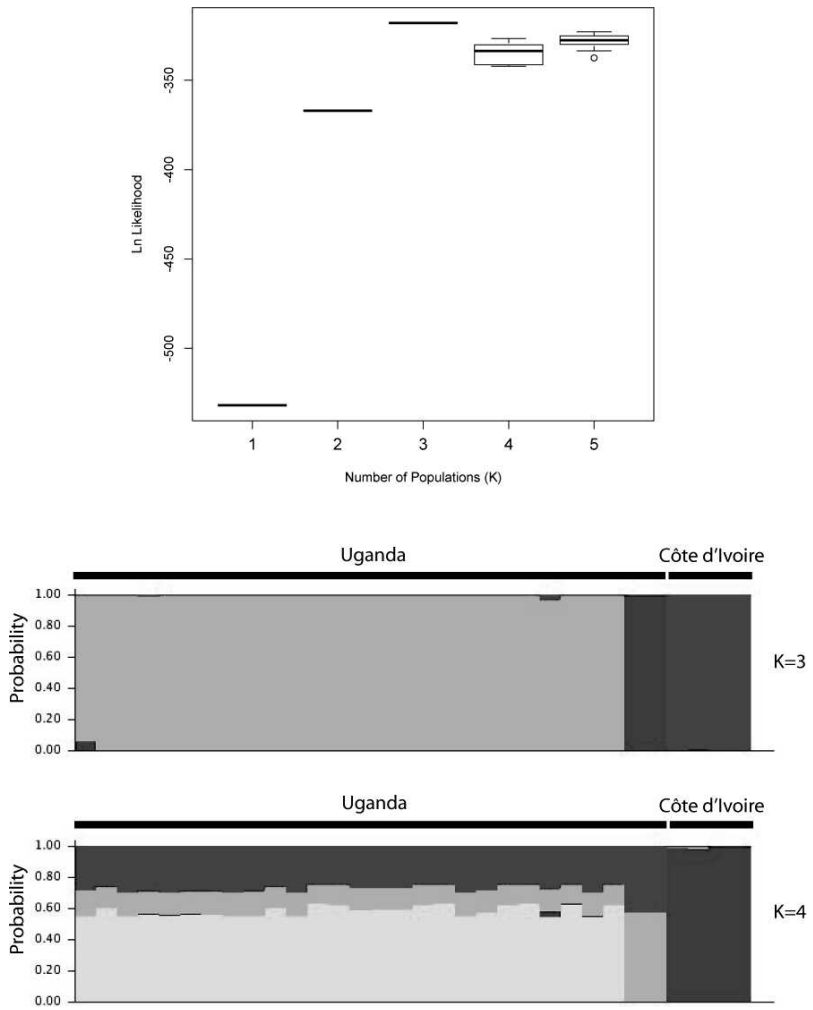

FIGURE 3. Box plots of ln likelihood scores from STRUCTURE results for 5 a priori settings of K (number of populations) from 1 to 5 populations and bar graphs from a single run of each $K = 3$ and $K = 4$. Each K was tested 10 times, and box plots were created from the 10 mean ln likelihood scores from all 10 independent trials. Box plots divide the results into 4 quartiles. The box contains the middle 50% of the values separated by the median. The whiskers extend 1.5 times the interquartile range (quartile3 − quartile1). Circles represent points outside of the 95% confidence interval. Because the results from each of the 10 runs were similar the box is very short and in some cases just looks like a single line. These results support 3 populations of lice.

Analysis of population structure

Tests for population structure were conducted using the variable loci that were not found to deviate significantly from Hardy–Weinberg expectations and the program STRUCTURE version 2.3.2 (Pritchard et al., 2000). STRUCTURE is a Bayesian clustering program that uses allele frequencies of multilocus genotypes to group individuals into populations. This program was run using an admixture model with correlated allele frequencies among populations. We ran STRUCTURE with 3 nested sets of data to test for structure within louse populations. In the preliminary analyses for each data set, we evaluated the number of clusters (K) from 1 to 10 to determine the appropriate number of populations (K) to use in each analysis. We then limited the runs based on these results. The first data set included all the lice from KNP and the 4 lice from TNP (n = 32); K values were tested from 1 to 5. We further subsampled this data set to determine whether there was structure within the KNP samples only. Therefore, the second run included only the KNP samples (n = 28), and K values ranged from 1 to 4. The third run included a single large clade of lice from KNP that was uncovered based on the analysis of COI data described below (n = 26); K values ranged from 1 to 3. Ten independent runs of each K were tested at 5×10^6 generations post 5×10^5 burnin generations. For each of these runs, values of K greater than those mentioned were not evaluated because the variance around the mean likelihood scores was increasing, and there were no new clusters forming.

