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# Host associations between laelapine mites (Mesostigmata: Laelapidae) and palustrine rodents in Paraguay: a study of host specificity and cryptic species

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## Abstract

Host specialization has contributed to the high diversity of laelapine mites associated with Neotropical rodents, but the lack of taxonomic development at the species-level has confounded study of the coevolutionary history of both host and ectoparasite groups. Morphometric comparisons of presumptive polyxenous laelapine species infesting a diverse assemblage of palustrine rodents in Paraguay clearly reveal that each host species is infested by a morphologically distinct mite population. The nominal taxa *Laelaps manguinhosi*, *Gigantolaelaps goyanensis*, and *G. mattogrossensis* may be composites of morphologically distinct but similar species with narrower host preferences. These results suggest that laelapine mites are primarily monoxenous, and that numerous currently unrecognized species may be discerned by standard morphometric techniques.

**Key words**: Acari, Ectoparasites, Host specificity, Laelapidae, Neotropics, Rodents

## Introduction

Laelapine mites (Mesostigmata: Laelapidae) are common associates of Neotropical rodents and marsupials (Tipton *et al.* 1966; Furman, 1972). When parasitic arthropods are sampled from the host skin and pelage, these mites are often abundant, rich in species, and diverse in assemblage (Dowling, 2006). The factors structuring host associations of laelapid mites and Neotropical rodents are still poorly understood, but in some genera host specificity is known to be remarkably high (Gettinger 1987, 1992, Gettinger & Ernest 1995, Gettinger & Owen 2000). In survey and inventory research, host distributions of laelapine mites clearly reflect specificity at higher taxonomic levels (e.g., *Gigantolaelaps* Fonseca only infests sigmodontine rodents of the Tribe Oryzomyini), suggesting that these mites may be distributed in parallel with the phylogenetic relationships of their hosts (Tipton *et al.* 1966, Furman 1972, Gettinger 1987, 1992, Gettinger & Owen 2000). But the host records of some laelapine mite species imply that associations may be polyxenous, with mites actively infesting a wide taxonomic range of rodents that share ecological time and space. It is important to evaluate these associations carefully and determine whether they represent active transfer of polyxenous mites or if these nominal laelapine species are composed of groups of morphologically similar laelapine species with narrower host preferences ("monoxeny").

In Paraguay, five species of Neotropical rodents and their associated mites were collected from palustrine habitats. These included four oryzomyine species—*Holochilus chacarius* Thomas, 1906, *Pseudoryzomys simplex* (Winge, 1887), *Nectomys squamipes* (Brants, 1827), *Sooretamys angouya* (Fischer, 1814)—and one akodontine species, *Scapteromys aquaticus* Thomas, 1920. *Nectomys*

 

*squamipes* were also collected from three Brazilian localities, and *H. chacarius* from a Brazilian and a Bolivian locality (Fig. 1). Preliminary examination of mites associated with these mammals revealed the presence of the laelapid genera *Laelaps* Koch and *Gigantolaelaps* Fonseca. One nominal mite species (*Laelaps manguinhosi* Fonseca) parasitized all five rodent species, while two nominal species of *Gigantolaelaps* (*G. mattogrosensis* Fonseca, *G. goyanensis* Fonseca) parasitized the oryzomyine rodents. No *Gigantolaelaps* were recovered from *S. aquaticus*. In this study, we employ multivariate analysis of morphometric data of mite populations sampled from five species of palustrine rodents, making comparisons between mites sampled from a single host individual, from multiple host individuals, and from species sampled from closely- and widely-separated localities. Using morphometric and host association data, our objectives are to explore and describe the morphological variation of *Laelaps* and *Gigantolaelaps* mites sampled from five rodent species that share a common, palustrine habitat.

## Materials and methods

During a large biodiversity survey of small mammals in Paraguay, rodents and small marsupials were captured in live traps, anesthetized, and brushed for ectoparasites before being prepared as standard museum specimens. In addition, this study includes mites from several host individuals collected in Bolivia and Brazil (Appendix 1, Fig. 1). Ectoparasite sampling techniques are described in Gettinger (1992); a rigorous sampling protocol was employed to minimize inter-host contamination of parasites. All mite specimens used in this study were mounted individually in Hoyer's medium, ringed in glyptal, and measured with a stage-calibrated ocular micrometer. All mite specimens were prepared, identified, and measured by DG. Identifications are based on the study of comparative specimens, including types (*L. manguinhosi, G. mattogrossensis*). Also, topotypes were collected for *L. manguinhosi* and *G. mattogrossensis* from the type host and type locality (Porto Joffre, Mato Grosso, Brazil). Adult female mites were used in all morphometric analyses, because it is the most abundant life stage in populations sampled from the host mammal, and it is the stage upon which laelapid mite taxonomy is based.

From each host individual, one or two adult female specimens were randomly selected for measurement and inclusion in the analyses. In total, 77 specimens of *Laelaps manguinhosi* and 50 of *Gigantolaelaps* spp. were used in this study (see Appendix 1). The *L. manguinhosi* were collected from five species of rodents from 18 Paraguayan localities, plus one Bolivian and four Brazilian localities, whereas *Gigantolaelaps* spp. were collected from four species of rodents from 14 localities in Paraguay (Fig. 1). Host voucher specimens from the following countries are deposited in the following institutions: Paraguay - Museo Nacional de Historia Natural del Paraguay, San Lorenzo, Paraguay, and The Museum of Texas Tech University, Lubbock, TX, USA; Brazil – The Sam Noble Museum of Natural History, Norman, OK, USA; Bolivia – American Museum of Natural History, New York, NY, USA. Mite vouchers are in the Harold W. Manter Laboratory of Parasitology, University of Nebraska-Lincoln, NE, USA.

Thirty-seven continuous characters were chosen to represent different regions of the laelapid body, some because they diagnose taxa, and others as representative descriptors of size and shape of the mite. General morphological terminology follows Krantz & Walter (2009). The body regions and characters included: Dorsal Shield—Dorsal shield length (DSL), Dorsal shield width at midlevel (DSW); Dorsal Chaetotaxy—Distance between j5 setae (j5-j5), Distance between z5 setae (z5-z5), Length of j5 (j5L), Length of z5 (z5L), Distance between J5 setae (J5-J5), Distance between Z5 setae (Z5-Z5), Length of J5 (J5L), Length of Z5 (Z5L); Gnathosoma—Distance between subcapitular setae (CAP-CAP), Length of subcapitular setae (CAPL), Length of inner hypostomal

