Comparative cophylogenetics of Australian phabine pigeons and doves (Aves: Columbidae) and their feather lice (Insecta: Phthiraptera) ☆

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ABSTRACT

Host–parasite coevolutionary histories can differ among multiple groups of parasites associated with the same group of hosts. For example, parasitic wing and body lice (Insecta: Phthiraptera) of New World pigeons and doves (Aves: Columbidae) differ in their cophylogenetic patterns, with body lice exhibiting higher phylogenetic congruence with their hosts than wing lice. In this study, we focus on the wing and body lice of Australian phabine pigeons and doves to determine whether the patterns in New World pigeons and doves are consistent with those of pigeons and doves from other regions. Using molecular sequence data for most phabine species and their lice, we estimated phylogenetic trees for all three groups (pigeons and doves, wing lice and body lice), and compared the phabine (host) tree with both parasite trees using multiple cophylogenetic methods. We found a pattern opposite to that found for New World pigeons and doves, with Australian wing lice showing congruence with their hosts, and body lice exhibiting a lack of congruence. There are no documented records of hippoboscid flies associated with Australian phabines, thus these lice may lack the opportunity to disperse among host species by attaching to hippoboscid flies (phoresis), which could explain these patterns. However, additional sampling for flies is needed to confirm this hypothesis. Large differences in body size among phabine pigeons and doves may also help to explain the congruence of the wing lice with their hosts. It may be more difficult for wing lice than body lice to switch among hosts that vary more dramatically in size. The results from this study highlight how host–parasite coevolutionary histories can vary by region, and how local factors can shape the relationship.

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1. Introduction

Parasitic organisms are ubiquitous in most biological systems. Their ability to occupy a variety of niches has resulted in great diversity and many independent transitions from free-living to parasitic lifestyles (Poulin and Morand, 2000; Poulin, 2011; Poulin and Randhawa, 2013). Some organisms parasitize many different hosts throughout their life cycles, and may even have a free-living life stage (Gandon and Poulin, 2004; Banks and Paterson, 2005; Belzile and Gosselin, 2015). Other parasites are more tightly associated with their hosts, spending their entire life cycle on a single host and being limited to a particular species or group of hosts (Rohde, 1979; Hafner et al., 1994; Hafner and Page, 1995; Proctor,

2003). In cases in which parasite reproduction is heavily linked to the host, the diversification patterns (phylogenies) of these obligate parasites may mirror those of their hosts. In these cases, when a host undergoes speciation, its obligate parasites may also cospeciate, causing the parasite phylogeny to be congruent with the host phylogeny (Fahrenholz, 1913; Eichler, 1948). However, this expectation is rarely observed in nature. Although some obligate parasite groups exhibit patterns of congruence with their host's phylogeny, most exhibit some level of incongruence generated by host switching, duplication or sorting events during their evolutionary history with their hosts (Page, 1994; Page and Charleston, 1998). The degree of incongruity can vary among different host groups, and even among different groups of parasites associated with the same group of hosts (Whiteman et al., 2007; Toon and Hughes, 2008; Bueter et al., 2009; Stefka et al., 2011).

The feather lice (Insecta: Phthiraptera: Philopteridae) of pigeons and doves (Aves: Columbidae) are an example of obligate parasites that have varying levels of congruence between host and parasite phylogenies. Pigeons and doves harbor two types (ecomorphs) of

feather lice: wing and body lice (Johnson et al., 2012). These two groups are not closely related, and their morphologies differ dramatically (Cruickshank et al., 2001). Wing lice are long and slender, and insert themselves between wing and tail feather barbs to avoid removal by host preening. In contrast, body lice are round and escape preening by burrowing into the downy feathers close to their host body (Clayton, 1991; Clayton et al., 2005, 2010). However, both types of lice eat the downy feathers of their hosts (Nelson and Murray, 1971). Comparative cophylogenetic analysis of wing and body lice from New World pigeons and doves indicates that body lice have a fairly congruent evolutionary history with their hosts, whereas wing lice exhibit less congruence and do not show evidence for cospeciation (Clayton and Johnson, 2003; Johnson and Clayton, 2004). The body lice of pigeons and doves are also more host-specific than wing lice, meaning that wing louse species are more often associated with multiple host species (Johnson et al., 2002). This difference may be due, in part, to the greater ability and incidence of wing lice using hippoboscid flies for transport (phoresis) within and among host species (Keirans, 1975; Harbison et al., 2008; Harbison and Clayton, 2011). Experimental studies have indicated that wing lice are much more likely than body lice to successfully switch hosts using this behavior (Harbison et al., 2009). Globally across Columbidae, both groups of lice do show significant congruence with the host phylogeny; however, it is unclear how much of this congruence is due to shared biogeographic patterns (Sweet et al., 2016). It is important, therefore, to examine congruence within additional biogeographic regions to determine whether patterns observed within New World taxa also hold for other regional host–parasite faunas.

In this study we focus on the wing and body lice of phabine pigeons and doves, a monophyletic group of birds from Australia and southeastern Asia (Johnson and Clayton, 2000b; Pereira et al., 2007). By exploring the cophylogenetic patterns of a distinct group of birds and their lice, we can test whether the patterns these taxa exhibit are similar to those exhibited by New World taxa. Phabines are a monophyletic group of 15 species in the genera *Phaps*, *Geophaps*, *Ocyphaps*, *Petrophassa*, *Geopelia* and *Leucosarcia* (Pereira et al., 2007). Most representatives are primarily terrestrial and prefer arid, open scrub, or dry forest habitats (Goodwin, 1983; Gibbs et al., 2001). However, some species (*Leucosarcia melanoleuca* and *Geopelia humeralis*) occupy more humid, wetter habitats. As with other terrestrial doves, phabines primarily forage on small seeds and fruits. All phabine wing lice belong to the genus *Columbicola* (Price et al., 2003) whereas phabine body lice are classified into three genera (*Campanulotes*, *Coloceras* and *Physconelloides*).

We sampled most representatives of phabines together with their wing and body lice, focusing particularly on species from continental Australia. From these samples we sequenced or used existing sequences of multiple molecular loci, and used these sequences to estimate molecular phylogenies for all three groups. We then performed several cophylogenetic analyses to test for congruence between the phylogenies of phabine pigeons and doves, and those of their wing and body lice.

