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Codivergence in Heteromyid Rodents (Rodentia: Heteromyidae) and Their Sucking Lice of the Genus *Fahrenholzia* (Phthiraptera: Anoplura)

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Abstract.—Although most studies of codivergence rely primarily on topological comparisons of host and parasite phylogenies, temporal assessments are necessary to determine if divergence events in host and parasite trees occurred contemporaneously. A combination of cophylogenetic analyses and comparisons of branch lengths are used in this study to understand the host-parasite association between heteromyid rodents (Rodentia: Heteromyidae) and their sucking lice of the genus *Fahrenholzia* (Phthiraptera: Anoplura). Cophylogenetic comparisons based on nucleotide substitutions in the mitochondrial COI gene reveal a significant, but not perfect, pattern of cophylogeny between heteromyids and their sucking lice. Regression analyses show a significant functional relationship between the lengths of analogous branches in the host and parasite trees, indicating that divergence events in hosts and parasites were approximately contemporaneous. Thus, the topological similarity observed between heteromyids and their lice is the result of codivergence. These analyses also show that the COI gene in lice is evolving two to three times faster than the same gene in their hosts (similar to the results of studies of other lice and their vertebrate hosts) and that divergence events in lice occurred shortly after host divergence. We recommend that future studies of codivergence include temporal comparisons and, when possible, use the same molecular marker(s) in hosts and parasites to achieve the greatest insight into the history of the host-parasite relationship. [Cophylogenetic methods; cophylogeny; cospeciation; host; molecular rates; parasite; symbiosis.]

Considering that approximately 1250 species of mammals are parasitized by roughly 1110 species of chewing or sucking lice (Insecta: Phthiraptera; Durden and Musser, 1994; Price et al., 2003), it is remarkable that only one mammal-louse association, that involving pocket gophers (Rodentia: Geomyidae) and their chewing lice (Phthiraptera: Ischnocera), has been studied in depth from a cophylogenetic perspective (see Hafner et al., 2003, and included references). Because molecular data obtained from pocket gophers and chewing lice have figured prominently in development of both theory and methods of cophylogenetic analysis (e.g., Hafner and Nadler, 1990; Page, 1990, 1994; Huelsenbeck and Rannala, 1997; Huelsenbeck et al., 1997; Charleston, 1998; Johnson et al., 2001), one might expect that studies of other mammal-louse associations would yield equally interesting and potentially useful data.

In this study, we test for congruent host and parasite trees (cophylogeny) between heteromyid rodents (Rodentia: Heteromyidae) and their parasitic sucking lice of the genus *Fahrenholzia* (Phthiraptera: Anoplura). The Heteromyidae includes 58 species divided into the subfamilies Dipodomysinae (kangaroo rats and kangaroo mice; *Dipodomys* and *Microdipodops*), Heteromyinae (spiny pocket mice; *Heteromys*, following the taxonomy of Hafner et al. [2007], which synonymizes *Liomys* and *Heteromys*), and Perognathinae (pocket mice; *Chaetodipus* and *Perognathus*). All sucking lice that parasitize heteromyids belong to the genus *Fahrenholzia*, and all 12 species of *Fahrenholzia* parasitize only heteromyids (Kim et al., 1986; Whitaker et al., 1993; Light and Hafner, 2007a). Although heteromyid rodents are closely related to pocket gophers and sucking lice are close relatives of chewing lice, the natural histories of these evolutionary partners differ considerably. Whereas pocket gophers are fossorial and asocial, heteromyid rodents forage above ground and show somewhat higher levels of sociality

(Jones, 1993). Although both groups of lice are permanent and obligate ectoparasites of mammals (and both are wingless), they have different population structures and different diets (Marshall, 1981). Chewing louse populations usually are large (often >300 lice per host) and infect all host individuals in a population of pocket gophers (Rust, 1974; M.S.H., personal observation). In contrast, sucking louse populations usually are small (<10 lice per host; J.E.L. personal observation) and prevalence on their heteromyid hosts generally is low (Light and Hafner, 2007a). As their name implies, chewing lice have chewing mouthparts and eat skin dander, whereas sucking lice have piercing-sucking mouthparts and feed on host blood (Marshall, 1981). These differences in natural history may have influenced both the kind and degree of coevolutionary interactions, including coadaptation and cospeciation, operating in these host-parasite assemblages.

Testing for Codivergence and Cospeciation

Throughout this paper, we use the term “cophylogeny” to describe a *pattern* (i.e., significantly similar topology in host and parasite trees) and “codivergence” (or “cospeciation”) to describe a *process* (i.e., contemporaneous divergence [or speciation] in coexisting hosts and parasites). We regard cospeciation as a special kind of codivergence in which the end products of the divergence process are considered separate species. Just as the process of repeated speciation in a lineage of organisms creates a pattern termed *phylogeny*, the process of repeated codivergence (or cospeciation) in a host-parasite assemblage can result in a pattern termed *cophylogeny*. Importantly, however, significantly similar patterns in host and parasite trees (cophylogeny) can result from processes other than codivergence (Page, 1991; Paterson and Banks, 2001; Ronquist and Liljeblad, 2001; Charleston

and Robertson, 2002; Page, 2003; Percy et al., 2004; de Vienne et al., 2007). For example, host switching, sorting events (extinction and lineage sorting), duplication events (speciation of the parasite independent of the host), and failure of the parasite to diverge when the host diverges, may happen in such a way as to cause the host and parasite trees to be congruent. Similarly, preferential colonization of related hosts by parasites can cause the parasite phylogeny to mimic the host phylogeny, even though parasite divergence may have occurred long after host divergence. For these reasons, it is vital to perform rigorous comparisons of host and parasite data sets to distinguish between cophylogeny resulting from codivergence and cophylogeny resulting from causes other than codivergence.

Host and parasite phylogenies can be assessed for similarity using distance-based, tree-based, and data-based methods. Distance-based methods, such as ParaFit (Legendre, 2001a; Legendre et al., 2002), determine if hosts and their parasites are associated randomly by comparing genetic distances from homologous gene regions for the associated taxa. Tree-based methods compare only the branching structure of host and parasite trees to determine if tree topologies are more similar than would be observed by chance. Commonly used tree-based methods include reconciliation analysis (TreeMap; Page, 1994; Charleston and Page, 2002) and generalized parsimony (TreeFitter; Ronquist and Nylin, 1990; Ronquist, 1995). If distance-based and tree-based methods show significant cophylogeny between associated taxa, then data-based methods can be used to determine the cause of topological incongruence (if any) between host and parasite trees. Data-based methods test whether the host and parasite data sets from homologous gene regions support the same tree. Commonly used data-based methods include the Kishino-Hasegawa test (KH test; Kishino and Hasegawa, 1989), the Shimodaira-Hasegawa test (SH test; Shimodaira and Hasegawa, 1999; Goldman et al., 2000), the likelihood-ratio test (LRT; Huelsenbeck et al., 1997, 2000), and the incongruence length difference test (ILD test; Johnson et al., 2001). Each of these cophylogenetic methods has advantages and disadvantages, but recent studies suggest that all three should be used together for optimal resolution of the evolutionary history of hosts and their parasites (Huysse and Volckaert, 2005; Hughes et al., 2007; Light and Hafner, 2007b).

Although distance-based, tree-based, and data-based methods offer a variety of ways to investigate host-parasite associations, these methods cannot distinguish between trees that are concordant as a result of codivergence and trees that are concordant for reasons unrelated to codivergence. This level of discrimination requires use of a combination of methods that compare not only topological similarities between host and parasite trees but also timing of putative codivergence events. Estimates of relative or absolute divergence times in host and parasite lineages are therefore a critical component of cophylogenetic studies because they provide a way to distinguish between codivergence and

other processes that could result in identical branching patterns in host and parasite trees.

MATERIALS AND METHODS

Host and Parasite Data

Rodent and louse specimens analyzed in this study were collected from throughout much of their respective geographic ranges (Fig. 1, Appendix 1). The parasites and hosts used in the cophylogenetic analysis are true associates; i.e., the lice used in the parasite analysis were taken directly from the heteromyid individuals used in the host analysis. There were only two instances in which DNA from the exact host specimen was not available. In one instance, the louse specimen was donated for analysis (*F. fairchildi* from locality 1 donated by L. Durden) and in the other instance, amplification of host DNA was not possible (*D. heermanni* from locality 23). In both instances, DNA from a heteromyid specimen of the appropriate host species was obtained from a nearby locality (Appendix 1).

In this study, we treat each terminal taxon as an independent evolutionary unit, and although some of these terminal taxa belong to the same species according to current taxonomy, many of our included taxa, especially the lice, may represent cryptic species (Light and Hafner, 2007a). Treatment of conspecific lineages as independent evolutionary units is common in the cospeciation literature (e.g., Page et al., 2004), but we will restrict our use of the term "cospeciation" to refer to codivergence events involving taxa that are currently recognized as separate species. In all other cases, we will use the more general term, "codivergence."