Tests for Hardy–Weinberg equilibrium and linkage disequilibrium

Tests for Hardy–Weinberg equilibrium were performed on the large clade from Figure 1 (n = 26). The program Cervus version 3.0 calculated observed and expected heterozygosities (Kalinowski et al., 2007). Hardy–Weinberg exact tests, probability of heterozygote deficiency, and the probability of gametic disequilibrium were calculated by Genepop version 4.0 (Raymond and Rousset, 1995).

RESULTS

Three major *Pedicinus* clades were found with the COI sequence data (Fig. 1), and these clades did not correspond to our putative species identifications. The majority of the KNP lice (*P. badii*) clustered together in a wide, shallow clade, but 2 lice formed a separate clade, and the 4 lice from TNP (*P. badii and P. pictus*) formed a third clade. The position of the smaller KNP

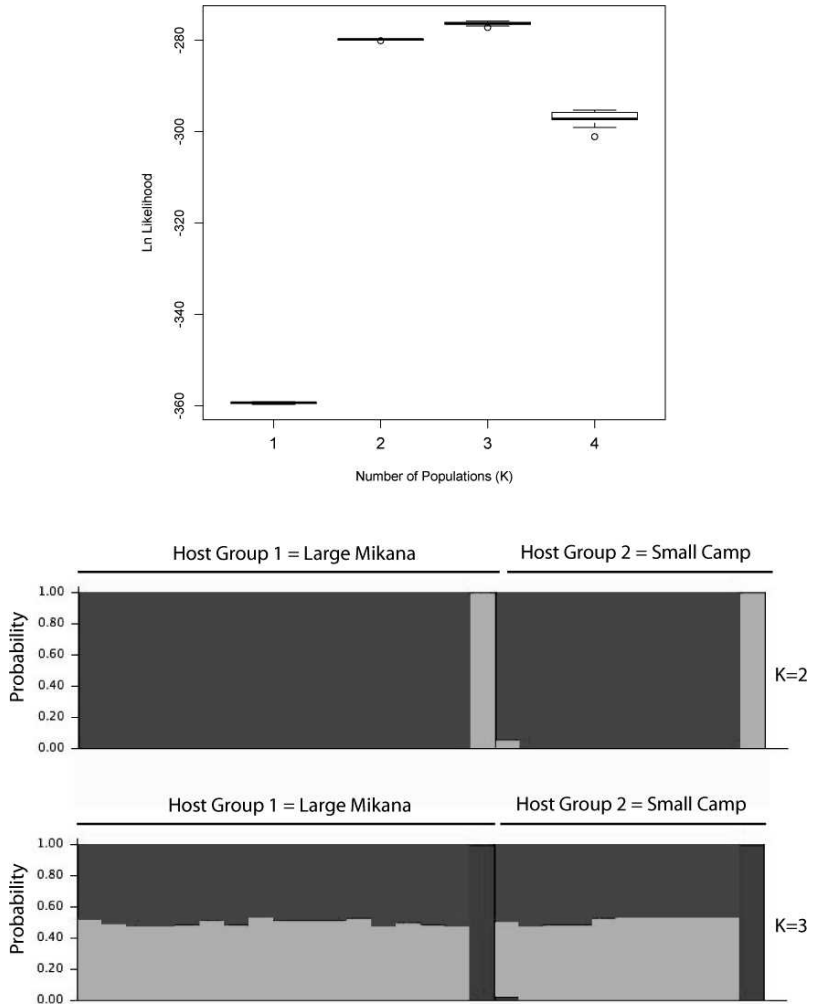

FIGURE 4. Box plots of ln likelihood scores from STRUCTURE for 4 a priori settings of K (number of populations) from 1 to 4 populations and bar graphs of a single run of each $K = 2$ and $K = 3$. Each K was tested 10 times, and box plots were created from the 10 mean ln likelihood scores from all 10 independent trials. These results support 2 populations of lice within KNP; as before, the likelihood score increases after $K = 2$. However, the program was unable to group individuals into more populations.

