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Unlocking the black box of feather louse diversity: A molecular phylogeny of the hyper-diverse genus *Brueelia* ☆

Sarah E. Bush [a,*], Jason D. Weckstein [b,1], Daniel R. Gustafsson [a], Julie Allen [c], Emily DiBlasi [a], Scott M. Shreve [c,2], Rachel Boldt [c], Heather R. Skeen [b,3], Kevin P. Johnson [c]

[a] Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA
[b] Field Museum of Natural History, Science and Education, Integrative Research Center, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA
[c] Illinois Natural History Survey, University of Illinois, 1816 South Oak Street, Champaign, IL 61820, USA

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ABSTRACT

Songbirds host one of the largest, and most poorly understood, groups of lice: the *Brueelia*-complex. The *Brueelia*-complex contains nearly one-tenth of all known louse species (Phthiraptera), and the genus *Brueelia* has over 300 species. To date, revisions have been confounded by extreme morphological variation, convergent evolution, and periodic movement of lice between unrelated hosts. Here we use Bayesian inference based on mitochondrial (COI) and nuclear (EF-1α) gene fragments to analyze the phylogenetic relationships among 333 individuals within the *Brueelia*-complex. We show that the genus *Brueelia*, as it is currently recognized, is paraphyletic. Many well-supported and morphologically unified clades within our phylogenetic reconstruction of *Brueelia* were previously described as genera. These genera should be recognized, and the erection of several new genera should be explored. We show that four distinct ecomorphs have evolved repeatedly within the *Brueelia*-complex, mirroring the evolutionary history of feather-lice across the entire order. We show that lice in the *Brueelia*-complex, with some notable exceptions, are extremely host specific and that the host family associations and geographic distributions of these lice are significantly correlated with our understanding of their phylogenetic history. Several ecological phenomena, including phoresis, may be responsible for the macroevolutionary patterns in this diverse group.

"Taxonomist's nightmare... evolutionist's delight"
[*MacIntyre (1967), after A.J. Cain*]

1. Introduction

In 2012 a British birder was the first person to see 9000 different species of birds (McCarthy, 2012). This impressive tally is roughly 85–90% of all known bird species. Although a few new species of birds are being discovered and described each year (Sangster and Luksenburg, 2015), it is estimated that over 95% of all bird species have already been described (Mayr, 1982). In short, birds are among the best known groups of organisms on the planet. Despite this knowledge, however, birds represent many additional layers of undiscovered diversity. Each bird species harbors a complex community of parasites and other symbionts, many of which are undescribed and understudied.

Songbirds (Passeriformes), the largest order of birds, are host to one of the largest, and most poorly understood groups of feather lice. The genus *Brueelia* Kéler 1936 has over 300 described species (Price et al., 2003; Cicchino, 2004; Rékási and Saxena, 2005; Valim and Palma, 2006, 2015; Cicchino and González-Acuña, 2008, 2009; Sychra et al., 2009, 2010a, 2010b; Valim and Weckstein, 2011; Najer et al., 2012a, 2012b, 2012c; Mey and Barker, 2014; Najer et al., 2014; Valim and Silveira, 2014), and thousands of slides with specimens of unidentified and undescribed species of *Brueelia* line the drawers of museum collections around the world.

Lice in the genus *Brueelia* are incredibly diverse. They vary enormously in body shape: from short, round, "head" louse ecomorphs, to long, thin, "wing" louse ecomorphs (Johnson et al., 2012). They

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* Corresponding author.
E-mail address: bush@biology.utah.edu (S.E. Bush).
[1] Current address: Department of Ornithology and Department of Biodiversity, Earth, and Environmental Sciences, Academy of Natural Sciences of Drexel University, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103, USA.
[2] Current address: Division of Mathematics and Natural Sciences, Brescia University, Owensboro, KY 42301, USA.
[3] Current address: Biology Department, Loyola University Chicago, Chicago, IL 60660, USA.

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vary in color from light to dark (Bush et al., 2010), and in pigmentation patterns from simple to complex. Indeed, the morphological diversity within *Brueelia* echoes the diversity among all feather lice in the order Phthiraptera. A thorough understanding of the macroevolutionary patterns within *Brueelia* promises to illuminate the ecological and evolutionary forces influencing diversity among lice in general. However, this tantalizing diversity is a quintessential example of a "taxonomist's nightmare. . . evolutionist's delight" (MacIntyre, 1967). Convergent evolution of similar morphological characteristics is known to occur among lice (Johnson et al., 2012), which suggests that taxonomy based solely on morphological characters may obscure our understanding of the phylogenetic relationships within this group.

Lice in the genus *Brueelia* are also perplexing from another perspective. Among the groups of lice studied thus far, host specificity tends to correlate with cospeciation (Clayton et al., 2004). Lice on gophers are extremely host specific, and show among the strongest patterns of cospeciation in any system. Similarly, body lice on doves are quite host specific, and show a significant degree of cospeciation with their hosts, whereas wing lice on the same hosts are less host specific and show significantly less cospeciation than body lice (Clayton and Johnson, 2003; Clayton et al., 2004). *Brueelia* are considered to be highly host specific, with over 85% of described species recorded from just a single host species (Price et al., 2003). Despite this apparent high degree of host specificity, however, a preliminary cophylogentic analysis did not support a hypothesis of cospeciation (Johnson et al., 2002a).

There are at least two plausible explanations for this pattern. First, while specificity is a necessary condition for cospeciation, it is not a sufficient condition. For example, herbivorous beetles in the genus *Belpharida* are specific to particular host plants (*Bursera*), yet the beetle phylogeny is not congruent with the phylogeny of the host plants (Becerra, 1997). This is, in part, because these insects are relatively mobile organisms and can move between different host plants. In contrast, most lice are relatively immobile, only moving between hosts during periods of direct contact (Clayton et al., in press). *Brueelia* species, however, are known to hitch rides on hippoboscid flies (Fig. 1). This phoretic behavior may provide these lice with opportunities to switch to and adapt to new host species. If phoresis between host species is rare, and gene flow is limited, then lice may specialize and become quite specific on the "new" host species. Thus, rare phoretic events over macroevolutionary time could simultaneously support high levels of host specificity while disrupting patterns of cospeciation at a coarse macroevolutionary time-scale.

Alternatively, the apparent host specificity of lice in the *Brueelia* complex may be a taxonomic artifact. Early louse taxonomists tended to describe new species on the basis of host associations, rather than on the basis of the lice themselves. This unfortunate practice has required synonymization of nearly 2000 species and subspecies of chewing lice in comparison to only 4464 valid species and subspecies (Price et al., 2003). Indeed, initial molecular studies of lice in this genus indicate that a single species of *Brueelia* can infest multiple host species across several distantly related host families (Johnson et al., 2002a). A comprehensive taxonomic revision, independent of louse morphology, and host associations, is needed to identify the ecological and evolutionary drivers of diversity in this group.

Here we provide a molecular based phylogenic reconstruction for lice in the genus *Brueelia* and related lice in the genera *Bizarrifrons*, *Buerelius*, *Meropoecus*, *Motmotnirmus*, and *Sturnidoecus*, which are core members of the "*Brueelia* complex" (Clay and Tandan, 1967; Ledger, 1980; Valim and Palma, 2012, 2015). These genera are primarily found on songbirds (Passeriformes), although a few species are known to occur on Coraciiformes (bee-eaters), Piciformes (woodpeckers, barbets, and toucans), Trogoniformes (trogons), and Cuculiformes (couas). Our sampling includes lice from all of these host groups. We use DNA sequences of nuclear (EF-1α) and mitochondrial (COI) genes to provide a phylogenetic reconstruction of a worldwide sampling of over 300 specimens of lice from the *Brueelia*-complex and related genera (Johnson et al., 2002a). This is the largest phylogenetic reconstruction for any group in the order Phthiraptera. We discuss these results in the context of prior generic classifications and recommend that several previously recognized genera be considered valid. We also discuss emerging patterns of host specificity, biogeography, morphology, and behavior that are intimated by our new understanding of the phylogenetic relationships of these feather lice.

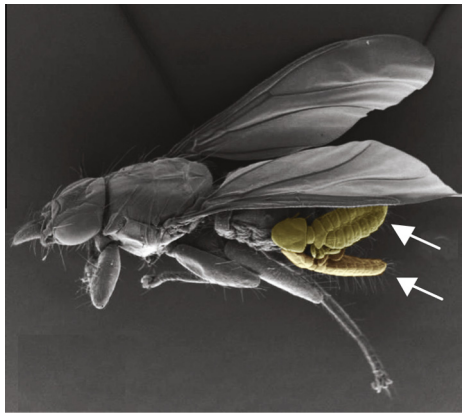

Fig. 1. Phoretic *Brueelia* sp. (arrows) hitching a ride on a hippoboscid fly. Fly collected from a blackbird *Turdus merula*. False-color SEM (SEM by V.S. Smith).

2. Materials and methods

2.1. Sampling

We sampled a total of 333 louse specimens belonging to the *Brueelia*-complex (see Table 1 in Bush et al., in press). These lice were sampled from 250 bird species belonging to 66 bird families and five orders (Passeriformes, Coraciiformes, Cuculiformes, Piciformes, and Trogoniformes). Sampled lice include 38 known species and 211 lice that represent either new species of lice or new host associations. These samples were collected from 23 countries and all continents except Antarctica. An additional 30 outgroup taxa for rooting the phylogeny were selected to represent nested sister taxonomic relationships within the family Philopteridae (Cruickshank et al., 2001; Johnson et al., 2001a; Smith et al., 2011). These 30 louse outgroup species were from 27 host species, in 17 host families, collected from 9 countries.

Lice were collected either from euthanized bird specimens using ethyl acetate fumigation or from live birds dusted with pyrethrum powder (Clayton and Drown, 2001; Bueter et al., 2009). Care was taken to make sure that individual hosts were kept separate at all times and to clean all working surfaces between fumigation. Lice were collected by the authors and colleagues during field-work conducted over several decades and were stored in vials of 95% ethanol, usually in ultracold (−80 °C) freezers.

2.2. DNA extraction, amplification and alignment

DNA was extracted from lice using either the Qiagen DNeasy micro-kit (Valencia, California, USA) following the manufacturer's protocol as described by Valim and Weckstein (2011), or the Qiagen DNeasy tissue kit (Valencia, California, USA) following the manufacture's protocol as described by Johnson et al. (2001b).

After DNA was extracted from individual lice, the exoskeletons were retained and mounted on microscope slides (Palma, 1978). These voucher slides were used to identify each specimen to genus using the keys in Price et al. (2003). Specific-level identifications were based on original descriptions, specific keys if possible, and comparison with identified slide mounted material. Voucher slides are deposited in the Illinois Natural History Survey Insect Collection (INHS), Price Institute for Parasite Research at the University of Utah (PIPeR), and Field Museum of Natural History (FMNH) (Table 1 in Bush et al., in press).

Portions of one mitochondrial (COI) and one nuclear gene (EF-1α) were selected because these genes have successfully resolved phylogenies of closely related groups of parasitic lice and more distantly related "bark lice" (Johnson et al., 2002c, 2003, 2004; Smith et al., 2004, 2011). We used PCR to amplify and sequence portions of the mitochondrial cytochrome oxidase I (COI; 379 bp) and the nuclear gene elongation factor 1a (EF-1α; 347 bp) using published amplification and sequencing protocols (Johnson et al., 2004; Smith et al., 2004). Purified PCR products were cycle sequenced using ABI Big Dye (Applied Biosystems, Foster City, California) and run on an ABI Prism 3730 DNA sequencer (Applied Biosystems). Raw sequence data was trimmed, edited, and reconciled using Sequencher 5.0.1 (Genecodes CO., Ann Arbor, Michigan) or Geneious (version 7.0.3, Biomatters LTD). Both genes are protein coding and therefore we were able to easily align them by eye according to codons. These aligned gene sequences were then concatenated for phylogenetic analysis (GenBank Accession Numbers original to this study KT892064–KT892646, for all other GenBank Accession Numbers see Table 1 of Bush et al., in press).

