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# Phylogenetics and host associations of *Fahrenholzia* sucking lice (Phthiraptera: Anoplura)

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**Abstract.** Mitochondrial and nuclear DNA sequence data were used to reconstruct phylogenetic relationships for eleven of the twelve currently recognized species of *Fahrenholzia*, lice found only on rodents of the family Heteromyidae. Field collections included twenty of the thirty-three known host associations and resulted in the discovery of four new associations. Phylogenetic analyses of the mitochondrial and nuclear datasets were in general agreement, resulting in a well-resolved *Fahrenholzia* phylogeny. Analyses supported the monophyly of lice parasitizing the host subfamily Heteromyinae (spiny pocket mice). Lice parasitizing the genera *Chaetodipus* (pocket mice) and *Perognathus* (silky pocket mice) each represent monophyletic lineages. Phylogenetic patterns and levels of genetic differentiation suggest that the widespread *Fahrenholzia pinnata* may contain several cryptic species. Cryptic species may exist also within the less widely distributed species, *Fahrenholzia microcephala* and *Fahrenholzia reducta*.

## Introduction

Lice (Insecta: Phthiraptera) are obligate and permanent parasites of birds and mammals. Presently, four suborders are recognized: the chewing louse suborders Amblycera, Ischnocera and Rhynchophthirina, and the sucking louse suborder Anoplura. Sucking lice are ectoparasites only of eutherian (placental) mammals. These highly specialized blood-sucking insects live in close association with their hosts, requiring the hosts to complete their life cycle (2–4 weeks; Marshall, 1981). Anoplurans are morphologically adapted for life on mammals; being wingless, dorsoventrally flattened, possessing a single tarsal claw on each leg to cling to host hair, and having piercing mouthparts for feeding. Sucking lice feed directly from host blood vessels (termed solenophagy) as often as every 3 h (Buxton, 1947; Hocking, 1971; Nelson *et al*., 1977), and heavy infestations of lice can cause host anaemia (Peterson *et al*., 1953; Nelson *et al*., 1975). Anoplurans are important also in human epidemiology, where they serve as vectors of the causative agents of epidemic diseases such as trench fever, relapsing fever and louse-borne typhus (Kim *et al*., 1986).

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By contrast with the numerous phylogenetic studies of chewing lice (for example, see Hafner *et al*., 1994; Page *et al*., 1995; Banks *et al*., 2006; and references cited therein), there have been relatively few studies investigating the relationships amongst sucking lice (Kim & Ludwig, 1978a, b; Kim, 1988; Yong *et al*., 2003; Reed *et al*., 2004). This disparity between the two louse groups probably reflects both their difference in species diversity (there are more than 4000 species of chewing lice but fewer than 600 species of sucking lice currently recognized) and the often observed high prevalence and abundance of chewing lice on their hosts (Nadler *et al*., 1990; Lindell *et al*., 2002). High species diversity, prevalence and abundance make chewing lice model organisms for co-speciation studies, accompanied by increasing phylogenetic investigations (for example, see Hafner *et al*., 2002; Weckstein, 2004; and references cited therein). In contrast, sucking lice have rarely been studied for cospeciation (e.g. Reed *et al*., 2004), yet their blood-feeding habits and high host specificity (Marshall, 1981; Kim *et al*., 1986) suggest their suitability for future cospeciation studies, provided that robust phylogenies of sucking lice are available.

Presently, the 530 described species of sucking lice are assigned to fifty genera in fifteen families (Kim & Ludwig, 1978a; Durden & Musser, 1994a, b; Durden & Webb, 1999). Approximately two-thirds of these species parasitize rodents (Kim, 1988), as do most species in the large and cosmopolitan family Polyplacidae. Polyplacid lice of the genus *Fahrenholzia*

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Kellogg & Ferris, 1915 are restricted to New World rodents of the family Heteromyidae, which includes roughly 55 species divided into the subfamilies Dipodomyinae (kangaroo rats and kangaroo mice), Heteromyinae (spiny pocket mice) and Perognathinae (pocket mice). Heteromyid rodents are parasitized only by *Fahrenholzia* sucking lice, and other lice found on heteromyids (Morlan & Hoff, 1957; Beer *et al*., 1959; Allred, 1970; Johnson, 1972) appear to be accidental occurrences (straggling lice or contamination by the investigator) and not true host associations.

Currently, twelve species of *Fahrenholzia* are recognized: *F. boleni* McDaniel, 1968, *F. ehrlichi* Johnson, 1962, *F. fairchildi* Johnson, 1962, *F. ferrisi* Werneck, 1952, *F. hertigi* Johnson, 1962, *F. microcephala* Ferris, 1922, *F. pinnata* Kellogg & Ferris, 1915, *F. reducta* Ferris, 1922, *F. schwartzi* Werneck, 1952, *F. texana* Stojanovich & Pratt, 1961, *F. tribulosa* Ferris, 1922 and *F. zacatecae* Ferris, 1922 (Stojanovich & Pratt, 1961; Johnson, 1962; McDaniel, 1968; Kim *et al*., 1986; Whitaker *et al*., 1993). Known host associations indicate that *F. fairchildi*, *F. ferrisi*, *F. pinnata* and *F. reducta* parasitize multiple heteromyid species (Kim *et al*., 1986; Thomas *et al*., 1990; Whitaker *et al*., 1993; Durden & Musser, 1994a, b), and the host species *Heteromys desmarestianus*, *Liomys irroratus* and *Perognathus parvus* are parasitized by multiple species of *Fahrenholzia*. Only half of the currently recognized heteromyid species are parasitized by sucking lice, but the remaining species are likely to have been unsurveyed.

Relationships between the twelve *Fahrenholzia* species are unknown, although morphological descriptions of these species indicate similarity between species of the *F. microcephala* group (*F. microcephala*, *F. ehrlichi*, *F. ferrisi*, *F. schwartzi*, *F. fairchildi* and *F. hertigi*; Johnson, 1962). Morphology also identifies several pairs of similar taxa that may be sister species, including *F. zacatecae* and *F. tribulosa*, *F. ehrlichi* and *F. microcephala*, *F. ferrisi* and *F. schwartzi*, *F. texana* and *F. fairchildi*, and *F. boleni* and *F. pinnata* (Stojanovich & Pratt, 1961; McDaniel, 1968; Kim *et al*., 1986). In this study, molecular data from both the mitochondrial and nuclear genomes are examined to elucidate the relationships between *Fahrenholzia* species.