population within the tree is uncertain and differed depending on the type of analysis done. For example, in a neighbor joining and maximum likelihood analysis, this clade clustered with the TNP lice (Fig. 1), whereas it clustered with the second KNP clade in a Bayesian analysis (data not shown). Despite these differences, the individuals within each clade remained the same in all analyses. The 3 clades in Figure 1 were then identified as 3 groups for population level analysis. The largest sample analyzed included all *Pedicinus* sp. lice (n = 32), the second sample only the KNP lice (n= 28), and the third sample only the large clade of KNP lice (n = 26).

Microsatellite analysis

From the human head louse genome, primers were designed for 96 microsatellite loci, (Table I). Unfortunately, the sequences revealed that the primers were not amplifying microsatellites but rather other unknown loci. From the 454 data, 109,899 reads were recovered, with an average size of 345 base pairs. Primers were designed for 63 microsatellite loci (Tables I, II). Of these, 38 were successfully amplified and then genotyped in 8 individuals to test for variability. Invertebrate genomes have been found to yield fewer amplifiable microsatellites from NGS data than vertebrates (Gardner et al., 2011), and our data fall within the range found for other invertebrates (Fig. 2). Twelve loci were identified as potentially variable from the initial 8 lice tested (Table I). All but 1 (PbTri6) of these primers also successfully amplified microsatellites in the *Pedicinus* sp. collected from the red colobus and black and white colobus from TNP.

Population structure

The results from STRUCTURE suggest that there are 3 populations of lice in the large data set that correspond with the results from the COI gene. Those populations included the 4 lice from the TNP, a larger KNP group of 26 individuals, and a smaller KNP group of 2 individuals. No further population structure was detected by running sub-structure analyses within each population (Figs. 3–5).

Tests for Hardy–Weinberg equilibrium and linkage disequilibrium

Tests for gametic disequilibrium and for deviations from Hardy–Weinberg expectations were conducted with the large

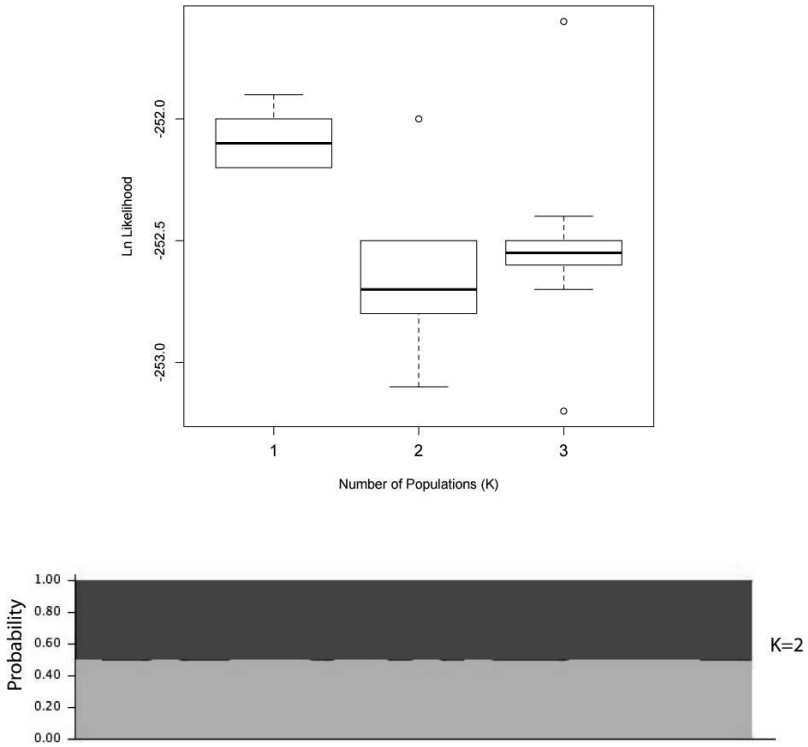

FIGURE 5. Box plots of ln likelihood scores from STRUCTURE for 3 a priori settings of K (number of populations) from 1 to 3 populations and bar graph from a single run of $K = 2$. Each K was tested 10 times, and box plots were created from the 10 mean ln likelihood scores from all 10 independent trials. These results support a single population of lice within KNP found across both host groups.