Host and parasite phylogenies were constructed using sequence data from the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. Genomic DNA was isolated from liver tissue of 43 heteromyid specimens (Appendix 1) using the DNeasy Tissue Kit (Qiagen, Valencia, California). PCR amplifications of the entire COI gene (1551 bp) were performed in 50 μ L reaction volumes using primers COI-5285f and COI-6929r (Spradling et al., 2004). The primers COI-5285f, COI-6929r, MCO-173f, MCO-1480r, and MCO-1345r (Hafner et al., 2007) were used to perform sequencing reactions. PCR cleanups and sequencing reactions were performed according to Hafner et al. (2005). Sequences were edited using Sequencher Version 4.1 (Gene Codes, 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 Maddison, 2000). Because there were several insertion/deletion events at the end of the COI gene, each sequence terminated with the same set of conserved amino acids by eliminating the stop codon and up to 9 bp of sequence upstream from the stop codon before phylogenetic analysis (Spradling et al., 2004; Hafner et al., 2007). Outgroup taxa consisted of four pocket gopher species, *Cratogeomys perotensis*, *Orthogeomys grandis*, *Pappogeomys bulleri*, and *Zygogeomys trichopus*, and sequences for the

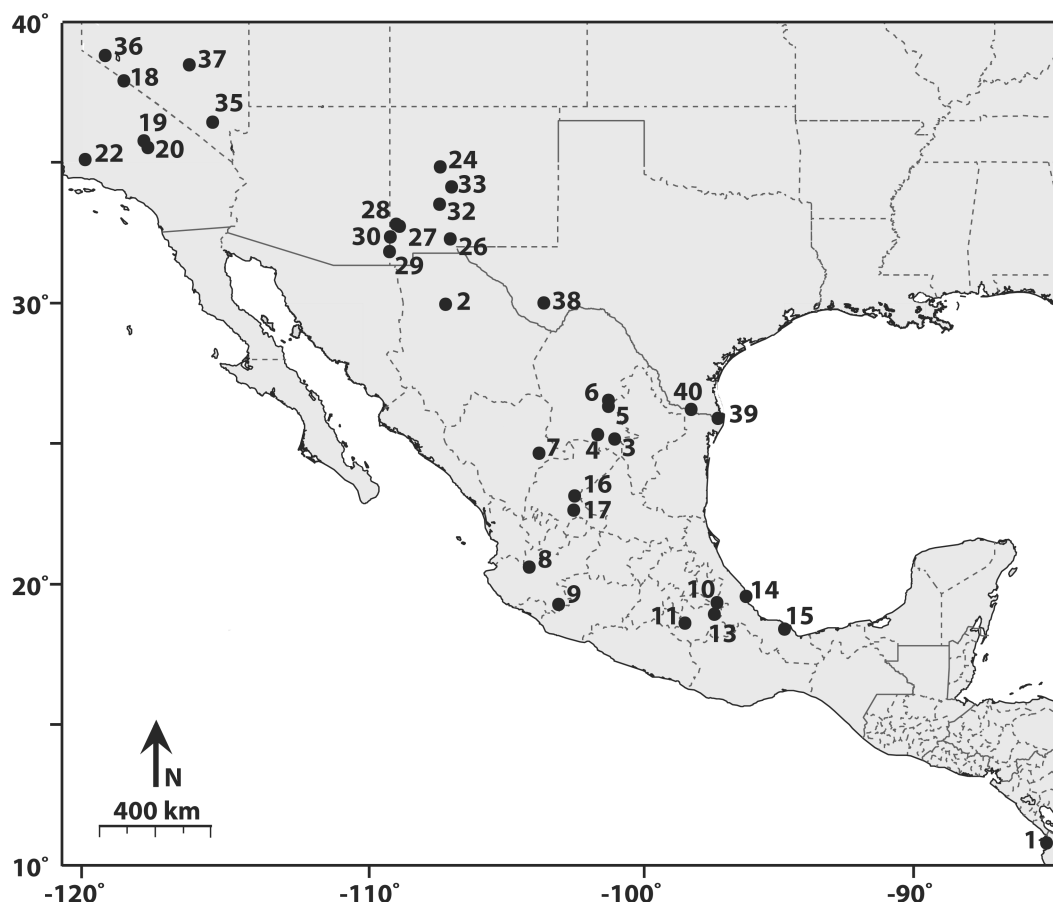


FIGURE 1. Geographical distributions of heteromyid and *Fahrenholzia* specimens used in the phylogenetic and cophylogenetic analyses (modified from Light and Hafner, 2007a). Numbers refer to louse collecting localities listed in Appendix 1.

homologous portion of the COI gene were downloaded from Genbank (accession numbers AY649478, AY331082, AY331084, and AY331087, respectively).

Fahrenholzia COI data (1011 bp) were collected for a previous study (Light and Hafner, 2007a), although six specimens from that study were omitted here because they were collected at localities that were nearly identical to those of other sampled specimens. These six taxa were located terminally on the COI tree and were identical or almost identical genetically to their closest relative. The louse parasitizing *Dipodomys deserti* was omitted because the host association of this louse was uncertain, and the lice parasitizing *Heteromys pictus* (*F. microcephala* 9—CNMA 39674 and *F. microcephala* 14—CNMA 41912) were treated as sister taxa based on prior analyses and morphological similarity (Light and Hafner, 2007a). All sequences are available in Genbank (Heteromyids: EF156837 to EF156839, EF156841, EF156842, EF156844, EF156845, EF156850, EF156854 to EF156858, EF156860, EF156865, EF156866, EU107432 to EU107518; *Fahrenholzia*: DQ324550 to DQ324601).

Phylogenetic Analyses

Relationships among heteromyid rodents are relatively well established based on analyses of multiple

molecular markers (e.g., Alexander and Riddle, 2005; Hafner et al., 2007). Because the mitochondrial gene used in this analysis (COI) lacks resolving power at the base of the heteromyid tree, our heteromyid analysis was constrained to adhere to the subfamily relationships determined by Hafner et al. (2007), who examined a combination of three mitochondrial markers (including the COI gene) and obtained good basal resolution. To generate the best ML tree for the heteromyids, 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). For the heteromyid COI data set, the general time-reversible (GTR) model, including among-site rate variation and invariable sites (GTR+I+ Γ ; Yang, 1994; Gu et al., 1995), was the best model of evolution according to the hLRT and the K81uf+I+ Γ model was the best model of evolution according to the AIC. Full heuristic ML and bootstrap searches (200 pseudoreplicates) were conducted using the preferred model in PAUP* 4.0b10 (Swofford, 2003).

ModelTest also was used to evaluate the relative fit of evolutionary models to the louse COI data set, and the GTR+I+ Γ model was chosen as the best model of evolution according to both hLRTs and the AIC. Full heuristic ML and bootstrap searches (300 pseudoreplicates) were conducted using the preferred model in PAUP* 4.0b10. Bayesian phylogenetic analyses also were performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). A general time-reversible model including invariable sites and among-site rate variation was used in the Bayesian analyses and model parameters were treated as unknown variables with uniform priors. 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. To avoid entrapment on local optima, two independent Bayesian analyses were run and log-likelihood scores were compared for convergence (Huelsenbeck and Bollback, 2001; Leaché and Reeder, 2002). Log-likelihood scores of sample points were plotted against generation time to assess stationarity and all burn-in points (the first 5000 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. All executable data files for the rodents and lice used in this study are available at TreeBASE (<http://www.treebase.org>; SN number S2029).

Cophylogenetic Analyses

Because of the large size of the host and parasite phylogenies (Figs. 2 and 3), tree-based analyses were performed on pruned topologies that included one representative of each host species and its respective parasite. Pruning did not change tree topology, and because tree-based analyses consider topology only, pruning had no effect on the analyses other than reducing computation time. Instances in which a host species was parasitized by more than one parasite species were treated in separate analyses. Multiple specimens of *F. pinnata*, *F. zacatecae*, and *F. reducta* were included in the pruned analyses because of the large numbers of host species parasitized by these louse species. Finally, *F. pinnata* specimens parasitizing *Perognathus flavus* were treated as monophyletic (Light and Hafner, 2007a).

Because some analyses of the full data set were not computationally feasible and because of basal disagreements between the host and parasite phylogenies, tree-based and data-based analyses were performed separately on the major host and parasite clades indicated in Figures 2 and 3. "Major clades" were defined as all monophyletic groups of hosts parasitized by monophyletic groups of lice for which at least five localities were sampled. All possible fully resolved trees were examined separately to account for polytomies in the host and parasite trees, including localities 19, 20, and 35 for the hosts and localities 2, 26, 32, and ([2, 26, 32], 6, and 33) for the

parasites. This treatment resulted in 21 fully resolved trees, each consisting of five taxa. There were two distinct topologies (*Dipodomys* clades A and B) among these 21 trees, one of which (*Dipodomys* clade A) was identical between hosts and parasites.

The distance-based method ParaFit (Legendre, 2001a; Legendre, et al., 2002) was used to test the null hypothesis of random association between host and parasite data sets. Distance matrices for heteromyids and sucking lice were derived from ML estimates of pairwise genetic distances using model parameters derived from both hLRTs and the AIC, as selected by ModelTest. The programs DistPCoA (Legendre and Anderson, 1998) and the R Package (Casgrain and Legendre, 2001) were used to convert distance matrices into principal coordinate matrices. Tests of random association were performed with 999 permutations globally across both matrices and for each individual host-parasite association.

The tree-based reconciliation method implemented in TreeMap 2.0 β (Page, 1990, 1994; Charleston and Page, 2002) was used to test for phylogenetic congruence between host and parasite trees. This method (implementing the jungles algorithm; Charleston, 1998) finds the least costly reconstruction of host-parasite relationships while maximizing the number of putative codivergence events. The default settings of TreeMap 2.0 β were used (assigning a cost of zero for codivergence events and a cost of 1 for host switches, losses, and duplications). The parasite tree was randomized 1000 times and the observed number of putative codivergence events was compared to the null distribution of codivergence events derived from this randomization procedure to determine whether the number of codivergence events recovered from the reconciliation analysis was significant.

Data-based cophylogenetic methods (LRT tests) were performed only if significant topological congruence was documented between sucking lice and their hosts based on prior analyses using distance-based and tree-based methods. Data-based methods were used to explore the causes of conflict (if any) between the host and parasite phylogenies by testing the hypothesis that the host and parasite data sets underlie identical topologies. If so, then topological differences in the host and parasite trees are most likely an artifact of the study, such as sampling error in one or both data sets (e.g., inadequate taxon sampling or insufficient number of informative sites). If, however, there is a significant difference between the topologies supported by the host and parasite data sets, then we infer that the topological differences we see are biologically meaningful and represent real historical events, such as host switching or parasite extinction (Huelsenbeck et al., 1997, 2003; Clark et al., 2000; Page, 2003; Jackson, 2004a, 2004b; Kawakita et al., 2004). Although data-based analyses can handle polytomies, they require a one-to-one association between host and parasite taxa. Thus, instances in which two louse species parasitize a single host were analyzed separately, first with one host-parasite pair, then with the other.

Likelihood-ratio tests (LRTs) were used to compare trees estimated from alternative host and parasite data

sets in both a parsimony and likelihood framework (Clark et al., 2000). Because of computational limitations, LRTs were performed on data sets for major clades only. The parsimony and likelihood scores obtained for the best host tree given the host data were compared to the score of the alternative parasite tree, also given the host data. Under likelihood criteria, the likelihood parameters of this alternative parasite tree were optimized for the host data to maximize the likelihood score (Clark et al., 2000). Similarly, the best parasite tree was compared to the score of the alternative host tree given the parasite data. The likelihood-ratio test statistic was used to determine the difference between the parasite and host trees, and significance was calculated using parametric bootstrapping in PAUP*4.0b10. 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 data sets (Huelsenbeck et al., 1997). The null distribution of likelihood scores was constructed by optimizing likelihood parameters for each data set 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 data sets (Monte Carlo simulation) using the optimized parameters and the constrained topology. Because SeqGen 1.3.2 requires fully resolved trees, the program TreeEdit v1.0a10 (<http://evolve.zoo.ox.ac.uk/software.html?id=treededit>) was used to resolve all polytomies present in the major louse clades and their respective hosts. The likelihood-ratio test statistic for the constrained and best trees for each of these simulated data sets was calculated, and a null distribution of test statistics was generated. The test statistic derived from the empirical data was then compared to the null distribution to determine if phylogenetic conflict existed between data sets.

