The Journal of
PARASITOLOGY

COPHYLOGENY ON A FINE SCALE: *GEOMYDOECUS* CHEWING LICE AND THEIR POCKET GOPHER HOSTS, *PAPPOGEOMYS BULLERI*

James W. Demastes, Theresa A. Spradling, Mark S. Hafner*, Gretchen R. Spies, David J. Hafner†, and Jessica E. Light‡
Department of Biology, University of Northern Iowa, Cedar Falls, Iowa, 50614. *e-mail: jim.demastes@uni.edu*

The Journal of the
American Society of
Parasitologists

J. Parasitol., 98(2), 2012, pp. 262–270

COPHYLOGENY ON A FINE SCALE: *GEOMYDOECUS* CHEWING LICE AND THEIR POCKET GOPHER HOSTS, *PAPPOGEOMYS BULLERI*

James W. Demastes, Theresa A. Spradling, Mark S. Hafner*, Gretchen R. Spies, David J. Hafner†, and Jessica E. Light‡
Department of Biology, University of Northern Iowa, Cedar Falls, Iowa, 50614. *e-mail: jim.demastes@uni.edu*

ABSTRACT: Many species of pocket gophers and their ectoparasitic chewing lice have broadly congruent phylogenies, indicating a history of frequent codivergence. For a variety of reasons, phylogenies of codiverging hosts and parasites are expected to be less congruent for more recently diverged taxa. This study is the first of its scale in the pocket gopher and chewing louse system, with its focus entirely on comparisons among populations within a single species of host and 3 chewing louse species in the *Geomydoecus bulleri* species complex. We examined mitochondrial DNA from a total of 46 specimens of *Geomydoecus* lice collected from 11 populations of the pocket gopher host, *Pappogeomys bulleri*. We also examined nuclear DNA from a subset of these chewing lice. Louse phylogenies were compared with a published pocket gopher phylogeny. Contrary to expectations, we observed a statistically significant degree of parallel cladogenesis in these closely related hosts and their parasites. We also observed a higher rate of evolution in chewing louse lineages than in their corresponding pocket gopher hosts. In addition, we found that 1 louse species (*Geomydoecus burti*) may not be a valid species, that subspecies within *G. bulleri* are not reciprocally monophyletic, and that morphological and genetic evidence support recognition of a new species of louse, *Geomydoecus pricei*.

Comparative study of host and parasite phylogenies can provide insight into the historical nature of an intimate biological association. Congruence in the phylogenies of hosts and their parasites is a pattern commonly acknowledged in parasitology. The phenomenon of ''parallel cladogenesis'' (Futuyma and Slatkin, 1983) is described by Fahrenholz's Rule: ''the natural classification of some groups of parasites corresponds with that of their hosts'' (Eichler, 1948). In some cases, this trend may be the result of strict-sense coevolution, or ''reciprocal adaptive responses between ecologically interacting species'' (Brooks and McLennan, 1991). However, in many host–parasite interactions, congruence in phylogenies is more likely the result of a shared biogeographic history that includes responses to the same fragmentation of ranges through vicariance and host dispersal, a process frequently called cospeciation, although actual speciation may or may not be a fundamental part of the process. Although the complex and idiosyncratic histories inherent in the evolution of 2 separate lineages usually prevent the pattern of cophylogeny from being perfect, a pattern of predominantly parallel cladogenesis nevertheless signals a long history of intimate ecological and evolutionary interactions between organisms within these lineages (Hafner and Nadler, 1990; Hafner and Page, 1995).

The field of cophylogenetics has grown dramatically over the past 2 decades, which is not surprising given the diversity of intriguing, long-term associations between organisms with disparate natural histories (e.g., Jackson et al., 2008; Jousselin et al., 2009; Shafer et al., 2009; Desai et al., 2010; Funaro et al., 2011; Johnson et al., 2011) and the advent of modern methods, including molecular methods to investigate these associations. In some cases, documenting widespread cophylogeny has led to tremendous opportunities for addressing comparative evolutionary questions involving very different organisms (e.g., Ochman and Wilson, 1987; Light and Hafner, 2007a; Kikuchi et al., 2009).

Received 26 June 2011; revised 5 October 2011; accepted 19 October 2011.
* Museum of Natural Science and Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803.
† Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico 87131.
‡ Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843.
DOI: 10.1645/GE-2904.1

Pocket gophers (Rodentia: Geomyidae) and their chewing lice (Phthiraptera: Trichodectidae) have been the subjects of considerable study with respect to cophylogeny (Hafner et al., 2003, and references therein; Light and Hafner, 2007a). Geomyid rodents have a strictly New World distribution, ranging from southern Canada to northern Colombia. They are fossorial and asocial, occurring in patchily distributed populations, with genetically distinct groups being largely parapatric (Daly and Patton, 1990; Hafner et al., 2003). Within the closed burrow system of pocket gophers live dozens of organisms that have specialized in this unique niche. In fact, many of these creatures are found nowhere else (Hubbel and Goff, 1940; Tishechkin and Cline, 2008). Among these pocket gopher–dependent organisms are 122 named species and subspecies of chewing lice (Phthiraptera: Trichodectidae). The vast majority of these chewing lice seem to be highly host-specific, occurring on a single pocket gopher species or subspecies. Most often, only 1 species of louse is found on each pocket gopher, although there are numerous cases in which 2 louse species, occasionally 3, reside on the same individual host (Hellenthal and Price, 1991). Chewing lice are wingless insects that feed on skin detritus of their hosts (Marshall, 1981). The very specializations that make chewing lice well suited for a subterranean existence on a solitary host also greatly reduce their ability to disperse. When this poor dispersal ability is coupled with the solitary nature of geomyids, the probability of colonizing a new host (host switching) is thought to be quite low. Hence, where the pocket gopher travels, the louse follows. This game of 'follow-the-leader' takes place across evolutionary timescales (Hafner et al., 1994; Light and Hafner, 2007a), making the pocket gopher–louse association a literal ''textbook case'' of cophylogeny (e.g., see Futuyma, 2005; Page and Holmes, 1998; Ridley, 2004).

Paterson et al. (2003) discussed the kinds of historical events that will affect the degree of congruence between phylogenies of symbiotic organisms. Whereas cospeciation (codivergence) yields congruent phylogenies, 4 kinds of events can result in varying degrees of phylogenetic incongruence: (1) parasite duplication (intrahost speciation); (2) parasite inertia (in which the host lineage diverges and the parasite lineage does not); (3) host switching; and (4) lineage sorting. Whereas sorting events are an intrinsic property of any host–parasite interaction, the asocial nature of pocket gophers and low vagility of chewing lice reduces the likelihood of host switching in this system. Gene trees that do

not match species trees for the parasite lineage, the host lineage, or both, can obscure an underlying pattern of cophylogeny. Moreover, an ancestral host population may carry multiple genetically divergent lineages of parasite, which can then be retained or lost in a stochastic manner on isolated host populations.

Recently diverged lineages of hosts and parasites are less likely to show cophylogeny than older lineages because recently diverged populations of hosts are more likely to share gene flow (reticulate evolution of the host populations), which increases the likelihood of host switching and detracts from the likelihood of observing parallel phylogenies (Nieberding and Olivieri, 2007). Successful transfer of parasites is more likely in hybridization events between intraspecific populations of hosts because the hosts are more similar (providing similar habitats for the parasites) and because there has been less time for the evolution of host specificity in the parasites. Incomplete lineage sorting, a potential problem for phylogenetic analyses of recently diverged taxa, particularly when effective population size is large (Maddison and Knowles, 2006), also may obscure an underlying pattern of cophylogeny. Because younger lineages have had less time for lineage sorting of parasites to occur, and because the genes we use to infer relationships in each lineage have had less time to coalesce, we are less likely to observe a pattern of similar evolutionary histories based on genetic analysis of more recently diverged pairs of host and parasite taxa (Hafner and Page, 1995; Rannala and Michalakis, 2003; Nieberding and Olivieri, 2007). Alternatively, one could argue that younger parasite lineages may show greater levels of phylogenetic congruence to their hosts because there has been less time for extinction of parasite lineages, but parasite extinction will not, by itself, obscure the pattern of cophylogeny unless it is coupled with colonization by a new parasite lineage.

