

MUSEUM SPECIMENS AND PHYLOGENIES ELUCIDATE ECOLOGY'S ROLE IN COEVOLUTIONARY ASSOCIATIONS BETWEEN MITES AND THEIR BEE HOSTS

Pavel B. Klimov,¹ Barry M. OConnor,² and L. Lacey Knowles³

University of Michigan, Museum of Zoology, 1109 Geddes Ave., Ann Arbor, Michigan 48109-1079

¹*E-mail: pklimov@umich.edu*

²*E-mail: bmoc@umich.edu*

³*E-mail: knowlesl@umich.edu*

Received December 26, 2006

Accepted February 19, 2007

Coevolutionary associations between hosts and symbionts (or parasites) are often reflected in correlated patterns of divergence as a consequence of limitations on dispersal and establishment on new hosts. Here we show that a phylogenetic correlation is observed between chaetodactylid mites and their hosts, the long-tongued bees; however, this association manifests itself in an atypical fashion. Recently derived mites tend to be associated with basal bee lineages, and vice versa, ruling out a process of cospeciation, and the existence of mites on multiple hosts also suggests ample opportunity for host shifts. An extensive survey of museum collections reveals a pattern of infrequent host shifts at a higher taxonomic level, and yet, frequent shifts at a lower level, which suggests that ecological constraints structure the coevolutionary history of the mites and bees. Certain bee traits, particularly aspects of their nesting behavior, provide a highly predictive framework for the observed pattern of host use, with 82.1% of taxa correctly classified. Thus, the museum survey and phylogenetic analyses provide a unique window into the central role ecology plays in this coevolutionary association. This role is apparent from two different perspectives—as (a) a constraining force evident in the historical processes underlying the significant correlation between the mite and bee phylogenies, as well as (b) by the highly nonrandom composition of bee taxa that serve as hosts to chaetodactylid mites.

KEY WORDS: Apidae, chaetodactylidae, coevolution, long-tongued bees, Megachilidae, mites, nest architecture.

The intimate interactions of symbionts (or parasites) with their hosts predicts not only the coevolution of symbiont and host biology, but also nonrandom associations among species. A strict history of cospeciation will generate a concordance between symbiont and host phylogenies (Fahrenholz's rule) (Eichler 1948; Klassen 1992; Peek et al. 1998; Clark et al. 2000; Lo et al. 2003; Degnan et al. 2004). However, a suite of factors influences the degree of phylogenetic concordance (Reed and Hafner 1997; Johnson et al. 2002; Rannala and Michalakis 2003; Ronquist 2003; Taylor and Purvis 2003; Quek et al. 2004; Ricklefs

et al. 2004; Smith et al. 2004; Banks et al. 2005), including shifts to new hosts or speciation of the symbiont on the same host (Clayton and Johnson 2003; Clayton et al. 2003; Clayton et al. 2004; Weckstein 2004).

Chaetodactylid mites represent a compelling group to investigate factors influencing divergence across their hosts. Chaetodactylids are obligate associates of solitary and facultatively social long-tongued bees (Apidae and Megachilidae), representing the most species-rich group among 30 other bee–mite lineages (reviewed Eickwort 1994). More than 200 species of chaetodactylid

mites live in bee nests feeding throughout bee development as either mutualists (feeding on nest waste), parasitoids (killing the bee egg or larvae), or as commensals or cleptoparasites (feeding on provisioned pollen) (Krombein 1962; Roubik 1987; Abrahamovich and Alzuet de 1990; Qu et al. 2002). During each bee generation, the nonfeeding immature mites disperse to new bee nests on the newly emerged adult bees. Successful dispersal to new nests is critical for the mites because the bees do not commonly reuse their old nests. The dispersing life-history stage of the mite (the deutonymph) is tightly synchronized with the last stages of its bee host's development. Moreover, some mites are carried in specialized pouches (acarinarium) of their bee hosts (Fain and Pauly 2001; Okabe and Makino 2002).

These striking adaptations suggest that the mites and bees have been involved in long-term coevolutionary interactions, and that shifts among distantly related bee hosts would be rare events. For example, the 170 mite species of the genus *Sennertia* (Klimov and OConnor) have remained strictly associated with the more than 500 species of carpenter bees (Michener 2000), even tracking the ancient dispersal (34–34.6 Mya) of Old World bee lineages to the New World (Leys et al. 2002). Nevertheless, highly nonrandom host shifts on unrelated hosts probably have occurred in the early stages of mite evolution.

Using a comparison of the chaetodactylid mite and long-tongued bee phylogenies, and an extensive survey of museum material, we investigate what factors have structured the historical associations of these symbionts/parasites and their hosts. Our goals were to (1) determine whether the evolutionary history of major clades of mites and bee supports a model of coevolutionary divergence, and (2) understand the underlying determinants of the observed phylogenetic associations, and specifically (a) what historical events (e.g., cospeciation, host switching, and speciation or extinction on the host (Johnson et al. 2003; Ronquist 2003) structure this symbiont/parasite–host assemblage, and (b) whether bee ecology predicts which potential hosts are likely to be part of the bee–mite coevolutionary association.

Materials and Methods

SPECIMENS AND DETERMINATION OF HOST ASSOCIATIONS

A thorough survey of museum specimens determined that most chaetodactylid mites are not associated with a single host species, but instead, exist on several closely related sympatric hosts. The only well-supported exception is *Sennertia americana*, which is exclusive to a single host; several poorly known species are also described from a single host, but may reflect the relative rarity of these species (e.g., species associated with *Chalepogenus*, *Ancylloscelis*, *Ptilothrix*, and *Diadasia*). The results of the survey on

host ranges of the different chaetodactylid mites are the focus of this study and are summarized at the generic level of the bees (Table 1).

Mites from roughly 1500 museum specimens of long-tongued bees from 18 museum collections in the United States and abroad were examined for mites, as were specimens freshly collected by the authors in North America and Africa. All major groups of short- and long-tongued bees were sampled, but only the long-tongued bees had chaetodactylid mites (Table 1). This survey yielded about 230 mite species that includes all currently known species and species groups, as well as a large number of undescribed taxa, with the exception of a few species of *Sennertia* for which their bee hosts were not available for inspection. The majority of these mite specimens (about 5000 slide-mounted specimens) are vouchered in the University of Michigan, Museum of Zoology (Klimov and OConnor 2003).

PHYLOGENETIC ANALYSES

Only the relationships among mite genera are considered (Table 1) for comparison with the phylogeny of the bee hosts because of constraints imposed by incomplete host phylogenies (Michener 2000) and to emphasize the unique cophylogenetic pattern observed at this level. Maximum parsimony analyses of 51 morphological characters (the appendix) from chaetodactylid heteromorphic deutonymphs was used to estimate the mite phylogeny were conducted in PAUP* 4.0b10 (Swofford 2002) using either equal character weights, or characters weighted according to the degree of homoplasy using Goloboff's concave weighting function (Goloboff 1993) with the constant of concavity (*k*) set to 2 (implied weights parsimony). A bootstrap majority rule consensus tree was calculated using the branch-and-bound algorithm and 10,000 bootstrap replicates. Bremer branch support or decay indices were also calculated using PAUP* with a command file generated in TreeRot.v2 (Sorenson 1999). A Bayesian analysis was also conducted; four chains (three hot, one cold) of 5×10^6 generations each with a burn-in of 6300 and a sampling frequency of 100 were used (MrBayes ver. 3.1.1 [Ronquist and Huelsenbeck 2003]). Five independent analyses were conducted to confirm convergence; all resulted in similar topologies. All analyses produced the same topology, except for unresolved relationships in *Achaetodactylus* in the two parsimony analyses. *Megacanestrinia* (family Canestriniidae) was selected as the outgroup for the mite phylogeny as Chaetodactylidae is most likely a basal group of the superfamily Hemisarcoptoidea (OConnor 1993). The influence of outgroup choice on the phylogeny and position of the root were thoroughly investigated and the topology of the tree was robust to various potential outgroups (54 astigmatid families, including Canestriniidae, Aeroglyphidae, Glycyphagidae, Winterschmidtidae, Hyadesiidae, and Algophagidae). The trees are deposited

