J. Parasitol., 100(6), 2014, pp. 873–877

Characterization of 17 Novel Polymorphic Microsatellite Loci in the Mammal Chewing Louse *Geomydoecus ewingi* (Insecta: Phthiraptera) for Population Genetic Analyses

C. E. Nessner, J. J. Andersen, M. A. Renshaw*, M. M. Giresi†, and J. E. Light, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843; *Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556; †Department of Biology, Texas A&M University, College Station, Texas 77843. Correspondence should be sent to: *jlight2@tamu.edu*

ABSTRACT: We report 17 novel microsatellite loci in the parasitic chewing louse *Geomydoecus ewingi*, a common parasite of the pocket gopher, *Geomys breviceps*. Thirty-three *G. ewingi* individuals from 1 geographic locality and 3 pocket gopher hosts (populations) were genotyped at each locus. The number of alleles per locus ranged from 3 to 13. Observed heterozygosity ranged from 0.182 to 0.788. Four to 6 loci per louse population fell outside of Hardy-Weinberg expectations (HWE) and examination of population structure also revealed substantial homozygote excess as well as significant structure among louse populations. These findings are likely the consequence of biological characteristics of the lice (low dispersal abilities, population bottlenecks, etc.), which can result in inbreeding. Notably, when all louse individuals were analyzed together as 1 population, a Wahlund effect was detected, supporting that louse populations are restricted to 1 host individual. The microsatellite markers characterized in this study will be useful in future studies exploring the population dynamics in host–parasite systems, potentially yielding a better understanding of the processes underlying symbiotic associations.

Lice (Insecta: Phthiraptera) are wingless insects that are obligate and permanent parasites of birds and mammals. Chewing lice belonging to the genus *Geomydoecus* (Ischnocera: Trichodectidae) are relatively well-known lice that parasitize only mammals, specifically pocket gophers in the rodent family Geomyidae. Pocket gophers are fossorial, spending the majority of their lives underground in elaborate burrow systems, rarely coming above ground (Sulentich et al., 1991). These rodents are highly modified morphologically for this fossorial lifestyle, having shortened, muscular forelimbs and large incisors and claws for digging (Stein, 2000). Because of their conservative morphology and resulting limited dispersal ability, pocket gophers have been documented as having long-term associations with coexisting organisms, specifically lice (Hafner et al., 2003).

Information regarding chewing lice, especially when used in conjunction with other data, can act as 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 (Whiteman and Parker, 2005; Nieberding and Olivieri, 2007). Gopher lice are confined to their hosts both in ecological and evolutionary time and this, in addition to the biology of pocket gophers, has helped make lice and their pocket gopher hosts model organisms for cospeciation studies (Hafner and Nadler, 1988; Demastes and Hafner, 1993; Hafner et al., 1994, 2003; Light and Hafner, 2008; Demastes et al., 2012). However, there have been few attempts to explore the microevolutionary processes of parasite populations, which likely play a crucial role in parasite speciation and the establishment and maintenance of host–parasite associations (Criscione et al., 2005; Huyse et al., 2005), occurring within and among parasite populations. Among lice, there have only been 2 studies investigating genetic variation at the population level: Nadler et al. (1990) and Barker et al. (1991). In their research, both Nadler et al. (1990) and Barker et al. (1991) used allozymes to examine genetic differentiation among populations, and although different louse taxa were used (*Geomydoecus actuosi* and *Heterodoxus octoseriatus*, respectively), both studies found substantial differentiation among host individuals. These findings indicate that louse populations likely are subdivided among individual hosts.

