



Cophylogeny and disparate rates of evolution in sympatric lineages of chewing lice on pocket gophers

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Received 23 March 2007; revised 20 August 2007; accepted 4 September 2007

Available online 12 September 2007

Abstract

Although molecular-based phylogenetic studies of hosts and parasites are increasingly common in the literature, no study to date has examined two congeneric lineages of parasites that live in sympatry on the same lineage of hosts. This study examines phylogenetic relationships among chewing lice (Phthiraptera: Trichodectidae) of the *Geomydoecus coronadoi* and *Geomydoecus mexicanus* species complexes and compares these to phylogenetic patterns in their hosts (pocket gophers of the rodent family Geomyidae). Sympatry of congeneric lice provides a natural experiment to test the hypothesis that closely related lineages of parasites will respond similarly to the same host. Sequence data from the mitochondrial COI and the nuclear EF-1 α genes confirm that the two louse complexes are reciprocally monophyletic and that individual clades within each species complex parasitize a different species of pocket gopher. Phylogenetic comparisons reveal that both louse complexes show a significant pattern of cophylogeny with their hosts. Comparisons of rates of nucleotide substitution at 4-fold degenerate sites in the COI gene indicate that both groups of lice have significantly higher basal mutation rates than their hosts. The two groups of lice have similar basal rates of mutation, but lice of the *G. coronadoi* complex show significantly elevated rates of nucleotide substitution at all sites. These rate differences are hypothesized to result from population-level phenomena, such as effective population size, founder effects, and drift, that influence rates of nucleotide substitution.

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Keywords: Codivergence; Cospeciation; *Cratogeomys*; Evolutionary rates; *Geomydoecus*; Phylogeny; Pocket gophers

1. Introduction

Perhaps the most fascinating aspect of symbiosis is the simple realization that distantly related and biologically dissimilar organisms can be intimately associated across millions of years of evolutionary time. Many symbiotic associations observed today have persisted in the face of major geologic and climatic upheavals including major extinction events that affected organisms worldwide. Most biologists who study symbioses are intrigued by the many possible evolutionary consequences of such interactions, including adaptations in both partners in direct response to each other's presence (termed *coadaptations*, *reciprocal*

adaptations, or *coevolution*; sensu Janzen, 1980). Such adaptations can change a relationship that began as an opportunistic or facultative association into an obligate relationship for one or both partners.

Although the word “symbiosis” often is misused as a synonym for “mutualism,” symbiotic associations can take several forms, including parasitism, commensalism, and mutualism. Symbiotic associations in which the parasite, commensal, or mutualist (depending on the kind of symbiosis involved) has limited dispersal abilities and is passed primarily or exclusively from a host to its offspring presents an unusual opportunity to study concurrent genetic divergence events (*codivergence*) in the host and its evolutionary partner (the “associate”). Because the associate is effectively stranded on the host lineage, codivergence can eventually lead to parallel speciation (*cospeciation*) in the host and parasite lineages. Over millennia, these processes of codivergence and cospeciation result in parallel phyloge-

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nies for the hosts and their associates, a pattern termed *cophylogeny*.

Testing for cophylogeny is the first step toward understanding codivergence, cospeciation, coadaptation, and general ecological relationships between associated taxa. Whereas some studies have found statistically significant cophylogeny between hosts and their symbiotic associates (e.g., Hafner and Nadler, 1988, 1990; Hafner et al., 1994; Peek et al., 1998; Hugot, 1999; Clark et al., 2000; Paterson et al., 2000; Hugot, 2003; Kawakita et al., 2004), others have not (Barker, 1991; Ronquist and Liljeblad, 2001; Des-Devises et al., 2002; Johnson et al., 2002; Quek et al., 2004; Weckstein, 2004). Additionally, cophylogeny, even when significant, rarely is perfect among all taxa in a comparison because historical events, such as host switching, sorting events (extinction and lineage sorting), duplication events (speciation of the associate independent of the host), and failure of the associate to diverge when the host diverges (“missing the boat”; Paterson and Gray, 1997) may disrupt perfect correspondence among taxa. By comparing the phylogenies (or the data upon which those phylogenies are based) of hosts and their associates, it is possible to determine if statistically significant cophylogeny is present and discriminate among the various historical events that may disrupt perfect host–parasite correspondence.

Phylogenetic comparisons of hosts and their symbiotic associates offer the potential for studies that extend well beyond simple documentation of cophylogeny. If data gathered about the host and its associate (and trees resulting from those data) are statistically independent, show significant cophylogeny, and are based on homologous molecular markers, then timing of cladogenetic events and possible differences in rate of molecular evolution in the hosts and associates can be estimated (Ochman and Wilson, 1987; Hafner and Nadler, 1990; Hafner et al., 1994). Such studies have the potential to elucidate broad evolutionary processes that influence rates of molecular evolution across distantly related taxa.

One of the best known symbiotic systems is that involving pocket gophers (Rodentia: Geomyidae) and their ectoparasitic lice (Insecta: Phthiraptera). The gopher–lice assemblage is one of only a few mammal–parasite systems that is known to exhibit significant cophylogeny (Hafner et al., 2003). Chewing lice are wingless and obligate ectoparasites that die soon after removal from their host, resulting in limited dispersal capabilities (Kellogg, 1913; Marshall, 1981). Pocket gophers are asocial mammals that live in small, isolated populations and have low effective rates of dispersal (Daly and Patton, 1990; Hafner et al., 2003). Viewed together, the lifestyles of gophers and lice have resulted in a low degree of host switching, a high degree of host specificity, and a high level of codivergence and cospeciation between these hosts and their parasites (Hopkins, 1949, 1957; Price and Hellenthal, 1981; Timm, 1983; Hellenthal and Price, 1984). It is not surprising, therefore, that most investigations of cophylogeny between various taxa of pocket gophers and their chewing lice have

found significant cophylogeny (Timm, 1983; Lyal, 1986, 1987; Hafner and Nadler, 1988, 1990; Demastes and Hafner, 1993; Hafner et al., 1994; Page et al., 1995).

Recent investigations of the Mexican pocket gopher, *Cratogeomys merriami*, revealed chromosomal, molecular, and morphological variation that corresponds to three major genetic clades within the taxon (Hafner et al., 2005). These clades correspond to three distinct species, *C. perotensis*, *C. merriami*, and *C. fulvescens* that comprise the *C. merriami* species group of the Trans-Mexican Volcanic Belt and Oriental Basin (Fig. 1). The *C. merriami* species group is parasitized by two louse species complexes in the genus *Geomydoecus*, the *G. coronadoi* complex (consisting of two louse species, *G. coronadoi* and *G. veracruzensis*) and the *G. mexicanus* complex (consisting of four louse species, *G. mexicanus*, *G. fulvescens*, *G. perotensis*, and *G. traubi*; Price and Hellenthal, 1989). These two louse complexes are morphologically distinct, and representatives of both the *G. coronadoi* and *G. mexicanus* species complexes are almost always found together on the same host individual (Fig. 1; Price and Hellenthal, 1989). Although each of the louse species is morphologically distinct, relationships among these louse species currently are unknown.

This study investigates relationships among the chewing lice that parasitize pocket gophers of the *Cratogeomys merriami* species group and incorporates tree-based, distance-based, and data-based methods of cophylogenetic analysis to investigate the historical association between these hosts and parasites. This study is the first to involve exhaustive sampling of all taxa within a monophyletic lineage of gophers and all taxa of lice parasitizing those gophers. As a result, this analysis is unlikely to suffer from phylogenetic sampling error, the consequences of which can be severe (Hafner and Page, 1995; Page, 1996). This also is the first study to explore from a phylogenetic perspective two congeneric lineages of chewing lice that parasitize a single lineage of pocket gophers. Thus, one louse lineage is a replicate of the other, providing the first opportunity to investigate potential differences in the way louse lineages interact with a common host lineage as well as potential differences in rates of evolution in sympatric lineages of lice.

2. Materials and methods

2.1. Sampling and molecular methods

Forty-one specimens of chewing lice (representing seven species from 16 localities) were collected directly from the pocket gopher specimens used in the study by Hafner et al. (2005; Fig. 1 and Table 1). Lice were brushed from the pelage of the pocket gophers immediately after euthanization of the host and stored at -70°C . Because male lice are more easily identified than female lice, only males were used in this study, the only exception being one female *Geomydoecus coronadoi* (from host LSUMZ 34344) used in the molecular analysis.

