Cophylogenetic patterns are uncorrelated between two lineages of parasites on the same hosts

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Free-living organisms are often host to multiple lineages of closely related parasites. Different lineages of obligate parasites living on the same hosts might potentially be expected to display similar cophylogenetic patterns. However, there are also reasons why these lineages might have different evolutionary histories (e.g. host switching, host geography). In the present study, we use mitochondrial and nuclear DNA sequence data to evaluate the cophylogenetic patterns between doves and their wing and body lice. Previous studies have found that the wing and body lice of doves have different levels of congruence between their phylogenetic histories. However, these studies are limited in scope, either taxonomically or geographically. We used both new and existing data to generate a worldwide and taxonomically diverse data set for doves and two independent groups of lice: wing and body lice. Using event and topology-based methods, we found that cophylogenetic patterns were not correlated between wing and body lice, even though both groups showed evidence of cospeciation with their hosts. These results indicate that external factors vary in their impact on different groups of parasites and also that broad sampling is critical for identifying patterns in cophylogenetic analyses. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **00**, 000–000.

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INTRODUCTION

Parasitic organisms are among the most abundant and diverse group of organisms on earth (Windsor, 1998; Poulin & Morand, 2000, 2004; Dobson *et al.*, 2008; Mora *et al.*, 2011). One of the mechanisms contributing to this diversity is cospeciation, the parallel speciation of two organisms with dependent life histories (Hafner & Nadler, 1990; Hafner *et al.*, 1994; Hafner & Page, 1995; Page, 2003; de Vienne *et al.*, 2013). Parasites that cospeciate with their hosts should exhibit congruent diversification patterns (Fahrenholz, 1913; Eichler, 1948). Although this congruence has been found in some instances (Hafner & Nadler, 1988; Page *et al.*, 2004; Hughes *et al.*, 2007), many host–parasite systems show discordant patterns. This indicates evolutionary processes that promote diversification in parasites independently of their hosts (Paterson *et al.*, 2000; Johnson *et al.*, 2002; Bruyndonckx *et al.*, 2009). For example, host

switching and parasite duplication (speciation within a host) may result in incongruent diversification patterns between hosts and their parasites (Page, 2003). Additionally, geography (Weckstein, 2004; Johnson *et al.*, 2007), host preference (Johnson, Bush & Clayton, 2005; Gorrell & Schulte-Hostedde, 2008), host-imposed selective pressures (Clayton *et al.*, 1999; Clayton & Walther, 2001; Waite, Henry & Clayton, 2012), competition between parasites (Bush & Malenke, 2008; Poulin, 2007; Johnson, Malenke & Clayton, 2009), and opportunities for host switching may influence the parasite diversification. In the present study, we generally refer to the patterns of diversification between hosts and their parasites, either congruent or incongruent, as 'cophylogenetic patterns'.

Free-living organisms often host many lineages of closely related parasites (Poulin, 1997). Comparisons of phylogenies of multiple parasite lineages with those of their hosts can address fundamental questions in host–parasite coevolution. For example, it is important to understand how different parasite lineages respond to host speciation events. Additionally,

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host ecology may shape cophylogenetic patterns in different ways for different parasite lineages (Page, 1994; Johnson & Clayton, 2003). The ectoparasitic lice (Insecta: Phthiraptera) parasitizing pigeons and doves (Aves: Columbidae) are ideal subjects for addressing such questions. Pigeons and doves are parasitized by two groups of feather lice: wing and body lice (Johnson & Clayton, 2003; Johnson, Shreve & Smith, 2012). Although both feed on abdominal downy feathers, members of these two groups have different mechanisms for escaping host preening (Rothschild & Clay, 1952; Nelson & Murray, 1971; Clayton *et al.*, 2005, 2010). To escape preening, the elongate wing lice insert themselves between the barbs of the wing feathers, whereas the rounder body lice burrow into feather down (Clayton, 1991; Clayton *et al.*, 1999) (Fig. 1). Although both of these groups of lice are in the same family (Philopteridae), wing and body lice parasitized doves independently, being relatively distantly related to each other (Cruickshank *et al.*, 2001). These two lineages can be treated as 'ecological replicates' that have different environmental limitations (Johnson & Clayton, 2003). Additionally, pigeons and doves are distributed worldwide and occupy a variety of ecological niches. Some groups, such as ground doves, exhibit terrestrial lifestyles and primarily feed on seeds. Other groups, such as the fruit doves, are primarily arboreal and feed on fruits (Goodwin, 1983; Gibbs, Cox & Cox, 2001). Because both groups of dove lice are found on most host species (Price *et al.*, 2003), it is possible to obtain a geographically extensive sample across the range of host niches for both groups of lice.

Despite both wing and body lice being distributed worldwide on many species of doves, wing lice are more likely than body lice to switch between host species as a result of ecological differences in dispersal capability. Although both are obligate parasites, wing lice are more mobile than the more host-specific body lice (Johnson *et al.*, 2002; Price *et al.*, 2003). Wing lice have been shown to 'hitchhike' on hippoboscid flies, which are generalist ectoparasites that often target doves (Harbison *et al.*, 2008; Harbison & Clayton, 2011). This hitchhiking behaviour, known as phoresy, may allow wing lice to rapidly move between hosts that may not normally interact. Body lice do not appear to utilize phoresy, and so they are unlikely to disperse between host individuals in this way (Harbison, Jacobsen & Clayton, 2009). However, body lice do show some evidence of host switching, which appears to be facilitated by host behaviours. For example, gregarious roosting and foraging bring different species of doves into contact and may facilitate the exchange of both wing and body lice (Harbison *et al.*, 2008; Johnson *et al.*, 2011a). Given this
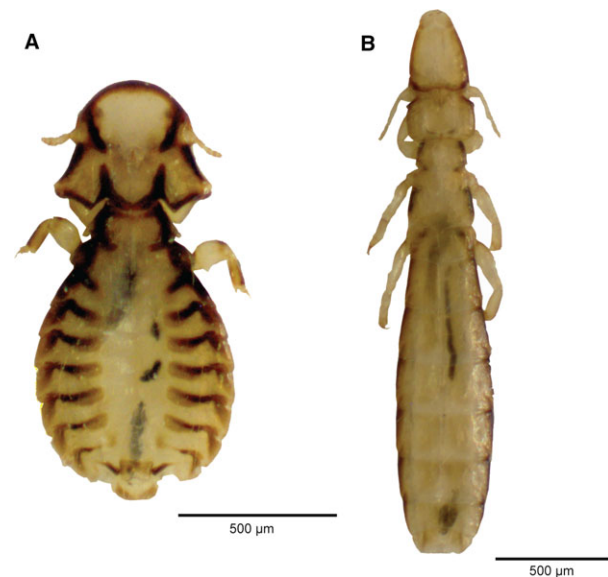

Figure 1. Photographs of (A) a body louse (*Physconelloides emersoni*) and (B) a wing louse (*Columbicola drowni*) from a black-winged ground dove (*Metriopelia melanoptera*). Scale indicated at the bottom right of each photograph.

knowledge, we expect that wing lice will be more likely to show phylogenetic patterns incongruent with their hosts. Previous taxonomically or geographically limited cophylogenetic studies have shown this to be the case (Clayton & Johnson, 2003; Johnson & Clayton, 2004). A study with broader sampling is needed to evaluate these patterns more thoroughly.

In the present study, we combined new and existing data from multiple studies to compare cophylogenetic patterns of wing and body lice on a worldwide scale. From this data set, we estimated phylogenetic trees for the doves and their associated wing and body lice. We used the resulting trees in cophylogenetic analyses, under both topology-based and event-based approaches.

MATERIAL AND METHODS

TAXON AND MARKER SELECTION

We obtained sequence data from NCBI-GenBank as deposited in previous studies. This includes pigeon and dove data from Johnson & Clayton (2000), Johnson (2004), Johnson & Weckstein (2011), Pereira *et al.* (2007), Sweet & Johnson (2015), and Johnson *et al.* (2001b); wing louse data from Johnson *et al.* (2007) and Johnson & Clayton (2004); and body louse data from Johnson *et al.* (2011a, b), Johnson & Clayton (2004), and Johnson *et al.* (2001a) (see Support-

ing information, Table S1). In instances where no GenBank data were available, we sequenced samples according to methods outlined in Johnson & Clayton (2000), Johnson *et al.* (2007, 2011b). Wing lice in this study belong to the genus *Columbicola*, whereas body lice are spread across the genera *Auricotes*, *Coloceras*, *Campanulotes*, and *Physconelloides*. We used *Aerodramus salangana* (swiflet) as the outgroup for the doves in accordance with the rooting of Johnson & Clayton (2000), *Oxylipeurus chiniri* (chachalaca louse) for wing lice in accordance with the rooting of Johnson *et al.* (2007), and *Stronglyocotes orbicularis* (tinamou louse), *Goniocotes talegallae* (brushturkey louse), and *Goniodes assimilis* (partridge louse) for body lice in accordance with the rooting of Johnson *et al.* (2011b).