Accordingly, previous studies of pocket gophers and chewing lice and of Neotropical figs and their pollinators have both shown less cospeciation at finer phylogenetic scales in lineages that appear to cospeciate on a larger scale (Demastes and Hafner, 1993; Demastes et al., 2003; Jackson et al., 2008). These observations, however, are far from exhaustive. As Huyse et al. (2005) suggest, studies of multiple host–parasite systems at multiple taxonomic levels are needed before drawing any conclusions regarding the relationship between taxonomic level and likelihood of cospeciation.

To date, there have been no cophylogenetic studies of pocket gophers and their chewing lice that have involved exhaustive sampling across an entire clade of chewing lice to reduce sampling error of parasite species. Hafner and Nadler (1988) and Hafner et al. (1994) sampled from a broad range of distantly related pocket gophers and their chewing lice. Demastes and Hafner (1993) sampled several species of pocket gophers and chewing lice in a particular geographic region (Texas and Louisiana). Light and Hafner (2007a) analyzed an extensive set of chewing lice (*Geomydoecus*) found on a single clade of pocket gophers (the *Cratogeomys merriami* group), which hosts 2 separate clades of chewing lice. Sampling involved in the Light and Hafner (2007a) study was extensive and exhaustive for the pocket gopher hosts, but it was incomplete for the louse lineages that resided on these hosts because the louse complexes have a widespread distribution including pocket gophers in the *Cratogeomys fumosus* species group to the west (Price and Hellenthal, 1989b; Hafner et al., 2004). Complete sampling will be difficult for many louse lineages given the widespread distribution of their species complexes.

Herein, we investigate phylogenetic relationships in chewing lice of the *Geomydoecus bulleri* species group (Price and Hellenthal, 1989a). Our sampling of the chewing lice is exhaustive, with the exception of 1 subspecies we were unable to collect. These chewing lice are found exclusively on species of the pocket gopher *Pappogeomys* (Hafner et al., 2009). In addition to the widespread species, *G. bulleri*, originally described by Price and Emerson (1971), Price and Hellenthal (1989a) described 2 additional species (*Geomydoecus burti* and *Geomydoecus nadleri*) to bring the total number of louse species in this species group to 3. Price and Emerson (1971) expressed some reservation about elevating *G. burti* to the species status in the absence of qualitative differences but did so based on a large number of quantitative morphological differences and a "low level of misidentification probability" (Price and Hellenthal, 1989a). Additionally, they described 3 subspecies within *G. bulleri*: *G. b. bulleri*, *G. b. melanuri*, and *G. b. intermedius*. Price and Hellenthal (1989a) found that variation among these 3 subspecies is in continuous characters (mainly width) that overlap somewhat in their ranges, and the subspecies name *intermedius* reflects the fact that measurements for these individuals fall between those of individuals in the other 2 subspecies. We have sampled all of these taxa, with the exception of 1 subspecies, *G. b. melanuri*.

Pappogeomys bulleri is the sole host species recognized within the genus (Hafner et al., 2009). This species has a distribution that encompasses west-central Mexico, including a variety of habitats ranging from coastal lowlands to >3,000 m (Fig. 1). Hafner et al. (2009) examined karyotypic data and DNA sequences from 2 mitochondrial genes and 1 nuclear gene in *P. bulleri*. Although sequence data suggested some potentially old divergences between some lineages, the divergence values are consistent with intraspecific variation in other pocket gophers; thus, Hafner et al. (2009) recognized 5 distinct intraspecific lineages as subspecies of *P. bulleri*. One of these, *Pappogeomys bulleri alcorni*, is the host of a louse, *Geomydoecus alcorni*, that is part of the *Geomydoecus mcgregori* complex, a complex commonly found on species of *Cratogeomys*. As such, *G. alcorni* represents 1 of only 3 cases in which members of a louse species complex are found on more than 1 genus of pocket gopher (Price and Emerson, 1971; Hellenthal and Price, 1991). By sampling chewing lice from the same host individuals described by Hafner et al. (2009), we reduce the likelihood of artificial sorting events caused by sampling error ("x events"; Paterson et al., 2003), but, given that the hosts are so recently diverged, we also invite the potentially confounding influences of processes such as incomplete sorting of louse populations, incomplete sorting of louse and/or pocket gopher alleles, increased potential for host switching, and reticulate host evolution. Any of these factors, alone or in combination, can obscure past cospeciation events or can prevent cospeciation from occurring in the first place (Demastes et al., 2003). Although previous studies of pocket gophers and chewing lice have explored these potentially confounding factors at the intrageneric level, the present study is the first to restrict its focus entirely to comparisons among populations within a single species of host.

MATERIALS AND METHODS

Specimens examined

We examined a total of 62 specimens of *Geomydoecus* collected from the same pocket gopher specimens (genus *Pappogeomys*) from 11 localities

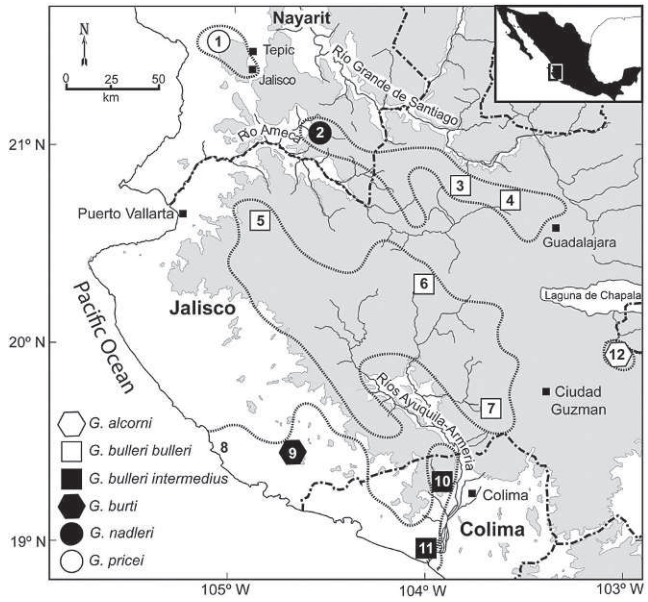

FIGURE 1. Collecting localities for pocket gophers and chewing lice (*Geomydoecus*) sampled for this study. Complete locality information is listed in Appendix I. Locality numbers correspond to those of Hafner et al. (2009). Locality 8 from Hafner et al. (2009) is mapped, but no chewing lice were available from this locality. Shapes indicate chewing louse (*Geomydoecus*) species. Shaded area indicates elevations above 1,000 m. Heavier dashed lines indicate state boundaries and lighter dashed lines show approximate distributions of 5 subspecies of *Pappogeomys bulleri* identified by Hafner et al. (2009).

analyzed by Hafner et al. (2009; Fig. 1; Appendix I). Chewing lice were not available from 1 locality (locality 8, Chamela, from Hafner et al., 2009). Following DNA isolation, voucher specimens were preserved following Cruickshank et al. (2001) and identified to species based on the taxonomic characters of Price and Emerson (1971) and Price and Hellenthal (1989a). The standard morphologic measurements of TW = temple width, HL = head length, PW = prothorax width, and TBL = total body length were obtained from digital micrographs using the program Motic Images Plus (version 2.0 ML, Motic, Richmond, British Columbia, Canada).