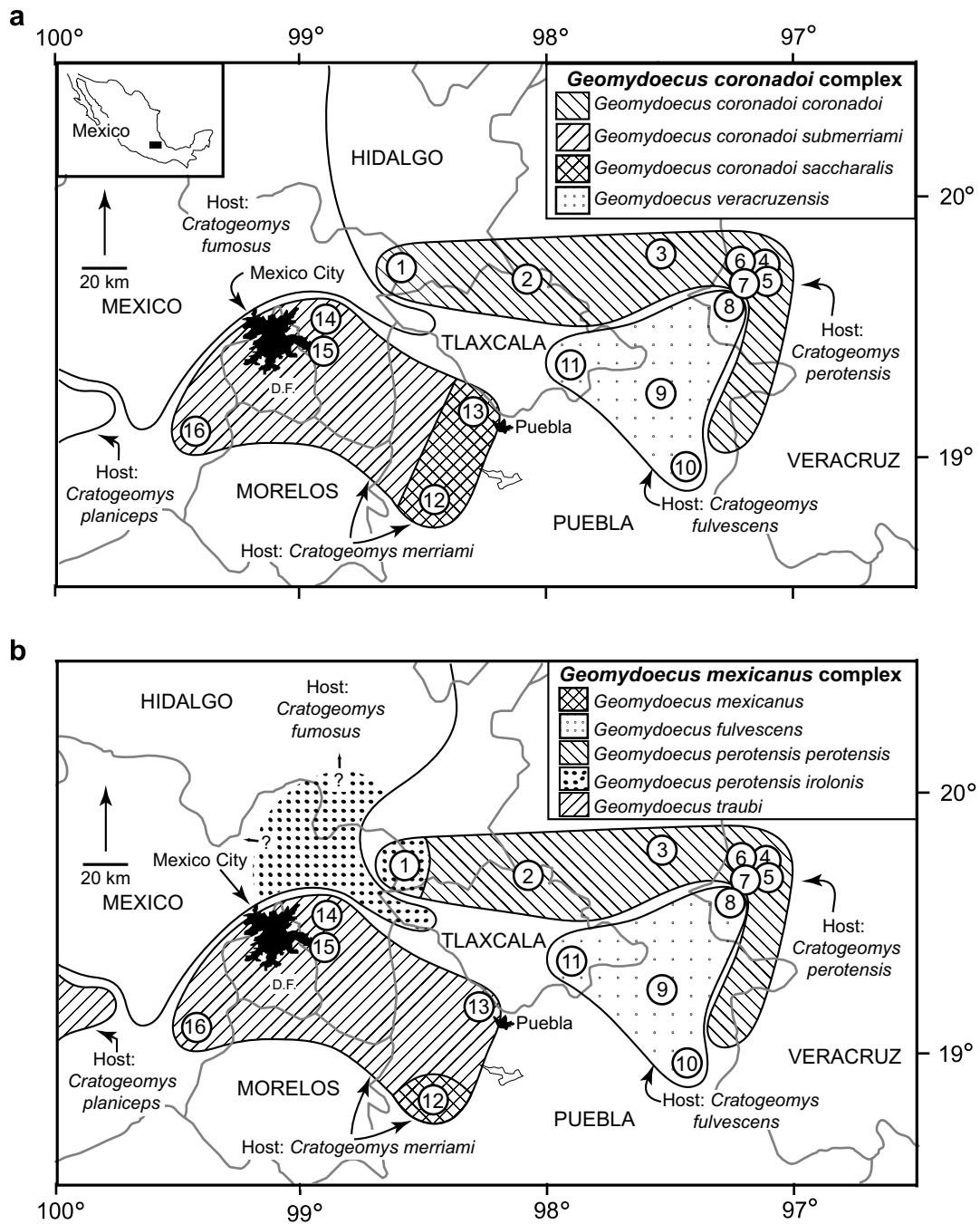


Fig. 1. Distribution of chewing lice (*Geomydoecus* sp.) in central Mexico. Louse geographic distributions are overlain on the geographic distribution of their hosts. Numbers refer to collecting localities listed in Table 1. (a) Geographic distribution of lice of the *Geomydoecus coronadoi* complex. (b) Geographic distribution of lice of the *Geomydoecus mexicanus* complex.

Before DNA was extracted, each louse was tentatively identified with the aid of a dissecting microscope and taxonomic keys from Price and Emerson (1971) and Price and Hellenthal (1989). Following DNA extraction, lice were mounted on slides using the method of Johnson and Clayton (2003), re-identified with the aid of a compound microscope, and retained as vouchers. Voucher specimens are currently housed at the Museum of Natural Science, Louisiana State University and will be deposited to the Price Institute of Phthiraptera (University of Utah).

Genomic DNA was isolated from each louse using the DNeasy Tissue Kit (Qiagen Inc., Valencia, California) according to louse-specific protocols (Cruikshank et al., 2001; Johnson and Clayton, 2003). The mitochondrial COI gene was amplified and sequenced in all louse specimens listed in Table 1 plus the outgroup taxon (two specimens of *Geomydoecus wernecki*, a parasite of *Cratogeomys fumosus*). PCR amplification and sequencing of a portion of the COI gene (1017 bp) was performed as in Light and Hafner (2007). A nuclear gene, Elongation Factor 1 Alpha

Table 1
Host (*Cratogeomys*) and parasite (*Geomydoecus*) taxa included in the phylogenetic and cophylogenetic analyses

Locality number, host species, and specimen number	<i>G. coronadoi</i> species complex	<i>G. mexicanus</i> species complex	Collection locality
1. <i>C. perotensis</i> LSUMZ 34344	<i>G. coronadoi</i>	<i>G. perotensis</i>	Hidalgo; Irolo, 2500 m
1. <i>C. perotensis</i> CNMA 41904	<i>G. coronadoi</i>	<i>G. perotensis</i>	Hidalgo; Irolo, 2500 m
2. <i>C. perotensis</i> CNMA 41905	<i>G. coronadoi</i>	<i>G. perotensis</i>	Tlaxcala; 8 km N Tlaxco, 2887 m
3. <i>C. perotensis</i> CNMA 41906	<i>G. coronadoi</i>	<i>G. perotensis</i>	Puebla; 2.5 km N Zaragoza, 2250 m
4. <i>C. perotensis</i> LSUMZ 36070	No louse collected	<i>G. perotensis</i>	Veracruz, Las Vigas, 7900 ft
5. <i>C. perotensis</i> LSUMZ 34903	<i>G. coronadoi</i>	<i>G. perotensis</i>	Veracruz; 2 km S Las Vigas, 2568 ft
6. <i>C. perotensis</i> CNMA 41909	No louse collected	<i>G. perotensis</i>	Veracruz; Cruz Blanca, 2450 m
7. <i>C. perotensis</i> CNMA 41910	<i>G. coronadoi</i>	<i>G. perotensis</i>	Veracruz; 9 km NE Perote, 2440 m
7. <i>C. perotensis</i> CNMA 41911	<i>G. coronadoi</i>	<i>G. perotensis</i>	Veracruz; 9 km NE Perote, 2440 m
8. <i>C. fulvescens</i> LSUMZ 36069	<i>G. veracruzensis</i>	<i>G. fulvescens</i>	Veracruz; 2 km NE Perote, 7900 ft
8. <i>C. fulvescens</i> CNMA 41824	<i>G. veracruzensis</i>	<i>G. fulvescens</i>	Veracruz; 2 km NE Perote, 7900 ft
8. <i>C. fulvescens</i> CNMA 41825	<i>G. veracruzensis</i>	<i>G. fulvescens</i>	Veracruz; 2 km NE Perote, 7900 ft
9. <i>C. fulvescens</i> CNMA 41907	No louse collected	<i>G. fulvescens</i>	Puebla; 1 km NW Zacatepec, 2380 m
10. <i>C. fulvescens</i> CNMA 41823	<i>G. veracruzensis</i>	<i>G. fulvescens</i>	Puebla; 1 km SE Ciudad Serdán, 2700 m
11. <i>C. fulvescens</i> CNMA 41908	<i>G. veracruzensis</i>	<i>G. fulvescens</i>	Tlaxcala; Huamantla, 2380 m
12. <i>C. merriami</i> LSUMZ 36067	<i>G. coronadoi</i>	<i>G. mexicanus</i>	Puebla, 1 km S Atlixco, 6300 ft
12. <i>C. merriami</i> LSUMZ 36068	<i>G. coronadoi</i>	<i>G. mexicanus</i>	Puebla, 1 km S Atlixco, 6300 ft
12. <i>C. merriami</i> LSUMZ 36293	<i>G. coronadoi</i>	<i>G. mexicanus</i>	Puebla, 1 km S Atlixco, 6300 ft
13. <i>C. merriami</i> CNMA 41826	<i>G. coronadoi</i>	<i>G. traubi</i>	Puebla; 1 km SE San Miguel Xoxtla, 2430 m
14. <i>C. merriami</i> LSUMZ 36065	<i>G. coronadoi</i>	<i>G. traubi</i>	México; 5 km SSW Texcoco, 7000 ft
15. <i>C. merriami</i> CNMA 41819	<i>G. coronadoi</i>	<i>G. traubi</i>	México; 15 km SSW Texcoco, 2253 m
16. <i>C. merriami</i> LSUMZ 36125	<i>G. coronadoi</i>	<i>G. traubi</i>	México; 2 km SE Coatepec, 8600 ft

Pocket gophers and chewing lice are grouped by locality (all localities are in Mexico; Fig. 1). All lice used in this study were collected directly from the host individuals used in Hafner et al. (2005). Museum acronyms are as follows: Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México (CNMA) and Louisiana State University Museum of Natural Science (LSUMZ).

(EF-1 α), also was sequenced to provide an additional hypothesis of louse relationships based on a molecular marker independent of the mitochondrial genome. EF-1 α was examined in 17 specimens representing all six species and the outgroup taxon *G. craigi* (hosted by *Thomomys talpoides*), whose sequence was downloaded from GenBank (Accession No. AF 545784). PCR amplification and sequencing of a portion of the EF-1 α gene (347 bp) was performed as in Light and Hafner (2007).

Prior to sequencing, amplified products were purified using either the QIAquick PCR Purification Kit or the QIAquick Gel Extraction Kit (Qiagen, Inc.). Amplified products were sequenced in both directions at the Museum of Natural Science, Louisiana State University. Each 10 μ l reaction included 1.6 μ l of BigDye™ (Applied Biosystems, Perkin-Elmer Corporation), 0.32 μ l of 10 μ M primer, 2.0 μ l of 5 \times ABI extension buffer, 4.08 μ l of ddH₂O, and 2 μ l of amplification product. Samples were sequenced for 24 cycles at 96 °C (20 s; 1 cycle) then 96 °C (12 s; 23 cycles), 50 °C (15 s), and 60 °C (4 min). These sequences were then purified with Centri-Sep spin columns (Princeton Separations) and were electrophoresed using an ABI Prism 377 Genetic Analyzer (Perkin-Elmer, Foster City, CA). Sequences were edited using Sequencher Version 4.1 (Gene Codes Corporation, Ann Arbor, Michigan), and aligned using Se-AL v2.0a11 (Rambaut, 1996). Primer sequences were removed and sequences were trimmed in reference to the translated protein sequence using Se-AL v2.01a11 (Rambaut, 1996) and MacClade 4.0 (Maddison and Madd-

ison, 2000). All sequences were submitted to GenBank (GenBank Accession Nos.: DQ200297–DQ200339 for COI and DQ200340–DQ200355 for EF-1 α).