2. Materials and methods

2.1. Sampling and sequencing

We obtained samples for 12 species of Australian phabine pigeons and doves and their wing (12 samples) and body lice (15 samples). For outgroup taxa, we used available GenBank sequences of *Columbina passerina*, *Zenaida macroura*, *Ptilinopus rivoli* and *Chalcophaps indica* for hosts, *Columbicola passerinae* (ex. Inca dove (*Columbina inca*)) for wing lice, and *Goniocotes talegallae* (ex.

black-billed brushturkey (*Talegalla fuscirostris*)) for body lice. Muscle tissue was extracted from birds collected in the field and stored at −80 °C. Lice were collected in the field with pyrethrin powder or fumigation protocols (Clayton and Drown, 2001) and stored in 95% ethanol at −80 °C. DNA was extracted from bird tissue using a Qiagen Blood and Tissue Kit (Qiagen, Valencia, CA, USA) with standard protocols. DNA was extracted from individual louse specimens using a modified Qiagen protocol, with louse specimens incubating in a proteinase K/buffer solution at 55 °C for ∼48 h. PCR was used to target genes for Sanger sequencing, using a Promega taq kit (Promega, Madison, WI, USA) according to recommended protocols. PCR products were purified with a Qiagen PCR Purification Kit according to standard protocols. For birds, 381 bp of the mitochondrial gene cytochrome oxidase subunit 1 (*Cox*1), 1,074 bp of NADH dehydrogenase subunit 2 (ND2), and 1,172 bp of the nuclear gene beta-fibrinogen intron 7 and flanking exon regions (FIB7) were sequenced. For wing lice, 383 bp of *Cox*1, 379 bp of 12S rRNA (12S), and 360 bp of the nuclear gene elongation factor 1α (EF-1α) were sequenced. For body lice, 383 bp of *Cox*1, 362 bp of EF-1α, and 553 bp of 16S rRNA (16S) were sequenced. Sequencing primers and amplification protocols were used as outlined in Johnson and Clayton (2000a,b), and Johnson et al. (2007, 2011b). Resulting PCR products were sequenced with an ABI Prism BigDye Terminator kit (Applied Biosystems, Foster City, CA, USA), and fragments were run on an AB 3730x capillary sequencer at the University of Illinois Roy J. Carver Biotechnology Center (Champaign, IL, USA). Resulting complementary chromatograms were manually resolved and primer sequences removed in Sequencher v.5.0.1 (Gene Codes, Ann Arbor, MI, USA) or Geneious v.8.1.2 (Biomatters, Auckland, NZ). We submitted all resulting sequence files to GenBank (Supplementary Table S1).

2.2. Phylogenetic analysis

All genes were aligned using the default parameters of the MAFFT plugin in Geneious (Katoh et al., 2002) and each resulting alignment was checked manually. For protein coding loci, alignments were trimmed to be within reading frame. Maximum-likelihood (ML) phylogenies were estimated using RAxML v.8.1.17 (Stamatakis, 2006) for each gene alignment, using 200 rapid bootstrap replicates (−f a) and GTR + Γ (GTRGAMMA) nucleotide substitution models. Finally, for each data set (doves, wing lice, and body lice) the gene alignments were concatenated in Geneious. PartitionFinder v.1.1.1 (Lanfear et al., 2012) was used to test for appropriate partitioning schemes and substitution models for the concatenated alignments, setting up potential partition schemes according to genes and using the corrected Akaike Information Criterion (AICc) to test for the best fitting substitution models (Sugiura, 1978). PartitionFinder searched through all possible models, and again only through models available in MrBayes. Partition and model results are listed in Table 1.

Partitioned ML and Bayesian analyses were run for the concatenated alignments in all three data sets. ML estimations were run in Garli v.2.0 (Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin, USA) with two searches of 500 bootstrap replicates, and summarizing the bootstrap trees using SumTrees in the DendroPy package (Sukumaran, J., Holder, M., 2008. SumTrees: Summarization of split support on phylogenetic trees v.1.0.2). Best trees for each concatenated alignment were also estimated using Garli. Bayesian estimations were run in MrBayes v.3.2 (Ronquist and Huelsenbeck, 2003). Two runs of four Markov chain Monte Carlo (MCMC) chains were run for 20 million generations, sampling every 1000 trees. Resulting .p files were viewed in Tracer

Table 1
Best fitting substitution models for each partition as estimated by PartitionFinder.

ML		MrBayes	
Partition	Model	Partition	Model
Phabine doves			
Cox1	GTR + I + Γ	Cox1	GTR + I + Γ
ND2	GTR + I + Γ	ND2	GTR + I + Γ
FIB7	HKY + I + Γ	FIB7	HKY + I + Γ
Wing lice			
12S	GTR + Γ	12S	GTR + Γ
Cox1	TrN + I + Γ	Cox1	GTR + I + Γ
EF-1α	K80 + Γ	EF-1α	K80 + Γ
Body lice			
16S	GTR + Γ	16S	GTR + Γ
Cox1	TIM + I + Γ	Cox1	GTR + Γ
EF-1α	HKY + I	EF-1α	HKY + I

ML, maximum likelihood; Cox1, mitochondrial cytochrome oxidase subunit 1; ND2, NADH dehydrogenase subunit 2; FIB7, nuclear beta-fibrinogen intron 7; EF-1α, elongation factor 1α; 12S, 12S rRNA; 16S, 16S rRNA.

v.1.5 (http://tree.bio.ed.ac.uk/software/tracer/) to assess parameter convergence, and .t files were analyzed in RWTY v.1.0.0 (Warren, D., Geneva, A., Lanfear, R., 2016. rwty: R We There Yet? Visualizing MCMC convergence in phylogenetics. v.1.0.0) to assess topological convergence. Based on these assessments the first 10% (2000) trees were discarded as a burnin.

2.3. Taxonomic assessment

Properly defining host and parasite taxonomic units is critical for cophylogenetic analysis. Because avian lice often harbor cryptic species, it was necessary to more objectively evaluate the number of species in the wing and body louse data sets. To this end we used distances values and the Automatic Barcode Gap Discovery (ABGD) method for wing and body louse Cox1 sequence data (Puillandre et al., 2012). Uncorrected pairwise distance matrices were generated from the wing and body louse Cox1 alignments using the "dist.dna" command in the R package *ape* (Paradis et al., 2004; R Development Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing.). The Cox1 alignment was then used as input for the web version of ABGD (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html), applying default Pmin, Pmax, and Steps parameters and adjusting the relative gap width to 1.0. ABGD was run with both Jukes-Cantor (JC) and Kimura80 (K80) models. Based on these taxonomic assessments, the louse trees were trimmed accordingly before using those as input for cophylogenetic analyses.