population (n = 26) from KNP. Tests for gametic disequilibrium yielded no significant values for all combinations of loci (Bonferroni corrected $P > 0.00075$). Of the 9 variable loci, only 2 fell outside of Hardy–Weinberg expectations because of heterozygote deficiency, i.e., PbTri8 and PbDi4 ($P < 0.004$). Table I lists the observed and expected heterozygosities (H_o and H_e, respectively). The minimum H_o was 0.000, and the maximum H_o was 0.667. The minimum H_e was 0.000, and maximum H_e was 0.625. Within our sample of 26 lice, locus PbTri6 was monomorphic. This is a sampling artifact, because genotyping more individuals yielded more alleles (data not shown). Overall, in this sample of 26 lice from KNP, 9 of the 12 loci were variable, each having between 2 and 6 alleles (Table I).

DISCUSSION

Here, we designed a microsatellite library for lice in the genus *Pedicinus* sp. that parasitize Old World monkeys. In particular, we collected lice from red colobus and black and white colobus from TNP, Côte d'Ivoire, and from red colobus in KNP, Uganda, to find variable microsatellite loci to facilitate studies on the lice as well as the behavior of primates.

Our COI data consistently found 3 clades; however, the relationships between the clades differed depending on the phylogenetic analysis done. This suggests that there is a short branch between all of the *Pedicinus* sp., and more loci are needed to resolve the relationships between these groups.

We found that primers designed from the human louse genome did not amplify microsatellites in *Pedicinus* sp. Although it is possible to cross-amplify microsatellites designed from the human genome in red colobus monkeys, 2 taxa with the same amount of divergence time as their lice, the same pattern is not shown in their lice. This may be due to the faster evolutionary rates in lice than in their hosts (Hafner et al., 1994).

From the 454 data, 12 variable loci were recovered and all but 1 locus, PbTri6, successfully amplified microsatellites in the *Pedicinus* sp. collected from the red colobus and black and white colobus from TNP, suggesting that these loci may be useful for population genetic studies of lice across the *Pedicinus* spp.

It seems that there are at least 2 genetically distinct populations of lice parasitizing the red colobus monkeys in KNP. The 2 populations are not structured by the social structure of the hosts; representatives from the 2 louse populations were found on each of the 2 red colobus social groups. These results suggest that the parasites are probably moving between host groups often enough to remove genetic signal from the host's behavior. However, our results suggest that there may be 2 different species of lice. Many species within *Pedicinus* parasitize more than 1 species of host (Durden and Musser, 1994) and may be more generalist in their host preferences. One of the 2 louse species on the red colobus may have originated on another species of primate and moved to the red colobus in KNP. Interestingly, 1 of the louse species is much more common on the red colobus, and the other species was found on only 2 individuals. If the rare louse evolved on a different host it, may be unable to outcompete the more common red colobus louse. The 2 louse species may move among red colobus and other primate species in KNP during interactions between the host species. Close interactions between different species of primates have been observed in KNP and could facilitate host switching in lice. For example, red colobus are often

seen interacting with red tail monkeys (*Cercopithecus ascanius*; Chapman and Chapman, 2000; J. Allen, pers. obs). There are at least 13 primate species in KNP, so further research is necessary to determine which species are the more common hosts to these lice, and how the lice are moving between the hosts. Although the 2 louse species do not correspond to the 2 behavioral groups of red colobus monkeys, the lice may provide information about the interactions of the red colobus with other primates within the park.