The objective of this research was to identify useful, codominant DNA-based genetic markers and examine population structure in the chewing louse *Geomydoecus ewingi*. Although several mitochondrial genes have successfully been used to examine cospeciation, and are commonly used markers for many louse molecular studies, mitochondrial genes do not evolve quickly enough in lice to be informative at the population level (Ascunce et al., 2013; C. E. Nessner, unpubl. data). Rapidly evolving markers such as microsatellites are more appropriate to address population level questions such as estimation of inbreeding, migration, relatedness, parentage, effective population size, and population assignment, among others (Criscione et al., 2007; Ascunce et al., 2013). Although there have been several recent studies identifying microsatellite markers in lice (Leo et al., 2005; McMeniman and Barker, 2006; Peters et al., 2009a, 2009b; Scholl et al., 2012; Ascunce et al., 2013), there are no known reports identifying variable microsatellite loci from mammalian chewing lice. Thus, designing microsatellite loci for pocket gopher chewing lice may provide markers that can be used to gain a better understanding of population dynamics in these host–parasite assemblages. Herein we describe microsatellite loci for the chewing louse *Geomydoecus ewingi*, a parasite of the Baird's pocket gopher (*Geomys breviceps*), and examine these loci for their utility in a population genetic context.

The protocol outlined in Welborn et al. (2012) was used to develop the enriched genomic microsatellite library for *G. ewingi*. Genomic DNA was isolated from a pooled sample of 50 individuals of *G. ewingi* with the use of the DNeasy Blood and Tissue Kit (QIAGEN Inc., Valencia, California). DNA fragments were hybridized with biotin-modified di-, tri-, and tetra-oligonucleotides, incubated with streptavin-coated magnetic M-280 Dynabeads (Invitrogen, Carlsbad, California), and rinsed. The quantity of this enriched DNA was increased via polymerase chain reaction (PCR) amplification and cleaned with a PCR purification kit (QIAGEN). Cleaned products were ligated into PCR 2.1 TOPO vectors (Invitrogen) and transformed in *Escherichia coli* (One Shot TOP10 Chemically Competent Cells, Invitrogen). Cells were dispersed onto X-Gal/LB/Agar plates treated with ampicillin and incubated overnight at 37 C. Positive clones (white) were sent to the University of Florida Interdisciplinary Center for Biotechnology Research Genomics Division (Gainesville, Florida) for sequencing with the M13 forward primer.

Sequences were edited with the use of SEQUENCHER 4.1 (Gene Codes) and screened for microsatellites. Primer sequences for unique microsatellite loci initially identified by the software package PHOBOS (Mayer, 2006, www.rub.de/spezzoo/cm/cm_phobos.htm) were developed with the use of Primer3 (http://frodo.wi.mit.edu/primer3/), and these loci were tested for amplification and polymorphisms across 16 louse individuals. Genomic DNA was extracted from individual lice with the use of the DNeasy Tissue Kit (QIAGEN). The abdomen of each louse was punctured and lacerated with a sterile insect pin prior to DNA extraction. The cleaved bodies were individually incubated overnight with proteinase K and a lysis buffer with extraction processes continuing the following day, after which the exoskeletons of the lice were removed for slide mounting as voucher specimens (Cruickshank et al., 2001). Manufactur-

DOI: 10.1645/13-415.1

TABLE I. Microsatellite marker information for 33 genotyped louse individuals, *Geomydoecus ewingi*, from 1 geographic locality in Brazos County, Texas.