Table 1. Distribution of chaetodactylid mites across their bee hosts showing their geographic affiliations. Aust, Australian region; Orient, Oriental region; Madag, Madagascar; Afr, Afrotropical region; Palear, Palearctic region; Near, Nearctic Region; Antill, the Greater and Lesser Antilles, excluding Trinidad; Arauc, Araucanian region (Michener 2000). Some associations that are marginally occurred in a region are omitted. Unusual finding of chaetodactylids on *Andrena*, *Halictus*, *Anthophora*, *Apis*, *Vespula*, Passalidae (Zachvatkin 1941; Chmielewski 1993; Haitlinger 1999), and *Bombus* (our data) are omitted. Cleptoparasites of the principal hosts (parenthesis) that may transfer chaetodactylids are also not included: *Stelis* (*Osmia*), sapygids *Polochrum* (*Xylocopa*), *Sapyga* (*Chelostoma*) (Zachvatkin 1941; Samšič 1973), and *Coelioxoides* (*Tetrapedia*) (our data).

Mite taxon	Bee taxon	Aust	Orient	Madag	Afr	Palear	Near	Neotr	Antill	Arauc
	Megachilidae									
	Lithurgini									
<i>Chaetodactylus</i>	<i>Lithurgus</i>	+	+	+	+	+	+	+	+	+
<i>Chaetodactylus</i>	<i>Trichothurgus</i>									+
<i>Chaetodactylus</i>	<i>Microthurge</i>							+		
	Osmiini									
<i>Chaetodactylus</i>	<i>Osmia</i>					+	+			
<i>Chaetodactylus</i>	<i>Hoplitis</i>						+			
<i>Chaetodactylus</i>	<i>Chelostoma</i>					+				
	Anthidiini									
<i>Chaetodactylus</i>	<i>Rhodanthidium</i>					+				
<i>Chaetodactylus</i>	<i>Anthidium</i>							+		+
	Megachilini									
<i>Chaetodactylus</i>	<i>Megachile</i>					+				
	Apidae (Xylocopinae)									
	Xylocopini									
<i>Sennertia</i>	<i>Xylocopa</i>	+	+	+	+	+	+	+	+	
	Ceratinini									
<i>Sennertia</i>	<i>Ceratina</i>		+		+	+	+	+		
<i>Achaetodactylus</i>	<i>Ceratina</i>				+					
	Apidae (Apinae)									
	Tapinotaspidini									
<i>Chaetodactylus</i>	<i>Chalepogenus</i>									+
	Tetrapediini									
<i>Roubikia</i>	<i>Tetrapedia</i>							+		
	Emphorini									
<i>Chaetodactylus</i>	<i>Melitoma</i>						+			
<i>Chaetodactylus</i>	<i>Diadasia</i>						+			+
<i>Chaetodactylus</i>	<i>Ptilothrix</i>									+
<i>Chaetodactylus</i>	<i>Ancyloscelis</i>							+		
	Centridini									
<i>Centriacarus</i>	<i>Centris</i>						+	+		

in TreeBase (SN3139). Taxon selection for the analyses above (Table 2) was done on the basis of a larger analysis representing all species groups and genera of chaetodactylids (46 species) (online Supplementary Figure S1).

The results from the morphology-based phylogenetic analysis (Fig. 1) were confirmed by a molecular phylogenetic analysis of nuclear protein-coding and ribosomal gene sequence data (1.1 kb of EF1- α , 1.8 kb of 18S, and 2.15 kb of domains 1–5 and 9–10 of 28S rDNA) for a subset of taxa (one species of *Achaetodactylus*, five species of *Chaetodactylus*, and seven species of *Sennertia*).

This analysis is part of a larger ongoing molecular phylogenetic project (P. Klimov and B. M. OConnor, unpubl. data); as with the morphological based analyses, robustness of the phylogenetic tree to different outgroups (specifically, representatives from 54 different astigmatid mite families) was confirmed with the molecular phylogenetic analyses as well.

As a phylogenetic estimate of the bee hosts, the generic topology of Roig-Alsina and Michener, analysis C (Roig-Alsina and Michener 1993) and the tribal phylogeny of Engel (2001) were used. These findings are widely accepted by bee systematists and

Table 2. Taxa used in the generic-level phylogenetic analysis of Chaetodactylidae. Early derivative mite lineages representing all known host associations at the generic level were selected for the phylogenetic analyses (except for little known derived species of *Chaetodactylus* associated with Emphorini and Tapinotaspidini, see Table 1. The taxon selection was based on the results of the 46-taxon phylogenetic analysis (online Supplemental Figure S3).

Taxon	Host	Collection locality
<i>Megacanestrinia</i> sp.	<i>Tefflus zanzibaricus</i>	Tanzania
<i>Centriacarus turbator</i>	<i>Centris (Heterocentris) vittata</i> , <i>C.</i> sp.	Brazil, Peru, Colombia, Panama, Mexico
<i>Centriacarus guahibo</i>	<i>Centris</i> sp.	Venezuela
<i>Roubikia panamensis</i>	<i>Tetrapedia diversipes</i> , <i>T. peckholtii</i> , <i>T.</i> sp., <i>Coelioxoides waltheriae</i> (cleptoparasite of <i>Tetrapedia</i>)	Panama, Mexico, French Guiana, Brazil, Bolivia
<i>Roubikia latebrosa</i>	<i>Tetrapedia</i> sp.	Peru
<i>Achaetodactylus ceratinae</i>	<i>Ceratina opaca</i>	South Africa
<i>Achaetodactylus leleupi</i>	<i>Ceratina diloensis</i>	Democratic Republic of Congo
<i>Ochaetodactylus decellei</i>	<i>Ceratina</i> sp., <i>C. spilota</i> , <i>C. aereola</i> , <i>C. excavata</i> (Fain 1981)	Tanzania, Cameroon, Democratic Republic of the Congo (Fain 1981)
<i>Chaetodactylus melitomae</i>	<i>Melitoma marginella</i> , <i>M. segmentaria</i> , <i>Melitoma</i> sp.	Mexico, Honduras
<i>Chaetodactylus ludwigi</i>	<i>Lithurgus dentipes</i> , <i>L. scabrosus</i> , <i>L. atratus</i>	Micronesia, Indonesia, New Caledonia, French Polynesia, South India
<i>Chaetodactylus osmiae</i>	<i>Osmia rufa</i> , <i>O. tricornis</i> , <i>O. fulviventris</i> , <i>O. cornuta</i>	France, Belgium, England, Germany, Hungary, Croatia, Spain
<i>Sennertia zhelochovtsevi</i>	<i>Xylocopa olivieri</i>	Greece
<i>Sennertia surinamensis</i>	<i>Ceratina chloris</i> , <i>C. laeta</i>	Suriname, French Guiana, Panama

largely match molecular data (unpublished phylogeny, Danforth, pers. comm., 2005); family-level relationships (Danforth et al. 2006); however, some relationships require further investigation given weak support and character conflict in the dataset (Roig-Alsina and Michener 1993).