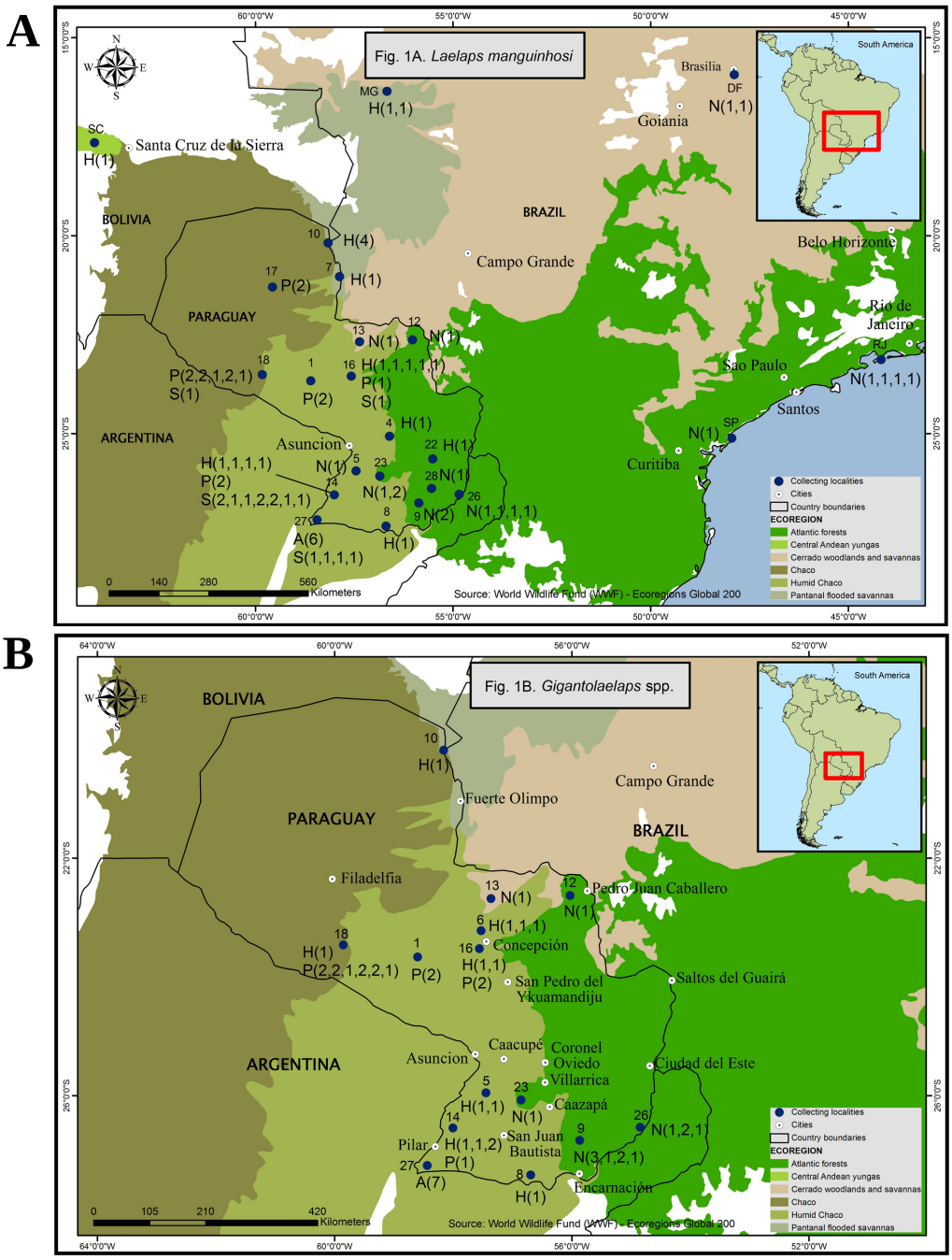


**FIGURE 1**. Maps indicating localities from which host rodents and mites were collected, and ecoregion associations). A, localities for *Laelaps manguinhosi*; B, localities for *Gigantolaelaps* spp. Letters beside locality code indicate rodent species (A, *Sooretamys angouya*; H, *Holochilus chacarius*; N, *Nectomys squamipes*; P, *Pseudoryzomys simplex*; S, *Scapteromys aquaticus*); the number of host individuals is indicated by the number of numbers in parentheses; and the number of mites examined from each of these individuals is indicated by the actual number. For precise localities, hosts, and mites collected, see Appendix 1. Paraguayan locality numbers are standardized to other publications pertaining to the "Mammals of Paraguay and their Ectoparasites" project (e.g., Willig et al., 2000; Dick and Gettinger, 2005; Graciolli et al., 2006; Goodin et al., 2006). Bolivian and Brazilian locality codes indicate the state or department (Bolivia: SC, Santa Cruz de la Sierra; Brazil: DF, Distrito Federal; MG, Mato Grosso; RJ, Rio de Janeiro; SP, São Paulo). Ecoregions based on World Wildlife Fund (WWF) Global 200 Ecoregions.

setae (INNL), Distance between subcapitular and inner hypostomal setae (CAP-INN); Sternal Shield—Length of sternal shield (SSL), Width of sternal shield at level of second sternal setae (SSW), Distance between first sternal setae (S1-S1), Distance between third sternal setae (S3-S3), Length of anterior sternal setae (S1L), Length of posterior sternal setae (S3L); Epigynial Shield Area—Length of epigynial shield (ESL), Distance between epigynial setae (E5-E5), Greatest width of epigynial shield (ESW), Length of poststernal setae (S4L), Length of epigynial setae (E5L); Anal Shield—Length of paranal setae (PARAL), Length of postanal seta (POSTL), Distance from postanal seta to anterior midline of anal shield (POST-EDGE), Distance between paranal setae (PARA-PARA), Greatest width of anal shield (ASW); Legs—Length of proximal seta coxa I (PROXCOX), Length of distal seta coxa I (DISTCOX), Length of posterior seta coxa II (POSTCOX2), Length of posterior seta coxa III (POSTCOX3), Length of posterior seta coxa IV (POSTCOX4), Length of anterior dorsal seta femur I (DFEM1L), Length of posterior dorsal seta genu I (DGEN1L).

Morphometric relationships were evaluated separately for *L. manguinhosi* and *Gigantolaelaps* spp. The NT-SYSpc multivariate statistical package (version 2.20, Applied Biostatistics Inc., 2005) was used to perform Unweighted Pair Group Method using Arithmetic Averages (UPGMA) clustering and Principal Component Analysis (PCA), and to display results of these analyses. For these analyses, each of the 37 linear measurement characters was standardized to a mean of zero and a standard error of one in order to mitigate the influence of size variation among characters on the phenetic relationships among individuals.

To evaluate relative variation among mites from the same host individual, mites from the same host species from geographically diverse localities, and mites from different host species, a matrix of average taxonomic distances (Sneath & Sokal 1973) among specimens was calculated from the matrix of standardized characters. A phenogram (UPGMA) was then constructed from the distance matrix.

Principal Component Analysis was used to further define and visualize the sample clusters, and to evaluate the contributions of individual characters to the phenetic differences among clusters. Eigenvectors were extracted from a pair-wise matrix of Pearson product-moment correlations of the standardized characters. The original matrix of standardized measurements was then projected onto the eigenvectors, and a two- or three-dimensional model was constructed based on these projections. These models enable visualization of inter-individual relationships in the two- or three-dimensional space that best represents the complete (37-dimensional) character space. In addition, a minimum spanning tree among the 77 (for *L. manguninhosi)* or 50 (*Gigantolaelaps)* individuals was calculated from inter-individual distances based on the standardized characters, and this tree was mapped onto the two- or three-dimensional model. This enhanced visualization of relative inter-individual distances and enabled detection of distortions in the relationships as depicted in the models.