Relative Timing of Divergence Events and Relative Rates of Evolution

Sequence data from homologous portions of the host and parasite COI gene were used to explore relative timing of putative codivergence events in heteromyid rodents and sucking lice by direct comparison of the lengths of all analogous tree branches (copaths; Page, 1996). Putative codivergence events were determined by TreeMap analyses of the host and parasite trees, and heteromyid and *Fahrenholzia* data sets were pruned to include only those taxa linked to analogous branches in the host and parasite trees (putative codiverging taxa). ModelTest was used to determine the best model of evolution for these taxa according to hLRTs and AIC criteria for hosts (GRT+I+ Γ and K81uf+I+ Γ , respectively) and parasites (TVM+I+ Γ and GTR+I+ Γ , respectively). Branch lengths were estimated following a full heuristic ML search constraining these taxa to fit the best heteromyid and sucking louse phylogenies (based on all data) using the preferred model of evolution in PAUP* 4.0b10.

Analogous branch lengths also were estimated for substitutions at third codon positions only. Because most nucleotide substitutions at third codon positions are synonymous and therefore less subject to purifying selection, they should provide an estimate of substitution rate that is closer to the actual mutation rate of the gene. Branch lengths were determined using the methodology described above, and models of evolution were selected by both hLRTs and AIC criteria for hosts (TrN+ Γ and GTR+ Γ , respectively) and parasites (HKY+ Γ and K81uf+ Γ , respectively). Likelihood-ratio tests were used to determine if data sets (including all nucleotide sites and third codon positions only) for the putative codiverging taxa showed a significant departure from clock-like behavior. To compare relative timing of divergence events, ML branch lengths as well as uncorrected p distance branch lengths were compared using nonparametric Model II regression analysis (Legendre, 2001b) and Kendall's nonparametric robust line-fit method (Kendall and Gibbons, 1990).

To compare evolutionary rates in the COI gene in rodents and lice, branch length comparisons (as described above) were restricted to terminal sister taxa of hosts that are parasitized by terminal sister taxa of lice. Terminal taxa were used to ensure statistical independence among the data points (nested nodes are not statistically independent) and reduce the likelihood of error resulting from substitution saturation, which increases with time. Analyses included all nucleotide substitutions, as well as substitutions at third codon positions only. Model II regression analysis was used to determine whether the parasite diverged before, coincident with, or after its host (as indicated by the y -intercept of the relationship) and if one associate evolved faster or slower than the other (as indicated by the slope of the relationship; Hafner and Nadler, 1990).

RESULTS

Host and Parasite Phylogenies

Although four basal nodes in the heteromyid rodent phylogeny (Fig. 2; subfamilies Heteromyinae, Perognathinae, Dipodominae, and Heteromyinae + Perognathinae) were constrained to match those in the more inclusive heteromyid phylogeny presented by Hafner et al. (2007), the unconstrained tree did not differ significantly from the constrained tree (KH and SH tests $P > 0.05$) and all unconstrained portions of the tree generated in this study are topologically identical to the tree in Hafner et al. (2007). Likewise, the phylogeny of the sucking lice examined in this study (Fig. 3) is topologically identical to the more inclusive louse phylogeny presented by Light and Hafner (2007a). The heteromyid tree (Fig. 2) and the louse tree (Fig. 3) show many obvious topological similarities. For example, pocket mice (*Chaetodipus* and *Perognathus*) and spiny pocket mice (*Heteromys*) are each parasitized by monophyletic groups of sucking lice (bootstrap support, however, is not strong for some of the louse clades). Although lice sampled from kangaroo rats (*Dipodomys*) are not depicted as

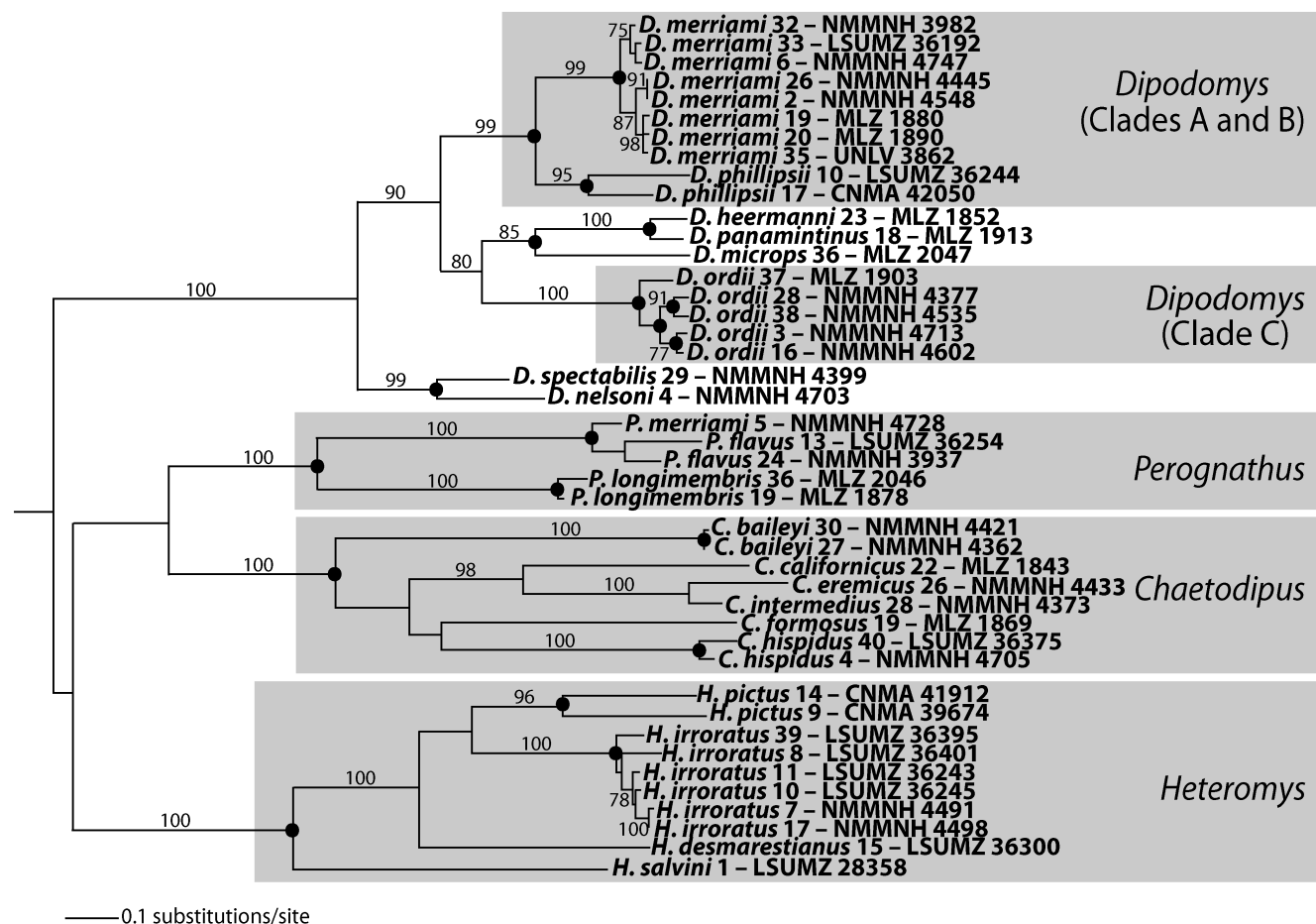


FIGURE 2. Maximum likelihood phylogram for the heteromyid rodent specimens from which sucking lice (Fig. 3) were sampled (outgroup taxa omitted). The phylogram is based on sequence data from the mitochondrial cytochrome *c* oxidase subunit I (COI) gene using the model of evolution chosen by the hierarchical likelihood-ratio test (GRT+I+Γ; Akaike information criterion results available upon request) and constrained to fit the basal heteromyid relationships determined by Hafner et al. (2007; see text). Maximum likelihood bootstrap support values greater than 75% are indicated at the nodes (constrained nodes lack bootstrap values). Species names are followed by locality number and museum specimen number (Appendix 1). Shaded boxes surround clades of rodents that are parasitized by monophyletic clades of lice. Shaded circles indicate nodes for which relative divergence times were estimated. Heteromyid taxonomy follows that of Hafner et al. (2007), who synonymized *Liomys* under *Heteromys*.

monophyletic in Figure 3, their apparent paraphyly involves only short and weakly supported branches that may reflect a lack of resolution at basal levels in the louse tree (Light and Hafner, 2007a).

Cophylogenetic Analyses

Global tests using ParaFit resulted in rejection of random association between host and parasite taxa ($P = 0.001$). Thirty-nine of the 44 tests of individual host-parasite pairs resulted in significant associations between heteromyid rodents and their *Fahrenholzia* lice ($P < 0.05$; Table 1). Nonsignificant associations included only those between *Perognathus* rodents and their lice.

Reconciliation analyses using TreeMap 2.0β detected significant congruence between the rodents and lice ($P < 0.001$; Table 1; Fig. 4). For comparisons within major clades, significant congruence was evident in the

Chaetodipus clade and two of the three *Dipodomys* clades but not in the *Heteromys* or *Perognathus* clades. Host and parasite phylogenies were not perfectly concordant, and reconciliation analysis attributed this lack of concordance to various combinations of parasite divergence (duplication), extinction, and host switching events (Table 2). Additional tree-based analyses performed using TreeFitter (Ronquist, 1998, 2000) also detected significant cophylogeny between heteromyid rodents and their sucking lice (data available on request).