For the doves, we used the mitochondrial loci cytochrome *c* oxidase subunit I (COI), ATP synthase F0 subunit 8 (ATP8), NADH dehydrogenase subunit 2 (ND2), and cytochrome *b* (Cytb), and nuclear locus beta-fibrinogen intron 7 (FIB7). For wing lice, we used mitochondrial loci COI and 12S ribosomal RNA (12S), and nuclear locus elongation factor 1α (EF-1α). For body lice, we used mitochondrial loci COI and 16S ribosomal RNA (16S), and nuclear locus EF-1α. These markers were chosen because the majority of our targeted taxa have this sequence information, therefore minimizing missing sequences in our final data matrix. We also excluded lice for which we did not have host DNA sequence data and vice versa. Thus, each host taxon had data for at least one associated wing and body louse.

Based on our criterion of only including host samples with both associated wing and body louse data, we had a finalized matrix of 52 different dove species, along with 43 associated wing and 49 body louse taxa (see Supporting information, Table S1). NCBI data yielded a complete or almost complete sampling of loci in the host, wing louse, and body louse data sets. For the loci Cytb, COI, ND2, and FIB7 in the birds, there were seven instances of missing data for a gene (4% of entire matrix). However, for the ATP8 locus, there were eighteen instances of missing data (37%). There were four instances of missing data for the three loci in the wing louse data (approximately 2%), and nine instances of missing data for the three loci in the body louse data (5%).

PHYLOGENETIC ANALYSIS

We aligned sequences for each locus and in each taxonomic group (doves, wing lice, and body lice) independently. All alignments were performed using MUSCLE (Edgar, 2004) and visualized using SEA-VIEW, version 4 (Gouy, Guindon & Gascuel, 2010). After inspecting the alignments, we concatenated the locus-based alignments into a single alignment for each group (doves, wing lice, and body lice) in SEA-VIEW. Using the concatenated data sets for each group, we estimated maximum likelihood trees in RAXML, version 7.0.4 (Stamatakis, 2006) using the GTR+I+Γ model of sequence evolution and 500 bootstrap replicates. We also estimated ultrametric Bayesian trees using BEAST, version 1.7.5 (Drummond *et al.*, 2012). For the BEAST analyses, we partitioned each concatenated alignment by locus and used JMODELTEST2 (Darriba *et al.*, 2012) to estimate the best-fitting substitution models for each locus according to the corrected Akaike information criterion (Sugiura, 1978). We treated all mitochondrial loci as a single locus in all three alignments. For wing lice and body lice, we applied a GTR+I+Γ model to the mitochondrial data, and a K80+I+Γ model to EF-1α. For the doves, we applied separate GTR+I+Γ models to the mitochondrial data and FIB7. In BEAST, we used a log-normal relaxed clock and a Yule speciation tree prior for all three partitioned data sets, and ran analyses for 20 million Markov chain Monte Carlo generations with sampling every 1000. We checked the resulting .log files in TRACER, version 1.4 (Rambaut & Drummond, 2007) and, from the trace plots, found that each analysis reached stationarity and had effective sample size values ≫ 200. Based on the trace files, we discarded the first 2000 trees (10%) as burn-in.

COPHYLOGENETIC ANALYSIS

Preparing trees for analysis

For phylogenetic analysis, we included multiple louse samples that were of the same species but were associated with different host species. However, because, in some cases, there was no evidence that these multi-host parasites were genetically distinct (see Supporting information, Fig. S1), we collapsed these down to a single terminal taxon for cophylogenetic analysis using MESQUITE, version 2.75 (Maddison & Maddison, 2011). We did this to avoid bias as a result of taxon duplication in our data set. We also removed outgroup taxa because their inclusion was for rooting the phylogenetic trees and not for cophylogenetic analysis. We used these trimmed trees for all subsequent analyses. In particular, we analyzed our data with both topology-based and event-based methods (de Vienne *et al.*, 2013).

Topology-based approach to test for cophylogenetic signal

For a topology-based comparison, we used PARAFIT (Legendre, Desdevises & Bazin, 2002) in the 'ape' package of R (Paradis, Claude & Strimmer, 2004). PARAFIT takes the host phylogeny, parasite

phylogeny, and association matrix as input and tests for a random association between the two groups of taxa by randomizing the association matrix. PARAFIT also tests for the contribution of each host–parasite association to the global statistic through two individual link statistics: ParaFitLink1 ('F1') and ParaFitLink2 ('F2'). F1 is a more conservative test and is generally preferred; however, F2 has greater power in some cases (Legendre, Desdevises & Bazin, 2002). We ran PARAFIT comparing the wing louse tree with the host tree and comparing the body louse tree with the host tree, and also for both the maximum likelihood trees from RAXML and the ultrametric trees from BEAST. We first converted our trees to patristic distance matrices using 'ape', and ran PARAFIT for 100 000 random permutations using the 'lingoes' correction for negative eigenvalues. We also used the alternative correction, 'calliez', although the results were almost identical. Therefore, we used 'lingoes' results in all subsequent analyses. In all PARAFIT analyses, we computed the F1 and F2 statistics for individual links.

To test whether cophylogenetic patterns may be correlated between the wing lice and body lice, we used contingency tables to partition the results of the individual link (i.e. host–parasite association) tests for each PARAFIT analysis. The contingency tables were 2×2 matrices, with wing lice results on the rows and body lice results on the columns. Each cell indicated whether a particular host had a significant linkage with its parasite species (indicating that this association contributes to topological similarity between the trees). In instances when the links for both the wing and body lice of a particular host were significant, we counted those associations as a single decision in the appropriate cell. If, on the other hand, the body louse had a significant linkage but the wing louse did not, we counted the associations as a single decision in a different cell. For instances where a host had multiple links for one louse type but did not have multiple links for the other louse type (e.g. one host species has multiple wing louse species but only one body louse species associated with it), we counted the single species host–parasite link to match the number of links in the corresponding louse type link. If a host species had one wing louse species but two body louse species associated with it (or vice versa), we counted the wing louse link twice to correspond to each of the body louse links.

PARAFIT produces *P*-values for each individual link test to provide a level of support for the contribution of that host–parasite association to the global statistic testing for random association between a group of hosts and their parasites. To correct for false discovery with multiple tests, we used the

Benjamini–Hochberg control of false discovery rate (Benjamini & Hochberg, 1995). We performed corrections in R assuming $\alpha = 0.05$. Using the corrected *P*-values, we tallied the individual test links in the cells of our contingency tables and used a Pearson's chi-squared test for independence for each contingency table to test for potentially correlated cophylogenetic patterns between the wing and body lice. A significant chi-squared result would indicate that cophylogenetic patterns in wing and body lice are correlated. That is, we tested the null hypothesis of whether the significant linkages of wing lice were independent of those for body lice over the same group of hosts. Because PARAFIT produces two individual link test statistics, we tallied the results and used a chi-squared test for both statistics. We also used a Fisher's exact test for each contingency table to test whether small sample sizes may affect the results of the chi-squared analysis. We performed the chi-squared tests and Fisher's exact tests in R.

Event-based approach to test for cophylogenetic signal

For an event-based approach, we used JANE, version 4.01 (Conow *et al.*, 2010). JANE uses a priori event costs to reconcile host and parasite phylogenies by minimizing the overall cost. We used this method for both the wing and body louse data sets, using the ultrametric trees that we generated from BEAST. We ran JANE with the genetic algorithm parameters set at 100 generations and with a population size of 100, and set the costs as default: 0 for cospeciation, 1 for duplication, 2 for duplication and host switch, 1 for loss, and 1 for failure to diverge. To test whether the resulting reconstruction cost is significantly lower than by chance, we randomized the tip associations 999 times. A significant result from this statistical test would indicate some level of phylogenetic congruence between host and parasite. Finally, we tested for the correlation of recovered cospeciation events from their placement on the host tree using a contingency table (*sensu* Johnson & Clayton, 2003).

Testing for taxonomic bias

Because our sample has a high proportion (10/15 representatives) of small New World ground doves (*Columbina*, *Claravis*, *Uropelia*, and *Metriopelia*) relative to other clades, our cophylogenetic analyses could potentially be affected by a taxonomic/clade representative bias. To test this idea, we removed the small New World ground dove links in 'ape'. Using this reduced data set, we ran PARAFIT for 100 000 iterations for both the phylogram and ultrametric trees, and applied both the F1 and F2 individual link tests. From the results of the individual link

tests, we checked for correlated cophylogenetic patterns between wing and body lice using contingency tables and Pearson's chi-squared tests as described above.

