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The phylogeny of the louse genus *Brueelia* does not reflect host phylogeny

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Lice (Insecta: Phthiraptera) are often considered a model group of parasites for studies of cospeciation because of their high host specificity, and louse species in the genus *Brueelia* are relatively host-specific. To test the extent of cospeciation, we reconstructed a phylogeny for *Brueelia* from nuclear (EF1α) and mitochondrial (COI) DNA sequences. This phylogeny was generally well resolved and supported. Two major clades within *Brueelia* (as well as several other lineages) were identified, and these corresponded to major morphological differences in the preantennal region of the head and sclerotization of the abdomen. However, the phylogeny of *Brueelia* showed little concordance to a published phylogeny of the hosts. In addition, we uncovered four cases (out of 15 species) of one species of *Brueelia* on two or more bird species. We argue that the high dispersal capabilities of *Brueelia* species, e.g. phoresis on hippoboscid flies, are a likely explanation for the incongruence between host and parasite phylogenies in this case.

ADDITIONAL KEYWORDS: Phthiraptera – ectoparasites – systematics – birds – passerines – cytochrome oxidase I – elongation factor-1α – cospeciation

INTRODUCTION

Phylogenetic studies of closely interacting organisms can reveal a pattern of shared evolutionary history, known as cospeciation. Cospeciation occurs when speciation in one group is accompanied by speciation in the other group. Repeated bouts of cospeciation can lead to significant congruence between the phylogenies of the two groups. Indeed, it is this congruence that is actually used to detect cospeciating groups.

Instances of cospeciation can be used to study relative rates of evolution or examine correlated evolution between interacting taxa. One parasite–host system that is particularly well suited to the study of cospeciation is lice (Insecta: Phthiraptera) and their hosts (mammals and birds). In general, lice are extremely host-specific; many louse species only occur on one species of host (Hopkins & Clay, 1952). In the extremely host-specific gopher lice (*Geomydoecus*), cospeciation is widespread (Hafner *et al*., 1994). A study of a less host-specific bird–louse system (*Dennyus* on swiftlets) revealed a more complicated cophylogenetic history, but still uncovered many instances of cospeciation (Page *et al*., 1998). More recent studies of two groups of dove lice (Johnson & Clayton, 2002) also revealed substantial cospeciation. Together these studies suggest that cospeciation between lice and their hosts may be widespread. However, the degree of cospeciation between lice and their hosts may vary between louse groups depending on the ecological details of the interaction. To assess this potential relationship, additional comparisons of louse and host phylogenies are needed. Here we examine the pattern of cophylogenetic history between species in the louse genus *Brueelia* and their avian hosts by constructing a molecular phylogeny for *Brueelia* and comparing it with published avian phylogenies.

Brueelia, containing over 260 described species, is one of the largest genera of lice in the suborder Ischnocera. In addition to the large number of species, *Brueelia* has a very broad host distribution for a genus of

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ischnoceran louse. While most ischnoceran genera are restricted to a single family or order of hosts, *Brueelia* occurs on at least 41 families of birds in at least four orders. It is one of the genera typically found on passerine songbirds (Passeriformes), the largest avian order. Despite widespread taxonomic distribution as a genus, species of *Brueelia* are extremely host specific. As currently described, approximately 90% of species of *Brueelia* occur on a single host. If host-specificity and cospeciation are related, we would predict a high level of cospeciation between *Brueelia* and their avian hosts.

While *Brueelia* seems to be relatively host-specific, other aspects of its biology, when compared to other groups of lice, suggest that dispersal between host species might be common. For example, lice are often described attached to ectoparasitic hippoboscid flies when collections of these flies are made from birds (Keirans, 1975). Hippoboscid flies can readily fly between hosts and often parasitize several host species. If such phoresis is a viable means of dispersal for lice between different host species, we might expect phoresis to break down patterns of cospeciation, because of a higher potential for host-switching. Over 80% of the records of louse phoresis on hippoboscid flies result from flies recovered from Passeriformes (the principal hosts of *Brueelia*). Of these records, the vast majority involve species of *Brueelia*, rather than other lice parasitic on passerines. If phoresis is a major dispersal route between host species for species of *Brueelia*, we might expect cospeciation in *Brueelia* to be minimal despite its relatively high host specificity. Thus, cophylogenetic analysis of species of *Brueelia* and their hosts provides an opportunity to assess the potential role of phoresis of lice for breaking down cospeciation.

While *Brueelia* might initially appear to be a good candidate for cophylogenetic analysis, several problematic issues surround this genus. These problems revolve around the taxonomy of the genus and the ramifications these problems create for species validity and inferred host-specificity. Because *Brueelia* is so speciose, no comprehensive revision of this genus has ever been conducted and there is no comprehensive key for identification of species. Revising large genera can be a difficult matter because of the large number of specimens that need to be examined and evaluated. Most revisions and species descriptions in this genus have only examined louse material from a suite of closely related hosts. For example, Ansari (1956, 1957) revised the species of *Brueelia* occurring on Corvidae (crows and jays, Passeriformes) and Dalgleish (1971) revised the species on Picidae (woodpeckers, Piciformes). Dalgleish (1971) separates the species on Picidae into two species groups corresponding to the same species groups recognized by Ansari (1956, 1957) for corvid *Brueelia*. This suggests that *Brueelia* on either Corvidae or Picidae (or both) are not monophyletic groups and that the use of host taxonomy in establishing the scope of a revision may be suspect. In addition, in this case at least, it appears that the phylogeny of *Brueelia* may not reflect host phylogeny. Clay (1951) also recognized this problem and suggested that genera separated from *Brueelia* by some authors on the basis of host occurrence are unlikely to be valid.

In the case of new species descriptions, species have often been described solely on the basis of their occurrence on a novel host. Ledger (1980) states, 'At present, the majority of *Brueelia* species described are not recognizable from their published descriptions . . . '. Many authors have suggested that this genus is in serious need of revision, but until subgroups of *Brueelia* can be identified such a revision will prove extremely difficult. In addition to comparing cophylogenetic patterns, an additional goal of our study is to begin to identify monophyletic groupings of species within *Brueelia* as an aid to future taxonomic revision. We examine some of the more distinct morphological characters of the species in our study. We assess the congruence of morphology with the molecular phylogeny, providing a basis for future morphologically based revisions.

We also reassess the level of host-specificity in *Brueelia* by sampling individuals from at least four species of hosts from several localities. If species of *Brueelia* are not as host-specific as indicated by previous host records, we would expect to find some species of lice on multiple hosts in the same locality. Well-sampled locales (> four host species) included South Africa, Mexico, and the Philippines. In making collections for this phylogenetic study, we encountered many new host records for *Brueelia*. Because we have not yet carried out a taxonomic revision of *Brueelia*, we refer to these possibly new, undescribed species of *Brueelia* simply by numbering them.

For phylogenetic analysis, we use sequences of both nuclear (elongation factor 1-alpha) and mitochondrial (cytochrome oxidase I) genes. We compare the evolutionary rates of these two genes in relation to their usefulness for resolving the phylogeny of this genus. Because phylogenies of different gene regions can differ, owing to differences in lineage sorting, hybridization, or differential rates (Bull *et al*., 1993; de Queiroz, 1993), we use this study as an opportunity to compare phylogenies from alternate gene regions.