2.2. Phylogenetic analysis

Many insects are known to show A–T bias in mitochondrial genes, especially at third positions (Jermiin and Crozier, 1994; Schwarz et al., 2004), and phylogenetic inference can be affected by base composition heterogeneity (Lockhart et al., 1994; Galtier and Gouy, 1998; Jansa and Weksler, 2004; Schwarz et al., 2004). Therefore, base composition bias was evaluated for the mitochondrial COI gene and COI codon positions across all taxa (Jansa and Weksler, 2004; Jansa et al., 2006). Departure from average base composition was determined for each taxon using a Chi-square (χ^2) test implemented in PAUP*b4.10 (Swofford, 2003). The incongruence length difference test (Farris et al., 1994) using a heuristic search with 100 random addition replicates (implemented as the partition homogeneity test in PAUP*4.0b10; Swofford, 2003) was used to determine if significant conflict exists among codon positions.

Phylogenetic analyses of the louse COI and EF-1 α datasets used maximum parsimony (MP), maximum likelihood (ML), and Bayesian approaches. Equally weighted maximum parsimony searches were performed with 100 random addition replicates and tree bisection-reconnection (TBR) branch swapping using PAUP*4.0b10 (Swofford, 2003).

To assess nodal support, nonparametric bootstrap analyses were performed (1000 pseudoreplicates and 10 random sequence additions; Felsenstein, 1985). Because EF-1 α was sampled for only a subset of taxa (see above), combined analyses using both COI and EF-1 α were not performed. All executable data files for the COI and EF-1 α genes are available at TreeBASE (<http://www.treebase.org>; SN number S1887).

To generate the best COI and EF-1 α ML trees, Modeltest (version 3.6; Posada and Crandall, 1998) was used to examine the fit of 56 models of nucleotide substitution to the sequence data. Models of evolution providing the best approximation of the data using the fewest parameters were chosen for subsequent analyses according to hierarchical likelihood ratio tests (hLRTs) and the Akaike Information Criterion (AIC; Huelsenbeck and Rannala, 1997; Posada and Buckley, 2004). The general time reversible (GTR) model, including among-site rate variation and invariable sites (GTR + I + Γ ; Gu et al., 1995; Yang, 1994), was chosen as the best model of evolution according to hLRTs of the louse COI dataset. Similarly, the K81uf model, again including among-site rate variation and invariable sites (K81uf + I + Γ), was chosen according to AIC for the COI dataset. The TrNef and the TrN + I models were chosen by hLRT and AIC, respectively, for the EF-1 α dataset. A full heuristic ML search was conducted using the successive-approximations approach with the preferred model in PAUP*4.0b10 (Swofford, 2003). A full heuristic bootstrap (200 pseudoreplicates) also was performed using the preferred model on a Beowulf cluster with eight alpha-processor nodes. Only the results of the hLRTs are presented here because both approaches selected similar models and phylogenetic analysis using these models of evolution yielded the same topology.

Partitioned and non-partitioned Bayesian phylogenetic analyses were performed using MrBayes 3.12 (Huelsenbeck and Ronquist, 2001). Partitioned analyses were performed on the codon positions of the COI dataset in an effort to avoid biased posterior probability estimates, parameter mismodeling, and potential systematic error (Brandley et al., 2005; Castoe et al., 2004; Castoe and Parkinson, 2006; Mueller et al., 2004). The GTR + I + Γ and the GTR models were used in COI and EF-1 α analyses, respectively, and model parameters were treated as unknown variables with uniform priors and were estimated as part of the analysis. Bayesian analyses were initiated with random starting trees, run for 10 million generations with four incrementally heated chains (Metropolis-coupled Markov chain Monte Carlo; Huelsenbeck and Ronquist, 2001), and sampled at intervals of 1000 generations. Two independent Bayesian analyses were run to avoid entrapment on local optima, and log-likelihood scores were compared for convergence (Huelsenbeck and Bollback, 2001; Leaché and Reeder, 2002). Stationarity was assessed by plotting the log likelihood scores of sample points against generation time and all burn-in points (the first 2500 trees) were discarded. The retained equilibrium samples were used to

generate a 50% majority rule consensus tree with the percentage of samples recovering any particular clade representing that clade's posterior probability (Huelsenbeck and Ronquist, 2001).

Alternative phylogenetic hypotheses were compared statistically using the Kishino–Hasegawa (KH) and the Shimodaira–Hasegawa tests (SH) as implemented in PAUP*4.0b10 (MP and ML analyses using resampling estimated log-likelihood [RELL] optimization and 1000 bootstrap replicates; Kishino and Hasegawa, 1989; Shimodaira and Hasegawa, 1999; Goldman et al., 2000).

2.3. Tests of cophylogeny

The host and parasite phylogenies used in the cophylogeny tests in this study were pruned so that only one representative from each locality was analyzed for phylogenetic congruence. Localities 4 and 5 (Table 1 and Fig. 1) were treated as one locality because of their close geographic proximity and the high genetic similarity between hosts (0.186% uncorrected p distance) and between parasites ($p = 0.098\%$). Because *G. coronadoi* specimens were not recovered from host specimens LSUMZ 36070 (*C. perotensis*), CNMA 41909 (*C. perotensis*), and CNMA 41907 (*C. fulvescens*), only 13 host–parasite comparisons were available for the *G. coronadoi* complex versus 15 comparisons for the *G. mexicanus* complex (Table 1).

The host phylogeny (obtained from Hafner et al., 2005) was not fully resolved for the *C. fulvescens* clade (Fig. 2). Because some cophylogenetic methods cannot handle polytomies, the three host and parasite taxa involved in this polytomy were treated individually in three separate analyses, each of which involved trees that were fully resolved. As a result, there were two cophylogeny analyses between lice belonging to the *G. coronadoi* complex and their hosts, and three cophylogeny analyses between lice belonging to the *G. mexicanus* complex and their hosts.

Tree-based methods, such as reconciliation analysis, compare only the branching structure of host and parasite trees to determine if more codivergence events are present than would be expected by chance. Reconciliation analysis was performed using TreeMap 2.0 β (Charleston and Page, 2002) to find the least costly reconstruction of host–parasite relationships while maximizing the number of codivergence events. The default settings of TreeMap 2.0 β were used (assigning a cost of zero for codivergence events, and a cost of one for host switches, losses, and duplications). To determine whether the number of codivergence events recovered from the reconciliation analysis was significant, the parasite tree was randomized 10,000 times and the observed number of codivergence events was compared to the null distribution of codivergence events derived from this randomization procedure.

Distance-based methods determine if the hosts and their parasites are associated randomly by comparing genetic distances from homologous gene regions for the associated taxa. The distance-based method ParaFit (Legendre,

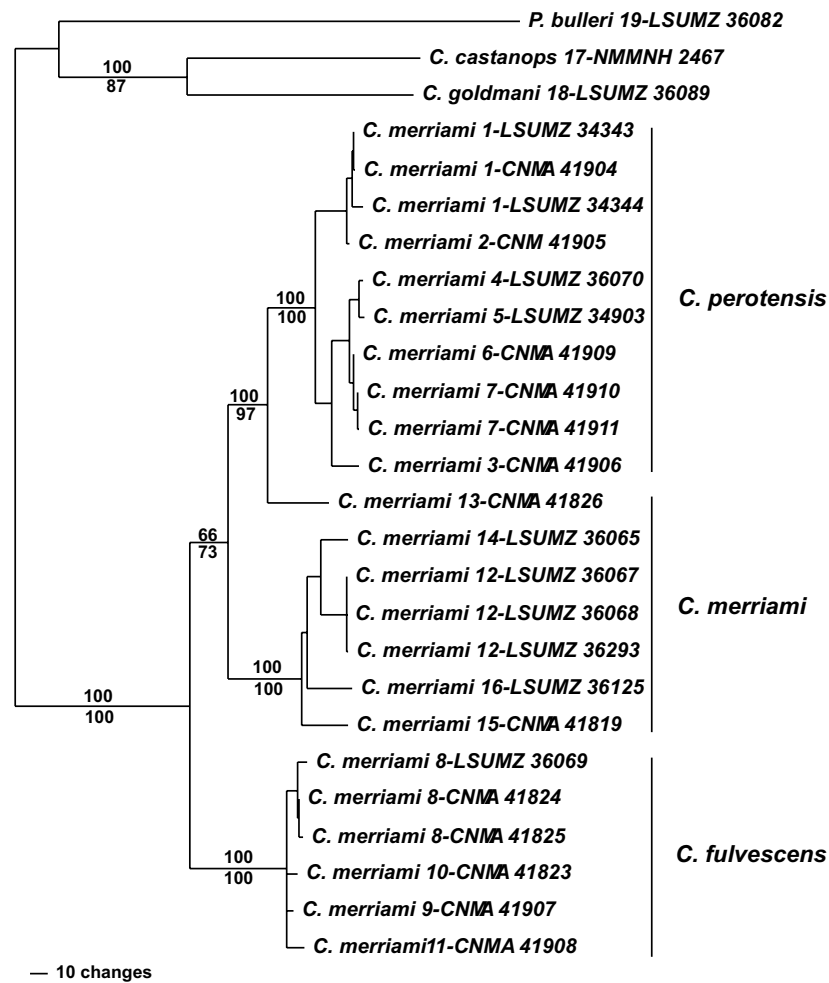


Fig. 2. Parsimony phylogram of the *Cratogeomys merriami* species group redrawn from Hafner et al. (2005). Species names are followed by locality numbers (Fig. 1 and Table 1) and host specimen numbers (Table 1). Phylogenetic analysis was based on two mtDNA genes, cytochrome *b* and cytochrome *c* oxidase subunit I, and the topology of the tree is corroborated by chromosomal and morphological evidence from the nuclear genome. Maximum likelihood and maximum parsimony bootstrap values are indicated above and below the nodes, respectively.