## Materials and methods

### *Louse specimens examined*

Sucking lice were collected from localities across the geographical range of the heteromyid hosts (Table 1, Fig. 1). Only one louse species known to parasitize heteromyid rodents, *F. schwartzi*, was not collected. Lice were obtained from hosts using one of two protocols, both of which involved complete isolation of host specimens to avoid potential louse contamination. In the first method, which yielded few lice, a stiff brush was used to remove lice from the pelage of the host immediately after the rodent was killed (Kim *et al*., 1986). A second protocol effectively yielding higher numbers of lice required skinning of the rodent in the field and freezing the skin in an airtight foil packet. Processing of the foil packet in the laboratory involved immersing skins individually in a 1% detergent solution and shaking vigorously to dislodge lice (Henry & McKeever, 1971; Clayton & Drown, 2001). The wash solution was then filtered, and lice were removed from the filter paper and stored at −70 °C. Lice were identified tentatively with the aid of a dissecting microscope and, after DNA extraction, were mounted on slides using Balsam and retained as vouchers. Voucher specimens of lice were prepared using the technique of Johnson & Clayton (2002), which enabled the extraction of whole genomic DNA from each louse whilst retaining the entire louse body as a voucher specimen. Voucher identifications were verified with the aid of dissecting and compound microscopes. Specimens, currently held by the authors, will be accessioned into the Price Institute for Phthirapteran Research at the University of Utah, Salt Lake City, Utah.

### *Amplification and sequencing of DNA*

Genomic DNA was isolated from the body of each louse using the DNeasy Tissue Kit (QIAGEN Inc., Valencia, California) according to louse-specific protocols (Cruickshank *et al*., 2001; Johnson & Clayton, 2002). Polymerase chain reaction (PCR) amplification and sequencing of a portion of the mitochondrial cytochrome *c* oxidase subunit I gene (COI; 1011 bp) was performed using combinations of the following primers: LCO1490, HCO2198 (Folmer *et al*., 1994), LCO1718 (Reed *et al*., 2004) and H7005 (Hafner *et al*., 1994). Double-stranded PCR amplifications were performed in 50-μl reaction volumes using primers LCO1490 with HCO2198, LCO1718 with H7005, or LCO1490 with H7005. Each reaction included 1.5 μl of each primer (20 μm), 8 μl of $MgCl_2$ (10 mm), 10 μl of a deoxynucleotide-triphosphate mixture (10 mm solution; dATP, dGTP, dCTP and dTTP, each 100 mm), 5 μl of 10 × *Taq* buffer and 0.4 μl of *Taq* DNA polymerase. The amplification protocol required an initial denaturation step of 94 °C for 1 min, followed by 40 PCR cycles of 94 °C (30 s), 45 °C (45 s) and 72 °C (45 s), and a final extension of 72 °C for 5 min.

A portion of the nuclear gene elongation factor-1α (EF-1α) was sequenced to provide an additional hypothesis of louse relationships based on a molecular marker independent of the mitochondrial genome. Fourteen specimens representing eleven *Fahrenholzia* species were examined for EF-1α (Table 1). PCR amplification and sequencing of 345 bp of the EF-1α gene were performed using the primers For3 and Cho10 (Danforth & Ji, 1998). One double-stranded PCR amplification was performed in a 50-μl reaction volume; the reaction included 2.5 μl of each primer (10 μm), 9 μl of $MgCl_2$ (10 mm), 10 μl of a deoxynucleotide-triphosphate mixture (10 mm solution; dATP, dGTP, dCTP and dTTP, each 100 mm), 5 μl of 10 × *Taq* buffer and 0.4 μl of *Taq* DNA polymerase. The amplification protocol required an initial denaturation step of 94 °C for 2 min,

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**Table 1.** Sucking louse (*Fahrenholzia*) taxa included in the phylogenetic analysis of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene and the nuclear elongation factor-1α (EF-1α) gene. Lice are grouped by country, state and host locality, and are mapped on Fig. 1. Asterisks indicate taxa included in the analysis of EF-1α. Museum acronyms and numbers are for host taxa, and 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 (MLZ), New Mexico Museum of Natural History (NMMNH) and University of Nevada Las Vegas (UNLV). Louse specimens lacking museum acronyms and numbers were donated for this study and do not have host voucher specimens.