METHODS

Collecting and Sample Preparation

We collected lice from hosts using the ethyl acetate fumigation method described by Clayton, Gregory & Price (1992). Individual hosts were kept separate at

all times in paper or plastic bags and care was taken to clean all working surfaces between host fumigation. Lice were stored either frozen at –70°C or in 95% ethanol at –20°C. Samples of *Brueelia* were collected from 21 host species (Table1). These samples were chosen to span the diversity of hosts on which *Brueelia* occurs. We extracted DNA from individual lice by removing the head from the body with a pair of jeweler's forceps. These parts were placed in an extraction buffer and DNA was extracted from individual lice using a Qiagen Tissue Extraction Kit. At the end of the digestion procedure, the head and the body of the louse were removed from the digestion buffer and reassembled in basalm on a microslide. This procedure, which does not damage fine structure, including setae, allows for morphological identification of louse specimens. Voucher slides are deposited in the Price Institute of Phthirapteran Research, University of Utah, Salt Lake City, Utah. Using other comparative slide material, we attempted to identify each species and also noted general morphological differences between species for comparison with our molecular phylogeny.

SEQUENCING

DNA extracts of individual lice were used in PCR amplifications of the mitochondrial cytochrome

Table 1. Lice sequenced in this study

Species†	Host	Host Order	Locality
***Brueelia* sp. 1**	***Ficedula hyperythra****	Passeriformes	Philippines
***Brueelia* sp. 1**	***Parus elegans***	Passeriformes	Philippines
***Brueelia* sp. 1**	***Sitta frontalis****	Passeriformes	Philippines
***Brueelia* sp. 1**	***Rhipidura nigrocinnamomea***	Passeriformes	Philippines
***Brueelia* sp. 2**	***Hypsipetes philippenis***	Passeriformes	Philippines
Brueelia sp. 3	*Trogon massena**	Trogoniformes	Mexico
Brueelia sp. 3	*Trogon melanocephalus*	Trogoniformes	Mexico
Brueelia sp. 4	*Megalaima monticola*	Piciformes	Borneo
Brueelia sp. 4	*Megalaima mystacophanos*	Piciformes	Borneo
***Brueelia* sp. 5**	***Coracina striata***	Passeriformes	Philippines
***Brueelia* sp. 6**	***Parus niger****	Passeriformes	South Africa
***Brueelia* sp. 7**	***Ploceus velatus***	Passeriformes	South Africa
***Brueelia* sp. 8**	***Cacicus haemorrhous***	Passeriformes	Brazil
***Brueelia* sp. 9**	***Carpodacus mexicanus***	Passeriformes	Utah
Brueelia sp. 10	*Melanerpes candidus*	Piciformes	Brazil
***Brueelia* sp. 11**	***Parisoma subcaeruleum***	Passeriformes	South Africa
***Brueelia* sp. 12**	***Pycnonotus nigricans***	Passeriformes	South Africa
Brueelia laticeps	*Andigena nigrirostris*	Piciformes	Peru
Brueelia laticeps	*Aulacorhynchus prasinus*	Piciformes	Peru
Brueelia moriona	***Cyanocorax morio***	Passeriformes	Mexico
Brueelia marginella	*Momotus momota*	Coraciiformes	Mexico
Formicaricola analoides	***Formicarius moniliger***	Passeriformes	Mexico
***Formicaphagus* sp.**	***Thamnophilus doliatus***	Passeriformes	Mexico
Paragoniocotes sp.	*Aratinga astec*	Psittaciformes	Mexico
Nyctibicola longirostris	*Nyctibius jamaicensis*	Caprimulgiformes	Mexico
Quadraceps punctatus	*Larus cirrocephalus*	Charadriiformes	South Africa
Saemundssonia lari	*Larus cirrocephalus*	Charadriiformes	South Africa
Rallicola columbiana	***Dendrocolaptes certhia***	Passeriformes	Mexico
Rallicola fuliginosa	***Dendrocincla anabatina***	Passeriformes	Mexico
***Penenirmus* sp.**	***Psaltriparus minimus***	Passeriformes	Utah
Physconelloides cubanus	*Geotrygon montana*	Columbiformes	Mexico

†numbered taxa represent new host records for *Brueelia*
*indicates host taxa from which lice were collected (and sequenced) from >1 host individual.
Passerines and their lice are indicated in bold.
Birds in this study from indicated host orders include Passeriformes (oscine and suboscine songbirds), Trogoniformes (trogons), Piciformes (barbets, woodpeckers, and toucans), Coraciiformes (motmots), Psittaciformes (parrots), Caprimulgiformes (nightjars), Charadriiformes (gulls), Columbiformes (doves).

oxidase I (COI) and nuclear elongation factor 1-alpha (EF1α) genes. We used the primers L6625 and H7005 (Hafner *et al*., 1994) to amplify COI, and EF1-For3 and EF1-Cho10 (Danforth & Ji, 1998) to amplify EF1α (reaction conditions described by Johnson & Clayton, 2000). We purified PCR products using a Qiagen PCR purification kit and used the amplification primers in sequencing reactions. DNA cycle sequencing was performed with *Taq* FS DNA polymerase using either ABI dRhodamine dye terminators or ABI Prism BigDye Terminators (Perkin-Elmer). DNA sequence data were collected with an ABI Prism 377 automated DNA sequencer (PE Applied Biosystems). We resolved complementary chromatograms using Sequencher 3.0 (GeneCodes). We also used Sequencher to align gene sequences across species (GenBank accession numbers AY149382–AY149439).

Phylogenetic analysis

We used a composite outgroup of several louse genera to root the tree for *Brueelia*. These genera were identified by Cruickshank *et al*. (2001) as close relatives of *Brueelia*. We also used these outgroup taxa as a test of *Brueelia* monophyly. Outgroup taxa included *Penenirmus*, *Rallicola* (2 sp.), *Quadraceps*, *Saemundssonia*, *Nyctibicola*, *Paragoniocotes*, *Formicaricola*, *Formicaphagus* and the entire tree was rooted on *Physconelloides*, a more distant outgroup (Table 1).

To assess the relative stability of trees to method of analysis, we used three different tree construction methods: parsimony, maximum likelihood, and neighbour-joining. We used PAUP* (Swofford, 2000) for these analyses. We first constructed trees for both genes independently using unordered parsimony. We conducted parsimony searches using 100 random addition replicates with TBR branch swapping. To test whether the phylogenetic signal between the nuclear and mitochondrial genes was in significant conflict, we performed a partition homogeneity test with 1000 replicates (Farris *et al*., 1994, 1995; Swofford, 2000). Because we observed significant conflict between genes over the phylogeny (see Results), we removed single taxa and repeated the partition homogeneity test with each taxon removed singly (Johnson, 1997; Johnson, Drown & Clayton, 2001). The conflict between genes was relatively weak and could be eliminated by the removal of a single taxon or differential weighting of gene regions (see Results), so we decided to proceed with a combined analysis. We constructed 1000 parsimony bootstrap replicates (Felsenstein, 1985) with the two genes independently, and combined, to evaluate relative support for branches in the tree.

We used the parsimony tree from combined searches to estimate the simplest maximum likelihood model that could not be rejected in favour of a more complex model under the framework of likelihood ratio tests outlined by Huelsenbeck & Crandall (1997). We used this likelihood model in heuristic searches with 100 random addition replicates and NNI branch swapping to find the most likely tree under the model. We used 100 bootstrap replicates with random taxon addition and NNI branch swapping to evaluate relative support for various nodes in this tree. As a final tree construction technique, we used neighbour-joining (NJ) with Kimura two-parameter distances (Kimura, 1980). We also performed 1000 bootstrap replicates in the NJ analysis.