2001a; Legendre et al., 2002) was used to assess the null hypothesis of random association between hosts and parasites. ParaFit is a permutation procedure that uses distance matrices, rather than tree topologies, to test for congruence between host and parasite phylogenies (Legendre et al., 1997). Although it does not estimate numbers of historical events, this program has an advantage over tree-based methods because it can accommodate uncertainty in tree topologies, multiple parasites per host lineage, and multiple hosts per parasite lineage. Distance matrices for pocket gophers and chewing lice were derived from ML estimates of pairwise genetic distances using model parameters derived from both hLRTs and the AIC as selected by Modeltest. Genetic distances were averaged for multiple specimens collected from the same locality. Distance matrices were converted to principal coordinate matrices using the programs DistPCoA (Legendre and Anderson, 1998) and the R Package (Casgrain and Legendre, 2001). Tests of random association were performed with 999 permutations globally across both matrices and for each individual host–parasite association.

If tree-based and distance-based methods show statistically significant cophylogeny between associated taxa, then data-based methods can be used to determine the cause of any topological incongruence between host and parasite trees. Data-based methods test the hypothesis that host and parasite datasets are consistent with an identical topology, thereby determining whether sampling error (rather than biological processes such as host switching or extinction) can account for observed differences between topologies (Clark et al., 2000; Page, 2003; Jackson, 2004a, 2004b; Kawakita et al., 2004). If the null hypothesis is not rejected, topological incongruence is assumed to be the result of sampling error (e.g., inadequate taxon sampling or too few informative sites). If the null hypothesis is rejected, it is assumed that historical events such as host switching or parasite extinction caused observed topological incongruence. Common data-based methods include the KH and SH tests (Kishino and Hasegawa, 1989; Shimodaira and Hasegawa, 1999; Goldman et al., 2000), likelihood ratio tests (LRTs; Huelsenbeck et al., 1997, 2000), and incon-

gruence length difference tests (ILD tests; Johnson et al., 2001).

KH and SH tests were used to compare trees estimated from alternative datasets in both a parsimony and likelihood framework (Peek et al., 1998; Clark et al., 2000). The likelihood and parsimony scores obtained for the best parasite tree given the parasite data were compared to the score of the alternative host tree, also given the parasite data. Under likelihood criteria, the likelihood parameters of this alternative host tree were optimized for the parasite data to maximize the likelihood score (Clark et al., 2000). Similarly, the best host tree was compared to the score of the alternative parasite tree given the host data. The differences between tree scores were determined using RELL optimization and 1000 nonparametric bootstrap replicates as implemented in PAUP*b4.10 (Swofford, 2003; Degnan et al., 2004).

The LRT was used to test whether sampling error could explain dataset heterogeneity. The likelihoods of the parasite and host datasets across alternative topologies (the parasite and host topologies) were obtained in the same manner as described above for the KH and SH tests. The difference between the parasite and host trees was determined using the likelihood-ratio test statistic, and the significance of this likelihood-ratio test statistic was calculated using parametric bootstrapping. The test statistic was then compared to a distribution of likelihood scores generated under the null hypothesis of identical topologies given the host and parasite datasets (Huelsenbeck et al., 1997). The null distribution of likelihood scores was constructed by optimizing likelihood parameters for each dataset given the constrained tree. The program SeqGen 1.3.2 (Rambaut and Grassly, 1997) using the graphical interface SG Runner 2.0 (T.P. Wilcox; <http://homepage.mac.com/tpwilcox/SGRUNNER/FileSharing8.html>) was used to generate 100 datasets (Monte Carlo simulation) using the optimized parameters and the constrained topology. The likelihood ratio test statistic for the constrained and best topologies for each of these simulated datasets was calculated, and a null distribution of test statistics was constructed. The test statistic derived from the empirical data was then compared to the null distribution to determine if phylogenetic conflict existed between datasets.

The ILD test (Johnson et al., 2001) was used to identify taxa causing incongruence between the host and parasite datasets. Invariable sites were removed in all ILD tests (Cunningham, 1997). Host and parasite datasets were treated as partitions and the ILD test was performed using 1000 replicates. A *p* value of 0.10 was used for significance testing to adjust for Type I error (Johnson et al., 2001), and a *p* value > 0.1 was interpreted as no significant incongruence between data partitions. In the case of rejection of the null hypothesis of strict codivergence in favor of incongruence, taxa were sequentially deleted to identify the source (or sources) of incongruence. First, single taxa (one host–parasite pair) were removed from the dataset and the ILD test was repeated. This procedure was repeated for

all single host–parasite associations, all possible couplets of hosts and their parasites, all possible triplets, etc., until there was no significant difference between data partitions. This process identified the taxa responsible for the incongruence between datasets. The ILD method (Johnson et al., 2001) can be continued by separating the incongruent taxa from the rest of the dataset and using reconciliation analyses to explore potential explanations for the incongruence. Because several other methods were used in this study to compare host and parasite topologies and datasets, the ILD method was stopped once the taxa responsible for dataset incongruence were identified.

2.4. Comparison of molecular rates

Comparison of molecular rates in hosts and parasites requires use of data from the same gene in both groups. These data provide identical characters to construct host and parasite phylogenies and a common scale to compare amounts of divergence (Lewontin, 1984; Page, 1993b; Hafner et al., 1994; Page, 1996; Page et al., 1998). The louse phylogeny generated in this study is based on 1017 bp of the COI gene, whereas the gopher phylogeny (from Hafner et al., 2005) was based on the entire COI gene (1551 bp). Accordingly, only the homologous 1017 bp of the gopher COI gene were used in the rate comparisons with lice, and these COI data were constrained to fit the best gopher tree based on the complete mtDNA dataset (Hafner et al., 2005).

Evolutionary rates were compared between both the two louse complexes and between gophers and their lice. Relative rates of nucleotide substitution (i.e., potential differences in branch lengths) in the two louse complexes were assessed by comparing mean patristic COI distances between all louse taxa and the outgroup using the nonparametric Kruskal–Wallis and Mann–Whitney *U* tests. Patristic distances were calculated using patristic distance data derived from ML phylogeny estimates using nucleotide substitution models selected using both hLRT and AIC criteria (GTR + I + Γ and K81uf + I + Γ , respectively).

Comparison of evolutionary rates in gophers and lice were performed using only 4-fold degenerate sites in the COI gene (Hafner et al., 1994). These sites provide the best estimate of basal mutation rates because of their presumed selective neutrality. The program MEGA3 (Kumar et al., 2004) was used to identify 4-fold degenerate sites in the pocket gopher and chewing louse datasets (using the vertebrate and invertebrate mtDNA codes for the gophers and the lice, respectively). These data were then used in phylogenetic reconstructions constrained to match the best pocket gopher and chewing louse topologies based on all data (treating the two louse complexes independently), and the trees were tested for significant departure from clock-like behavior. In these tests, ML branch lengths were calculated for gopher and louse phylogenies, both with and without imposing a molecular clock constraint, and the resulting trees were compared by likelihood ratio tests. A

significant difference between the constrained and unconstrained trees results in rejection of the molecular clock.

Given significant cophylogeny and clock-like rate variation, sequence data were used to explore relative timing of divergence or speciation events and rate of molecular evolution in gophers and lice by direct comparison of the lengths of analogous branches in the host and parasite trees. Copath analysis (Page, 1996) was used to identify putative analogous branches (“copaths”) in the host and parasite trees according to the following rules. First, those taxa identified by the ILD test (Johnson et al., 2001) as causes of incongruence between the host and parasite datasets were removed from the analysis. Second, because much of the uncertainty in phylogenetic analyses involves deep branches, only terminal and subterminal branches were compared in the host and parasite trees. Lastly, to increase the statistical power of the tests, molecular rate comparisons were made only for copath solutions that contained six or more pairs of potentially cospeciating taxa. Outgroup taxa were not included in these comparisons.

Before host and parasite branches were compared, Mantel tests were used to compare distance matrices (uncorrected p distances) for all possible combinations of potentially cospeciating host and parasite taxa identified in the copath analysis to test for significant association independent of phylogeny (Hafner and Nadler, 1988, 1990; Page, 1991). Branch lengths for all host–parasite combinations that showed a significant association (Mantel tests $p < 0.05$) were estimated in an ML framework. Branch lengths were averaged for those localities from which multiple hosts and their lice were collected. Estimated branch lengths were compared for all combinations of cospeciating hosts and parasites using Model II regression analysis (Legendre, 2001b) to determine if one associate evolved faster or slower than the other (as indicated by the slope of the relationship) and whether the parasite diverged before, coincident with, or after its host (as indicated by the y -intercept of the relationship; Hafner and Nadler, 1990).

3. Results

3.1. Phylogenetic analysis

No louse taxon differed significantly in base composition from the expected value for either the first, second, or third codon positions of the mitochondrial COI gene ($\chi^2 < 17.18$, $df = 3$; $p > 0.05$). Third positions showed a small amount of A–T bias, but the bias was not significantly more than that observed at first and second positions. Third codon positions also were saturated (data not shown), but phylogenetic analyses removing third positions had no effect on basal relationships among the lice in either species complex (data not shown). The ILD test revealed no conflict between COI codon positions ($p = 1.00$) so all positions were combined in subsequent phylogenetic analyses.