All data-based analyses (LRTs) revealed significant differences ($P < 0.05$; data not shown) between the host and parasite data sets (the *Heteromys* and *Perognathus* clades were not included in the LRTs because neither taxon showed significant concordance in the tree-based tests of cophylogeny). Additional data-based analyses (KH and SH tests) were concordant with the results from the LRTs (data available on request). The null hypothesis that the data sets underlie identical topologies is therefore

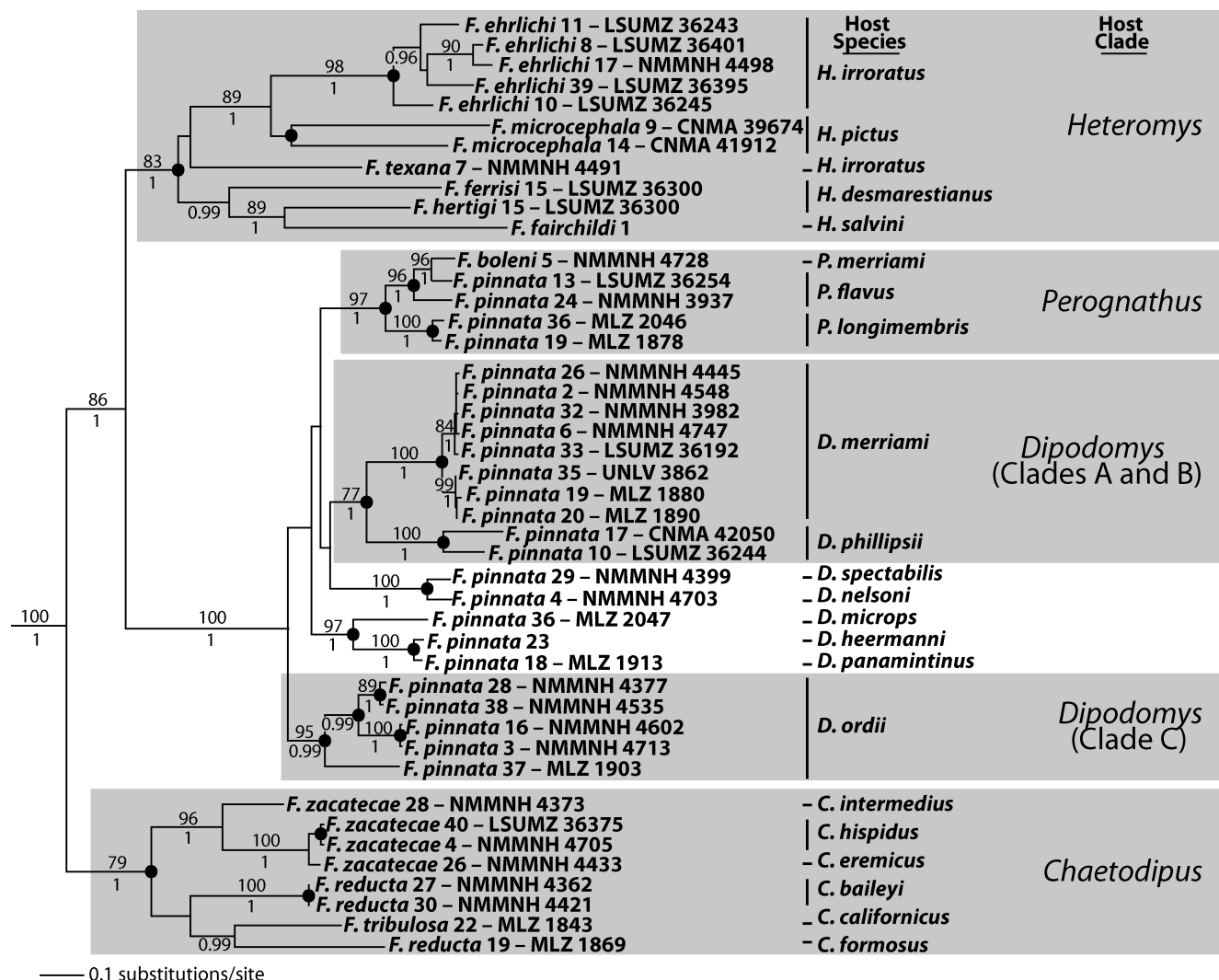


FIGURE 3. Maximum likelihood phylogram for lice of the genus *Fahrenholzia* based on sequence data from the mitochondrial cytochrome c oxidase subunit I (COI) gene. Maximum likelihood bootstrap support values greater than 75% and Bayesian posterior probability values greater than 95% are indicated above and below the nodes, respectively. Species names are followed by locality number and museum specimen number for the host (Appendix 1). Host associations are listed to the right of the phylogram. Shaded boxes surround clades of lice that parasitize monophyletic clades of rodents. Shaded circles indicate nodes for which relative divergence times were estimated.

rejected, meaning that the differences between the host and parasite trees likely result from biological causes, not sampling error.

Relative Timing of Divergence Events and Relative Rates of Evolution

Likelihood-ratio tests of all nucleotide substitutions (1017 in the rodents, and 1011 in the lice) and substitutions at third codon positions only (339 and 337, respectively) for the pruned host and parasite trees (including only putative codiverging taxa) showed that rates of substitution did not depart significantly from a molecular clock ($P > 0.05$).

All Model II regression analyses showed a significant relationship between branch lengths in the host and parasite trees (Kendall's tau $[\tau]$, $P < 0.001$; Table 3). These

tests included all combinations of models (hLRT, AIC, or uncorrected p distances), sequence data (all substitutions or third codon positions only), and taxa (all putative codiverging taxa in the host and parasite trees or terminal taxa only).

Model II regression analyses using all sequence data and all branches linked to analogous nodes (shaded circles at nodes in Fig. 5) showed a significant relationship between host and parasite branch lengths (Table 3; Fig. 6a). Regression analyses restricted to terminal pairs of host and parasite taxa (asterisks at nodes in Fig. 5) yielded slopes ranging from 1.78 to 2.80 (all significantly greater than 1.0) regardless of the model used or data set analyzed (Table 3; Fig. 6b). These results show that nucleotide substitutions in the COI gene are occurring at approximately two to three times the rate in sucking lice compared to heteromyid rodents. The y -intercepts in

TABLE 1. Summary of results from distance-based and tree-based analyses of cophylogeny in heteromyid rodents and their parasitic sucking lice. Detailed results from TreeFitter analyses are available upon request. "Yes" indicates that significant congruence was detected when the data set (designated by host clade in left-hand column) was analyzed using the specified method. Distance-based (ParaFit) analyses of individual host-parasite pairs are shown. Distance-based analyses performed on all data in the full host and parasite trees (global tests of association; not shown) also were statistically significant ($P < 0.001$; see text).

	Distance-based analyses ParaFit ^a	Tree-based analyses	
		TreeMap	TreeFitter
Pruned comparison	—	Yes	Yes
Within <i>Heteromys</i>	Yes	No	No
Within <i>Dipodomys</i> clade A	Yes	Yes	Yes
Within <i>Dipodomys</i> clade B	Yes	No	No
Within <i>Dipodomys</i> clade C	Yes	Yes	Yes ^b
Within <i>Perognathus</i>	No	No	No
Within <i>Chaetodipus</i>	Yes	Yes	Yes

^aPairwise comparisons.

^bTopological congruence between host and parasite phylogenies was marginally significant between *Dipodomys ordii* and their lice ($0.05 < P < 0.07$).

all regression analyses restricted to terminal taxa were negative and significantly different from zero (range -0.02 to -0.23 ; Table 3), indicating presence of a time lag between host divergence events and parasite divergence events (Hafner and Nadler, 1990).

DISCUSSION

Tests of Cophylogeny

The host and parasite trees used in these analyses are based on sequence data from a single mitochondrial gene and, as a result, represent gene trees rather than species phylogenies. However, our COI-based rodent and louse trees closely resemble trees based on morphology and other nuclear-encoded characters (e.g., Genoways and Brown, 1993; Light and Hafner, 2007a; J.E.L., unpublished data), and much of our study is focused at the species level and above, thus reducing the likelihood of incomplete lineage sorting.

All distance-based and tree-based tests revealed significant topological similarity between the phylogenies for heteromyid rodents and their sucking lice of the genus