ANALYSES OF HISTORICAL ASSOCIATIONS BETWEEN MITES AND THEIR HOSTS

A test for a significant correlation between the phylogenies of the mites and bee hosts was conducted with PARAFIT (Legendre et al. 2002); this approach accommodates the association of mite taxa on more than one host, where taxon distances are used in the test, rather than topology. Correlation was evaluated by determining whether the degree of association between species distances (computed from a principal coordinate analysis of the patristic distance matrices from the host [Roig-Alsina and Michener 1993; Engel 2001] and parasite phylogenies) (Fig. 1, excluding the out-group) and an incidence matrix that describes the associations between mites and their hosts (Table 2) differs significantly from expectations of random association between mites and hosts (for details see Legendre et al. 2002). The program DISTPCoA (Legendre and Anderson 1999) was used to transform patristic matrices to principal coordinates. Permutation tests (9999 randomizations) were used to assess the probability that the detected coevolutionary correlation differs significantly from that expected by chance.

The program TREEFITTER (Ronquist 1995, 2003) was used to examine what historical processes might have generated the

observed correlation between the mite and bee histories. Possible historical scenarios were investigated by evaluating the cost space of the four events: cospeciation, speciation within a host lineage, host shifts, and extinction (which are referred to as codivergence, duplication, switching, and sorting, respectively in the program; Ronquist [2003]). General cost optimization was performed in which the cost of each event was inversely related to the likelihood of the event and the cost was varied incrementally within a specified range (i.e., between 0 to 10 for all events, except for extinction, for which a range of 0.5–4.0 was considered) (see Ronquist [2003]). The significance of each historical scenario was evaluated against the null hypothesis that such a combination of events was statistically indistinguishable from a pattern arising by chance using randomization tests. Phylogenetically significant scenarios were identified as those for which the probability of observing a particular number of events (e.g., Table 3) was $P < 0.05$ as assessed by 10,000 permutations of both mite- and host-tree terminals. For this test, the topology of the host tree used in the analysis was the same as that of Roig-Alsina and Michener (1993) with a few additions of bee tribes from recent smaller scale phylogenetic analyses (none of which is chaetodactylid hosts).

Four associations of *Chaetodactylus* with Emphorini and Tapinotaspidini (Table 1) were excluded. They constitute only 1.8% of all known associations at the species level, comprise only derived mite taxa (see online Supplemental Fig. S1) collected from a single bee species each and may be accidental.

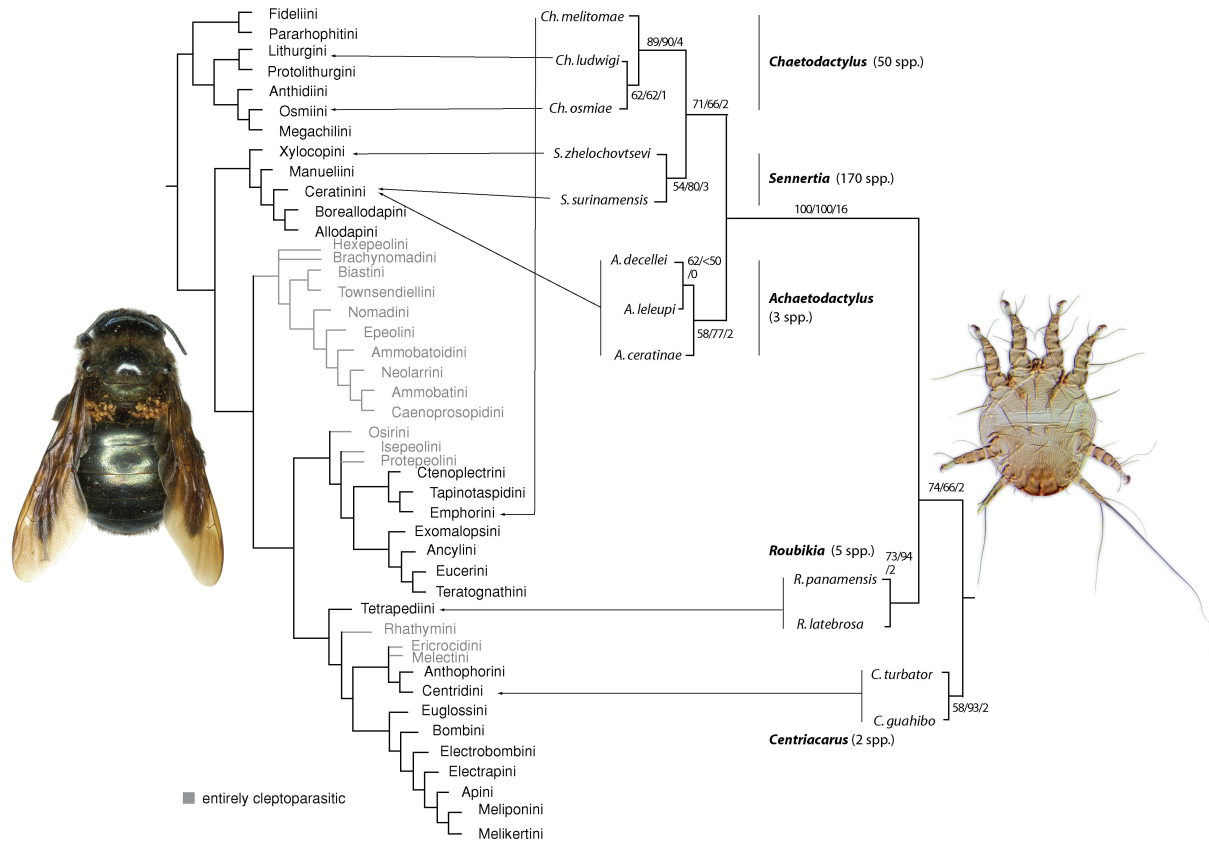


Figure 1. Phylogenetic relationships within the family Chaetodactylidae based on Bayesian analysis of morphological data (on the right) of 12 representative taxa (Table 2) selected on the basis of a larger analyses (Fig. S1); posterior probabilities, bootstrap values, and Bremer indices are shown. A phylogeny of the long-tongued bees (Engel 2001) is shown on the left. Links between mites and their bee hosts are shown (a few other links formed by rare and relatively derived mite species are given in Table 1; their exclusion is justified by phylogenetic analyses presented in supplemental material available online). Note that the chaetodactylid phylogeny is drawn “upside-down” for ease of showing the inverse phylogenetic correlation.

ANALYSES OF THE ECOLOGICAL DETERMINANTS OF THE MITE AND BEE ASSEMBLAGES

Four aspects of bee biology that may affect the suitability of a particular taxon as a chaetodactylid host were considered: nest construction site, the arrangement of cells within a nest, the provisioning of cells, and the degree of sociality characterizing the

bee taxa. These data were collected primarily from Radchenko and Pesenko (1994), Radchenko (1996), and Michener (2000), references cited therein, and more recent publications (online Supplementary Table S1). In a few cases, data were extrapolated from other species when the trait appeared to be similar across the genus.

Table 3. The six significant models (as shown in Fig. 2B) that are consistent with the observed correlation between the mite and bee phylogenies, and the number of invoked historical events specified under a particular model (cospeciation, speciation within a host lineage, host shifts, and extinction); see Figure 2B for the distribution of costs for host switching and speciation within host associated with each model.