Where the PCA did not differentiate well between clusters, Analysis of Variance (ANOVA) and Multivariate Analysis of Variance (MANOVA) were used to test the statistical validity of those clusters. All of the quantitative variables characterizing the populations and individuals exhibited varying extent and form of departure from normal distributions (i.e., had skewed and/or kurtotic distributions). Because linear models such as ANOVA are sensitive to departures from normality, character values were transformed to normalized rank variables, using the formula of Blom (1958:145), and these transformed variables were used in the ANOVA and MANOVA analyses. Because most of these analyses were of unbalanced design, tests for significant differences among group means were based on comparisons of least-squared means. All statistical analyses were done using SAS 9.2 (SAS Institute, Cary, North Carolina). Significance levels reported throughout text and tables are: *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $0.001 \geq P$.

## Results

*Laelaps manguinhosi* — A distance phenogram (Fig. 2) indicates that the mites from each host species form discrete clusters. Within each of these clusters, mites from the same host individual do not tend to cluster together, nor is any geographic pattern apparent. Thus, the only phenotypic pattern seen among these 77 *L. manguinhosi* is a strict clustering according to host species.

In the PCA for *Laelaps manguinhosi* mites, 4 components had standardized eigenvalues > 1.0, indicating that they carried important phenetic information. Loadings of the 37 standardized characters onto components 1–4 are given in Table 1. Component 1, which accounts for 58.6% of the total variation, is a size-related component, with 33 of the 37 characters loading strongly and positively. In contrast, component 2 (15.6% of the variation) has the heaviest loadings of the other 4 characters, as well as one other character which loads nearly equally on components 1 and 2. CAPL, POST-EDGE, and POSTCOX4 load positively, whereas j5–j5 and POSTCOX3 are negatively associated. Although components 3 and 4 have eigenvalues greater than 1.0 (indicating that they carry important additional information regarding mite phenetic relationships), neither has any character loading most heavily on it.

Projection of the 77 individual *L. manguinhosi* onto the first two principal components (Fig. 3A), resulted in clear separation of four host-associated groups of mites. Mites collected from *Scapteromys* and *Pseudoryzomys* are smaller (found to the left on component 1), while those from the other three hosts are larger. Component 2 separates *Pseudoryzomys* from *Scapteromys* mites, and *Sooretamys* from *Nectomys* and *Holochilus.*

Although clusters of mites from *Holochilus* and *Nectomys* show overlap in both components 1 and 2, component 3 (Fig. 3B) provides clear separation between these two groups. Univariate and Multivariate Analyses of Variance (ANOVA, MANOVA) were used to further evaluate the mites from these two hosts. The multivariate assessment of these 40 specimens indicated that mites from the two hosts are statistically different ($F = 7472.4$; DF = 37, 2; $P < 0.0001$, Wilks' Lambda). Table 2 shows the character means and ranges, as well as F-values and significance levels, for mites from *Holochilus* and *Nectomys*. Of the 37 characters, 14 do not vary significantly between mites from the two hosts. Of the other 23, 18 are larger in mites from *Nectomys*, and 5 in mites from *Holochilus*. This independence of character relationships between mites from the two host species further supports the recognition of these as two independent lineages of *Laelaps* mites.

*Gigantolaelaps* spp. — As with the phenogram of *Laelaps* mites, specimens from each oryzomyine host species form a discrete cluster (Fig. 4). *Scapteromys aquaticus* does not have an associated *Gigantolaelaps*. Also similar to the *Laelaps* specimens, *Gigantolaelaps* mites from the same host individual do not cluster together, nor is any consistent geographic pattern apparent within the four host-associated clusters. Thus, the only phenotypic pattern seen among these 50 *Gigantolaelaps* mites is a strict clustering according to host species.

In the PCA for *Gigantolaelaps* mites, 5 components had standardized eigenvalues > 1.0, indicating that they carried important phenetic information. Loadings of the 37 standardized characters onto principal components 1–5 are given in Table 1. Component 1 accounted for 35.9% of the variation, and 20 characters loaded heavily on this component. Of these 20, 13 loaded positively and 7 negatively, indicating that size is not an important component of variation in *Gigantolaelaps* mites. Component 2 (28.9% of the variation) had 14 components loading heavily (including 3 with loadings nearly equal to those on component 1), 12 of which were positive; component 3 (11.0%) had 5 (1 shared with component 2), 3 of which were positive; component 4 (4.2%), 1 character (negative); component 5 (2.9%) had 1 (shared with component 1, positive).

