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**Cophylogenetic analysis of New World ground-doves (Aves: Columbidae) and their
parasitic wing lice (Insecta: Phthiraptera: *Columbicola*)**

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25 **ABSTRACT**

26 Hosts-parasite interactions are plentiful and diverse, and understanding the
27 patterns of these interactions can provide great insight into the evolutionary history of the
28 organisms involved. Estimating the phylogenetic relationships of a group of parasites and
29 comparing them to that of their hosts can indicate how factors such as host or parasite life
30 history, biogeography, or climate affect evolutionary patterns. In this study we compare
31 the phylogeny generated for a clade of parasitic chewing lice (Insecta: Phthiraptera)
32 within the genus *Columbicola* to that of their hosts, the small New World ground-doves
33 (Aves: Columbidae). We sampled lice from the majority of host species, including
34 samples from multiple geographic locations. From these samples we sequenced
35 mitochondrial and nuclear loci for the lice, and used these data to estimate phylogenetic
36 trees and population networks. After estimating the appropriate number of operational
37 taxonomic units (OTUs) for the lice, we used cophylogenetic analyses to compare the
38 louse phylogeny to an existing host phylogeny. Our phylogenetic analysis recovered
39 significant structure within the louse clade, including evidence for potentially cryptic
40 species. All cophylogenetic analyses indicated an overall congruence between the host
41 and parasite trees. However, we only recovered a single cospeciation event. This finding
42 suggests that certain branches in the trees are driving the signal of congruence. In
43 particular, lice with the highest levels of congruence are associated with high Andean
44 species of ground-doves that are well separated altitudinally from other related taxa.
45 Other host-parasite associations are not as congruent, and these often involved
46 widespread louse taxa. These widespread lice did, however, have significant

phylogeographic structure, and their phylogenetic relationships are perhaps best explained by biogeographic patterns. Overall these results indicate that both host phylogeny and biogeography can be simultaneously important in influence the patterns of diversification of parasites.

Key words: cophylogenetic analysis, cospeciation, phylogeny, wing lice, doves

1. Introduction

Parasites and their hosts form intricate and often complex evolutionary relationships. Untangling the narrative of how hosts and parasites interact and what factors are important for shaping their evolutionary patterns is a challenging task. Many parasites utilize multiple unrelated hosts, or are associated with different hosts during different life stages (Morand et al., 1995; Bartholomew et al., 1997; Parker et al., 2003). Hosts also often harbor multiple types of closely related parasites (Poulin, 1997; Bruydonckx et al., 2009; Prugnolle et al., 2010; Colinet et al., 2013). However, reconstructing the evolutionary history of these interactions can provide novel biological insight (Poulin, 2011). Comparing the evolutionary trees of hosts and associated parasites is a way to test for factors influencing joint patterns of host and parasite diversification (Page, 2003). Congruence between host and parasite trees indicates that cospeciation may be important, suggesting parasites are strongly associated with their hosts, whereas incongruence is a sign of host switching or other cophylogenetic events (e.g. parasite duplication; Page, 1994). The cophylogenetic patterns revealed by these comparisons can also provide a starting point for testing hypotheses about what biotic and/or abiotic factors dictate the observed patterns of congruence. For example, host habitat (Krasnov et al., 1997), host/parasite behavior (Clayton et al., 2010), biogeography (Weckstein, 2004), and climate (Feder et al., 1993) could all play important roles in shaping an interaction.

Targeting host-parasite systems where the parasites have permanent and obligate relationships to a group of hosts can simplify the “untangling” process (Fahrenholz, 1913; Eichler, 1948; Hafner and Page, 1995). Ectoparasitic feather lice (Insecta:

Ischnocera) of pigeons and doves (Aves: Columbidae) (hereafter referred to only as “doves”) are an ideal system for this purpose (Johnson and Clayton, 2004). Dove lice are widespread, fairly host specific (an average of 1.7 louse species per dove host taxon), and spend their entire life cycle on the host (Price et al., 2003; Marshall, 1981). Doves are host to two types of distantly related feather lice – wing lice and body lice. Dove wing lice are in a single genus (*Columbicola*), whereas dove body lice are in multiple genera (*Auricotes*, *Coloceras*, *Campanulotes*, *Kodocephalon*, and *Physconelloides*). Although both types of lice parasitize the same group of hosts and are often found together on a single individual, previous analysis indicates the two groups have different evolutionary histories with their hosts (Clayton and Johnson, 2003; Johnson and Clayton, 2004; Johnson et al., 2002). Body lice showed strong phylogenetic congruence with their hosts, which is expected for host-specific parasites and implies cospeciation between the two groups of organisms. Wing lice did not exhibit a similar patterns of congruence, instead showing evidence of multiple host-switches between dove hosts. More frequent host switching may be due to wing lice using phoresis (“hitchhiking”) behavior with generalist parasitic hippoboscids to move between host species (Harbison et al., 2008; Harbison and Clayton, 2011).

However, these initial cophylogenetic studies had limited taxonomic and geographic sampling. A recent study with a more extensive taxonomic representation of both doves and their lice showed different patterns, with wing lice showing strong evidence for overall phylogenetic congruence with their hosts (Sweet et al., 2016). These results imply that taxonomic and geographic scale of sampling could greatly affect the results of a cophylogenetic study. Clade-limited host switching, when parasites

preferentially switch among closely related hosts, can also mislead results by producing a false signal of phylogenetic congruence (Charleston and Robertson, 2002; Sorenson et al., 2004; de Vienne et al., 2007). In consideration of these issues, it is therefore important to study systems with as complete a taxonomic representation as possible. Since this may be less feasible for higher taxonomic groupings (e.g. the over 300 species of Columbidae), it is necessary to focus on cophylogenetic patterns in specific clades in order to obtain near complete taxonomic sampling. Targeting a specific clade of hosts and their parasites, with comprehensive sampling of multiple individuals per taxon, also provides the opportunity to sample from multiple host populations in different geographic locations.