Comparison with host tree

We used trees from Sibley & Ahlquist (1990) to construct a host tree for the relevant host taxa in this study. While we are aware that aspects of Sibley & Ahlquist's (1990) study are controversial (Lanyon, 1992), the host phylogeny used here generally reflects previous classification and is generally consistent with analysis of a number of avian cyt *b* sequences (Johnson, 2001), as well as nuclear gene sequences for birds (Barker, Barrowclough & Groth, 2002). We compared the host tree with trees for *Brueelia* from each of the three phylogenetic analysis methods (parsimony, likelihood, neighbour-joining). In this comparison, we used only *Brueelia*, *Formicaricola*, *Formicaphagus*, *Paragoniocotes* and *Nyctibicola*, excluding more distant outgroups. We used reconciliation analysis (Page, 1990a) as implemented in TreeMap (Page, 1995) to conduct these comparisons. This method identifies possible cospeciation events between host and parasite trees, and considers similarities between trees as possible instances of cospeciation. For each analysis we compared the number of cospeciation events recovered in the tree reconciliations to a null distribution derived from randomizing the parasite tree 1000 times, and counting the number of 'cospeciation' events for each randomized tree (Page, 1990b, 1995). This analysis can be used to compare the reconstructed number of cospeciation events to what might be expected by chance, thus yielding a *P*-value.

Morphology

Clay (1951) identified six different forms of the shape and pattern of the dorsal preantennal head structures, with some species exhibiting what Clay considered to be a specialized head form. Specifically, these differences involve the shape of the dorsal marginal carina (a heavily sclerotized margin at the leading edge of the head) and the presence and shape of the dorsal anterior head plate (a heavily sclerotized plate just posterior to the marginal carina), as well as the presence

and location of sutures in the dorsal anterior portion of the head capsule. For each of our voucher specimens, we coded the preantennal dorsal head pattern according to Clay's (1951) classification (Fig. 1). We found one head pattern not described by Clay that we coded as a separate character state (D). We mapped the character states of head pattern over each of the three trees described above. We also noted that the degree of sclerotization and pigmentation of the abdomen varied considerably between species. We identified six forms of this sclerotization and pigmentation (Fig. 2) and coded each species based on this categorization. We assessed whether these two morphological characters contained significant phylogenetic signal by randomizing character states to taxa 1000 times and comparing the observed number of steps to this null distribution (Maddison & Slatkin, 1991). We also examined whether morphology tended to change when species of *Brueelia* occur on non-passerine hosts. We determined whether changes in morphological character state were associated with occurrence on a non-passerine host more than expected by chance using a concentrated changes test (Maddison, 1990) implemented in MacClade (Maddison & Maddison, 1992).

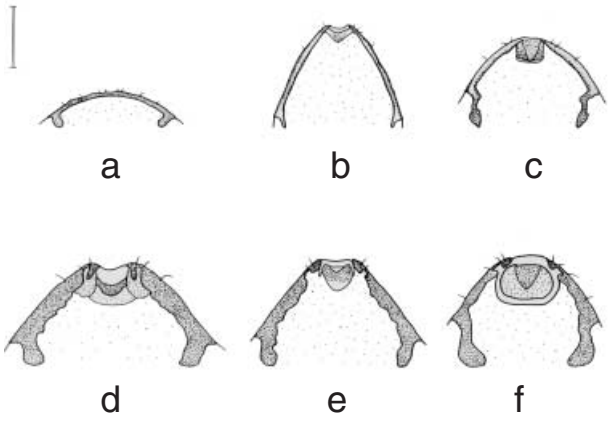

Figure 1. Preantennal dorsal head patterns of species of *Brueelia* with the following character states: (a) marginal carina complete, no dorsal anterior head plate; (b) marginal carina complete with an anterior hyaline margin, anterior portion indented with a triangular-shaped area of sclerotization within indentation; (c) marginal carina complete, with a sclerotized triangle within a simple dorsal anterior head plate, suture at posterior edge of head plate; (d) marginal carina shows partial lateral interruptions, with a broad anterior hyaline margin, dorsal anterior head plate continuous with the rest of the head showing a distinctly sclerotized band along the anterior portion and bordered by a pair of weakly sclerotized plates; (e) marginal carina with partial lateral interruptions and complete medial interruptions formed by a pair of sutures originating in the anterior hyaline margin, sutures run along either side of a moderately sclerotized dorsal anterior head plate, but leave the plate continuous with the remainder of the head's dorsal sclerotization; (f) marginal carina with partial lateral interruptions, dorsal anterior head plate completely encircled by sutures, isolating it from the rest of the dorsal head sclerotization. Scale bar = 0.1 mm.

RESULTS

Sequence Variation

Within a species of *Brueelia* (as indicated in Table 1), there was no variation in either COI or EF1α sequences. Between species of *Brueelia*, uncorrected sequence divergence for COI ranged from 9.7% to 23.1%. For these same comparisons, divergences for EF1α ranged from 0.6% to 9.8%. Of 389 sites for COI, 199 (51.2%) were variable and of these 184 (47.3% of all sites) were potentially phylogenetically informative. Of 348 sites for EF1α, 111 (31.9%) were variable and 91 (26.1% of all sites) were potentially phylogenetically informative. We estimated the relative rate of substitution between the two genes by plotting pairwise divergences in COI against those for EF1α

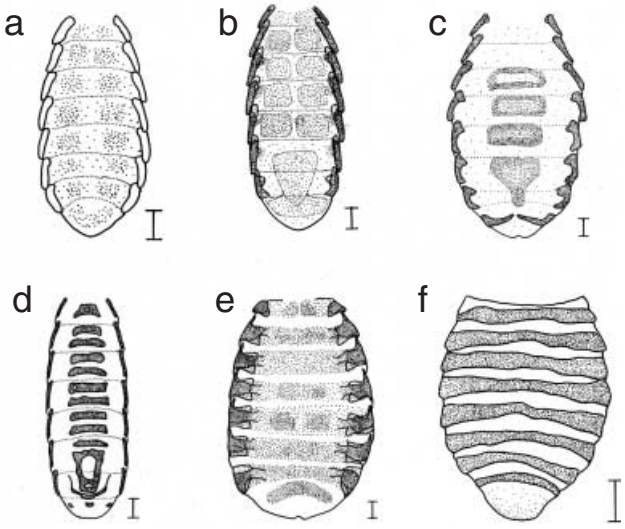

Figure 2. Abdominal sclerotization and pigmentation of species of *Brueelia* with the following character states: (a) abdomen weakly sclerotized with non-pigmented pleurites; (b) abdomen weakly sclerotized with moderate to darkly pigmented pleurites; (c) abdomen weakly sclerotized with several wide diffuse dark bars along the median, pleurites darkly pigmented; (d) abdomen weakly sclerotized with numerous well-defined dark bars along the median, pleurites darkly pigmented; (e) abdomen with moderately sclerotized, lightly pigmented bands, sclerites wrap around lateral edges of abdominal segments; (f) abdomen with interspersed sclerotized and-non-sclerotized bands. Scale bars = 0.1 mm.

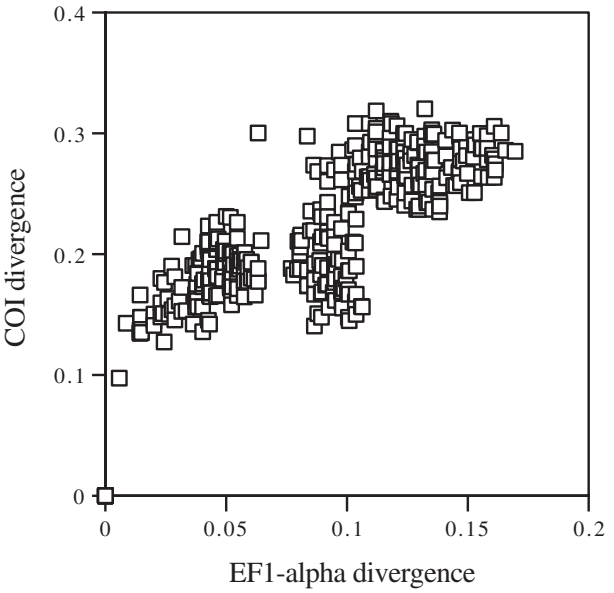

Figure 3. Plot of pairwise uncorrected percent divergences in the COI gene against those for the EF1α gene. The initial slope of the relationship is an estimate of the relative substitution rate between the two genes.