Lice are obligate and, in some cases, host-specific, permanent ectoparasites. These insects only move among hosts during direct host-to-host contact. Some species of lice are found on more than 1 type of mammal, whereas others are so host specific that they are only found on 1 host species (Johnson et al., 2002). Great apes usually have a single species of louse (with the exception of humans, who have 2). In chewing lice, the presence of more than 1 species of louse on a single host is common (Reed et al., 2000; Johnson and Clayton, 2003). Here, we found 2 genetically distinct lice (probably different species) on the same population of monkeys. Future research could determine whether both types of lice can be found on the same host, and if so, whether they partition the host space like some species of chewing lice (Reed and Hafner, 1997, Reed et al., 2000).

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LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model Identification. IEEE Transactions on Automatic Control **19:** 716–723.

Chapman, C. A., and L. J. Chapman. 2000. Interdemic variation in mixed-species association patterns: Common diurnal primates of Kibale National Park, Uganda. Behavioural Ecology and Sociobiology **47:** 129–139.

Durden, , L. A., and G. G. Musser. 1994. The mammalian hosts of the sucking lice (Anoplura) of the world: A host-parasite list. Bulletin of the Society for Vector Ecology **19:** 130–168.

Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research **32:** 1792–1797.

Ferris, G. F. 1934. Contributions toward a monograph of the sucking lice Part VII. Stanford University Publications, University Series, Biological Sciences **2:** 471–526.

Faircloth, B. C. 2008. MSATCOMMANDER: Detection of microsatellite repeat arrays and automated, locus-specific primer design. Molecular Ecology Resources **8:** 92–94.

Galtier, N., M. Gouy, and C. Gautier. 1996. SEAVIEW and PHYLO_win: Two graphic tools for sequence alignment and molecular phylogeny. Computational Applied Bioscience **12:** 543–548.

Gardner, M. G., A. J. Fitch, T. Bertozzi, and A. J. Lowe. 2011. Rise of the machines –recommendations for ecologists when using next generation sequencing for microsatellite development. Molecular Ecology Resources Molecular Ecology Resources **11:** 1093–1101.

Goldberg, T. L., D. M. Sintasath, C. A. Chapman, K. M. Cameron, W. B. Karesh, S. Tang, N. D. Wolfe, I. B. Rwego, N. Ting, and W. M. Switzer. 2009. Coinfection of Ugandan red colobus (*Procolobus* [*Piliocolobus*] *rufomitratus tephrosceles*) with novel, divergent, Delta-Lenti- and Spumaretroviruses. Journal of Virology **83:** 11318–11329.

Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology **52:** 696–704.

Hafner, M. S., P. D. Sudman, F. X. Villablanca, T. A. Spradling, J. W. Demastes, and S. A. Nadler. 1994. Disparate rates of molecular evolution in cospeciation hosts and parasites. Science **265:** 1087–1090.

Hasegawa, M., K. Kishino, and T. Yano. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution **22:** 160–174.

Kuhn, H., and H. W. Ludwig. 1964. *Pedicinus badii* n. sp., eine laus de stummelaffen *Procolobus badius badius* (Hoplopleuridae, Anoplura). Senckenbergiana Biologica **45:** 145–149.

Holbrook, J. F., D. Stabley, and K. Sol-Church. 2005. Exploring whole genome amplification as a DNA recovery tool for molecular genetic studies. Journal of Biomolecular Techniques **16:** 125–133.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics **17:** 754–755.

Johnson, K. P., and D. H. Clayton. 2003. The biology ecology and evolution of chewing lice. *In* The chewing lice: World checklist and biological overview, R. D. Price, R. A. Hellenthall, R. L. Palma, K. P. Johnson, and D. H. Clayton (eds.). Illinois Natural History Survey Special Publication 24, Illinois Natural History Survey, Champaign, Illinois, 501 p.

———, B. L. Williams, D. M. Drown, R. J. Adams, and D. H. Clayton. 2002. The population genetics of host specificity: Genetic differentiation in dove lice (Insecta: Phthiraptera). Molecular Ecology **11:** 25–38.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology **16:** 1099–1106.