| Locality number and locality | *Fahrenholzia* species | Host species |
|---|---|---|
| Costa Rica | | |
| 1. Guanacaste; Santa Rosa National Park | *F. fairchildi* 1* | *Liomys salvini* |
| Mexico | | |
| 2. Chihuahua: 6 mi NW Ricardo Flores Magón | *F. pinnata* 2 – NMMNH 4548 | *Dipodomys merriami* |
| 3. Coahuila: 2 mi E Agua Nueva | *F. pinnata* 3 – NMMNH 4714 | *Dipodomys ordii* |
| 4. Coahuila: 5 km S, 16 km W General Cepeda | *F. pinnata* 4 – NMMNH 4703 | *Dipodomys nelsoni* |
| 4. Coahuila: 5 km S, 16 km W General Cepeda | *F. zacatecae* 4 – NMMNH 4705 | *Chaetodipus hispidus* |
| 5. Coahuila: Plan de Guadalupe | *F. boleni* 5 – NMMNH 4728* | *Perognathus merriami* |
| 6. Coahuila: 2 km S Santa Teresa | *F. pinnata* 6 – NMMNH 4747 | *Dipodomys merriami* |
| 7. Durango: Hacienda Atotonilco | *F. texana* 7 – NMMNH 4491 | *Liomys irroratus* |
| 7. Durango: Hacienda Atotonilco | *F. texana* 7 – NMMNH 4491* | *Liomys irroratus* |
| 8. Jalisco: 16 km NNE Ameca | *F. ehrlichi* 8 – LSUMZ 36401 | *Liomys irroratus* |
| 9. Jalisco: 4.5 km SW Jilotlán | *F. microcephala* 9 – CNMA 39674* | *Liomys pictus* |
| 10. Puebla: 11 km (by road) SW Alchichica | *F. pinnata* 10 – LSUMZ 36244 | *Dipodomys phillipsii* |
| 10. Puebla: 11 km (by road) SW Alchichica | *F. ehrlichi* 10 – LSUMZ 36245 | *Liomys irroratus* |
| 11. Puebla: 3 km (by road) NE Tilapa | *F. ehrlichi* 11 – LSUMZ 36243 | *Liomys irroratus* |
| 12. Puebla: 6 km N Tilapa | *F. ehrlichi* 12 – CNMA 41832* | *Liomys irroratus* |
| 13. Puebla: 3.1 km SW El Veladero | *F. pinnata* 13 – LSUMZ 36254 | *Perognathus flavus* |
| 14. Veracruz: Biological Station La Mancha | *F. microcephala* 14 – CNMA 41912 | *Liomys pictus* |
| 15. Veracruz: 8 km ENE Catemaco | *F. hertigi* 15 – LSUMZ 36300* | *Heteromys desmarestianus* |
| 15. Veracruz: 8 km ENE Catemaco | *F. ferrisi* 15 – LSUMZ 36300* | *Heteromys desmarestianus* |
| 16. Zacatecas: 1 mi SE Bañon | *F. pinnata* 16 – NMMNH 4602 | *Dipodomys ordii* |
| 17. Zacatecas: 2 mi E San Jeronimo | *F. pinnata* 17 – NMMNH 4496 | *Dipodomys phillipsii* |
| 17. Zacatecas: 2 mi E San Jeronimo | *F. ehrlichi* 17 – NMMNH 4498 | *Liomys irroratus* |
| U.S.A.: California | | |
| 18. Mono Co. 5 mi N Benton | *F. pinnata* 18 – MLZ 1913 | *Dipodomys panamintinus* |
| 19. San Bernardino Co. 8.9 mi N, 1.1 mi E Red Mountain | *F. reducta* 19 – MLZ 1869* | *Chaetodipus formosus* |
| 19. San Bernardino Co. 8.9 mi N, 1.1 mi E Red Mountain | *F. pinnata* 19 – MLZ 1878 | *Perognathus longimembris* |
| 19. San Bernardino Co. 8.9 mi N, 1.1 mi E Red Mountain | *F. pinnata* 19 – MLZ 1880 | *Dipodomys merriami* |
| 20. San Bernardino Co. 3.2 mi S, 3.7 mi W Westend | *F. pinnata* 20 – MLZ 1890 | *Dipodomys merriami* |
| 21. San Bernardino Co. 9.7 mi S, 9.2 mi W Westend | *F. pinnata* 21 – MLZ 1892 | *Dipodomys merriami* |
| 22. San Luis Obispo Co. 15.9 mi S, 7.2 mi E Simmler | *F. tribulosa* 22 – MLZ 1843* | *Chaetodipus californicus* |
| 23. San Luis Obispo Co. 15 mi S, 8.2 mi E Simmler | *F. tribulosa* 23 – MLZ 1855 | *Chaetodipus californicus* |
| 23. Fresno Co. | *F. pinnata* 23 | *Dipodomys heermanni* |
| U.S.A.: New Mexico | | |
| 24. Cibola Co. 8.5 mi S, 5 mi W Correo | *F. pinnata* 24 – NMMNH 3937 | *Perognathus flavus* |
| 25. Cibola Co. 4 mi S, 1.5 mi W Correo | *F. pinnata* 25 – NMMNH 3945 | *Perognathus flavus* |
| 26. Doña Ana Co. 1 mi S jct. I-10 & Picacho Ave,W Las Cruces | *F. zacatecae* 26 – NMMNH 4433 | *Chaetodipus eremicus* |
| 26. Doña Ana Co. 1 mi S jct. I-10 & Picacho Ave,W Las Cruces | *F. pinnata* 26 – NMMNH 4445 | *Dipodomys merriami* |
| 27. Grant Co. 1.7 mi N, 0.5 mi E Redrock | *F. reducta* 27 – NMMNH 4362 | *Chaetodipus baileyi* |
| 28. Grant Co. 2.6 mi N, 1.8 mi E Redrock | *F. zacatecae* 28 – NMMNH 4373* | *Chaetodipus intermedius* |
| 28. Grant Co. 2.6 mi N, 1.8 mi E Redrock | *F. pinnata* 28 – NMMNH 4377* | *Dipodomys ordii* |
| 29. Hidalgo Co. 6 mi SE Portal (Cochise Co., Arizona) | *F. pinnata* 29 – NMMNH 4399 | *Dipodomys spectabilis* |
| 30. Hidalgo Co. Doubtful Canyon, 8 mi N,1 mi W Steins | *F. reducta* 30 – NMMNH 4421 | *Chaetodipus baileyi* |
| 31. Hidalgo Co. Doubtful Canyon, 8 mi N,0.5 mi W Steins | *F. reducta* 31 – NMMNH 4427 | *Chaetodipus baileyi* |
| 32. Socorro Co. 13 mi S, 13 mi W San Marcial | *F. pinnata* 32 – NMMNH 3982 | *Dipodomys merriami* |
| 33. Socorro Co. 5 mi N, 2 mi E Socorro | *F. pinnata* 33 – LSUMZ 36192 | *Dipodomys merriami* |
| 34. Socorro Co. 4.5 mi N, 1 mi E Socorro | *F. pinnata* 34 – LSUMZ 36198* | *Dipodomys merriami* |
| U.S.A.: Nevada | | |
| 35. Clark Co. Corn Creek Desert Wildlife Refuge | *F. pinnata* 35 – UNLV 3886* | *Dipodomys deserti* |
| 35. Clark Co. Corn Creek Desert Wildlife Refuge | *F. pinnata* 35 – UNLV 3882* | *Dipodomys merriami* |
| 36. Lyon Co. 10.3 mi S, 2.2 E Yerington | *F. pinnata* 36 – MLZ 2046* | *Perognathus longimembris* |
| 36. Lyon Co. 10.3 mi S, 2.2 E Yerington | *F. pinnata* 36 – MLZ 2047 | *Dipodomys microps* |
| 37. Nye Co. 19.2 mi N, 13.4 mi E Warm Springs | *F. pinnata* 37 – MLZ 1903 | *Dipodomys ordii* |

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**Table 1.** Continued.

| Locality number and locality | *Fahrenholzia* species | Host species |
|---|---|---|
| U.S.A.: Texas | | |
| 38. Brewster Co. Elephant Mountain WMA | *F. pinnata* 38 – NMMNH 4535 | *Dipodomys ordii* |
| 39. Cameron Co. 8.8 mi E Brownsville (on Hwy 4) | *F. ehrlichi* 39 – LSUMZ 36395 | *Liomys irroratus* |
| 40. Hidalgo Co. Mission, 2519 Inspiration Road | *F. zacatecae* 40 – LSUMZ 36375 | *Chaetodipus hispidus* |

followed by 29 PCR cycles of 94 °C (1 min), 46 °C (55 s) and 72 °C (1 min), and a final extension of 72 °C for 5 min.

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, Baton Rouge, Louisiana. Each 10-μl reaction included 1.6 μl of BigDye™ (Applied Biosystems, Perkin-Elmer Corporation, Foster City, California), 0.32 μl of 10 μm primer, 2.0 μl of 5 × ABI extension buffer, 4.08 μl of double-distilled $H_2O$ and 2 μl of amplification product. Samples were sequenced for 24 cycles at 96 °C (20 s; 1 cycle), followed by 96 °C (12 s; 23 cycles), 50 °C (15 s) and 60 °C (4 min). The sequences were then purified using Centri-Sep spin columns (Princeton Separations, Inc., Adelphia, New Jersey) and were electrophoresed using an ABI Prism 377 Genetic Analyser (Perkin-Elmer Corporation). Sequences were edited using Sequencher Version 4.1 (Gene Codes Corporation, Ann Arbor, Michigan) and aligned using Se-Al version 2.0a11 (http://evolve.zps.ox.ac.uk/Se-Al/Se-Al.html). Primer sequences were removed and sequences were trimmed with reference to the translated protein sequence using Se-Al version 2.01a11 and MacClade 4.0 (Maddison & Maddison, 2000). Only partial sequences for the COI gene were obtained for five *F. pinnata* specimens (localities 10, 13, 21, 33 and 37; Table 1, Fig. 1). Outgroup taxa in the analysis of the COI data consisted of two louse specimens belonging to the genus *Polyplax* (*P. auricularis* and *P. borealis*), a closely related member of the family Polyplacidae (J. E. Light, unpublished data). *P. auricularis* was used as the outgroup taxon in the analysis of the EF-1α data. In phylogenetic analyses, outgroup taxa were not designated a priori so that monophyly of the ingroup (*Fahrenholzia*) could be tested. All sequences were submitted to GenBank (COI, DQ324548–DQ324601; EF-1α, DQ324602–DQ324616, DQ683190).