(Fig. 3). These comparisons indicated that COI evolves approximately five (or more) times faster than EF1α based on the slope of the initial linear portion of substitution accumulation (Sturmbauer & Meyer, 1992). While EF1α showed no insertions or deletions, COI exhibited at least two indel events. *Brueelia* and *Paragoniocotes* showed a deletion of the 23rd codon of our COI sequences relative to other outgroups. In addition, *Brueelia* species 1–3 parasitizing *Trogon*, *Hypsipetes*, *Sitta*, *Rhipidura*, *Ficedula* and *Parus elegans* all contained an insertion of two codons (6 bp) at the 91st and 92nd codons of our aligned COI sequences. These sites were coded as missing in the taxa exhibiting these indels in our analysis.

PHYLOGENY

Analysis of gene regions independently with unordered parsimony produced differing tree topologies (not shown). For COI, eight nodes received bootstrap support greater than 50%. For EF1α, 15 nodes received bootstrap support greater than 50%. In only one case did a node receiving greater than 50% bootstrap support for COI (59%) conflict with a node receiving greater than 50% support for EF1α (92%). A partition homogeneity test (Farris *et al*., 1994, 1995; Swofford, 2000) on these data indicated marginally significant conflict between data sets over the phylogeny ($P = 0.025$). Removal of single species, one at a time, produced ten cases where removal of a single species resulted in a *P*-value greater than 0.05. In two of these cases (*Brueelia marginella* and *Formicaricola analoides*), the *P*-value exceeded 0.35, while in the remaining eight, the *P*-value was 0.15 or less. Thus, removal of either *Brueelia marginella* or *Formicaricola analoides* appeared to remove most of the conflict between gene regions. These two species were not involved in the conflicting bootstrap support but were placed in quite different places in the COI and EF1α trees.

In some cases, conflict between molecular data sets may result from a difference in relative rates of evolution (Bull *et al*., 1993; Chippendale & Wiens, 1994; Dolphin *et al*., 2000; Barker & Lutzoni, 2002). COI appears to be evolving at a much more rapid rate than EF1α (Fig. 3). Weighting EF1α sequences five times over COI resulted in a *P*-value of 0.105 for the partition homogeneity test, indicating that conflict between data sets might be attributable to differential rates of evolution between genes. In addition, weighting transversions over transitions by 2 : 1 for both genes results in a *P*-value for the partition homogeneity test of 0.20. Thus, phylogenetic methods that take into account differential rates of evolution, such as maximum likelihood, would be most appropriate to accommodate these differences. Under a conditional combination framework (Bull *et al*., 1993), data sets should be analysed separately if significant conflict between them exists. However, in our case, the conflict was only marginal and could be eliminated by removal of a single taxon or by differential weighting (Chippendale & Wiens, 1994). Thus we chose to conduct combined analyses to best estimate the tree topology for *Brueelia*.

Combined parsimony analysis produced six trees in two islands (consensus Fig. 4). This tree has 14 nodes supported at the 50% bootstrap level. This number was less than EF1α alone (15 nodes) but greater than COI alone (eight nodes), and these differences are likely to be attributable to the combination of a data set with high homoplasy (COI, RC = 0.141) and a data set with lower homoplasy (EF1α, RC = 0.332; Johnson & Clayton, 2000). This tree indicated two major groups within *Brueelia* (one with three species [clade A, spp. 1–3] and one of seven species [clade B, spp. 6–12]). These groups had relatively strong bootstrap support (89% and 83%, respectively). The monophyly of clade A is also supported by the two codon insertion in COI. It is unclear if *Brueelia* is monophyletic in the unordered parsimony analysis, but bootstrap support for monophyly of *Brueelia* plus *Paragoniocotes*, *Formicaricola* and *Formicaphagus* is high (94%).

Because of the dramatic rate difference between genes, maximum likelihood models that take into account rate heterogeneity may provide a better estimate of the phylogeny than unordered parsimony. Using likelihood ratio tests (Huelsenbeck & Crandall,

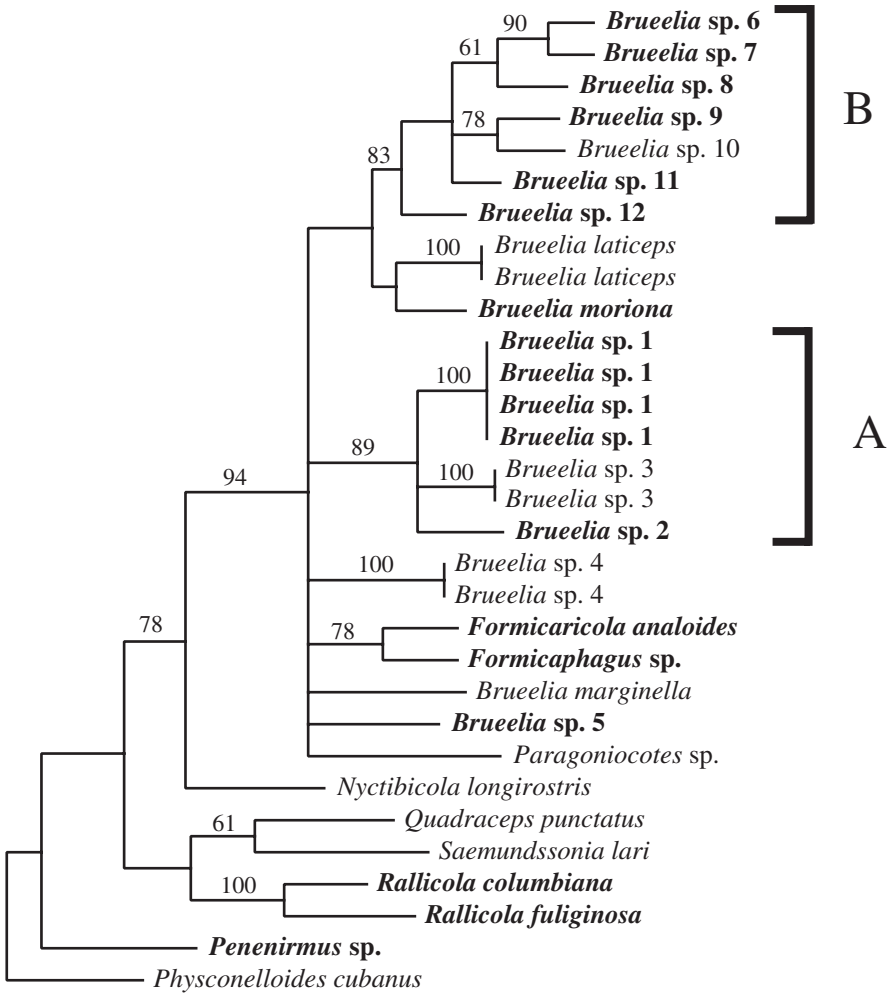

Figure 4. Strict consensus of six trees for *Brueelia* and outgroups resulting from unordered parsimony analysis of combined COI and EF1α DNA sequences. Tree length = 1473, rescaled consistency index = 0.175. Branch lengths are proportional to the number of reconstructed changes. Numbers above branches indicate bootstrap support from 1000 bootstrap replicates. Unnumbered nodes received <50% bootstrap support. Species occurring on Passeriformes are indicated in bold. Two well-supported clades (A and B) are labelled as they are referred to in the text.