To this end, we focus on the cophylogenetic patterns between small New World ground-doves and their wing lice (*Columbicola*). Small New World ground-doves are a clade of four genera (*Claravis*, *Columbina*, *Metriopelia*, and *Uropelia*) and 17 species within Columbidae (Johnson and Clayton, 2000; Shapiro et al., 2002; Pereira et al., 2007). Representatives of the clade are small-bodied birds that primarily forage on grass seeds and prefer open scrubby habitat, although species in the genus *Claravis* are found in forested areas (Goodwin, 1983; Gibbs et al., 2003). The group has a broad geographic distribution, extending throughout the southern United States, Central America, and most of South America, although many species have more localized ranges. Four species of *Columbicola* are known to parasitize small New World ground-doves (*C. passerinae*, *C. altamimiae*, *C. drowni*, and *C. gymnopelia*). These lice form a monophyletic group within *Columbicola* (Johnson et al., 2007). However, previous phylogenetic studies on *Columbicola* have detected additional lineages within the ground-dove wing lice clade,

perhaps indicative of cryptic species (Johnson et al., 2002; Johnson et al., 2007). Parasites often have simplified morphological features, which make cryptic species a relatively common phenomenon (Poulin and Morand, 2000; Jousson et al., 2000; Lafferty and Kuris, 2002; Miura et al., 2005; Detwiler et al., 2010). For cophylogenetic analyses it is important to properly identify parasite species, as misrepresenting the number of tips on a parasite (or host) tree can alter the outcome of an analysis (Refrégier et al., 2008; de Vienne et al., 2013; Martinez-Aquino, 2016).

In this study, we use mitochondrial and nuclear data from multiple geographic representatives of each species of small New World ground-dove *Columbicola* to infer a robust phylogeny of the clade. Based on our phylogenetic analysis we identify potential cryptic lineages/species in this group, and use these results to aid us in providing an adequate parasite species tree for subsequent cophylogenetic analyses. We compare this tree to a published tree for their hosts. We also explore the phylogeographic patterns of ground-dove *Columbicola*, particularly focusing on the widespread species *C. passerinae*. We use this as a basis to test whether the ground-dove *Columbicola* phylogeny is significantly structured according to host biogeography rather than to host phylogeny.

2. Methods

2.1. Data collection

Louse specimens were collected from hosts using fumigation or pyrethrin powdering protocols (Clayton and Drown, 2001), then immediately placed in 95% ethanol and stored long-term at -80 C. We extracted DNA from individual lice using a Qiagen Blood and Tissue Kit (Qiagen, Valencia, CA, USA) according to standard

protocol and modified according to Johnson et al. (2003), with lice incubating in digestion buffer at 55 °C for ~48 hours. After DNA extraction all lice exoskeletons were slide-mounted and saved as voucher specimens. Using polymerase chain reaction (PCR), we targeted the mitochondrial locus cytochrome oxidase subunit 1 (CO1) and the nuclear loci elongation factor 1 α (EF-1 α), transmembrane emp24 domain-containing protein 6 (TMEDE6), and a hypothetical protein (HYP). For PCR reactions, we used NEB 5X Master Mix (New England Biolabs, Ipswich, MA, USA) and the manufacturer's protocol for 25 μ L reactions (5 μ L 5X Master Mix, 0.2 μ M forward and reverse primers). We used primers H7005 and L6625 for CO1 (Hafner et al., 1994), Ef1 and Cho-10 for EF-1 α (Danforth and Ji, 1998), BR69-295L and BR69-429R for TMEDE6, and BR50-181L and BR50-621R for HYP (Sweet et al., 2014). Our thermal cycler protocols followed Johnson et al. (2001) and Sweet et al. (2014). We purified the resulting PCR products with ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA, USA) according to standard protocol, and sequenced them using an ABI Prism BigDye Terminator kit (Applied Biosystems, Foster City, CA, USA). Fragments were then run on an AB 3730x capillary sequencer (Applied Biosystems, Foster City, CA, USA) at the University of Illinois Roy J. Carver Biotechnology Center (Champaign, IL, USA). We used Geneious v.8.1.2 (Biomatters) to manually resolve the resulting complementary chromatograms and remove primer sequences. We submitted all novel sequences to GenBank (Table 1). We also utilized existing GenBank data for COI and EF-1 α generated in Johnson et al. (2007) and Johnson et al. (2002). For an outgroup taxon, we included sequence data from the rock pigeon wing louse *C. columbae*. In total, we sequenced 51 *Columbicola* samples from 13 host species.

2.2. Phylogenetic analysis

We aligned each locus using default gap parameters in the Geneious MUSCLE plugin (Edgar, 2004), and checked each alignment by eye in Geneious. We concatenated all four alignments, and used PartitionFinder v.1.1.1. (Lanfear, 2012) to search for the most appropriate gene partitions and substitution models under the corrected Akaike Information Criterion (AICc, Sugiura, 1978). We ran two PartitionFinder searches, one searching through all 56 models in PartitionFinder and another only searching through models applicable in MrBayes.

We then ran both maximum likelihood (ML) and Bayesian phylogenetic reconstruction methods. For ML, we ran a partitioned analysis in Garli v.2.0. Based on our PartitionFinder results, we applied a TVM+G substitution model to COI, a TrN+I+G model to HYP, and a TrNef+G model to an EF-1 α /TMEDE6 partition. We ran Garli using two searches of 500 bootstrap replicates, and summarized resulting bootstrap trees using Sumtrees v.3.3.1 (Sukumaran and Holder, 2008). For the Bayesian analysis, we used MrBayes v.3.2 (Ronquist and Huelsenbeck, 2003). Based on our MrBayes-specific model search, we applied a GTR+G model to COI, a K80 model to EF-1 α , and a GTR+I+G model to a HYP/TMEDE6 partition. We ran 20 million generations of Markov Chain Monte Carlo (MCMC) for 2 runs of 4 chains each, sampling every 1,000 trees. To assess parameter convergence we viewed trace files in Tracer v.1.5 (Rambaut and Drummond, 2007), and ran .t files with the R package RWTY v.1.0.0 (Warren et al., 2016) to assess topological congruence. Based on these assessments, we discarded the first 10% (2,000 trees) as a burnin.