To examine non-geographical variation in louse sequences, six specimens each of *F. ehrlichi* (locality 12), *F. reducta* (locality 19) and *F. zacatecae* (locality 26) were sequenced for the mitochondrial COI gene. Each louse specimen was collected from a different host individual at each locality. All sequences were submitted to GenBank (DQ324567, DQ324570, DQ324591, DQ324617–DQ324631).

### *Molecular phylogenetic analysis*

Phylogenetic congruence of the louse COI and EF-1α datasets was evaluated using the partition homogeneity test (Farris *et al.*, 1994) in PAUP* 4.0b10 (Swofford, 2002). One thousand partition replicates were analysed by maximum parsimony (MP) [heuristic search option with random addition replicates and tree bisection–reconnection (TBR) branch swapping].

Phylogenetic analyses were conducted on the louse molecular datasets using MP, maximum likelihood (ML) and Bayesian approaches. Equally weighted MP heuristic searches were performed on the COI and COI + EF-1α datasets with 100 random addition replicates and TBR branch swapping using PAUP* 4.0b10. A branch-and-bound search with simple addition was performed on the EF-1α dataset. Non-parametric bootstrap analyses (1000 pseudoreplicates and ten random sequence additions per replicate) were performed to assess nodal support (Felsenstein, 1985). All executable data files and trees for the COI, EF-1α and combined COI + EF-1α datasets were submitted to TreeBASE (http://www.treebase.org; study accession number S1588).

Modeltest (Version 3.6; Posada & Crandall, 1998) was used to examine the fit of fifty-six models of nucleotide substitution to the sequence data. In the analysis of the COI data, the GTR (General Time Reversible) model, including between-site rate variation and invariant sites (GTR + I + Γ; Yang, 1994; Gu *et al.*, 1995), was chosen as the best model of evolution according to both the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC; Huelsenbeck & Rannala, 1997; Posada & Buckley, 2004). The TrNef (Equal-Frequency Tamura-Nei) + Γ model was chosen by both the hLRT and AIC for the EF-1α dataset, and the TVM (Transversional) + I + Γ and GTR + I + Γ models were chosen by the hLRT and AIC, respectively, for the COI + EF-1α dataset. A full heuristic ML search was conducted using the preferred model in PAUP* 4.0b10 (Swofford, 2002). A full heuristic bootstrap (200 pseudoreplicates) was performed using the preferred model. Only the results of the hLRTs are presented here, because both approaches selected similar models and phylogenetic analyses using these models of evolution yielded the same topology.

The COI and EF-1α data were treated individually and as separate partitions in the Bayesian analyses, which were performed using MrBayes 2.01 and 3.1.2 (Huelsenbeck & Ronquist, 2001). The GTR + I + Γ model was used in all analyses, and model parameters, which were treated as unknown variables with uniform priors, were estimated by the analysis. Bayesian analyses were initiated with random starting trees, run for $2 \times 10^6$ generations with four incrementally heated chains (Metropolis-coupled Markov chain Monte Carlo; Huelsenbeck & Ronquist, 2001) and

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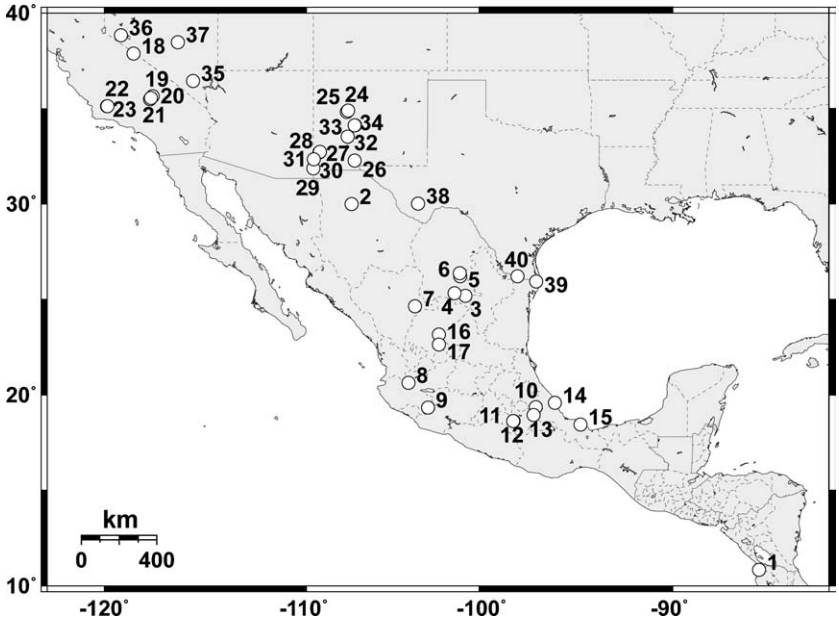


**Fig. 1.** Geographical distribution of sucking louse (*Fahrenholzia*) specimens used in the phylogenetic analyses. Numbers refer to collecting localities listed in Table 1.

sampled at intervals of 100 generations. Two independent Bayesian analyses were run to avoid entrapment on local optima, log-likelihood scores were compared for convergence (Huelsenbeck & Bollback, 2001; Leaché & Reeder, 2002) 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 (PP) (Huelsenbeck & Ronquist, 2001).

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

## Results

### *Host associations*

The hosts *Dipodomys californicus*, *D. elator*, *Perognathus inornatus*, *Heteromys anomalus*, *H. goldmani*, *H. gaumeri* and *Liomys adspersus* were not collected during this study; thus, the lice parasitizing these rodents (Table 2) were not sampled. Although specimens of *Chaetodipus nelsoni* ($n$ = 20), *C. penicillatus* ($n$ = 8), *D. agilis* ($n$ = 2), *P. flavescens* ($n$ = 1), *P. parvus* ($n$ = 2), *Microdipodops megacephalus* ($n$ = 63) and *M. pallidus* ($n$ = 38) were collected, no lice were observed on these specimens (Table 2). Additional heteromyid species not known previously to support lice were sampled, yielding several new host records, including *F. pinnata* from *D. panamintinus* and *D. nelsoni*, and *F. zacatecae* from *C. intermedius* and *C. eremicus*.