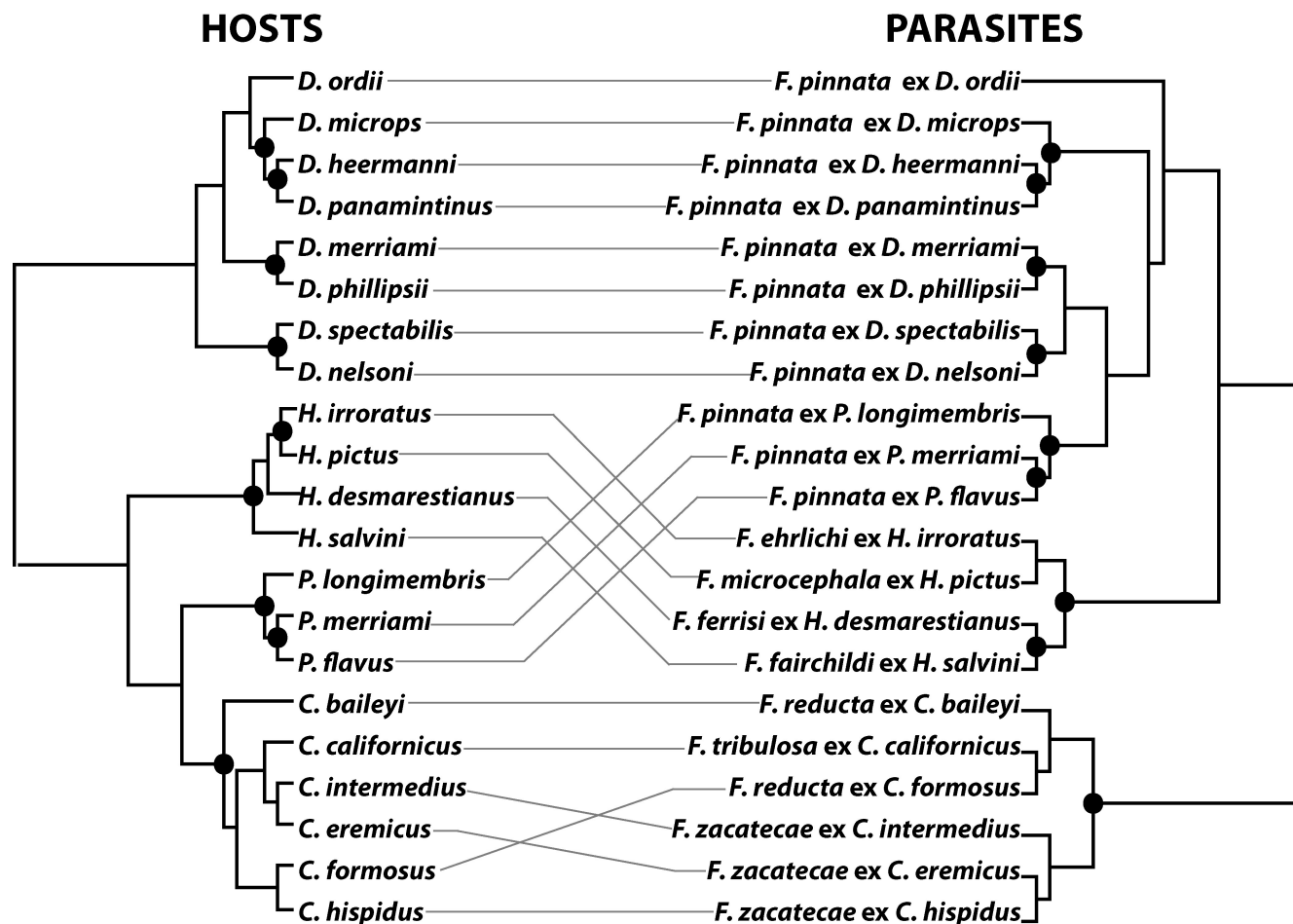


FIGURE 4. Results of reconciliation analysis (TreeMap 2.0β) for heteromyid rodents and their ectoparasitic lice of the genus *Fahrenholzia*. Gray lines between taxa indicate host-parasite associations. Shaded circles at nodes indicate instances of perfect cophylogeny (i.e., putative codivergence events). The number of reconstructed codivergence events (Table 2) was greater than expected by chance ($P < 0.001$).

TABLE 2. Results of TreeMap analyses comparing pruned trees (see text and Fig. 4) and major clades (shaded boxes in Figs. 2 and 3) in heteromyid rodents and their sucking lice of the genus *Fahrenholzia*. Columns show the cost, number of each event type necessary to reconcile the host and parasite trees, and number of solutions (equally probable reconstructions).

Comparison	Cost	Codivergence	Duplication	Extinction	Host switching	Solutions
Pruned comparison	38	26 ^a	14	23	1	1
<i>Heteromys</i> comparison	21	8	10	6	5	8
<i>Dipodomys</i> comparison A	0	8 ^a	0	0	0	1
<i>Dipodomys</i> comparison B	4	6	2	1	1	2
<i>Dipodomys</i> comparison C	0	8 ^a	0	0	0	1
<i>Perognathus</i> comparison	4	8	2	1	1	2
<i>Chaetodipus</i> comparison	9	8 ^a	6	0	3	1

^a The observed value of codivergence events between host and parasite trees is significantly greater than chance ($P < 0.05$).

Fahrenholzia (Table 1). Concordance between host and parasite trees and data sets was not absolute, however, and varied within the major clades of rodents and lice examined. In general, evidence for cophylogeny was strongest in kangaroo rats (*Dipodomys*) and chaetodipine pocket mice (*Chaetodipus*) and weakest in spiny pocket mice (*Heteromys*) and silky pocket mice (*Perognathus*). Differences in patterns of congruence across the host and parasite trees cannot be explained by host phylogeny alone, given that *Chaetodipus* (significant cophylogeny) is sister to *Perognathus* (no cophylogeny). Neither can the differences be explained by parasite phylogeny alone—the two clades of lice that show cophylogeny with *Dipodomys* and *Chaetodipus* hosts are not sister clades within *Fahrenholzia*. There are no known behavioral or ecological differences that might promote or impede louse transfer in clades that show cophylogeny compared to those that do not. At this point, we can only postulate that cophylogeny between heteromyid rodents and their sucking lice is driven by low vagility of the lice coupled with generally asocial behavior of the hosts (Eisenberg, 1963). Causes of among-clade differences in degree of cophylogeny remain unknown.

Relative Timing of Divergence Events

As mentioned earlier, host and parasite phylogenies can show significantly similar, even identical, branching patterns for reasons other than codivergence or cospeciation. Thus, documentation of significant cophylogeny between heteromyid rodents and their sucking lice is necessary, but not sufficient, to conclude that the two lineages have a shared history of codivergence. For this, we need to show a temporal linkage between divergence events in the host and parasite phylogenies; in other words, we must address the question of whether divergence events occurred not only in the same *pattern* but also at the same *time* in the hosts and parasites.

One approach is to use dated fossils to calibrate the host and parasite trees and then compare estimated divergence dates of putative codivergence events in the two trees. Although heteromyid rodents are known from multiple dated fossils (Reeder, 1956; Wahlert, 1993), the louse fossil record is almost nonexistent (Dagleish et al., 2006; Smith et al., 2007). Cross-calibration (use of a calibration point in one tree to calibrate the other) has been used in several studies of cophylogeny (e.g., Percy et al., 2004; Sorenson et al., 2004; Switzer et al., 2005; Reed et al., 2007), but this approach is inherently circular if estimated

TABLE 3. Results of Model II regression analysis comparing estimated branch lengths in the heteromyid phylogeny to corresponding branch lengths in the phylogeny of their sucking lice to investigate relative timing of divergence events and rate of molecular evolution in hosts and parasites. Analogous host and parasite branches were compared for all corresponding nodes (shaded circles at nodes in Figs. 2, 3, 5) and for terminal sister taxa of hosts parasitized by terminal sister taxa of lice. Branch lengths were estimated using the model of evolution selected by the hierarchical likelihood-ratio test (hLRT; Akaike information criterion results available upon request) as well as uncorrected p distances (pdist), using all codon positions and third-codon positions only (see text). Slopes of the regressions reflect rate of nucleotide substitution in the sucking lice relative to their hosts, and y -intercepts indicate whether lice diverged before (intercept > 0), after (intercept < 0), or coincident with (slope not significantly different from zero) their hosts (Hafner and Nadler, 1990). Ninety-five percent confidence intervals (CIs) are given for both the slope of the regressions and the y -intercept. The slope of each regression was estimated by Kendall's nonparametric robust line-fit method (Kendall and Gibbons, 1990) and significance was determined by use of Kendall's tau (τ). Two of these comparisons (indicated by asterisks) are shown graphically in Figure 6.

Comparison	Slope (95% CI)	Y-intercept (95% CI)	Kendall's τ
All codon positions			
hLRT: All analogous branches	2.91 (2.06, 4.68)	-0.09 (-0.22, -0.03)	0.575 ($P < 0.001$)
Terminal pairs only	2.50 (1.88, 3.56)	-0.06 (-0.12, -0.02)	0.680 ($P < 0.001$)
pdist: All analogous branches	2.53 (1.91, 3.61)	-0.03 (-0.06, -0.01)	0.637 ($P < 0.001$)
Terminal pairs only	2.18 (1.68, 3.00)	-0.02 (-0.04, -0.01)	0.765 ($P < 0.001$)
Third codon positions only			
hLRT: All analogous branches	3.36 (2.37, 5.48)	-0.37 (-0.91, -0.12)	0.545 ($P < 0.001$)
*Terminal pairs only	2.80 (2.19, 3.78)	-0.23 (-0.42, -0.12)	0.752 ($P < 0.001$)
pdist: All analogous branches	2.13 (1.57, 3.14)	-0.07 (-0.15, -0.02)	0.570 ($P < 0.001$)
Terminal pairs only	1.78 (1.40, 2.36)	-0.05 (-0.09, -0.02)	0.725 ($P < 0.001$)

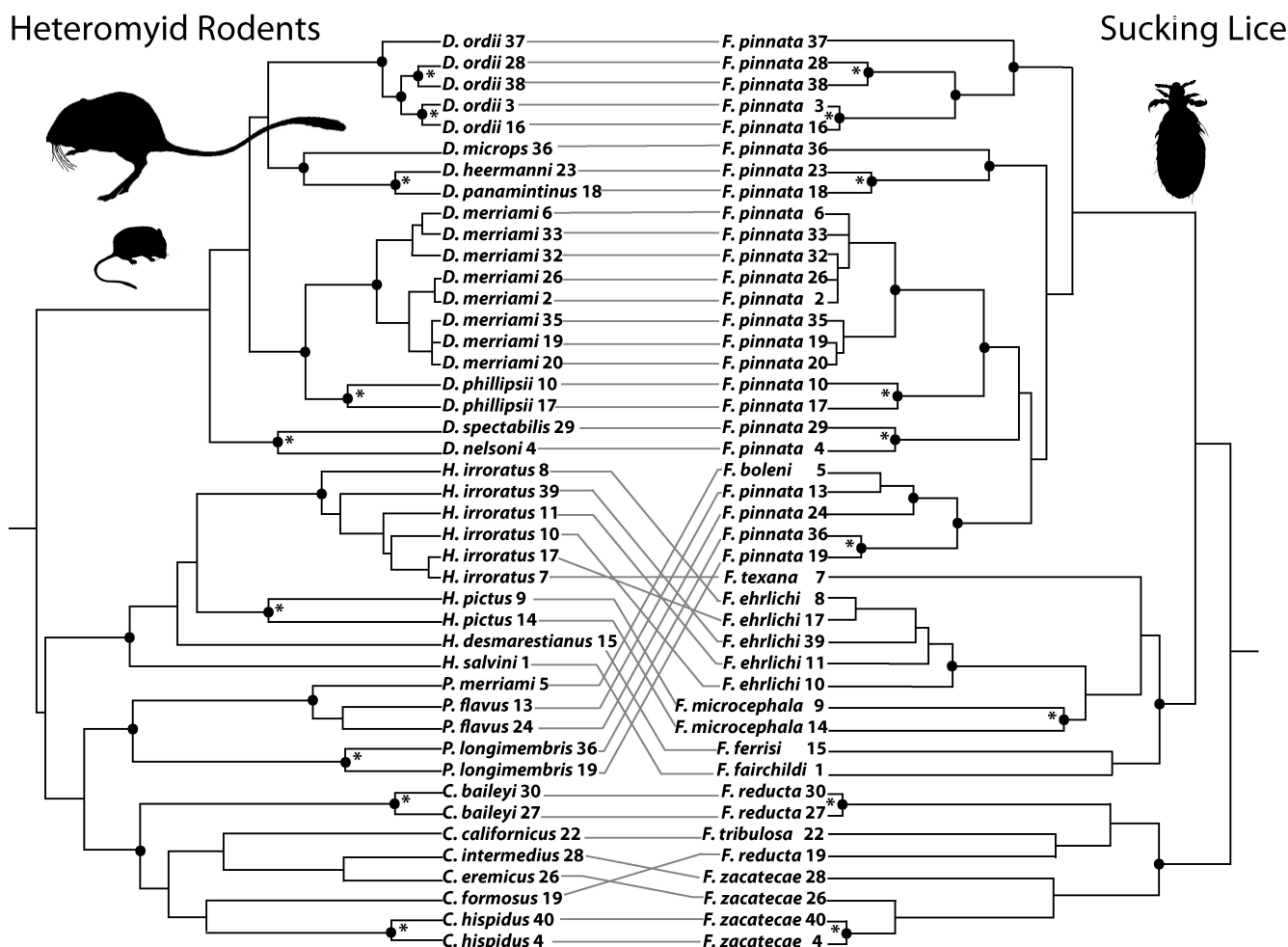


FIGURE 5. Comparison of cladograms for heteromyid rodents and their sucking lice of the genus *Fahrenholzia* (parasite tree does not include *F. hertigi*; see text). Gray lines link parasites with the hosts from which they were sampled. Shaded circles and asterisks indicate nodes for which relative divergence times and evolutionary rates were estimated, respectively. Species names are followed by locality number (Fig. 1), and museum specimen numbers are available in Appendix 1.

dates are then used to document codivergence or cospeciation.