2.3. OTU analysis

We used several methods for identifying potential cryptic lineages/species. First, we computed uncorrected pairwise distances for the COI sequences. We computed the distance matrix using the “dist.dna” command in the *ape* package (Paradis et al., 2004) in R (R Development Core Team 2015). Second, we used the COI sequences to infer a median-joining (MJ) network (Bandelt et al., 1999) in PopART v.1.7 (Leigh and Bryant, 2015). We set epsilon = 0 and only included sequence data for *Columbicola passerinae*, since previous studies have identified this species as potentially harboring multiple cryptic lineages (Johnson et al., 2007). Third, we used the online version of the Automatic Barcode Gap Discovery (ABGD) method (<http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html>, Puillandre et al., 2012) to partition the number of possible groups in our data set. We ran the ABGD analysis with the COI alignment applying both K2P and Jukes-Cantor (JC) distance models, and used default Pmin, Pmax, gap width, and steps values. Finally, we used the Generalized Mixed Yule Coalescent (GMYC) approach to species delimitation (Fujisawa and Barraclough, 2013). Since this approach requires ultrametric and bifurcating gene trees, we estimated the gene tree phylogeny of COI using BEAST v.1.8.2 (Drummond et al., 2012). We ran the MCMC analysis for 20 million generations using a GTR+G substitution model, an uncorrelated lognormal relaxed clock, and a Yule speciation processes tree prior. We sampled trees every 1,000 generations, and discarded the first 10% as a burn-in based on plots and ESS values from Tracer. From the post-burn-in tree samples we constructed a maximum clade credibility (MCC) tree using TreeAnnotator. With the MCC COI tree as

an input, we implemented GMYC in the R package *splits* (Ezard et al., 2009) using the single threshold setting.

2.4. Cophylogenetic analysis

Using our small New World ground-dove *Columbicola* phylogeny (see Figure 1) and information from our species/lineage analyses, we conducted both event-based and distance-based cophylogenetic analyses. For all methods, we used the host phylogeny generated by Sweet and Johnson (2015). We also pruned the louse phylogeny so that each species was represented by a single tip, and removed the outgroup taxon.

For the distance-based cophylogenetic analyses, we used ParaFit (Legendre et al., 2002) and PACo (Balbuena et al., 2013). Both methods assess overall congruence between the host and parasite phylogenies, as well as the relative contribution of individual host-parasite links (associations) to the overall congruence. However, ParaFit assesses whether or not parasites are randomly associated with their hosts, whereas PACo assess the dependence of the parasite phylogeny on the host phylogeny through a residual sum of square goodness-of-fit test. For both analyses, we converted the host and parasite phylogenies to patristic distance matrices using the “cophenetic” command in *ape*, and sorted each distance matrix according to the host-parasite association matrix. We ran ParaFit for 100,000 permutations in the R package *ape* using the Cailliez correction for negative eigenvalues and testing for the contribution of each individual link using the ParaFitLink1 test. Since ParaFit runs multiple tests to calculate p-values for each individual link, it is necessary to correct the raw output. Using R, we corrected individual link p-values using the Benjamini-Hochberg correction for false discovery rate

(Benjamini and Hochberg, 1995). We also ran PACo for 100,000 permutations with the R packages *ape* and *vegan* (Oksanen et al., 2016), and used the jackknife method to estimate the importance of each individual link to the overall sum of squares score.

For an event-based approach, we used Jane v4 (Conow et al., 2010). We used default settings for the Genetic Algorithm parameters (100 generations, population size of 100) and event costs (0 cospeciation, 1 duplication, 2 duplication and host switch, 1 loss, and 1 failure to diverge). After solving for the most optimal solutions, we tested whether our best score was lower than expected by chance by randomizing the tip mappings 999 times. If the randomization procedure indicates our best score from the data is lower than by chance, this would indicate some level of congruence between the host and parasite phylogenies.

2.5. Testing for biogeographic structure

To test if our inferred louse phylogeny is significantly structured according to host biogeography, we used the Maddison-Slatkin test (Maddison and Slatkin, 1991). We coded lice as being associated with hosts in one of the following regions: southern United States/northern Central America (north of the Isthmus of Tehuantepec), southern Central America (south of the Isthmus of Tehuantepec), Andes Mountains, South America west of the Andes (trans-Andes), or South America east of the Andes (cis-Andes). We used the best ML tree as an input, but removed duplicate taxa to avoid biasing our test results. In this context, we considered two louse taxa as duplicate if they were from the same host species, geographic region (according to our coding), and were separated by short branch lengths (uncorrected CO1 distance values $< 0.1\%$), being relatively genetically indistinct.

We also removed the outgroup taxon. We implemented the Maddison-Slatkin procedure using an R script that randomly assigns character states (geographic regions) 999 times and calculates the parsimony score for each assignment (Bush et al., 2016, script from <https://github.com/juliema/publications/tree/master/BrueeliaMS>).

3. RESULTS

3.1. Phylogenetic analysis

We sequenced 378 bp of the CO1 locus (131 variable sites, 115 parsimony-informative sites), 345 bp of EF-1 α (32 variable sites, 15 parsimony-informative sites), 407 bp of HYP (71 variable sites, 26 parsimony-informative sites), and 219 bp of TMEDE6 (28 variable sites, 4 parsimony-informative sites). The final concatenated alignment was 1,349 bp in length, with ~36% missing data (Table 1). The ML and Bayesian phylogenetic analyses inferred similar trees (Figure 1). After the 10% burn-in all parameters and topologies from the Bayesian analysis had ESS values <200 or average standard deviation of split frequencies <0.01, thus indicating the MCMC runs had converged to stationarity. Monophyly of all four *Columbicola* species were recovered with good support from both analyses (>80 bootstrap [BS] and >0.95 posterior probability [PP]).