1997), we determined that a model incorporating unequal base frequencies, six substitution types, and rate heterogeneity ($\alpha = 0.20$) was the best fit model. Random addition searches (100 replicates) with NNI branch swapping produced a single most likely tree (Fig. 5). This tree was recovered in 45 of the 100 replicates so this search algorithm appeared to be efficient. In this tree, 17 nodes received over 50% support by bootstrap replicates. The maximum likelihood tree also recovered the three-species (clade A) and seven-species (clade B) groups within *Brueelia*. In addition, with the exception of *Brueelia marginella* from *Momotus* (a non-passerine), *Brueelia* is monophyletic with a sister relationship to a clade containing *Paragoniocotes*, *Formicaricola* and *Formicaphagus*. Bootstrap support for monophyly of this clade plus all of *Brueelia* is relatively high (72%).

Neighbour-joining analysis of Kimura two-parameter distances recovered monophyly of *Brueelia* (Fig. 6) although bootstrap support was less than 50%. The two large subgroups (A and B) of *Brueelia* identified in the parsimony and likelihood analyses were also recovered by neighbour-joining analysis. Like the previous trees, neighbour-joining analysis indicated a sister relationship between *Formicaricola* and *Formicaphagus*, and these in combination with *Paragoniocotes* and all of *Brueelia* formed a well supported clade (bootstrap 98%).

Comparison with host tree

Regardless of the tree for *Brueelia* selected (Figs 4–6), there is little concordance between the phylogeny of *Brueelia* and host phylogeny. For example, two species

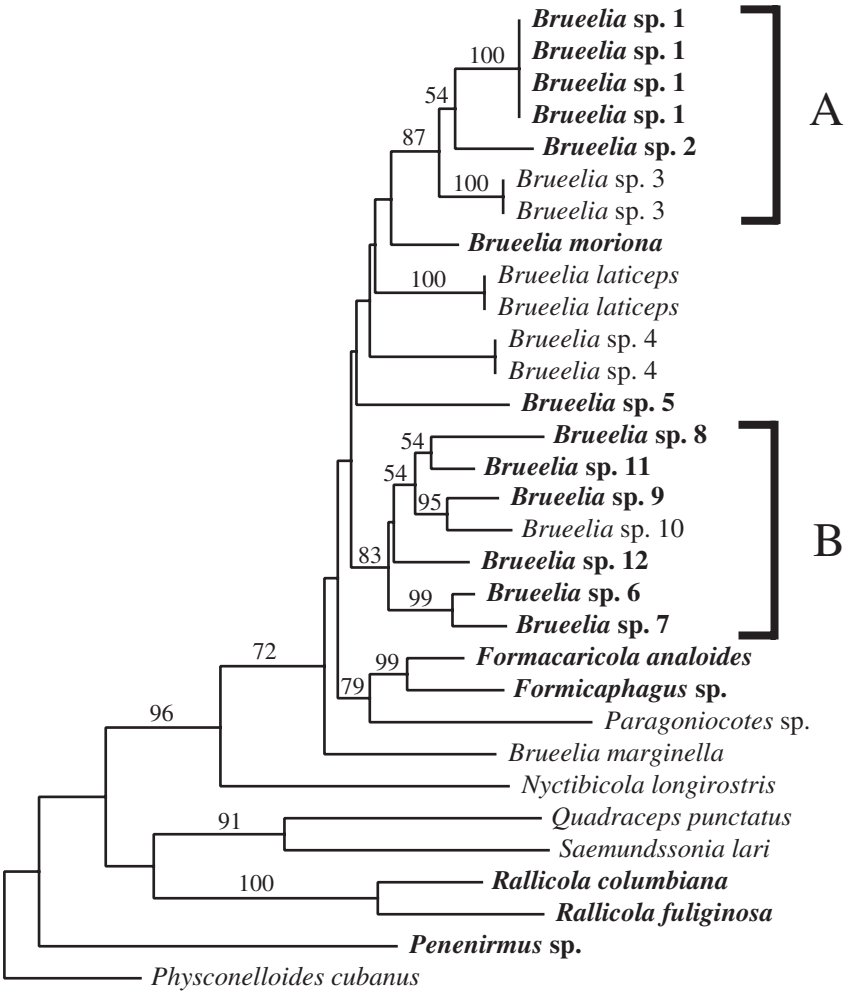

Figure 5. Tree for *Brueelia* and outgroups resulting from 100 random addition maximum likelihood searches using combined COI and EF1α DNA sequences (L = 6800.04). Model includes empirical base frequencies, rate heterogeneity according to a gamma distribution (shape parameter = 0.20, partitioned into eight rate categories), and six substitution categories, A-C = 0.548, A-G = 11.08, A-T = 2.55, C-G = 1.47, C-T = 5.22, G-T = 1. Branch lengths are proportional to maximum likelihood estimated branch lengths under the model. Numbers above branches indicate bootstrap support from 100 bootstrap replicates. Unnumbered nodes received <50% bootstrap support. Species occurring on Passeriformes are indicated in bold. Two well-supported clades (A and B) are labelled as they are referred to in the text.

of *Brueelia* from the host genus *Parus* fall in two distinctly separate clades (Fig. 7). *Brueelia* from Piciformes (toucans, barbets and woodpeckers) appear in three separate places in the tree, often having lice from Passeriformes (songbirds) as their relatives. Likewise, *Brueelia* from Trogoniformes (trogons) are imbedded within passerine lice. Reconciliation analysis (Page, 1990a) of the three *Brueelia* trees (parsimony, likelihood, neighbour-joining, Figs 4–6) with the host tree (Sibley & Ahlquist, 1990) recovered seven cospeciation events in each case. However, seven cospeciation events were not beyond that expected by chance when the parasite tree was randomized (*P*-values ranging from 0.20 to 0.26). Thus, even after accounting for uncertainties in tree topology for *Brueelia*, parasite phylogeny is essentially random with respect to the host phylogeny. The only terminal cospeciation event evident is between *Formicaricola* and *Formicaphagus*, parasites of suboscine passerines. No terminal sister taxa of *Brueelia* are found on a terminal sister pair of hosts.