2.3. Phylogenetic analyses

The final sequence alignment was analyzed using PartitionFinder (v1.1.1; Lanfear et al., 2012, 2014), an open source python script that selects the best-fit partitioning schemes and models of molecular evolution for phylogenetic analysis. We tested whether the two genes (COI, EF-1α) should be analyzed together under the same model and parameters or as two separate partitions. We tested only these two partitions because separating each of these genes by codon would only provide 100 bps for each partition, a very small amount of sequence for estimating parameters and would likely result in over-parameterization. The PartitionFinder analysis found that a single partition and GTR+I+G model of molecular evolution best fit the data, using both AICc and BIC criterion. Using these parameters, which were estimated as part of the analysis, and a flat Dirichlet prior for state frequencies, we ran a Bayesian analysis in MrBayes 3.2.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012) for 10,000,000 generations. Each Bayesian analysis included two parallel runs, each with four Markov chains, to ensure that our analyses were not stuck at local optima (Huelsenbeck and Bollback, 2001). Markov chains were sampled every 500 generations, yielding 20,000 parameter point-estimates. We used these 20,000 point-estimates minus the burn-in generations (500 point-estimates, 250,000 generations) to create a 50% majority-rule consensus tree and calculated Bayesian posterior probabilities to assess nodal support. We rooted the Bayesian tree using a nested set of sister taxa within the family Philopteridae (Cruickshank et al., 2001; Johnson et al., 2004, 2012; Smith et al., 2004).

2.4. Operational Taxonomic Units (OTUs)

The number of OTUs was calculated using a single locus, the COI dataset, with two methods. First, OTUs were estimated using Mothur (Schloss et al., 2009), which takes into account sequence divergence. We used a cutoff of 5% for each OTU, and in lice values over this are generally associated with different species, whereas values under 5% generally carry little additional biological information (such as distinct patterns of host association) (Johnson et al., 2002b, 2007; Weckstein, 2004; Price et al., 2008; Price and Johnson, 2009). Second, OTUs were estimated with bGMYC, a coalescent method (Reid and Carstens, 2012) that takes into account phylogenetic uncertainty and determines a threshold for which the branching in the phylogenetic tree switches from interspecific to intraspecific. We used BEAST (Drummond and

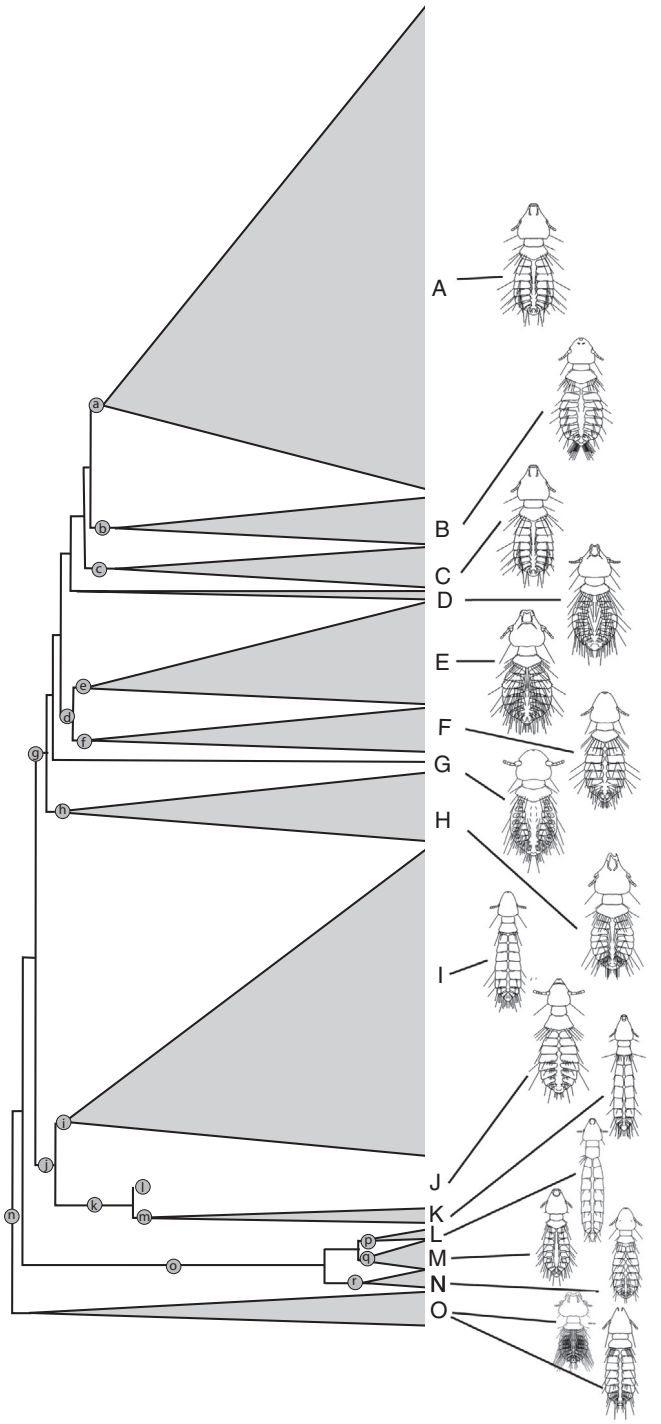

Fig. 2. Phylogenetic and graphical overview of clades in the *Brueelia* complex. Bullets indicate the presence of morphological characters unique to designated clades. These characters are defined in Table 1.

Rambaut, 2007) with the original COI alignment to obtain ultrametric trees using a GTR+G model of molecular evolution for 50 million generations, the burn-in was set at 20% and 100 trees were randomly selected from the post burn-in distribution for use in the bGMYC analysis. The threshold between intra and interspecific branching was calculated using the R package bGMYC (Reid and Carstens, 2012) running each tree for 5000 generations removing 2000 as burn-in and results calculated across the trees and the number of OTUs estimated based on the intra-interspecific threshold.

2.5. Test for phylogenetic signal with respect to host family and geographic distribution

We recorded the associated host family (Clements et al., 2014; Dickinson et al., 2014) and bioregion (see map, Olson et al., 2001; Fig. 3a) for each louse OTU. Several OTUs were associated with more than one host family or bioregion. To account for these polymorphic characters we randomly sampled 100 trees from the post-burnin Mr. Bayes analysis, and pruned each tree to represent only one taxon per OTU selected at random. Thus, our trees only contained one character per OTU, as each individual taxon was associated with only a single character. However, polymorphic characters were incorporated in the analyses because a single taxon was selected at random from each OTU for each tree; therefore, it is likely that all possible characters associated with each OTU are represented in the analyses. For each character (host family and bioregion) we ran a Maddison and Slatkin (1991) randomization procedure as a test of phylogenetic signal with Perl and R (Perl scripts and R code is available at www.github.com/juliema/publications). Specifically, we randomly assigned character states to taxa for each OTU tree 999 times and calculated the parsimony scores for each random assignment. We calculated the parsimony score for the true character states and determined whether the empirical parsimony score was significantly different than the random distribution. Significance indicates that the character in question is significantly conserved with respect to the tree topology as compared to random assignment of character states. This was done for all 100 randomly sampled OTU trees with: (1) host family as the character, (2) with bioregion as a character and (3) with host family and bioregion as a combined character. In total these analyses included 300 Maddison–Slatkin tests.

3. Results

The phylogenetic tree resulting from Bayesian analysis of the two gene regions identified several major clades, many of which are unified by gross morphology (Fig. 2, Table 1). Within clades, there was often strong phylogenetic resolution and support (Fig. 3a–f). However, the relationships among many of these clades were not very highly supported (see clade by clade results below). The OTU analyses of the 333 ingroup taxa indicated that there are between 114 and 166 operational taxonomic units in this dataset (Table 1 in Bush et al., in press). Based on the 5% species delimitation cutoff, Mothur calculated 166 OTUs and bGMYC calculated 114 OTUs. The majority of the OTUs identified in each analysis correspond well with the clades identified in the tree.

Tests that explored patterns of host association and geographic distribution among bioregions indicate a significant amount of phylogenetic signal in these characters, suggesting that these characters are more conserved with respect to the phylogenetic tree than expected by chance. Maddison and Slatkin tests for phylogenetic signal with respect to louse associations with host family, bioregion, and host family × bioregion were all significant ($p < 0.05$ in all cases), indicating that host associations and geographical distributions are significantly conserved.

Table 1
Key morphological characters that apply to clades of lice from the *Brueelia* complex. Letters refer to labeled bullets in Fig. 2.

	Morphological characters unique to designated clades
a	Marginal carina interrupted laterally and submedianly, connected by dorsal pre-antennal suture that may be transversally continuous posterior to dorsal anterior plate. Mesomere small compared to other genital elements, typically more or less triangular. Parameral heads axe-shaped or rectangular. Abdomen not very setose
b	Sternites modified antero-laterally into thickened bars.[a] Pleurites with complex anterior ends. Terminal abdominal segment very setose in male. Marginal carina uninterrupted. Male subgenital plate with subsidiary lateral plates. Mesomere either minute, triangular, or large and shield-shaped. Parameres elongated distally
c	As Clade A, but head elongately trapezoidal, and mesomere generally larger proportional to other genital elements. Not easily separable from Clade A on morphological grounds
d	Mesomere thickened distally, either transversally continuous, convergent along lateral margins of mesomeral lobes, divergent along median margins of lobes. Or along both margins of the lobes, but not transversally continuous
e	Head broadly triangular. Tergites generally with transversally continuous rows of setae. Marginal carina interrupted laterally and submedianly, connected by dorsal pre-antennal suture that is transversally continuous posterior to dorsal anterior plate; this plate has a flat or slightly concave posterior margin. Coni very prominent. Male genitalia very variable between species groups
f	Marginal carina not interrupted, but displaced medianly. Mesomeral lobes generally fused distally. Prominent rugose nodi on distal mesomere
g	AS3 absent. Extrusor muscles as parallel or convergent lines on mesomere extending onto basal plate,[a] male with *spa* on at least some tergites,[a] parameres with folded heads overlapping with mesomere, mesomere overlapping with basal plate
h	Mesomere much extended anteriorly
i	Marginal carina un-interrupted[a]; no dorsal pre-antennal suture[a]; *spa* present on at least some male tergites
j	Parameral heads cup-shaped, blunt or digitate; mesomere with scaly, hairy, or brushlike distal margin; parameres triangular (but may be extended distally); tergal setae missing on at least segments II–III; *stp* on at least some male tergites[a]
k	Marginal carina interrupted at least laterally, dorsal pre-antennal suture present
l	Dorsal anterior suture transversal; marginal carina interrupted only laterally, but modified into wide interior plate at frons; abdomen teardrop-shaped. Female subgenital plate may be divided transversally
m	Marginal carina interrupted laterally and submarginally; dorsal pre-antennal suture continuous with both interruptions and may entirely encircle a dorsal anterior plate
n	No setae on anterior end of tergite II, male tergites II–IX divided medianly,[a] female tergites II–VIII divided medianly[a]; at most one *mts* macroseta[a]
o	Parameral heads small, blunt; proximal mesomere flat or rounded; *as3* present; parameral heads folded into V- or heart-shapes[a]
p	Parameres lobe-like, typically with large pore in distal half; *ss* absent on anterior tergites[a]; anterior end of head typically with distinct nail-shaped thickening; *pns* microseta
q	Marginal carina interrupted laterally and submedianly; all Post-Nodal Setae microsetae; parameral heads large, heart-shaped; *ss* on all tergites; *pns* microseta
r	Splayed mesomeres with serrated distal margins; marginal carina uninterrupted or interrupted only submedianly; parameral heads small, pointed; *pns* long[a]; proximal mesomere fishtail-shaped; *ss* on all tergites

[a] A few taxa do not exhibit this character. Terminology of head characters and setae follows Clay (1951), Mey and Barker (2014); abdominal chaetotaxy follows Cicchino and Castro (1996).