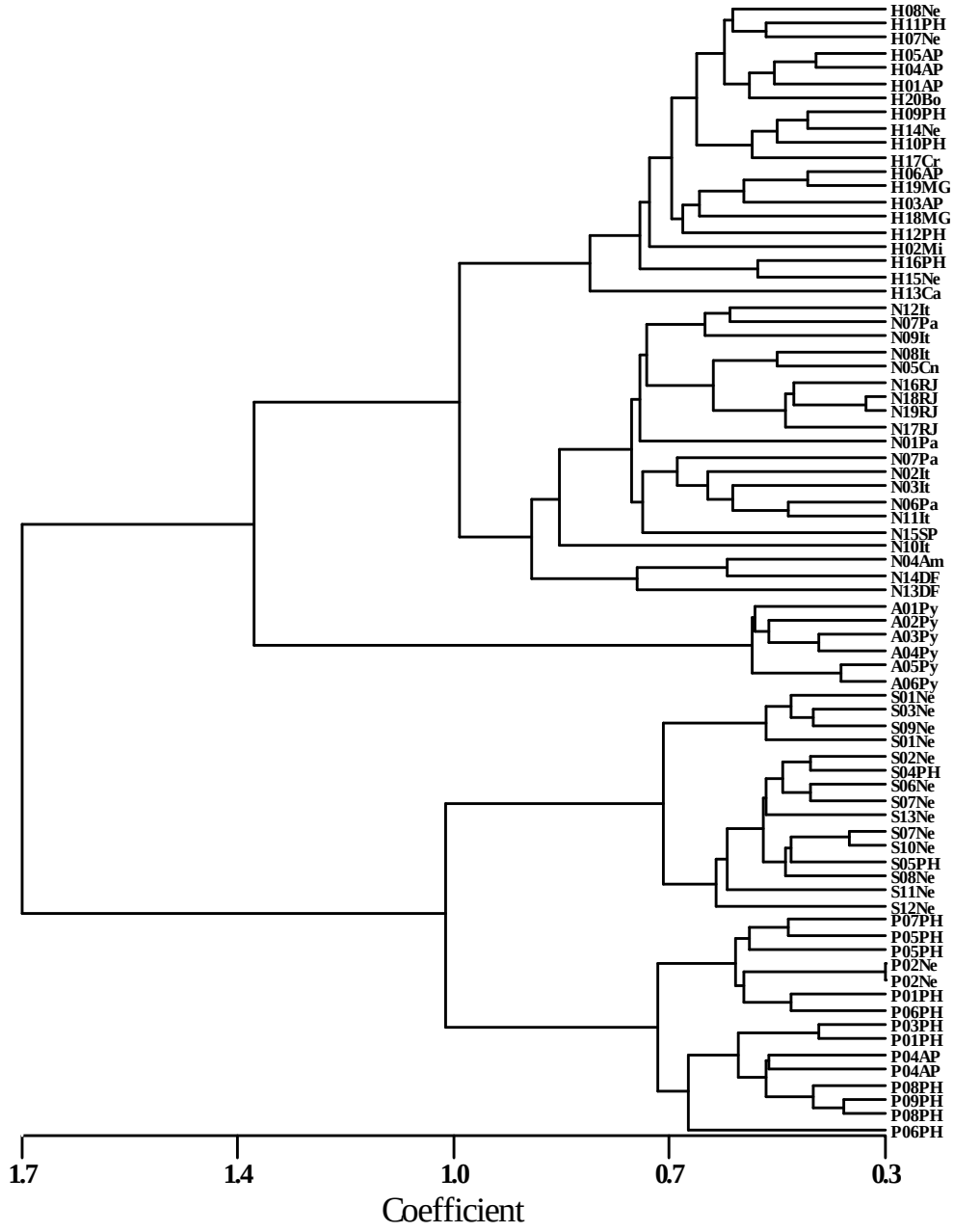


**FIGURE 2**. Phenogram of *Laelaps manguinhosi* relationships, calculated from a dissimilarity metric based on standardized characters. In the OTU labels, capital letters indicate host rodent species (A, *Sooretamys angouya*; H, *Holochilus chacarius*; N, *Nectomys squamipes*; P, *Pseudoryzomys simplex*; S, *Scapteromys aquaticus*); the next two-digit (or letter) code indicates the locality (see Fig. 1, Appendix1); and the final two-digit code differentiates among individual hosts collected from that locality. A final lower-case letter differentiates among mites collected from a particular host individual.

Projections of the 50 individual *Gigantolaelaps* mites onto the first three principal components are shown in Fig. 5. Component 1 separates mites into three host-associated groups: *Pseudoryzomys*,

*Holochilus* + *Sooretamys*, and *Nectomys*. *Holochilus* mites are clearly distinct from *Sooretamys* mites in both components 2 and 3.

**TABLE 1**. Character loadings of 37 standardized characters on principal components with standardized eigenvalues ≥1, for *Laelaps manguinhosi* and *Gigantolaelaps* spp. Loading values are printed in bold face for the component(s) they are most strongly associated with (whether positively or negatively).

| | *Laelaps manguinhosi* | | | | *Gigantolaelaps* spp. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Character | PC 1 | PC 2 | PC 3 | PC 4 | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 |
| DSL | **0.95** | 0.11 | 0.20 | 0.02 | 0.39 | **0.88** | 0.05 | 0.00 | 0.09 |
| DSW | **0.95** | 0.09 | 0.15 | 0.18 | -0.14 | **0.94** | 0.07 | 0.07 | 0.10 |
| j5-j5 | -0.13 | **-0.73** | -0.30 | 0.05 | **0.92** | -0.02 | -0.26 | 0.02 | 0.07 |
| Z5-z5 | **0.61** | -0.43 | -0.09 | 0.45 | **0.51** | **0.51** | 0.30 | 0.09 | 0.32 |
| j5L | **0.78** | 0.49 | 0.27 | 0.11 | -0.62 | **0.72** | -0.22 | 0.06 | -0.04 |
| Z5L | **0.86** | 0.40 | 0.21 | 0.05 | -0.45 | **0.82** | -0.15 | -0.01 | -0.12 |
| J5-J5 | **0.71** | 0.39 | -0.27 | -0.22 | 0.12 | **-0.61** | **0.57** | -0.17 | 0.02 |
| Z5-Z5 | **0.90** | -0.06 | -0.21 | -0.06 | -0.25 | -0.20 | **0.82** | -0.05 | -0.10 |
| J5L | **0.70** | 0.41 | -0.33 | -0.19 | 0.23 | 0.03 | **-0.60** | -0.30 | -0.36 |
| Z5L | **0.90** | 0.11 | -0.02 | 0.10 | **-0.63** | 0.57 | -0.21 | -0.12 | -0.16 |
| CAP-CAP | **0.75** | -0.28 | -0.08 | -0.49 | **0.62** | -0.20 | -0.08 | -0.07 | 0.06 |
| CAPL | 0.11 | **0.86** | -0.32 | 0.11 | 0.45 | **0.80** | 0.26 | 0.17 | -0.01 |
| INNL | **0.63** | 0.56 | -0.43 | -0.03 | **0.84** | 0.27 | -0.32 | -0.12 | 0.04 |
| CAP-INN | **0.89** | 0.00 | -0.03 | -0.26 | **0.86** | -0.22 | -0.35 | -0.09 | 0.06 |
| SSL | **0.83** | 0.07 | 0.49 | 0.09 | **0.67** | **0.63** | 0.28 | 0.07 | 0.06 |
| SSW | **0.85** | -0.30 | -0.28 | 0.08 | **-0.70** | 0.57 | -0.02 | -0.17 | 0.02 |
| S1-S1 | **0.75** | -0.47 | -0.08 | -0.21 | **-0.48** | 0.26 | 0.27 | -0.29 | **0.53** |
| S3-S3 | **0.87** | -0.22 | -0.14 | 0.07 | **-0.89** | 0.30 | -0.02 | -0.15 | 0.08 |
| S1l | **0.86** | -0.29 | -0.04 | -0.04 | **0.58** | -0.19 | 0.39 | -0.40 | -0.38 |
| S3L | **0.90** | -0.13 | 0.22 | 0.02 | **0.70** | 0.38 | 0.32 | -0.06 | -0.23 |
| ESL | **0.89** | -0.05 | 0.36 | -0.02 | 0.61 | **0.71** | -0.10 | 0.00 | 0.14 |
| E5-E5 | **0.84** | -0.07 | 0.16 | 0.00 | **-0.82** | 0.24 | 0.03 | -0.17 | 0.11 |
| ESW | **0.88** | -0.35 | -0.06 | -0.08 | -0.14 | 0.31 | **-0.71** | -0.40 | 0.11 |
| S4L | **0.80** | 0.28 | 0.06 | -0.25 | 0.20 | 0.23 | **0.77** | -0.34 | -0.16 |
| E5L | **0.86** | 0.09 | -0.05 | -0.34 | **-0.59** | 0.45 | 0.12 | -0.42 | -0.14 |
| PARAL | **0.70** | 0.32 | -0.17 | -0.14 | -0.16 | **0.90** | 0.28 | -0.01 | -0.11 |
| POSTL | **0.79** | 0.40 | 0.26 | -0.17 | **-0.74** | 0.58 | -0.16 | -0.13 | -0.01 |
| POST-EDGE | **0.63** | **0.61** | 0.23 | 0.26 | 0.41 | -0.05 | 0.12 | **-0.63** | 0.25 |
| PARA-PARA | **0.77** | 0.23 | 0.17 | 0.30 | **0.55** | **-0.57** | 0.16 | -0.24 | 0.25 |
| ASW | **0.85** | 0.16 | -0.12 | 0.31 | 0.30 | **0.74** | -0.29 | -0.14 | -0.02 |
| PROXCOX | **0.65** | -0.56 | 0.21 | 0.06 | **0.87** | 0.23 | -0.27 | 0.04 | -0.01 |
| DISTCOX | **0.56** | -0.33 | -0.38 | 0.51 | -0.30 | **0.80** | 0.39 | 0.18 | -0.03 |
| POSTCOX2 | **0.90** | -0.23 | 0.00 | 0.03 | **0.80** | 0.38 | -0.28 | -0.10 | -0.09 |
| POSTCOX3 | 0.51 | **-0.63** | -0.06 | 0.03 | 0.32 | **0.89** | 0.11 | 0.13 | -0.02 |
| POSTCOX4 | 0.29 | **0.67** | -0.54 | 0.17 | **0.79** | 0.38 | 0.24 | 0.05 | -0.11 |
| DFEM1L | **0.77** | -0.48 | -0.20 | -0.19 | **0.78** | 0.44 | 0.10 | 0.03 | 0.14 |
| DGEN1L | **0.74** | -0.46 | -0.13 | 0.14 | **0.80** | 0.23 | 0.03 | -0.10 | -0.03 |
| Eigenvalue | 21.69 | 5.78 | 2.10 | 1.58 | 13.27 | 10.68 | 4.07 | 1.55 | 1.08 |
| Percentage | 58.6 | 15.6 | 5.7 | 4.3 | 35.9 | 28.9 | 11.0 | 4.2 | 2.9 |
| Cum. Perc. | 58.6 | 74.2 | 79.9 | 84.2 | 35.86 | 64.73 | 75.73 | 79.93 | 82.86 |