MORPHOLOGY

The shape and structure of the marginal carina and dorsal anterior head plate (Fig. 1) contained signifi-

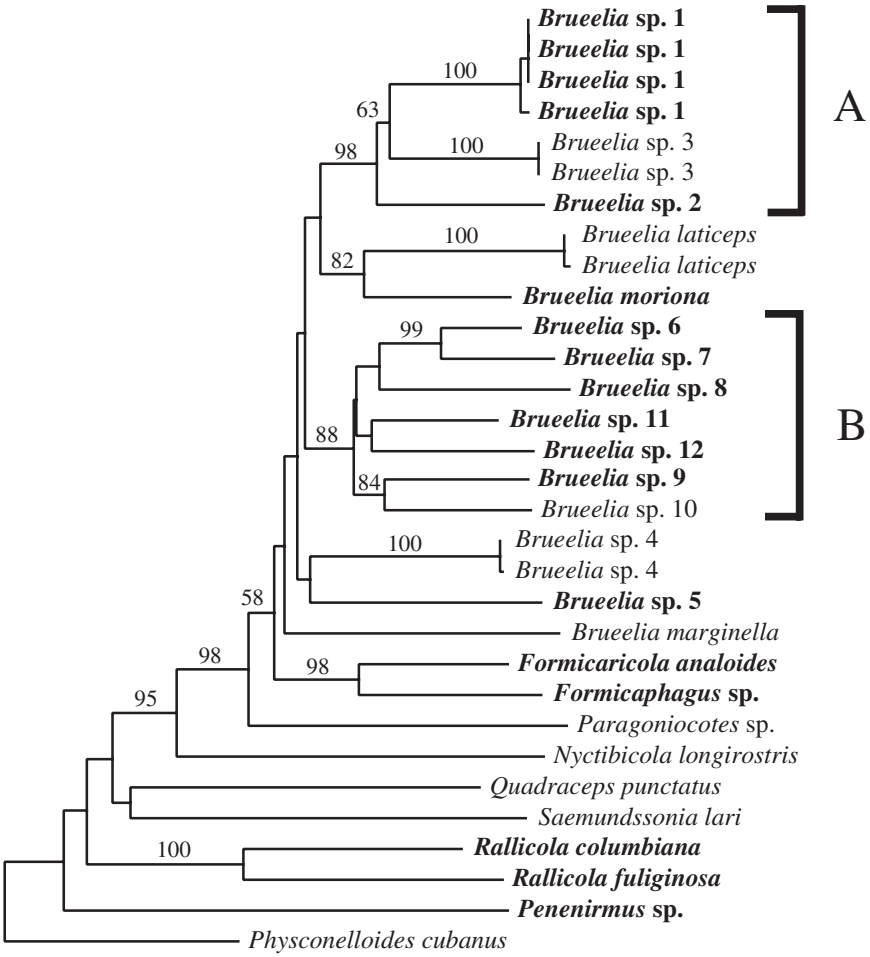

Figure 6. Tree for *Brueelia* and outgroups resulting from neighbour-joining analysis of Kimura two-parameter distances (Kimura, 1980) using combined COI and EF1α DNA sequences. Branch lengths are proportional to Kimura two-parameter distances. Numbers above branches indicate bootstrap support from 1000 bootstrap replicates. Unnumbered nodes received <50% bootstrap support. Species occurring on Passeriformes are indicated in bold. Two well-supported clades (A and B) are labelled as they are referred to in the text.

cant phylogenetic signal ($P < 0.002$) in relation to all trees (see Fig. 8 for reconstruction over maximum likelihood tree). The degree and pattern of abdominal sclerotization and pigmentation (Fig. 2) showed less phylogenetic conservation (Fig. 9), and this conservation was only significant over the unordered parsimony tree ($P = 0.04$). There was a tendency for morphology to change in *Brueelia* lineages that parasitize non-passerine hosts. For head structure, concentrated changes tests (Maddison, 1990) indicated a significant association between morphological change and host occurrence over the parsimony and likelihood trees ($P = 0.004$ and 0.028, respectively), but this relationship was not significant over the NJ tree ($P = 0.26$). Similarly, abdominal sclerotization showed a significant correlation over the parsimony and NJ trees ($P = 0.004$) but not over the likelihood tree ($P = 0.15$).

DISCUSSION

Sequences of the nuclear EF1α gene and the mitochondrial COI gene produced a generally well resolved tree for the bird louse genus *Brueelia*. Considered separately, EF1α produced a more strongly supported tree, compared to COI. This difference may relate to a difference in the evolutionary rates and corresponding levels of homoplasy in the two genes. While comparisons of these two data sets indicated some degree of incongruence, generally this was weak and could be

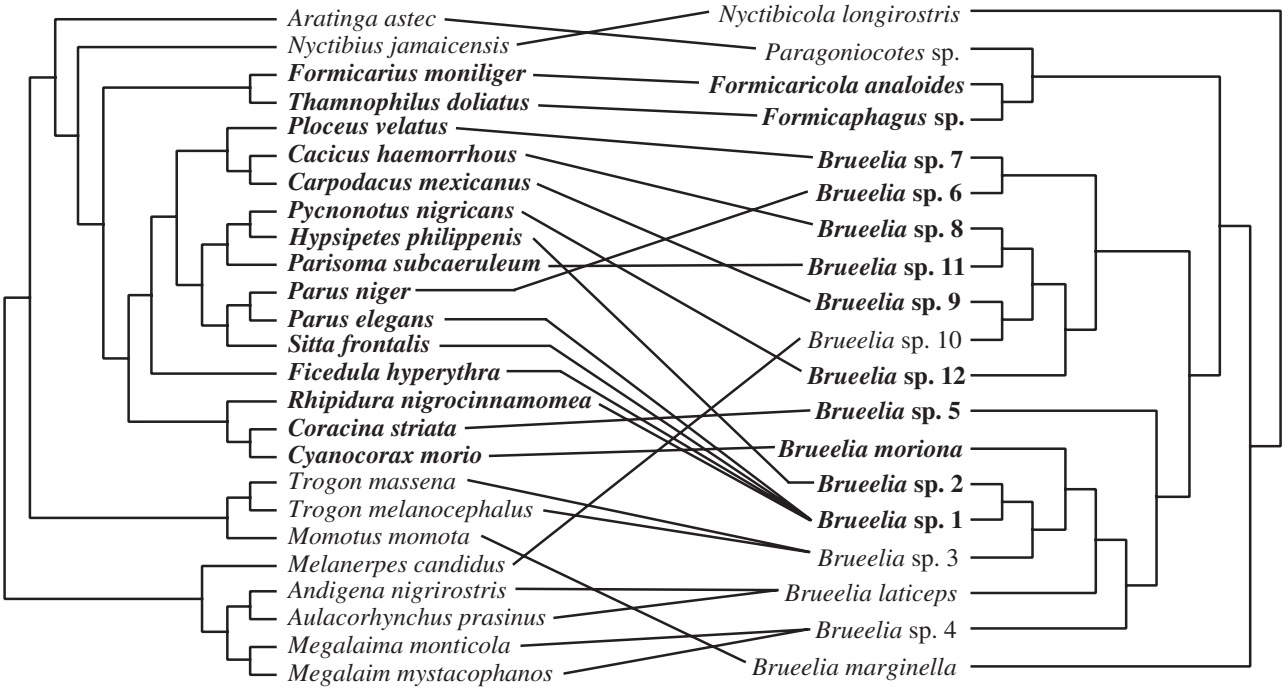

Figure 7. Comparison of host and parasite trees for birds and lice (*Brueelia* and outgroups). Lines represent host associations. Passerines and their lice are indicated in bold. The maximum likelihood tree for *Brueelia* (Fig. 5) is depicted. The host tree is taken from Sibley & Ahlquist (1990).

eliminated by differential weighting or removal of a single taxon from the analysis. Genes that differ dramatically in rates of molecular evolution may cause incongruence between data sets (Bull *et al*., 1993; Chippendale & Wiens, 1994). However, in our analysis, this incongruence appeared to involve weakly supported nodes, suggesting that the rate difference itself is probably the source of incongruence, and not any real underlying difference in the phylogenies from the two genes.