3.2. OTU analyses

The OTU analyses indicated there are five taxa in this clade of *Columbicola*. This includes the three species found on *Metriopelia* ground-doves – *C. altamimiae*, *C. drowni*, and *C. gymnopeliae*. The uncorrected p-distances had an average distance of

13.43% between samples of *C. altamimiae* and all other ingroup samples, 14.31% between samples of *C. drowni* and all other ingroup samples, and 13.88% between samples of *C. gymnopoliae* and all other ingroup samples (Table S1). The ABGD analysis also recovered those three taxa as separate taxonomic units. The GMYC analysis did not recover these three taxa as separate units, instead grouping all three into a single taxonomic unit. However, GMYC separated them from the fourth species in the clade, *C. passerinae*. All OTU analyses indicated *C. passerinae* should be considered two taxa (here labeled *C. passerinae* 1 and *C. passerinae* 2). Both the ABGD (with both K2P and JC distance models) and GMYC analyses recovered two separate taxonomic units within *C. passerinae*, and those two groups had an average uncorrected p-distance of 9.30% between them. In addition, the median-joining network indicated there are 23 steps between the two *C. passerinae* groups (Figure 2). There was some genetic distinctiveness between the *C. passerinae* parasitizing *Columbina passerina* and *Co. inca* from the United States/Mexico and *Co. talpacoti* from Panama, which together differed by an average of 1.80% in COI. However, the ABGD and GMYC analyses did not recover this group as a distinct unit. Additionally, the MJ network placed lice from *Co. talpacoti* from Panama as embedded within *C. passerinae* 1 (Figure 2).

3.3. Cophylogenetic analysis

Our cophylogenetic analyses based on five *Columbicola* taxa indicated some level of congruence between the host and parasite phylogenies. Both distance-based tests were significant across the entire data set (PACo global $P = 0.002$, ParaFit global $P = 0.003$), thus rejecting the independence of the host and parasite phylogenies. The ParaFitLink1

test recovered five host-parasite links as significantly contributing to the global score after correcting for multiple tests ($\alpha = 0.05$) (Figure 3, Table 2). ParaFit also calculates a second individual link statistic (ParaFitLink2), but ParaFitLink1 is better suited for scenarios with widespread parasites (Legendre et al., 2002, Dhimi et al., 2013, Perez-Escobar et al., 2015 [Supporting information]). The individual jackknife link test in PACo recovered three host-parasite links with the 95% confidence intervals of their squared residuals lower than the median global squared residual (Figure 4). All three links were between *Metriopelia* ground-doves and their lice.

The event-based method of Jane also recovered a global signal of congruence across the whole data set. The observed cost was 27, which was significantly lower than by chance ($P = 0.049$). The event reconstruction recovered one cospeciation event, between *M. melanoptera* and *M. ceciliae* and their lice, *C. drowni* and *C. gymnopeliae* (Figure 3). Jane also recovered three duplications, fifteen losses, nine failures to diverge, and no host switches (Table 3).

3.4. Phylogeographic patterns and biogeographic structure

Lice from *C. passerinae* 1 group neatly according to biogeography in both the phylogeny and MJ network. There are distinct groups of lice from southern United States/northern Central America, southern Central America, and cis-Andean South America (Figures 1 and 2). Lice from *C. passerinae* 2 also tend to group by biogeography, but the patterns are less well-defined than in *C. passerinae* 1. Lice from *Co. cruziana* and *Co. buckleyi*, both restricted to South America's northwest coast, fall within *C. passerinae* 2. However, all lice from *Cl. pretiosa* are also within this lineage,

including samples from Mexico and Brazil. Lice parasitizing *Co. talpacoti* sampled from Brazil also fall within the lineage. In total, three of our four host biogeographic regions are represented in *C. passerinae* 2. However, lice from *C. passerinae* 2 seem to primarily parasitize hosts from trans-Andean South America, given that all lice from this region group with the lineage (Figures 1 and 2).

Randomization of biogeography over the *Columbicola* phylogeny with the Maddison-Slatkin test indicated significant phylogenetic conservation of biogeography. After trimming the louse phylogeny to remove duplicate samples, there were six observed character state transitions (Figure S1). None of the randomizations resulted in equal or fewer state transitions than the observed value ($P < 0.001$).

4. DISCUSSION

Our phylogenetic analyses of ground-dove wing lice (*Columbicola*) revealed patterns of diversification concordant at some level with both host phylogeny and biogeography within this group. In particular, biogeographic distribution, separate from host phylogeny, appears to play a major role in patterns of louse diversification. More specifically, some lineages of lice are associated with multiple distantly related hosts, but these lice tend to occur together in the same biogeographic region.

Some of our phylogenetic results agreed with previous, less extensively sampled studies (Clayton and Johnson, 2003; Johnson et al., 2007). In particular, we recovered two distinct clades within *C. passerinae* (*C. passerinae* 1 and *C. passerinae* 2), and *C. gymnopeliae* + *C. drowni* as sister to *C. passerinae*. We also recovered *C. altamimiae* as sister to the rest of the clade. Increased taxon sampling indicated even more structure

within the clade. We found some evidence for genetic differentiation within *C. passerinae* 1, with lice from *Co. passerina*, *Co. inca*, and *Co. talpacoti* forming a distinct group. However, our OTU analyses and MJ network did not recover these lice as representing a distinct taxon, so this likely represents population level structuring. We recovered *C. drowni* as the sister species to *C. gymnopeliae*, although this relationship was not well supported (53 BS/0.75 PP). All three species of *Metriopelia* doves included in this study have unique species of lice previously described based on morphological data (Eichler, 1953; Clayton and Price, 1999). Each louse species is generally associated with one species of *Metriopelia*. *Columbicola altamimiae* has been recorded on both *M. aymara* (the primary host) and *M. melanoptera* (Price et al., 2003), but we were unable to document this by our sampling. Our study provides further evidence for the genetic distinctiveness of each species of *Metriopelia* wing louse. All three species (*C. altamimiae*, *C. drowni*, and *C. gymnopeliae*) are separated from the rest of the ground-dove wing louse clade by long branches and have large CO1 uncorrected distances between them and other taxa (13.43% - 14.31%).