RESULTS

Maximum likelihood and Bayesian phylogenetic analyses with RAXML and BEAST produced trees largely in agreement with previous studies using these data. However, several of the basal nodes for all dove and louse trees were not well supported. The global PARAFIT statistics were significant for both the wing and body lice data sets ($P < 0.001$) (Table 1). This was true for patristic distances from both the phylogram and ultrametric trees. Although each dataset indicated strong support for a global nonrandom association between host and parasite trees, a subset of individual host–parasite links (i.e. host–parasite associations) contribute to this signal. Because PARAFIT can also test each link by recalculating the global PARAFIT statistic with the link removed, we can obtain a better understanding of

how certain links contribute to the global statistic. A significant individual link statistic means that the global PARAFIT statistic decreased in value when that particular link was removed, and therefore indicates that the link represents an important component of the overall host–parasite relationship (Legendre et al., 2002). PARAFIT also produces two different individual link statistics (F1 and F2). Here, we report the results from both tests. The F1 phylogram results included 40 significant wing louse–host links and 33 significant body louse–host links after correcting for multiple comparisons, whereas the F2 phylogram results indicated 43 significant wing louse–host links and 55 significant body louse–host links after correction (Table 1). The F1 ultrametric results did not have any significant body or wing louse links after correction, whereas the F2 ultrametric results indicated 19 significant wing louse–host links after correction and no significant body louse–host links after correction (Table 1). Several links were significant before correction ($\alpha = 0.05$) but not significant after correction. The specific host–parasite links and associated P-values of both individual link statistics for the phylogram and ultrametric trees are provided in Table 2.

Most of the chi-squared tests of independence of significant linkages between wing and body lice performed on the contingency tables were not significant or were not applicable (Table 3). The only significant test was from the PARAFIT phylogram F1 results ($P = 0.002$). The P-values from the other chi-squared tests were all > 0.3. Fisher's exact tests yielded similar P-values.

Our JANE analyses recovered 15 nodes of cospeciation among the wing lice and their hosts, and 22 nodes of cospeciation among the body lice and their hosts (Table 4). The specific nodes recovered as cospeciation events in both data sets are indicated in Figs 2 and 3. The placement of these events on the host tree is not correlated between wing and body lice (Table 3), suggesting that these two parasite lineages diversify independently in response to host diversification. The total reconstruction cost was 84 for the wing lice and 79 for the body lice. In both analyses, none of the costs from 999 random tip associations were equal to or lower than these original reconstruction costs ($P = 0.0$).

In our PARAFIT analyses with the small New World ground dove tips and links removed, our global statistics were significant in all cases ($P < 0.0001$). However, the corrected individual link statistics differed from the full data set results (Table 1). For the phylogram trees, wing lice had 12 significant links for both the F1 and F2 statistics, whereas the body lice had 27 and 33, respectively. The ultrametric trees also had 12 significant wing

Table 1. Summary of the results from PARAFIT for the full wing and body louse data set and the partial (excluding small New World ground doves) data set

	Phylogram	Ultrametric
PARAFIT full		
Wing	ParaFitGlobal = 1.947	ParaFitGlobal = 6.043
	$P = 0.00001$	$P = 0.00001$
F1 links	40	0
F2 links	43	19
Body	ParaFitGlobal = 0.276	ParaFitGlobal = 6.138
	$P = 0.00001$	$P = 0.00007$
F1 links	33	0
F2 links	55	0
PARAFIT partial		
Wing	ParaFitGlobal = 471.8	ParaFitGlobal = 4.219
	$P = 0.00002$	$P = 0.00003$
F1 links	12	12
F2 links	12	12
Body	ParaFitGlobal = 0.132	ParaFitGlobal = 5.134
	$P = 0.00001$	$P = 0.00001$
F1 links	27	30
F2 links	33	31

The global PARAFIT statistics and associated P-values are indicated for the results from PARAFIT. F1 and F2 links refer to the number of significant ParaFitLink1 and ParaFitLink2 statistics, respectively, after correcting for false discovery rate with the Benjamini–Hochberg correction.

Table 2. PARAFIT individual link statistic *P*-values for both the ParaFitLink1 (F1) and ParaFitLink2 (F2) statistics of the full data set

Wing lice	Body lice	Phylogram		Ultrametric		Phylogram		Ultrametric	
		F1	F2	F1	F2	F1	F2	F1	F2
Claravis pretiosa	*Claravis pretiosa*	0.00008*	0.00007*	0.02570	0.02190	0.00011*	0.00001*	0.02856	0.02592
Uropelia campestris	*Uropelia campestris*	0.00001*	0.00001*	0.01579	0.01328*	0.00001*	0.00001*	0.01723	0.01527
Metriopelia cecliae	*Metriopelia cecliae*	0.00002*	0.00002*	0.02864	0.02461	0.00001*	0.00001*	0.01943	0.01699
Metriopelia melanoptera	*Metriopelia melanoptera*	0.00002*	0.00002*	0.02841	0.02421	0.00001*	0.00001*	0.01714	0.01495
Columbina cruziana	*Columbina cruziana*	0.00001*	0.00001*	0.04662	0.04067	0.00001*	0.00001*	0.02524	0.02303
Columbina picui	*Columbina picui*	0.00001*	0.00001*	0.01189	0.01011*	0.00001*	0.00001*	0.02481	0.02277
Columbina inca	*Columbina inca*	0.00001*	0.00001*	0.01152	0.00987*	0.00001*	0.00001*	0.06328	0.05695
Columbina passerina	*Columbina passerina*	0.00001*	0.00001*	0.01110	0.00958*	0.00001*	0.00001*	0.02342	0.02106
Columbina minuta	*Columbina minuta*	0.00001*	0.00001*	0.01074	0.00918*	0.00001*	0.00001*	0.03633	0.03301
Columbina buckleyi	*Columbina buckleyi*	0.00001*	0.00001*	0.08411	0.07502	0.00001*	0.00001*	0.02381	0.02178
Geopelia placida	*Geopelia placida*	0.01956*	0.01421*	0.07200	0.06285	0.03328	0.00008*	0.51637	0.50952
Geopelia humeralis	*Geopelia humeralis*	0.02365*	0.01787*	0.07796	0.06852	0.06950	0.00008*	0.52301	0.51534
Geopelia cuneata	*Geopelia humeralis*	0.01475*	0.01006*	0.98455	0.98682	0.06891	0.00023*	0.56601	0.55829
Ocyphaps lophotes	*Geopelia cuneata*	0.00108*	0.00053*	0.00502	0.00399*	0.02827	0.00006*	0.38403	0.37433
Geophaps plumifera	*Ocyphaps lophotes*	0.00056*	0.00030*	0.00223	0.00170*	0.55195	0.17624	0.69007	0.68750
Geophaps smithii	*Geophaps plumifera*	0.00022*	0.00007*	0.00231	0.00179*	0.02017*	0.00001*	0.03660	0.03291
Geophaps scripta	*Geophaps smithii*	0.00028*	0.00015*	0.00324	0.00256*	0.01292*	0.00001*	0.03678	0.03353
Phaps elegans	*Geophaps smithii*	0.00003*	0.00002*	0.00569	0.00460*	0.01096*	0.00001*	0.29318	0.27887
Phaps historionica	*Geophaps scripta*	0.00205*	0.00114*	0.00566	0.00459*	0.03457	0.00001*	0.28342	0.27100
Phaps chalcoptera	*Phaps elegans*	0.00013*	0.00008*	0.01099	0.00929*	0.03434	0.00003*	0.33949	0.32789
Petrophassa albipennis	*Phaps historionica*	0.00001*	0.00001*	0.09712	0.08609	0.36331	0.02395*	0.85158	0.85319
Petrophassa rufipennis	*Phaps chalcoptera*	0.00003*	0.00001*	0.47186	0.45759	0.00771*	0.00001*	0.08144	0.07461
Turtur tympanistria	*Phaps chalcoptera*	0.11891	0.09854	0.28731	0.27249	0.12494	0.00118*	0.62715	0.62295
Turtur brehmeri	*Petrophassa albipennis*	0.04250	0.02994*	0.35844	0.34415	0.00020*	0.00001*	0.22198	0.21032
Chalcophaps indica	*Petrophassa rufipennis*	0.06037	0.04672	0.20758	0.19174	0.00069*	0.00001*	0.20205	0.19010
Chalcophaps stephani	*Turtur tympanistria*	0.06646	0.05275	0.21715	0.20112	0.17887	0.00470*	0.26607	0.25424
Phapitreron leucotis	*Turtur brehmeri*	0.04800	0.03508*	0.08231	0.07290	0.13412	0.00330*	0.53348	0.52524
Treron waalia	*Chalcophaps indica*	0.42768	0.38297	0.48008	0.47061	0.04962	0.00009*	0.20788	0.19813
Lopholaimus antarcticus	*Chalcophaps indica*	0.11849	0.10004	0.04238	0.03614	0.12411	0.00203*	0.34677	0.33532
Ducula rufigaster	*Chalcophaps stephani*	0.08872	0.07267	0.04223	0.03619	0.05824	0.00015*	0.20217	0.19201
Ptilinopus rivoli	*Chalcophaps stephani*	0.08741	0.07161	0.98733	0.98908	0.14097	0.00300*	0.34242	0.33096
Geotrygon montana	*Phapitreron leucotis*	0.11946	0.10241	0.01893	0.01615	0.17388	0.00436*	0.19630	0.18541
Leptotila plumbiscens	*Treron waalia*	0.01707*	0.01231*	0.11572	0.10485	0.41405	0.05609	0.33430	0.32301
Leptotila plumbiscens	*Lopholaimus antarcticus*	0.09522	0.07936	0.01835	0.01564*	0.62149	0.04019*	0.17732	0.16627
Leptotila jamaicensis	*Ducula rufigaster*	0.01413*	0.01003*	0.10823	0.09812	0.24254	0.00200*	0.24748	0.23622
Leptotila verreauxi	*Ptilinopus rivoli*	0.01579*	0.01117*	0.14646	0.13503	0.25603	0.00314*	0.29547	0.28400
Leptotila verreauxi	*Geotrygon montana*	0.09957	0.08351	0.01861	0.01602*	0.06264	0.00040*	0.00711	0.00591