Locus	Forward sequence (5′–3′)	Reverse sequence (5′–3′)	Repeat motif
Gew35	TTCACGCTTTTGCATCACAT	GGAATGGAAGTTACGACTACGC	$(CA)_{18}$
Gew39*	GGGAGGAGTGAAAAATAGAAAGC	TTCCGAAGGAACGTTACAGG	$(CA)_{11}$
Gew40	GGTTTATGACACCGGTCACG	TCGACGACTTACTGGGTTGG	$(GA)_{7}$
Gew41*	TGGGCATTGCTAAGAAGTCC	TCAGTTCATTTGATGTTTTGTCG	$(AG)_{11}$
Gew43*	TTCGATTCTTTCGCGTTTCT	GCAATTCGATCGTTTATTTCG	$(CAT)_{6}$
Gew44	TTCTCACTCGAAAAATTTAATGC	TGTTGTTTTGCCAACGGTTA	$(TC)_{13}$
Gew47*	ACCACAAGGGGATTTTCTGG	TCACAGCCTCATTTTCTACGG	$(GA)_{10}$
Gew51*	AGCCAAACCCAGATTTACCG	TTTAAATTCCCCTCCCTAACG	$(CA)_{10}$
Gew52	GTTTGCTGTTGCCATTTCG	AAAGGAAGCAGAGACTGAATGC	$(CTT)_{5}$
Gew54*	GGTCGAAGGAATTTAAACATAAGC	GCGTCTGAAGTGAAGATTTACG	$(CT)_{7}$
Gew55	AAGCGGCAGATAAATTAAAGACC	CATTCCCGTTTAACCATTTCC	$(GAAT)_{5}$
Gew56	GGAACCGATTGTAATGAGACC	GTTTTCGCTAACAGGACTCG	$(ATTT)_{4}$
Gew57	AATTCGCCTCAGGTTGAGC	TCGGCAAAGATGGTAAAACC	$(TCTT)_{5}$
Gew58*	CAATTTTTCCTCGCCTCTCC	GACAGGAAAAGATGCGAAGC	$(TCA)_{6}$
Gew59	CGATTCTCTTTTTCTTTTTACTTCTGG	AAAAAGCCGAGAAAAACTGG	$(ATC)_{9}$
Gew60	AGTTCCGTGCAACTCATGG	GGACAAATTCCGCAAAAAGG	$(ATC)_{10}$
Gew62*	CCGGGATGATGTTTAACTCC	TTCAAGCCTTCATTTTCACG	$(CAT)_{17}$

* Indicates potential null alleles at loci as indicated by Micro-checker due to homozygote excess.

er's recommendations were followed for the remainder of the extraction process except that the total DNA elution volume was 60 µl. PCR amplifications of microsatellites used unlabeled 5′ (forward) and 3′ (reverse) primers and a fluorescently labeled 5′-tail-sequence primer following Karlsson et al. (2008) and Boutin-Ganache et al. (2001). All PCRs were performed in 10-µl reactions containing 3.7 µl Emerald Master Mix (Takara Bio Inc., Mountain View, California), 4.25 µl water, 0.05 µl forward primer (10 µM) with an additional 0.5 µl fluorescently labeled tail primer (6-FAM; 5′-GCCTCGTTTATCAGATGTGGA-3′; 10 µM), 0.5-µl reverse primer (10 µM), and 1 µl DNA. Amplified PCR products were multiplexed when possible (loci grouped according to allele size), combined with 400 HD Rox size-standard DNA ladder (Applied Biosystems, Foster City, California), loaded on a polyacrylamide gel, and electrophoresed on an ABI PRISM 377 DNA Sequencher (Applied Biosystems). Sizes of microsatellite fragments were visualized in GENESCAN v. 3.1.2 (Applied Biosystems) and assessed with the use of GENOTYPER v. 2.5 (Applied Biosystems). In total, 17 loci amplified successfully and were polymorphic (Table I).

TABLE II. Microsatellite genetic diversity information per locus for 33 genotyped louse individuals, *Geomydoecus ewingi*. Abbreviations: N, number of individuals with data for each locus; NA, number of alleles expressed for each locus; H_S, within-population gene diversity; H_O, observed heterozygosity; HWE, probability of conforming to Hardy-Weinberg equilibrium.

Locus	N	NA	Range	H_S	H_O	HWE
Gew35	33	9	161–181	0.739	0.697	0.026
Gew39	33	10	203–225	0.821	0.515	0.002*
Gew40	33	11	256–300	0.792	0.788	0.116
Gew41	33	5	178–190	0.588	0.182	0.000*
Gew43	33	4	238–247	0.635	0.273	0.000*
Gew44	33	7	200–222	0.779	0.667	0.001*
Gew47	33	8	256–274	0.777	0.455	0.000*
Gew51	33	7	169–185	0.821	0.606	0.000*
Gew52	33	3	193–199	0.477	0.364	0.054
Gew54	33	13	244–284	0.833	0.697	0.007
Gew55	32	5	163–183	0.622	0.533	0.443
Gew56	33	4	240–252	0.539	0.606	0.881
Gew57	33	4	224–236	0.658	0.576	0.173
Gew58	33	5	201–213	0.559	0.303	0.000*
Gew59	32	6	259–277	0.647	0.600	0.844
Gew60	32	6	207–228	0.709	0.661	0.283
Gew62	33	9	172–208	0.761	0.424	0.000*

* Significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction (Rice, 1989).