Two analyses (parsimony and likelihood) suggested that *Brueelia* is not monophyletic. However, neighbour-joining analysis produced a tree in which *Brueelia* was monophyletic. In the maximum likelihood analysis, *B. marginella* was placed outside the rest of *Brueelia*. This species is a parasite of Coraciiformes (a motmot in this case), while most other species of *Brueelia* in our study are parasites of Passeriformes (songbirds) or Piciformes (toucans, barbets and woodpeckers). *Brueelia marginella* may be a representative of an earlier radiation of *Brueelia* on Coraciiformes. Two genera confined to suboscine Passeriformes (*Formicaricola* and *Formicaphagus*) appear to be closely related to, if not imbedded within, *Brueelia*. These two genera are found on only two families of birds: Formicariidae (antbirds) and Conopophagidae (gnateaters). Despite extensive sampling efforts (Clayton *et al*., 1992), no species of *Brueelia* has been recorded from these two families of birds, but *Brueelia* has been recovered from a number of other families of suboscine Passeriformes. Thus, it appears that *Formicaricola* and *Formicaphagus* are the equivalent of *Brueelia* on the Formicariidae and Conopophagidae. The close relationship of *Paragoniocotes* to *Brueelia* is surprising given that most other workers do not consider these two genera to be close relatives (Eichler, 1963); however, bootstrap support for this relationship was high (Figs 4–6). In addition, a close relationship between *Brueelia* and *Paragoniocotes* is further supported in an analysis of EF1α sequences for 52 Ischnoceran genera (Cruickshank *et al*., 2001). Further work involving more representatives of *Formicaricola*, *Formicaphagus*, and *Paragoniocotes* is needed to resolve the relationships between these taxa and *Brueelia*.

Our analysis divides *Brueelia* into two major groups, each receiving strong support. These groups generally reflect major morphological differences within *Brueelia*. For example, *Brueelia* sp. 6–12 (clade B) are united by possessing a marginal carina that is complete, but with a hyaline margin and a slight indentation of the carina (Figs 1b,8). In contrast to these two major groupings, several representatives of *Brueelia*

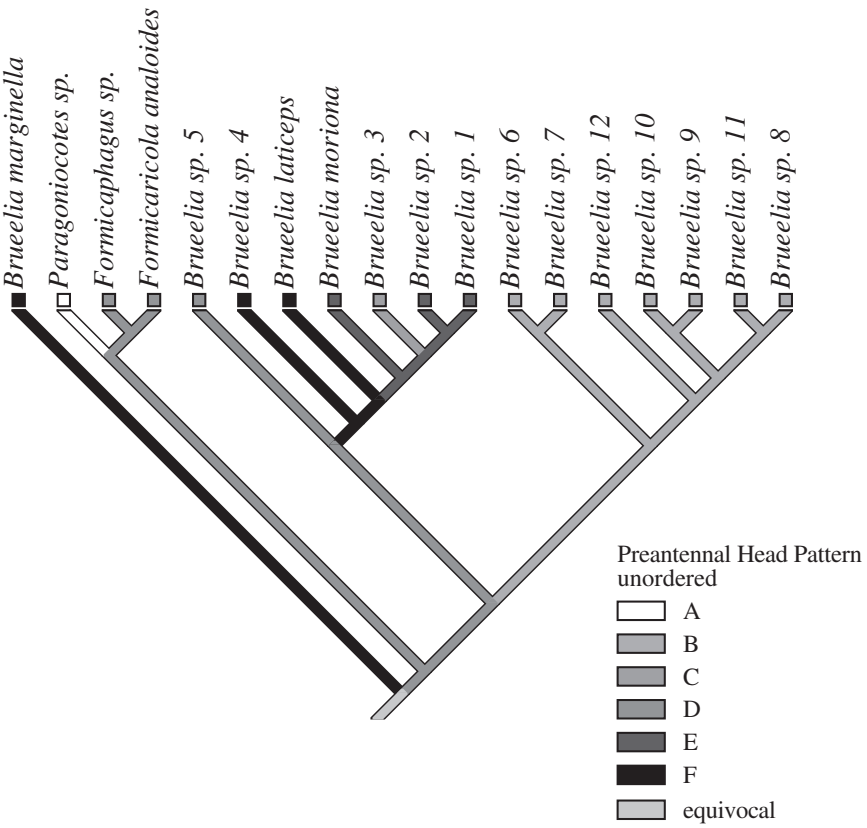

Figure 8. Parsimony reconstruction (ACCTRAN) of preantennal dorsal head patterns in *Brueelia* and close relatives (CI = 0.83). Letters correspond to the character states indicated in the legend of Fig. 1.

included in our study could not be placed with strong support. Some of these species possess unique (in our study) morphological characteristics of the shape of the marginal carina or pattern of abdominal sclerotization. Often these unique morphological character states seem to be associated with occurrence on non-passerine hosts. Perhaps differences in host morphology or ecology select for differences in louse morphology. Differences in feather structure or in host defence need to be examined in these host groups in more detail to determine the possible cause of this apparent pattern. Further sampling of species with these morphological character states may reveal other major groups within *Brueelia* corresponding to these major differences in morphology.

The phylogeny of *Brueelia* shows little relation to host phylogeny. The lack of association between parasite and host phylogeny does not appear to be a result of uncertainty in the estimation of the phylogeny of *Brueelia*. All methods of phylogenetic analysis produced a similar cophylogenetic result. Another concern is that the host phylogeny is incorrect, breaking down the congruence between host and parasite phylogenies. While the phylogeny of Sibley & Ahlquist (1990) is controversial (Lanyon, 1992), phylogenetic analysis of mitochondrial cytochrome *b* sequences (Johnson, 2001) and nuclear gene sequences (Barker *et al*., 2002) for a large number of avian taxa produces similar relationships and identifies similar major groupings of birds. Further examination of host–parasite relationships in our analysis also indicates that it is unlikely that an incorrect host phylogeny could be a major contributing factor to incongruence between host and parasite trees. For example, lice on the host genus *Parus* are in two very different clades of the *Brueelia* tree. In addition, species parasitic on Piciformes appear in several different places in the phylogeny of *Brueelia*. Thus, even using simple host classification, there appears to be little correspondence between the phylogeny of *Brueelia* and the phylogeny of its hosts.

The lack of strong phylogenetic structure in relation to host phylogeny suggests that species of *Brueelia* are capable of dispersing between unrelated hosts. Since lice spend their entire lifecycle on the body of the host, opportunities for dispersal are limited. Most transmis-

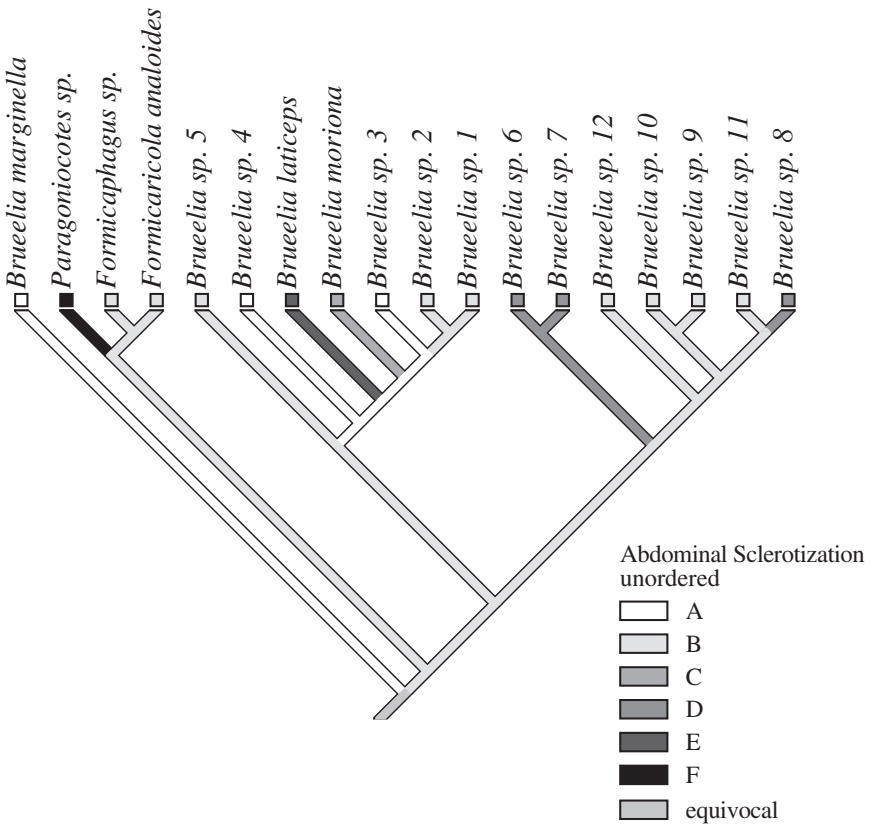

Figure 9. Parsimony reconstruction (ACCTRAN) of the degree of abdominal sclerotization and pigmentation of the abdomen in *Brueelia* and close relatives (CI = 0.63). Letters correspond to the character states indicated in the legend of Fig. 2.