Historical processes	Models					
	1	2	3	4	5	6
Cospeciation	0	1	2	3	3	3
Speciation within a host lineage	5	5	5	5	7	8
Host shifts	6	5	4	3	1	0
Extinction	0	3	7	12	26	35
Total number of events	11	14	18	23	37	46
Total cost	3.3–16.5	19.5–24.75	24.6–31.8	28.5–50.9	33.15–49.9	35–39

Logistic regression analysis was used to investigate how well these traits predict whether chaetodactylid mites will (or will not) be associated with a particular bee taxon. The fit of the data to the model was evaluated using a likelihood-ratio test. The predictive power of the model (i.e., the contribution of host biological traits to the observed pattern of bee-mite associations) was evaluated with the program SPSS version 11.0.4 (2005) by calculating the posterior probabilities for each bee taxon and estimating the percentage of correctly predicted associations.

Results

INVERSE CORRELATION BETWEEN MITE AND BEE PHYLOGENIES

A significant ($P = 0.029$) inverse correlation between the phylogenies of the mites and bees was detected (Fig. 1) (based on analyses from the program PARAFIT [Legendre et al. 2002]), but it is not the pattern expected from a history of cospeciation (Eichler 1948; Klassen 1992; Peek et al. 1998; Clark et al. 2000; Lo et al. 2003; Degnan et al. 2004). Recently derived mites are not associated with recently derived hosts. Instead the converse is observed. This intriguing pattern of bee-mite associations at the level of bee tribes creates an unprecedented case of a reverse “codivergence” that violates Fahrenholz’s rule and is obviously not caused by cospeciation.

FACTORS UNDERLYING THE BEE-MITE ASSOCIATIONS

Different historical scenarios, involving cospeciation, host shifts, speciation within a host lineage, and/or extinction (Ronquist 1995,

2003), might account for the correlation between the mite and bee-host phylogenies. These historical scenarios (i.e., models) were explored using a general cost-optimization procedure (Fig. 2A). Costs were assigned and varied incrementally to each of the four historical processes (i.e., cospeciation, host shifts, speciation within a host lineage, and extinction), where the cost assigned to each event was inversely related to the likelihood of the process, to produce a cost space surface for the number of specific historical events that would have to be invoked in order to produce the observed association between the mite and bee phylogenies under different models (Ronquist 2003).

Six significant models (Table 3) were identified from the general cost-optimization procedure as having a probability of less than 5% of being generated by chance (Fig. 2B), as assessed by permutations of both mite and host taxa across the respective trees. However, these models are not necessarily biologically equivalent. Some models can be rejected as unlikely because of the excessive number of historical events required to generate the observed correlation between mite and bee phylogenies and high total cost. For example, the absence of host shifts postulated by model 6 (Table 3) requires invoking 35 extinctions, which results in a relative high total cost for the model (lower, right corner of Fig. 2B). Consideration of both the total costs and number of individual events required to produce the observed mite-bee assemblage identifies a model of speciation within hosts and host switching as more parsimonious than the other scenarios; a difference of 11 total events (model 1, Table 3, vs. 14, 18, 25, 37, and 46 events for models 2, 3, 4, 5, and 6, respectively).

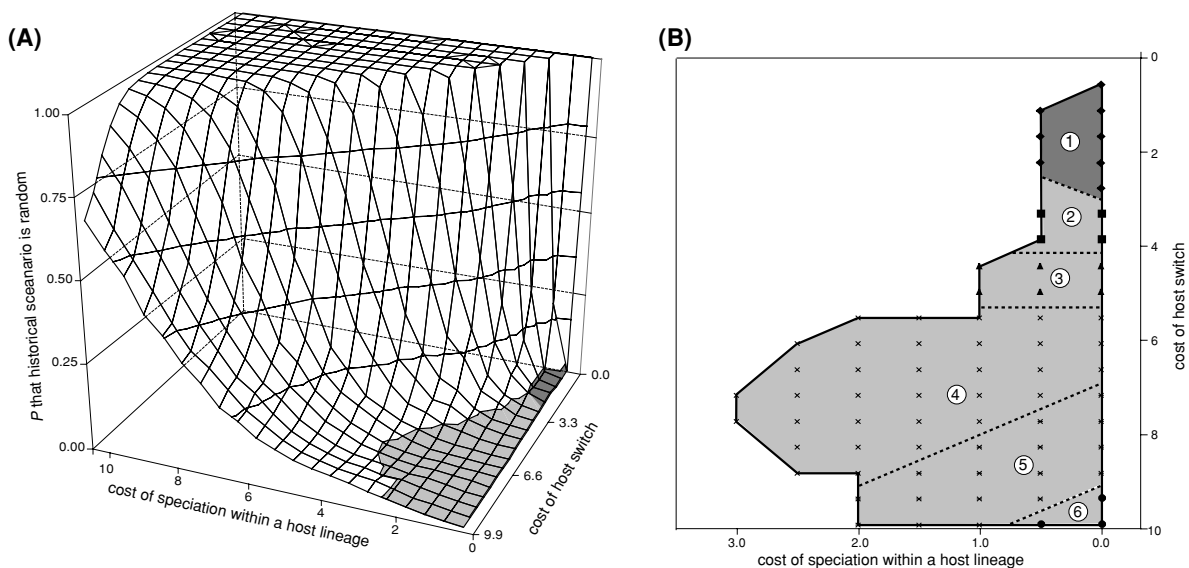


Figure 2. Exploration of the cost space to evaluate the different processes underlying the observed coevolutionary association of chaetodactylid and bees: (A) P -values were estimated from 10,000 random permutations of both host and symbiont terminals with a codivergence and extinction cost of 0 and 1, respectively; significant values ($P < 0.05$) are shaded and shown in detail on the right (B), where the dashed lines demarcate the cost space that corresponds with the specific suite of historical events represented in the six significant models (as given in Table 3).

Table 4. Classification accuracy of the four-variable logistic regression analysis, indicating the analyses high classification accuracy of predicting the presence or absence of chaetodactylid mites in the nest of a long-tongued bee; the four-variables used in the model were nest construction site, cell arrangement, cell provision, and the degree of sociality.

Mites	Inferences from model		
	Absent	Present	Accuracy (%)
Absent	20	4	83.3
Present	3	12	80.0
Overall			82.1

Because model 1 (speciation within hosts and host switching) is the most parsimonious and biologically meaningful (many species of chaetodactylids are known to exist on different hosts and several species of the mites can be associated with a single host species), we consider it as the most preferred historical scenario for this association.

A significant relationship between the presence of chaetodactylid mites and specific ecological aspects of the bees was detected by the logistic regression analysis ($P = 0.024$). The classification accuracy of bees as potential hosts based on four bee traits (nesting site, cell arrangement, cell provisioning, and sociality; see online Supplemental Table S1) is 82.1%, indicating the importance of the traits (Table 4). Any combination of bee traits, where one or more were removed, resulted in a decreased predictive power, suggesting all four aspects of bee ecology structure the coevolutionary assemblage of these symbionts/parasites and hosts. For relative contribution of each variable to the model see online Supplementary Table S1; logistic regression coefficients (online Supplementary Table S2) can be used to calculate the probability of mite association with any given long-tongued bee.

Discussion

We propose that ecology has had a predominant influence on the history of coevolutionary associations between chaetodactylid mites and long-tongued bees, affecting both the opportunity and probability of successful host shifts, and thereby dictating which bees are potential hosts (Michener 2000). One expectation resulting from the dependency of the mites on their bee hosts for food, habitat, and a means of dispersal (Krombein 1962; Abrahamovich and Alzuet de 1990) is the significant correlation between the mite and bee phylogenies, as with other symbiont/parasite–host assemblages (Reed and Hafner 1997; Peek et al. 1998; Clark et al. 2000; Lo et al. 2003; Degnan et al. 2004). Indeed, such a correlation is observed but it is inverse (Fig. 1). The signature of ecological constraint in this case manifests itself in an atypical fashion.