Table 2. *Continued*

Wing lice	Phylogram		Ultrametric		Body lice	Phylogram		Ultrametric	
	F1	F2	F1	F2		F1	F2	F1	F2
Zenaida asiatica	0.09108	0.07665	0.05169	0.04492	*Leptotila plumbiscens*	0.00975*	0.00001*	0.00565	0.00479
Zenaida macroura	0.03140	0.02398*	0.25981	0.24584	*Leptotila jamaicensis*	0.00930*	0.00001*	0.00580	0.00489
Zenaida macroura	0.10867	0.09123	0.08665	0.07844	*Leptotila verreauxi*	0.01108*	0.00001*	0.00966	0.00855
Zenaida auriculata	0.02731*	0.02027*	0.17294	0.15933	*Zenaida asiatica*	0.01177*	0.00001*	0.05639	0.05150
Zenaida galapagoensis	0.09795	0.08200	0.07817	0.07110	*Zenaida macroura*	0.00088*	0.00001*	0.05379	0.04903
Reinwardtoena reinwardtii	0.68163	0.63206	0.95386	0.95765	*Zenaida auriculata*	0.00087*	0.00001*	0.06820	0.06275
Macropygia ruficeps	0.51641	0.45329	0.95706	0.96126	*Zenaida galapagoensis*	0.00097*	0.00001*	0.08349	0.07734
Patagioenas fasciata	0.06957	0.05661	0.10090	0.09013	*Reinwardtoena reinwardtii*	0.39229	0.02314*	0.04714	0.04294
Patagioenas speciosa	0.03658	0.02829*	0.01522	0.01241*	*Macropygia ruficeps*	0.36144	0.02511*	0.04741	0.04286
Patagioenas subvinacea	0.02743*	0.02096*	0.35100	0.33643	*Patagioenas fasciata*	0.04900	0.00009*	0.28717	0.27382
Patagioenas plumbea	0.02122*	0.01559*	0.01519	0.01262*	*Patagioenas speciosa*	0.02749	0.00002*	0.89469	0.89269
Columba palumbus	0.01750*	0.01290*	0.00771	0.00584*	*Patagioenas subvinacea*	0.01469*	0.00001*	0.95666	0.95928
Columba livia	0.01276*	0.00883*	0.00718	0.00542*	*Patagioenas plumbea*	0.01227*	0.00001*	0.95821	0.96038
Columba guinea	0.01806*	0.01303*	0.16711	0.15326	*Columba palumbus*	0.50342	0.10344	0.18510	0.17503
Streptopelia semitorquata	0.03015*	0.02086*	0.03887	0.03341	*Columba livia*	0.46886	0.10415	0.18246	0.17266
Streptopelia decaocto	0.01284*	0.00881*	0.14090	0.12848	*Columba guinea*	0.07331	0.00052*	0.02042	0.01810
Streptopelia vinacea	0.01850*	0.01367*	0.05554	0.04879	*Streptopelia semitorquata*	0.01911*	0.00002*	0.20881	0.19817
Streptopelia capicola	0.01685*	0.01239*	0.05499	0.04831	*Streptopelia decaocto*	0.84719	0.83298	0.79868	0.79914
					Streptopelia decaocto	0.00641*	0.00001*	0.00549	0.00484
					Streptopelia vinacea	0.00368*	0.00001*	0.00556	0.00488
					Streptopelia capicola	0.00320*	0.00001*	0.00747	0.00656

*Significant after correcting for false discovery rate ($\alpha = 0.05$). Hosts are listed left of the *P*-values. Hosts listed more than once indicate multiple species of lice associated with that particular host.

Table 3. Summary of the contingency table results from PARAFIT individual link statistics and cospeciation events recovered in JANE for the full data set and from PARAFIT statistics for the partial (excluding small New World ground doves) data set

Wing/body	No/No	No/Yes	Yes/No	Yes/Yes	Chi-squared *P*-value
Full					
Phylogram F1	14	5	12	28	0.002
Phylogram F2	1	16	4	39	1
Ultrametric F1	61	0	0	0	NA
Ultrametric F2	42	0	19	0	NA
JANE	23	14	6	8	0.3547
Partial					
Phylogram F1	20	19	4	8	0.4481
Phylogram F2	14	25	4	8	1
Ultrametric F1	17	22	4	8	0.767
Ultrametric F2	16	23	4	8	0.8893

Both ParaFitLink1 (F1) and ParaFitLink2 (F2) individual link statistics are reported for PARAFIT. Values indicate total tallies for a particular cell of the contingency table. PARAFIT values indicate the number of individual link statistics in that category after correcting for false discovery rate. JANE values indicate the number of cospeciation and/or noncospeciation events as recovered on the host phylogeny. Results from Pearson's chi-squared tests for each contingency table are listed to the right. NA, not available.

Table 4. Summary of the results from JANE for the wing and body louse data sets

	Cospeciations	Duplications	Duplications and host switches	Losses	Failures to diverge
Wing	15	4	23	22	12
Body	22	1	25	19	9

Data are the number of events that resulted in the lowest reconstruction cost, based on the default cost parameters. Specific events are listed in the top row.

louse links for both the F1 and F2 statistics, whereas body lice had 30 and 31, respectively. Pearson's chi-squared tests on the contingency tables were not significant ($P > 0.45$ in all cases) (Table 3). The specific links and associated *P*-values from cophylogenetic analyses on the reduced data set are provided in Table 5.

DISCUSSION

The present study aimed to determine whether wing and body lice (either or both) have phylogenetic histories congruent with their dove hosts or with each other. If both types of lice are affected similarly by host speciation events, we might expect their cophylogenetic patterns to be similar. However, we failed to identify significant evidence indicating that wing and body lice have similar phylogenetic histories. Despite a lack of correlated patterns between wing and body lice of specific host–parasite links, both the wing and body louse data sets individually showed evidence of cospeciation with their hosts.

The chi-squared tests based on the contingency tables failed to reject the null hypothesis of independence of cophylogenetic patterns in wing and body lice in all but one case. These results indicate that dove wings and body lice have unique and independent evolutionary histories. This is consistent with previous smaller scale studies of both louse groups and can potentially be explained by differences in life history between wing and body lice (Clayton & Johnson, 2003; Johnson & Clayton, 2003, 2004; Johnson *et al.*, 2003).