Of the 347 bp of EF-1 α examined, six were potentially parsimony informative. Parsimony analysis of the EF-1 α gene resulted in one most parsimonious tree (not shown; length 14, CI = 1.00, RI = 1.00, RC = 1.00) in which only the two louse species complexes (*Geomydoecus coronadoi* and *G. mexicanus*) were resolved (bootstrap support = 99 for the *G. coronadoi* complex and bootstrap = 75 for the *G. mexicanus* complex). ML and Bayesian analyses resulted in the same topology with comparable support values. The EF-1 α data provided no resolution within either of the two species complexes (data available upon request and from TreeBase).

Of the 1017 bp of the COI gene examined in the lice, 394 bp were potentially parsimony informative. Parsimony analysis of the COI gene produced 160 equally parsimonious trees (Fig. 3; length = 490, CI = 0.641, RI = 0.939, RC = 0.602). Monophyly of the two species complexes was supported with high bootstrap support (≥ 90 ; Fig. 3), and within each louse complex, three monophyletic clades corresponding to the three host clades (*Cratogeomys perotensis*, *C. merriami*, and *C. fulvescens*) were identified. In the *G. coronadoi* complex, lice parasitizing *C. fulvescens* and *C. merriami* were each monophyletic (bootstrap support = 100 and 98, respectively; Fig. 3), whereas lice parasitizing *C. perotensis* were not completely resolved. Two of the three louse clades in the *G. mexicanus* complex received high bootstrap support (≥ 90), whereas support for the third clade (containing the species *G. mexicanus*, *G. traubi*, and one of seven populations of *G. perotensis*) was low (bootstrap support = 66).

ML and Bayesian analyses yielded identical trees (Fig. 4) that were only slightly different from the topology generated by parsimony analysis (Fig. 3). These topological differences were not significant by SH tests (MP versus Bayesian topology $p = 0.394$; MP versus ML topology $p = 0.430$; and ML versus Bayesian topology $p = 0.282$).

3.2. Tests of cophylogeny

Reconciliation analyses using TreeMap 2.0 β detected significant cophylogeny between both the *Geomydoecus coronadoi* and *G. mexicanus* species complexes and their pocket gopher hosts (Fig. 5). Reconciliation analysis of the *G. coronadoi* complex found two equally probable reconstructions with 16 codivergence events, six duplications, three extinctions, three host switches, and a net cost of 12. The 16 reconstructed codivergence events were greater than expected by chance ($p < 0.001$). Reconciliation analysis of the *G. mexicanus* complex found six equally probable reconstructions with 16 codivergence events, eight duplications, four extinctions, four host switches, and a net cost of 16. The 16 reconstructed codivergence events were greater than expected by chance ($p < 0.002$).

Global tests of cophylogeny using ParaFit resulted in rejection of random association between host and parasite taxa ($p = 0.03$) when both species complexes were included in the analysis. Random association between hosts and

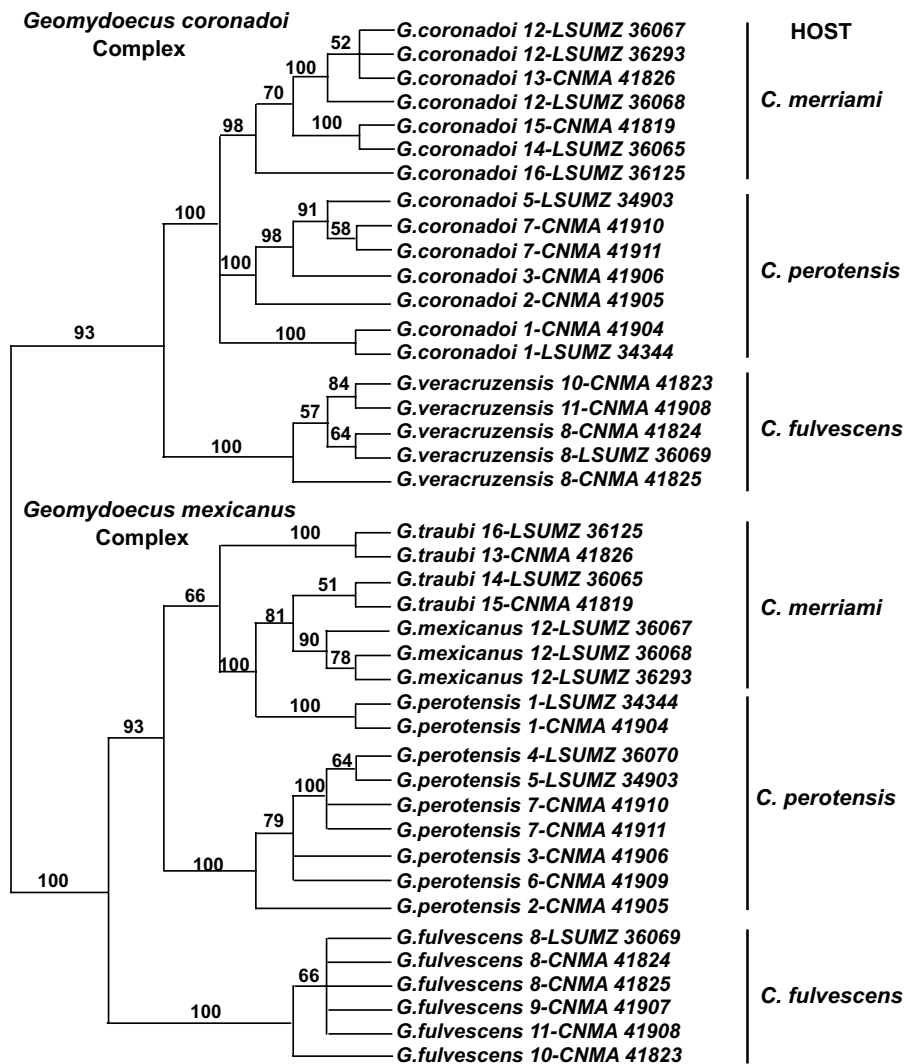


Fig. 3. Cladogram resulting from parsimony analysis of the COI gene for the *Geomydoecus coronadoi* (top) and *G. mexicanus* (bottom) species complexes. Bootstrap support values are indicated above the nodes. Species names are followed by locality numbers (Fig. 1 and Table 1) and host specimen numbers (Table 1). Host associations are listed to the right of the cladogram.

parasites also was rejected in tests of only the *G. coronadoi* complex and their hosts ($p = 0.001$) and tests of only the *G. mexicanus* complex and their hosts ($p = 0.002$). All individual associations between lice in the *G. coronadoi* species complex and their hosts were significant ($p < 0.05$), except for the taxa parasitizing *C. merriami* (Fig. 1, localities 12–16). All individual associations between lice in the *G. mexicanus* species complex and their hosts were significant ($p < 0.05$), except for the louse parasitizing *C. perotensis* from locality three and the lice parasitizing *C. merriami* from localities 12–15 (Fig. 1).

Data-based methods were performed to determine the causes of topological incongruence between pocket gopher and louse trees. KH, SH, and LRT analyses all revealed significant differences between the host and parasite datasets, thereby rejecting the null hypothesis that observed topological differences were caused by sampling error. Thus, differences between host and parasite phylogenies

were the result of other historical events, such as host switching, parasite extinction, or parasite speciation (see below). Results of these tests did not vary with outgroup choice or when analyses were performed using ML parameters determined by the hLRT or the AIC. ILD tests showed a significant difference between host and parasite data partitions, and it was necessary to remove localities 1, 13, and 15 (Table 1 and Fig. 5) to render the difference between data partitions nonsignificant in the *G. coronadoi* analysis. Similarly, it was necessary to remove localities 1, 6–9, 11, and 16 (Table 1 and Fig. 5) to eliminate the significant difference between data partitions in the *G. mexicanus* analysis.

3.3. Comparison of molecular rates

There was significant heterogeneity in mean ML patristic distance between the two louse complexes (Mann–Whit-