To assess variability of the 17 polymorphic loci, 3 *G. breviceps* pocket gophers (where each pocket gopher is a louse population) and their lice were collected from within 0.5 km of each other from 1 locality in Brazos County, Texas (Texas A&M University's Riverside Campus, a 1,900-acre campus adjacent to State Highway 47 and Highway 21, west of Bryan, Texas). Pocket gophers collected for this study were treated humanely according to the guidelines of the Texas A&M University Animal Care and Use Committee and the American Society of Mammalogists (Sikes et al., 2011). Specimen voucher information for hosts and lice is available from Texas A&M University's Biodiversity Research and Teaching Collections. PCRs for the 17 polymorphic loci were performed on a total of 33 *G. ewingi* individuals (11 lice per pocket gopher host) as described above.

For each louse population (all lice from 1 pocket gopher host individual), FSTAT V2.9.3 (Goudet, 2002) was used to calculate estimates of genetic variation for each locus (within population gene diversity H_S and observed heterozygosity H_O). The 17 microsatellite loci were highly variable, with H_S and H_O ranging from 0.477 to 0.833 and 0.182 to 0.788, respectively, per locus (Table II). Genotypic linkage disequilibrium (LD) also was measured for each population between all pairs of loci with the use of Genepop v. 2.5 (Rousset, 2008; Markov chain parameters: 5,000 dememorizations; 1,000 batches; 1,000 iterations) and a sequential Bonferroni method to correct for multiple tests (Rice, 1989). LD was not detected between any loci among populations. Both F_{IS} (average per locus and within hosts) and F_{ST} (among populations) were measured in FSTAT to evaluate overall population structure (Table III). Results indicate that there was a significant deficit of heterozygotes for lice within hosts (average within-host $F_{IS} = 0.24$; $P < 0.001$) and significant genetic structure for lice among hosts ($F_{ST} = 0.0672$; $P < 0.001$). These results indicate not only significant homozygote excess and some level of inbreeding, but also significant structure among populations.

Arlequin v. 3.5 (Excoffier et al., 2005) was used to calculate the probability of conformance to Hardy-Weinberg equilibrium (HWE) at each locus for each louse population (100,000 randomizations). The number of loci deviating from HWE was minor (4–6 loci per population) and varied per population (Table III). To assess loci out of HWE further, Micro-Checker (Van Oosterhout et al., 2004) was used to test for null

TABLE III. Microsatellite *F* statistics per locus and infrapopulation for 33 genotyped louse individuals, *Geomydoecus ewingi*. HWE, probability of conforming to Hardy-Weinberg equilibrium.