First, the temporal disjunction between the diversification of the mite and bee lineages as evidenced by the bee and mite topologies rules out a process of cospeciation (Ronquist 2003). Second, the existence of mite species on multiple related hosts indicates there are ample opportunities for host shifts. The museum survey and the phylogenetic analyses identify two ways in which ecology has constrained this coevolutionary history—interestingly, the non-random association between the mites and bees (Fig. 1) provides evidence for ecological constraint in terms of the infrequency of shifts among distantly related hosts and their frequency among closely related hosts (Table 1).

Most of the mite genera and species groups are associated with hosts from a single bee tribe, with the notable exception of *Chaetodactylus* (see below). Conservatism in the pattern of host use evident at this higher taxonomic level (Fig. 1) does not apparently reflect the lack of opportunities for host shifting at lower taxonomic levels. For example, species of *Sennertia* have experienced multiple host shifts within and between the host genera *Ceratina* and *Xylocopa* and these were followed by speciation events. All this without any obvious mechanism of transfer between host taxa. These bees differ in nesting sites and lack a common hymenopteran cleptoparasite (a potential means of dispersal among host species). Ecological constraints imposed by similarities in life-cycles of these bees may structure patterns of host shifting in these mite genera. The absence of *Sennertia* mites on numerous alternative and sympatric hosts supports the hypothesized constraint of mite dispersal imposed by the life-cycle of its host, as opposed to geography explaining patterns of host shifts.

Mites in the genus *Chaetodactylus* represent an interesting contrast to the pattern of host conservatism, and have shifted onto many unrelated and different bee tribes in the families Megachilidae and Apidae, with particular mite species still associated with closely related hosts (Fig. 1; Table 1). Many unrelated hosts of *Chaetodactylus* exhibit similar ecologies, as evidenced by the relatively high accuracy of the predictive classification based on aspects of bee ecology (Table 4). The broad host range of *Chaetodactylus* may be explained by two particular characteristics of this mite genus. These mites most likely experience increased opportunities for host shifting as a result of an inert deutonymph, a cyst-like life-history stage that can survive off the host. By remaining in the nest cavity, the mite is able to infest the next bees to reuse the cavity (Krombein 1962). For example, the nests of the hosts of the *C. osmiae* species group (i.e., several taxa of bees in the genus *Osmia*) are reused by other bee taxa, providing means for dispersal between different bee species. Antagonistic interactions of *Chaetodactylus* with its host may also incur evolutionary pressures driving the utilization of new unrelated hosts. Species in this mite genus often kill the developing bee larvae (van Lith 1957; Krombein 1962; Qu et al. 2002). Phylogenetic reconstruction of the genus (Klimov and OConnor; online Supplementary

Fig. S1) shows that associations with Apidae (except for *Melittoma*) (Table 1) are clearly secondary and resulted from host shifts from unrelated megachilid hosts.

How do host shifts occur in mites that typically inhabit nests of a single bee are dependent on their host for dispersal to new nests, and therefore a single host species? Various mechanisms could provide opportunities for host shifting, which include nest supersedure (i.e., the take over of a nest partly provisioned by a different individual of the same or different species), utilization of a shared entrance to intraspecific or interspecific nest tunnels, and hibernating aggregations (Rust 1974; Linsley et al. 1980; Gerling et al. 1989; McCorquodale and Owen 1994; Hogendoorn 1996). Host shifts may also occur from an incidental transfer on flowers (e.g., *Sennertia*) or with loose dirt collected as nest material (e.g., *Roubikia*) (Roubik 1987; Vicidomini 1996). Lastly, hymenopteran cleptoparasites attacking multiple host species may facilitate movement of mites among populations and different host species (Zachvatkin 1941; Samšičák 1973; Munster-Swendsen and Calabuig 2000). For example, deutonymphs of *C. krombeini* and *C. reaumuri* were found on the cleptoparasites *Stelis montana* and *S. murina*, respectively (Klimov and OConnor, in press; Türk and Türk 1957). Transfer among their hymenopteran hosts via cleptoparasites is known to influence the host ranges of the unrelated mite genera *Vidia* (OConnor and Eickwort 1988) and *Parasitellus* (Richards and Richards 1976), and dispersal on parasitic hippoboscid flies is similarly considered a major cause of incongruence between phylogenies of the louse genus *Brueelia* and their avian hosts (Johnson et al. 2002). Although it is not possible to determine how prevalent one mode of transfer might be over another, irrespective of the specific mechanism involved in a host shift, the success of such events in chaetodactylid mites is highly predictable based on aspects of bee ecology (Table 4).

Host characteristics play a critical role in structuring the host-mite assemblage (Table 4), no doubt by influencing the opportunity and probability of success of a host shift. Properties of the bee nests can significantly constrain mite dispersal. Cell partitions constructed by the majority of bees are impenetrable for mites and do not allow them to move across the brood; the mites die if the bee in an infested cell dies early (Krombein 1962, 1967; Michener 2000). Consequently, chaetodactylids are associated with bees where the dispersal ecology of the mites is not limited by nest architecture. For example, in nests with cells arranged in a linear sequence, bees in the inner cells usually complete development sooner and break through partitions of the outermost cells to emerge (Skaife 1952; Krombein 1962; Linsley et al. 1980) and cross-contaminate other members of the nest. Not surprisingly, chaetodactylids are associated with bees exhibiting these qualities as opposed to bees with independent emergence of broods (e.g., branching nests or nests composed of clusters of cells, see online Supplementary Table S1). In addition to the bee traits that

would foster a host-mite association, certain developmental and biological characteristics of hosts that would negatively impact the mite could also contribute to the observed coevolutionary patterns. For example, the bee tribes Allodapini and Ceratinini are closely related, with similar nest architectures (excluding the absence of cell partitions in the former), but only the Ceratinini provision their cells prior to laying eggs. Chaetodactylids are not associated with bee lineages of the Allodapini whereas they do exist on the Ceratinini (Table 1, online Supplementary Table S1), suggesting that progressive feeding of larvae, rather than mass provisioning, may make them unsuitable hosts for the mites. Bees in both the Apini and Bombini lay their eggs with a little or no associated food, and neither hosts any chaetodactylids, although it possible that the production of different castes in highly eusocial bees might also reduce the chances of mite dispersal and contribute to the complete absence of chaetodactylids.

Conclusions

The extensive museum survey and phylogenetic analyses provide a unique window into how ecological constraint has shaped the coevolutionary associations of chaetodactylid mites and their hosts, the long-tongued bees. The temporal disjunction between the diversification of the mite and bee lineages rules out a process of cospeciation. Moreover, although the existence of mites on multiple hosts species suggests ample opportunity for dispersal among hosts, the infrequency of host shifts at one level of taxonomic resolution and their frequency at another suggest how ecological characteristics of the bees affect both the opportunity for dispersal and the probability of successful infestation of the mites. When certain characteristics of the bees and, in particular, aspects of their nesting behavior are considered, a highly predictive framework for this coevolutionary association emerges, reflecting the critical role ecology plays in governing the distribution of mites across the bee hosts.