The global PARAFIT statistic testing for random host–parasite association was significant for both wing and body louse phylogenies individually. Additionally, the JANE event reconstruction costs were significantly lower than by chance. This indicates that, at some level, both body and wing lice show congruent phylogenetic patterns with their hosts. Congruence between body lice and their dove hosts was expected. Previous studies based on event-based methods showed strong patterns of cospeciation between body lice and their hosts (Clayton & Johnson, 2003). However, the wing lice sampled in the
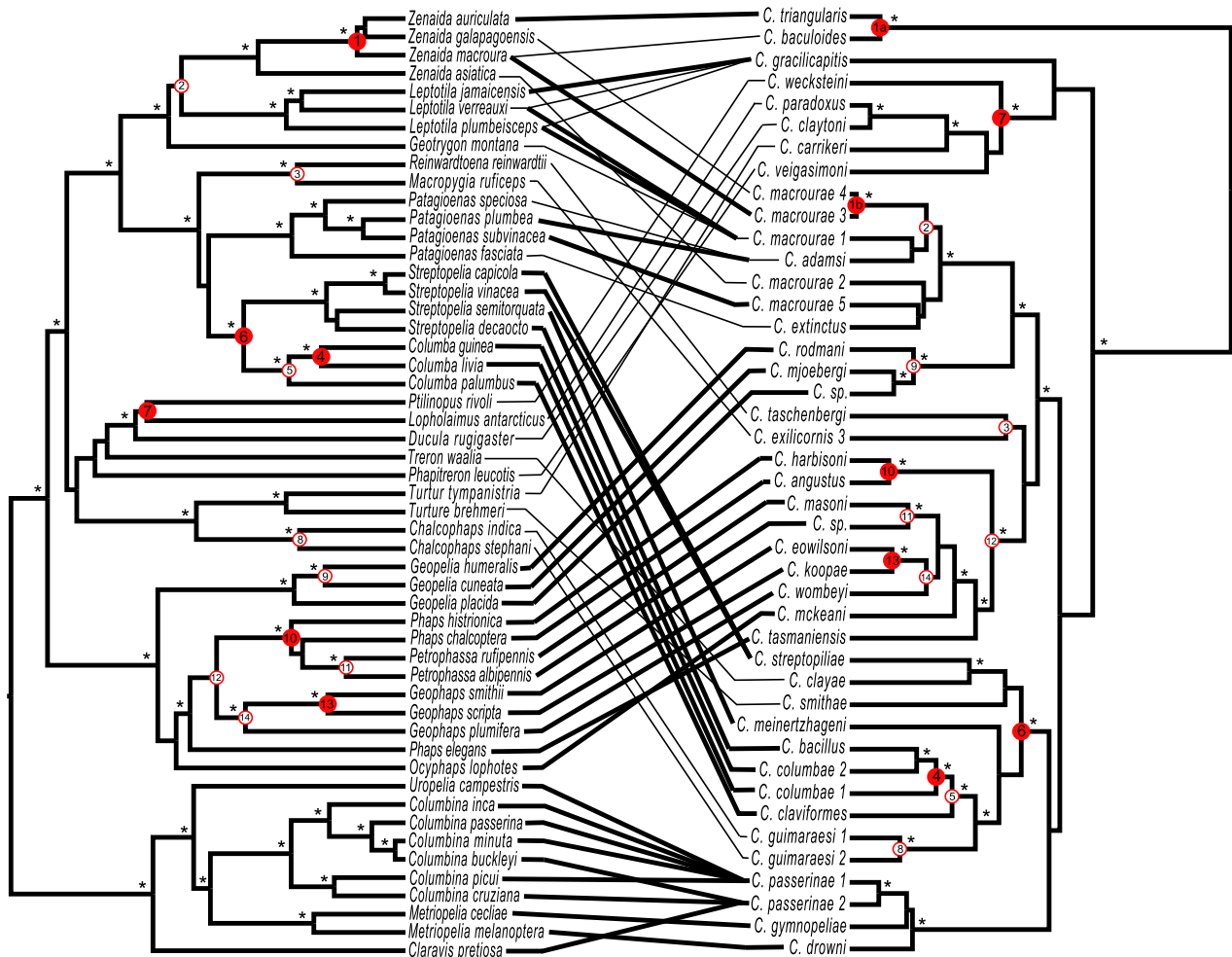
Figure 2. Tanglegram showing the associations between dove wing lice (right) and their hosts (left). Phylogenies were generated using BEAST, version 1.7.5 (Drummond *et al.*, 2012). Asterisks (*) indicate posterior probabilities (PP) ≥ 0.95. Circles at nodes indicate cospeciation events as recovered by JANE, version 4 (Conow *et al.*, 2010). Cospeciation events are numbered starting from the top of the host phylogeny, with matching numbers on corresponding speciation events indicated on the wing louse phylogeny. Open circles indicate recovered cospeciation events shared by wing and body lice. Bold lines between host and parasite indicate a significant link as recovered by the PARAFIT (Legendre *et al.*, 2002) F1 statistic using the phylogram topology.

present study also showed evidence for cospeciation with their hosts. Although previous event-based results have recovered some cospeciation events within this group, the overall patterns indictate a lack of cospeciation over larger time scales (Johnson *et al.*, 2003). However, when taking into account a broad geographical and taxonomic sample, both wing and body lice appear to have undergone some level of cospeciation with their hosts. Having a more extensive sample and therefore more branches on phylogenetic trees provides greater statistical power. This probably allowed us to detect a cophylogenetic signal that was obscured in studies with limited samples, an issue also discussed by Hughes *et al.* (2007). This could be the case particularly if the

smaller samples are biased towards a particular geographical region or host group (Jackson *et al.*, 2008).

By contrast to the global PARAFIT statistics, which indicated overall host–parasite congruence in all cases, the individual link statistics of the lice differed among tree type (phylogram vs. ultrametric) and link statistic (F1 vs. F2). Neither wing, nor body lice showed consistency in the number of significant links among the different analyses. For example, more wing louse links were significant in the phylogram F1 analysis, whereas more body louse links were significant in the phylogram F2 analysis. In the ultrametric F1 statistic, none of the links showed significance. Several links in this analysis initially showed significant *P*-values, although these became
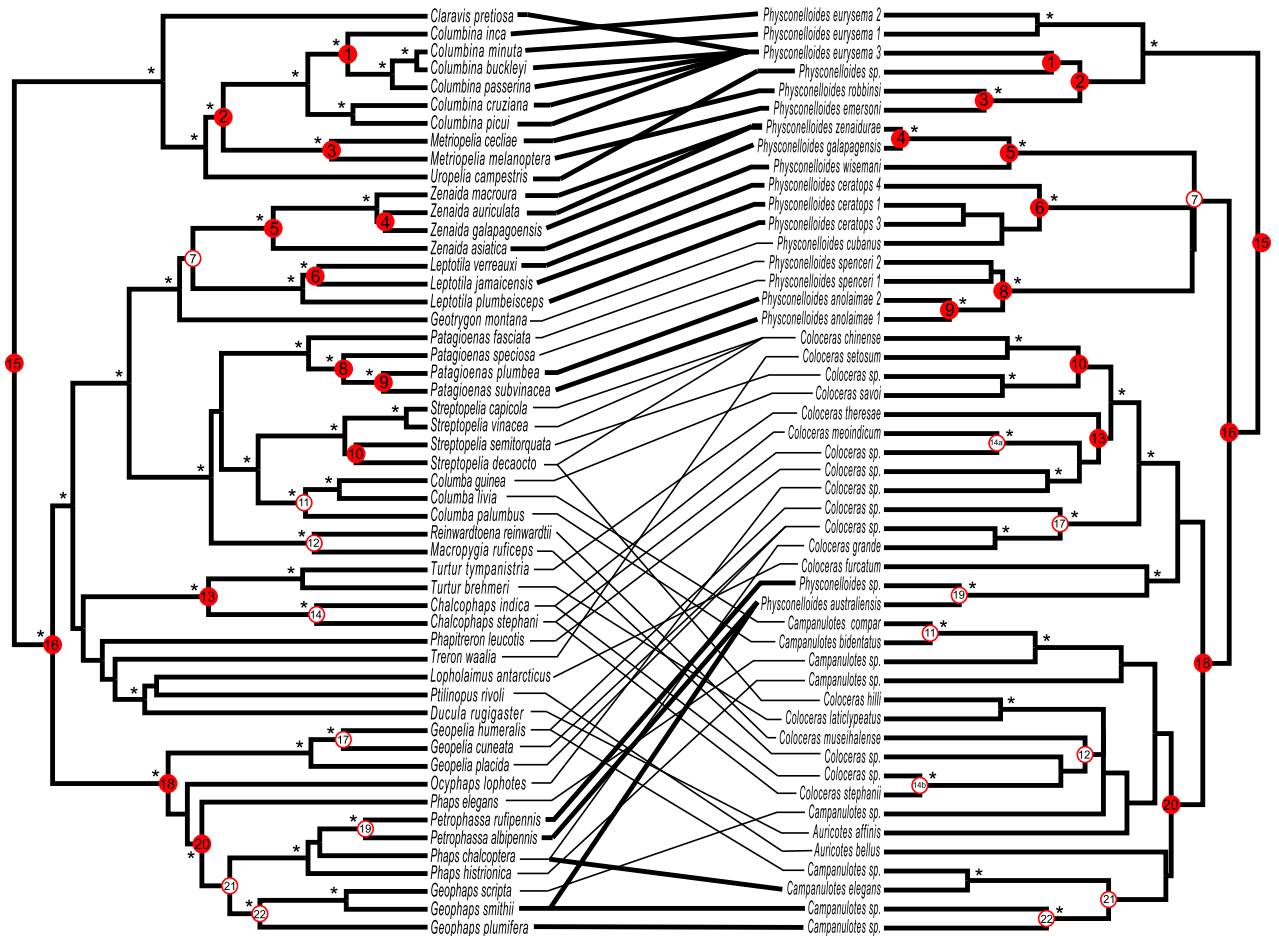
Figure 3. Tanglegram showing the associations between dove body lice (right) and their hosts (left). Phylogenies were generated using BEAST, version 1.7.5 (Drummond *et al.*, 2012). Asterisks (*) indicate posterior probabilities (PP) ≥ 0.95. Circles at nodes indicate cospeciation events as recovered by JANE, version 4 (Conow *et al.*, 2010). Cospeciation events are numbered starting from the top of the host phylogeny, with matching numbers on corresponding speciation events indicated on the body louse phylogeny. Open circles indicate recovered cospeciation events shared by wing and body lice. Bold lines between host and parasite indicate a significant link as recovered by the PARAFIT (Legendre *et al.*, 2002) F1 statistic using the phylogram topology.

nonsignificant after we corrected for multiple tests (Table 2). The instances of more significant wing louse links than body louse links are somewhat surprising. As discussed above, previous work has indicated that body lice have stronger phylogenetic congruence with their hosts, and so we might expect them to have more significant individual links than wing lice.