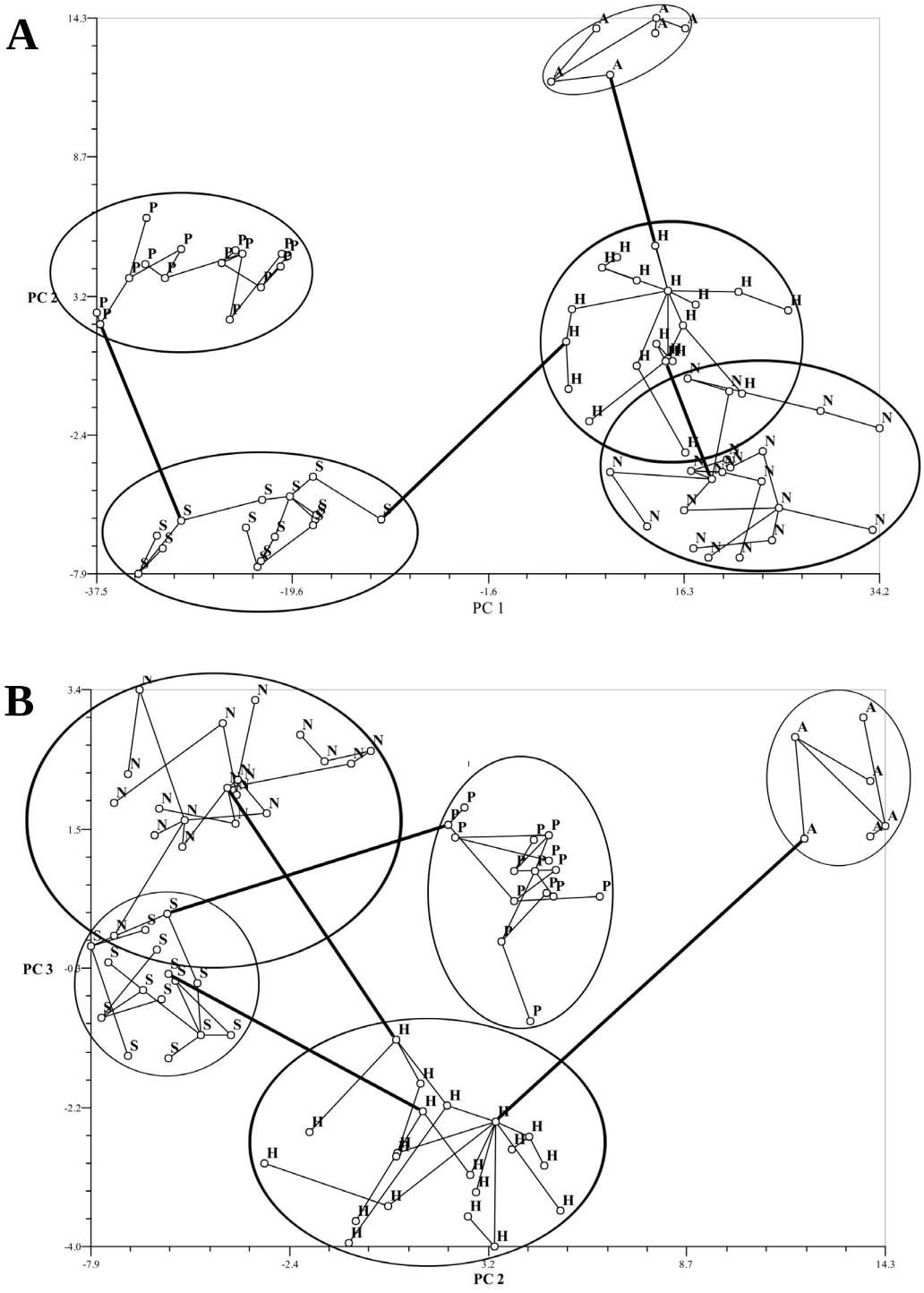


**FIGURE 3**. Two-dimensional plots resulting from principal component analysis of *Laelaps manguinhosi* measurement data. Fig. 3A, PC 1 x PC 2; Fig. 3B, PC 2 x PC 3. Letters indicate host rodent species (A, *Sooretamys angouya*; H, *Holochilus chacarius*; N, *Nectomys squamipes*; P, *Pseudoryzomys simplex*; S, *Scapteromys aquaticus*). A minimum spanning tree is superimposed to further indicate relationships among individuals and groups; thicker segments of the MST are those connecting the host-groups.

**TABLE 2.** Results of ANOVA and MANOVA analyses of *Laelaps manguinhosi* mites from *Holochilus chacarius* and *Nectomys squamipes*. DF = 37, 2 for MANOVA, DF = 1 for all ANOVAs. Statistical significance of F values indicated by: *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $0.001 \geq P$. Means in bold face are significantly greater than comparable mean from other host species.