### *Phylogenetic analysis*

Little sequence variation was observed in the COI gene in multiple individuals of *F. ehrlichi* (average uncorrected $p$ distance, 0.4%), *F. reducta* (1.5%) and *F. zacatecae* (0.2%) collected from single localities. Within-species sequence variation was 25.7% within *F. microcephala*, 17.3% within *F. pinnata* plus *F. boleni* (see 'Discussion'), 13.1% within *F. irroratus*, 11.6% within *F. reducta*, 9.7% within *F. zacatecae* and 0.4% within *F. tribulosa*. Of the 1011 bp of the COI gene examined, 514 bp was potentially parsimony informative. MP analysis of the COI gene produced 36 equally parsimonious trees [length, 3932; consistency index (CI), 0.286; retention index (RI), 0.657; rescaled consistency index (RC), 0.188]. MP, ML and Bayesian analyses resulted in slightly different topologies because of a lack of resolution at the basal nodes. However, all analyses showed strong support for the monophyly of *Fahrenholzia* (MP bootstrap, 89; ML bootstrap, 100; Bayesian PP = 1) and the monophyly of a clade of lice restricted to the host genera *Dipodomys* and *Perognathus* (*F. pinnata* and *F. boleni*; MP bootstrap, 100; ML bootstrap, 100; Bayesian PP = 1; Fig. 2). Phylogenetic analyses also supported a monophyletic clade of lice parasitizing the host genus *Chaetodipus* (*F. zacatecae*, *F. reducta* and *F. tribulosa*; MP bootstrap, 93; ML bootstrap, 61; Bayesian PP = 1; Fig. 2). ML (bootstrap, 83) and Bayesian (PP = 1) analyses supported a monophyletic clade of lice parasitizing the host subfamily Heteromyinae (the *F. microcephala* group; Johnson, 1962). Strong support (ML bootstrap, 90; Bayesian PP = 1) was found for a sister relationship between lice parasitizing the host subfamilies Heteromyinae and Dipodomyinae plus *Perognathus* (Fig. 2).

Of the 345 bp of the EF-1α gene examined, thirty-nine sites were potentially parsimony informative. Parsimony analysis of the EF-1α gene resulted in twelve equally

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**Table 2.** Host associations for twelve species of *Fahrenholzia* sucking lice (Kim *et al*., 1986; Thomas *et al*., 1990; Whitaker *et al*., 1993; Durden & Musser, 1994a, b). Potential hosts were either brushed or washed to obtain lice. Lice from some of the localities were not included in the phylogenetic analyses. Prevalence estimates at the bottom of the table do not include host species from which lice remain unreported. Host genera are as follows: *C*., *Chaetodipus*; *D*., *Dipodomys*; *H*., *Heteromys*; *L*., *Liomys*; *M*., *Microdipodops*; *P*., *Perognathus*. An asterisk identifies the type host for each louse species.

| Louse species | Host species | Number of hosts examined (with lice) | Number of localities examined (with lice) | Locality numbers (Fig. 1, Table 1) | Known host association? |
|---|---|---|---|---|---|
| *F. boleni* | *P. merriami** | 8 (1) | 4 (1) | 5 | Yes |
| *F. ehrlichi* | *L. irroratus** | 23 (21) | 9 (9) | 7, 8, 10–12, 17, 39 | Yes |
| *F. fairchildi* | *L. adspersus* | 0 | – | – | Host not examined |
| *F. fairchildi* | *L. salvini* | 11 (11) | 1 (1) | 1 | Yes |
| *F. fairchildi* | *H. desmarestianus** | 1 (0) | 1 (0) | – | Yes |
| *F. ferrisi* | *H. desmarestianus* | 1 (1) | 1 (1) | 15 | Yes |
| *F. ferrisi* | *H. goldmani** | 0 | – | – | Host not examined |
| *F. ferrisi* | *H. gaumeri* | 0 | – | – | Host not examined |
| *F. hertigi* | *H. desmarestianus** | 1 (1) | 1 (1) | 15 | Yes |
| *F. microcephala* | *L. pictus** | 5 (2) | 5 (2) | 9, 14 | Yes |
| *F. pinnata* | *D. californicus** | 0 | – | – | Host not examined |
| *F. pinnata* | *D. deserti* | 6 (2) | 3 (2) | 35 | Yes |
| *F. pinnata* | *D. elator* | 0 | – | – | Host not examined |
| *F. pinnata* | *D. heermanni* | 21 (3) | 3 (2) | 23 | Yes |
| *F. pinnata* | *D. merriami* | 77 (26) | 31 (13) | 2, 6, 19–21, 26,32–35 | Yes |
| *F. pinnata* | *D. microps* | 4 (3) | 2 (2) | 36 | Yes |
| *F. pinnata* | *D. nelsoni* | 5 (1) | 3 (1) | 4 | New host association |
| *F. pinnata* | *D. ordii* | 15 (8) | 10 (5) | 3, 16, 28, 37, 38 | Yes |
| *F. pinnata* | *D. panamintinus* | 10 (3) | 3 (2) | 18 | New host association |
| *F. pinnata* | *D. phillipsii* | 4 (2) | 2 (2) | 10, 17 | Yes |
| *F. pinnata* | *D. spectabilis* | 7 (1) | 1 (1) | 29 | Yes |
| *F. pinnata* | *M. megacephalus* | 63 (0) | 18 (0) | – | Yes |
| *F. pinnata* | *C. penicillatus* | 8 (0) | 4 (0) | – | Yes |
| *F. pinnata* | *P. flavescens* | 1 (0) | 1 (0) | – | Yes |
| *F. pinnata* | *P. flavus* | 52 (5) | 14 (3) | 13, 24, 25 | Yes |
| *F. pinnata* | *P. inornatus* | 0 | – | – | Host not examined |
| *F. pinnata* | *P. longimembris* | 15 (6) | 5 (3) | 19, 36 | Yes |
| *F. pinnata* | *P. parvus* | 2 (0) | 2 (0) | – | Yes |
| *F. reducta* | *C. baileyi* | 12 (4) | 6 (3) | 27, 30, 31 | Yes |
| *F. reducta* | *C. formosus** | 8 (8) | 1 (1) | 19 | Yes |
| *F. reducta* | *P. parvus* | 2 (0) | 2 (0) | – | Yes |
| *F. texana* | *L. irroratus** | 9 (1) | 4 (1) | 7 | Yes |
| *F. tribulosa* | *C. californicus** | 8 (2) | 2 (2) | 22, 23 | Yes |
| *F. schwartzi* | *H. anomalus** | 0 | – | – | Host not examined |
| *F. zacatecae* | *C. eremicus* | 20 (8) | 9 (1) | 26 | New host association |
| *F. zacatecae* | *C. hispidus** | 6 (2) | 6 (2) | 4, 40 | Yes |
| *F. zacatecae* | *C. intermedius* | 26 (4) | 11 (1) | 28 | New host association |
| Unknown | *D. agilis* | 2 (0) | 2 (0) | – | Unknown |
| Unknown | *C. nelsoni* | 20 (0) | 12 (0) | – | Unknown |
| Unknown | *M. pallidus* | 38 (0) | 14 (0) | – | Unknown |
| Prevalence of hosts with lice | | 29.2% | | | |
| Prevalence of localities with lice | | | 37.6% | | |

parsimonious trees (length, 107; CI, 0.850; RI, 0.887; RC, 0.754). Results of the MP, ML and Bayesian analyses did not conflict strongly with each other or with the results from the COI analyses (trees available on TreeBASE; study accession number S1588). However, only the parsimony branch-and-bound search supported a sister relationship (MP bootstrap, 65) between lice parasitizing the host subfamilies Heteromyinae and Dipodomyinae plus *Perognathus*. In addition, all analyses of EF-1α showed moderate support for a sister relationship between *F. texana* and *F. ferrisi* (MP bootstrap, 75; ML bootstrap, 60; Bayesian PP = 0.87), a relationship that was not evident in the analysis of the COI data (Fig. 2).