2.4. Cophylogenetic analysis

We implemented both distance-based and event-based cophylogenetic methods. For distance-based analysis we used ParaFit (Legendre et al., 2002). This method takes host and parasite distances matrices and a host–parasite association matrix as input to test for congruence between the two trees. It also tests for the contribution of each individual link to the global patterns. For ParaFit, the best host and parasite trees from the Garli analyses were converted to patristic distance matrices using the "cophenetic" command in *ape*, and the resulting distance matrices were ordered according to the association matrix. ParaFit was then run for 999 permutations with the Cailliez correction for negative eigenvalues, and the contribution of each individual link to the global metric was tested with the ParaFitLink1 and ParaFitLink2 statistics. Because the individual link statistics are multiple tests, false discovery rate was corrected for using the Benjamini–Hochberg cor-

rection (Benjamini and Hochberg, 1995). To account for poorly supported relationships, ParaFit was also run as described above with 50% majority-rule host and parasite consensus trees. Consensus trees were generated with SumTrees from the Garli bootstrap analyses.

For the event-based analysis, we used Jane v.4 (Conow et al., 2010). Instead of testing for global congruence and individual link contributions, Jane is a genetic algorithm (GA) that seeks to reconstruct evolutionary events (e.g. cospeciation, host switches) at the nodes and branches of the host and parasite trees. Jane reconstructed events using the recommended GA parameters of population size twice the number of generations (number of generations = 500, population size = 1000) and default event costs (cospeciation = 0, duplication = 1, duplication and host switch = 2, loss = 1, and failure to diverge = 1). Jane also randomized the tip mappings 999 times to test for the probability of obtaining our observed overall cost. An observed cost significantly lower than the randomized costs would indicate global host–parasite congruence.

To test whether lice switch between hosts of similar size, we reconstructed ancestral host body size using the "ace" command in *ape*, implementing the ML method under the Brownian motion model for continuous traits. The best ML phabine phylogeny from Garli was used as the input tree. Body sizes were assigned to the tree tips as the average mass (g) from Gibbs et al. (2001). After running the state reconstruction, the absolute difference in average host size was calculated between two host nodes/tips involved in a host switch based on the Jane analyses. These values were then averaged separately for the wing and body louse switches.

3. Results

The body louse matrix was 88% complete, with three samples missing EF-1α and 16S sequences. There were no missing data for the wing lice or phabine hosts (Supplementary Table S1). Statistics on individual loci are indicated in Table 2. The concatenated dove alignment was 2627 bp, concatenated body louse alignment 1298 bp, and concatenated wing louse alignment 1122 bp.

3.1. Phylogenetic analysis

Likelihood and Bayesian analyses of the birds (Fig. 1) provided strong support for monophyly of the phabines and of most genera (*Geophaps*, *Petrophassa* and *Geopelia*), and moderate support for monophyly of *Phaps*. Relationships among genera were weakly

Table 2
Summary statistics for each locus sequenced for phabine pigeons and doves, phabine wing lice, and phabine body lice.

Locus	Length (bp)	Variable sites	Parsimony-informative sites
Phabine doves			
*Cox*1	381	121	92
ND2	1074	501	343
FIB7	1172	248	56
Wing lice			
12S	379	170	107
*Cox*1	383	157	134
EF-1α	360	84	39
Body lice			
16S	553	221	144
*Cox*1	383	142	117
EF-1α	362	29	14

*Cox*1, mitochondrial cytochrome oxidase subunit 1; ND2, NADH dehydrogenase subunit 2; FIB7, nuclear beta-fibrinogen intron 7; EF-1α, elongation factor 1α; 12S, 12S rRNA; 16S, 16S rRNA.

supported. The wing louse phylogenies (Fig. 2) indicated, with the exception of *Geophaps*, that lice from the same host genus formed monophyletic groups, with support ranging from rather weak (*Phaps*) to strong (*Geopelia*, *Petrophassa*). Relationships among these major groups of wing lice were generally poorly supported. In contrast to the phylogeny of wing lice, the phylogeny of body lice (Fig. 3) did not contain any monophyletic groups of lice confined to a single host genus. In addition, even within clades, the phylogeny of body lice was relatively uncorrelated with host taxonomy.

All individual gene trees from each data set did not have any well-supported conflicting nodes (Supplementary Figs. S1–S3). For all three concatenated data sets, the partitioned ML and Bayesian analyses estimated similar tree topologies. In all cases, the MCMC chains from the Bayesian analyses had parameter effective sample size (ESS) values >200 and average S.D. of split frequencies <0.01, indicating that the analyses converged to stationarity.