| | *Holochilus* | | | *Nectomys* | | | | |
|---|---|---|---|---|---|---|---|---|
| Character | Mean | Minimum | Maximum | Mean | Minimum | Maximum | *F*-value | *P* |
| DSL | 605.4 | 582 | 635 | **637.3** | 612 | 666 | 38.9 | *** |
| DSW | 400.1 | 384 | 418 | **416.7** | 400 | 431 | 28.5 | *** |
| j5-j5 | 49.8 | 48 | 50.2 | 62.4 | 46 | 53 | 0.2 | NS |
| Z5-z5 | 112.2 | 108 | 117 | 114.2 | 108 | 120 | 3.6 | NS |
| j5L | 56.1 | 53 | 59 | **59.2** | 53 | 66 | 6.5 | * |
| Z5L | 63.6 | 60 | 67 | **67.0** | 63 | 75 | 11.0 | ** |
| J5-J5 | **66.1** | 62 | 70 | 62.4 | 58 | 65 | 29.6 | *** |
| Z5-Z5 | 107.2 | 101 | 114 | 106.0 | 100 | 110 | 1.3 | NS |
| J5L | **49.0** | 45 | 55 | 44.8 | 39 | 48 | 37.7 | *** |
| Z5L | 95.4 | 90 | 102 | 96.3 | 91 | 102 | 0.7 | NS |
| CAP-CAP | 62.7 | 59 | 66 | **64.4** | 60 | 68 | 6.2 | * |
| CAPL | **16.6** | 14 | 18 | 13.4 | 13 | 15 | 87.9 | *** |
| INNL | **38.0** | 35 | 43 | 32.2 | 28 | 35 | 67.1 | *** |
| CAP-INN | 46.2 | 45 | 59 | 46.8 | 45 | 49 | 1.3 | NS |
| SSL | 95.6 | 89 | 100 | **108.4** | 102 | 117 | 73.5 | *** |
| SSW | 153.4 | 145 | 160 | 152.8 | 149 | 156 | 0.7 | NS |
| S1-S1 | 69.8 | 66 | 75 | **72.0** | 68 | 75 | 9.7 | ** |
| S3-S3 | 141.8 | 138 | 149 | 142.6 | 136 | 147 | 0.5 | NS |
| S1l | 85.8 | 81 | 90 | **89.3** | 80 | 100 | 7.5 | ** |
| S3L | 100.6 | 94 | 105 | **108.8** | 102 | 119 | 48.6 | *** |
| ESL | 141.8 | 133 | 150 | **162.8** | 155 | 170 | 72.8 | *** |
| E5-E5 | 89.3 | 84 | 92 | **92.6** | 88 | 99 | 14.6 | *** |
| ESW | 173.6 | 163 | 183 | **179.1** | 171 | 188 | 15.2 | *** |
| S4L | 100.0 | 97 | 105 | 100.8 | 93 | 113 | 0.1 | NS |
| E5L | 94.9 | 90 | 100 | 95.5 | 85 | 105 | 0.3 | NS |
| PARAL | 41.9 | 36 | 48 | 40.6 | 33 | 45 | 2.0 | NS |
| POSTL | 72.8 | 68 | 78 | **76.4** | 68 | 84 | 9.7 | ** |
| POST-EDGE | 73.6 | 70 | 77 | 74.2 | 70 | 79 | 0.4 | NS |
| PARA-PARA | 33.1 | 29 | 35 | **34.2** | 31 | 35 | 5.8 | * |
| ASW | 106.4 | 100 | 111 | 104.5 | 99 | 110 | 3.9 | NS |
| PROXCOX | 32.8 | 30 | 35 | **36.4** | 34 | 40 | 50.8 | *** |
| DISTCOX | 29.0 | 27 | 31 | 28.5 | 25 | 33 | 1.2 | NS |
| POSTCOX2 | 44.6 | 42 | 46 | **46.8** | 44 | 51 | 16.3 | *** |
| POSTCOX3 | 28.1 | 25 | 33 | **29.6** | 28 | 33 | 8.8 | ** |
| POSTCOX4 | **24.8** | 23 | 28 | 19.7 | 18 | 21 | 83.7 | *** |
| DFEM1L | 60.3 | 58 | 63 | **61.7** | 58 | 65 | 7.9 | ** |
| DGEN1L | 49.6 | 48 | 52 | 51.1 | 44 | 56 | 3.8 | NS |
| MANOVA | | | | | | | 7472.4 | *** |

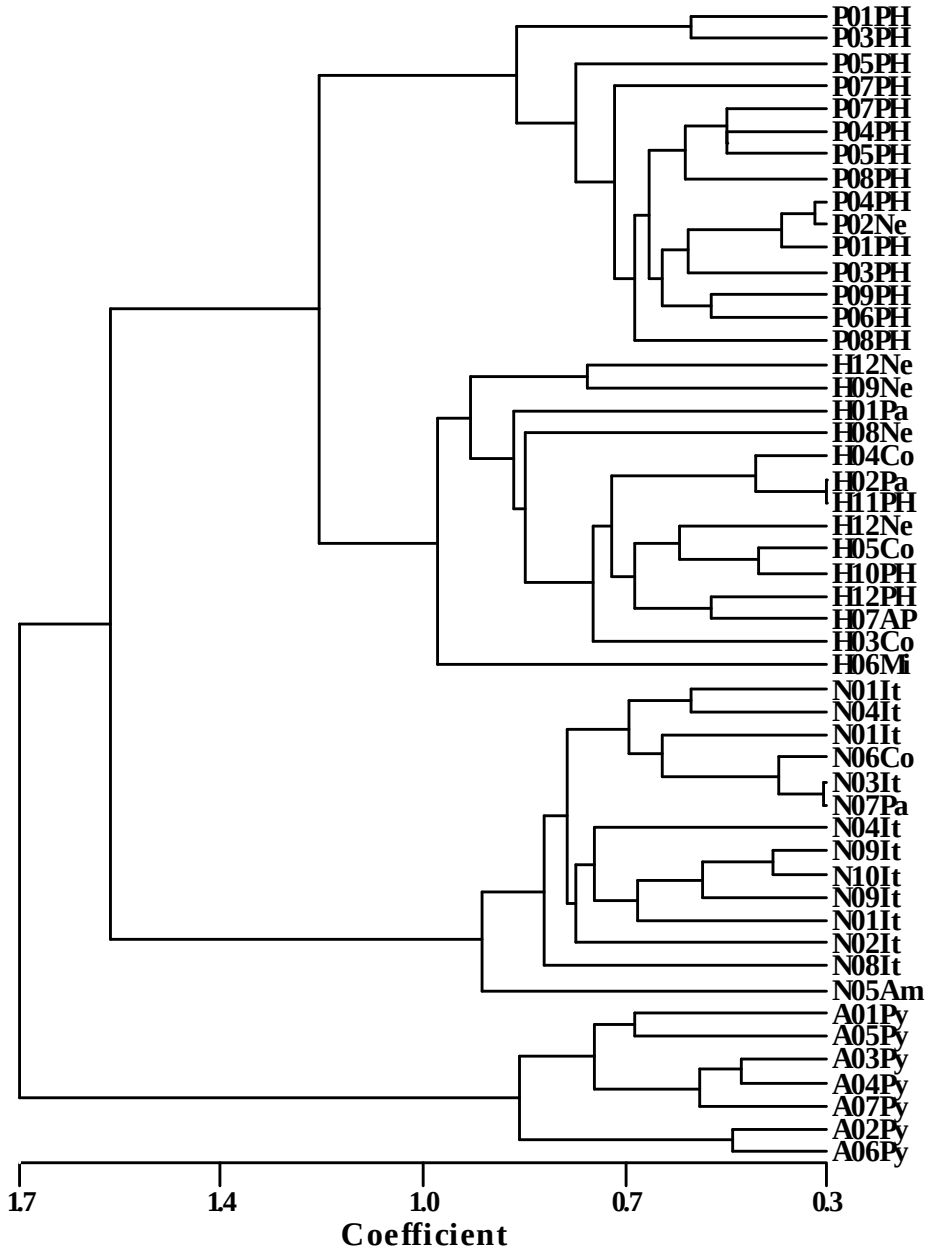


**FIGURE 4**. Phenogram of *Gigantolaelaps* spp. relationships, calculated from a dissimilarity metric based on standardized characters. In the OTU labels, capital letters indicate host rodent species (A, *Sooretamys angouya*; H, *Holochilus chacarius*; N, *Nectomys squamipes*; P, *Pseudoryzomys simplex*); the next two-digit (or letter) code indicates the locality (see Fig. 1, Appendix1); and the final two-digit code differentiates among individual hosts collected from that locality. A final lower-case letter differentiates among mites collected from a particular host individual.