Analysis of mitochondrial DNA

Mitochondrial DNA sequence data were collected for 45 chewing lice. DNA extraction, amplification, and sequencing for chewing lice followed Light and Hafner (2007a). Genomic DNA was extracted (DNeasy Tissue Kit, Qiagen, Valencia, California) from individual chewing lice following the manufacturer's protocol, with a final elution of 30 µl for each sample. Extractions were amplified by polymerase chain reaction (PCR) for overlapping regions of the mitochondrial cytochrome *c* oxidase subunit I (CO*I*) gene. Primers used were LCO1490, HCO2198 (Folmer et al., 1994), and 2 primers designed for *G. bulleri*: L450 (5′-AAATCTTTGAGTTGA-GAAGTCTAAGTATGA-3′) and H1019 (5′-GCTAAAACTAACC-CGGTCATGCCCCC-3′). The number of PCR cycles and annealing temperatures for each primer pair were as follows: LCO1490 with HCO2198, 40 cycles at 45 C; L450 with H1019, 45 cycle sat 45 C; and LCO1490 with H1019, 45 cycles at 45 C. Resulting amplified fragments were prepared for sequencing using Exosap-it (USB, Cleveland, Ohio), and sequencing was performed at Iowa State University's DNA Facility (Ames, Iowa) using their ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California). All sequenced regions received at least 2× coverage by sequencing with both PCR primers. Sequences were submitted to GenBank (GenBank Accession Nos. JF342595–JF342641). Outgroup taxa for phylogenetic analyses (detailed below) included *Geomydoecus mexicanus* (DQ200317), *Geomydoecus veracruzensis* (DQ200338), and *Geomydoecus wernecki* (DQ200298), all taken from Light and Hafner (2007a).

Analysis of louse phylogeny

An initial phylogenetic analysis was performed using 46 ingroup individuals and 546 base pairs (bp) of sequence from amplification primers LCO1490 and HCO2198 to ensure that chewing lice from the same localities formed monophyletic clades (all executable data files and trees for this study: Treebase Accession No. S11705). Following this procedure, single representatives were chosen for tests of cophylogeny that corresponded to the pocket gopher hosts reported in Hafner et al. (2009). An additional 357 bp of CO*I* sequence data were generated using primers L450 with H1019 or LCO1490 with H1019 for these individuals, yielding a total of 903 bp of data for the analyses of cophylogeny. For both datasets, uncorrected sequence divergence values (p) were calculated in PAUP* version 4.0b10 (Swofford, 2002).

Modeltest version 3.7 (Posada and Crandall, 1998) was used to select nucleotide substitution models for maximum likelihood and Bayesian analyses. Based on AIC criteria, GTR + I + G models were used for both louse datasets (546 and 903 bp), although different parameters were estimated in each case.

Louse phylogenetic analyses were conducted using PAUP* version 4.0b10 for maximum likelihood (ML) and parsimony (equal weights). Node support was estimated using nonparametric bootstrap replicates (500 for ML and 1,000 for parsimony) generated using random taxon-addition in a heuristic search using tree-bisection–reconnection branch swapping. MrBayes version 3.1.1 (Ronquist and Huelsenbeck, 2003) was used for Bayesian analyses. Bayesian analysis consisted of paired runs of 4 Markov-Chain Monte Carlo analyses, each using default settings and iterated for 10^7 generations sampled every 100 generations and discarding the initial 500 trees sampled.

Analysis of nuclear DNA

Representative louse individuals were selected from each mtDNA clade to assess the level of differentiation in a portion of the nuclear gene, elongation factor-1α (EF-1α). Amplification and sequencing of 11 individuals followed Light and Hafner (2007b) using the primers For3 and Cho10 (Danforth and Ji, 1998), resulting in 226 bp of DNA sequence data. PCR amplification used 40 cycles with 45 C annealing temperature. Sequencing reactions were performed at Iowa State University's DNA Facility. Sequences were aligned and heterozygosity was evaluated by eye using Sequencher 4.1.2 software (Gene Codes Corporation, Ann Arbor, Michigan). No heterozygotes were observed. Given the paucity of genetic variation at this locus, analysis consisted simply of calculating uncorrected percentage sequence divergence (p) by hand. Sequences were submitted to GenBank (GenBank Accession Nos. JF342643–JF342653). Other louse sequences from the *G. mexicanus* and *Geomydoecus coronadoi* species complexes used for comparison were obtained from GenBank (DQ 200340, DQ200344, DQ200345, and DQ200355; Light and Hafner, 2007a).

Tests of cophylogeny

The overall approach taken to test for cophylogeny followed the methods of Light and Hafner (2007a). The host phylogeny was taken from Hafner et al. (2009) and pruned to include taxa hosting chewing lice from the *G. bulleri* species complex present in this study. The phylogenies of the hosts and parasites were compared with a test for a history of widespread cospeciation. Reconciliation analysis was performed using TreeMap 2.0β (Charleston and Page, 2002), using the default options for assigning costs (zero cost for a codivergence event and a cost of 1 for host switches, duplications, or losses). Significance was determined by randomization of the parasite tree (n = 10,000) and comparison of the resulting null distribution of codivergence events to number of codivergence events estimated from comparison of the data-based host and parasite trees.

Distance-based methods of testing for cophylogeny are topology free and compare genetic distances for homologous gene regions for the associated host and parasite taxa. Host gene sequences were taken from Hafner et al. (2009; EU880352, EU880353, EU880355–EU880357, EU880363, EU880364, EU880367, and EU880370–EU880372). To allow for comparison of homologous gene regions, pocket gopher sequences were trimmed to the homologous 903-bp region of CO*I*, and maximum likelihood distances were calculated using the GTR + G model selected by Modeltest using AIC criteria. Parasite distance matrices were calculated using the GTR + I + G model, also selected by Modeltest based on AIC

criteria. The null hypothesis of random association between the host and parasite distance matrices was tested using Mantel tests as implemented in the R Package of programs (Casgrain and Legendre, 2001). Probabilities were calculated using both the approximate (Z) and standardized (r) statistics. The standardized statistic (r) was calculated using 5,000 permutations. A second distance-based approach using the program Parafit (Legendre, 2001b) also tested for random association between the host and parasite distance matrices, with distance matrices being converted to principal coordinate matrices using the R Package (Casgrain and Legendre, 2001). Tests of random association were conducted with 999 permutations globally and across each pair of associated host and parasite.

Comparison of molecular rates

Past studies have revealed marked differences in the rate of accumulated mutations between chewing lice and pocket gophers (Hafner et al., 1994; Hafner and Page, 1995). Herein, homologous 903-bp regions of CO*I* were compared between pocket gophers and chewing lice to test for significant differences in rates of evolution. Rate comparisons involved only 4-fold degenerate sites to minimize the potential effect of selection on estimated basal mutation rates (Hafner et al., 2003). The program Mega3 (Kumar et al., 2004) was used to identify 4-fold degenerate sites using the appropriate mtDNA translational codes.

For comparisons of analogous host and parasite branch lengths to be meaningful, a local molecular clock must be present (Page and Hafner, 1996). To test for clock-like behavior of the data within each data set, trees were constructed with and without a molecular clock enforced; scores were compared using likelihood-ratio tests.

Significant cophylogeny and clock-like variation of rates allows for the comparison of analogous branches to test for significant rate differences between cospeciating pairs of hosts and parasites. A co-path analysis (Page, 1996) was performed to identify analogous branches (co-paths) between the hosts and parasites. Branches involving non-cospeciating taxa (including outgroups) were omitted from rate comparisons. User trees including only cospeciating taxa were enforced for all branch length comparisons. Branch lengths for 4-fold degenerate sites were calculated on parsimony trees (using both Acctran and Deltran character-state optimization), on minimum evolution trees built using p-distances, and on maximum likelihood trees built using the models selected for 4-fold data only (GTR models for both pocket gophers and chewing lice; Modeltest, version 3.7; AIC criteria). Estimated branch lengths for all co-paths were compared using Wilcoxon sign-rank tests and using Model II regression analysis with a permutation test for testing the significance of the slopes (Legendre, 2001a) to determine whether significant rate differences were present between parasites and hosts (Hafner and Nadler, 1990).