Locus	Number of alleles	F_{IS} per locus	F_{IS} per host	HWE
Gew35		0.057		
Host 1	6		0.136	0.631
Host 2	5		0.007	0.736
Host 3	6		0.027	0.031†
Gew39*		0.373		
Host 1	6		0.681	0.00†
Host 2	5		0.200	0.099
Host 3	7		0.218	0.483
Gew40		0.006		
Host 1	7		0.027	0.881
Host 2	5		−0.277	0.244
Host 3	9		0.188	0.158
Gew41*		0.691		
Host 1	3		0.701	0.006†
Host 2	2		1.000	0.001†
Host 3	5		0.437	0.022†
Gew43*		0.570		
Host 1	3		0.685	0.009†
Host 2	4		0.470	0.041†
Host 3	3		0.574	0.038†
Gew44		0.144		
Host 1	5		0.518	0.006†
Host 2	6		−0.099	0.965
Host 3	7		0.036	0.328
Gew47*		0.415		
Host 1	6		0.487	0.023†
Host 2	6		0.181	0.276
Host 3	5		0.570	0.001†
Gew51*		0.262		
Host 1	6		0.259	0.001†
Host 2	5		0.186	0.263
Host 3	6		0.337	0.009†
Gew52		0.238		
Host 1	3		0.441	0.121
Host 2	3		−0.212	1.000
Host 3	3		0.452	0.278
Gew54		0.164		
Host 1	11		0.204	0.221
Host 2	4		0.136	0.117
Host 3	6		0.144	0.623
Gew55		0.150		
Host 1	5		0.161	0.623
Host 2	3		−0.137	1.000
Host 3	4		0.338	0.228
Gew56		−0.124		
Host 1	4		−0.143	1.000
Host 2	3		−0.163	1.000
Host 3	3		−0.077	0.811
Gew57		0.124		
Host 1	4		0.259	0.129
Host 2	3		−0.085	1.000
Host 3	4		0.176	0.058
Gew58*		0.458		
Host 1	5		0.221	0.349
Host 2	2		1.000	0.007†
Host 3	4		0.441	0.111
Gew59		0.077		
Host 1	4		0.020	0.401
Host 2	4		−0.021	0.147
Host 3	4		0.216	0.259
Gew60		0.071		
Host 1	6		0.000	0.799
Host 2	4		0.123	0.548
Host 3	5		0.101	0.401
Gew62*		0.442		
Host 1	8		0.184	0.202
Host 2	5		0.603	0.004†
Host 3	6		0.613	0.001†

* FSTAT results showing significant variation among individuals (F_{IS}; $P < 0.001$)

† Significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction (Rice 1989).

alleles in each louse population. The presence of null alleles is indicated if the combined probability test shows there is an overall significant excess of homozygotes, and when this excess is evenly distributed across the homozygote classes (Van Oosterhout et al., 2004). Possible null alleles were detected at 4 loci by the general excess of homozygotes for most allele size classes. Although these possible null alleles may in fact really be null alleles and interpreted as a technical error, Micro-Checker may also be detecting biological factors such as the heterozygote deficiency due to inbreeding. Five percent of the PCRs were repeated, and no inconsistent results were found. We therefore believe that null alleles for these 4 loci are unlikely. Further supporting this conclusion, all of these microsatellite loci successfully amplified and were polymorphic in *Geomydoecus subgeomydis*, a close relative of *G. ewingi* and ca. 12% genetically divergent (uncorrected *p* distance for the mitochondrial cytochrome oxidase *c* subunit I gene; C. E. Nessner, unpubl. data).

Internal relatedness (IR) and individual homozygosity weighted by locus (HL) were calculated with the IR macro (Amos, 2005) to determine level of louse inbreeding, where high positive values indicate inbreeding and negative values indicate a highly outbred ancestry (Amos et al., 2001; Aparicio et al., 2006). IR and HL were calculated defining the lice from each pocket gopher host as a separate population (11 lice from each of 3 pocket gophers). For each population, IR and HL were positive, ranging from 0.009 to 0.678 (IR) and 0.291 to 0.768 (HL). These results indicate that there are high levels of inbreeding among pocket gopher lice on each host individual.