3.2. Cophylogenetic analysis

Analysis of the uncorrected p-distances of *Cox*1 and ABGD indicated in two cases that two body louse samples should be collapsed to a single taxon: *Coloceras* sp. from *Geopelia placida* and *Coloceras* sp. from *Geopelia cuneata*, and *Physconelloides australiensis* from *Petrophasa albipennis* and *Geophaps smithii*. The latter result agrees with current taxonomic treatment of these lice (Price et al., 2003; Johnson et al., 2011b). The *Cox*1 sequences within each pair were identical (Supplementary Table S2), and the ABGD analysis likewise indicated that each pair should be considered a single taxon. We did not find any support for collapsing tips of the wing louse phylogeny. Mean uncorrected p-distance
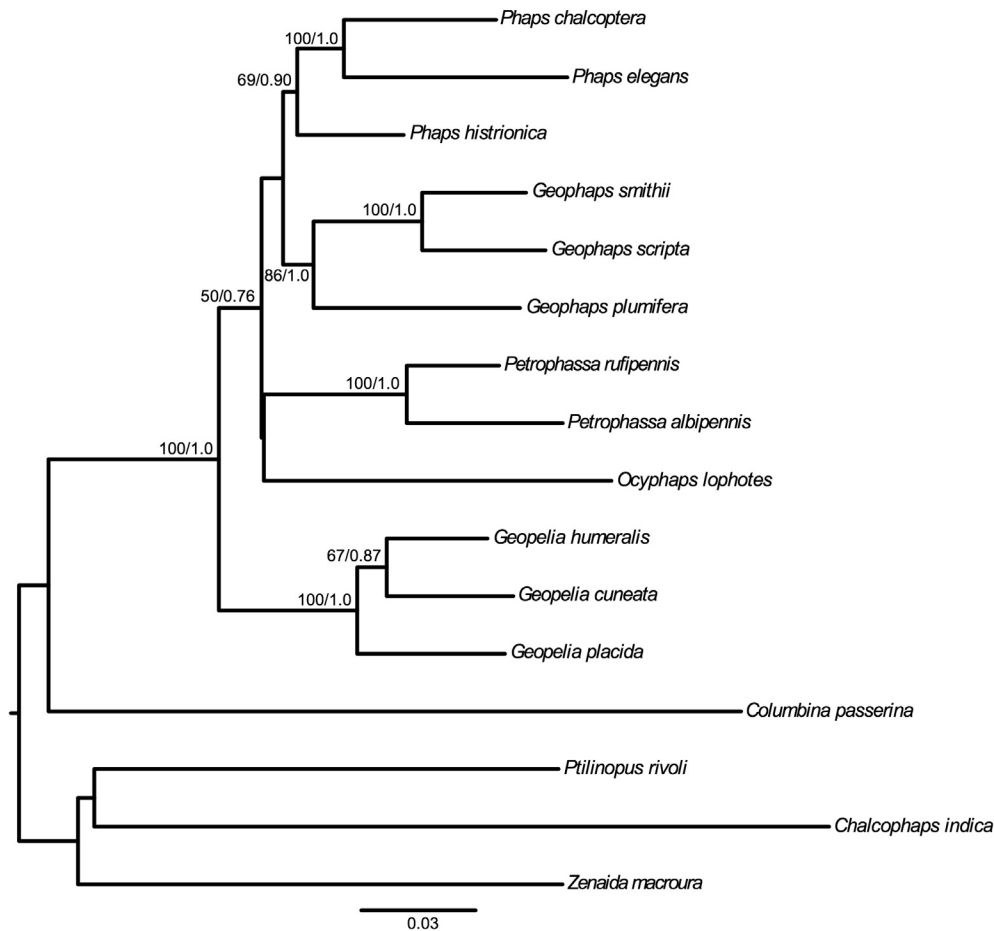
Fig. 1. Best maximum likelihood phylogeny of phabine pigeons and doves. Values at nodes are bootstrap values from Garli and posterior probability values from MrBayes (bootstrap/posterior probability). Only values >50 bootstrap/>0.50 posterior probability support are indicated. Branch lengths are nucleotide substitutions per site, as indicated by the scale bar.
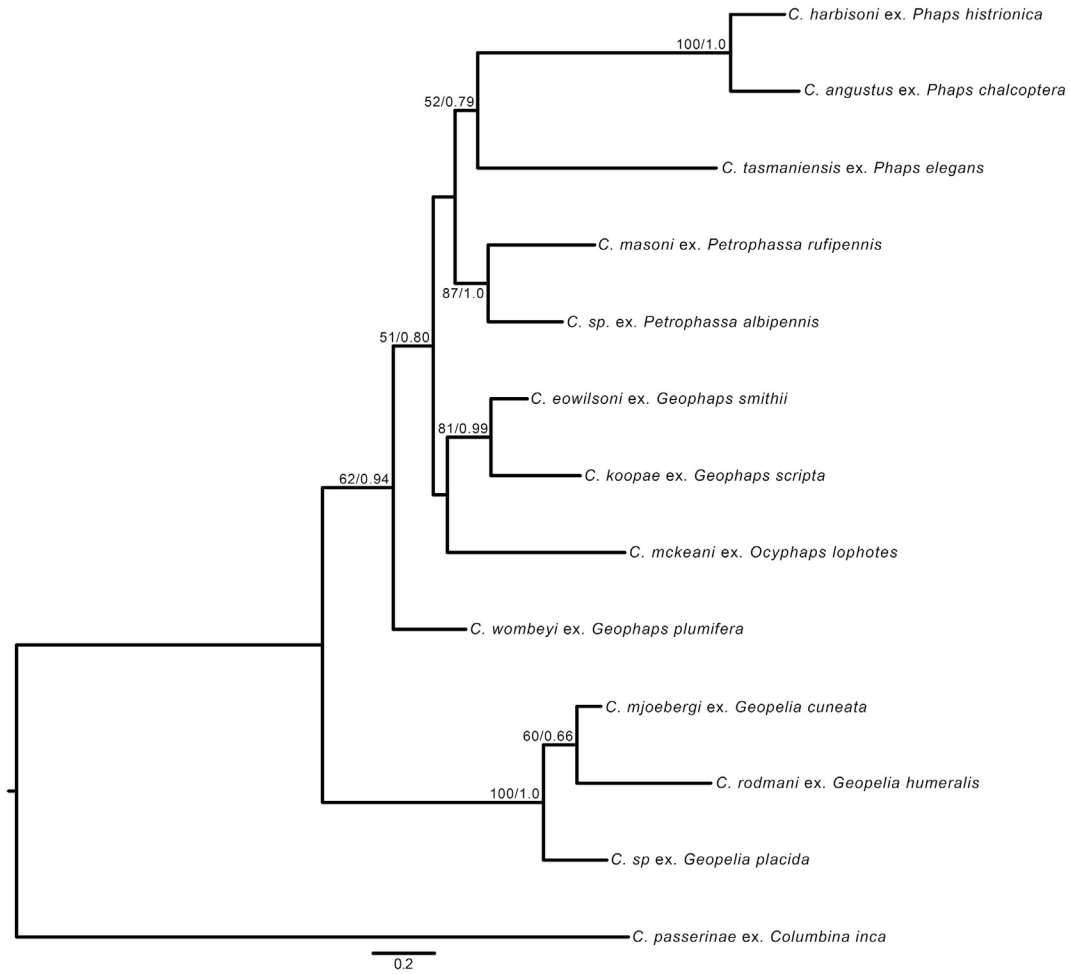
Fig. 2. Best maximum likelihood phylogeny of wing lice from phabine pigeons and doves (*C.*, *Columbicola*). Values at nodes are bootstrap values from Garli and posterior probability values from MrBayes (bootstrap/posterior probability). Only values >50 bootstrap/>0.50 posterior probability support are indicated. Branch lengths are nucleotide substitutions per site, as indicated by the scale bar.

between all pairs of taxa was >12% (Supplementary Table S3). Most species of wing lice in our dataset have been previously described (only two samples are undescribed species), in contrast to our body louse data set (11 samples are undescribed species).