Poorly resolved backbones of the phylogenies (see Supporting information, Fig. S1) could be a possible explanation for the varying individual link statistic results. This could be a primary cause of the disagreement between the phylogram and ultrametric results. Because PARAFIT takes topology and branch lengths (patristic distances) into consideration, differences between ultrametric and non-ultrametric trees

in relative patristic distances could account for these differences.

Alternatively, clade representation biases could be driving cophylogenetic signals. Our data set includes 10 of 15 representatives of the small New World ground dove clade and their lice, which represents the most thorough sampling representation of a clade in our data set. The hosts, their wing lice, and their body lice have all been shown to be monophyletic (Cruickshank *et al.*, 2001; Johnson *et al.*, 2007, 2011b; Pereira *et al.*, 2007). In both the F1 and F2 PARAFIT analysis, every link from this clade contributed to the overall pattern of nonrandom associations. Because the hosts and their lice are in monophyletic clades, and we were able to include strong taxon sample representation of these groups,

Table 5. PARAFIT individual link statistic *P*-values for both the ParaFitLink1 (F1) and ParaFitLink2 (F2) statistics of the partial (excluding small New World ground doves) data set

Wing lice	Phylogram		Ultrametric		Body lice	Phylogram		Ultrametric	
	F1	F2	F1	F2		F1	F2	F1	F2
Geopelia placida	0.00689*	0.00689*	0.00044*	0.00030*	Geopelia placida	0.00159*	0.00014*	0.00017*	0.00010*
Geopelia humeralis	0.00882*	0.00881*	0.00062*	0.00048*	Geopelia humeralis	0.01493*	0.00296*	0.00038*	0.00025*
Geopelia cuneata	0.00529*	0.00529*	0.97899	0.98119	Geopelia humeralis	0.00322*	0.00046*	0.00028*	0.00021*
Ocyphaps lophotes	0.00806*	0.00805*	0.00069*	0.00057*	Geopelia cuneata	0.00106*	0.00015*	0.00015*	0.00012*
Geophaps plumifera	0.01338	0.01337	0.00033*	0.00025*	Ocyphaps lophotes	0.98095	0.98148	0.00155*	0.00107*
Geophaps smithii	0.00367*	0.00367*	0.00035*	0.00028*	Geophaps plumifera	0.00993*	0.00162*	0.00078*	0.00059*
Geophaps scripta	0.00321*	0.00321*	0.00056*	0.00049*	Geophaps smithii	0.00548*	0.00088*	0.14195	0.11651
Phaps elegans	0.00122*	0.00121*	0.00061*	0.00050*	Geophaps smithii	0.00175*	0.00020*	0.00069*	0.00052*
Phaps historionica	0.01011*	0.01011*	0.00068*	0.00058*	Geophaps scripta	0.54828	0.39861	0.50612	0.49294
Phaps chalcoptera	0.98669	0.98669	0.92363	0.92597	Phaps elegans	0.29108	0.14308	0.43060	0.41543
Petrophassa albipennis	0.00036*	0.00036*	0.00091*	0.00073*	Phaps historionica	0.90047	0.92751	0.73173	0.72954
Petrophassa rufipennis	0.00083*	0.00083*	0.00653*	0.00547*	Phaps chalcoptera	0.00020*	0.00004*	0.00212*	0.00145*
Turtur tympanistria	0.10294	0.10293	0.76565	0.76732	Phaps chalcoptera	0.00240*	0.00033*	0.00147*	0.00106*
Turtur brehmeri	0.11686	0.11684	0.28719	0.27598	Petrophassa albipennis	0.00001*	0.00001*	0.00158*	0.00131*
Chalcophaps indica	0.03282	0.03280	0.06931	0.06326	Petrophassa rufipennis	0.00011*	0.00002*	0.00354*	0.00266*
Chalcophaps stephani	0.03989	0.03989	0.06940	0.06321	Turtur tympanistria	0.37395	0.18811	0.01058*	0.00809*
Phapitreron leucotis	0.10146	0.10144	0.16257	0.15303	Turtur brehmeri	0.05223	0.01466*	0.43786	0.41939
Treron waalia	0.42496	0.42495	0.54868	0.54396	Chalcophaps indica	0.03481	0.00862*	0.16752	0.15235
Lopholaimus antarcticus	0.21807	0.21805	0.09336	0.08558	Chalcophaps indica	0.26451	0.11194	0.89698	0.90062
Ducula rufigaster	0.13632	0.13632	0.09364	0.08582	Chalcophaps stephani	0.04826	0.01226*	0.23149	0.21464
Ptilinopus rivoli	0.13266	0.13265	0.17689	0.16746	Chalcophaps stephani	0.25180	0.11168	0.89360	0.89649
Geotrygon montana	0.21255	0.21254	0.07552	0.06918	Phapitreron leucotis	0.12873	0.04992	0.59081	0.58004
Leptotila plumbiscens	0.08902	0.08899	0.25736	0.24790	Treron waalia	0.78962	0.79540	0.99065	0.99206
Leptotila plumbiscens	0.14086	0.14084	0.09413	0.08715	Lopholaimus antarcticus	0.70867	0.67833	0.50855	0.49539
Leptotila jamaicensis	0.07381	0.07377	0.18292	0.17346	Ducula rufigaster	0.21583	0.10358	0.21074	0.19447
Leptotila verreauxi	0.08507	0.08506	0.28267	0.27358	Ptilinopus rivoli	0.22241	0.10969	0.24347	0.22563
Leptotila verreauxi	0.14746	0.14744	0.13489	0.12628	Geotrygon montana	0.05016	0.01240*	0.00953*	0.00706*
Zenaida asiatica	0.94321	0.94321	0.22428	0.21358	Leptotila plumbiscens	0.00698*	0.00085*	0.00603*	0.00409*
Zenaida macroura	0.18222	0.18221	0.37579	0.36634	Leptotila jamaicensis	0.00467*	0.00060*	0.00677*	0.00498*
Zenaida macroura	0.13859	0.13857	0.03719	0.03284	Leptotila verreauxi	0.01772*	0.00285*	0.01842*	0.01434*
Zenaida auriculata	0.14541	0.14541	0.32555	0.31615	Zenaida asiatica	0.06730	0.01925*	0.11565	0.10233

Table 5. *Continued*

Wing lice

Wing lice	Phylogram		Ultrametric	
	F1	F2	F1	F2
Zenaida galapagoensis	0.12229	0.12228	0.03254	0.02892
Reinwardtoena reinwardtii	0.65526	0.65524	0.91886	0.92173
Macropygia ruficeps	0.18765	0.18763	0.66010	0.65607
Patagioenas fasciata	0.15042	0.15042	0.07117	0.06465
Patagioenas speciosa	0.10359	0.10358	0.01453	0.01255
Patagioenas subvinacea	0.97986	0.97986	0.10580	0.09755
Patagioenas plumbea	0.07594	0.07594	0.02538	0.02205
Columba palumbus	0.14230	0.14227	0.08380	0.07309
Columba livia	0.04722	0.04722	0.07566	0.06580
Columba guinea	0.05858	0.05855	0.52160	0.51535
Streptopelia semitorquata	0.03934	0.03934	0.23935	0.22740
Streptopelia decaocto	0.05605	0.05605	0.50478	0.49799
Streptopelia vinacea	0.03009	0.03009	0.13042	0.12199
Streptopelia capicola	0.02854	0.02853	0.13205	0.12331

Body lice

Body lice	Phylogram		Ultrametric	
	F1	F2	F1	F2
Zenaida macroura	0.00106*	0.00007*	0.00699*	0.00537*
Zenaida auriculata	0.00095*	0.00009*	0.00712*	0.00560*
Zenaida galapagoensis	0.00641*	0.00080*	0.03422	0.02821*
Reinwardtoena reinwardtii	0.33631	0.19851	0.64246	0.63398
Macropygia ruficeps	0.33480	0.19545	0.85180	0.85395
Patagioenas fasciata	0.02949	0.00717	0.00037*	0.00029*
Patagioenas speciosa	0.02108*	0.00382*	0.00046*	0.00028*
Patagioenas subvinacea	0.00508*	0.00077*	0.00069*	0.00050*
Patagioenas plumbea	0.00399*	0.00068*	0.00217*	0.00150*
Columba palumbus	0.78127	0.72592	0.19800	0.18036
Columba livia	0.99839	0.99981	0.18988	0.17219
Columba guinea	0.12180	0.04572	0.00140*	0.00099*
Streptopelia semitorquata	0.07935	0.02283*	0.02707*	0.02190*
Streptopelia decaocto	0.70843	0.67464	0.92208	0.92600
Streptopelia decaocto	0.00650*	0.00100*	0.00012*	0.00007*
Streptopelia vinacea	0.00387*	0.00052*	0.00010*	0.00009*
Streptopelia capicola	0.00307*	0.00041*	0.00011*	0.00007*

*Significant after correcting for false discovery rate ($\alpha = 0.05$). Hosts are listed left of the *P*-values. Hosts listed more than once indicate multiple species of lice associated with that particular host.

the results are perhaps a result of congruence between whole clades rather than between specific links within each clade. If the relationships between the clades contribute significantly to the global statistic, removing a single host–parasite link from a clade would alter the global statistic. Because this is how PARAFIT calculates the individual link statistics, each link in the small New World ground dove clade could potentially be significant.