The partition homogeneity test did not detect significant heterogeneity between the COI and EF-1α datasets ($P = 0.996$), and so these data were pooled for a combined analysis of all taxa for which EF-1α sequences were available. Of the 1356 bp examined in the combined analysis, 498

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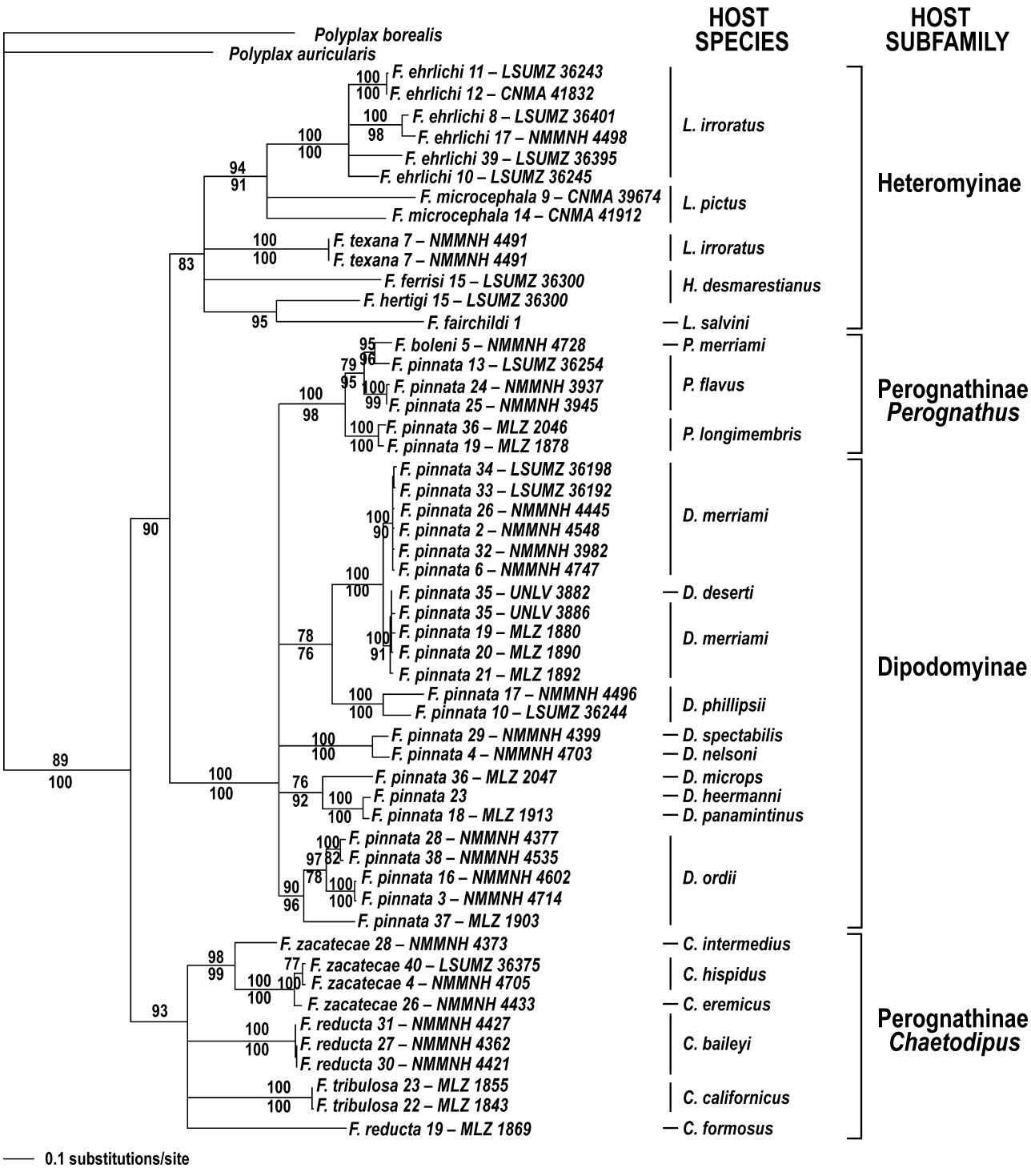


**Fig. 2.** Maximum likelihood phylogram resulting from the analysis of the cytochrome *c* oxidase subunit I (COI) gene for 52 *Fahrenholzia* specimens (Table 1). Maximum parsimony and maximum likelihood bootstrap support values greater than 75 are indicated above and below the nodes, respectively, and all nodes receiving support values less than 75 are collapsed. Species names are followed by the locality number and museum specimen number for the host (Fig. 1, Table 1). Host associations are listed to the right of the cladogram. Abbreviations for host genera are as follows: *C.*, *Chaetodipus*; *D.*, *Dipodomys*; *H.*, *Heteromys*; *L.*, *Liomys*; *P.*, *Perognathus*.