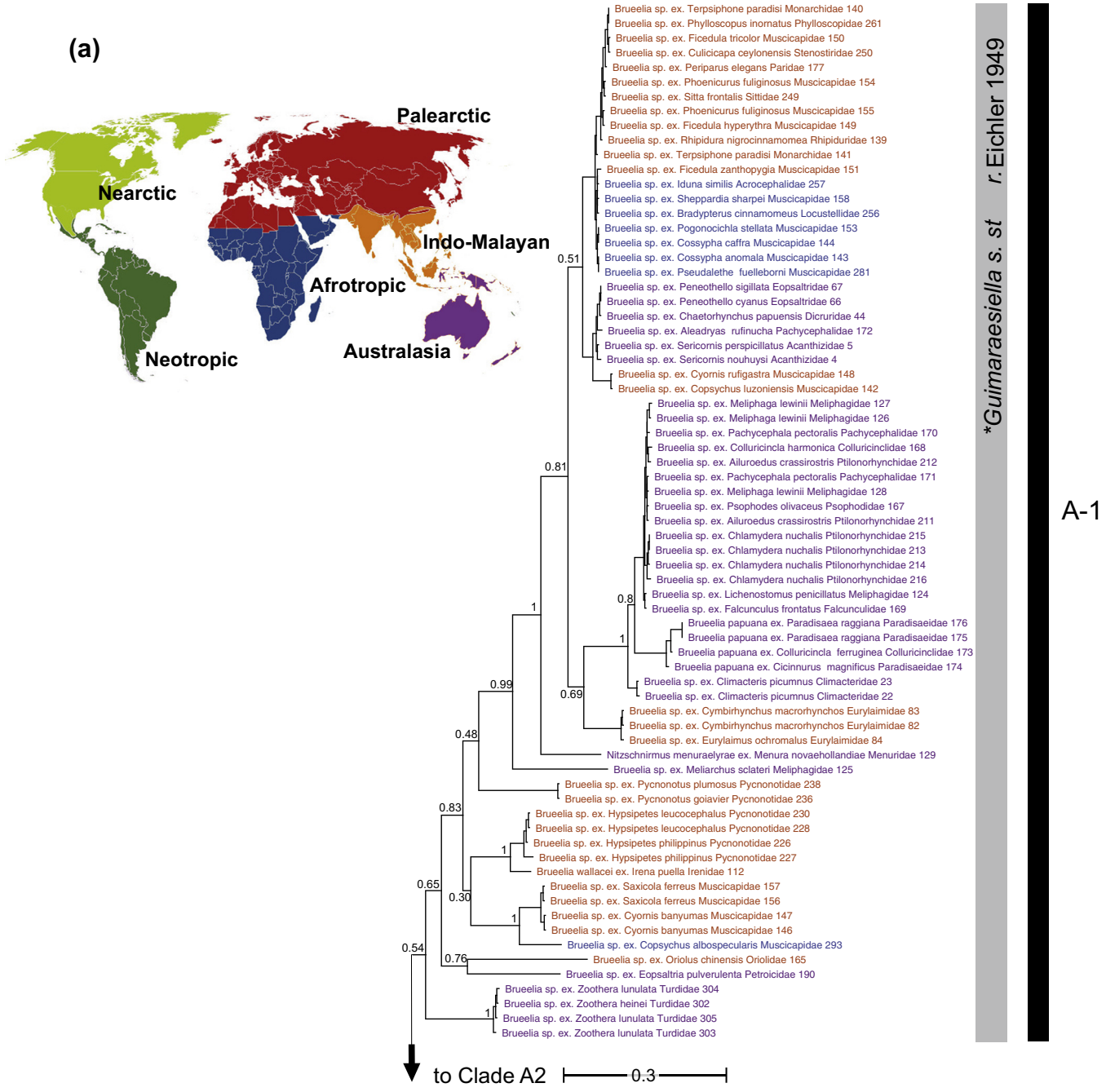

Fig. 3. Consensus tree from Bayesian analysis of combined COI and EF-1α sequences for *Brueelia*-complex species and outgroup taxa. Branches proportional to substitutions per site for the consensus tree (scale indicated). Numbers associated with nodes are posterior probabilities for the clade from a 10 million generation MCMC analysis, sampled every 1000 generations and excluding the first 1 million generations as burn-in (values <0.5, and values associated with short terminal branches not shown here; all support values >0.5 are shown in Fig. 2, Bush et al., in press). Text color refers to the geographic bioregion (map) where the specimen was collected. Numbers after taxonomic names refer to Table 1 in Bush et al., (in press). Louse taxonomy follows the classification of Price et al. (2003) and subsequent publications. Taxonomy indicated in the vertical gray bars indicate generic classifications; ∗ indicates alternate, historical treatments of genera that are considered junior synonyms of *Brueelia* by Price et al. (2003); † indicates genera that have been recognized as valid in taxonomic studies published after Price et al. (2003), see results for details. Host taxonomy follows Clements et al. (2014) and Dickinson et al. (2014): host genus, species, and family are all indicated. Tree partitioned into six portions (a–f). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.1. Clade A (Fig. 3a and b)

This strongly supported clade includes taxa that are morphologically similar to the type species of *Guimaraesiella* Eichler 1949. Although Price et al. (2003) considered this genus synonymous with *Brueelia*, species within Clade A form a strongly supported monophyletic group based on both genetic and morphological characters. The lice in this genus exhibit variable head characters, yet, they are unified by some pre-antennal head characters, abdominal chaetotaxy, and the shape and structure of the male genitalia (Table 1a). This clade also includes a specimen recently placed in a new genus, *Nitzschnirmus menuraelyrae* (Mey and Barker, 2014) (#129, Table 1 in Bush et al., in press). Given the molecular and morphological distinctions of this clade, the resurrection of the genus *Guimaraesiella* is warranted, which would make *Nitzschnirmus* a junior synonym of *Guimaraesiella*.

Clade A is distributed worldwide; however, within Clade A there are monophyletic units that are geographically restricted.

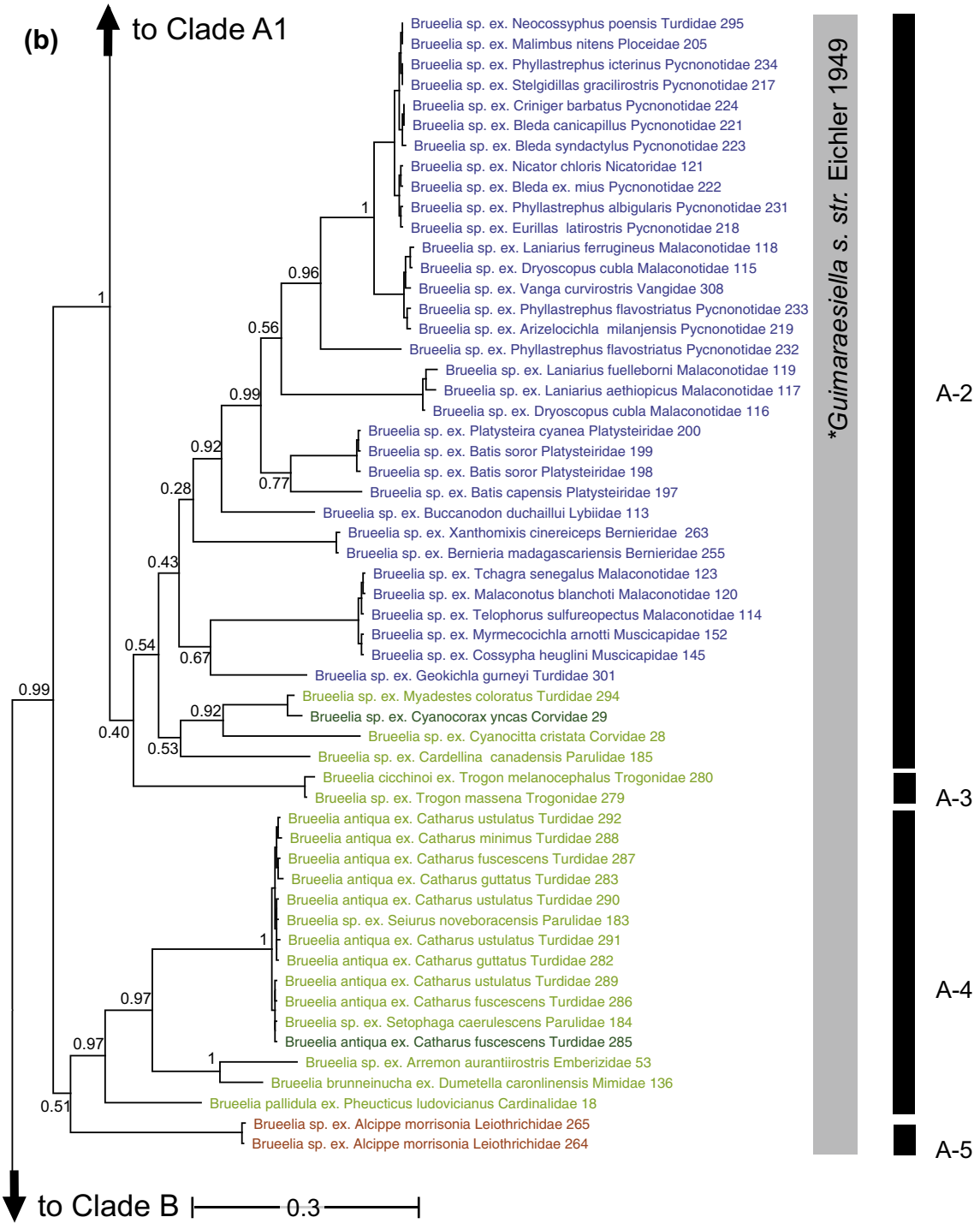

Fig. 3 (*continued*)

Clade A-1 is restricted to the Old World. Most of the taxa in this clade are from Indo-Malaya and Australasia, but a few taxa are from the Afrotropics. Samples from mainland Africa generally cluster together; however, they are genetically extremely close (<2% COI divergence) to samples from the Indo-Malayan region. The only remaining African taxon in Clade A-1 is a louse found on *Copsychus albospecularis*, a flycatcher from Madagascar, and this louse appears in a strongly supported clade with lice from Indo-Malayan Bulbuls and Old World Flycatchers.