Our PARAFIT analyses with the small New World ground doves removed indicate that some level of taxonomic bias may be a reality in our data set. Although our global PARAFIT statistics were once again significant in the reduced data set, the results from the individual link tests were more consistent with previous studies. Body lice had at least twice as many significant links as wing lice in all scenarios (Table 1). Additionally, the results were fairly consistent among tree types (phylogram and ultrametric) and test statistics (F1 and F2) (Table 5). In general, the full data sets were not nearly as consistent, which indicates the small New World ground doves and associated lice were driving the results, possibly because of a clade representation bias.

Signals of host–parasite cospeciation in a taxonomically biased sample may be primarily attributable to clade-limited host switching, where parasites utilizing a geographically, ecologically, and/or phylogenetically similar group of hosts preferentially switches within that particular host group. This can produce a false signal of host–parasite phylogenetic congruence (de Vienne, Giraud & Shykoff, 2007). Similar effects have been observed in primate viruses (Charleston & Robertson, 2002) and brood parasitic finches (Sorenson, Balakrishnan & Payne, 2004). Small New World ground doves are in a monophyletic group and are similar in size, and most forage for small seeds in brushy habitat (Gibbs et al., 2001; Sweet & Johnson, 2015). Because of these shared attributes, the wing and body lice of these doves may be able to switch within the host clade, although they are limited in switching to hosts outside of the clade as a result of host body size or the habitat proximity of the host species. Although these lice are switching hosts, the switching events are limited to the small New World ground dove clade, perhaps contributing to host–parasite congruence in the absence of strict cospeciation.

The results from JANE differed from the results from PARAFIT (Figs 2, 3; Tables 3, 4). However, JANE is an event-based method, and so the results from PARAFIT are not completely analogous. Event-based analyses reconcile host and parasite phylogenies by reconstructing cospeciation and duplication events at nodes and sorting and host-switching events along branches, rather than estimating the statistical significance of particular host–parasite associations. The results from JANE are more consistent with previous research, with more cospeciation events being recovered in the body louse analysis (22) than the wing louse analysis (15). The results from JANE are also more consistent with the results from PARAFIT with respect to the analyses without small New World ground doves and their lice. If the ground dove/lice clades are indeed biasing the results from PARAFIT, then the results from JANE (i.e. event-based) might give a more accurate portrayal of the evolutionary history within these groups. It is likely that event-based methods such as JANE are more resistant to clade representation biases because JANE reconstructs events along every node and branch of the tree, even within clades.

EXTERNAL FACTORS DRIVING COPHYLOGENETIC PATTERNS

Although we found no evidence of significantly correlated cophylogenetic patterns between dove wings and body lice, the worldwide sampling highlights external factors potentially associated with cophylogenetic patterns. For example, a stronger signal of cospeciation in most of the body louse data sets alludes to phoresis behaviour in wing lice, as described previously (Harbison et al., 2008, 2009; Harbison & Clayton, 2011). The results of the present study show that his phenomenon could be operating at a worldwide scale.

Many of the host species consistently showing evidence of cospeciation with both their wing and body lice are phabines native to Australia and/or New Guinea. The phabines are a clade that includes Geopelia doves, Geophaps pigeons, Petrophassa rock pigeons, Phaps (bronzewings), and Ocyphaps lophotes (crested pigeon). Although the hosts are native to the same region, geography alone does not explain these patterns because some Australian species did not have evidence of cospeciation with their parasites [e.g. Lopholaimus antarcticus (topknot pigeon)]. As with the small New World ground doves and their lice, clade-limited host switching may play a role in generating these patterns of cospeciation. Similar to small New World ground doves, Australian phabines are terrestrial foraging birds that prefer open, scrubby habitat (Gibbs et al., 2001). These habitat preferences may limit opportunities for phabine lice to switch to hosts outside of the clade. However, in contrast to the small New World ground doves and their lice, phabine body lice are not monophyletic. In addition, our event-based analyses recovered several nodes of cospeciation in the phabine clade, whereas only a few nodes of cospeciation were recovered in the small New World ground dove clade. Taken together, these two

differences indicate that clade-limited host switching may be less of a factor in the Australasian phabine clade, and that any signal of cospeciation originates from actual topological congruence between phabines and their lice.

CONCLUSIONS

Based on our results from both topology-based and event-based cophylogenetic analyses, there is no strong evidence for correlated cophylogenetic patterns between the wing and body lice of pigeons and doves. Despite finding no overall correlation, we did identify potentially interesting patterns within smaller groups. Because neither the wing lice, nor body lice showed perfect patterns of cospeciation with their hosts, we would expect external factors to shape the observed patterns of parasitism. As proposed in previous studies, differences in the ability to switch hosts because of differences in the use of hippoboscid flies for phoresis may drive differences between wing and body lice. However, geography, host life history, and host phylogeny are all important factors shaping the relationship between host and parasite.

Unlike previous studies, we found that both wing and body lice had evidence for cospeciation with their hosts and that body lice did not have substantially more associations contributing to this signal than wing lice. However, when we removed the small New World ground doves and their associated lice from the PARAFIT analyses, the results are more in line with previous studies and predictions from ecological differences. The results were also more consistent across analyses, which was not the case with the ground dove data included. Such results highlight the importance of considering phylogenetic scale and taxa representation in cophylogenetic analysis. The results drawn from subsets of these taxa may show varying patterns dependent on the sampling level.

Host–parasite interactions are complex systems. Understanding how different factors influence the dynamics of host–parasite relationships may ultimately depend on the scale and density of taxonomic sampling. With a large and geographically extensive data set of pigeons and doves and their wing and body lice, we were able to reveal cophylogenetic patterns previously hidden by less representative sampling and, in doing so, further our understanding of the possible life-history and geographical factors driving the patterns. In addition, we highlight the possible pitfalls of cophylogenetic analyses and demonstrate the importance of identifying the proper level of taxon sampling and relative clade representation in such studies.

REFERENCES

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society* **57:** 289–300.

Bruyndonckx N, Dubey S, Ruedi M, Christe P. 2009. Molecular cophylogenetic relationships between European bats and their ectoparasitic mites (Acari, Spinturnicidae). *Molecular Phylogenetics and Evolution* **51:** 227–237.

Bush SE, Malenke JR. 2008. Host defence mediates interspecific competition in ectoparasites. *Journal of Animal Ecology* **77:** 558–564.

Charleston MA, Robertson DL. 2002. Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. *Systematic Biology* **51:** 528–535.

Clayton DH. 1991. Coevolution of avian grooming and ectoparasite avoidance. In: Loye JE, Zuk M, eds. *Bird–parasite interactions: ecology, evolution, and behavior*. Oxford: Oxford University Press, 258–289.

Clayton DH, Johnson KP. 2003. Linking coevolutionary history to ecological process: doves and lice. *Evolution* **57:** 2335–2341.

Clayton DH, Walther BA. 2001. Influence of host ecology and morphology on the diversity of Neotropical bird lice. *Oikos* **94:** 455–467.

Clayton DH, Lee P, Tompkins D, Brodie I. 1999. Reciprocal natural selection on host–parasite phenotypes. *American Naturalist* **154:** 261–270.

Clayton DH, Moyer BR, Bush SE, Jones TG, Gardiner DW, Rhodes BB, Goller F. 2005. Adaptive significance of avian beak morphology for ectoparasite control. *Proceedings of the the Royal Society of London Series B, Biological Sciences* **272:** 811–817.

Clayton DH, Koop JAH, Harbison CW, Moyer BR, Bush SE. 2010. How birds combat ectoparasites. *The Open Ornithology Journal* **3:** 41–71.

Conow C, Fielder D, Ovadia Y, Libeskind-Hadas R. 2010. Jane: a new tool for the cophylogeny reconstruction problem. *Algorithms for Molecular Biology* **5:** 16.

Cruickshank RH, Johnson KP, Smith VS, Adams RJ, Clayton DH, Page RD. 2001. Phylogenetic analysis of partial sequences of elongation factor 1alpha identifies major groups of lice (Insecta: Phthiraptera). *Molecular Phylogenetics and Evolution* **19:** 202–215.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9:** 772.

Dobson A, Lafferty KD, Kuris AM, Hechinger RF, Jetz W. 2008. Colloquium paper: homage to Linnaeus: how many parasites? How many hosts? *Proceedings of the National Academy of Sciences of the United States of America* **105:** 11482–11489.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32:** 1792–1797.

Eichler WD. 1948. Some rules in ectoparasitism. *Annals and Magazine of Natural History* **1:** 588–598.

Fahrenholz H. 1913. Ectoparasiten und abstammungslehre. *Zoologischer Anzeiger* **41:** 371–374.

Gibbs DE, Cox E, Cox J. 2001. *Pigeons and doves: a guide to the pigeons and doves of the world*. Crowborough: Pica Press.

Goodwin D. 1983. *Pigeons and doves of the world*, 3rd edn. Ithaca, NY: Cornell University Press.