Using the most likely host and parasite species phylogenies as input, ParaFit did not reject the null hypothesis of a random association between phabines and their body lice (ParaFitGlobal = 0.041, *P* = 0.069). The individual link tests (ParaFitLink1 and ParaFitLink2) did not recover any significant links after correcting for false discovery rate with the Benjamini–Hochberg correction (Table 3). ParaFit also indicated random association using the 50% majority rule consensus trees (ParaFitGlobal = 0.023, *P* = 0.081), and did not recover any significant links after correction (Supplementary Table S4). The Jane event-based reconstruction recovered only three potential cospeciation events between phabines and their body lice: one cospeciation event at the *G. cuneata*/*G. humeralis* split, one at the *Geophaps scripta*/*G. smithii* split, and a third at the *Phaps*/*Geophaps* split (Fig. 4). Jane also recovered eight host-switching events, one duplication, four losses and two failures to diverge, for a total cost of 23 (Table 4). Other reconstructions with an identical total cost recovered zero duplications, nine host switches, three losses and two failures to diverge. The Jane randomization test indicated the observed cost was not lower than by chance (*P* = 0.161), suggesting no congruence between the phylogenetic trees of phabine body lice and their hosts. From the ancestral state reconstruction of host size, the average absolute

difference in host size between phabine nodes/tips involved in body louse host switches was 111.69 g.

Comparing the most likely phabine host and wing louse phylogenies, ParaFit indicated global phylogenetic congruence between the two groups. The ParaFitGlobal test indicated a non-random host–parasite association (ParaFitGlobal = 0.322, *P* = 0.005), and the ParaFit individual link tests included three significant links after correction, all between *Geopelia* doves and their wing lice (Table 5). ParaFit also indicated a significant global association between the 50% majority-rule consensus trees (ParaFitGlobal = 0.496, *P* = 0.004), and significant links between *Geopelia* and their wing lice. In addition, the consensus analysis recovered a significant link between *Ocyphaps lophotes* and its wing louse (Supplementary Table S5). Similarly, Jane recovered eight cospeciation events between phabines and their wing lice (Fig. 5). The reconstruction recovered cospeciation events at both *Geopelia* splits, one at the *Phaps chalcoptera*/*Phaps elegans* split, at the *G. smithii*/ *G. scripta* split, at the *Petrophassa rufipennis*/*P. albipennis* split, the *Petrophassa*/*Ocyphaps* split, and at the two deepest nodes. Jane also recovered three host-switching events and two losses between phabines and their wing lice, for a total cost of 8 (Table 4). The average absolute difference in host size for phabine nodes/tips involved in wing louse host switches was 41.67 g. The Jane randomization test indicated that the observed cost was significantly lower than by chance (*P* < 0.001), suggesting a history of repeated cospeciation in this group of lice.
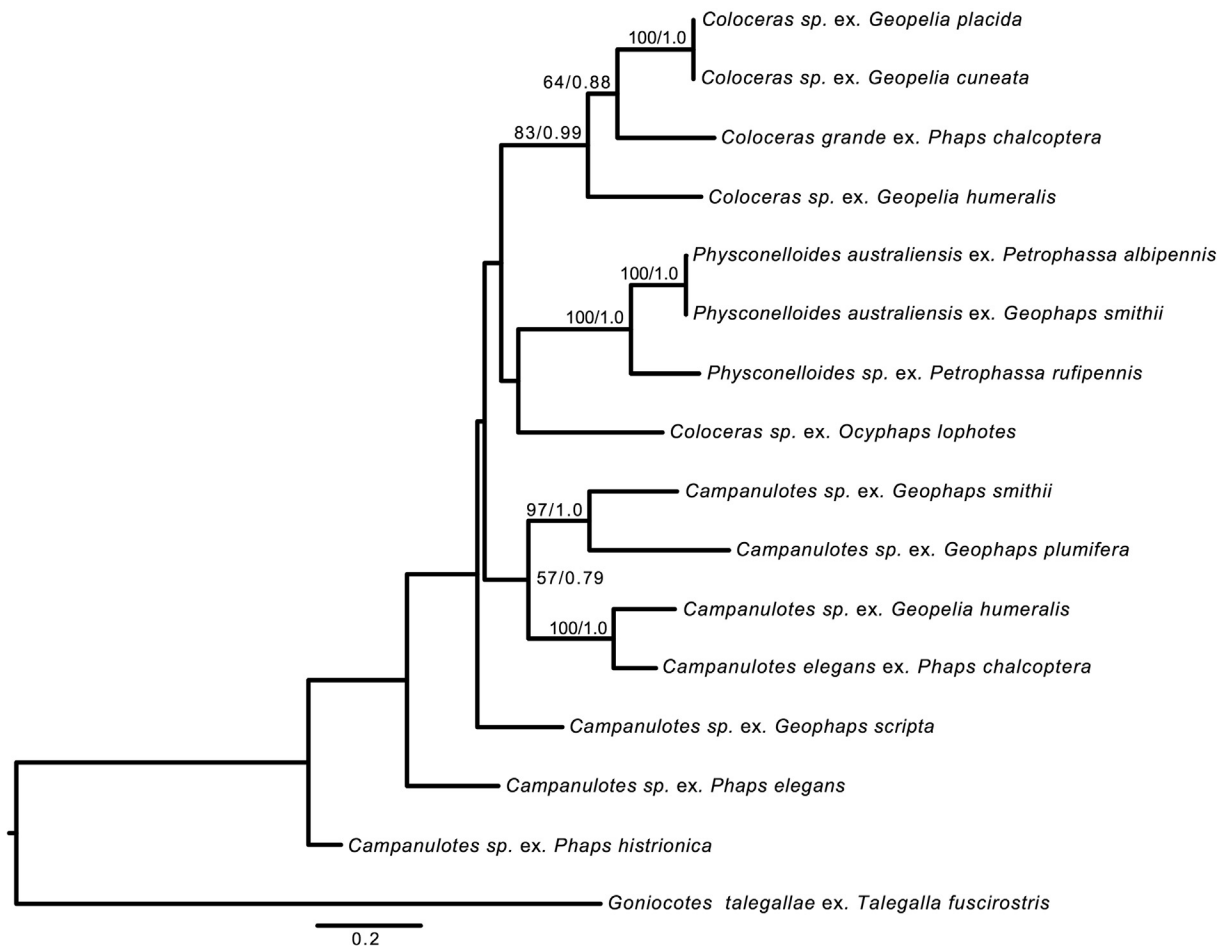
Fig. 3. Best maximum likelihood phylogeny of body lice from phabine pigeons and doves. Values at nodes are bootstrap values from Garli and posterior probability values from MrBayes (bootstrap/posterior probability). Only values >50 bootstrap/>0.50 posterior probability support are indicated. Branch lengths are nucleotide substitutions per site, as indicated by the scale bar.