Kirkness, E. F., B. J. Haas, S. Weillin, H. R. Braig, M. A. Perotti, J. M. Clark, S. H. Lee, H. M. Robertson, R. C. Kennedy, E. Elhaik et al. 2010. Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. Proceedings of the National Academy of Sciences of the United States of America **107:** 12168–12173.

Kittler, R., M. Kayser, and M. Stoneking. 2003. Molecular evolution of *Pediculus humanus* and the origin of clothing. Current Biology **13:** 1414–1417.

———, ———, and ———. 2004. Erratum: Molecular evolution of *Pediculus humanus* and the origin of clothing. Current Biology **14:** 2309.

Leendertz, S. A. J., S. Junglen, C. Hedemann, A. Goffe, S. Calvignac, C. Boesch, and F. H. Leendertz. 2010. High prevalence, coinfection rate and genetic diversity of retroviruses in wild red colobus monkeys (*Piliocolobus badius badius*) in Taï National Park, Côte d'Ivoire. **84:** 7427–7436.

Light, J. E., V. S. Smith, J. M. Allen, L. A. Durden, and D. L. Reed. 2010. Evolutionary history of mammalian sucking lice (Pthiraptera: Anoplura). BMC Evolutionary Biology **10:** 292.

Nieberding, C. M., and I. Olivieri. 2007. Parasites: Proxies for host geneology and ecology? Trends in Ecology and Evolution **22:** 156–165.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution **25:** 1253–1256.

Pritchard, J. K., M. Stephens, and P. Donnelley. Inference of population structure using mulitlocus genotype data. Genetics **155:** 945–959.

Raymond, M., and F. Rousset. 1995. Genepop (version 1.2) population genetics software for exact tests and ecumenicism. Journal of Heredity **86:** 248–249.

Reed, D. L., S. Allen, M. S. Hafner, and M. Babin. 2000. Spatial partitioning of host habitat by ectoparasitic chewing lice of the genera *Geomydoecus* and *Thomomydoecus* (Phthiraptera: Trichodectidae). Journal of Parasitology **86:** 951–955.

———, and M. S. Hafner. 1997. Host specificity of chewing lice on pocket gophers: A potential mechanism for cospeciation. Journal of Mammalogy **78:** 655–660.

———, V. S. Smith, S. L. Hammond, A. R. Rogers and D. H. Clayton. 2004. Genetic analysis of lice supports direct contact between modern and archaic humans, PLoS Biology **2:** 1972–1983.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:** 1572–1574.

ROZEN, S., AND H. J. SKALETSKY. 2000. Primer3 on the WWW for general users and for biologist programmers. *In* Bioinformatics methods and protocols: Methods in molecular biology, S. Krawetz and S. Misener (eds.). Humana Press, Totowa, New Jersey, p. 365–386.

SCHUELKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments. A poor man's approach to genotyping for research and high-throughput diagnostics. Nature Biotechnology **18:** 223–234.

STAMATAKIS, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22:** 2688–2690.

STRUHSAKER, T. T. 1975. The red colobus monkey. University of Chicago Press, Chicago, Illinois, 311 p.

———. 2010. The red colobus monkeys: Variation in demography, behavior and ecology of endangered species. Oxford University Press, Oxford, U.K. 343 p.

SWOFFORD, D. L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

TAVARÉ, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lecture on Mathematics in the Life Sciences **17:** 58–83.

TOMBAK, K. J., A. REID, C. A. CHAPMAN, J. M. ROTHMAN, C. A. JOHNSON, AND R. REYNA-HURTADO. 2011. Patch depletion behavior differs between sympatric folivorous primates. Primates **53**: 57–64.

TOUPS, M. A., A. KITCHEN, J. E. LIGHT, AND D. L. REED. 2010. Origin of clothing lice indicates early clothing use by anatomically modern humans in Africa. Molecular Biology and Evolution **28:** 29–32.

WHITEMAN, N. K., AND P. G. PARKER. 2005. Using parasites to infer host population history: A new rationale for parasite conservation. Animal Conservation **8:** 175–181.

YANG, Z. 1996. Among-site rate variation and its impact on phylogenetic analyses. Trends in Ecology and Evolution **11:** 367–371.