Clade A-2+3 (Fig. 3b) contains a large monophyletic clade of lice from Africa, and smaller monophyletic clades from the New World, although neither of these have strong support. The separation and distinction of Clade A-1 and Clade A-2+3 is strongly supported by molecular data (100% posterior probability); yet, an initial morphological examination did not reveal any characteristics that clearly discriminate taxa in A-1 relative to A-2+3. There are, however, several strongly supported groups within in Clade A that have distinct morphological features. Although morphologically similar to *Guimaraesiella* s. str., the lice on trogons (Clade A-3; Fig. 3b) have unique pre-antennal head characteristics, (see Valim and Weckstein, 2011), and the lice on fulvettas (Clade A-5; Fig. 3b) have unique male genitalic characteristics. In each of these clades, very

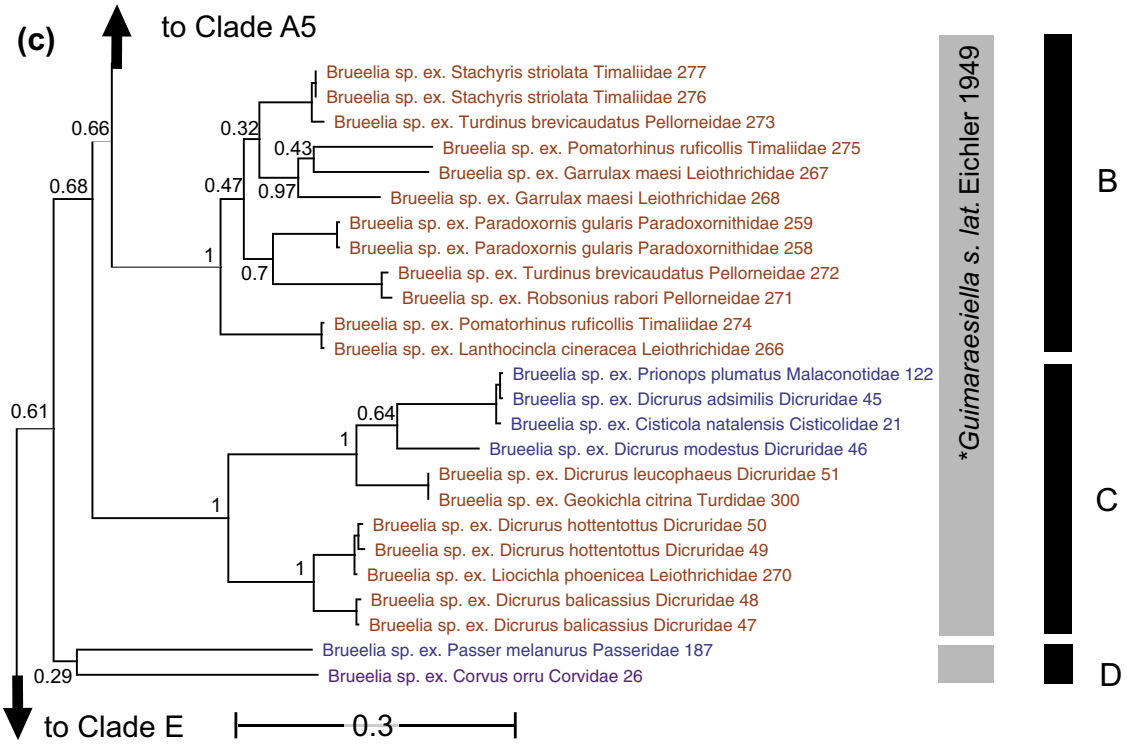

Fig. 3 (*continued*)

few lice were available for molecular analyses and their exact placement within Clade A is not well supported.

The lice on Nearctic songbirds (Clade A-4; Fig. 3b) form a strongly supported clade (97% posterior probability) based on molecular data, and these lice all share a dorsal pre-antennal head suture that completely separates the dorsal anterior plate from the main head plate. This character is ubiquitous in the New World, but is only rarely found in Old World taxa [e.g. in *Brueelia myiophoneae* (Clay, 1936)]. Most (10 out of 15) of the lice in this clade are found on thrushes (Turdidae); however, lice in this group are also found on four other passerine families: Mimidae, and more distantly related Parulidae, Cardinalidae, and Emberizidae (Cracraft and Barker, 2009).

3.2. Clades B–D (Fig. 3c)

Clade B is a strongly supported clade of lice geographically restricted to Indo-Malaya, and found only on Old World babblers (Timaliidae). Lice in Clade B share some chaetotaxy characters with lice in Clade A, but these chaetotaxy characters are somewhat variable within Clade A. However, striking differences in preantennal head structure and male genitalia separate lice in Clade B from other lice in the *Brueelia*-complex (Table 1b). This clade is placed as the sister group to Clade A (although this is weakly supported).

Morphologically, lice in Clade C are very similar to lice in Clade A. Subtle characteristics of the head unite this group (Table 1c), but these taxa have thoracic and genitalic characters that are extremely similar to those of *Guimaraesiella* s. str. (Clade A). Lice in this clade are restricted to the Old World. They are found largely on drongos (Dicuridae), but they also occur on other passerine families (Turdidae, Cisticolidae, and Malaconotidae).

Clade D is poorly supported. Both of the sequenced specimens were nymphs, which makes morphological characterization difficult. However, the sample from *Passer melanurus* appears to represent the genus *Rostrinirmus* Zlotorzycka, 1964, whereas the sample from *Corvus orru* likely represents *Corvonirmus* Eichler, 1944. Morphological differences in the preantennal head and abdominal chaetotaxy suggests that these two genera are not closely related. Additional samples for morphological and molecular work are sorely needed to sort out the phylogenetic placement and host specificity of these taxa.

Clades B, C, and D most likely represent distinct genera. However, additional taxonomic work is needed to formally address the taxonomic status of Clades B and C, and more specimens are needed to resolve the taxonomic status of Clade D.

3.3. Clades E–H (Fig. 3d)

Clade E is a strongly supported clade that contains lice in the genus *Sturnidoecus* Eichler 1944. These lice are unified by several morphological characters (Table 1e), including a distinctly round body with a broad triangular head, a phenotype typical of "head" lice (Johnson et al., 2012). Within Clade E several strongly supported monophyletic clades appear to be restricted to particular host families and/or geographic regions. For example, one clade includes lice from most Indo-Malayan thrushes (Turdidae), and another clade includes most lice from Nearctic thrushes. Lice from African weavers also group together into two distinct clades. As a whole, *Sturnidoecus* spp. in our study parasitize hosts from four different avian families: Sturnidae, Turdidae, Ploceidae, and Malaconotidae that diverged roughly 50 mya (Cracraft and Barker, 2009). Described species of *Sturnidoecus* are known from an additional 12 passeriform families, for which we did not have specimens for molecular work (Price et al., 2003). Thus, additional sampling of *Sturnidoecus* spp. is required before rigorous conclusions about radiations of lice in this genus across host families can be made.

Clade F is a strongly supported clade as is its position as a sister to *Sturnidoecus* (Clade E; 93% posterior probability). Although no described species from the previously recognized genus *Olivinirmus* Zlotorzycka, 1964 were sequenced in this study,

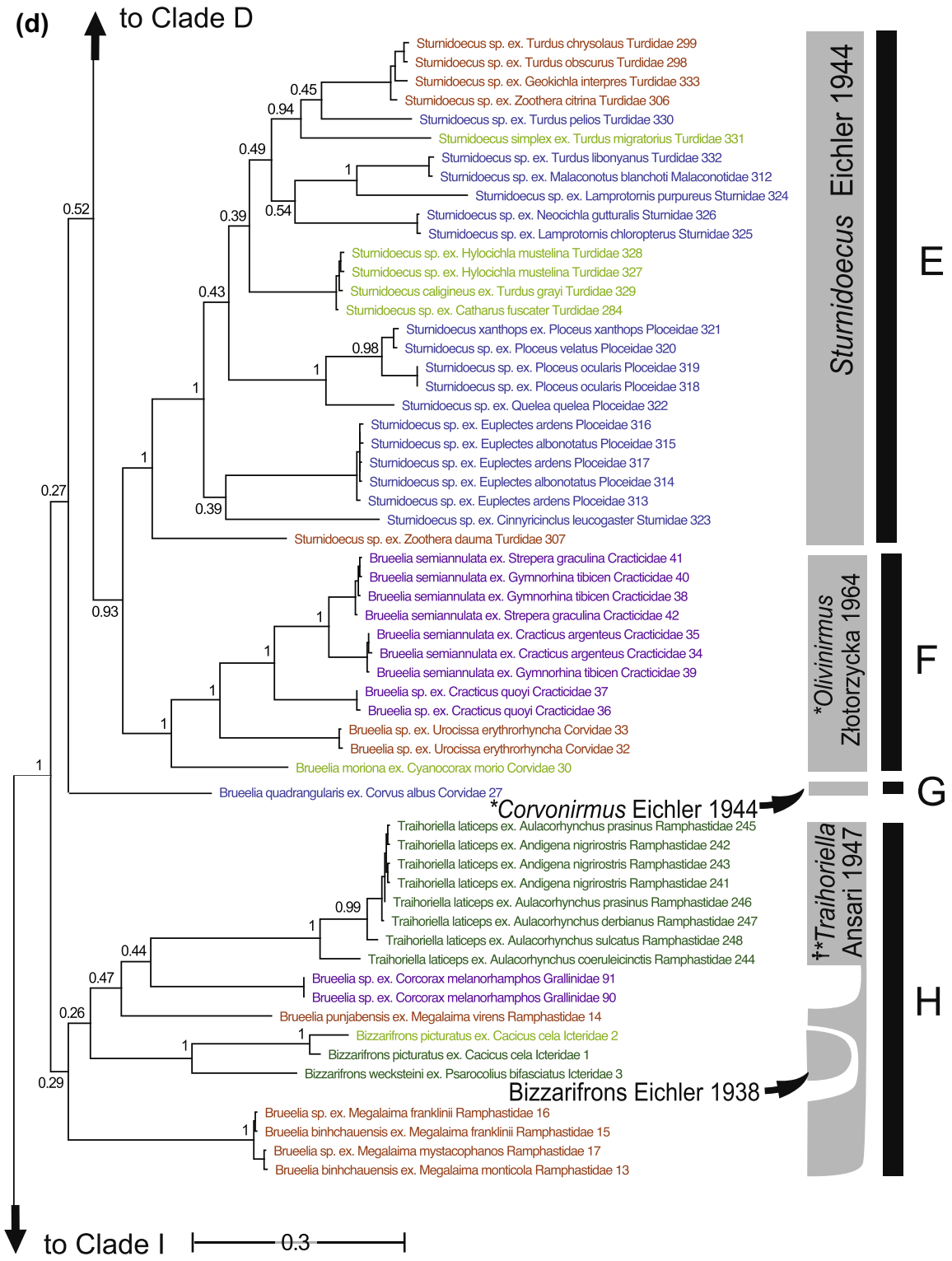

Fig. 3 (*continued*)

the shape of the male genitalic mesomere and pre-antennal head of the taxa in Clade F are unique (Table 1f) and are most similar to the generic description of *Olivinirmus*, which was considered a synonym of *Brueelia* by Ledger (1980). Given the observed molecular support, the resurrection of the genus *Olivinirmus* should be considered pending sequencing of described *Olivinirmus* spp. or formal taxonomic comparisons of the taxa in this study with *Olivinirmus glandarii* (Denny, 1842), the type species of *Olivinirmus*.