Table 3
ParaFit individual link test statistics and *P* values for phabine pigeons and doves and their body lice.

Host	Body louse	ParaFitLink1 Stat	ParaFitLink1 *P* value	ParaFitLink2 Stat	ParaFitLink2 *P* value
Geopelia cuneata	*Coloceras* sp.	0.0089	0.0185	0.0057	0.0186
Geopelia humeralis	*Campanulotes* sp.	−0.0012	0.9443	−0.0008	0.9448
Geopelia humeralis	*Coloceras* sp.	0.0074	0.0397	0.0047	0.0396
Geopelia placida	*Coloceras* sp.	0.0086	0.0207	0.0055	0.0205
Geophaps plumifera	*Coloceras* sp.	0.0048	0.2435	0.0031	0.2398
Geophaps scripta	*Campanulotes* sp.	0.0009	0.2649	0.0006	0.2615
Geophaps smithii	*Physconelloides australiensis*	<0.0001	0.7487	<0.0001	0.7487
Geophaps smithii	*Campanulotes* sp.	0.0030	0.2890	0.0019	0.2869
Ocyphaps lophotes	*Coloceras* sp.	0.0039	0.0398	0.0025	0.0381
Petrophassa albipennis	*Physconelloides australiensis*	0.0067	0.0446	0.0043	0.0442
Petrophassa rufipennis	*Physconelloides* sp.	0.0059	0.1090	0.0038	0.1063
Phaps chalcoptera	*Campanulotes elegans*	0.0004	0.6990	0.0003	0.6983
Phaps chalcoptera	*Coloceras grande*	−0.0019	0.9486	−0.0012	0.9493
Phaps elegans	*Campanulotes* sp.	0.0036	0.1527	0.0023	0.1503
Phaps histrionica	*Campanulotes* sp.	0.0015	0.6804	0.0010	0.6785

4. Discussion

Comparisons of molecular phylogenies for Australian phabine pigeons and doves and their wing and body louse parasites revealed that the phylogeny of wing lice was highly congruent with that of their hosts, whereas the phylogeny of body lice was not. These results were consistent with both best and consensus trees, indicating the pattern is not an artifact of poor topology support. This result stands in dramatic contrast to pat-

terns found for New World pigeons and doves and their lice (Clayton and Johnson, 2003; Johnson and Clayton, 2004), in which the phylogeny of body lice generally matched that of their hosts while the phylogeny of wing lice did not. However, in a study with a worldwide sample of pigeons and doves, both wing and body lice showed evidence of cophylogenetic congruence with their hosts (Sweet et al., 2016). The differences between the New World and Australian studies suggest that biogeographic differences may exist in factors that promote congruence in wing
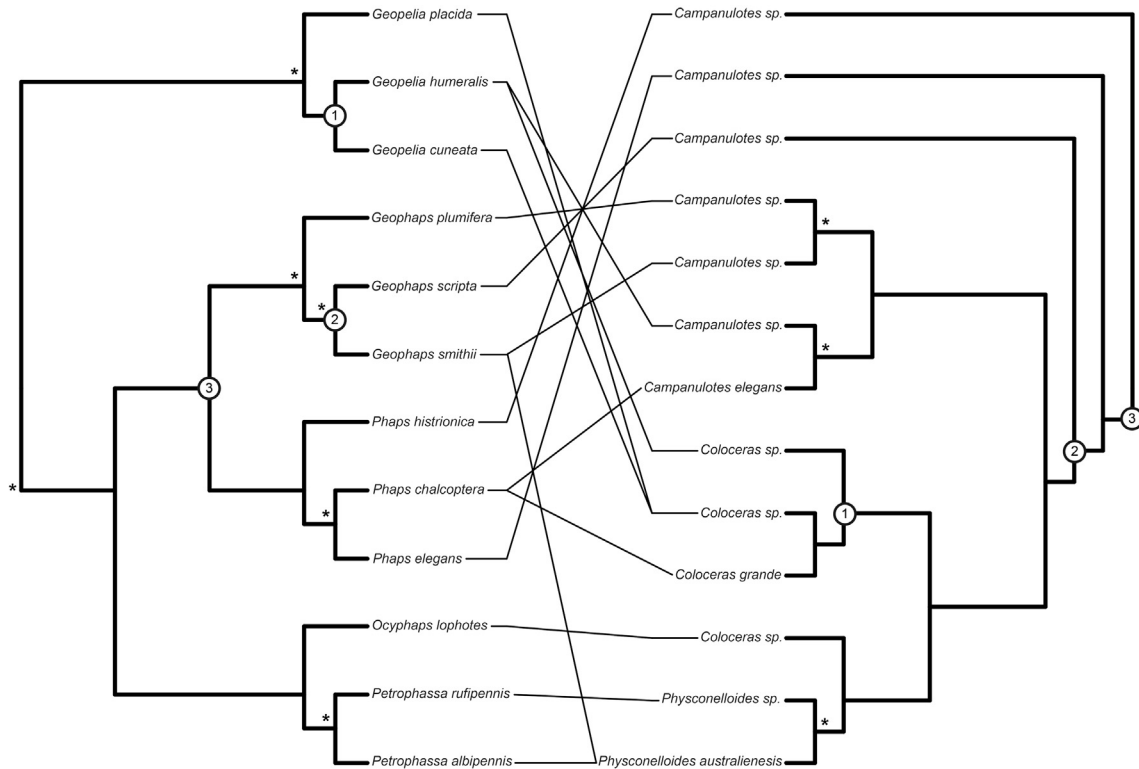
Fig. 4. Tanglegram between phabine pigeons and doves (left) and their body lice (right). Topologies are the best maximum likelihood trees from Garli. Branches with >75 bootstrap/0.95 posterior probability support are indicated with asterisks (∗). Circles over nodes indicate cospeciation events as recovered by Jane, with matching numbers indicating corresponding events in the host and parasite phylogenies.