RESULTS

Analysis of louse phylogeny

All chewing lice from localities 2–7, 10, and 11 (Fig. 1) were readily assigned to either *G. nadleri* or *G. bulleri* based on morphology. In contrast, chewing lice from locality 9, which is only 11 km north of the type locality of *G. burti* (Price and Hellenthal, 1989a), could not be neatly classified as either *G. bulleri* or *G. burti* based on morphology (using measurements for temple width, prothorax width, and head length). As noted previously, Price and Hellenthal (1989a) expressed reservations about elevating *G. burti* to the species level; there were no diagnostic qualitative characters for the species, and the mensural characters they used to distinguish *G. burti* from *G. bulleri* were broadly overlapping. For example, temple width was reported by Price and Hellenthal (1989a) to range between 0.470–0.490 mm in *G. b. melanuri* and 0.485–0.535 in *G. burti*. Each of 6 male chewing lice examined from locality 9 for these 3 characters showed a mixture of measurements, some of which were consistent with *G. burti* (9 measurements), some of which were consistent with *G. b. melanuri* (6 measurements), and some of which were ambiguous given the overlapping ranges of measurements reported by Price and Hellenthal (1989a; 3 measurements). Although it is possible that we did not sample *G. burti* in the present study, we are reasonably confident that the chewing lice from locality 9 are what Price and Hellenthal (1989a) referred to as "*G. burti*," given the morphology of the chewing lice and the geographic proximity of their collection locality to that of the G. *burti* type locality.

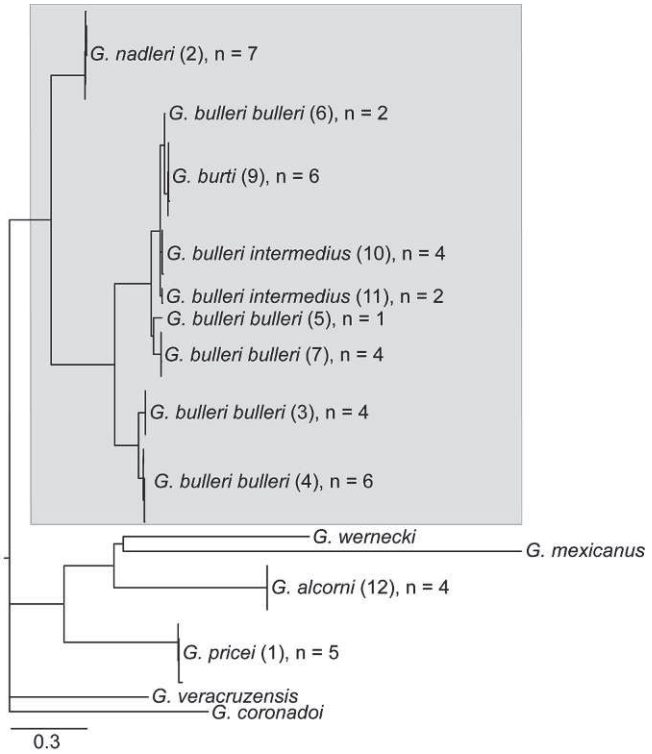

FIGURE 2. Maximum likelihood phylogram for *Geomydoecus* based on 546 bp of mitochondrial CO*I*. The *Geomydoecus bulleri* species group is indicated by shading. Numbers in parentheses after each taxon name indicate sampling locality (as in Appendix I and Fig. 1) followed by sample size (n).

Parsimony, ML, and Bayesian analyses of the 546-bp mtDNA dataset (211 parsimony-informative characters) indicated that chewing louse individuals from each locality formed distinct, well-supported clades, with little sequence divergence within each clade (0–0.6% sequence divergence in most populations, and a maximum of 1.4% uncorrected sequence divergence within locality 1; Fig. 2). Based on this result, a representative individual was chosen from each of these clades and included in further sequencing, resulting in a dataset with 903 bp of CO*I* data for 11 chewing lice. This dataset contained 306 parsimony-informative characters, and all 3 methods of analyses (parsimony, ML, and Bayesian) produced identical tree topologies, although support as indicated by bootstrapping was weak at some nodes (Fig. 3).

All genetic analyses strongly supported the monophyly of the *bulleri* species group (shaded area in Fig. 3) as described by Price and Hellenthal (1989a). Current taxonomy within the clade was not supported by mtDNA data, however, since placement of *G. burti* in the tree renders *G. bulleri* paraphyletic, and the subspecies *G. b. bulleri* and *G. b. intermedius* are not reciprocally monophyletic.

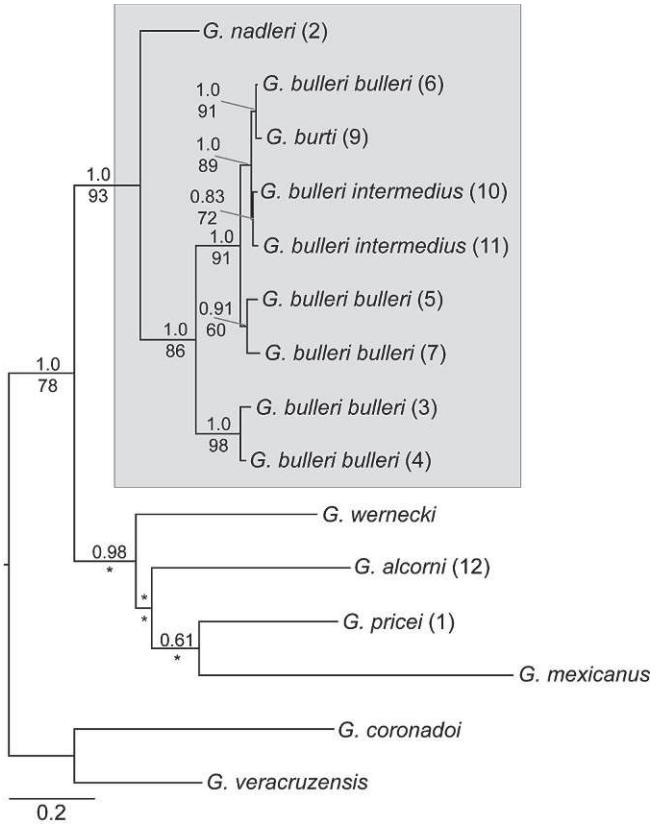

FIGURE 3. Maximum likelihood phylogram for *Geomydoecus* based on 903 bp of mitochondrial CO*I*. The *Geomydoecus bulleri* species group is indicated by shading. Bayesian posterior probabilities (above) and maximum likelihood bootstrap values (below) are listed on nodes with support >60%. Parsimony support values are available upon request. Numbers in parentheses after each taxon name indicate sampling locality (as in Appendix I and Fig. 1).

Chewing lice collected from the state of Nayarit (La Libertad, locality 1; Fig. 1) were expected to be *G. nadleri* based on locality information, but this was not the case. Instead, these individuals are very similar morphologically to *G. alcorni* (locality 12), a member of the *mcgregori* species complex. However, chewing lice from locality 1 are genetically unique, not showing a close phylogenetic relationship to either *G. alcorni* or *G. bulleri* (Fig. 3). Chewing lice from locality 1 show a large genetic distance from *G. alcorni* ($P = 18.3\%$) and from chewing lice belonging to the *bulleri* species complex (average $P = 19.8\%$). This genetic divergence is roughly equivalent to that between *G. alcorni* and the *bulleri* species complex ($P = 20.0\%$). In contrast, uncorrected CO*I* sequence divergence between *G. nadleri* and other members of the *bulleri* species complex (excluding the louse from locality 1) averages only 15.3%, and within *G. bulleri* and *G. burti* (localities 3–7, 9–11) sequence divergence ranges from 1.5% to 12.8% (Table I). Therefore, CO*I* sequences indicate that chewing lice from locality 1 are not *G. nadleri*, nor are they likely members of the *bulleri* species complex.