In the absence of louse fossils to calibrate the louse tree, another approach is to use relative dating methods to explore possible temporal linkages between the rodent and louse trees. Relative dating was used in Hafner and Nadler's (1990) analysis of phylogenetic congruence in pocket gophers and chewing lice, and this approach was examined in depth by Page (1991). Page (1991) emphasized that the nodes of a phylogenetic tree often are autocorrelated, with the level of autocorrelation depending on tree balance; i.e., nodes of highly unbalanced (or pectinate) trees show high autocorrelation, whereas nodes of balanced trees show lower levels of autocorrelation. Thus, if the host and parasite trees being compared are highly unbalanced, there may be a significant relationship between the relative node heights (or branch lengths) in the two trees simply because of the autocorrelation among nodes in each of the separate trees.

The heteromyid rodent and sucking louse trees (Figs. 2 and 3) are well balanced and, therefore, less likely to

show high levels of autocorrelation (Page, 1991). Comparison of branch lengths for putative codiverging taxa (shaded circles at nodes in Fig. 5) shows a highly significant relationship between analogous host and parasite branches (Table 3; Fig. 6a). This comparison remained significant when different models of molecular evolution and different data partitions (all substitutions and substitutions at third codon positions only) were used. However, not knowing the degree to which autocorrelation may affect the relationship between branch lengths in the host and parasite trees, we avoided the potential problem of autocorrelation by restricting the analysis of divergence times to terminal sister taxa of hosts that are parasitized by terminal sister taxa of lice (asterisks at nodes in Fig. 5). These statistically independent nodes also showed a significant relationship in the Model II regression analyses of host and parasite branch lengths (Table 3; Fig. 6b).

The branches of terminal sister taxa of hosts (and of parasites) may be similar in length because of the close phylogenetic relationship between these taxa. To address

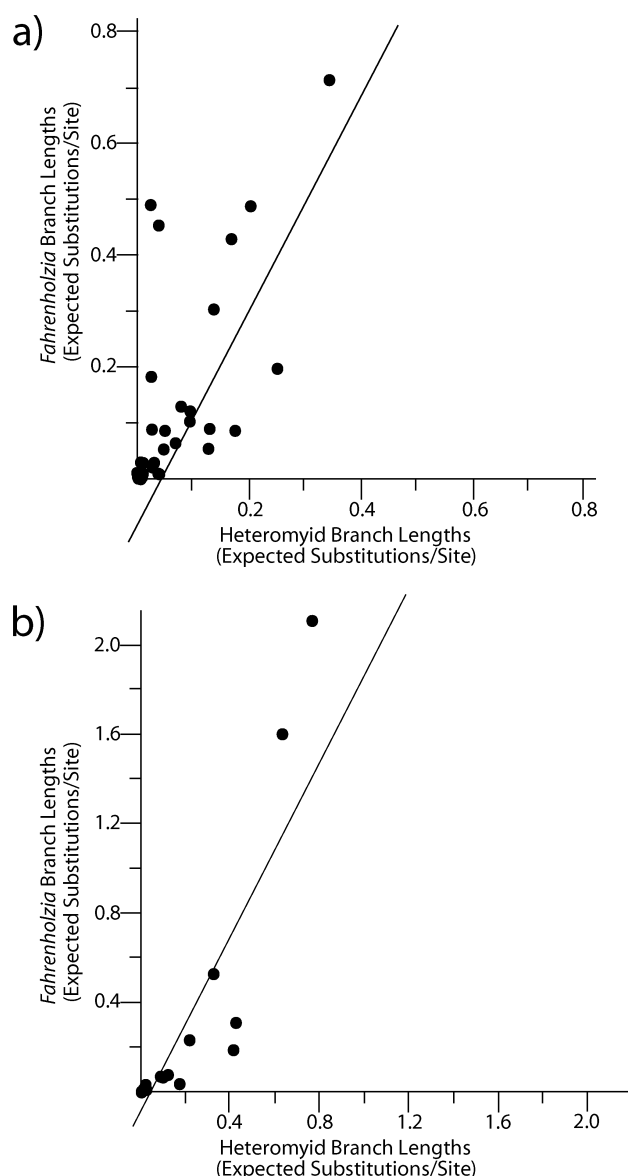


FIGURE 6. Bivariate plot of branch lengths for two of the comparisons listed in Table 3. (a) Plot of analogous host and parasite branches based on all nucleotide substitutions. The relationship between the variables is highly significant (Kendall's $\tau = 0.566$, $P < 0.001$), indicating that divergence events in the hosts and parasites were approximately contemporaneous. The relationship remains significant ($\tau = 0.551$, $P < 0.001$) when the outlying point is removed. (b) Plot of analogous host and parasite branches for terminal sister taxa of hosts parasitized by terminal sister taxa of lice based on nucleotide substitutions at third-codon positions only. The relationship between the variables is highly significant ($\tau = 0.752$, $P < 0.001$). The slope of the line (Model II regression analysis) is 2.80, with a y -intercept significantly less than zero (-0.23). This indicates that the rate of synonymous substitutions in this gene region is roughly two to three times faster in sucking lice compared to their hosts and that parasite divergence was, on average, slightly delayed relative to host divergence.

the possibility of phylogenetic bias in our analysis of branch lengths, we repeated the regression analyses using only one branch (selected randomly) from each pair of terminal sister taxa of hosts and compared it to the corresponding branch from the parasites. Although this

method reduced the number of branch length comparisons by half (only one, rather than two, comparisons per pair of terminal taxa), the resulting regression coefficients were still highly significant (data available on request).

How Contemporaneous are "Contemporaneous Codivergence Events"?

Significant results in the Model II regression analyses (Table 3) show that divergence events in the rodents and lice were approximately contemporaneous. If they were *exactly* contemporaneous, we would expect the y -intercept of the regression plot to pass through zero, regardless of the slope of the line. If, however, divergence in the lice consistently lagged behind divergence in the rodents by a roughly consistent amount of time, then we would expect the y -intercept to be significantly less than zero (i.e., the hosts always show a small amount of genetic divergence before the parasites begin diverging).

The y -intercepts in all four regression analyses restricted to terminal taxa (Table 3) are significantly less than zero (i.e., the 95% confidence interval of the intercept does not include zero). Individual intercepts range from -0.02 to -0.23 (depending on model and data partition), with a mean of -0.09 . Our conclusion is that divergence events in sucking lice consistently lag behind divergence events in heteromyid rodents. Because dated heteromyid fossils are available to calibrate the heteromyid COI tree, we can make a rough estimate of the length of the delay (in years) between rodent divergence and louse divergence. The oldest fossil *Dipodomys* dates from 12.5 to 15.9 million years ago (Ma; Reeder, 1956; Hafner et al., 2007). When considering only model-corrected substitutions at third codon positions (these data should provide an estimate of substitution rate that is closer to the actual mutation rate of the gene; see above), the mean patristic distance between the basal *Dipodomys* node in Figure 2 and all terminal *Dipodomys* taxa is 1.512 expected substitutions per site (ESS). Because these COI sites are evolving in a roughly clock-like manner, we can estimate an average rate of substitution by dividing the total amount of change (1.512 ESS) by the minimum and maximum fossil dates (12.5 and 15.9 Ma). This yields an average rate of change between 0.0951 and 0.1210 ESS/my over the past 12.5 to 15.9 million years (Myr). We can do the same for the oldest fossil perognathine (*Perognathus* + *Chaetodipus*), which dates from 20 to 22 Ma (James, 1963; Hafner et al., 2007). The mean patristic distance between the basal perognathine node in Figure 2 and all terminal perognathine taxa (again, considering substitutions at third codon positions only) is 2.164 ESS. Thus, these sites in the COI gene of perognathines have changed at an estimated average rate of 0.0984 to 0.1082 ESS/my over the past 20 to 22 Myr. Although they were calculated using different fossil calibrations, the range of rate estimates for perognathines is similar to (and contained within) the range of rate estimates for *Dipodomys*. In a bivariate plot of host and parasite branch lengths (e.g.,

Fig. 6), the x -intercept of the line is proportional to the time lag between host and parasite codivergence. We can estimate the x -intercept in our rodent-louse data from the regression equations (Table 3; hLRT model for third codon positions for terminal taxa only—length of terminal branches is less likely to be underestimated due to saturation). Doing this yields minimum and maximum x -intercept estimates of 0.0317 ESS and 0.1918 ESS, respectively. These values can then be converted into years by dividing them by the maximum and minimum rate estimates for *Dipodomys* and perognathines calculated above, which yields estimates of from 262,000 to 2,016,800 years between rodent and louse divergence events.

Our estimate of the time lag between host and parasite divergence events is potentially subject to several sources of error. For example, the long period of time over which average rates of substitution are estimated in heteromyids (up to 22 Ma) raises the possibility that our evolutionary models do not correct sufficiently for substitution saturation in our sequence data. If saturation remains in the data set, then our rate estimates will be lower than the true rate of COI evolution in *Dipodomys* and perognathines, which would inflate our estimate of the time lag. Also, because we have evidence that the lice are evolving more rapidly than their hosts, it is possible that louse divergences are more seriously underestimated than host divergences, which would have the effect of lowering the y -intercept and thus inflating our estimate of the time lag. Similarly, estimates of slopes and intercepts in the Model II regression analyses are crude, as evidenced by the spread of points around the lines in Figure 6 and the large confidence intervals in Table 3. Finally, we are relying on fossils that may be incorrectly placed in the heteromyid phylogeny, and the age estimates of these fossils also may be crude (Hafner et al., 2007).