"Clade" G is a single louse from *Corvus albus* (Corvidae) that fits the description of the genus *Corvonirmus* erected by Eichler (1944). This genus was considered synonymous with *Brueelia* by Hopkins and Clay (1952). The position of this sample is sister to Clades A–F; however, this relationship is poorly supported. Future

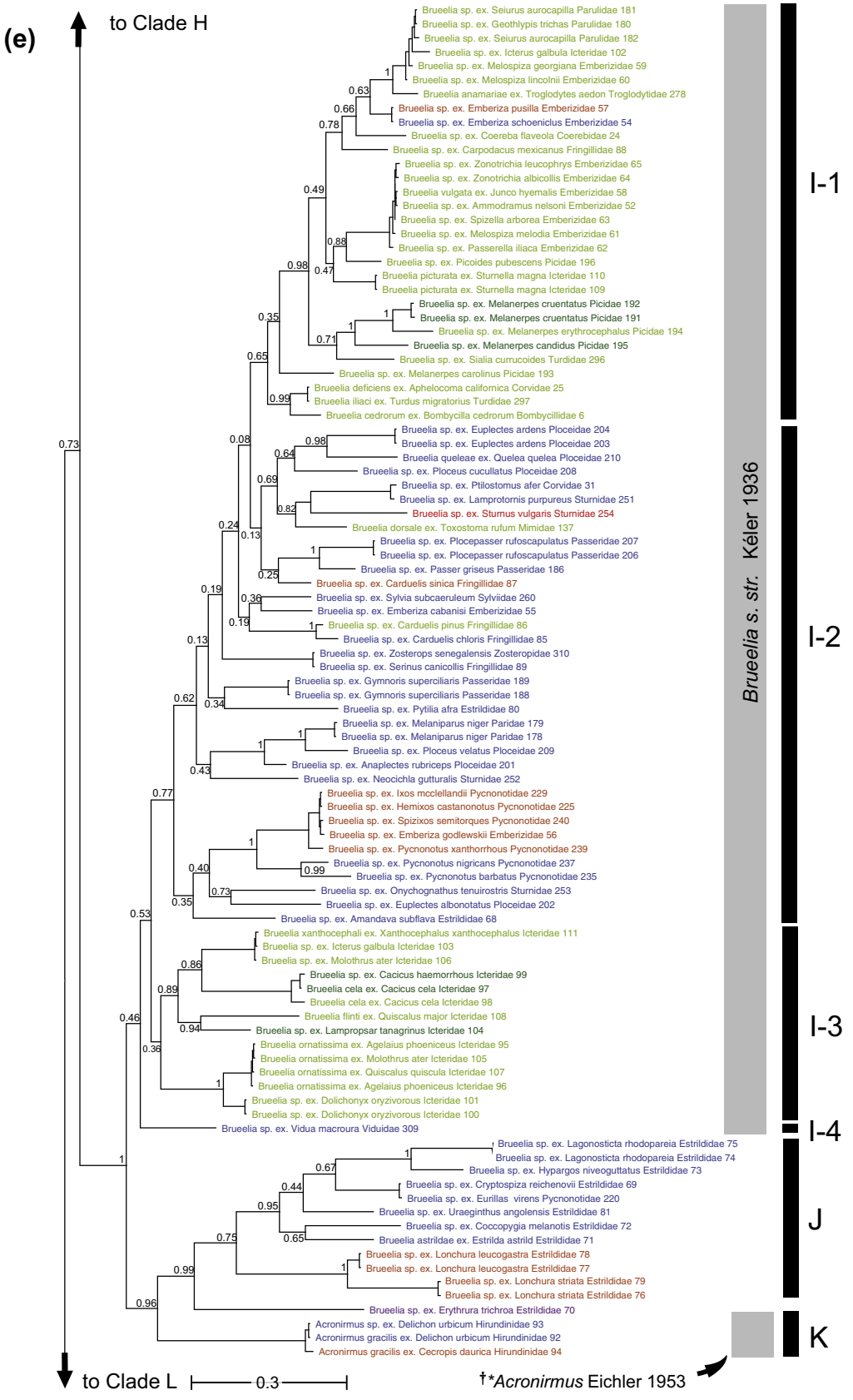

Fig. 3 (*continued*)

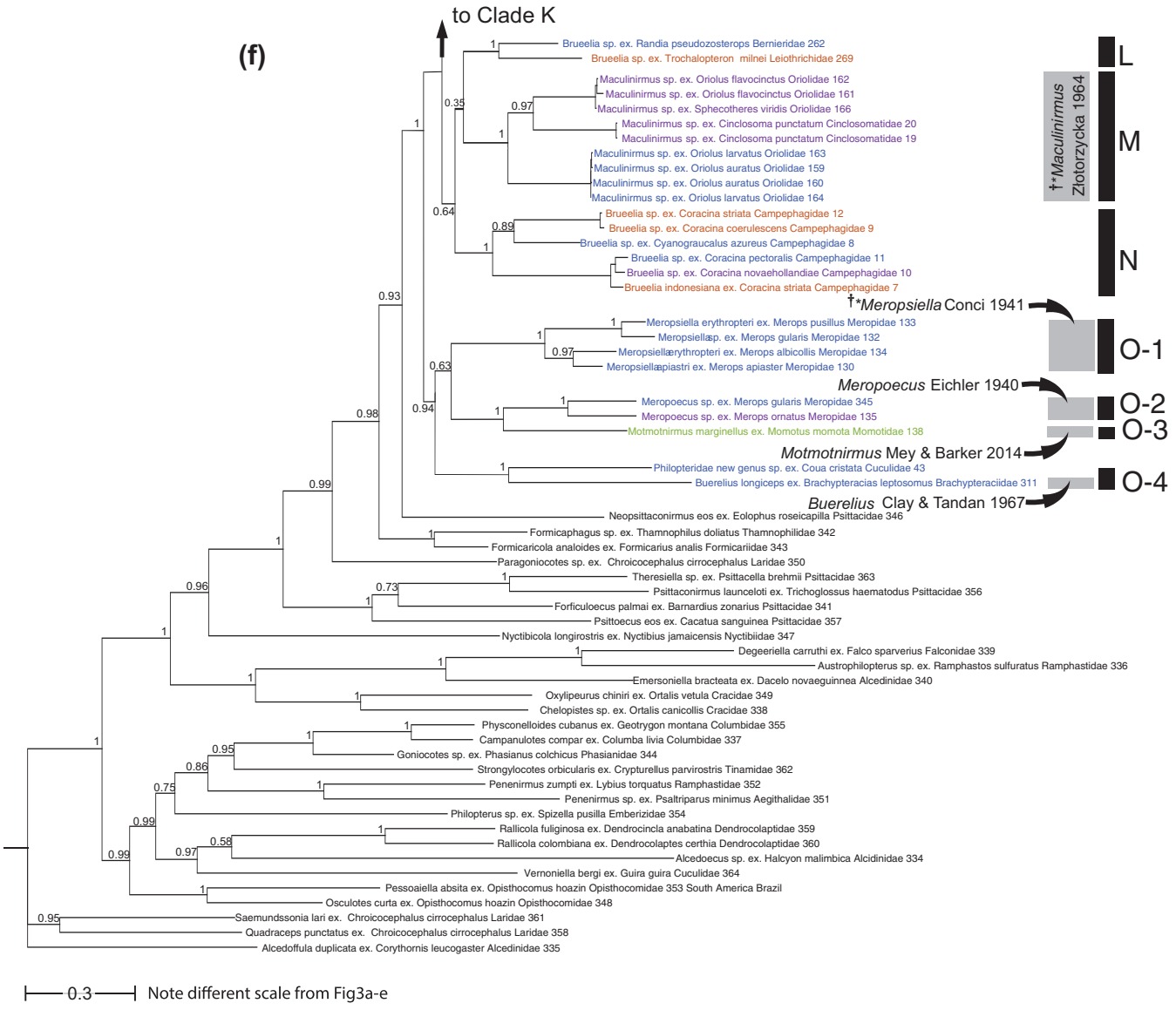

Fig. 3 (*continued*)

collections of fresh, sequenceable, material of described *Corvonirmus* spp. will greatly improve our understanding of how *Corvonirmus* is related to other genera within the *Brueelia* complex, and whether the resurrection of this genus is warranted.

Clade H is a poorly supported clade that contains lice from hosts in the orders Passeriformes and Piciformes. Ansari (1947) erected the genus *Traihoriella*, which included lice on toucans and barbets (Ramphastidae). This genus was later considered a synonym of *Brueelia* by Hopkins and Clay (1952). More recently, however, *Traihoriella* was considered a valid genus by Mey and Barker (2014) and Valim and Palma (2015). Lice from toucans form a strongly supported clade. Similarly, lice from most (4 out of 5) of the barbets (*Megalaima*) form a strongly supported clade. Morphologically, lice from toucans and barbets are quite similar, but they can be separated from each other by head shape and abdominal chaetotaxy: lice on toucans have broad, bell-shaped heads, whereas those on barbets are more roundly triangular. Within Clade H, there is also a strongly supported clade of three taxa found on New World Icteridae; these are all lice in the currently recognized genus *Bizarrifrons* Eichler, 1939. Lice in this genus all have an asymmetrical pre-antennal area, which easily separates them from all other taxa included in this study. Other songbird lice in Clade H are from Australian white-winged choughs (*Corcorax melanorhamphos*). Morphologically, these lice are no more similar to *Traihoriella* and *Bizarrifrons* than they are to other lice in Clades A–H. Indeed, lice within Clade H represent two distinct ecomorphs. Unfortunately, there is little support for the basal nodes within Clade H. Greater sampling is needed to resolve the molecular relationships of these morphologically disparate taxa.

3.4. Clade I (Fig. 3e)

Clade I contains 81 samples that belong to *Brueelia sensu stricto*. This clade is found world-wide, with the exception of Australasia, and it is found on 18 families of songbirds (Passeriformes: Bombycillidae, Coerebidae, Corvidae, Emberizidae, Estrilidae, Fringillidae, Icteridae, Mimidae, Paridae, Parulidae, Passeridae, Ploceidae, Pycnonotidae, Sturnidae, Sylviidae, Troglodytidae, Turdidae, Zosteropidae) and on woodpeckers (Piciformes: Picidae). Morphologically, lice in this genus are separated from other lice in the *Brueelia* complex by distinct pre-antennal and chaetotaxy characteristics (Table 1i).

Clade I-1, although not strongly supported by molecular data, is morphologically unified by the presence of a ventral anterior plate located in the feeding canal. This group is largely restricted to the New World, but two taxa were collected in the Old World (#54 and #57), from host species that are known to occur, albeit rarely, in the New World (*Emberiza schoeniclus* and *Emberiza pusilla*) (Lepage, 2015). Lice in this clade occur mostly on related passerine families in the parvorder Passerida (Cracraft and Barker, 2009), but they also occur on the distantly related passerine (*Aphelocoma*: Corvidae) and on woodpeckers in the distantly related order Piciformes.

I-2 is a paraphyletic grade of lice that are largely restricted to Old World hosts in the parvorder Passerida. Two geographic exceptions are lice from Nearactic hosts within Passerida (#86 and #137). Notably, associations of lice with particular host families are not phylogenetically conserved along this part of the tree. The morphology and relationships of these species are generally poorly known, and the majority of the species are undescribed. Poor molecular support of basal nodes with this grade prevents rigorous interpretation of the relationships between these taxa.

Clade I-3 contains lice that form the species group *B. ornatissima* (Cicchino and Castro, 1996). This group is restricted to New World blackbirds (Icteridae). Species in this group are morphologically similar to other *Brueelia* s. str., but are easily recognized by strikingly complex pigmentation patterns. Cicchino and Castro (1996) further divided the *B. ornatissima* species group into "*cela*" and "*amazonae*" subgroups, separated by different pigmentation characteristics. The two well-supported clades with within *B. ornatissima* may reflect this morphological split, but additional morphological work on the undescribed species in this clade is needed to confirm the monophyly of the group and subgroups proposed by Cicchino and Castro (1996).