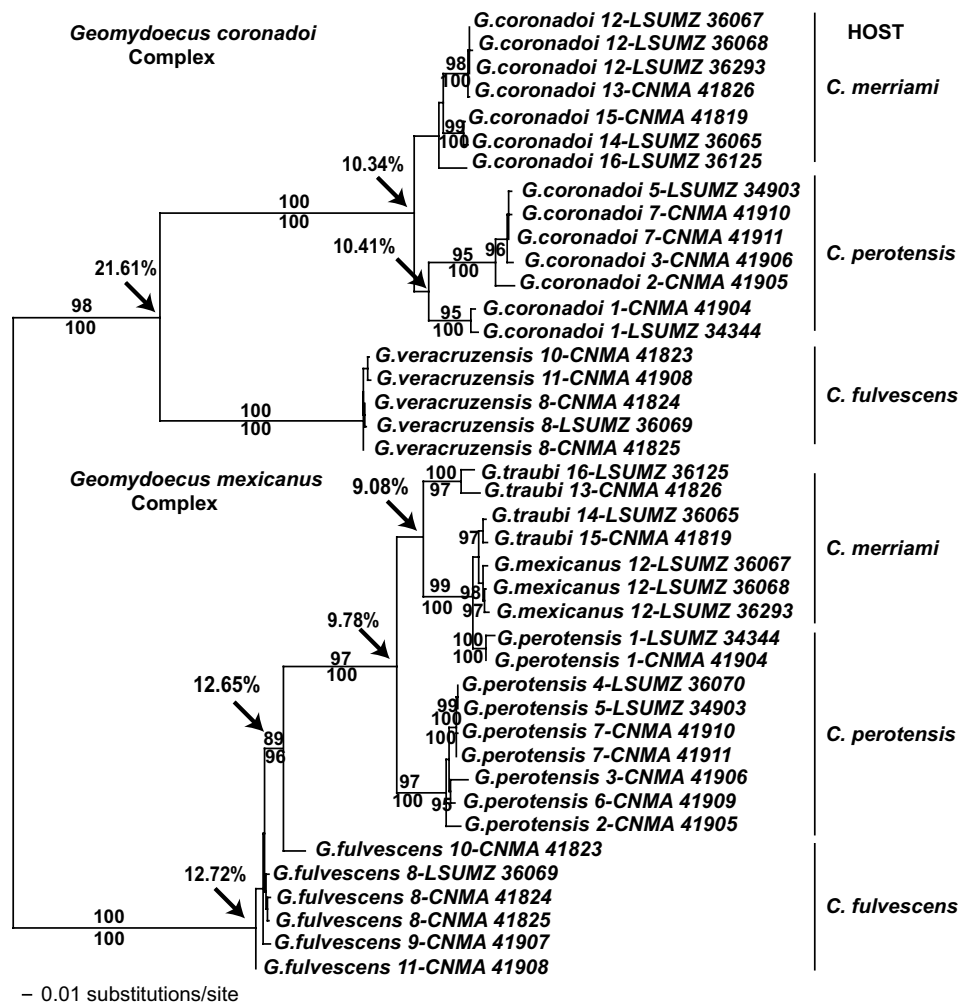


Fig. 4. Phylogram resulting from maximum likelihood analyses of the COI gene for the *Geomydoecus coronadoi* (top) and *G. mexicanus* (bottom) species complexes. Maximum likelihood bootstrap support values greater than 75 are shown above the nodes, and Bayesian posterior probabilities greater than 95 are indicated below the nodes. Average uncorrected p distances are indicated at several basal nodes. Species names are followed by locality numbers (Fig. 1 and Table 1) and host specimen numbers (Table 1). Host associations are listed to the right of the phylogram.

ney $U = 83$, $p = 0.001$), with mean distances in the *G. coronadoi* complex being significantly greater than those in the *G. mexicanus* complex (Fig. 4). Overall, lice of the *G. coronadoi* complex show increased rates of substitution compared to lice of the *G. mexicanus* complex and the gopher hosts (Table 2).

There were 167 and 141 4-fold degenerate sites for pocket gophers and chewing lice, respectively, with more sequence differences in the *G. coronadoi* complex compared to either the *G. mexicanus* complex or their hosts (Table 2). Likelihood ratio tests revealed that 4-fold degenerate sites for pocket gophers and chewing lice did not depart significantly from a molecular clock ($p > 0.05$). Thus, analogous branches in the phylogenies of codiverging hosts and parasites should be proportional in length (i.e., fit a linear model) because they have diverged at a more-or-less constant rate (but potentially different rates in the hosts and parasites) for the same length of time since the codivergence event.

After removal of taxa causing incongruence between the host and parasite datasets, cophath analysis (Page, 1996) resulted in a total of 108 combinations of potentially codiverging taxa, and Fig. 6 shows the results of one of 20 cophath analyses in the *G. coronadoi* species complex. Mantel tests revealed a significant relationship ($p < 0.05$) between genetic distance matrices for taxa involved in all cophath solutions. Because branch structure in the gopher and louse trees was not identical (Fig. 5), many of the 108 cophath solutions necessarily involve taxa that do not have a history of codivergence. Thus, a globally significant relationship exists between host and parasite genetic distance matrices despite the high probability that non-codiverging taxa remain in many, if not most, of the putative cophath solutions.

Model II regression analysis of estimated branch lengths for hosts and parasites was used to identify cophath solutions with the best fit to the linear model predicted by codivergence with a molecular clock. Solutions with the

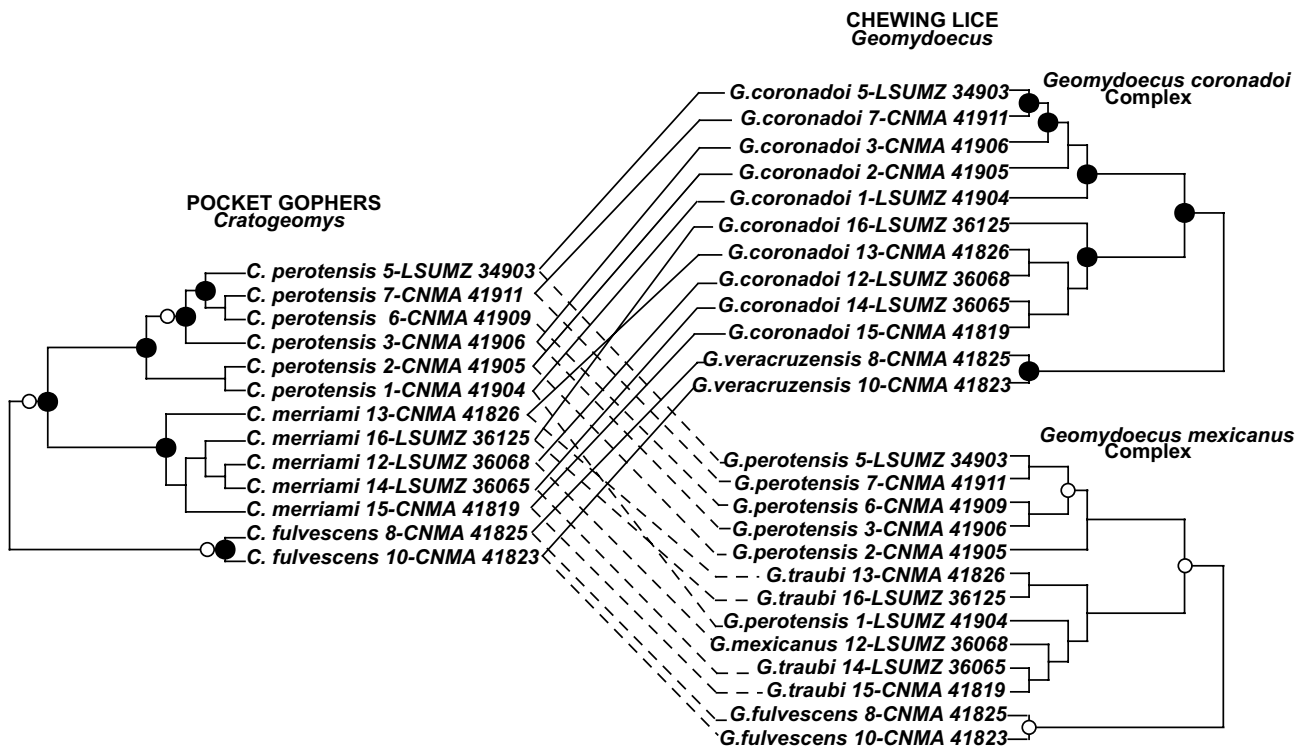


Fig. 5. Results of reconciliation analysis of the *G. coronadoi* (top) and *G. mexicanus* (bottom) complexes and their pocket gopher hosts using TreeMap 2.0β. Numbers of louse specimens per species complex were not identical because representatives of the *G. coronadoi* complex were not collected from all gopher hosts (Table 1). Localities 4 and 5 (Fig. 1 and Table 1) were treated as one locality because of their close geographic proximity and high genetic similarity between hosts and parasites. Solid and dashed lines between the louse and gopher taxa indicate host-parasite associations. Black and white dots at nodes indicate instances of perfect cophylogeny for the *G. coronadoi* and *G. mexicanus* complexes, respectively. The number of reconstructed cospeciation events between lice of both the *G. coronadoi* and *G. mexicanus* complexes and the gopher hosts was greater than expected by chance ($p < 0.001$ and 0.002 , respectively).

Table 2

Observed percent of differences (means ± 1SD) in various characteristics of the COI nucleic acid sequence from pocket gophers and their ectoparasitic chewing lice calculated using MacSequence (version 4.2, D.A. Good, unpublished program)

	Percent of sequence difference ^a (±1SD)		
	Gophers	<i>G. coronadoi</i> species complex	<i>G. mexicanus</i> species complex
First position transitions	0.31 (0.18)	1.58 (1.03)	0.58 (0.33)
First position transversions	0.01 (0.03)	0.35 (0.28)	0.18 (0.15)
Second position transitions	0.02 (0.04)	0.32 (0.29)	0.00 (0.00)
Second position transversions	0.00 (0.00)	0.10 (0.10)	0.04 (0.07)
Third position transitions	2.43 (1.17)	6.93 (3.43)	5.89 (2.64)
Third position transversions	0.58 (0.41)	3.77 (3.02)	2.31 (1.52)
Total differences	3.34 (1.68)	13.00 (7.87)	8.98 (4.35)
Silent nucleotide differences	3.21 (1.62)	11.53 (6.61)	8.53 (4.17)
Replacement nucleotide differences	0.13 (0.11)	1.48 (1.35)	0.46 (0.31)
Amino acid differences	0.36 (0.29)	3.60 (2.82)	1.26 (0.82)
Percent sequence differences at 4-fold degenerate sites	9.55 (5.44)	38.14 (22.27)	30.65 (15.17)

^a Means and standard deviations based on all pairwise comparisons.

highest correlation coefficient of determination ($r^2 > 0.50$; obtained from Model II regression output) are shown in Table 3. All *G. coronadoi* solutions have slopes significantly greater than 1.0 ($p < 0.032$ for the least-squares regression coefficient), and point estimates of the slopes ranged from 3.42 to 4.02. Point estimates for slopes for the *G. mexicanus* solutions ranged from 1.46 to 1.97 (Table 3) and the lower limit of the confidence interval in all of the comparisons

included the value of 1.0 (equal rates of substitution). In all solutions for both louse complexes, the y -intercept was not significantly different from zero (Table 3), indicating that divergence events in the hosts and parasites were approximately contemporaneous (Hafner and Nadler, 1990).