Analysis of the nuclear gene, EF-1α, revealed little genetic variation. Six *G. nadleri* individuals from locality 2, all individuals of *G. bulleri* sampled for EF-1α (localities 3, 7, and 11), and the outgroups *Geomydoecus fulvescens* and *G. mexicanus* had identical EF-1α nucleotide sequences. *Geomydoecus fulvescens* and *G. mexicanus* are members of the *G. mexicanus* species complex, which hints at a possible relationship between the *G. bulleri* and *G. mexicanus* species complexes. *Geomydoecus alcorni* (locality 12), a member of the *G. mcgregori* species complex, had 1 unique nucleotide character state (a transition) relative to the above samples ($P = 0.4\%$). The louse from La Libertad (locality 1) had another autapomorphic transition, rendering it unique compared with all *G. bulleri*, *G. mexicanus*, and *G. mcgregori* species-complex members studied to date. In contrast, *G. veracruzensis* and *G. coronadoi* of the *G. coronadoi* complex had matching EF-1α nucleotide sequences that differed by 5 nucleotides relative to the *G. bulleri*, *G. mcgregori*, and *G. mexicanus* species complexes (uncorrected $P = 2.2$–2.6%).

Tests of cophylogeny

Cophylogeny analysis was restricted to chewing lice of the *G. bulleri* species complex and their hosts. Potential cophylogeny involving *G. alcorni* and chewing lice from locality 1 will be explored in more depth as part of an ongoing macroevolutionary study that includes greater representation of *Cratogeomys* and *Pappogeomys* spp. pocket gophers and their chewing lice.

Reconciliation analysis using TreeMap for the *G. bulleri* species-complex chewing lice and their hosts revealed a significant pattern of cophylogeny (Fig. 4; $P = 0.003$). The optimally reconciled trees contained 12 cospeciation events, 4 duplications, 1 loss, and 2 host switches, with a total cost of 7.0. Distance-based analysis using a Mantel test likewise revealed significant congruence between the host and parasite distance matrices. Both the approximate Mantel test and the permutation-based standardized test were highly significant ($P = 0.00004$ and $P = 0.005$, respectively). Parafit analysis also yielded a global probability for significant congruence of 0.003. Three individual host–parasite links, involving localities 6, 9, and 10, were found to have non-significant probabilities of congruence.

Comparison of molecular rates

Analysis of the 903-bp datasets revealed 153, 4-fold degenerate sites in the pocket gopher dataset and 139 in the louse dataset. Likelihood-ratio tests did not detect a significant departure from clock-like behavior in datasets consisting exclusively of 4-fold degenerate sites ($P = 0.38$ for the chewing lice; $P = 0.078$ for the pocket gophers). Chewing lice and pocket gophers showed significantly different rates of evolution when homologous branch lengths (Fig. 4) were compared using Wilcoxon sign-rank tests for branches from parsimony trees (Acctran character-state optimization, $P = 0.04$, and Deltran character-state optimization, $P = 0.05$) and minimum evolution trees (*P*-distances, $P = 0.05$), with the sum of all branch lengths in the louse tree being 1.8–1.9 times longer than in the corresponding gopher tree. Maximum likelihood branch lengths showed the same pattern of elevated rate of evolution in chewing lice (total of branch lengths in chewing lice were 2.3-fold higher than in pocket gophers), but this difference was not statistically significant (Wilcoxon sign-rank test, $P = 0.20$). Tracing homologous branches from any cospeciating taxon through the base of the tree to any other distantly related taxon, e.g., pocket gophers from populations 10 and 3 and their lice, indicated that chewing lice have on average 2.7–4.4 times faster rates of substitution at 4-fold degenerate sites.

TABLE I. Uncorrected percentage sequence divergence (p) for *Geomydoecus bulleri* species group lice. Population numbers 2–11 correspond with numbers used in the text.

Population and louse species		2	3	4	5	6	7	9	10	11
2	*G. nadleri*	—								
3	*G. bulleri*	0.152	—							
4	*G. bulleri*	0.146	0.033	—						
5	*G. bulleri*	0.155	0.124	0.123	—					
6	*G. bulleri*	0.155	0.128	0.130	0.057	—				
7	*G. bulleri*	0.153	0.124	0.124	0.047	0.064	—			
9	*G. burti*	0.155	0.125	0.128	0.063	0.015	0.069	—		
10	*G. bulleri*	0.157	0.127	0.130	0.063	0.025	0.065	0.034	—	
11	*G. bulleri*	0.155	0.124	0.128	0.062	0.027	0.062	0.031	0.021	—

For each tree type, Type II regression analysis of louse branch lengths against pocket gopher branch lengths yielded a scatter of points with a slope that was not significantly different from zero.

DESCRIPTION

Geomydoecus pricei n. sp.

(Fig. 5)

General diagnosis: Morphologically similar to *G. alcorni* (Price and Emerson, 1971; Price and Hellenthal, 1989b) but found nearer to geographic range of *G. nadleri* and *G. bulleri* (Fig. 1; Price and Hellenthal, 1989a).

Male (holotype and 14 paratypes): Very similar to *G. alcorni* (Price and Emerson, 1971; Price and Hellenthal 1989b). Both inner and outer marginal setae on temple short and spiniform with longer submarginal setae between; endomeral plate elongate triangle with apical "shoulders" (width = 0.062–0.726 mm), genital sac prominent with 6 large spines, parameral arch with shallow v-shaped indentation in medioanterior margin. Temple width (TW) 0.423–0.477 mm, head length (HL) 0.290–0.337, prothorax width (PW) 0.301–0.333, total body length (TBL) 1.279–1.373.

Female (allotype and 12 paratypes): Very similar to *G. alcorni*. Short outer dorsal head setae, longer submarginal temple setae between marginal temple setae, subgenital plate transverse (not lobate), genital sac with loops forming continuous transverse arches. TW 0.454–0.468 mm, HL 0.296–0.321, PW 0.305–0.352, TBL 1.069–1.302.

Taxonomic summary

Type host: Pappogeomys bulleri nayaritensis (Goldman, 1939).

Type locality: Nayarit: La Libertad, 10 km NE Jalcocotán; 1,034 m elevation.

Symbiotype: CNMA 43263.

Paratype locality: Nayarit: Jalisco, 1524 m (USNM 88129).

Type material: Nayarit: La Libertad, 10 km NE Jalcocotán, 1,034 m elevation. Holotype ♂ (labeled MSH 1689.5), allotype ♀ (labeled MSH 1689.P3), paratypes 10 ♂♂, 9 ♀♀ (including allotype); also, Nayarit: Jalisco, 1,524 m elevation. 4 ♂♂, 3 ♀♀ (labeled USNM 88129). All type specimens are deposited in the University of Minnesota Insect Collection (UMSP 110901–UMSP 110919).

Etymology: This species is named in honor of Roger D. Price in recognition of his outstanding leadership in the fields of chewing louse taxonomy and cospeciation.

Range: Specimens known only from type and paratype localities. Likely common on *P. b. nayaritensis* found throughout the northeastern edge of the species range.