3.5. Clades J–K

Clade J is a strongly supported clade of lice that are largely restricted to Old World finches (Estrildidae). Clade K is a small, but well supported clade of lice on swallows and martins (Hirundinidae). The sister relationships of Clade I, and Clades J + K, are supported morphologically as well as from the molecular data (Table 1i). Gross differences in morphology are apparent; lice in Clade J generally have a much rounder, almost tear-drop shaped body, whereas lice in Clade K have long, slender forms such as the "wing" lice in Johnson et al. (2012). These two clades most likely represent distinct genera, but additional taxonomic work is needed to formally address the taxonomic status of Clade J. Lice in Clade K belong to the genus *Acronirmus* (Eichler, 1953), which was considered synonymous with *Brueelia* by Price et al. (2003). Lice in this clade were placed in the genus *Hirundiniella* Carriker 1963, (a taxonomic treatment recently cited by Mey (2009), Mey and Barker (2014) and Valim and Palma (2015)) but this name is a junior synonym of *Acronirmus* Eichler 1953. Based on both molecular and morphological data the resurrection of the genus *Acronirmus* is warranted for Clade K.

3.6. Clades L–N

Clades L–N form a monophyletic group that is poorly supported with molecular data, but the taxa are united by similar male genitalic structures (Table 1o). Clade L is a small but strongly supported clade that is further unified by similarities in male genitalia (Table 1p). The relative phylogenetic arrangement of Clades L, M, and N is not well resolved, but morphological similarities suggest that Clades M and N are more closely related. Lice in Clade L have long, slender abdomens (Fig. 2), whereas lice in clades M and N have more ovate body forms reminiscent of the "head" and "generalist" ecomorphs (Fig. 4).

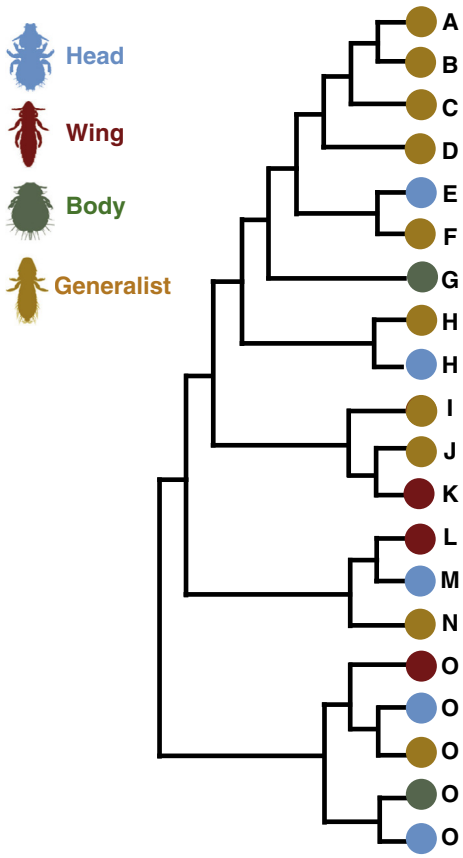

Fig. 4. Distribution of four louse ecomorphs across the *Brueelia*-complex. Head lice have oval bodies with large, triangular heads. The broad temples of head lice support large muscles attached to the mandibles, which enhance their grip, and presumably help these lice to avoid getting dislodged by a scratching host (Clay, 1949). Scratching is the principle host defense against head lice. Other ecomorphs are susceptible to being removed by the bird's bill during preening. Wing lice have long slender bodies, and long legs. They spend most of their time on the large flight feathers of the host's wings or tail, where they insert themselves between adjacent feather barbs to avoid preening (Clayton, 1991; Bush et al., 2006). Body lice have oval bodies and round heads. They live in the abdominal contour feathers, where they avoid preening by dropping between adjacent feathers, or by burrowing into the downy portions of feathers (Clayton, 1991). Generalist lice have intermediate body shapes. They can be found on most regions of the host's body, where they escape from preening by running quickly.

Clade M is a strongly supported clade of lice restricted to Old World orioles (Oriolidae) and quail-thrushes (Cinclosomatidae). These lice have morphologically similar heads and male genitalia (Table 1q). Lice in Clade M are morphologically most similar to the genus *Maculinirmus* Zlotorzycka 1964, which was considered a synonym of *Brueelia* by Ledger (1980). More recently, however, *Maculinirmus* is considered a valid genus by and Mey and Barker (2014) and Valim and Palma (2015). Molecular support for this monophyletic clade suggests that the resurrection of *Maculinirmus* may be warranted.

Clade N is a strongly supported clade of Old World lice restricted to cuckoo-shrikes (Campephagidae). Although lice in this clade are similar in many respects to Clade M, they are morphologically distinct in both head and male genitalic characters (Table 1r) and may represent a new genus.

3.7. Clade O

Clade O is a strongly supported clade of lice that occur on non-passerine hosts. There are no obvious morphological characters that unite the extremely variable taxa in this clade. Four taxa from

bee-eaters form the strongly supported Clade O-1; these lice are representatives of the genus *Meropsiella* Conci 1941a, which was later considered synonymous with *Brueelia* by Hopkins and Clay (1952) and Price et al. (2003). More recently, however, *Meropsiella* has been treated as a valid genus by Mey and Barker (2014) and Valim and Palma (2015). Taxa belonging to the currently recognized genus *Meropoecus* Eichler 1940 form Clade O-2. The single sequenced specimen of *Motmotnirmus* Mey and Barker 2014 (Clade O-3) is sister to *Meropoecus*. Clade O-4 includes the single sequenced specimen from *Buerelius* Clay and Tandan 1967, and an undescribed species from *Coua cristata* (#43), which shares some genitalic similarities with *Buerelius* spp. but it is dissimilar with respect to head characters, abdominal chaetotaxy, and other somatic characters. Although many of the nodes in this clade are strongly supported, additional sampling of lice from of these distinct morphological groups would increase the confidence for interpretations of the evolutionary history among these disparate taxa.

4. Discussion

Our phylogeny supports the monophyly of the *Brueelia*-complex (100% posterior probability, including the currently recognized genera *Brueelia*, *Buerelius*, *Bizarrifrons*, *Sturnidoecus*, and *Meropoecus*) (Clay and Tandan, 1967; Ledger, 1980; Price et al., 2003; Valim and Palma, 2012). Together members of this complex form a larger clade (100% posterior probability) with the genera *Forficuloecus* Conci 1941b, *Formicaricola* Carriker 1957, *Formicaphagus* Carriker 1957, *Neopsittaconirmus* Conci 1942, *Paragoniocotes* Cummings 1916, *Psittaconirmus* Harrison 1915, *Psittoecus* Conci 1942, and *Theresiella* Guimarães 1971. Previous taxonomists have variably considered the genera *Formicaricola* and *Formicaphagus* to be part of the *Brueelia*-complex (Clay and Tandan, 1967; Valim and Palma, 2012, 2015; Mey and Barker, 2014). However, based on our tree it appears that these genera may be nested within a clade of lice associated with parrots and thus are not strongly allied with the remainder of the *Brueelia*-complex. A more thorough molecular and morphological study of the species in these and related genera is necessary to determine the taxonomic limits of the *Brueelia*-complex.

Our phylogeny also indicates that the genus *Brueelia* as recognized by Price et al., 2003 is not monophyletic; the currently recognized genera *Bizzarrifrons*, *Buerelius*, *Meropoecus*, *Motmotnirmus*, and *Sturnidoecus* are firmly nested within *Brueelia* s. lat. (Price et al., 2003). Thus, a monophyletic definition of *Brueelia* would either need to be expanded to include the very distinct morphologies of these genera, or be limited to one or a few of the clades named here. We advocate the latter, as shared-derived morphological characters can be identified for well-supported clades in the molecular phylogeny (Fig. 2, Table 1). Indeed, many of these morphological characters were identified and used by previous taxonomists as generic level characters. Our molecular data suggests that re-elevation of the following genera would be warranted: *Guimaraesiella* Eichler 1949, *Olivinirmus* Zlotorzycka 1964, *Acronirmus* Eichler 1953, *Maculinirmus* Zlotorzycka 1964, *Meropsiella* Conci 1941a. Three undescribed, yet well-supported, clades are also apparent: Clades J, L, and N. In the future, formal alpha taxonomic work will likely lead to the description of specimens in these clades as new genera. For the previously recognized genera *Corvonirmus* Eichler 1944, *Rostrinirmus* Zlotorzycka 1964, and *Traihoriella* Ansari 1947, the molecular data is inconclusive, mainly because of limited taxon sampling of these groups. The sequencing of additional material, as well as a more thorough morphological examination of preserved specimens may clarify our understanding of the phylogenetic relationships of these groups within the *Brueelia*-complex.

The *Brueelia*-complex includes many clades with very different gross morphologies, and many of these clades align with previously recognized genera (Figs. 2 and 3). These lice can be divided into four common "ecomorphs" that specialize on different microhabitats of the host: head lice, wing lice, body lice, and generalist lice (Clay, 1949). These ecomorphs have evolved repeatedly across the order Phthiraptera (Johnson et al., 2012), and our phylogenetic reconstruction indicates that these forms have also evolved repeatedly within the *Brueelia*-complex (Fig. 4). The variation in morphology among these ecomorphs is thought to be associated with the different ways in which these lice escape from host defense (preening) on different microhabitats of the host. Note, however, that the characterization of lice in the *Brueelia*-complex as different ecomorphs is based primarily on gross morphology. More research is needed to confirm whether similar "ecomorphs" do, in fact, behave similarly and use similar microhabitats on the surface of the host. Indeed, studies that examine the microhabitat preferences and behavior of these lice would considerably further our understanding of the evolution of these divergent forms within the *Brueelia*-complex and across the order Phthiraptera.

With 333 ingroup samples and 31 outgroup taxa, this is the largest phylogenetic reconstruction for any group in the order Phthiraptera. Only 73 (22%) of these samples could be assigned to formally described species (37 of them), which is less than 15% of all described species in the *Brueelia*-complex. The remaining 78% are either undescribed species of lice or new host associations for which a formal species assignment could not be conducted in the absence of a complete species level revision of the group. The paucity of described species in this large data set indicates that this group of lice is vastly under-sampled even with the large number of individuals ($n = 333$) included in this study. Alpha-taxonomic work on the thousands of unidentified specimens in museum collections as well as future collecting efforts are likely to reveal many more species in this group of lice.

The genus *Brueelia*, as recognized by Price et al. (2003), is so speciose that there has been no comprehensive revision of this genus. Instead taxonomists have attempted to revise groups of *Brueelia* from related hosts, e.g. *Brueelia* from Picidae (Dalgleish, 1971) and Corvidae (Ansari, 1956, 1957). This practice has been questioned because it assumes lice are specific to a particular host family (Johnson et al., 2002). While this assumption may be true among many genera of lice (Hafner and Nadler, 1990), Johnson et al.'s (2002) study of a small group of lice in the genus *Brueelia* found that some lice within *Brueelia* are shared among distantly related hosts. Indeed, their study showed that "*Brueelia* sp. 1" (Clade A-1) was found on birds in four distantly related passerine families: Muscicapidae, Paridae, Rhipiduridae, and Sittidae. This material was included in our study (specimens #149, 177, 139, and 249 respectively), and we confirm that these specimens are closely related to each other as well as to specimens from several other host species. Our 5% OTU analysis lumps these specimens along with 14 others into one OTU (5% OTU #1, Table 1 in Bush et al., in press) that parasitizes 11 different host families within the order Passeriformes. The bGMYC OTU analysis considers this OTU even less specific and groups lice from 27 host species and 17 host families in a single OTU (bGMYC OTU#114, Table 1 in Bush et al., in press). This species is clearly one of the most extreme cases, as most species in the *Brueelia*-complex are only found on a single host species. Indeed, the percentage of species in the *Brueelia*-complex with only one recorded host species is over 85% in Price et al. (2003). In comparison, the OTU analyses of our data set indicate that 72.3% and 55.3% of the OTUs are found on only one host species for 5% OTU and bGMYC OTU analyses respectively.