Despite these caveats, our estimate of the time lag may be realistic, and the period of delay between host and parasite codivergence events may be quite long if gene flow continues in the parasites long after their hosts have diverged. This scenario, termed “failure to speciate” (Johnson et al., 2003), was invoked by Banks and Paterson (2005) to explain the existence of “multi-host parasites” (a single parasite species that occurs on multiple host species), which are common in the genus *Fahrenholzia* (Fig. 5). Although it is difficult to imagine a cause-and-effect relationship between divergence events that happened more than 200,000 years apart, we have no context in which to evaluate what constitutes a “long” time lag. Existence of a time lag, large or small, certainly does not falsify the hypothesis of codivergence—in fact, the length of the time lag could not be estimated without first assuming codivergence—but the traditional view of cospeciation as “contemporaneous speciation events” may need to be broadened to include divergence events in parasites that occur long after divergence events in their hosts. As pointed out by Banks and Paterson (2005), failure to speciate supports association by descent.

Relative Rates of Evolution in Rodents and Lice

If rates of nucleotide substitution in the COI gene in co-diverging hosts and parasites are approximately equal, then corresponding host and parasite branch lengths will be approximately equal and the slope of a bivariate plot of analogous branch lengths will not be significantly different from 1.0. However, if one of the two partners is evolving more rapidly than the other, then the first partner's branches will be consistently longer and the slope of the line will be significantly different from 1.0. The slopes in all four regression analyses restricted to terminal taxa (Table 3) are significantly greater than 1.0 (i.e., the 95% confidence interval of the slope does not include 1.0). Individual slopes range from 1.78 to 2.80 (depending on model and data partition), with a mean of 2.32. Our conclusion is that the COI gene in sucking lice is evolving approximately two to three times faster than the same gene in heteromyid rodents.

In the only other study of evolutionary rates in sucking lice, Reed et al. (2004) showed that mitochondrial genes (COI and cytochrome *b* [Cyt-*b*]) in primate sucking lice (families Pediculidae, Pedicinidae, and Pthiridae) were evolving approximately twice as fast as control region sequences in their primate hosts. In several studies of evolutionary rates in chewing lice (Ischnocera), mitochondrial genes (COI, Cyt-*b*, and 12S) in the parasites were shown to evolve roughly two to four times faster than the same genes in their vertebrate hosts (Hafner et al., 1994; Page, 1996; Page et al., 1998, 2004; Paterson and Banks, 2001; Light and Hafner, 2007b). Thus, all evidence available to date is consistent with the proposition that mitochondrial genes in both chewing and sucking lice (Insecta: Phthiraptera) are evolving roughly two to four times faster than the same genes in their vertebrate hosts.

There are many fundamental biological differences between insects and their vertebrate hosts that could explain the molecular rate differences observed in this and the other studies cited above. However, before seeking biological causes for rate differences, researchers may want to consider potential stochastic causes, such as genetic drift. The fact that colonization of a new host individual by a parasite often involves a population bottleneck, coupled with the fact that most parasites experience a level of population structuring not experienced by their hosts (i.e., a population of hosts may support many individual populations of parasites, one per host), may increase the likelihood of genetic drift causing accelerated rates of loss and fixation of alleles in parasites relative to their hosts.

Documentation of Codivergence

In this study, we have used multiple methods to show statistically significant correspondence between branch structure in the phylogenies of heteromyid rodents and sucking lice (Table 1). Because evolutionary events other than codivergence or cospeciation may have caused significant topological concordance between the host and parasite trees (e.g., resource tracking, sequential colonization, or an assortment of historical processes such

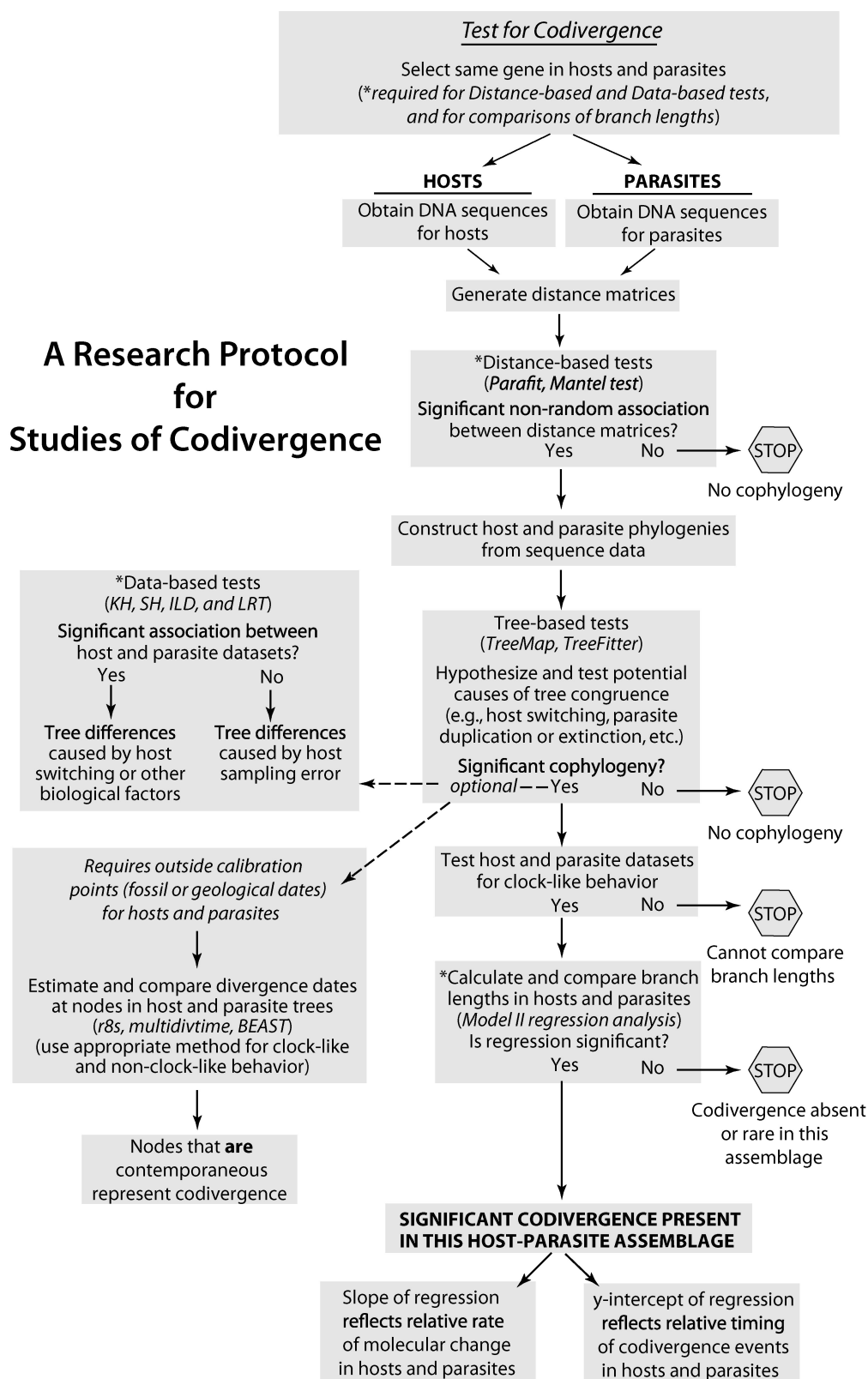


FIGURE 7. Schematic of a research protocol for examining codivergence in symbiotic organisms. Choice of methods will vary depending on whether the same (indicated by an asterisk) or different genes are used to construct host and parasite phylogenies.

as host switching, parasite extinction, or parasite speciation), we examined relative timing of putative codivergence events in the two trees. As a proxy for time, we used branch lengths, which were significantly related in the host and parasite trees. The only plausible cause for this significant relationship, other than codivergence, is autocorrelation among branch lengths in each of the two trees, which we removed by examining only terminal pairs of putatively codiverging hosts and parasites. The combined power of these two tests, one based on pattern, the other on timing, leads to a strong inference of codivergence in this host-parasite assemblage.

Future Directions in the Study of Codivergence

The research protocol used in our analysis involves a series of linked analyses, each of which tests a different null hypothesis of host-parasite, insect-plant, or other symbiotic association (Huelsenbeck et al., 2003). The sequence in which these tests are conducted, which we have shown schematically in Figure 7, is important because the results of earlier tests provide the rationale for either stopping the analysis or conducting additional tests. Researchers who use morphological data or sequence different genes in the hosts and parasites can use tree-based analyses to test the null hypothesis that host and parasite topologies are identical. If dated fossils are available to calibrate the host and parasite trees, these researchers also can compare estimated divergence dates in host and parasite trees (constructed using molecular data) to test the null hypothesis that putative speciation times in the two groups were approximately contemporaneous.

Studies comparing the same gene in hosts and parasites have the potential to provide considerably more insight into the history of a host-parasite association. Such studies can incorporate the above tests, but they also can use distance-based and data-based methods to explore the host-parasite association in greater detail. Distance-based methods test the null hypothesis that the host and parasite data sets are randomly associated; if they are, then cophylogeny is rejected at an early stage in the analysis (Fig. 7). If significant cophylogeny is documented using distance-based and tree-based methods, data-based analyses can be performed to help distinguish between random and biologically meaningful causes for discordance between host and parasite phylogenies.

Perhaps the most important advantage of sequencing the same gene in the hosts and parasites is that these data can be used to compare analogous branch lengths in the host and parasite trees to test for temporal concordance of putative codivergence events without the need for fossil or geological calibration of the trees. Analyses based on relative, rather than absolute, time bypass the many uncertainties associated with fossils (Rutschmann et al., 2007), including the variance associated with dating of fossils, questionable position of the fossil in the phylogeny, and absence of dated fossils altogether. Branch length comparisons based on the same gene test the null hypothesis that codivergence events were exactly con-

temporaneous in hosts and parasites and also test the hypothesis that rates of molecular change in the hosts and parasites have been identical throughout the history of their association. Whether these hypotheses are accepted or rejected, the results of these tests provide fascinating insight into the history of a host-parasite relationship—insights that could not be discovered by any other means.