ACKNOWLEDGMENTS

We would like to acknowledge the curators of 18 museums who provided access to host bee specimens and mite collections used in this study, including personal thanks for the help and hospitality during our visits to J. G. Rozen and J. S. Ascher (American Museum of Natural History, New York), T. Griswold (USDA—Bee Biology and Systematics Laboratory, Logan, Utah), B. V. Brown (Natural History Museum of Los Angeles County), C. Michener and the late B. Alexander (University of Kansas, Lawrence), and W. Pulawski (California Academy of Sciences, San Francisco). We also thank P. Legendre (Universite de Montreal, Canada) for his very useful assistance with ParaFit, C. Michener, J. Rozen, and J. Ascher for useful comments on the manuscript and, especially, its bee component, and identification of some critical host taxa, M. Terzo (Université de Mons-Hainaut, Belgium) for sharing his unpublished data on phylogeny of *Ceratina*, J. Bosch (Universitat Autònoma de Barcelona, Spain) for providing information on the nest architecture of *Rhodanthidium sticticum*,

and G. Hammond (University of Michigan) for his valuable comments on earlier drafts of the manuscript. We also extend our appreciation to J. Dykema, J. Diesel, and R. Tao (undergraduate assistants at the University of Michigan) for their help in mounting, labeling, and databasing mite specimens. This work was supported by grants from the National Science Foundation, DEB-0118766 (PEET) and the United States Department of Agriculture (CSREES #2002-35302-12654).

LITERATURE CITED

- Abrahamovich, A. H., and A. B. Alzuet de. 1990. Tipos de asociación entre acaros e Hymenópteros. I: *Sennertia* (A.) *splendidulae* Alzuet y Abrahamovich, 1988 (Acarina: Chaetodactylidae) comensal y forético de *Xylocopa* (S.) *splendula splendidula* Lepeletier, 1841 (Hymenoptera: Anthophoridae). *Turrialba* 40:319–322.
- Banks, J. C., R. L. Palma, and A. M. Paterson. 2005. Cophylogenetic relationships between penguins and their chewing lice. *J. Evol. Biol.* 19:156–166.
- Chmielewski, W. 1993. *Biologia Chaetodactylus osmiae* (Duf., 1866) (Acarida, Chaetodactylidae - pylkozernego rozkruska foretycznie zwiazanego z pszczolami samotnymi (Apoidea). *Pszczelnicze Zeszyty Naukowe* 37:133–143.
- Clark, M. A., N. A. Moran, P. Baumann, and J. J. Wernegreen. 2000. Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (Uroleucon) and pitfalls of testing for phylogenetic congruence. *Evolution* 54:517–525.
- Clayton, D. H., S. Al-Tamimi, and K. Johnson. 2003. The ecological basis of coevolutionary history. Pp. 310–341 in R. D. M. Page, ed. *Tangled trees: phylogeny, cospeciation, and coevolution*. Univ. Chicago Press, Chicago.
- Clayton, D. H., S. E. Bush, and K. P. Johnson. 2004. Ecology of congruence: past meets present. *Syst. Biol.* 53:165–173.
- Clayton, D. H., and K. P. Johnson. 2003. Linking coevolutionary history to ecological process: doves and lice. *Evolution* 57:2335–2341.
- Danforth, B. N., S. Sipes, J. Fang, and S. G. Brady. 2006. The history of early bee diversification based on five genes plus morphology. *Proc. Natl Acad. Sci. USA* 103:15118–15123.
- Degnan, P. H., A. B. Lazarus, C. D. Brock, and J. J. Wernegreen. 2004. Host-symbiont stability and fast evolutionary rates in an ant-bacterium association: cospeciation of *Camponotus* species and their endosymbionts, *Candidatus Blochmannia*. *Syst. Biol.* 53:95–110.
- Eichler, W. 1948. Some rules on ectoparasitism. *Ann. Mag. Nat. Hist.* 12:588–598.
- Eickwort, G. C. 1994. Evolution and life-history patterns of mites associated with bees. Pp. 218–251 in M. A. Houck, ed. *Mites: ecological and evolutionary analyses of life-history patterns*. Chapman and Hall, London.
- Engel, M. S. 2001. A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera). *Bull. Am. Mus. Nat. Hist.* 259:1–192.
- Fain, A. 1981. Notes on the hypopi of the genus *Chaetodactylus* Rondani, 1866 (Acari, Chaetodactylidae). *Bull. Inst. R. Sci. Nat. Belg. Entomol.* 53:1–9.
- Fain, A., and A. Pauly. 2001. Notes on phoretic deutonymphs of mites (Acari) associated with Old World Megachilidae and Anthophoridae (Insecta Hymenoptera), mainly from Madagascar I. Families Chaetodactylidae, Acaridae, Histiostomatidae and Winterschmidtidae (Astigmata). *Belg. J. Entomol.* 3:125–142.
- Gerling, D., H. H. W. Velthuis, and A. Hefetz. 1989. Bionomics of the large carpenter bees of the genus *Xylocopa*. *Annu. Rev. Entomol.* 34:163–190.
- Goloboff, P. A. 1993. Estimating character weights during tree search. *Cladistics* 9:83–91.
- Haitlinger, R. 1999. *Sennertia herminae* sp. n. (Acari, Astigmata: Chaetodactylidae) a phoretic mite associated with *Xylocopa* sp. (Hymenoptera: Anthophoridae) from Madagascar. *Folia Entomol. Hung.* 60:57–59.
- Hogendoorn, K. 1996. Socio-economics of brood destruction during supercedure in the carpenter bee *Xylocopa pubescens*. *J. Evol. Biol.* 9:931–952.
- Johnson, K. P., R. J. Adams, and D. H. Clayton. 2002. The phylogeny of the louse genus *Brueelia* does not reflect host phylogeny. *Biol. J. Linn. Soc. Lond.* 77:233–247.
- Johnson, K. P., R. J. Adams, R. D. M. Page, and D. H. Clayton. 2003. When do parasites fail to speciate in response to host speciation? *Syst. Biol.* 52:37–47.
- Klassen, G. J. 1992. Coevolution: a history of the macroevolutionary approach to studying host–parasite associations. *J. Parasitol.* 78:573–587.
- Klimov, P. B., and B. M. OConnor. 2003. Mites associated with bees, University of Michigan. Available at http://141.211.243.61/bee_mites/index.html.
- Klimov, P. B., and B. M. OConnor. Morphology, evolution and host associations of beeassociated mites of the family Chaetodactylidae (Acari: Astigmata), with monographic revision of North American taxa. *Misc. Publ. Mus. Zool. Univ. Mich.* 198. *In Press*.
- Krombein, K. V. 1962. Natural history of Plummers Island, Maryland. XVI. Biological Notes on *Chaetodactylus krombeini* Baker, a parasitic mite of the megachilid bee, *Osmia* (*Osmia*) *lignaria* Say (Acarina, Chaetodactylidae). *Proc. Biol. Soc. Wash.* 75:237–250.
- . 1967. *Trap-nesting wasps and bees: life histories, nests, and associates*. Smithsonian Institution Press, Washington, DC.
- Legendre, P., and M. J. Anderson. 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecol. Monogr.* 69:1–24.
- Legendre, P., Y. Desdevises and E. Bazin 2002. A statistical test for host–parasite coevolution. *Syst. Biol.* 51:217–234.
- Leys, R., S. J. B. Cooper, and M. P. Schwarz. 2002. Molecular phylogeny and historical biogeography of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae). *Biol. J. Linn. Soc. Lond.* 77:249–266.
- Linsley, E. G., J. W. MacSwain, and C. D. Michener. 1980. *Nesting Biology and Associates of Melitoma* (Hymenoptera, Anthophoridae). Univ. Calif. Publ. Entomol. 90:1–45.
- Lo, N., C. Bandi, H. Watanabe, C. Nalepa, and T. Beninati. 2003. Evidence for coeladogenesis between diverse dictyopteran lineages and their intracellular endosymbionts. *Mol. Biol. Evol.* 20:907–913.
- McCorquodale, D. B., and R. E. Owen. 1994. Laying sequence, diploid males, and nest usurpation in the leafcutter bee, *Megachile rotundata* (Hymenoptera: Megachilidae). *J. Insect Behav.* 7:731–738.
- Michener, C. D. 2000. *The bees of the world*. John Hopkins Univ. Press, Baltimore and London.
- Munster-Swendsen, M., and I. Calabuig. 2000. Interaction between the solitary bee *Chelostoma florissomme* and its nest parasite *Sapyga clavicornis*—empty cells reduce the impact of parasites. *Ecol. Entomol.* 25:63–70.
- OConnor, B. M. 1993. Generic relationships in the Chaetodactylidae (Acari: Astigmata) with description of a new genus. *Acarologia* 34:345–362.
- OConnor, B. M., and G. C. Eickwort. 1988. Morphology, ontogeny and systematics of the genus *Vidia* (Acari: Winterschmidtidae). *Acarologia* 29:147–174.
- Okabe, K., and S. Makino. 2002. Phoretic mite fauna on the large carpenter bee *Xylocopa appendiculata circumvolans* (Hymenoptera: Apidae) with descriptions of its acarinarium on both sexes. *J. Acarol. Soc. Japan* 11:73–84.
- Peek, A. S., R. A. Feldman, R. A. Lutz, and R. C. Vrijenhoek. 1998. Cospeciation of chemoautotrophic bacteria and deep sea clams. *Proc. Natl Acad. Sci. USA* 95:9962–9966.