Taxonomic revisions of lice that are circumscribed by a host family are also erroneous if lice on related hosts are not closely related. This pattern frequently occurs in the *Brueelia*-complex. For example, lice on jays and crows (Corvidae) occur in four distantly related clades and lice on thrushes (Turdidae) occur in at least eight different clades (Fig. 3). In short, our data indicates that revisionary work on lice in the *Brueelia*-complex cannot be accurately divided into groups based on the host family with which they are associated. Thus, revisionary taxonomy would benefit from a more holistic approach including both molecular and morphological data.

Why is the *Brueelia*-complex so diverse? One argument that has been suggested is that species richness of this group is a taxonomic artifact, generated by a tendency of taxonomist to over-describe species of lice in this group (Johnson et al., 2002). This hypothesis can be addressed by comparing the taxonomic treatments of Price et al. (2003) and the results of the two species delimitation methods (bGMYC and 5% OTU for the 73 taxa that are shared by Price et al. (2003) and our molecular data set. Price et al. (2003) treats these taxa as 37 species with a mean host specificity of 1.49 (1 sd ± 1.07) hosts per louse species. Our bGMYC OTU analysis indicates that these are best delimited as 34 OTUs with a mean host specificity of 1.53 (1 sd ± 1.02) hosts per OTU, and the 5% OTU analysis indicated that these taxa are best delimited as 43 OTUs with a mean host specificity of 1.35 (1 sd ± 0.78) hosts per OTU. Host specificity does not differ significantly among these three taxonomic treatments (Kruskal Wallis, df = 2, $\chi^2 = 0.45$, $P = 0.80$); moreover, all of these taxonomic treatments reflect very high levels of host specificity, all with $\leqslant$1.53 hosts per louse species. Thus, the patterns of diversity and high host specificity without cospeciation, as noted by Johnson et al. (2002), cannot be attributed to over-described species; instead ecological factors should be considered to explain these phenomenon.

What ecological processes are likely to influence the macroevolutionary patterns in the *Brueelia*-complex? Although we provide evidence that alpha-taxonomic work relying on host-family associations can lead to erroneous groupings among taxa in the *Brueelia*-complex (as suggested by Johnson et al. (2002)), we did find a significant correlation between host-family associations and the phylogenetic tree. This is, in part, a matter of scale. Smaller clades within the complex are often restricted to one or a few host families. At this scale, limited dispersal of lice among different host species may lead to cospeciation via shared vicariance events and/or louse adaptations to particular types of hosts may prevent them from establishing successfully on more distantly related hosts. Experiments testing whether lice that are naturally restricted to one host family can establish on "novel" hosts are needed to understand patterns of diversification in this system.

We also found that biogeographic region had significant phylogenetic signal. This pattern is not entirely independent of host-family, as many avian host families are restricted to particular biogeographic regions. It is, however, interesting to note that in most cases where louse OTUs are found on multiple host families, the families are from the same geographic region. This pattern in particular suggests that ecological factors such as variation in dispersal could be very important to some groups within the *Brueelia*-complex. Unlike most other groups of lice, members of the *Brueelia*-complex commonly use phoretic dispersal by attaching to hippoboscid flies (Fig. 1). In fact, 88% of all recorded phoretic events are lice currently recognized as *Brueelia* spp. or *Sturnidoecus* spp. (reviewed by Harbison (2008) and Harbison and Clayton (2011)). Indeed, records of phoresis are known from species of lice associated with clades: A, E, F, G, and I.

Phoresis is a particularly interesting form of horizontal dispersal because hippoboscid flies are very mobile and may visit multiple host species. This provides an opportunity for phoretic lice to move between different host species. In a survey of documented cases, Harbison (2008) found that, within the genus *Brueelia*, species that are phoretic are significantly less host specific than species that are not phoretic (38% of phoretic spp. occurred on more than on host species whereas only 11% of non-phoretic spp. occurred on more than one host species). It is possible that phoretic dispersal is a key innovation that allows lice in the *Brueelia*-complex to disperse to, and then radiate on new host families. This pattern of "escape and radiate co-evolution" (Ehrlich and Raven, 1964) has been described for herbivorous insects that specialize on host plants (Becerra, 1997), but it has not been suggested for ectoparasites. In the future, cophylogenetic comparisons of lice and their hosts are needed to test hypotheses about the nature of coevolution in this system.

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References

Ansari, M.A.R., 1947. Mallophaga (Ischnocera) infesting birds in the Punjab (India). Proc. Nat. Inst. Sci. India 13, 255–303.
Ansari, M.A.R., 1956. A revision of *Brueelia* (Mallophaga) species infesting the Corvidae, Part I. Bull. Brit. Mus. Nat. Hist., London 4, 371–406.
Ansari, M.A.R., 1957. A revision of the *Brueelia* (Mallophaga) species infesting the Corvidae. Part II. Brit. Mus. Nat. Hist., London 5, 145–182.
Becerra, J.X., 1997. Insects on plants: macroevolutionary chemical trends in host use. Science 276, 253–256.
Bueter, C., Weckstein, J.D., Johnson, K.P., Bates, J.M., Gordon, C.E., 2009. Comparative phylogenetic histories of two louse genera found on *Catharus* thrushes and other birds. J. Parasitol. 95, 295–307.
Bush, S.E., Sohn, E., Clayton, D.H., 2006. Ecomorphology of parasite attachment: experiments with feather lice. J. Parasitol. 92, 25–31.
Bush, S.E., Kim, D., Reed, M., Clayton, D.H., 2010. Evolution of cryptic coloration in ectoparasites. Am. Nat. 176, 529–535.
Bush, S.E., Weckstein, J.D., Gustafsson, D.R., Allen, J., DiBlasi, E., Shreve, S.M., Boldt, R., Skeen, H.R., Johnson, K.P., in press. Data supporting a molecular phylogeny of the hyper-diverse genus *Brueelia*. Data in Brief.
Carriker Jr., M.A., 1957. Studies in neotropical Mallophaga. XVI: bird lice of the suborder Ischnocera. Proc. US Nat. Mus. 106, 409–439.
Carriker Jr., M.A., 1963. Neotropical Mallophaga (Insecta) miscellany, No. 13. Rev. Bras. Biol. 23, 293–316.
Cicchino, A.C., 2004. Adición a la revisión de las especies del género *Brueelia* Kéler, 1936 (Phthiraptera: Philopteridae) parásitas de Icterinae. Las especies parásitas del género *Agelaius* Vieillot, 1816 (Aves: Passeriformes: Fringillidae). Bol. Mus. Nac. Hist. Nat. Parag. 15, 66–81.
Cicchino, A.C., Castro, D.C., 1996. Revisión preliminar de las especies del género *Brueelia* Kéler, 1936 (Phthirapter, Philopteridae) parásitas de Icterinae (Aves, Passeriformes, Fringillidae). Graellsia 52, 3–30.
Cicchino, A.C., González-Acuña, D., 2008. Two new species of *Brueelia* (Phthiraptera: Philopteridas s. l.) parasitic on two species of *Phrygilus* (Aves: Emberizidae) from Chile. Neotrop. Entomol. 37, 301–304.
Cicchino, A.C., González-Acuña, D., 2009. A new species of *Brueelia* Kéler (Phthiraptera: Philopteridae) parasitic on the Common Diuca-Finch, *Diuca diuca diuca* (Aves: Emberizidae) in Chile. Neotrop. Entomol. 38, 504–507.