For the sake of comparison, all 33 louse individuals were also analyzed as 1 population in Arlequin v. 3.5 (Excoffier et al., 2005). When these analyses were performed, these louse individuals showed significant deviation from HWE and of the 17 variable loci, 8 fell outside of Hardy-Weinberg expectations after Bonferroni correction: Gew39, Gew41, Gew43, Gew44, Gew47, Gew51, Gew58, and Gew62 (Table II). Seven of these loci (all except Gew44) in addition to Gew54 were identified as possible null alleles in Micro-Checker (Van Oosterhout et al., 2004). Analyzing all lice as 1 population therefore results in a substantial increase in heterozygote deficiencies. Additionally, results from the AMOVA analysis across all louse individuals and loci indicate significant deficit of heterozygotes ($F_{IS} = 0.273$; $P < 0.001$). Positive values of F_{IS}, with an

average of 0.239 across loci, are consistent with the homozygote excess observed in HWE analyses. With the exception of Gew56, all loci are characterized by homozygote excess, and 10 of these comparisons were statistically significant. IR and HL also were calculated across the entire geographic sample to allow comparisons of individuals from different hosts or populations. IR and HL values were relatively high, ranging from 0.075 to 0.659 (IR) and 0.212 to 0.770 (HL), suggesting some level of inbreeding. These findings in combination indicate that analyzing all 33 louse individuals together results in a Wahlund effect (Nadler et al., 1990; Selkoe and Toonen, 2006), where genetically distinct groups of lice are artificially grouped together. This confirms early findings that louse populations are subdivided among individual hosts (Nadler et al., 1990; Barker et al., 1991). This research further supports that for parasites with life histories that predispose them to multiple generations on a host and limited dispersal among hosts, care must be taken when defining populations in genetic and ecological analyses (Criscione et al., 2005).

Biological aspects of a parasite may lead to deviations from Hardy-Weinberg expectations (Criscione et al., 2005; Criscione, 2008; Dharmarajan et al., 2011). A variety of studies have shown that heterozygote deficiencies are not uncommon for parasite populations (Nadler et al., 1990; Plantard and Porte, 2004; Leo et al., 2005; Criscione et al., 2007; Guzinski et al., 2009; Dharmarajan et al., 2010; Kempf et al., 2010). The majority of the analyses reported here support the commonality of heterozygote deficiency in lice. Heterozygote deficiencies are consistent with the extremely low dispersal abilities of *G. ewingi*. Given the low probability of opportunities for lice to colonize new hosts, the homozygote excess observed in this study is likely the result of inbreeding within hosts. Based upon the combined biological characteristics of pocket gophers (solitary lifestyle) and chewing lice (low vagility), it may be expected that the potential for louse colonization of new hosts is limited (Nadler et al., 1990; Hafner and Page, 1995; Demastes et al., 2012), and nonrandom mating may be occurring within this louse species, increasing the probability of inbreeding. Furthermore, a reduction in expected heterozygosity (i.e., gene diversity) may occur during times of population bottlenecking when louse populations are recently founded by a small number of lice (Nadler et al., 1990; Nadler, 1995; Leo et al., 2005). Several recent population genetics studies of lice and other ectoparasites have reported similar findings of heterozygote deficiencies (Dharmarajan et al., 2011; Veracx et al., 2012; Ascunce et al., 2013), supporting the commonness of this phenomena in these organisms.

We would like to thank E. Birkner, J. Gold, D. Portnoy, J. Puritz, and S. Welborn for their assistance throughout this research. The Light, Voelker, and Conway labs at Texas A&M University, and J. Puritz and T. Spradling made helpful comments on earlier drafts of this manuscript. This is publication number 1460 of the Biodiversity Research and Teaching Collections and number 241 of the Center for Biosystematics and Biodiversity, both at Texas A&M University.

LITERATURE CITED

Amos, W. 2005. IR macro N4. Available at: http://www.zoo.cam.ac.uk/directory/william-amos. Accessed 7 July 2013.

———, J. W. Wilmer, K. Fullard, T. M. Burg, J. P. Croxall, D. Bloch, and T. Coulson. 2001. The influence of parental relatedness on reproductive success. Proceedings of the Royal Society of London, Biological Sciences **268:** 2021–2027.

Aparicio, J. M., J. Ortego, and P. J. Cordero. 2006. What should we weigh to estimate heterozygosity, alleles or loci? Molecular Ecology **15:** 4659–4665.