Table 4
Results from the Jane event-based cophylogenetic reconstruction between phabine pigeons and doves and their lice.

	Cospeciations	Duplications	Host switches	Losses	Failures to diverge	total cost
Body lice	3	1	8	4	2	23
	3	0	9	3	2	23
Wing lice	8	0	3	2	0	8

Table 5
ParaFit individual link test statistics and *P* values for phabine pigeons and doves and their wing lice (*Columbicola*).

| Host	Wing louse	ParaFitLink1 Stat	ParaFitLink1 *P* value	ParaFitLink2 Stat	ParaFitLink2 *P* value
Geopelia cuneata	*C. mjoebergi*	0.1074	0.0044[a]	0.0012	0.0044[a]
Geopelia humeralis	*C. rodmani*	0.1253	0.0095[a]	0.0014	0.0094[a]
Geopelia placida	*C. sp.*	0.0995	0.0096[a]	0.0011	0.0096[a]
Geophaps plumifera	*C. wombeyi*	0.0020	0.4710	<0.0001	0.4700
Geophaps scripta	*C. koopae*	0.0178	0.0444	0.0002	0.0442
Geophaps smithii	*C. eowilsoni*	0.0116	0.0551	0.0001	0.0544
Ocyphaps lophotes	*C. mckeani*	0.0232	0.0514	0.0003	0.0511
Petrophassa albipennis	*C. sp.*	0.0156	0.0453	0.0002	0.0447
Petrophassa rufipennis	*C. masoni*	0.0155	0.1072	0.0002	0.1064
Phaps chalcoptera	*C. angustus*	0.0388	0.1699	0.0004	0.1691
Phaps elegans	*C. tasmaniensis*	0.0352	0.0548	0.0004	0.0540
Phaps histrionica	*C. harbisoni*	0.0195	0.4623	0.0002	0.4611

[a] Statistically significant after correction ($\alpha = 0.05$).

and body lice. Thus, local congruence in some cases may be driving congruence at the global scale. Pigeons and doves are widespread birds, thriving in a variety of ecosystems in every continent other than Antarctica. Due to this geographical and ecological diversity, the evolutionary patterns exhibited by pigeons and doves, and their parasitic lice, may differ among different groups of hosts, especially because parasite diversification can be heavily affected by external factors (e.g. ecology or geography)

(Paterson et al., 2000; Weckstein, 2004; Bush and Malenke, 2008; Bruyndonckx et al., 2009).

Regional differences in congruence may reflect regional differences in the abundance of hippoboscid flies, which wing lice can use to disperse between host species (Harbison et al., 2008). Phabine pigeons and doves are a well-defined group within Columbidae, and most species live in arid scrub or forest on the Australian continent. A reduced abundance of hippoboscid flies in arid rather
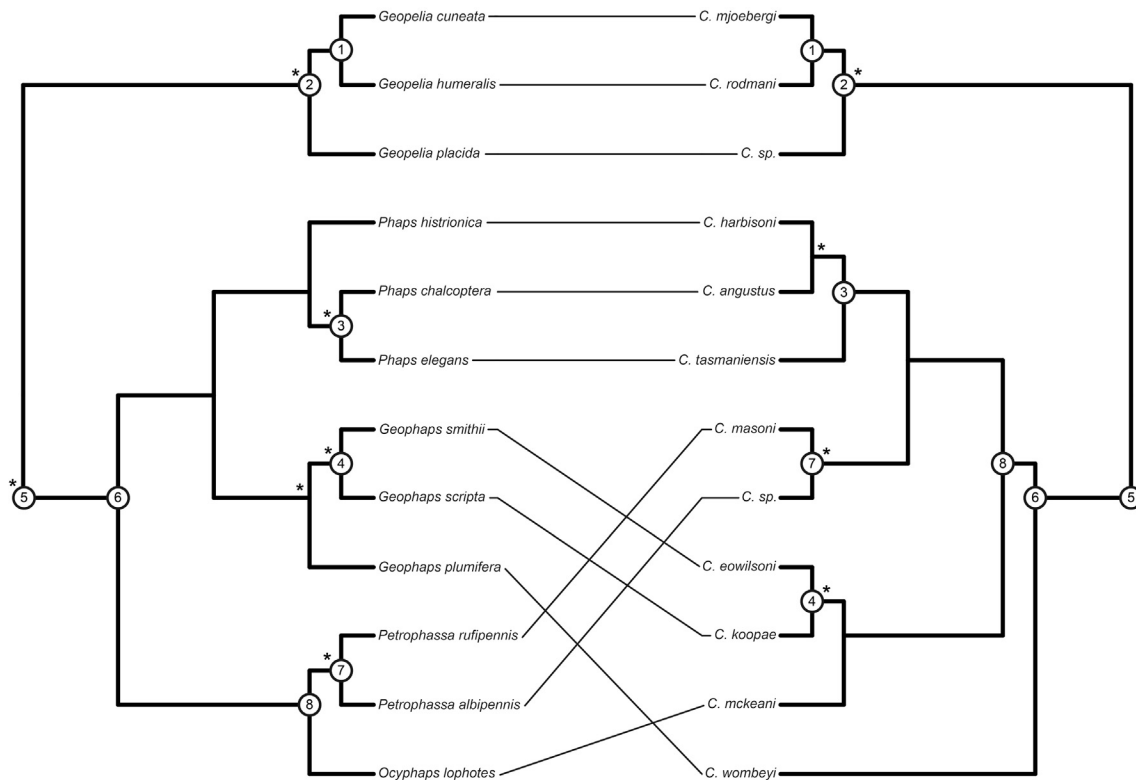
Fig. 5. Tanglegram between phabine pigeons and doves (left) and their wing lice (right). Topologies are the best maximum likelihood trees from Garli. Branches with >75 bootstrap/0.95 posterior probability support are indicated with asterisks (∗). Circles over nodes indicate cospeciation events as recovered by Jane, with matching numbers indicating corresponding events in the host and parasite phylogenies.

than humid regions could explain the congruence of the wing louse phylogeny with hosts in Australia compared with the incongruence with hosts in the New World. It is also possible that hipphoboscid flies rarely parasitize phabines in arid Australia. Although hippoboscid flies have been recorded from other Australian birds and from phabine hosts in the Philippines, we are unaware of any published records of hippoboscid flies associated with Australian phabines, while there are many records from New World pigeons and doves (Maa, 1963, 1969, 1980; Proctor and Jones, 2004; Toon and Hughes, 2008). This difference may be due to sampling effort, so it will be important to sample additional parasites on pigeons and doves in Australia.