Clay, T., 1936. New species of Mallophaga recorded from Asiatic birds. Proc. Zool. Soc. Lond. 4, 905–914.
Clay, T., 1949. Some problems in the evolution of a group of ectoparasites. Evolution 3, 279–299.
Clay, T., 1951. An introduction to a classification of the avian Ischnocera (Mallophaga): Part I. Trans. Roy. Entomol. Soc. Lond. 102, 171–194.
Clay, T., Tandan, B.K., 1967. A new genus and species of Philopteridae (Mallophaga). Proc. Roy. Entomol. Soc. Lond. B 36, 34–40.
Clayton, D.H., 1991. Coevolution of avian grooming and ectoparasites avoidance. In: Loye, J.E., Zuk, M. (Eds.), Bird Parasite Interactions: Ecology, Evolution and Behavior. Oxford Ornithology Series 2, pp. 259–287.
Clayton, D.H., Drown, D.M., 2001. Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). J. Parasit. 87, 1291–1300.
Clayton, D.H., Johnson, K.P., 2003. Linking coevolutionary history to ecological process: doves and lice. Evolution 57, 2335–2341.
Clayton, D.H., Bush, S.E., Johnson, K.P., 2004. Ecology of congruence: past meets present. Syst. Biol. 53, 165–173.
Clayton, D.H., Bush, S.E., Johnson, K.P., 2015. Coevolution of Life on Hosts: Integrating Ecology and History. University of Chicago Press. Chicago and London (in press).
Clements, J.F., Schulenberg, T.S., Iliff, M.J., Roberson, D., Fredericks, T.A., Sullivan, B. L., Wood, C.L., 2014. The eBird/Clements Checklist of Birds of the World: Version 6.8. Downloaded from http://www.birds.cornell.edu/clementschecklist/download/.
Conci, C., 1941a. Due nuovi generi e tre nuove specie di Mallofagi dei *Meropes*. Boll. Soc. Entomol. Ital. 73, 99–107.
Conci, C., 1941b. Nuovi generi di Mallofagi (nota preliminare). Boll. Soc. Entomol. Ital. 73, 126–127.
Conci, C., 1942. I Mallofagi deglie Psittaciformes. Boll. Soc. Entomol. Ital. 74, 33–41.
Cracraft, J., Barker, K., 2009. Passerine birds (Passeriformes). In: Hedges, S.B., Kumar, S. (Eds.), The Timetree of Life. Oxford Press, pp. 1–10.
Cruickshank, R.H., Johnson, K.P., Smith, V.S., Adams, R.J., Clayton, D.H., Page, R.D.M., 2001. Phylogenetic analysis of partial sequences of Elongation Factor 1*a* identifies major groups of lice (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 19, 202–215.
Cummings, B.F., 1916. New species of lice. Ann. Mag. Nat. Hist. 8, 90–107.
Dalgleish, R.C., 1971. The *Brueelia* (Mallophaga: Ischnocera) of the Picidae (Aves: Piciformes). J. NY Entomol. Soc. 79, 139–146.
Denny, H., 1842. Monographia Anoplurorum Britannie; or, an Essay on the British Species of Parasitic Insects. Henry G. Bohn, London.
Dickinson, E.C., Remsen Jr., J.V., Christidis, L. (Eds.), 2014. The Howard and Moore Complete Checklist of the Birds of the World, fourth ed., vols. 1–2. Aves Press Ltd.
Drummond, A.J., Rambaut, A., 2007. Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214. http://dx.doi.org/10.1186/1471-2148-7-214.
Ehrlich, P.R., Raven, P.H., 1964. Butterflies and plants: a study in coevolution. Evolution 18, 586–608.
Eichler, W., 1940. Notulae Mallophagologicae. IV. Neue Gattungen und höhere Einheiten von Kletterfederlingen. Zool. Anz. 130, 97–130.
Eichler, W., 1944. Notulae Mallophagologicae. XI. Acht neue Gattungen der Nirmi und Docophori. Stett. Entomol. Zeit. 105, 80–82.
Eichler, W., 1949. Phthirapterorum nova genera. Boll. Soc. Entomol. Ital. 79, 11–13.
Eichler, W., 1953. Von Alexander Koenig gesammelte Federlinge. I. Von Singvögeln und Spechten. Bonn. Zool. Beitr. 4, 333–343.
Guimarães, L.R., 1971. A new genus and two new species of parrot Mallophaga (Philopteridae) from New Guinea. Pap. Avol. Zool., São Paolo 24, 213–219.
Hafner, M.S., Nadler, S.A., 1990. Cospeciation in host-parasite assemblages: comparative analysis of rates of evolution and timing of cospeciation events. Syst. Biol. 39, 192–204.
Harbison, C.W., 2008. Ecology and Evolution of Transmission in Feather-feeding Lice (Phthiraptera: Ischnocera). University of Utah, Dissertation, Department of Biology.
Harbison, C.W., Clayton, D.H., 2011. Community interactions govern host-switching with implications for host–parasite coevolutionary history. Proceed. Nat. Acad. Sci. 108, 9525–9529.
Harrison, L., 1915. On a new family and five new genera of Mallophaga. Parasitology 7, 383–407.
Hopkins, G.H.E., Clay, T., 1952. A check list of the genera and species of mallophaga. Brit. Mus. Nat. Hist., London.
Huelsenbeck, J.P., Bollback, J.P., 2001. Empirical and hierarchical Bayesian estimation of ancestral states. Syst. Biol. 50, 351–366.
Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Johnson, K.P., Moyle, R.G., Witt, C.C., Faucett, R.C., Weckstein, J.D., 2001a. Phylogenetic relationships in the louse genus *Penenirmus* based on nuclear (EF-1α) and mitochondrial (COI) DNA segments. Syst. Entomol. 26, 491–497.
Johnson, K.P., Adams, R.J., Clayton, D.H., 2001b. Molecular systematics of Goniodidae (Insecta: Phthiraptera). J. Parasitol. 87, 862–869.
Johnson, K.P., Adams, R.J., Clayton, D.H., 2002a. The phylogeny of the louse genus *Brueelia* does not reflect host phylogeny. Biol. J. Linn. Soc. 77, 233–247. http://dx.doi.org/10.1046/j.1095-8312.2002.00107.x.
Johnson, K.P., Williams, B.L., Drown, D.M., Adams, R.J., Clayton, D.H., 2002b. The population genetics of host specificity: genetic differentiation in dove lice (Insecta: Phthiraptera). Mol. Ecol. 11, 25–38.
Johnson, K.P., Weckstein, J.D., Witt, C.C., Faucett, R.C., Moyle, R.G., 2002c. The perils of using host relationships in parasite taxonomy: phylogeny of the *Degeeriella* complex. Mol. Phylogenet. Evol. 23, 150–157.
Johnson, K.P., Cruickshank, R.H., Adams, R.J., Smith, V.S., Page, R.D.M., Clayton, D.H., 2003. Dramatically elevated rate of mitochondrial substitution in lice (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 26, 231–242.
Johnson, K.P., Yoshizawa, K., Smith, V.S., 2004. Multiple origins of parasitism in lice. Proc. R. Soc. Lond. B 271, 1771–1776. http://dx.doi.org/10.1098/rspb.2004.2798.
Johnson, K.P., Reed, D.L., Hammond-Parker, S.L., Kim, D., Clayton, D.H., 2007. Phylogenetic analysis of nuclear and mitochondrial genes supports species groups for *Columbicola* (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 45, 506–518.
Johnson, K.P., Shreve, S.M., Smith, V.S., 2012. Repeated adaptive divergence of microhabitat specialization in avian feather lice. BMC Biol. 10, 52. http://dx.doi.org/10.1186/1741-7007-10-52.
Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.
Lanfear, R., Calcott, B., Kainer, D., Mayer, C., Stamatakis, A., 2014. Selecting optimal partitioning schemes for phylogenomic datasets. BMC Evol. Biol. 14, 82.
Ledger, J. 1980. The Arthropod Parasites of Vertebrates in Africa South of the Sahara, vol. IV. Phthiraptera (Insecta).
Lepage, D., 2015. Avibase: The World Bird Database. Bird Studies Canada.
MacIntyre, G.T., 1967. Foramen pseudovale and quasi-mammals. Evolution 21, 834–841.
Maddison, W.P., Slatkin, M., 1991. Null models for the number of evolutionary steps in a character on a phylogenetic tree. Evolution 45, 1184–1197.
Mayr, E., 1982. The Growth of Biological Thought: Diversity, Evolution, and Inheritance. Harvard University Press.
McCarthy, M., 2012. World's greatest birdwatcher sets a new record – then hangs up his binoculars. The Independent [London] October 15, 2012.
Mey, E., 2009. Zweiter europäischer Nachweis von Coloceras tovornikae Tendeiro (Phthiraptera, Ischnocera) und weitere Federlingsbefunde bei Pskow (Russland). Entomologische Nachrichten und Berichte 53, 169–180.
Mey, E., Barker, S.C., 2014. Eine neue auf den Feenvögeln (Irenidae) lebende Brueelia-Art (Insecta, Phthiraptera, Ischnocera, Philopteridae), nebst Anmerkungen zur Gattung Brueelia Kéler, 1936 sensu lato. Rudolstädter Nat. Hist. Schr. 19, 73–114.
Najer, T., Sychra, O., Literák, I., Procházka, P., Capek, M., Koubek, P., 2012a. Chewing lice (Phthiraptera) from wild birds in Senegal, with descriptions of three new species of the genera *Brueelia* and *Philopteroides*. Acta Parasitol. 57, 90–98.
Najer, T., Sychra, O., Hung, N.M., Capek, M., Podzemny, P., Literak, I., 2012b. New species and new records of chewing lice (Phthiraptera: Amblycera and Ischnocera) from bulbuls (Passeriformes: Pycnonotidae) in Vietnam. Zootaxa 3357, 37–48.
Najer, T., Sychra, O., Hung, N.M., Capek, M., Podzemny, P., Literak, I., 2012c. Chewing lice (Phthiraptera: Amblycera, Ischnocera) from wild passerines (Aves: Passeriformes) in northern Vietnam, with descriptions of three new species. Zootaxa 3530, 59–73.
Najer, T., Sychra, O., Kounek, F., Papousek, I., Hung, N.M., 2014. Chewing lice (Phthiraptera: Amblycera and Ischnocera) from wild birds in southern Vietnam, with descriptions of two new species. Zootaxa 3755, 419–433.
Olson, D.M. et al., 2001. Terrestrial ecoregions of the world: a new map of life on earth a new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. Bioscience 51 (11), 933–938.
Palma, R.L., 1978. Slide-mounting of lice: a detailed description of the Canada balsam technique. N. Zeal. Entomol. 6, 432–436.
Price, R.D., Johnson, K.P., 2009. Five new species of *Myrsidea* Waterston (Phthiraptera: Menoponidae) from tanagers (Passeriformes: Thraupidae) in Panama. Zootaxa 2200, 61–68.
Price, R.D., Hellenthal, R.A. Palma, R.L., Johnson, K.P., Clayton, D.H., 2003. The Chewing Lice. World Checklist and Biological Overview. Illinois Natural History Survey.
Price, R.D., Johnson, K.P., Palma, R.L., 2008. A review of the genus *Forficuloecus* Conci (Phthiraptera: Philopteridae) from parrots (Psittaciformes: Psittacidae), with descriptions of four new species. Zootaxa 1859, 49–62.
Reid, N.M., Carstens, B.C., 2012. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. BMC Evol. Biol. 12, 196. http://dx.doi.org/10.1186/1471-2148-12-196.
Rékási, J., Saxena, A.K., 2005. A new Phthiraptera species (Philopteridae) from the Red Avadavat (*Amandava amandava*). Aquila 112, 87–93.
Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Ronquist, F., Teslenko, M., Mark, P.V.D., Ayres, D.L., Darling, A., Hohana, S.H., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. Syst. Biol. 61, 1–4.
Sangster, G., Luksenburg, J.A., 2015. Declining rates of species described per taxonomist: slowdown of progress or a side-effect of improved quality in taxonomy? Syst. Biol. 64, 144–151.
Schloss, P.D., Westcott, S.L., Rayabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakely, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing

and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.
Smith, V.S., Page, R.D.M., Johnson, K.P., 2004. 2004 Data incongruence and the problem of avian louse phylogeny. Zool. Scr. 30, 239–259. http://dx.doi.org/10.1111/j.0300-3256..00149.x.
Smith, V.S., Ford, T., Johnson, K.P., Johnson, P.C.D., Yoshizawa, K., Light, J.E., 2011. Multiple lineages of lice pass through the K-Pg boundary. Biol. Lett. 7, 782–785.
Sychra, O., Literák, I., Hung, N.M., Podzemny, P., 2009. Chewing lice from wild passerines (Aves, Passeriformes) from Vietnam, with description of a new species of the genus *Brueelia* (Phthiraptera, Ischnocera, Philopteridae). Acta Parasitol. 54, 154–157.
Sychra, O., Literák, I., Najer, T., Capek, M., Koubek, P., Procházka, P., 2010a. Chewing lice (Insecta: Phthiraptera) from estrildid finches (Aves: Passeriformes: Estrildidae) and louse flies (Insecta: Diptera: Hippoboscidae) from birds in Senegal, with descriptions of three new species of the genus *Brueelia*. Zootaxa 2714, 59–68.
Sychra, O., Barlev, E., Literák, I., Capek, M., Koubek, P., Procházka, P., 2010b. The chewing lice (Phthiraptera) of Red-billed Quelea (*Quelea quelea*) in Senegal, with a description of a new species. Afr. Entomol. 18, 17–22.
Valim, M.P., Palma, R.L., 2006. A new species of *Brueelia* Kéler, 1936 (Phthiraptera: ischnocera: Philopteridae) from the blue-back grassquit (Aves: Passeriformes: Emberizidae) in Brazil. Zootaxa 1153, 27–32.
Valim, M.P., Palma, R.L., 2012. Redescriptions of two species and descriptions of three new species of the louse genus *Bizarrifrons* Eichler, 1938 (Phthiraptera: Ischnocera: Philopteridae). Zootaxa 3273, 28–50.
Valim, M.P., Palma, R.L., 2015. A new genus and two new species of feather lice (Phthiraptera: Ischnocera: Philopteridae) from New Zealand endemic passerines (Aves: Passeriformes). Zootaxa 3926, 480–498.
Valim, M.P., Silveira, L.F., 2014. A new species and five new records of chewing lice (Insecta: Phthiraptera: Ischnocera) from an isolated population of the solitary tinamou *Tinamus solitarius* (Aves: Tinamiformes). Zootaxa 3838 (1), 127–142.
Valim, M.P., Weckstein, J.D., 2011. Two new species of *Brueelia* Kéler, 1936 (Ischnocera, Philopteridae) parasitic on Neotropical trogons (Aves, Trogoniformes). ZooKeys 128, 1–13.
Weckstein, J.D., 2004. Biogeography explains cophylogenetic patterns in toucan chewing lice. Syst. Biol. 53, 154–164.
Zlotorzycka, J., 1964. Mallophaga parasitizing Passeriformes and Pici. II. Brueeliinae. Acta Parasitologica Polonica 12, 239–282.