The phylogenetic structure and host specificity of *Metriopelia* wing lice is perhaps reflective of their hosts' life history. *Metriopelia* doves are high Andean species, living in paramo and puna grasslands generally above 2,000 meters. Due to the high elevation and extreme conditions, these regions have different habitats compared to the neighboring lowlands and cloud forest (Szumik et al., 2012). Inhabitants of these regions are well-adapted to the environment, and have therefore become isolated from other taxa in adjacent regions (Lloyd et al., 2010). This lack of species overlap is especially evident in avian taxa (Fjeldsa et al., 2012). The isolation of *Metriopelia* from other genera of

small New World ground-doves is reflected by the phylogenetic distinctiveness of their wing lice. While all other species of ground-doves share one or two species of wing lice with other lowland ground dove species, *Metriopelia* dove lice are in distinct lineages. Interestingly, we did not recover lice from *Metriopelia* as monophyletic, with lice from *M. aymara* coming out as sister to the rest of the ingroup. This indicates ground-dove *Columbicola* may have originated before major central Andean uplift (16-9 mya; Garzione et al., 2014), when *Metriopelia* likely became isolated from other ground-dove taxa (Sweet and Johnson, 2015).

Our results also indicate significant host-specificity among *Metriopelia* wing lice, consistent with known association records (Price et al., 2003). Each species of *Metriopelia* dove included in this study has a unique species of *Columbicola* associated with it, which indicates the lice have been isolated on their hosts for a long period of time. If this was not the case, we might expect the three louse species to be separated by short branch lengths and smaller COI pairwise distances, or for individual species to occur on multiple host species (i.e. not have fully sorted). All three dove species have range overlap, but have some variation in altitudinal preference. *M. aymara* tends to live at higher altitudes (2,800 - >5,000 meters) than the other two species. This habitat difference could result in hosts rarely coming in contact, therefore isolating their parasites. Host size differences may also be important for explaining the phylogenetic patterns in these lice, particularly in lice from *M. melanoptera* and *M. ceciliae*. Experimental work has indicated that host size differences can limit host switching in dove wing lice (Clayton et al., 2003; Johnson et al., 2005). If a new host is too small or large, wing lice are not able to effectively avoid preening behavior and are thus not able

to establish viable populations. *M. melanoptera* (113-125 grams) are considerably larger than *M. ceciliae* (51-67 grams) (Gibbs et al., 2001).

4.1. Cophylogenetic congruence

All three cophylogenetic analysis methods, including both topology-based and event-based methods, recovered a global signal of congruence between the host and parasite phylogenies. This indicates some level of cospeciation between the two groups of organisms, which might seem surprising given the general patterns of the phylogeny and host associations (Figure 3). These results also differ from earlier cophylogenetic studies on dove wing lice, which indicated a lack of congruence between the two groups (Clayton and Johnson, 2003). However, more recent studies with greater taxonomic representation also recovered evidence of cospeciation between doves and their wing lice (Sweet et al., 2016).

The signal of congruence is probably driven by the *Metriopelia* associations. The only cospeciation event recovered by Jane is between *M. melanoptera* + *M. ceciliae* and their lice, and ParaFit recovered both of those links as significantly contributing to the overall congruence. In the PACo analysis, the three *Metriopelia* host-parasite links had squared residual values that were lower than the other links and 95% confidence intervals below the median squared residual value, which indicates phylogenetic congruence between those taxa. As discussed above, *Metriopelia* doves are generally isolated from other species in the ground-dove clade, and based on the one-to-one relationships with their wing lice are probably generally isolated from one another. Over evolutionary time, a lack of opportunity to switch hosts could not only result in distinct lineages of parasites,

but also in cospeciation with their hosts. Interestingly, removing the *Columbicola passerinae* lineages from the louse phylogeny would result in a perfectly congruent relationship between *Metriopelia* and their wing lice. Furthermore, the basal lineages of the louse phylogeny (*C. altamimiae*) is associated with *M. aymara*, which is nested within the ground-dove phylogeny. This finding indicates ground-dove *Columbicola* possibly switched from ancestral *Metriopelia* doves to other ground-doves before Andean uplift isolated the groups, although Jane did not recover any host switching events. Since *M. aymara* is the basal lineage of *Metriopelia* and is usually found at higher elevations than other *Metriopelia*, it is possible this species first became isolated due to rapid uplift over several million years. This could explain why *C. altamimiae* is the earliest diverging species of ground-dove wing louse.

Conversely, the two lineages of *Columbicola passerinae* do not appear congruent with their hosts. We did not recover any cospeciation events between these lice and their hosts. Most of the individual links did not significantly contribute to the global ParaFit score and had high squared residuals from the PACo jackknife test. Taken together, these results suggest a lack of cospeciation between these two louse lineages and their hosts over evolutionary time. Both lineages are widespread, with *C. passerinae* 1 associated with seven ground-dove species and *C. passerinae* 2 associated with four. This distribution indicates recent host-switching or ongoing gene flow between the louse populations on these different host species. Small New World ground-doves generally prefer open scrubby habitat, and many of the lowland species (non-*Metriopelia*) are known to forage in mixed flocks (Dias 2006). Host proximity could allow for ongoing

gene flow between louse populations on different host species that are geographically proximal.

4.2. Phylogeographic structure of *Columbicola passerinae*

Although most of the ground-dove *Columbicola* phylogeny does not appear to be predicted by the hosts' phylogeny, particularly within *C. passerinae*, there is still significant phylogenetic structure within the clade. Previous work in other host-parasite systems have found that host biogeography, rather than host phylogeny, can be a better predictor of parasite evolutionary patterns (Weckstein, 2004; Johnson et al., 2007; Bush et al., 2016). After assigning host biogeography to the tips of our parasite phylogeny and using the Maddison-Slatkin character randomization test, we found the louse phylogeny is significantly structured according to host biogeography. Given these results and the results of our cophylogenetic analysis, this indicates host biogeography is very important for shaping evolutionary patterns in ground-dove wing lice. A similar pattern was recovered in a broad phylogenetic study of the *Columbicola* genus, which indicates host biogeography is an important factor at both a global and local scale (Johnson et al., 2007). If parasites are able to switch hosts but are limited to a group of similar hosts, as is the case with ground-dove wing lice, then the lice are likely to switch to hosts in close proximity. This could especially be the case in mixed foraging flocks. Since ground-doves are non-migratory and generally do not travel long distances within their ranges, over time the evolutionary patterns of their parasites would diverge according to geographic regions.