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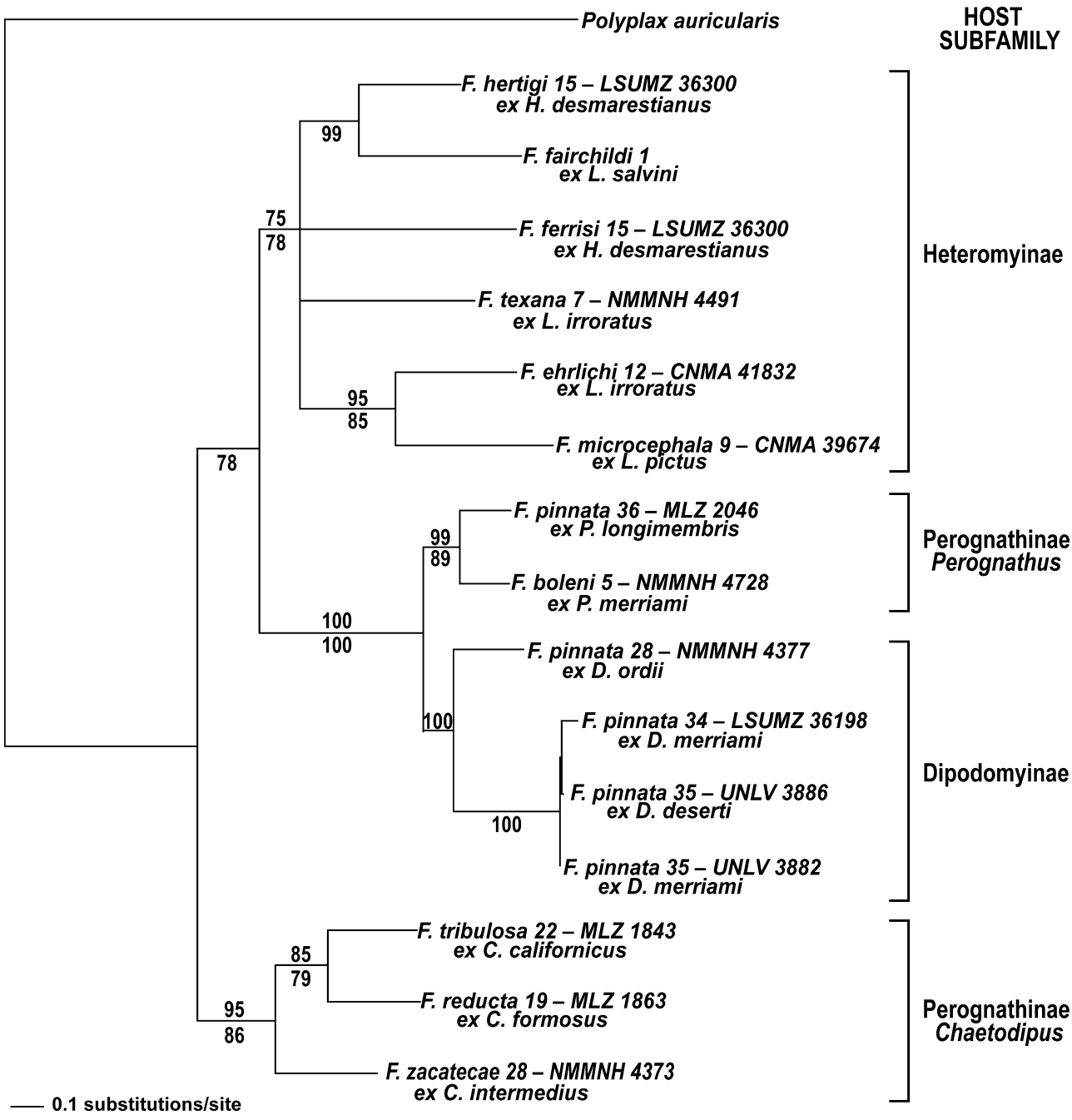


**Fig. 3.** Maximum likelihood phylogram resulting from the analysis of the combined cytochrome *c* oxidase subunit I (COI) and elongation factor-1α (EF-1α) sequence data for sucking lice of the genus *Fahrenholzia*. Maximum parsimony and maximum likelihood bootstrap support values greater than 75 are indicated above and below the nodes, respectively, and all nodes receiving support values less than 75 are collapsed. Abbreviations for host genera are as follows: *C*., *Chaetodipus*; *D*., *Dipodomys*; *H*., *Heteromys*; *L*., *Liomys*; *P*., *Perognathus*.

bp was potentially parsimony informative, and parsimony analysis resulted in one most-parsimonious tree (length, 2193; CI, 0.482; RI, 0.455; RC, 0.220). Phylogenetic analyses of the combined data (Fig. 3) did not disagree strongly with each other or with the results from the separate COI and EF-1α analyses. However, only the Bayesian analysis strongly supported a sister relationship between lice parasitizing the host subfamilies Heteromyinae and Dipodomyinae plus *Perognathus* (Bayesian PP = 1), and a sister relationship between *F. texana* and *F. ferrisi* (PP = 0.97).

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## Discussion

The extensive sampling undertaken in this study reveals that the prevalence of sucking lice on their heteromyid hosts is generally low (29.2% of hosts examined for lice were parasitized; Table 2). This is in contrast with chewing lice of mammals, which usually are found on all individuals in a host population (Nadler *et al*., 1990). On the basis of the number of localities and specimens sampled in this study, it is likely that *C. nelsoni* ($n = 20$ specimens examined from eleven localities) and *M. pallidus* ($n = 38$ from fourteen localities) do not harbour sucking lice. In addition, *M. megacephalus* is a reported host of *F. pinnata* (Ferris, 1916), but the sixty-three specimens sampled from fourteen localities showed no lice. Possibly, *F. pinnata* previously parasitized *M. megacephalus*, but, more likely, Ferris's (1916) report was the result of specimen contamination. Ferris (1916) listed *F. pinnata* from skins of *M. megacephalus*, but provided no museum number for either the host or the louse. Subsequent publications list *M. megacephalus* as a host of *F. pinnata* by reference to Ferris's (1916) publication (for example Ferris, 1922, 1951; McDaniel, 1968; Kim *et al*., 1986; Whitaker *et al*., 1993; Durden & Musser, 1994a, b). Thus, no study subsequent to 1916 has found *F. pinnata* parasitizing *M. megacephalus*, and our study suggests that both extant species of *Microdipodops* (*M. pallidus* and *M. megacephalus*) are not parasitized by sucking lice. Two new host associations for *F. pinnata,* both on species of *Dipodomys*, were found in this study (Table 2), suggesting that additional host associations for this genus will be discovered with continued sampling.

Within-locality genetic variation in *Fahrenholzia* was low (< 1.5%), indicating that a single louse specimen per locality was a reasonable genetic representative of the entire population, and therefore adequate for use in phylogenetic analyses. Although the results of the COI, EF-1α and combined analyses yielded similar trees, the COI dataset included a broader sampling of taxa, more sequence data and was generally more informative phylogenetically. Therefore, relationships between *Fahrenholzia* species are discussed below primarily with reference to the mitochondrial COI data (Fig. 2).

### *F. boleni and F. pinnata*

*Fahrenholzia boleni*, which parasitizes only *Perognathus merriami*, is nested deeply within the *F. pinnata* specimens parasitizing other species of *Perognathus* (Figs 2, 3). Constraining all *F. pinnata* specimens to be monophyletic to the exclusion of *F. boleni* yielded a tree whose likelihood score was significantly worse than the best tree (Fig. 2; KH and SH tests $P < 0.001$). Similarly, forcing the monophyly of the *F. pinnata* specimens that parasitize *Perognathus* (again excluding *F. boleni*) also resulted in trees that were significantly worse than the best tree (Fig. 2; KH and SH tests $P < 0.04$). The *F. boleni* specimen examined in this study is 9–15% and 0% genetically divergent (uncorrected *p* distances for the COI and EF-1α genes, respectively) from *F. pinnata* specimens that parasitize other species of *Perognathus*. These values are considerably lower than those measured between other *Fahrenholzia* species examined in this study (COI gene: mean divergence, 25.9%; range, 19.7–29.0%; EF-1α gene: mean divergence, 5.0%; range, 0.3–7.9%), and indicate conspecificity of *F. boleni* and the *F. pinnata* specimens that parasitize *Perognathus*.