A Mantel test comparing genetic distance matrices for the two louse complexes showed a significant association

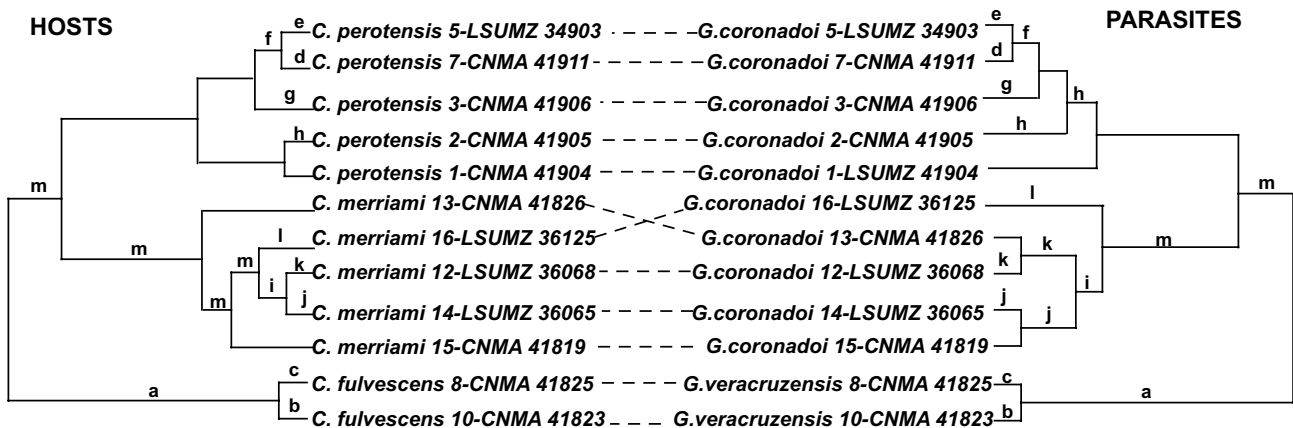


Fig. 6. One of 20 possible copath solutions for the *Geomydoecus coronadoi* species complex showing branches used in the comparison of evolutionary rates between pocket gophers and chewing lice. Dashed lines indicate host–parasite associations. Only potentially cospeciating host–parasite pairs were used in the rate analyses because these taxa are assumed to have experienced an equivalent amount of time since divergence. Only terminal and subterminal branches were used in the rate comparisons (see text), so branches labeled a, i, and m were omitted from the analysis.

Table 3

Results of Model II regression analysis comparing estimated branch lengths in the pocket gopher phylogeny to corresponding branch lengths in the phylogeny of their chewing lice

Comparison	Slope	95% CI of slope	r^2	y-intercept	95% CI of y-intercept
Comparisons of pocket gophers with lice of the <i>Geomydoecus coronadoi</i> complex					
hLRT	4.02	[2.12, 21.33]	0.588	−0.020	[−0.258, 0.006]
	3.79	[1.94, 24.76]	0.566	−0.011	[−0.284, 0.013]
AIC	3.62	[1.94, 15.70]	0.611	−0.016	[−0.190, 0.008]
	3.42	[1.78, 17.11]	0.590	−0.008	[−0.195, 0.014]
Comparisons of pocket gophers with lice of the <i>Geomydoecus mexicanus</i> complex					
hLRT	1.51	[0.89, 2.96]	0.763	0.008	[−0.010, 0.016]
	1.97	[0.99, 6.61]	0.760	0.003	[−0.050, 0.014]
	1.91	[0.91, 7.48]	0.735	0.002	[−0.061, 0.014]
	1.49	[0.65, 5.15]	0.731	0.008	[−0.051, 0.021]
AIC	1.48	[0.88, 2.87]	0.768	0.008	[−0.010, 0.016]
	1.88	[0.94, 6.21]	0.760	0.003	[−0.014, 0.026]
	1.83	[0.86, 7.17]	0.731	0.003	[−0.059, 0.014]
	1.46	[0.64, 4.84]	0.737	0.008	[−0.046, 0.020]

All phylogenies were based on homologous COI sequences in the pocket gophers and lice. Branch lengths were estimated using models of evolution selected by the hierarchical likelihood ratio test (hLRT) and the Akaike Information Criterion (AIC). Because there were multiple solutions in the copath analyses (Page, 1996; Fig. 6 shows one such solution), multiple comparisons were required between each louse complex and the hosts. Only those with $r^2 > 0.50$ are shown (see text). Slopes of the regression reflect relative rate of nucleotide substitution in the chewing lice relative to their hosts, and y-intercepts of the regression indicate whether lice speciated before (slope > 0), after (slope < 0), or coincident with (slope not significantly different from zero) their hosts (Hafner and Nadler, 1990).

between the matrices ($p < 0.001$), however direct comparison of analogous branches based on 4-fold degenerate sites only and using the Model II regression analysis found no coefficient of determination > 0.50 . The weakness of the association between branch lengths in the two louse complexes precludes interpretation of slope point estimates.

4. Discussion

4.1. Phylogenetic analyses

Phylogenetic analysis of both nuclear and mitochondrial genes support the species complexes originally described by

Price and Hellenthal (1989) on the basis of morphology. Although Price and Hellenthal (1989) did not comment on louse relationships, a morphological analysis by Page et al. (1995) suggested that the *Geomydoecus coronadoi* and *G. mexicanus* species complexes were not sister groups within the family Trichodectidae. Page et al. (1995) acknowledged, however, that their morphological data might contain a considerable amount of homoplasy. A molecular–phylogenetic analysis of louse relationships involving all louse taxa parasitizing pocket gophers is currently underway and is specifically designed to test the sister relationship of the *G. coronadoi* and *G. mexicanus* species complexes.

Within the *G. coronadoi* species complex, all phylogenetic analyses (ML, MP, and Bayesian) identify a monophyletic and strongly supported *G. veracruzensis* clade (Figs. 3 and 4). Louse collections made for this analysis verify that *G. veracruzensis* parasitizes only *Cratogeomys fulvescens*, corroborating previous host records (Price and Hellenthal, 1989). Two clades of *G. coronadoi* (Figs. 3 and 4) each parasitize different species of pocket gophers (Fig. 1a). Lice of the subspecies *G. c. saccharalis* and *G. c. submerriami* (Price and Hellenthal, 1989) are found only on *C. merriami* hosts, whereas lice of the subspecies *G. c. coronadoi* are restricted to *C. perotensis* hosts (Fig. 1a). *Geomydoecus c. saccharalis* (Fig. 1a, localities 12 and 13) is the only monophyletic *G. coronadoi* subspecies (Figs. 3 and 4). Lice from locality 1 (Irolo) are, on average, 10.43% divergent from other *G. coronadoi* lice parasitizing *C. perotensis* (Fig. 4). Although the lice from Irolo key morphologically to *G. coronadoi*, it is possible that they represent a cryptic species.

Within the *G. mexicanus* complex, *G. fulvescens* is the only louse that parasitizes *C. fulvescens* (Fig. 1b). This louse species is morphologically distinct from other representatives of the *G. mexicanus* complex, and although parsimony analysis strongly supports monophyly of the *G. fulvescens* clade (Fig. 3), ML and Bayesian analyses do not (Fig. 4). Statistical comparisons between the parsimony, ML and Bayesian trees, however, found no significant difference between the topologies, and outgroup selection had no effect on the resulting MP or ML topologies (data not shown). The morphological distinctiveness of *G. fulvescens* (Price and Emerson, 1971; Price and Hellenthal, 1989) coupled with the fact that the specimen from locality 10 is genetically more similar to other *G. fulvescens* than it is to other lice in the *G. mexicanus* species complex, argue in favor of *G. fulvescens* monophyly.

The louse subspecies *G. perotensis irolonis* (Fig. 1b, locality 1) and *G. p. perotensis* (localities 2–7) are difficult to distinguish morphologically (Price and Hellenthal, 1989) and parasitize the same host species (*C. perotensis*), yet they do not form a monophyletic unit based on mtDNA data (Figs. 3 and 4). Instead, lice from Irolo cluster with lice parasitizing the gopher species *C. merriami* (Figs. 3 and 4) and are roughly 10% genetically divergent from other lice parasitizing *C. perotensis*. When *G. perotensis* lice were constrained to form a monophyletic clade in MP and ML phylogenetic analyses, the resulting trees were significantly different from the trees in Figs. 3 and 4 (KH test [MP] $p = 0.0009$; KH test [ML] $p = 0.004$; SH test [ML] $p = 0.002$). Although specimens of both subspecies key morphologically to *G. perotensis*, it is clear that *G. p. irolonis* and *G. p. perotensis* represent distinct mtDNA lineages and may be cryptic species. It also is possible that the lice from Irolo have retained an ancestral haplotype similar to those of *G. mexicanus* and *G. traubi* and may cluster with these specimens because of incomplete lineage sorting. Interestingly, *G. p. irolonis* also parasitizes pocket gophers outside the *C. merriami* group (*C. fumosus*; Fig. 1b) so it is

possible that these gopher species are in contact and exchanging lice. Examination of nuclear markers with greater resolution than the fragment of EF-1 α used in this study and additional studies of gopher and louse contact zones and host associations will be necessary to determine the taxonomic status of these louse populations.