Qu, D., Y. Maeta, M. Goubara, K. J. Nakatsuka, J. Kozo, and K. Kenji. 2002. Reproductive strategy in the two species of cleptoparasitic astigmatid mites, *Chaetodactylus nipponicus* and *Tortonia* sp. (Acari: Chaetodactylidae and Suidasiidae), infesting *Osmia cornifrons* (Hymenoptera: Megachilidae). I. Invasion/infestation patterns and partial use of the host food. *Jpn. J. Entomol.* 5:121–141.

Quek, S. -P., S. J. Davies, T. Itino, and N. E. Pierce. 2004. Codiversification in an ant-plant mutualism: stem texture and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). *Evolution* 58:554–570.

Radchenko, V. G. 1996. Evolution of nest building in bees (Hymenoptera, Apoidea). *Entomol. Rev.* 75:20–32.

Radchenko, V. G., and Y. A. Pesenko. 1994. *Biologiya pchel* (Hymenoptera: Apoidea) [= Biology of bees (Hymenoptera: Apoidea)]. Russian Academy of Sciences, St. Petersburg.

Rannala, B., and Y. Michalakis. 2003. Population genetics and cospeciation: from process to pattern. Pp. 120–143 in R. D. M. Page, ed. *Tangled trees: phylogeny, cospeciation, and coevolution*. Univ. Chicago Press, Chicago.

Reed, D. L., and M. S. Hafner. 1997. Host specificity of chewing lice on pocket gophers: a potential mechanism for cospeciation. *J. Mammal.* 78:655–660.

Richards, L. A., and K. W. Richards. 1976. Parasitid mites associated with bumblebees in Alberta, Canada (Acarina: Parasitidae; Hymenoptera: Apidae). II. Biology. *Univ. Kans. Sci. Bull.* 51:1–18.

Ricklefs, R. E., S. M. Fallon, and E. Bermingham. 2004. Evolutionary relationships, zcospeciation, and host switching in avian malaria parasites. *Syst. Biol.* 53:111–119.

Roig-Alsina, A., and C. D. Michener. 1993. Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). *Univ. Kans. Sci. Bull.* 55

Ronquist, F. 1995. Reconstructing the history of host-parasite associations using generalised parsimony. *Cladistics* 11:73–89.

———. 2003. Parsimony analysis of coevolving species associations. Pp. 22–64 in R. D. M. Page, ed. *Tangled trees: phylogeny, cospeciation, and coevolution*. Univ. Chicago Press, Chicago.

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.

Roubik, D. W. 1987. Notes on the biology of anthophorid bee *Tetrapedia* and the mite *Chaetodactylus panamensis* Baker. Roubik and Delfinado-Baker (Acari: Chaetodactylidae). *Int. J. Acarol.* 13:75–76.

Rust, R. W. 1974. The systematics and biology of the genus *Osmia*, subgenera *Osmia*, *Chalcosmia*, and *Cephalosmia* (Hymenoptera: Megachilidae). *Wasmann J. Biol.* 32:1–93.

Samšičák, K. 1973. Zwei neue Arten der Gattung *Chaetodactylus* Rondani, 1866 (Acari, Acaroidea). *Zool. Anz.* 190:400–404.

Skaife, S. H. 1952. The yellow-banded carpenter bee *Mesotrichia caffra* Linn., and its symbiotic mite, *Dinogamasus braunsi* Vitzthum. *J. Entomol. Soc. South. Afr.* 15:63–76.

Smith, V. S., P. R. D. M., and K. P. Johnson. 2004. Data incongruence and the problem of avian louse phylogeny. *Zool. Scr.* 33:239–259.

Sorenson, M. D. 1999. TreeRot. Boston University, Boston, MA.

SPSS. 2005. SPSS for Macintosh. SPSS Inc., Chicago, IL.

Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.

Taylor, J., and A. Purvis. 2003. Have mammals and their chewing lice diversified in parallel. Pp. 240–261 in R. D. M. Page, ed. *Tangled trees: phylogeny, cospeciation, and coevolution*. Univ. Chicago Press, Chicago.

Türk, E., and F. Türk. 1957. Systematik und Ökologie der Tyroglyphiden Mitteleuropas. Pp. 4–384 in H. -J. Stammer, ed. *Beiträge zur Systematik und Ökologie mitteleuropäischer Acarina*. Band 1. Tyroglyphidae und Tarsonemini. Akademische Verlagsgesellschaft Geest & Portig K.-G., Leipzig.

van Lith, J. P. 1957. On the behaviour of *Chaetodactylus* mites (Acar., Tyr.) in the nests of *Osmia rufa* L. and *Chelostoma florissomme* (L.) (Apidae, Megachilidae). *Entomol. Berichten* 17:197–198.

Vicidomini, S. 1996. *Biologia di Xylocopa (Xylocopa) violacea* (L., 1758) (Hymenoptera: Apidae): interazione con *Sennertia (Sennertia) cerambycina* (Acari Chaetodactylidae). *Boll. Zool. Agrar. Bachic.* 28:71–76.

Weckstein, J. D. 2004. Biogeography explains cophylogenetic patterns in toucan chewing lice. *Syst. Biol.* 53:154–164.

Zachvatkin, A. A. 1941. Tiroglifoidnue kleshchi Tyroglyphoidea [= Tyroglyphoid mites Tyroglyphoidea]. Pp. 1–475 in S. A. Zernov, ed. *Fauna SSSR: Paukoobraznye*. Akademiya Nauk SSSR, Moscow-Leningrad.

Associate Editor: W. O. McMillan

Appendix. Data matrix of morphological characters (based on the character list below) used in phylogenetic reconstruction of Chaetodactylidae relationships: ?, unknown and –, nonapplicable characters; characters 4, 10, and 32 are uninformative. This is an exemplar subset of a larger matrix containing 46 chaetodactylid taxa (Fig. S1 available online).