Remarks

Geomydoecus pricei n. sp. represents a genetically distinct lineage based on DNA sequences from the mitochondrial gene, cytochrome *c* oxidase subunit I (CO*I*), and the nuclear gene, elongation factor-1α (EF-1α). Among *Geomydoecus*, *G. pricei* is morphologically most similar to *G. alcorni* (Price and Emerson, 1971; Price and Hellenthal, 1989b) despite geographic separation of approximately 240 km. (Fig. 1). Males can be differentiated from specimens of *G. alcorni* based upon a shallower indentation in the anterior margin of the parameral arch (Fig. 5). Females have narrower heads (TW 0.454–0.468) than those of *G. alcorni*, (TW 0.475–0.485). Males of *G. pricei* can be distinguished readily from nearby *G. nadleri* (also hosted by *P. b. nayaritensis*) by the smoothly tapering, triangular shape of the endomeral plate (the plate of *G. nadleri* possesses lateral margins with an uneven, stepped taper) and the notch in the medioanterior margin of the parameral arch versus the smooth margin of *G. nadleri*. Females of *G. pricei* lack the submarginal temple setae between marginal setae as in *G. nadleri* and possess only loops on their genital sacs, whereas *G. nadleri* possesses predominantly transverse lines (Price and Hellenthal, 1989a). *Geomydoecus umbrini* is hosted by *Thomomys umbrinus* and also occurs near the range of *G. pricei*. The endomeral plates of *G. umbrini* have nearly equal sides (equilateral) rather than being posteriorly elongated as in *G. pricei*. The parameral arches of *G. umbrini* are gracile and lack the indentation in the medioanterior margin. Females of *G. pricei* and *G. umbrini* are quite similar, with *G. umbrini* having "flatter" genital plate loops, and with submarginal temple setae shorter than marginal temple setae (submarginal temple setae are longer in *G. pricei*).

DISCUSSION

Louse phylogeny and taxonomy

The mitochondrial data presented herein are not fully concordant with the present taxonomy for chewing lice. Price and Hellenthal (1989a) pointed out that no qualitative characters separate *G. burti* from *G. bulleri*, but they elevated *G. burti* to species level based on the number of quantitative differences and the apparent reliability of these characters. Given the geographic proximity of *G. burti* to *G. bulleri*, the morphological similarity of

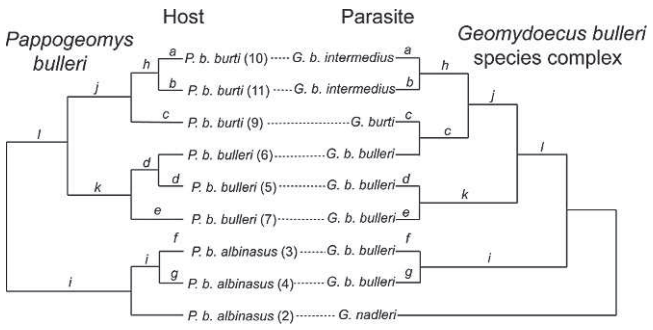

FIGURE 4. Tanglegrams illustrating cospeciation in *Pappogeomys bulleri* and their chewing lice (*Geomydoecus bulleri* species complex). TreeMap (2.0β) revealed a significant pattern of cophylogeny ($P = 0.003$). Letters on branches indicate analogous branches in the co-path analysis. Dashed lines between pocket gopher and chewing louse taxa indicate host–parasite associations.

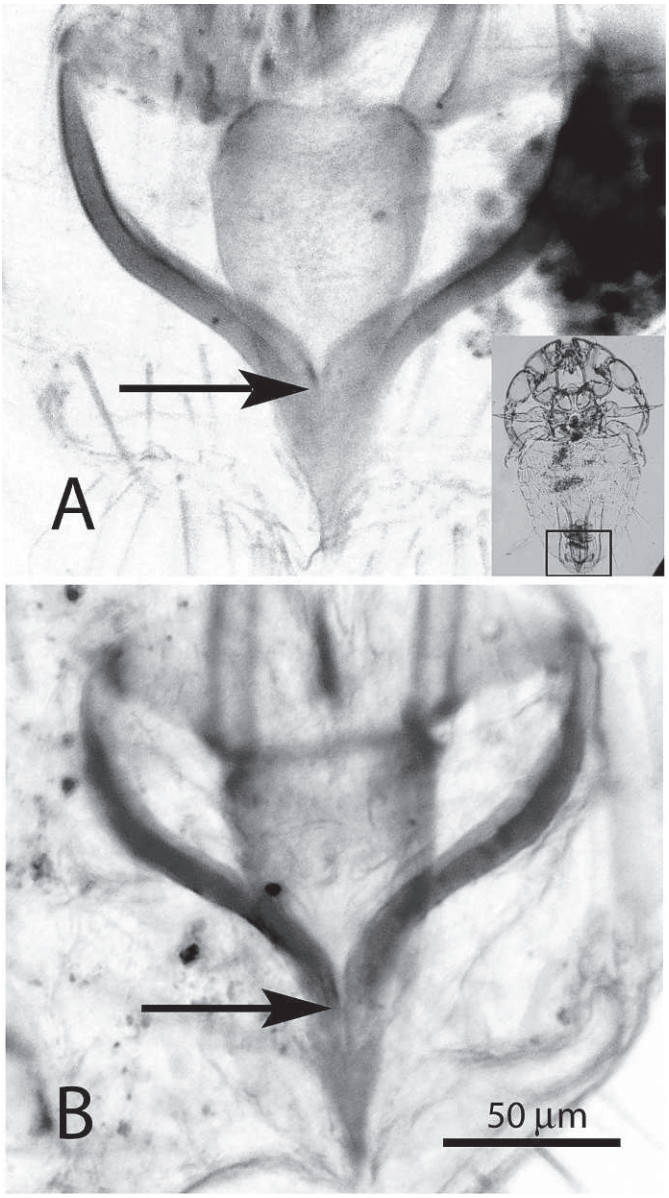

FIGURE 5. Male genitalia of the newly described species, (**A**) *Geomydoecus pricei*, and (**B**) the morphologically similar *Geomydoecus alcorni*. Note the shallower indentation in the parameral arch of *G. pricei* relative to *G. alcorni* (indicated by arrow).

the 2 taxa, and the genetic similarity ($P = 1.6\%$ between *G. burti* and *G. bulleri* of locality 6), the most parsimonious taxonomic solution may be to consider all chewing lice from the Sierra Madre del Sur region (Localities 5–7 and 9–11; Figs. 1–3) a single taxon, i.e., *G. bulleri*. Therefore, at this point, it appears that the *G. bulleri* species group comprises only 2 well-supported species of chewing lice, *G. bulleri* and *G. nadleri*. Likewise, subspecific designations within *G. bulleri* deserve further consideration when specimens of *G. b. melanuri* become available for genetic analysis. As it stands now, *G. b. bulleri* appears to be a paraphyletic taxon that includes *G. b. intermedius* within it, and morphological differentiation among the 3 subspecies is slight and overlapping.

Chewing lice collected in Nayarit from near La Libertad (locality 1; Figs. 1–3) did not resemble *G. nadleri* (locality 2) morphologically or genetically, or any other louse from the *G. bulleri* species complex. Moreover, the La Libertad lice did not resemble any other described pocket gopher louse, save *G. alcorni*, a member of the *G. mcgregori* species group found predominantly on *Cratogeomys* spp. pocket gophers to the east. Because these chewing lice are genetically distinct from *G. alcorni* (18.3% uncorrected sequence divergence; Fig. 3), it is likely that they represent a cryptic species. The only consistent diagnostic character for males of *G. alcorni* reported by Price and Hellenthal (1989b) is a deep indentation in the anterior margin of the parameral arch. Specimens from Nayarit (locality 1) also possess an indentation in the parameral arch, but the indentation is roughly 50% shallower than that of *G. alcorni* (Fig. 5). Additionally, females from this locality have smaller temple widths (0.454–0.468 mm) than females of *G. alcorni* (0.475–0.485 mm). Despite the morphological similarity between *G. alcorni* and the chewing lice from locality 1, there is a high degree of CO*I* sequence differentiation ($P = 18.3\%$). Therefore, the diagnostic morphological character in male individuals and the temple widths of females from locality 1, together with the large genetic divergence from other taxa, support recognition of a new species, *G. pricei*.