Geomydoecus traubi from localities 13 and 16 (Fig. 1b) are, on average, 8.59% divergent from *G. traubi* lice from localities 14 and 15 (Figs. 3 and 4). When specimens of *G. traubi* were constrained to group together in MP and ML phylogenetic analyses, the resulting trees were significantly different from the trees in Figs. 3 and 4 (KH test [MP] $p = 0.0001$; KH test [ML] $p = 0.005$; SH test [ML] $p = 0.004$). Although specimens of these two clades key morphologically to *G. traubi*, they clearly represent distinct mtDNA lineages and therefore potential cryptic species. Similar to the situation with lice from Irolo (see above), *G. traubi* is also known to parasitize a distantly related gopher species (*C. planiceps*; Fig. 1b) and the gopher hosts may be in contact and exchanging lice.

4.2. Tests of cophylogeny

4.2.1. The problem of multiple lineages

Page (1993a) introduced what he termed “the problem of multiple lineages,” by which he referred to the potential presence of multiple, paralogous genes (in molecular studies), species (in biogeography), or parasites (in studies of cophylogeny) that, coupled with inadequate sampling, could result in a misleading picture of the history of the molecule, geographic region, or host–parasite assemblage, respectively. Hafner and Page (1995) discussed this potential problem with reference to the gopher–louse assemblage and stressed that future studies should include exhaustive sampling of gopher and louse clades. This is the first study of gophers and lice to use exhaustive sampling of extant clades and perhaps not coincidentally, it is the first study to detect and analyze multiple lineages of lice on a single lineage of gophers. Exhaustive sampling of extant clades cannot compensate for absence of extinct clades, but it reduces the likelihood that phylogenetic reconstructions and estimates of rate differences will suffer from sampling error.

4.2.2. Comparative utility of cophylogenetic methods

Tree-based, distance-based, and data-based methods were all informative regarding the historical associations among the pocket gophers and chewing lice examined in this study. The tree-based reconciliation analysis detected a significant pattern of cophylogeny between the two louse lineages and their gopher hosts. Results from another tree-based method, generalized parsimony (TreeFitter 1.0; Ronquist, 1998, 2000), were similar to those of the reconciliation analysis even though these tree-based methods have different null hypotheses (results available upon request). The distance-based method used in this study (ParaFit) likewise revealed a significant nonrandom association

between host and parasite datasets and also identified host–parasite pairs that appear to be associated randomly, presumably as a result of a process other than codivergence such as host switching. Because distance-based methods are computationally feasible for large datasets and because they test for cophylogeny both over the entire dataset and for individual host–parasite pairs, these methods offer a different and useful perspective in cophylogenetic analyses (compared to tree-based methods) and should be included in future studies (Huysse and Volckaert, 2005).

Three of the data-based methods used to compare the hosts and parasites in this study (KH, SH, and LRT) confirmed that at least some of the differences in the datasets did not result from sampling error, but rather historical processes such as host switching or extinction (Clark et al., 2000; Page, 2003; Jackson, 2004a, 2004b; Kawakita et al., 2004). Because they treat the data differently, the distance-based ParaFit analysis and the data-based ILD test identified overlapping, yet different sets of taxa presumably responsible for incongruence between the host and parasite trees. Many of these taxa responsible for topological incongruence are easily identified by visual inspection of the host and parasite trees (Fig. 5). In general, data-based methods are computationally intensive, but increased use of parallel computing in phylogenetics may render data-based methods computationally feasible in future studies analyzing large datasets.

4.3. Timing of codivergence events and comparison of molecular rates

Relative timing of divergence events in hosts and parasites is reflected in the direction (positive or negative) and magnitude of the y -intercept in Model II regression analyses of estimated branch lengths (Hafner and Nadler, 1990; Hafner et al., 1994). The y -intercept indicates whether speciation in the parasite occurred before host divergence (intercept > 0), after host divergence (intercept < 0), or synchronous with host divergence (intercept not significantly different from zero). All regression analyses of estimated branch lengths in gophers and lice in this study showed intercepts that were not significantly different from zero (Table 3), indicating that cladogenic events in the gophers and lice were approximately synchronous. Synchronous cladogenic events are expected between codiverging hosts and their parasites, especially if the parasites have severely limited dispersal abilities and high host specificity, as do the chewing lice of mammals (Page, 1996; Page et al., 1998; Paterson et al., 2000).

Relative rates of nucleotide substitution in host and parasite mtDNA sequences are indicated by the slope in Model II regression analyses of branch length data (Table 3; Hafner and Nadler, 1990; Hafner et al., 1994). Regression analyses (Table 3) show that lice of the *G. coronadoi* complex are evolving significantly faster than their hosts at the COI locus, with the magnitude of these slopes sug-

gesting a 3- to 4-fold rate difference. Lice of the *G. mexicanus* complex appear to be evolving 1.5–2 times faster than their hosts (Table 3), but because the confidence intervals of the slopes slightly overlap 1.0 these results are, at best, only marginally significant. This marginal significance likely is an artifact of sampling error resulting from the low number of codivergence events observed in the *G. mexicanus* species complex (Fig. 5).

Direct comparison of branch lengths (calculated from 4-fold degenerate sites only) in the *G. coronadoi* and *G. mexicanus* species complexes showed no evidence for a significant rate difference, suggesting that the two louse complexes have similar basal rates of mutation in the COI gene. However, nonparametric tests involving all substitution types indicate that lice of the *G. coronadoi* complex have significantly greater patristic distances to the outgroup taxon. This evidence, coupled with the data on frequency of substitution types (Table 2), supports our contention that lice of the *G. coronadoi* complex are evolving faster than lice of the *G. mexicanus* complex at the COI locus.

Prior to this study, a consensus was emerging that mitochondrial genes of chewing lice evolve approximately 2–5 times faster than mitochondrial genes of their hosts. This was based on studies of a different lineage of pocket gophers and their lice (2.5 rate difference in the COI gene; Hafner et al., 1994), swiftlets and their lice (2.6 rate difference in the Cytb gene; Page et al., 1998), and seabirds and their lice (5.5 rate difference in the 12S rRNA gene; Paterson et al., 2000). This study reports a similar 3- to 4-fold rate difference at the COI locus between chewing lice of the *G. coronadoi* complex and their hosts, and a marginally significant 1.5- to 2-fold rate difference between lice of the *G. mexicanus* complex and their hosts (Table 3). We cannot explain why mitochondrial sequences in chewing lice evolve more rapidly than the homologous sequences in their hosts, and the myriad biological differences between insects and mammals likely will make the search for causal factors difficult. Differences in generation time, metabolic rate, DNA base composition, mitochondrial gene order, and evolution of the parasitic lifestyle have all been suggested as possible causes of observed rate differences between organisms (Brown and Simpson, 1982; Clayton, 1982; Tanaka and Ozawa, 1994; Wu and Li, 1985; Martin and Palumbi, 1993; Rand, 1994; Downton and Austin, 1995), but this list of potential causes is but a tiny subset of the many biological differences between insects and mammals. We predict that future studies will show that a combination of these and other factors are responsible for observed rate differences, and we further predict that the particular combination of causal factors will vary across taxa, geography, and time.

Perhaps more interesting in this study is the absence of a rate difference in basal mutation rate (estimated from 4-fold degenerate sites) between the two louse species complexes, but presence of a significant rate difference when all other substitution types are considered (Table 2).

Explaining this rate difference would seem to be a less daunting challenge than explaining the rate difference between pocket gophers and lice because of the many biological similarities between these congeneric louse complexes. If, for the sake of argument, we assume that the close relationship among these species of lice controls for major differences in generation time, metabolic rate, and other factors that influence basal mutation rate, then we are left with two possible explanations for the observed rate difference: either the COI genes in the two louse species complexes are under different selective constraints, or effective population size, frequency and severity of founder events, or other population-level phenomena have affected the two lineages of lice differently over time (Demastes and Hafner, 1993; Hafner et al., 1994; Spradling, 1997; Page et al., 1998; Demastes et al., 2003). Nadler et al. (1990) found moderate levels of genetic differentiation among conspecific louse populations living on different gophers in the same host population, suggesting that founder events and potential differences in effective population size may cause genetic differences that are measurable over small spatial and temporal scales. Although effective size of louse populations was not measured in this study, census size of *G. coronadoi* populations was markedly smaller than census size of *G. mexicanus* populations (JEL, personal observations), suggesting that lice of the faster evolving *G. coronadoi* complex may experience more frequent or severe founder events or population bottlenecks than do lice of the slower evolving *G. mexicanus* complex. Testing this hypothesis will first require evidence that generation time, metabolic rate, and other life history parameters that influence basal mutation rate are, in fact, reasonably constant across louse taxa. Additional evidence showing that extant populations of lice in the *G. coronadoi* complex have significantly more genetic structuring across hosts and host populations than do extant populations of lice in the *G. mexicanus* complex would document that the conditions necessary for relatively rapid evolution exist in *G. coronadoi* populations.

Acknowledgments

We thank our Mexican collaborator, F. Cervantes, and his students R. Castro, R. Rojas, M. Aquino, N. Ramírez, J. Fernández, and S. Ramírez for their hospitality and helpful assistance collecting gophers and lice in Mexico. S. Brant, J. Demboski, D. Hafner, J. Hafner, A. Leaché, and T. Spradling also helped collect gopher and louse specimens and provided insightful discussions of this research. S. Brant was instrumental in obtaining louse data and we thank N. Crochet for obtaining DNA sequences. J. Demastes and G. Hasty provided helpful comments on earlier drafts of this manuscript. This work was funded by NSF grants DEB-043869 (to M.S.H.) and DBI-0400797 (a NSF Multiuser Equipment Grant for The Museum of Natural Science's capillary sequencer).

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