Ascunce, M. S., M. A. Toups, G. Kassu, J. Fane, K. Scholl, and D. L. Reed. 2013. Nuclear genetic diversity in human lice (*Pediculus humanus*) reveals continental differences and high inbreeding among worldwide populations. PLoS One **8:** e57619.

Barker, S. C., D. A. Briscoe, R. L. Close, and P. Dallas. 1991. Genetic variation in the *Heterodoxus ostoseriatus* group (Phthiraptera): A test of Price's model of parasite evolution. International Journal for Parasitology **21:** 555–563.

Boutin-Ganache, I., M. Raposo, M. Raymond, and C. E. Deschepper. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques **31:** 24–27.

Criscione, C. D. 2008. Parasite co-structure: Broad and local scale approaches. Parasite **15:** 439–443.

———, J. Anderson, K. Raby, D. Sudimack, J. Subedi, D. R. Rai, R. P. Upadhayay, B. Jha, S. Williams-Blangero, and T. J. C. Anderson. 2007. Microsatellite markers for the human nematode parasite *Ascaris lumbricoides*: development and assessment of utility. Journal of Parasitology **93:** 704–708.

———, R. Poulin, and M. S. Blouin. 2005. Molecular ecology of parasites: Elucidating ecological and microevolutionary processes. Molecular Ecology **14:** 2247–2257.

Cruickshank, R. H., K. P. Johnson, V. S. Smith, R. J. Adams, D. H. Clayton, and R. D. M. Page. 2001. Phylogenetic analysis of partial sequences of elongation factor 1α identifies major groups of lice (Insecta: Phthiraptera). Molecular Phylogenetics and Evolution **19:** 202–215.

Demastes, J. W., and M. S. Hafner. 1993. Cospeciation of pocket gophers (*Geomys*) and their chewing lice (*Geomydoecus*). Journal of Mammalogy **74:** 521–530.

———, T. A. Spradling, M. S. Hafner, G. R. Spies, D. J. Hafner, and J. E. Light. 2012. Cophylogeny on a fine scale: *Geomydoecus* chewing lice and their pocket gopher hosts, *Pappogeomys bulleri*. Journal of Parasitology **98:** 262–270.

Dharmarajan, G., J. C. Beasley, and O. E. Rhodes. 2010. Spatial and temporal factors affecting parasite genotypes encountered by hosts: empirical data from American dog ticks (*Dermacentor variabilis*) parasitising raccoons (*Procyon lotor*). International Journal for Parasitology **40:** 787–795.

———, ———, and ———. 2011. Heterozygote deficiencies in parasite populations: An evaluation of interrelated hypotheses in the raccoon tick, *Ixodes texanus*. Heredity **106:** 253–260.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics **1:** 47–50.

Goudet, J. 2002. FSTAT: A program to estimate and test gene diversities and fixation indices. Version 2.9.3.2. Available at: http://www.unil.ch/izea/softwares/fstat.html. Accessed 3 May 2014.

Guzinski, J., C. M. Bull, S. C. Donnellan, and M. G. Gardner. 2009. Molecular genetic data provide support for a model of transmission dynamics in an Australian reptile tick, *Bothriocroton hydrosauri*. Molecular Ecology **18:** 227–234.

Hafner, M. S., J. W. Demastes, T. A. Spradling, and D. L. Reed. 2003. Cophylogeny between pocket gophers and chewing lice. *In* Tangled trees, R. D. M. Page (ed.). University of Chicago Press, Chicago, Illinois, p. 195–220.

———, and S. A. Nadler. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. Nature **332:** 258–259.

———, and R. D. M. Page. 1995. Molecular phylogenies and host–parasite cospeciation: Gophers and lice as a model system. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences **349:** 77–83.

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

Huyse, T., R. Poulin, and A. Théron. 2005. Speciation in parasites: A population genetics approach. Trends in Parasitology **21:** 469–475.

Karlsson, S., M. A. Renshaw, C. E. Rexroad, and J. R. Gold. 2008. PCR primers for 100 microsatellites in red drum (*Sciaenops ocellatus*). Molecular Ecology Resources **8:** 393–398.