*Fahrenholzia pinnata* specimens that parasitize *Perognathus* are 19.7% and 1.2% divergent (for the COI and EF-1α genes, respectively) from *F. pinnata* specimens parasitizing *Dipodomys*. Morphological examination of all *F. pinnata* specimens that parasitize *Perognathus* revealed several features shared with *F. boleni* that were not shared with *F. pinnata* specimens that parasitize *Dipodomys* (J. E. Light, unpublished data). Therefore, the subtle morphological characters traditionally used to distinguish *F. boleni* from *F. pinnata* (i.e. lobes on antennal segments, rounded lobes of genitalia and concave anterior margin of sternal plate; McDaniel, 1968; Kim *et al*., 1986) may not diagnose monophyletic lineages. Together, the genetic data and morphological observations suggest that the lice parasitizing *Perognathus* (*F. pinnata* and *F. boleni*) may represent a cryptic species distinct from the *F. pinnata* lice parasitizing *Dipodomys*. If future broader sampling supports this hypothesis, lice parasitizing *Perognathus* would be recognized collectively as *F. boleni*. This would restrict *F. pinnata* to hosts of the genus *Dipodomys*, given that our sampling suggests that *F. pinnata* is probably not a parasite of *Microdipodops* (*contra* Ferris, 1916) and may not be a parasite of *Chaetodipus* (*contra* Morlan & Hoff, 1957; Table 2).

Although they appear morphologically identical, *F. pinnata* lice parasitizing *D. ordii* are approximately 19% and 1.5% genetically divergent (for the COI and EF-1α genes, respectively) from *F. pinnata* lice parasitizing other species of *Dipodomys*. This high genetic divergence for the COI gene may signal another cryptic species within *F. pinnata*, although better phylogenetic resolution will be necessary to test this hypothesis

### *F. ehrlichi, F. microcephala and F. texana*

Phylogenetic analysis indicates a sister relationship between *F. ehrlichi* and *F. microcephala* (Figs 2, 3). The two *F. microcephala* specimens, however, are highly divergent genetically (approximately 26%) and appear as sister taxa only in the Bayesian analysis of the COI dataset (PP = 53). Although these two specimens of *F. microcephala* are from the extreme eastern and north-western limits of their host range (*c*. 750 km apart), their extremely high genetic divergence is probably evidence of cryptic species. Additional sampling of *F. microcephala* from geographically intermediate localities will be needed to resolve this issue.

The phylogenetic relationships of *F. texana* remain unclear. *F. texana* and *F. ehrlichi* occur on the same host (*Liomys irroratus*; Table 2), and both were collected from a single *L. irroratus* specimen at locality 7 (Table 1, Fig. 1). These louse specimens, however, are not closely related (approximately

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25% genetically divergent for the COI gene; J. E. Light, unpublished data), supporting their recognition as separate species. Mitochondrial (Fig. 2) and nuclear (Fig. 3) data analyses do not resolve *F. texana* relationships beyond including this taxon within the group of lice parasitizing rodents of the subfamily Heteromyinae (in agreement with Johnson, 1962). Further analyses, including additional specimens, will help to resolve the relationships between *F. texana* and other lice parasitizing heteromyine rodents.

### *F. hertigi, F. ferrisi and F. fairchildi*

Genetic divergence between *F. hertigi, F. ferrisi* and *F. fairchildi* is high for the COI gene, averaging 23% for the uncorrected *p* distance (2.1% for the EF-1α gene). *F. hertigi* and *F. ferrisi* were collected from the same host specimen (*H. desmarestianus* from locality 15) and are genetically and morphologically distinct. A sister relationship between *F. hertigi* and *F. fairchildi* (ML bootstrap, 95) is supported, even though these two species parasitize different host genera (Figs 2, 3). Recent evidence suggests that *H. desmarestianus* (the host of *F. hertigi* and *F. ferrisi*) and *L. salvini* (the host of *F. fairchildi*) may be more closely related to each other than *L. salvini* is to other species of *Liomys* (Anderson *et al.*, 2006), providing indirect support for the louse relationships shown in Fig. 2.

### *F. reducta, F. tribulosa and F. zacatecae*

Sucking lice parasitizing *Chaetodipus* (*F. reducta*, *F. tribulosa* and *F. zacatecae*) comprise a morphologically and genetically distinct group. Genetic divergence between the *F. reducta* specimen parasitizing *C. formosus* (*F. reducta* from locality 19) and other *F. reducta* specimens parasitizing *C. baileyi* (Fig. 2) is very high (approximately 23% for the COI gene), suggesting that these may represent two distinct louse species. If so, the louse parasitizing *C. formosus* (the type host of *F. reducta*; Ferris, 1922) would retain the species epithet *reducta*. Trees resulting from constraint analyses forcing *F. reducta* to be monophyletic were not significantly worse than the best tree (all analyses $P > 0.07$), leaving the question of *F. reducta* monophyly unresolved on the basis of these data.

Although the four *F. zacatecae* samples collected for this analysis were obtained from three host species (*C. eremicus*, *C. hispidus* and *C. intermedius*) from four localities that span approximately 1100 km from New Mexico to north-central Mexico, the average genetic divergence measured between the *F. zacatecae* specimens was only 9.7% for the COI gene. Because this study identified two new host associations for *F. zacatecae* (*C. eremicus* and *C. intermedius*; Table 2), it is likely that continued sampling of *Chaetodipus* species will reveal additional host associations for *F. zacatecae*.

### *F. schwartzi*

Only one *Fahrenholzia* species, *F. schwartzi*, was not included in this analysis. Multiple attempts to amplify DNA from dried museum specimens failed. *F. schwartzi* is known only from the South American host *H. anomalus*, and previous morphological work has suggested that this species is most similar to *F. ferrisi* (Johnson, 1962), which also parasitizes *Heteromys*.

### *Cryptic species*

Although multiple cryptic species of *Fahrenholzia* are evident from this analysis, a formal description of these species must await a thorough morphological analysis and, in some cases, confirmation by examination of additional nuclear genes. Several of these potential species also need to be sampled more extensively to clarify their geographical, genetic and host boundaries.

## Conclusion

This study provides strong evidence from both mitochondrial and nuclear genes supporting the monophyly of the sucking louse genus *Fahrenholzia*. These sucking lice parasitize a monophyletic lineage of rodents (Heteromyidae), and monophyletic lineages within the louse genus *Fahrenholzia* parasitize heteromyid lineages that are themselves monophyletic. These lineages include the subfamily Heteromyinae and the genera *Perognathus* and *Chaetodipus*. Furthermore, monophyly of the sucking lice that parasitize *Dipodomys* is supported by nuclear data (although this relationship is unresolved in the analysis of mitochondrial data). Given the generally high level of correspondence between clades of lice and clades of heteromyid rodents, it is appropriate to undertake a statistical comparison of their phylogenies to assess the degree of co-phylogeny in this host–parasite assemblage. However, this comparison must await a well-resolved phylogeny of the Heteromyidae based on molecular data, which is currently in progress (J. Hafner, personal communication, Occidental College, Los Angeles, CA). Meanwhile, the results of this study show that sucking lice, much like their relatives the chewing lice, hold considerable promise for future studies of co-speciation between these parasitic insects and their mammalian hosts.

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