We see early indications of genetic divergence from our phylogenetic and network analyses. Ground-dove wing lice form distinct groups based on hosts primarily from southern United States/northern Central America, southern Central America, trans-Andean South America, cis-Andean South America, or Andean highlands. Further evidence for this phylogeographic structure comes from lice that parasitize the same host species falling in different clades, but being grouped with lice from the same geographic region rather than host species. For example, *C. passerinae* from *Co. talpacoti* in Panama form a distinct group, whereas lice off of *Co. talpacoti* from Brazil group with lice from *Cl. pretiosa* also from Brazil. Also, lice from *Co. passerina* from Brazil group with lice from other cis-Andean South American hosts, and *C. passerina* lice sampled from hosts in Mexico/United States group with other lice from that region. Lice from these widespread host species that group according to geographic region are likely from different subspecies of host. Over time, these host subspecies may continue to diverge from each other and show similar conserved phylogeographic structure. Lice have much shorter generation times and faster substitution rates than their hosts, so geographic structure can potentially be more easily detected in lice than in their hosts (Johnson et al. 2014). Future phylogeographic analysis of widespread ground-dove species is needed to explore these patterns.

Columbicola passerinae 2, however, does not conform as strictly to this phylogeographic pattern. Although there is some phylogeographic structure within the lineage, a louse from trans-Andean Peru (ex. *Co. buckleyi*) is imbedded within lice from Brazil and Mexico (ex. *Co. talpacoti* and *Cl. pretiosa*), thus breaking up the conserved structure. The presence of both cis- and trans-Andean representatives within *C.*

passerinae 2 indicates the lice are able to move around or across the Andes. Given the range restrictions of *Co. buckleyi* and *Co. cruziana*, perhaps the lice are using *Cl. pretiosa* as a “bridge species.” *Claravis pretiosa* has a range that extends to both sides of the Andes and can move considerably during certain seasons (Gibbs et al., 2001; Piratelli and Blake, 2006). Although they are more arboreal and tend to forage in pairs, they are also known to forage at forest edges in open scrub close to other ground-dove species, including *Co. cruziana* and *Co. buckleyi* (Skutch, 1959; Parker et al., 1995; pers. obs.). *Claravis pretiosa* are not present in the high Andes, so wing lice in the *Co. passerinae* 2 clade are likely moving north around the Andes and into eastern South America via *Cl. pretiosa*. The lice are then able to switch to other hosts in that region of the continent. Interestingly, *Co. talpacoti* is the only other host species from east of the Andes with lice in *Co. passerinae* 2. It is possible that *C. passerinae* 2 is rare on eastern dove species, and that more thorough sampling will reveal other ground-dove species hosting this wing louse lineage. It is also possible that *C. passerinae* 2 are preferentially switching from *Cl. pretiosa* to *Co. talpacoti*. Alternatively, the existence of *C. passerinae* 2 on *Co. talpacoti* could be a reflection of the recent evolutionary history of this species. *Co. talpacoti* and *Co. buckleyi* are sister taxa that diverged very recently, with *Co. buckleyi* originally considered a subspecies of *Co. talpacoti* (Meyer de Schauensee, 1970). *Columbina talpacoti* likely shared lice with *Co. buckleyi* before the two species diverged, and could have retained this shared lineage until the present. Because wing lice from *Co. buckleyi* are so similar to lice from *Co. talpacoti*, this could be evidence that the two host species still have some contact, which would explain why a louse from *Co. buckleyi* (a trans-Andean species) is parasitized by a cis-Andean louse.

Despite less consistent phylogeographic patterns in *C. passerinae* 2, the fact that there is any conserved phylogeographic structure within *C. passerinae* indicates barriers for dispersal. If the primary mode of host-switching/dispersal is via phoresis using parasitic hippoboscids, it is also possible the flies have limited ranges due to geographical or ecological barriers. Hippoboscids have been recorded from small New World ground-doves throughout the doves' range (Maa, 1969), but if the flies have a restricted range or limited gene flow this would also limit louse dispersal. Additional work focused on the phylogeography of hippoboscids is needed to test whether the flies exhibit similar patterns to the lice.

4.3. Conclusions

By focusing on a small clade of doves and their associated wing lice (also in a monophyletic group), we were able to sample lice from most host species, including multiple louse samples per host species. This approach allowed us to uncover cophylogenetic and phylogeographic patterns that would be obscured in broader-scale studies, thereby further untangling some of the evolutionary history of this host-parasite system. In particular, the results of this study indicate that biogeography and host life-history are important factors for shaping host-parasite evolutionary patterns, particularly for systems involving permanent parasites. Although permanent parasites are tightly tied to their hosts, host phylogeny is rarely the primary predictor of parasite diversification. Identifying what external factors are promoting parasite diversification is crucial for understanding host-parasite interactions.

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TABLES

Table 1. *Columbicola* samples used in this study. Sequence data indicated by the associated GenBank accession numbers. Dashes (-) indicate missing data. Haplotypes for *C. passerinae* samples refer to Figure 2.