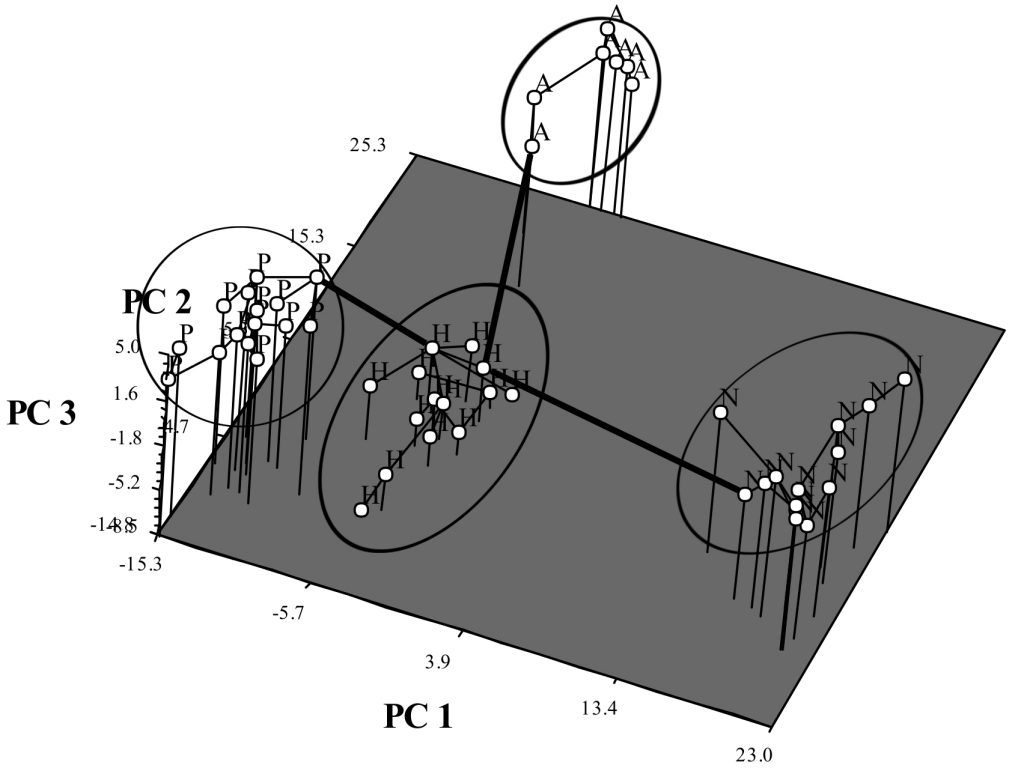


**FIGURE 5**. 3-D model resulting from principal component analyses of *Gigantolaelaps* spp. measurement data, showing principal components 1–3. Letters indicate host rodent species (A, *Sooretamys angouya*; H, *Holochilus chacarius*; N, *Nectomys squamipes*; P, *Pseudoryzomys simplex.* A minimum spanning tree is superimposed to further indicate relationships among individuals and groups; thicker segments of the MST are those connecting the host-groups.

## Discussion

Traditional morphometric techniques are an important tool for separating morphologically similar species of laelapines associated with different mammalian hosts (Gettinger & Owen 2000). Although genetic techniques are often employed to help define species boundaries, their utility relies on relatively fresh specimens suitable for DNA amplification. Specimens suitable for such analyses are often unavailable, and often crucial historical specimens must be ignored. Here, we apply PCA as a simple ordination tool to identify patterns of distribution among mite populations, and to express these data graphically in a way that clearly reveals the morphometric similarities and differences among mite individuals.

These studies suggest that the host-species specialization of laelapine mites infesting small mammals has promoted high diversity of these ectoparasites in the Neotropics. However, the lack of taxonomic resolution for these mites has precluded rigorous tests of this hypothesis. Here we show that sympatric populations of nominal laelapine species occupying phylogenetically distinct yet ecologically associated hosts display clear and concordant differences in morphology. Furthermore, these morphometric results point to the need for substantial reinterpretations of laelapine ecology and biogeography.

Although *Laelaps manguinhosi* has been described as a polyxenous ectoparasite that "would undoubtedly infest, at least temporarily, almost any warm-blooded animal with which it came in contact" (Furman 1972), our results refute this observation. Epidemiologically, polyxenous ectoparasites have great potential for serving as vectors of parasites and diseases between different host species in the mammalian community, and between sylvatic and domestic hosts (including man). Careful scrutiny of the larger inventories of neotropical laelapine mites (Fonseca 1958, Tipton *et al*. 1966, Furman 1972) may reveal that their associations are not polyxenous at all, but rather "pleioxenous" (associated with multiple, phylogenetically related host species) or indeed monoxenous. If so, this would imply that laelapine mites may have more closely cospeciated with mammalian hosts than has previously been recognized.

Laelapine mites are small and incapable of active dispersal over great distances. Without a phoretic associate, they are generally incapable of infesting alternative hosts outside the particular small mammal community in which they occur naturally. The presence of the same nominal laelapine species associated with five distinct rodent species from palustrine habitats in Paraguay allowed us to carry out comparative studies among host-mite populations and assess the accuracy of the assumption that these are polyxenous mite species infesting an array of mammalian host species in similar habitats. Because small mammals that are close phylogenetically rarely occur in sympatry, we would expect these host-sharing exchange events to occur somewhat at random within ecologically associated mammals. We have increased the robustness of this comparison by including more than a single mite genus in the analysis (*Laelaps* and *Gigantolaelaps*). The concordance of the morphometric patterns is striking, and further implies that these species are components of cospeciated mite communities.

The observation of size-free variation does not support the hypothesis that allometric differences alone are responsible for the morphometric variation in mite populations infesting different host species in Paraguay. If these populations were conspecific, we would expect shape differences to be minimal and insignificant. Our observation of the influence of shape differences in geographically sympatric populations implies that they are reproductively isolated, and host specificity is maintained by a consistent ecological barrier to dispersal among different host species.

The morphometric analysis of *Gigantolaelaps* spp. reveals a very different kind of character variation than that in the *Laelaps manguinhosi* data; PC1 is not a distinctive size component. However, this may be due to an unusual morphological characteristic of these large laelapines. Apparently with age, these mites accrue a granular thickening of the epicuticle along the external border of the major shields, expressing a kind of pseudo-allometric growth. Because specimens were not chosen to represent any specific age-group other than "adult," the variation among mite individuals is randomly increased, disguising general body size differences among host-mite populations. An analysis of more homogeneous samples (choosing only teneral individuals, for instance) may increase the character loading on the first principal component.