sion in lice appears to be vertical between parent and offspring (Clay, 1949; Lee & Clayton, 1995). However, opportunities for horizontal transmission among individuals of the same species of host may occur during copulation (Hillgarth, 1996), allopreening, or other incidental contact between host individuals of the same species (Clay, 1949; Marshall, 1981). Opportunities for dispersal to individual hosts of different species are probably even more limited in most groups of lice. However, we found four cases where a single species of *Brueelia* was found on two or more species of hosts. These shared species of lice occurred on species of (1) trogons, (2) barbets, (3) toucans, and (4) a suite of four Philippine passerine species (*Ficedula hyperythra*, *Parus elegans*, *Sitta frontalis* and *Rhipidura nigrocinnamomea*). Trogons, barbets and toucans are all hole-nesting species, and in each case the birds involved are both closely related and live in the same habitats. Broader taxon and geographical sampling would be needed to distinguish the roles of these two factors. In the other case, three of the four passerine species in the Philippines that share a species of *Brueelia* nest in holes, but these host species are generally distantly related. These observations suggest that lice may be able to disperse between species if they remain in the nest between occupation of a hole-nest by one host species and a different host species.

Competition between species of birds for nest holes can be extremely intense. For example, competition between species of *Parus* and *Ficedula* for nest holes often results in mortality of *Ficedula* and takeover of the nest hole by *Parus* (Merilä & Wiggins, 1995). Interestingly, these two host genera share a species of *Brueelia* (sp. 1) in the Philippines. Interspecific takeovers of nests may be an opportunity for dispersal of lice between host species. Time off the host in the nest by a louse could be minimal given the intensity of competition for nests. In support of this, there are several records in the literature of species of *Brueelia* being recovered from nests of birds (Nordberg, 1936). In addition, louse eggs attached to feathers deposited in the nest may hatch after an interspecific nest takeover, providing an opportunity for dispersal to a different host species. Another possibility is that contact

resulting from fights over nest holes provides an opportunity for louse dispersal between host species. Dispersal of lice between species of hosts facilitated by hole-nesting is generally considered to play a minor role in the breakdown of host specificity (e.g. Clay, 1949). However, studies of owl lice (*Strigiphilus*) suggest a more important role for nest-mediated transfer (Clayton, 1990). Examination of the ability of lice or louse eggs to survive in hole-nests is needed to further assess the role of this dispersal mechanism. Despite these cases of species of *Brueelia* parasitizing multiple host species, in most cases we found species of *Brueelia* on only one host species, and this accords with the high specificity for species of this genus identified by previous alpha taxonomic host associations.

While dispersal between host species via opportunities provided by hole-nesting might explain the lack of specificity in several species of *Brueelia*, the overall pattern of incongruence between parasite and host phylogenies is more difficult to explain by this mechanism, because most passerines do not nest in holes. Another possible mechanism for dispersal of lice between host species is by phoresis (hitchhiking) on hippoboscid flies (Insecta: Hippoboscidae). Collections of hippoboscid flies often reveal attached lice (reviewed by Keirans, 1975). There are many records of this type of phoresy in the literature, and some have suggested that phoresy may play an important role in the dispersal of lice both within and between host species (Clay, 1949; de L. Brooke & Nakamura, 1998). Since hippoboscid flies are generally not as host specific as lice (Marshall, 1981), dispersal of lice between species of hosts may be possible by this mechanism. Over 80% of the records of louse phoresis appear to involve species of *Brueelia* (Keirans, 1975). If phoresis of *Brueelia* on flies is common, as these records indicate, opportunities for dispersal of species of *Brueelia* between host species may be high. This phenomenon might explain why the phylogeny of *Brueelia* shows little evidence of cospeciation, while phylogenies of groups of non-phoretic lice indicate considerable cospeciation(Hafner *et al*., 1994; Page *et al*.,1998).

How can one explain the relatively high host-specificity of species of *Brueelia* on the one hand, but the lack of cospeciation on the other? Host-specificity can arise from either the inability to disperse among host species, or the inability to survive on foreign host species. Perfect cospeciation requires complete host specificity, but the converse is not necessarily true. For example, if a host-specific louse disperses to a novel host, but there is not continued gene flow, the population on the novel host is likely to speciate. This type of host-switch would result in two completely host-specific sister species of lice on distantly related hosts. We might expect this phenomenon to be common in a case where phoresis provides a limited number of opportunities for dispersal between host species. If host-switching with speciation is common, phylogenies of hosts and parasites could be quite incongruent, but at the same time parasites could still be relatively host-specific.

Our results implicate a potential major role of phoresis in breaking down cospeciation in *Brueelia*. Phoresis of ischnoceran lice, such as *Brueelia*, is well documented, but phoresis of amblyceran lice is believed to be very minimal (if at all) because of a difference in mouthparts needed for gripping the hippoboscid fly. A comparison of phylogenies of non-phoretic amblyceran passerine lice (e.g. *Myrsidea*) with those of birds might provide insight on whether the lack of cospeciation in *Brueelia* might be due to an increased incidence of phoresy, or rather is a general pattern of passerine louse phylogenies.

In addition to a potential higher rate of dispersal in *Brueelia*, this genus might be better able to establish on novel hosts to which it has dispersed. Clay (1949) considers *Brueelia* a very generalized genus of louse. Individuals of *Brueelia* can be found throughout the plumage of their hosts. In contrast, other genera of ischnoceran lice on Passeriformes, such as *Philopterus*, appear to occupy more specialized microhabitats on the host. *Philopterus* is generally confined to the head feathers of the host (Clay, 1949) and has a very characteristic rounded body shape. Assuming species that are specialized to a particular microhabitat on the host find it more difficult to survive on foreign hosts, this may also cause a difference between groups in the degree of cospeciation. Further comparisons of host and parasite phylogenies for generalist and specialist lice are needed to document whether this is the case. Transfer experiments of generalist and specialist lice to foreign hosts would shed light on the relative contributions of limitation to dispersal vs. establishment on foreign hosts to these patterns.

In summary, we suggest that the high potential for dispersal of *Brueelia* between host species contributes to the lack of significant cospeciation in this genus. In other louse groups, interspecific dispersal is likely to be lower. Phoresis on hippoboscid flies does not occur in gopher lice (*Geomydoecus*) or swift lice (*Dennyus*), both of which show significant cospeciation with their respective hosts. Cophylogenetic studies of additional louse and host groups are needed to better understand what ecological factors might contribute to patterns of cospeciation vs. patterns of incongruence between host and parasite trees.

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