In addition to ecological factors, geography may be an important factor governing diversification of phabine wing lice. In particular, there are several cases of clear allopatric codivergences of wing lice with their phabine hosts. For example, two pairs of sister species of phabines (*G. smithii* + *G. scripta* and *P. rufipennis* + *P. albipennis*) are allopatric and appear to have speciated in response to biogeographic barriers. Their wing lice, which are host-specific, also cospeciated according to this allopatric divergence. Host specificity and the lack of dispersal to other host species in the same regions reinforces the pattern of cospeciation in this case. Biogeographic barriers are important for determining cophylogenetic structure, and can either promote congruence, as in phabine wing lice, or promote parasite diversification independent of host speciation. For example, the Andes mountains (Sweet and Johnson, 2016) and Amazonian rivers (Weckstein, 2004) can explain diversification patterns in various groups of bird lice, despite incongruent patterns between many of the host–parasite associations.

Host body size may also be an important factor in reinforcing cospeciation of wing lice with their hosts by limiting host-switching (Clayton et al., 2003, 2016). The body size of wing lice

is closely correlated with that of their hosts, whereas the size of body lice is not (Johnson et al., 2005). Host preening defenses prevent wing lice from switching to hosts much larger or smaller than their usual host. In particular, wing lice must be of the appropriate size to fit between the feather barbs of the primary feathers to escape from host preening. This constraint may have been important in the codivergence of wing lice associated with the genus *Geopelia*. The three *Geopelia* doves represented in our data set have overlapping geographic distributions, yet vary in body size. The sister species *G. humeralis* (110–160 g) and *G. cuneata* (23–37 g) exhibit the greatest difference in size. *Geopelia placida* (36–60 g), which is sister to the other two species, is intermediate in body size (Gibbs et al., 2001). This variability in host size may reinforce phylogenetic congruence between *Geopelia* doves and their wing lice, as lice may not be able to switch to a related host even if the species co-occur.

While there is considerable evidence for cospeciation between phabines and their wing lice, there is also evidence for host-switching. Such events were likely facilitated by similarities in host size and by host geographic overlap, both of which can make it easier for lice to switch host species. For example, Jane recovered a host-switching event from *P. chalcoptera* to *Phaps histrionica*. Body sizes (*P. chalcoptera*: 230–390 g, *P. histrionica*: 260–320 g) and geographic ranges (*P. chalcoptera* widespread throughout Australia; *P. histrionica* primarily in the northern interior of Australia; Gibbs et al., 2001) of these two species overlap considerably.

While ecological and geographic factors may be important for generating congruence between Australian phabines and their wing lice, the same is not true for these hosts and their body lice, which do not appear to have a congruent evolutionary history. Body lice are not known to switch hosts effectively using phoresy on hippoboscid flies (Harbison et al., 2009), and are more often

shared among host species that forage on the ground than among those that forage in the canopy (Johnson et al., 2011a). It may be that dispersal among hosts on the ground is the primary mode of host-switching for phabine body lice, particularly since their hosts are primarily terrestrial. Two species of body lice were found on two different host species (Fig. 4), suggesting that these lice are able to disperse in ecological time among different host species.

Across species, there is much less of a match between the size of body lice and that of their hosts. For example, Jane recovered body lice from the small *G. cuneata* switching to the considerably larger *P. chalcoptera*. This is consistent with previous research, which found that body louse size is not correlated with host size (Johnson et al., 2005). The average differences in host body size between pairs of hosts involved in host switches support this notion. Even when including inferred ancestral host sizes, hosts had a much higher absolute average difference in body size for body louse switches compared with wing louse switches. Unlike wing lice, body lice burrow through the downy feathers to avoid preening, so their mechanism of escape is less tied to host body size. This may facilitate host-switching if there is a dispersal opportunity. A species of phabine can host multiple species of body lice that differ dramatically in size. As with wing lice, host distributional overlap may be an important factor for host-switching by body lice. Jane recovered several host switches along the lineage of body lice from *G. smithii*, which has a relatively small distribution in Australia. All of the host switches, however, involve other species of phabines (*P. rufipennis*, *O. lophotes*, *Geophaps plumifera* and *G. humeralis*) whose ranges overlap that of *G. smithii*. If body lice are indeed switching hosts primarily via ground contact, geographic proximity is necessary for dispersal to a new host species.

Previous studies of the wing and body lice of pigeons and doves in the New World have indicated that body lice exhibit more congruent cophylogenetic patterns with their hosts than do wing lice. However, our study revealed the opposite pattern, with wing lice of Australian phabine pigeons and doves exhibiting more phylogenetic congruence with their hosts than phabine body lice. This result highlights the importance of focusing cophylogenetic analyses on specific groups and biogeographic regions. A broader taxonomic and geographic focus, such as the entire pigeon and dove family (Columbidae) and its lice, can provide insight into general patterns in a group, but will mask narrower patterns if sampling is limited.

The drastic variation in cophylogenetic patterns between the New World dove and Australian phabine systems suggest regional differences in factors that shape these host–parasite interactions. For example, the lack of rampant host-switching in phabine wing lice may indicate that their hosts lack associated parasitic hippoboscid flies that wing lice of other species of pigeons and doves use as a means to switch hosts. This should be investigated with further sampling. Other factors including climate, host body size and host distribution may also influence cophylogenetic patterns. Although phabines are only a moderately diverse group of Columbidae confined to a particular geographic region, comparisons of their phylogeny with those of their lice provide valuable insight into the processes of parasite diversification and host–parasite coevolution.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2016.12.003.

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