The literature to date suggests that contemporaneous speciation in associated taxa (cospeciation) may be an uncommon phenomenon in nature and, as Lopez-Vaamonde et al. (2006) suggest, cospeciation may be the exception, rather than the rule, in plant-herbivore associations. Significant cophylogeny has been documented using distance-based and tree-based methods in only a few symbiotic associations, and in many of these instances, estimates of divergence times have shown that presumed codivergence events are temporally implausible (e.g., Percy et al., 2004; Sorenson et al., 2004; Lopez-Vaamonde, 2006). Very few researchers, including Ronsted et al. (2005), Switzer et al. (2005), and ourselves, have been fortunate enough to study an association in which estimates of relative or absolute divergence times document temporal plausibility of putative codivergence events identified in tree-based analyses. We find it is somehow fitting that the genus of louse examined in this study, which was named in honor of the pioneer anopluran specialist, Heinrich Fahrenholz, shows a relationship with its hosts that is generally consistent with Fahrenholz's rule: *Parasite phylogeny mirrors host phylogeny* (Eichler, 1942).

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APPENDIX 1. Heteromyid rodent and *Fahrenholzia* louse taxa included in the phylogenetic and cophylogenetic analyses. Lice are grouped by country, state, and host locality and are mapped in Figure 1. The two instances in which the exact host specimen was not available (see text) are noted by asterisks. Abbreviations for host genera are as follows: *C* = *Chaetodipus*; *D* = *Dipodomys*; *H* = *Heteromys* (following the taxonomy of Hafner et al. [2007], which synonymizes *Liomys* with *Heteromys*); and *P* = *Perognathus*. Museum acronyms for host taxa are as follows: Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México (CNMA), Louisiana State University Museum of Natural Science (LSUMZ), Moore Laboratory of Zoology, Occidental College (MLZ), New Mexico Museum of Natural History (NMMNH), and University of Nevada Las Vegas (UNLV).

Locality number and locality	Host species	Fahrenholzia species
Costa Rica		
1. Host: Puntarenas; 5 km S, 6 km W Esparza Louse: Guanacaste; Santa Rosa National Park	<i>H. salvini</i> 1—LSUMZ 28358	<i>F. fairchildi</i> 1*
Mexico		
2. Chihuahua; 6 mi NW Ricardo Flores Magón	<i>D. merriami</i> 2—NMMNH 4548	<i>F. pinnata</i> 2—NMMNH 4548
3. Coahuila; 2 mi E Agua Nueva	<i>D. ordii</i> 3—NMMNH 4713	<i>F. pinnata</i> 3—NMMNH 4713
4. Coahuila; 5 km S, 16 km W General Cepeda	<i>D. nelsoni</i> 4—NMMNH 4703	<i>F. pinnata</i> 4—NMMNH 4703
4. Coahuila; 5 km S, 16 km W General Cepeda	<i>C. hispidus</i> 4—NMMNH 4705	<i>F. zacatecae</i> 4—NMMNH 4705
5. Coahuila; Plan de Guadalupe	<i>P. merriami</i> 5—NMMNH 4728	<i>F. boleni</i> 5—NMMNH 4728
6. Coahuila; 2 km S Santa Teresa	<i>D. merriami</i> 6—NMMNH 4747	<i>F. pinnata</i> 6—NMMNH 4747
7. Durango; Hda. Atotonilco	<i>H. irroratus</i> 7—NMMNH 4491	<i>F. texana</i> 7—NMMNH 4491
8. Jalisco; 16 km NNE Ameca	<i>H. irroratus</i> 8—LSUMZ 36401	<i>F. ehrlichi</i> 8—LSUMZ 36401
9. Jalisco; 4.5 km SW Jilotlán	<i>H. pictus</i> 9—CNMA 39674	<i>F. microcephala</i> 9—CNMA 39674
10. Puebla; 11 km (by road) SW Alchichica	<i>D. phillipsii</i> 10—LSUMZ 36244	<i>F. pinnata</i> 10—LSUMZ 36244
10. Puebla; 11 km (by road) SW Alchichica	<i>H. irroratus</i> 10—LSUMZ 36245	<i>F. ehrlichi</i> 10—LSUMZ 36245
11. Puebla; 3 km (by road) NE Tilapa	<i>H. irroratus</i> 11—LSUMZ 36243	<i>F. ehrlichi</i> 11—LSUMZ 36243
13. Puebla; 3.1 km SW El Veladero	<i>P. flavus</i> 13—LSUMZ 36254	<i>F. pinnata</i> 13—LSUMZ 36254
14. Veracruz; Biological Station La Mancha	<i>H. pictus</i> 14—CNMA 41912	<i>F. microcephala</i> 14—CNMA 41912
15. Veracruz; 8 km ENE Catemaco	<i>H. desmarestianus</i> 15—LSUMZ 36300	<i>F. hertigi</i> 15—LSUMZ 36300
15. Veracruz; 8 km ENE Catemaco	<i>H. desmarestianus</i> 15—LSUMZ 36300	<i>F. ferrisi</i> 15—LSUMZ 36300
16. Zacatecas; 1 mi SE Bañón	<i>D. ordii</i> 16—NMMNH 4602	<i>F. pinnata</i> 16—NMMNH 4602
17. Zacatecas; 2 mi E San Jerónimo	<i>D. phillipsii</i> 17—CNMA 42050	<i>F. pinnata</i> 17—CNMA 42050
17. Zacatecas; 2 mi E San Jerónimo	<i>H. irroratus</i> 17—NMMNH 4498	<i>F. ehrlichi</i> 17—NMMNH 4498
United States: California		
18. Mono Co.; 5 mi N Benton	<i>D. panamintinus</i> 18—MLZ 1913	<i>F. pinnata</i> 18—MLZ 1913
19. San Bernardino Co.; 8.9 mi N, 1.1 E Red Mountain	<i>C. formosus</i> 19—MLZ 1869	<i>F. reducta</i> 19—MLZ 1869
19. San Bernardino Co.; 8.9 mi N, 1.1 E Red Mountain	<i>P. longimembris</i> 19—MLZ 1878	<i>F. pinnata</i> 19—MLZ 1878
19. San Bernardino Co.; 8.9 mi N, 1.1 E Red Mountain	<i>D. merriami</i> 19—MLZ 1880	<i>F. pinnata</i> 19—MLZ 1880
20. San Bernardino Co.; 3.2 mi S, 3.7 mi W Westend	<i>D. merriami</i> 20—MLZ 1890	<i>F. pinnata</i> 20—MLZ 1890
22. San Luis Obispo Co.; 15.9 mi S, 7.2 mi E Simmler	<i>C. californicus</i> 22—MLZ 1843	<i>F. tribulosa</i> 22—MLZ 1843
23. Host: San Luis Obispo Co.; 15 mi S, 8.2 mi E Simmler Louse: Fresno Co.	<i>D. heermanni</i> 23—MLZ 1852	<i>F. pinnata</i> 23*
United States: New Mexico		
24. Cibola Co.; 8.5 mi S, 5 mi W Correo	<i>P. flavus</i> 24—NMMNH 3937	<i>F. pinnata</i> 24—NMMNH 3937
26. Doña Ana Co.; W. Las Cruces, 1 mi S jct. I-10 & Picacho Ave	<i>C. eremicus</i> 26—NMMNH 4433	<i>F. zacatecae</i> 26—NMMNH 4433
26. Doña Ana Co.; W. Las Cruces, 1 mi S jct. I-10 & Picacho Ave	<i>D. merriami</i> 26—NMMNH 4445	<i>F. pinnata</i> 26—NMMNH 4445
27. Grant Co.; 1.7 mi N, 0.5 mi E Redrock	<i>C. baileyi</i> 27—NMMNH 4362	<i>F. reducta</i> 27—NMMNH 4362
28. Grant Co.; 2.6 mi N, 1.8 mi E Redrock	<i>C. intermedius</i> 28—NMMNH 4373	<i>F. zacatecae</i> 28—NMMNH 4373
28. Grant Co.; 2.6 mi N, 1.8 mi E Redrock	<i>D. ordii</i> 28—NMMNH 4377	<i>F. pinnata</i> 28—NMMNH 4377
29. Hidalgo Co.; 6 mi SE Portal, (Cochise Co., Arizona)	<i>D. spectabilis</i> 29—NMMNH 4399	<i>F. pinnata</i> 29—NMMNH 4399
30. Hidalgo Co.; Doubtful Canyon, 8 mi N, 1 mi W Steins	<i>C. baileyi</i> 30—NMMNH 4421	<i>F. reducta</i> 30—NMMNH 4421
32. Socorro Co.; 13 mi S, 13 mi W San Marcial	<i>D. merriami</i> 32—NMMNH 3982	<i>F. pinnata</i> 32—NMMNH 3982
33. Socorro Co.; 5 mi N, 2 mi E Socorro	<i>D. merriami</i> 33—LSUMZ 36192	<i>F. pinnata</i> 33—LSUMZ 36192
United States: Nevada		
35. Clark Co.; Corn Creek Desert Wildlife Refuge	<i>D. merriami</i> 35—UNLV 3862	<i>F. pinnata</i> 35—UNLV 3862
36. Lyon Co.; 10.3 mi S, 2.2 E Yerington	<i>P. longimembris</i> 36—MLZ 2046	<i>F. pinnata</i> 36—MLZ 2046
36. Lyon Co.; 10.3 mi S, 2.2 E Yerington	<i>D. microps</i> 36—MLZ 2047	<i>F. pinnata</i> 36—MLZ 2047
37. Nye Co.; 19.2 mi N, 13.4 mi E Warm Springs	<i>D. ordii</i> 37—MLZ 1903	<i>F. pinnata</i> 37—MLZ 1903
United States: Texas		
38. Brewster Co.; Elephant Mountain WMA	<i>D. ordii</i> 38—NMMNH 4535	<i>F. pinnata</i> 38—NMMNH 4535
39. Cameron Co.; 8.8 mi E Brownsville (on Hwy 4)	<i>H. irroratus</i> 39—LSUMZ 36395	<i>F. ehrlichi</i> 39—LSUMZ 36395
40. Hidalgo Co.; Mission, 2519 Inspiration Road	<i>C. hispidus</i> 40—LSUMZ 36375	<i>F. zacatecae</i> 40—LSUMZ 36375