With the exception of *Scapteromys*, all the host species in this study are classified within the rodent tribe Oryzomyini, and were grouped within "clade D" of Weksler's (2006) phylogenetic tree based on molecular and morphological data. In Paraguay, each of these four oryzomyine rodents were infested with a single species of *Gigantolaelaps* and *Laelaps*. Of the 18 species of *Gigantolaelaps* presently recognized, only three species (*G. goyanensis*, *G. mattogrossensis*, and *G. vitzthumi*) share two meristic characters, tibia IV with 10 setae (all other known species with holotrichous 11), and deutosternum with 11–12 rows of teeth. The *Laelaps* associated with these four rodent species all clearly key to *L. manguinhosi* in Furman's (1972) key to the South American species of *Laelaps*. The data presented here are concordant with the phylogenetic hypotheses presented in Weksler (2006) and Weksler et al, (2006) and implies that these mites have coevolved with their oryzomyine rodent hosts.

During 25 years of research in the neotropics, the senior author has never found a carefully sampled oryzomyine rodent species to be uninfested by *Gigantolaelaps* (some with two species) and *Laelaps* (one to four species). Given 114 species of oryzomyine rodents recognized in the most recent Mammal Species of the World (Carleton & Musser, 2005), and assuming each is infested with one or more species of *Gigantolaelaps* and *Laelaps*, we would predict a fauna of between 200-300 (or more) species of these two genera alone infesting neotropical oryzomyine rodents. However, the importance of our research is not simply that a large number of unrecognized mite species likely exist in the neotropics, but that monoxenous ectoparasites are ecologically very different from generalized polyxenous ectoparasites. Our results strongly indicate that laelapine mite species are primarily monoxenous, and suggests that they may be of considerable value in deciphering the alpha (and perhaps higher-level) systematics of their rodent hosts.

Although we suspect that the morphometrically distinct mite populations revealed in this study are reproductively isolated on their respective hosts, we have stopped short of formally describing new species. The approaching biodiversity crisis underscores the need to describe new species when they are encountered, but there should not be a rush to describe new taxa without the appropriate tests. There are several ways that our results could by clarified and reinforced, including: 1) comparison of DNA sequence data, 2) host-exchange experiments (Esberard et al. 2005, Dick et al. 2009), 3) inclusion of males and immature mite stases as independent morphological tests, and 4) geographic tests with mites infesting sympatric host species.

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**Appendix 1.**—Locality name, coordinates, department, rodent species, rodent specimen number, and mites (and number of mites, if >1) collected (G, *Gigantolaelaps* spp.; M, *Laelaps manguinhosi*). TK numbers represent unique field catalog numbers, each associated with a single rodent specimen plus all ancillary materials (e.g., ectoparasites, frozen tissues). See Fig. 1 for localities and their ecoregional associations.

**BOLIVIA: (SC)** (17°39'S, 64°04'W), Depto. Santa Cruz de la Sierra: *H. chacarius*: BOL41 (M); **BRAZIL: (DF)** (15°56'S, 47°53'W), Distrito Federal: *N. squamipes*: DF166 (M), DF200 (M); **(MG)** (16°21'S, 56°40'W), Matto Grosso: *H. chacarius*: PAN54 (M), PAN65 (M); **(RJ)** (23°08'S, 44°10'W), Rio de Janeiro: *N. squamipes*: RIO14 (M), RIO17 (M), RIO29 (M), RIO31 (M); **(SP)** (25°07'S, 47°57'W), Sao Paulo: *N. squamipes*: SP845 (M); **PARAGUAY: (1) Estancia La Victoria** (23°39.03'S, 58°34.79'W), Depto. Presidente Hayes: *P. simplex*: TK60118 (2G, 2M); **(4) Estancia Sombrero** (25°03'S, 56°40'W), Depto. Cordillera: *H. chacarius*: TK64834 (M); **(5) Lago Ypoá** (26°01.35'S, 57°28.73'W**),** Depto. Paraguarí: *H. chacarius*: TK60569 (G), TK60583 (G), *N. squamipes*: TK60553 (M); **(6) Estancia Cerrito** (23°15'S, 57°30'W), Depto. Concepción: *H. chacarius*: TK60656 (G), TK60657 (G), TK60658 (G); **(7) Fuerte Olimpo** (21°02'S, 57°52'W), Depto. Alto Paraguay: *H. chacarius*: TK60683 (M); **(8) Isla Yacyretá (**27°24.49'S, 56°45.79'W), Depto. Misiones: *H. chacarius*: TK60852 (G, M); **(9) Parque San Rafael** (26°45.32'S, 55°51.83'W), Depto Itapúa: *N. squamipes*: TK60890 (3G), TK60932 (G), TK60953 (2G, M), TK60978 (G, M); **(10) Estancia Doña Julia** (20°11'S, 58°90'W), Depto. Alto Paraguay: *H. chacarius*: TK61113 (M), TK61138 (G, M), TK61139 (M), TK61152 (M); **(12) Cerro Corá** (22°37.20'S, 56°02.62'W), Depto. Amambay; *N. squamipes*: TK61427 (G, M); **(13) Parque Nacional Serrania San Luis** (22°40'S, 57°21'W), Depto. Concepción; *N. sqamipes*: TK61633 (G, M); **(14) Estancia Yacaré** (26°39.49'S, 58°04.07'W**),** Depto. Ñeembucú: *H. chacarius*: TK61649 (G, M), TK61651 (G, M), TK64352 (M), TK64538 (G), TK64383 (2G, M); *P. simplex*: TK61744 (G, 2M); *S. aquaticus*: TK61724 (3M), TK61727 (M), TK61765 (M), TK64379 (2M), TK64386 (2M0, TK64388 (M), TK64423 (M); **(16) Estancia Loma Porá** (23°33.15'S, 57°34.30'W), Depto. Presidente Hayes; *H. chacarius*: TK61935 (G, M), TK61941 (M), TK61956 (M), TK61958 (M), TK64538 (M); *P. simplex*: TK61992 (2G, M); *S. aquaticus*: TK61933 (M); **(17) Laguna Placenta** (21°08.62'S, 59°24.86'W), Depto. Alto Paraguay: *P. simplex*: TK62425 (2M); **(18) Estancia Samaklay** (23°28.81'S, 59°48.43'W), Depto. Presidente Hayes: *H. chacarius*: TK62660 (G); *P. simplex*: TK62518 (G, 2M), TK62526 (2G, 2M), TK62527 (G, M), TK62576 (2G, 2M), TK62577 (2G, M), TK65005 (G); *S. aquaticus*: TK62165 (M); **(22) Estancia Golondrina** (24°34'S, 55°29'W), Depto. Caazapá: *H. chacarius*: TK63712 (M); **(23) Parque Nacional Ybycuí (**26°05'S, 56°51'W), Depto. Paraguarí: *N. squamipes*: TK63841 (G, M), TK63877 (2M); **(26) Ape Aimé** (26°32'S, 54°50'W), Depto. Itapúa: *N. squamipes*: TK65823 (M), TK65856 (G, M), TK65973 (2G, M), TK65974 (G), TK65975 (M); **(27) Estancia San José** (27°10'S, 58°24'W), Depto. Ñeembucú: *S. angouya*: TK66106 (7G, 6M); *S. aquaticus*: TK66037 (M), TK66053 (M), TK66054 (M), TK66067 (M); **(28) Estancia Parabel (**26°21'S, 55°31'W) Depto. Itapúa: *N. squamipes*: TK66435 (M).

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