Other researchers apparently have noted the morphological similarity between *G. alcorni* and chewing lice from Nayarit. Voucher specimens in the University of Minnesota Insect Collection include chewing louse specimens from a pocket gopher (*P. b. nayaritensis*, USNM 88129) that was collected in 1897 in the state of Nayarit near the town of Jalisco, approximately 30 km from locality 1 of this study. These chewing lice (5 males, 3 females, and 25 nymphs) initially were labeled *G. bulleri* (likely based on locality), then re-labeled as *G. alcorni*, most likely by leading chewing louse taxonomist, Roger D. Price, while at the University of Minnesota, although this cannot be confirmed. Our reexamination of the 5 male specimens shows these chewing lice to have the shallow indentation in the anterior margin of the parameral arch characteristic of *G. pricei*, and the female specimens have temple widths that fall within the range of measurements diagnostic for *G. pricei*.

Results of our analysis of nuclear DNA in these chewing lice are consistent with relationships based on mtDNA (Fig. 3), as well as relationships proposed by Page et al. (1995) based on morphology. Our nuclear DNA sequences suggest that the *G. bulleri* species complex (*G. bulleri* and *G. nadleri*) is closely related to the *G. mcgregori* (including *G. alcorni*) and *G. mexicanus* (including *G. fulvescens* and *G. mexicanus*) species complexes. Together, these species complexes appear to be more distantly related to chewing lice of the *G. coronadoi* species complex (*G. veracruzensis* and *G. coronadoi*). The position of the new species (*G. pricei*) within these louse complexes currently is unknown, but broader taxonomic sampling and additional nuclear DNA evidence should help resolve this issue. For now, it appears safe to say that this new species does not belong to the *G. coronadoi* complex, which ranges across much of the Mexican Transverse Volcanic Range and broadly overlaps the range of the *G. mcgregori* complex (Price and Hellenthal, 1989b).

Cophylogeny, rates, and evolutionary scale

The present study is the only example of statistically significant cophylogeny that involves only conspecific populations of pocket

gophers and their chewing lice. Other pocket gopher and chewing louse studies have approached the microevolutionary level but are best described as intrageneric (Demastes and Hafner, 1993; Spradling, 1997). Theory predicts that reticulate evolution, sorting events, and other population-level phenomena likely will obscure an underlying pattern of cophylogeny in recently diverged taxa (Hafner and Page, 1995; Rannala and Michalakis, 2003; Nieberding and Olivieri, 2007). Accordingly, our discovery here of a pattern of cophylogeny is somewhat surprising and offers an interesting system with which to compare host and parasite molecular evolution.

Past studies of codiverged pocket gophers and their chewing lice have documented a 2- to 4-fold higher rate of molecular evolution in chewing lice compared with their pocket gopher hosts (Hafner et al., 1994; Page, 1996; Light and Hafner, 2007a). A similarly high relative rate of neutral character evolution is evident even in the closely related populations examined herein. Given the variety of pocket gopher and chewing louse lineages examined at this point, including the host genera *Orthogeomys* (Hafner et al., 1994), *Cratogeomys* (Light and Hafner, 2007a), and now *Pappogeomys*, this trend appears to be a robust one, albeit difficult to interpret given the many biological differences between these mammals and their insect parasites (Light and Hafner, 2007a).

ACKNOWLEDGMENTS

We thank our Mexican collaborator, Fernando A. Cervantes, and his students Rosa Castro, Rubén Rojas, Lázaro Guevara, and Jésica Arcangeli for their hospitality and helpful field assistance in Mexico. We thank Paul Tinerella of the University of Minnesota Insect Collection and Michael Engel of the Kansas University Entomology Collection for graciously supplying loans of *Geomydoecus alcorni* for our examination. We thank Frederico Silva and Samantha Dow for assistance in the laboratory; the University of Northern Iowa Student Opportunities for Academic Research fund and the Intercollegiate Academic Fund supported their portion of the research. This research was supported by National Science Foundation grants 0236957 (to D. J. Hafner) and 0343869 (to M. S. Hafner). We are grateful to 2 anonymous reviewers for their contributions to the clarity of this manuscript.

LITERATURE CITED

Brooks, D. R., and D. A. McLennan. 1991. Phylogeny, ecology, and behavior. University of Chicago Press, Chicago, Illinois, 434 p.

Casgrain, P., and P. Legendre. 2001. The R Package for multivariate and spatial analysis, version 4.0 d6—User's manual. Département de Sciences Biologiques, Université de Montréal, Montreal, Quebec, Canada, 41 p.

Charleston, M. A., and R. D. M. Page. 2002. Treemap (v 2.0). Applications for Apple Macintosh. University of Oxford, Oxford, U.K., http://taxonomy.zoology.gla.ac.uk/rod/treemap.html.

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.

Daly, J. C., and J. L. Patton. 1990. Dispersal, gene flow, and allelic diversity between local populations of *Thomomys bottae* pocket gophers in the coastal regions of California. Evolution **44:** 1283–1294.

Danforth, B. N., and S. Ji. 1998. Elongation factor-1 alpha occurs as two copies in bees: Implications for phylogenetic analysis of EF-1 alpha sequences in insects. Molecular Biology and Evolution **5:** 225–235.

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, and M. S. Hafner. 2003. Effects of spatial and temporal scale on cospeciation. *In* Tangled trees: Phylogeny, cospeciation, and coevolution, R. D. M. Page (ed.). The University of Chicago Press, Chicago, Illinois, p. 221–239.

Desai, M. S., J. F. H. Strassert, K. Meuser, H. Hertel, W. Ikeda-Ohtsubo, R. Radek, and A. Brune. 2010. Strict cospeciation of devescovinid flagellates and *Bacteroidales* ectosymbionts in the gut of dry-wood termites (Kalotermitidae). Environmental Microbiology **12:** 2120–2132.

Eichler, W. 1948. Some rules in ectoparasitism. Annals and Magazine of Natural History, Series 12 **1:** 588–598.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology **3:** 294–299.

Funaro, C. F., D. J. C. Kronauer, C. S. Moreau, B. Goldman-Huertas, N. E. Pierce, and J. A. Russell. 2011. Army ants harbor a host-specific clade of *Entomoplasmatales* bacteria. Applied and Environmental Microbiology **77:** 346–350.

Futuyma, D. J. 2005. Evolution. Sinauer Associates, Sunderland, Massachusetts, 603 p.

———, and M. Slatkin. 1983. Introduction. *In* Coevolution, D. J. Futuyma and M. Slatkin (eds.). Sinauer Associates, Inc., Sunderland, Massachusetts, p. 1–13.

Goldman, E. A. 1939. The pocket gophers of the genus *Pappogeomys*. Journal of Mammalogy **20:** 93–98.

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

———, D. J. Hafner, J. W. Demastes, T. A. Spradling, G. L. Hasty, and J. E. Light. 2009. Evolutionary relationships of pocket gophers of the genus *Pappogeomys* (Rodentia: Geomyidae). Journal of Mammalogy **90:** 47–56.

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

———, and ———. 1990. Cospeciation in host-parasite assemblages: Comparative analysis of rates of evolution and timing of cospeciation events. Systematic Zoology **39:** 192–204.

———, 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, B **349:** 77–83.

———, T. A. Spradling, J. E. Light, D. J. Hafner, and J. R. Demboski. 2004. Systematic revision of pocket gophers of the *Cratogeomys gymnurus* species group. Journal of Mammalogy **85:** 1170–1183.

———, 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.

Hellenthal, R. A., and R. D. Price. 1991. Biosystematics of the chewing lice of pocket gophers. Annual Review of Entomology **36:** 185–203.