Kempf, F., K. D. McCoy, and T. De Meeûs. 2010. Wahlund effects and sex-biased dispersal in *Ixodes ricinus*, the European vector of Lyme borreliosis: New tools for old data. Infection, Genetics and Evolution **10:** 989–997.

Leo, N. P., J. M. Hughes, X. Yang, S. K. S. Poudel, W. G. Brogdon, and S. C. Barker. 2005. The head and body lice of humans are genetically distinct (Insecta: Phthiraptera, Pediculidae): Evidence from double infestations. Heredity **95:** 34–40.

Light, J. E., and M. S. Hafner. 2008. Codivergence in heteromyid rodents (Rodentia: Heteromyidae) and their sucking lice of the genus *Fahrenholzia* (Phthiraptera: Anoplura). Systematic Biology **57:** 449–465.

Mayer, C. 2006. Phobos 3.3.11. Available at: http://www.rub.de/spezzoo/cm/cm_phobos.htm. Accessed 10 October 2011.

McMeniman, C. J., and S. C. Barker. 2006. Transmission ratio distortion in the human body louse, *Pediculus humanus* (Insecta: Phthiraptera). Heredity **96:** 63–68.

Nadler, S. A. 1995. Microevolution and the genetic structure of parasite populations. Journal of Parasitology **81:** 395–403.

———, M. S. Hafner, J. C. Hafner, and D. J. Hafner. 1990. Genetic differentiation among chewing louse populations (Mallophaga: Trichodectidae) in a pocket gopher contact zone (Rodentia: Geomyidae). Evolution **44:** 942–951.

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

Peters, M. B., C. Hagen, N. K. Whiteman, P. G. Parker, and T. C. Glenn. 2009a. Characterization of 10 microsatellite loci in an avian louse, *Degeeriella regalis* (Phthiraptera: Ischnocera: Philopteridae). Molecular Ecology Resources **9**: 882–884.

———, N. K. Whiteman, C. Hagen, P. G. Parker, and T. C. Glenn. 2009b. Eight polymorphic microsatellite markers isolated from the widespread avian louse *Colpocephalum turbinatum* (Phthiraptera: Amblycera: Menoponidae). Molecular Ecology Resources **9:** 910–912.

Plantard, O., and C. Porte. 2004. Population genetic structure of the sugar beet cyst nematode *Heterodera schachtii*: A gonochoristic and amphimictic species with highly inbred but weakly differentiated populations. Molecular Ecology **13:** 33–41.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution **43:** 223–225.

Rousset, F. 2008. GENEPOP '007: A complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources **8:** 103–106.

Scholl, K., J. M. Allen, F. H. Leendertz, C. A. Chapman, and D. L. Reed. 2012. Variable microsatellite loci for population genetic analysis of Old World monkey lice (*Pedicinus* sp.). Journal of Parasitology **98:** 930–937.

Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. Ecology Letters **9:** 615–629.

Sikes, R. S., W. L., Gannon, and the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy **92:** 235–253.

Stein, B. 2000. Morphology of subterranean rodents. *In* Life underground: The biology of subterranean rodents, E. A. Lacey, J. L. Patton, and G. N. Cameron (eds.). University of Chicago Press, Chicago, Illinois., p. 19–61.

Sulentich, J. M., L. R. Williams, and G. N. Cameron. 1991. *Geomys breviceps*. Mammalian Species **338:** 1–4.

Veracx, A., R. Rivet, K. D. McCoy, P. Brouqui, and D. Raoult. 2012. Evidence that head and body lice on homeless persons have the same genotype. PLoS One **7:** e45903.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. Micro-Checker: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes **4:** 535–538.

Welborn, S. R., M. A. Renshaw, and J. E. Light. 2012. Characterization of 10 polymorphic loci in the Baird's pocket gopher (*Geomys breviceps*) and cross-amplification in other gopher species. Conservation Genetics Resources **4:** 467–469.

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.