Gorrell JC, Schulte-Hostedde AI. 2008. Patterns of parasitism and body size in red squirrels (*Tamiasciurus hudsonicus*). *Canadian Journal of Zoology* **86:** 99–107.

Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27:** 221–224.

Hafner MS, Nadler SA. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. *Nature* **332:** 258–259.

Hafner MS, Nadler SA. 1990. Cospeciation in host-parasite assemblages: comparative analysis of rates of evolution and timing of cospeciation events. *Systematic Biology* **39:** 192–204.

Hafner MS, Page RD. 1995. Molecular phylogenies and host-parasite cospeciation: gophers and lice as a model system. *Philosophical Transactions of the Royal Society of London* **349:** 77–83.

Hafner MS, Sudman PD, Villablanca FX, Spradling TA, Demastes JW, Nadler SA. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science* **265:** 1087–1090.

Harbison CW, Clayton DH. 2011. Community interactions govern host-switching with implications for host-parasite coevolutionary history. *Proceedings of the National Academy of Sciences of the United States of America* **108:** 9525–9529.

Harbison CW, Bush SE, Malenke JR, Clayton DH. 2008. Comparative transmission dynamics of competing parasite species. *Ecology* **89:** 3186–3194.

Harbison CW, Jacobsen MV, Clayton DH. 2009. A hitchhiker's guide to parasite transmission: the phoretic behaviour of feather lice. *International Journal for Parasitology* **39:** 569–575.

Hughes J, Kennedy M, Johnson KP, Palma RL, Page RDM. 2007. Multiple cophylogenetic analyses reveal frequent cospeciation between pelecaniform birds and Pectinopygus lice. *Systematic Biology* **56:** 232–251.

Jackson AP, Machado CA, Robbins N, Herre EA. 2008. Multi-locus phylogenetic analysis of neotropical figs does not support co-speciation with the pollinators : the importance of systematic scale in fig/wasp cophylogenetic studies. *Symbiosis* **45:** 16.

Johnson KP. 2004. Deletion bias in avian introns over evolutionary timescales. *Molecular Biology and Evolution* **21:** 599–602.

Johnson KP, Clayton DH. 2000. A molecular phylogeny of the dove genus *Zenaida*: mitochondrial and nuclear DNA sequences. *The Condor* **102:** 864–870.

Johnson KP, Clayton DH. 2003. Coevolutionary history of ecological replicates: comparing phylogenies of wing and body lice to Columbiform hosts. In: Page RDM, ed. *Tangled trees: phylogeny, cospeciation, and coevolution*. Chicago, IL: University of Chicago Press, 260–286.

Johnson KP, Clayton DH. 2004. Untangling coevolutionary history. *Systematic Biology* **53:** 92–94.

Johnson KP, Weckstein JD. 2011. The Central American land bridge as an engine of diversification in New World doves. *Journal of Biogeography* **38:** 1069–1076.

Johnson KP, Adams RJ, Clayton DH. 2001a. Molecular systematics of Goniodidae (Insecta: Phthiraptera). *The Journal of Parasitology* **87:** 862–869.

Johnson KP, Kort DS, Dinwoodey K, Mateman AC, Cate CT, Lessells CM, Clayton DH. 2001b. A molecular phylogeny of the dove genera *Streptopelia* and *Columba*. *The Auk* **118:** 874–887.

Johnson KP, Williams BL, Drown DM, Adams RJ, Clayton DH. 2002. The population genetics of host specificity: genetic differentiation in dove lice (Insecta: Phthiraptera). *Molecular Ecology* **11:** 25–38.

Johnson KP, Adams RJ, Page RDM, Clayton DH. 2003. When do parasites fail to speciate in response to host speciation? *Systematic Biology* **52:** 37–47.

Johnson KP, Bush SE, Clayton DH. 2005. Correlated evolution of host and parasite body size: tests of Harrison's Rule using birds and lice. *Evolution* **59:** 1744–1753.

Johnson KP, Reed DL, Hammond Parker SL, Kim D, Clayton DH. 2007. Phylogenetic analysis of nuclear and mitochondrial genes supports species groups for Columbicola (Insecta: Phthiraptera). *Molecular Phylogenetics and Evolution* **45:** 506–518.

Johnson KP, Malenke JR, Clayton DH. 2009. Competition promotes the evolution of host generalists in obligate parasites. *Proceedings of the the Royal Society of London Series B, Biological Sciences* **276:** 3921–3926.

Johnson KP, Weckstein JD, Bush SE, Clayton DH. 2011a. The evolution of host specificity in dove body lice. *Parasitology* **138:** 1730–1736.

Johnson KP, Weckstein JD, Meyer MJ, Clayton DH. 2011b. There and back again: switching between host orders by avian body lice (Ischnocera: Goniodidae). *Biological Journal of the Linnean Society* **102:** 614–625.

Johnson KP, Shreve SM, Smith VS. 2012. Repeated adaptive divergence of microhabitat specialization in avian feather lice. *BMC Biology* **10:** 52.

Legendre P, Desdevises Y, Bazin E. 2002. A statistical test for host–parasite coevolution. *Systematic Biology* **51:** 217–234.

Maddison WP, Maddison DR. 2011. Mesquite: a modular system for evolutionary analysis. Available at: http://mesquiteproject.org

Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B. 2011. How many species are there on earth and in the ocean? *PLoS Biology* **9:** 1–8.

Nelson BC, Murray MD. 1971. The distribution of Mallophaga on the domestic pigeon (*Columba livia*). *International Journal for Parasitology* **1:** 21–29.

Page RDM. 1994. Parallel phylogenies: reconstructing the history of host–parasite assemblages. *Cladistics* **10:** 155–173.

Page RDM. 2003. *Tangled trees: phylogeny, cospeciation, and coevolution*. Chicago, IL: University of Chicago Press.

Page RDM, Cruickshank RH, Dickens M, Furness RW, Kennedy M, Palma RL, Smith VS. 2004. Phylogeny of 'Philoceanus complex' seabird lice (Phthiraptera: Ischnocera) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **30:** 633–652.

Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20:** 289–290.

Paterson AM, Wallis GP, Wallis LJ, Gray RD. 2000. Seabird and louse coevolution: complex histories revealed by 12S and rRNA sequences and reconciliation analyses. *Systematic Biology* **49:** 383–399.

Pereira SL, Johnson KP, Clayton DH, Baker AJ. 2007. Mitochondrial and nuclear DNA sequences support a Cretaceous origin of Columbiformes and a dispersal-driven radiation in the Paleocene. *Systematic Biology* **56:** 656–672.

Poulin R. 1997. Species richness of parasite assemlages: evolution and patterns. *Annual Review of Ecology and Systematics* **28:** 341–358.

Poulin R. 2007. Are there general laws in parasite ecology? *Parasitology* **134:** 763–776.

Poulin R, Morand S. 2000. The diversity of parasites. *The Quarterly Review of Biology* **75:** 277–293.

Poulin R, Morand S. 2004. *Parasite biodiversity*. Washington, DC: Smithsonian Institution Press.

Price RD, Hellenthal RA, Palma RL, Johnson KP, Clayton DH. 2003. *The chewing lice: world checklist and biological overview*. Champaign, IL: Illinois Natural History Survey.

Rambaut A, Drummond AJ. 2007. Tracer, Vol. 1.4. Available at: http://tree.bio.ed.ac.uk/software/tracer/

Rothschild M, Clay T. 1952. *Fleas, flukes, and cuckoos: a study of bird parasites*, 3rd edn. London: Collins.

Sorenson MD, Balakrishnan CN, Payne RB. 2004. Clade-limited colonization in brood parasitic finches (*Vidua* spp.). *Systematic Biology* **53:** 140–153.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Sugiura N. 1978. Further analysts of the data by akaike' s information criterion and the finite corrections. *Communications in Statistics – Theory and Methods* **7:** 13–26.

Sweet AD, Johnson KP. 2015. Patterns of diversification in small New World ground doves are consistent with major geologic events. *The Auk* **132:** 300–312.

de Vienne DM, Giraud T, Shykoff JA. 2007. When can host shifts produce congruent host and parasite phylogenies? A simulation approach. *Journal of Evolutionary Biology* **20:** 1428–1438.

de Vienne DM, Refregier G, Lopez-Villavicencio M, Tellier A, Hood ME, Giraud T. 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist* **198:** 347–385.

Waite JL, Henry AR, Clayton DH. 2012. How effective is preening against mobile ectoparasites? An experimental test with pigeons and hippoboscid flies. *International Journal for Parasitology* **42:** 463–467.

Weckstein JD. 2004. Biogeography explains cophylogenetic patterns in toucan chewing lice. *Systematic Biology* **53:** 154–164.

Windsor DA. 1998. Most of the species on Earth are parasites. *International Journal for Parasitology* **28:** 1939–1941.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Maximum likelihood phylogeny of (A) doves, (B) dove wing lice, and (C) dove body lice.
Table S1. Sampling matrices for doves and their (A) wing lice and (B) body lice (left: hosts; right: associated louse samples).