<u>Number</u>	<u><i>Columbicola</i> species</u>	<u>Haplotype</u>	<u>Voucher</u>	<u>Host</u>	<u>Locality</u>	<u>Host Voucher</u>	<u>CO1</u>	<u>Efla</u>	<u>HYP</u>	<u>TMEDE6</u>
1	<i>passerinae</i> 2	1	Copsr.9.29.1998.3	<i>Claravis pretiosa</i>	Mexico	CO-23	KX528475	KX528509	-	-
2	<i>passerinae</i> 2	6	Cosp.Clpre.11.24.2014.10	<i>Claravis pretiosa</i>	Brazil	6413	KX528476	-	KX532199	-
3	<i>passerinae</i> 2	4	Cosp.Clpre.11.24.2014.9	<i>Claravis pretiosa</i>	Brazil	5178	KX528477	-	KX532200	-
4	<i>passerinae</i> 2	1	Cosp.Clpre.2.1.1999.2	<i>Claravis pretiosa</i>	Mexico	CO-14	AF414733 ¹	-	-	-
5	<i>passerinae</i> 2	1	Cosp.Clpre.2.1.1999.6	<i>Claravis pretiosa</i>	Mexico	CO-23	KX528478	KX528511	-	-
6	<i>passerinae</i> 2	2	Copas.Cobuc.9.4.2013.10	<i>Columbina buckleyi</i>	Peru	REW169	KX528479	-	KX532192	KX532221
7	<i>passerinae</i> 2	5	Copas.Cobuc.9.4.2013.9	<i>Columbina buckleyi</i>	Peru	REW187	KX528466	-	KX532193	KX532222
8	<i>passerinae</i> 2	2	Cosp.Cobuc.10.27.2003.4	<i>Columbina buckleyi</i>	Peru	REW169	KX528467	-	KX532201	KX532228
9	<i>passerinae</i> 2	2	Cosp.Cobuc.7.27.2004.7	<i>Columbina buckleyi</i>	Peru	REW187	KX528480	KX528512	-	-
10	<i>passerinae</i> 2	2	Cosp.Cocru.10.27.2003.3	<i>Columbina cruziana</i>	Peru	REW86	KX528481	-	KX532202	-
11	<i>passerinae</i> 2	2	Cosp.Cocru.4.9.2014.4	<i>Columbina cruziana</i>	Peru	REW85	KX528482	KX528513	-	KX532229
12	<i>passerinae</i> 2	2	Cosp.Cocru.4.9.2014.5	<i>Columbina cruziana</i>	Peru	REW86	KX528483	KX528514	KX532203	KX532230
13	<i>passerinae</i> 2	2	Cosp.Cocru.7.27.2004.4	<i>Columbina cruziana</i>	Peru	REW87	KX528484	KX528515	-	-
14	<i>passerinae</i> 1	8	Copsr.9.21.1999.3	<i>Columbina inca</i>	USA	I136	AF414727 ¹	-	-	-
15	<i>passerinae</i> 1	8	Copsr.9.29.1998.6	<i>Columbina inca</i>	USA	115	AF414725 ¹	KX528510	-	-
16	<i>passerinae</i> 1	13	Copas.Comin.9.4.2013.1	<i>Columbina minuta</i>	Peru	JLK258	KX528468	-	KX532194	-
17	<i>passerinae</i> 1	15	Copas.Comin.9.4.2013.8	<i>Columbina minuta</i>	Peru	REW201	KX528469	-	KX532195	KX532223
18	<i>passerinae</i> 1	10	Copsr.11.24.2003.1	<i>Columbina passerina</i>	USA	299	KX528473	KX528506	KX532198	KX532227
19	<i>passerinae</i> 1	11	Copsr.2.1.1999.12	<i>Columbina passerina</i>	USA	93, 101	AF414729 ¹	-	-	-
20	<i>passerinae</i> 1	10	Copsr.2.1.1999.3	<i>Columbina passerina</i>	Mexico	CO-9	AF414727 ¹	-	-	-
21	<i>passerinae</i> 1	8	Copsr.9.14.1999.7	<i>Columbina passerina</i>	USA	G181	KX528474	KX528507	-	-

22	<i>passerinae</i> 1	9	Copsr.9.21.1999.1	<i>Columbina passerina</i>	USA	G280	AF414731 ¹	-	-	-
23	<i>passerinae</i> 1	8	Copsr.9.29.1998.2	<i>Columbina passerina</i>	Mexico	CO-11	AF414728 ¹	KX528508	-	-
24	<i>passerinae</i> 1	12	Cosp.Copas.11.24.2014.7	<i>Columbina passerina</i>	Brazil	5049	KX528486	-	KX532204	-
25	<i>passerinae</i> 1	12	Cosp.Copas.11.24.2014.8	<i>Columbina passerina</i>	Brazil	5051	KX528487	-	-	-
26	<i>passerinae</i> 1	12	Copas.Copic.9.4.2013.4	<i>Columbina picui</i>	Argentina	KGM459	KX528470	-	-	KX532224
27	<i>passerinae</i> 1	12	Cosp.Copic.1.20.2003.7	<i>Columbina picui</i>	Argentina	KGM292	KX528488	KX528516	-	-
28	<i>passerinae</i> 1	12	Cosp.Copic.1.8.2003.3	<i>Columbina picui</i>	Argentina	KGM459	KX528489	KX528517	-	-
29	<i>passerinae</i> 1	12	Cosp.Cosqu.11.24.2014.1	<i>Columbina squammata</i>	Brazil	4073	KX528490	-	KX532205	-
30	<i>passerinae</i> 1	16	Cosp.Cosqu.11.24.2014.4	<i>Columbina squammata</i>	Brazil	4781	KX528491	-	-	-
31	<i>passerinae</i> 1	7	Copas.Cotal.4.9.2014.10	<i>Columbina talpacoti</i>	Panama	JMD732	KX528471	KX528504	KX532196	KX532225
32	<i>passerinae</i> 1	7	Copas.Cotal.4.9.2014.9	<i>Columbina talpacoti</i>	Panama	GMS1870	KX528472	KX528505	KX532197	KX532226
33	<i>passerinae</i> 2	3	Cosp.Cotal.11.24.2014.11	<i>Columbina talpacoti</i>	Brazil	6471	KX528492	KX528518	KX532206	-
34	<i>passerinae</i> 2	3	Cosp.Cotal.11.24.2014.12	<i>Columbina talpacoti</i>	Brazil	6473	KX528493	-	KX532207	-
35	<i>altamimiae</i>		Coalt.Meayr.9.4.2013.2	<i>Metriopelia aymara</i>	Argentina	KGM431	KX528463	KX528503	KX532188	KX532218
36	<i>altamimiae</i>		Cosp.Meaym.4.9.2014.12	<i>Metriopelia aymara</i>	Argentina	KGM1148	KX528494	KX528519	KX532208	KX532231
37	<i>altamimiae</i>		Cosp.Meaym.4.9.2014.7	<i>Metriopelia aymara</i>	Peru	REW291	KX528495	KX528520	KX532209	-
38	<i>gymnopoliae</i>		Cogym.10.5.1999.12	<i>Metriopelia ceciliae</i>	Peru	RCF952	AY151009 ¹	AY151021 ¹	-	-
39	<i>gymnopoliae</i>		Cogym.Mecec.9.4.2013.12	<i>Metriopelia ceciliae</i>	Peru	REW150	KX528464	-	KX532190	KX532219
40	<i>gymnopoliae</i>		Cogym.Mecec.9.4.2013.7	<i>Metriopelia ceciliae</i>	Peru	REW192	KX528465	-	KX532191	KX532220
41	<i>gymnopoliae</i>		Cosp.Mecec.4.9.2014.3	<i>Metriopelia ceciliae</i>	Peru	REW153	KX528496	-	KX532210	KX532232
42	<i>gymnopoliae</i>		Cosp.Mecec.7.27.2004.5	<i>Metriopelia ceciliae</i>	Peru	REW192	EF679008 ¹	EF679143 ¹	KX532211	-
43	<i>drowni</i>		Coalt.1.8.2003.4	<i>Metriopelia melanoptera</i>	Argentina	KGM511	EF679018 ¹	EF679153 ¹	KX532187	-
44	<i>drowni</i>		Cosp.Memel.1.8.2003.2	<i>Metriopelia melanoptera</i>	Argentina	KGM514	EF679017 ¹	EF679152 ¹	KX532212	-
45	<i>drowni</i>		Cosp.Memel.4.9.2014.6	<i>Metriopelia melanoptera</i>	Peru	REW282	KX528497	KX528521	KX532213	KX532233
46	<i>drowni</i>		Cosp.Memel.4.9.2014.8	<i>Metriopelia melanoptera</i>	Peru	REW319	KX528498	-	KX532214	KX532234
47	<i>drowni</i>		Cosp.Memel.9.4.2013.3	<i>Metriopelia melanoptera</i>	Argentina	KGM444	KX528499	-	KX532215	KX532235