Hubbel, T. H., and C. C. Goff. 1940. Florida pocket gopher burrows and their arthropod inhabitants. Proceedings of the Florida Academy of Science **4:** 127–166.

Huyse, T., R. Poulin, and A. Théron. 2005. Speciation in parasites: A population genetics approach. Trends in Parasitology **21:** 467–475.

Jackson, A. P., C. A. Machado, N. Robbins, and E. A. Herre. 2008. Multi-locus phylogenetic analysis of neotropical figs does not support co-speciation with the pollinators: The importance of systematic scale in fig/wasp cophylogenetic studies. Symbiosis **45:** 57–72.

Johnson, K. P., J. D. Weckstein, M. J. Meyer, and D. H. Clayton. 2011. There and back again: Switching between host orders by avian body lice (Ischnocera: Gnoiodidae). Biological Journal of the Linnean Society **102:** 614–625.

Jousselin, E., Y. Desdevises, and A. Couer d'acier. 2009. Fine-scale cospeciation between *Brachucaudus* and *Buchnera aphidicola*: Bacterial genome helps define species and evolutionary relationships in aphids. Proceedings of the Royal Society, B **276:** 187–196.

Kikuchi, Y., T. Hosokawa, N. Nikoh, X.-Y. Meng, Y. Kamagata, and T. Fukatsu. 2009. Host symbiont co-speciation and reductive genome evolution in gut symbiotic bacteria of acanthosomatid stink bugs. BMC Biology **7:** 1–22.

Kumar, S., K. Tamura, and M. Nei. 2004. Mega3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics **5:** 150–163.

LEGENDRE, P. 2001a. Model II regression—User's guide. Département de Sciences Biologiques, Université de Montréal, Montreal, Quebec, Canada, 23 p.

———. 2001b. Test of host–parasite coevolution: Program ParaFit user's guide, Département de Sciences Biologiques, Université de Montréal, Montreal, Quebec, Canada, 10 p.

LIGHT, J. E., AND M. S. HAFNER. 2007a. Cophylogeny and disparate rates of evolution in sympatric lineages of chewing lice on pocket gophers. Molecular Phylogenetics and Evolution **45:** 997–1013.

———, AND ———. 2007b. Phylogenetics and host associations of *Fahrenholzia* sucking lice (Phthiraptera: Anoplura). Systematic Entomology **32:** 359–370.

MADDISON, W. P., AND L. L. KNOWLES. 2006. Inferring phylogeny despite incomplete lineage sorting. Systematic Biology **55:** 21–30.

MARSHALL, A. G. 1981. The ecology of ectoparasitic insects. Academic Press, London, U.K., 459 p.

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

OCHMAN, H., AND A. C. WILSON. 1987. Evolution in bacteria-evidence for a universal substitution rate in cellular genomes. Journal of Molecular Evolution **26:** 74–86.

PAGE, R. D. M. 1996. Temporal congruence revisited: Comparison of mitochondrial DNA sequence divergence in cospeciating pocket gophers and their chewing lice. Systematic Biology **45:** 151–167.

———, AND M. S. HAFNER. 1996. Molecular phylogenies and host-parasite cospeciation: Gophers and lice as a model system. *In* New uses for new phylogenies, P. H. Harvey, A. J. L. Brown, J. Maynard Smith, and S. Nee (eds.). Oxford University Press, Oxford, U.K., p. 255–270.

———, AND E. C. HOLMES. 1998. Molecular evolution: A phylogenetic approach. Blackwell Scientific, Oxford, U.K., 352 p.

———, R. D. PRICE, AND R. A. HELLENTHAL. 1995. Phylogeny of *Geomydoecus* and *Thomomydoecus* pocket gopher lice (Phthiraptera: Trichodectidae) inferred from cladistic analysis of adult and first instar morphology. Systematic Entomology **20:** 129–143.

PATERSON, A. M., R. L. PALMA, AND R. D. GRAY. 2003. Drowning on arrival, missing the boat, and x-events: How likely are sorting events? *In* Tangled trees: Phylogeny, cospeciation, and coevolution, R. D. M. Page (ed.). The University of Chicago Press, Chicago, Illinois, p. 287–309.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics **14:** 817–818.

PRICE, R. D., AND K. C. EMERSON. 1971. A revision of the genus *Geomydoecus* (Mallophaga: Trichodectidae) of the New World pocket gophers (Rodentia: Geomyidae). Journal of Medical Entomology **8:** 228–257.

———, AND R. A. HELLENTHAL. 1989a. *Geomydoecus bulleri* complex (Mallophaga: Trichodectidae) from Buller's pocket gopher, *Pappogeomys bulleri* (Rodentia: Geomyidae), in west-central Mexico. Annals of the Entomological Society of America **82:** 279–285.

———, AND ———. 1989b. *Geomydoecus* (Mallophaga: Trichodectidae) from *Pappogeomys* and *Zygogeomys* pocket gophers (Rodentia: Geomyidae) in Central America. Journal of Medical Entomology **26:** 385–401.

RANNALA, B., AND Y. MICHALAKIS. 2003. Population genetics and cospeciation: From process to pattern. *In* Tangled trees: Phylogeny, cospeciation, and coevolution, R. D. M. Page (ed.). The University of Chicago Press, Chicago, Illinois, p. 120–143.

RIDLEY, M. 2004. Evolution, 3rd ed. Blackwell Publishing, Malden, Massachusetts, 751 p.

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

SHAFER, A. B. A., G. R. WILLIAMS, D. SHUTLER, R. E. L. ROGERS, AND D. T. STEWART. 2009. Cophylogeny of *Nosema* (Hymenoptera: Apidae) suggests both cospeciation and a host-switch. Journal of Parasitology **95:** 198–203.

SPRADLING, T. A. 1997. Relative rates of molecular evolution in rodents and their symbionts. Ph.D. Dissertation. Department of Biology, Louisiana State University, Baton Rouge, Louisiana, 174 p.

SWOFFORD, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, Massachusetts.

TISHECHKIN, A. K., AND A. R. CLINE. 2008. The beetle (Coleoptera) fauna of pocket gopher burrows in Louisiana. Proceedings of the Entomological Society of Washington **110:** 331–339.

APPENDIX I

Specimens examined: Host specimens of *P. bulleri* are housed in the Mammal Collection of Louisiana State University Museum of Natural Science (LSUMZ) or in the Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México (CNMA). All specimens were collected in Mexico. Locality numbers (in parentheses) correspond to those of Hafner et al. (2009). Chewing lice were not available from locality 8 (Chamela) of Hafner et al. (2009). Louse sample sizes (in brackets) indicate the number of individuals used in the 546-bp CO*I* analysis. **Colima:** (10) 1 km SE El Mixcoate, 530 m (CNMA 43269 [n = 4]); (11) 4 km S Armería, 10 m (CNMA 41924 [1], CNMA 41925 [1]); **Jalisco:** (3) Cerro Tequila, 7 mi S, 2 mi W Tequila, 2,900 m (LSUMZ 36082 [4]); (4) 1 km SW La Primavera, 1,585 m (LSUMZ 36565 [6]); (5) 9 mi. NW Mascota, 1,300 m (LSUMZ 36580 [1]); (6) 20 km S Ameca, 2223 m (LSUMZ 36581 [2]); (7) El Jazmín, 1,763 m (CNMA 43265 [1], CNMA 43266 [2], CNMA 43267 [1]); (9) 8.6 km (by road) SW La Huerta, 374 m (LSUMZ 36562 [6]); (12) 4 mi W (6.4km) Mazamitla (CNMA 44499 [4]); **Nayarit:** (1) La Libertad, 10 km NE Jalcocotán, 1,034 m (CNMA 43263 [5]); (2) 8 km W Ahuacatlán, 1,000 m (CNMA 41927–41933 [1 louse each]).