	1	10	20	30	40	50
<i>Centriacarus turbator</i>	000000101100001000001000000000000000001010000100001					
<i>Centriacarus guahibo</i>	000100101100001000001000000000000000001010000100001					
<i>Roubikia panamensis</i>	001-000011000010000000000000001101100002010100100101					
<i>Roubikia latebrosa</i>	001-000010000010000000000000001101100002010100100101					
<i>Achaetodactylus ceratinae</i>	111-01000110111110111000010112101211112101111111111					
<i>Achaetodactylus leleupi</i>	111-01000110111110111000010112101211112101111111111					
<i>Ochaetodactylus decellei</i>	111-?1000110111110111000010112111211112101111111111					
<i>Chaetodactylus melitomae</i>	0100100011111111101111110012101211112101111111110					
<i>Chaetodactylus ludwigi</i>	0100100011111110110111111101210121111210111111110					
<i>Chaetodactylus osmiae</i>	0100100011111110110111111111210121111210111111110					
<i>Sennertia zhelochovtsevi</i>	011-1--11111111110111110111112101211112101111011111					

CHARACTER LIST

1. Gnathosomal solenidion: 0, present; 1, absent
2. Setae on free palpomeres: 0, present; 1, absent
3. Free palpomeres: 0, present; 1, absent
4. Free palpomeres: 0, longer than width at base; 1, shorter than width at base
5. Alveoli *ve*: 0, dorsal, distinctly anterior to *se*; 1, dorsal, approximately at level of *se*; 2, ventral
6. Prodorsal shield striation: 0, longitudinal anteriorly and transverse posteriorly; 1, longitudinal; 2, absent
7. Posterior edge of prodorsal shield: 0, longer than lateral edges; 1, shorter than lateral edges
8. Prodorsal shield: 0, present; 1, absent
9. Setae *se* situated: 0, on prodorsal shield; 1, on soft cuticle
10. Setae *si*: 0, about twice or more longer than *se*; 1, less than twice longer than *se*
11. Setae *c*₂ situated: 0, on same transverse level as *c*₁; 1, distinctly anterior to level of *c*₁
12. Setae *e*₂ situated: 0, on hysterosomal shield; 1, outside hysterosomal shield or touch it
13. Setae *1a* and *3a*: 0, touching posterior borders of respective coxal fields and filiform (conoids in outgroup; 1, Setae *1a* and *3a* not touching posterior borders of respective coxal fields, if touching then inflated and elongated
14. Cupules *ia* situated: 0, on hysterosomal shield; 1, outside hysterosomal shield
15. Cupules *im* situated: 0, at level of acetabules III, approximately at middle of line between setae *d*₂ and *e*₂; 1, distinctly posterior to acetabules III, situated off line between *d*₂ and *e*₂
16. Cupules *im*: 0, ventral, ventro-lateral; 1, dorsal
17. Cupules *ip* are: 0, anterior to setae *f*₂; 1, posterior to setae *f*₂
18. Cupules *ih* situated: 0, on sides of attachment organ; 1, incorporated into lateral sclerotized borders of attachment organ
19. Posterior part of posterior apodemes of coxal fields II: 0, not displaced posteriorly to anterior apodemes III; 1, displaced posteriorly to anterior apodemes III
20. Coxal fields III: 0, enclosed; 1, open
21. Coxal fields IV: 0, enclosed; 1, open
22. Transverse medial extension of posterior apodemes IV: 0, well developed; 1, absent
23. Anterior extension of posterior apodemes IV: 0, present, connecting with anterior apodeme III; 1, absent or not connecting
24. Ventral longitudinal sclerites of progenital chamber at posterior part: 0, conspicuous; 1, inconspicuous
25. Ventral longitudinal sclerites of progenital chamber at anterior part: 0, conspicuous; 1, inconspicuous
26. Posterior and lateral cuticular suckers: 0, present; 1, absent
27. Suckers *ad*₃ (excluding transparent margin; 0, larger than inner unsclerotized area of suckers *ad*₁+*ad*₂; 1, smaller or nearly equal to inner unsclerotized area of suckers *ad*₁+*ad*₂)
28. Anterior cuticular suckers: 0, present; 1, absent or vestigial
29. Bases of anterior cuticular suckers: 0, inserted on separate apodeme (may touch or overlap postero-lateral sclerotized border of the attachment organ; 1, incorporated to the border
30. Apodemes of *ps*₁: 0, separated; 1, partially fused anteriorly; 2, completely fused
31. Setae *wa* and *f* I–II: 0, *wa* I–II submedial, *f* I–II apical, near tarsal apices; 1, *wa* I–II apical or subapical, *f* I–II at level or proximal to *wa* I–II and far from tarsal apices
32. Solenidion w2: 0, present; 1, absent
33. Empodial claws I–III: 0, not twisted; 1, twisted
34. Dorsal cuticular folds of ambulacra I–III: 0, absent; 1, weakly developed, with distal part smaller than proximal; 2, well developed, with distal part distinctly larger than any of proximal folds
35. Condyliphores of tarsi I–III: 0, weakly developed, almost symmetrical; 1, well developed, distinctly assymetrical— anterior longer, posterior shorter, incorporated into postero-lateral lobe
36. Supporting sclerites of condyliphores (latero-apical sclerites of tarsus; 0, not distinct from the tarsus, not connected by dorsal bridge; 1, distinct from the tarsus, connected by dorsal bridge
37. Disto-dorsal lobe of distal part of the caruncle: 0, absent; 1, present, well developed
38. Dorsal condylar plate of femur-tibia joint: 0, broad; 1, absent or indistinct
39. Tarsi I–II with: 0, eight setae (*e* present; 1) 7 setae (*e* absent, *p* and *q* present); 2, 5 setae (*e*, *p*, and *q* absent)
40. Tarsal setae *ra* and *la* I–II: 0, foliate; 1, simple or spiniform
41. Genua seta *cG* I: 0, distinctly shorter than genu I and unmodified; 1, longer or slightly shorter than genu I and modified
42. Genua setae: 0, *cG* I longer than *cG* II; 1, *cG* I–II subequal
43. Tarsal setae *q* III: 0, present; 1, absent
44. Tarsal setae *w*, *r*, and *p* III: 0, present; 1, absent
45. Tarsal seta *s* III: 0, foliate; 1, simple
46. Sigma III: 0, present; 1, absent, represented by alveola
47. Tarsus IV with: 0, 8 setae (*s*, *p*, *q* present); 1, maximum five setae (*s*, *p*, *q* always absent)
48. Tarsal setae *e*, *f* IV: 0, foliate or slightly lanceolate; 1, simple or absent
49. Tarsal setae *w* IV: 0, longer than leg IV; 1, distinctly shorter than leg IV or absent
50. Tibial setae *kT* IV: 0, present; 1, absent
51. Solenidion phi IV: 0, present; 1, absent, represented by alveola

Supplementary Material

The following supplementary material is available for this article:

Table S1. Aspects of host biology, and in particular nest architecture, considered to investigate the factors influencing associations between the chaetodactylid mites and their bee-hosts. The variable “cell construction material” was not included in the analysis because of difficulties with uniform coding and a possibility of model overfitting. Cleptoparasitic bees were also not included because they do not have chaetodactylids by definition.

Table S2. Logistic regression model for prediction of the presence of chaetodactylid mites in the nests of long-tongued bees (raw data from Table S1).

Figure S1. Phylogenetic relationships of Chaetodactylidae reconstructed by maximum parsimony analysis of morphological data; Bremer indices are shown.

This material is available as part of the online article from:

<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1558-5646.2007.00119.x>

(This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.