48	<i>drowni</i>		Cosp.Memel.9.4.2013.6	<i>Metriopelia melanoptera</i>	Bolivia	KGM512	KX528500	-	KX532216	KX532236
49	<i>passerinae</i> 1	12	Cosp.Urcam.10.12.1999.5	<i>Uropelia campestris</i>	Bolivia	CCW925	KX528501	KX528522	KX532217	-
50	<i>passerinae</i> 1	14	Cosp.Urcam.11.24.2014.5	<i>Uropelia campestris</i>	Brazil	4789	KX528502	-	-	-
51	<i>columbae</i>		Cocol.6.29.1998.3	<i>Columba livia</i>		4 Col	EF678962 ¹	EF679097 ¹	KX532189	KX532237

¹ Previously published sequence

Table 2. Results from ParaFit analysis on ground-doves and their wing lice. The global test results and each individual link test results are indicated. For the individual link test, values with an asterisk (*) indicate significance after the Benjamini-Hochberg correction ($\alpha = 0.05$).

ParaFitGlobal = 20.663		P-value = 0.003
<u>Host</u>	<u>Columbicola species</u>	<u>ParaFitLink1 P-values</u>
<i>Claravis pretiosa</i>	<i>C. passerinae</i> 2	0.960
<i>Columbina buckeyi</i>	<i>C. passerinae</i> 2	0.057
<i>Columbina cruziana</i>	<i>C. passerinae</i> 2	0.200
<i>Columbina inca</i>	<i>C. passerinae</i> 1	0.024
<i>Columbina minuta</i>	<i>C. passerinae</i> 1	0.014*
<i>Columbina passerinae</i>	<i>C. passerinae</i> 1	0.016*
<i>Columbina picui</i>	<i>C. passerinae</i> 1	0.115
<i>Columbina squammata</i>	<i>C. passerinae</i> 1	0.024
<i>Columbina talpacoti</i>	<i>C. passerinae</i> 1	0.013*
<i>Columbina talpacoti</i>	<i>C. passerinae</i> 2	0.056
<i>Metriopelia aymara</i>	<i>C. altamimiae</i>	0.031
<i>Metriopelia ceciliae</i>	<i>C. gymnopeliae</i>	0.015*
<i>Metriopelia melanoptera</i>	<i>C. drowni</i>	0.011*
<i>Uropelia campestris</i>	<i>C. passerinae</i> 1	0.966

Table 3. Summary of the Jane v. 4 results for New World ground-doves and their wing lice. Cospeciation event indicated in Figure 3.

Cospeciations	Duplications	Duplications and Host Switches	Losses	Failures to Diverge
1	3	0	15	9

FIGURES

Figure 1. Maximum likelihood topology of New World ground-dove wing lice *Columbicola*, from a partitioned concatenated analysis of CO1, EF-1 α , TMEDE6, and HYP sequences. Values at nodes indicate ML bootstrap (BS) support values followed by Bayesian posterior probabilities (PP). Support is only indicated on nodes with ≥ 50 BS/ ≥ 0.50 PP values, and nodes with ≥ 95 BS/ ≥ 0.95 PP support are indicated with asterisks (*). Scale bar indicates nucleotide substitutions per site. Species as recovered by OTU analysis are indicated to the right of the figure. Individual samples are colored according to the geographic region where the sample was collected, as indicated on the map.

Figure 2. Median-joining (MJ) network estimated from CO1 sequences of *Columbicola passerinae* samples. The two distinct lineages within *C. passerinae* are indicated at the bottom of the figure. Nodes correspond to unique haplotypes, and are numbered according to Table 1. The size of each node indicates the relative number of individuals in each haplotype. Nodes are colored according to host species as indicated in the upper-left of the figure. Inferred ancestral nodes are colored black. Haplotypes are grouped with colored lines according to geographic region of sampling, and are colored as in Figure 1.

Figure 3. Tanglegram of small New World ground-doves and their associated *Columbicola* wing lice. Red circles indicate a cospeciation event as recovered by Jane. Red lines indicate significant host-parasite links estimated by the ParaFitLink1 test after correcting for multiple tests.

Figure 4. Squared residuals from the PACo jackknife test for individual host-parasite links. Median squared residual value indicated with the horizontal dotted line. Host-parasite associations that were significant according to the corrected ParaFitLink1 test results are indicated by asterisks (*).

Figure %

Figure 8

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Figure(

*Graphical Abstract

“*C. passerinae* 1”

“*C. passerinae* 2”

C. drowni

C. gymnopoliae

C. altamimiae