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Article

Cophylogenetic assessment of New World warblers (Parulidae) and their symbiotic feather mites (Proctophyllodidae)

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Host–symbiont relationships are ubiquitous in nature, yet evolutionary and ecological processes that shape these intricate associations are often poorly understood. All orders of birds engage in symbioses with feather mites, which are ectosymbiotic arthropods that spend their entire life on hosts. Due to their permanent obligatory association with hosts, limited dispersal and primarily vertical transmission, we hypothesized that the cospeciation between feather mites and hosts within one avian family (Parulidae) would be perfect (strict cospeciation). We assessed cophylogenetic patterns and tested for congruence between species in two confamiliar feather mite genera (Proctophyllodidae: *Proctophyllodes*, *Amerodectes*) found on 13 species of migratory warblers (and one other closely related migratory species) in the eastern United States. Based on COI sequence data, we found three *Proctophyllodes* lineages and six *Amerodectes* lineages. Distance- and event-based cophylogenetic analyses suggested different cophylogenetic trajectories of the two mite genera, and although some associations were significant, there was little overall evidence supporting strict cospeciation. Host switching is likely responsible for incongruent phylogenies. In one case, we documented prairie warblers *Setophaga discolor* harboring two mite species of the same genus. Most interestingly, we found strong evidence that host ecology may influence the likelihood of host switching occurring. For example, we documented relatively distantly related ground-nesting hosts (ovenbird *Seiurus aurocapilla* and Kentucky warbler *Geothlypis formosa*) sharing a single mite species, while other birds are shrub/canopy or cavity nesters. Overall, our results suggest that cospeciation is not the case for feather mites and parulid hosts at this fine phylogenetic scale, and raise the question if cospeciation applies for other symbiotic systems involving hosts that have complex life histories. We also provide preliminary evidence that incorporating host ecological traits into cophylogenetic analyses may be useful for understanding how symbiotic systems have evolved.

Keywords: Acari, birds, coevolution, cophylogenetic analysis, cospeciation, host–symbiont, symbiosis

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The dynamic associations between hosts and their symbionts have intrigued evolutionary ecologists for over a century (Klassen 1992). Over this time, research has made it clear that hosts and symbionts may evolve and diverge in concert, when disruption of gene flow between formerly reproductively connected host populations leads to corresponding disruption of gene flow between symbiont populations. Reproductive isolation of symbionts is expected to be greatest if a symbiont is completely dependent on its host (i.e. obligate) and does not have a dispersal and/or free-living stage (i.e. permanent). Thus, hosts and their permanent obligate symbionts are often considered to be classic examples of cospeciation (Clayton et al. 2004, 2016. Morelli and Spicer 2007). Reciprocal adaptive selection that eventually leads to changes in allele frequencies over time, or "strict" coevolution (de Vienne et al. 2013), may also be expected between hosts and permanent obligate symbionts. Cases where divergence of hosts and symbionts is concurrent and interdependent, or symbionts are "tracking" host divergence without influencing host evolution, this is considered cospeciation (de Vienne et al. 2013). To test for the process of cospeciation, one can construct and compare phylogenies of hosts and symbionts (cophylogenetic analyses; Martínez-Aquino 2016). Cophylogenetic analyses that result in perfect topological congruence between host and symbiont phylogenies provide evidence for strict cospeciation, which is also reflected in 'Fahrenholz's rule' (symbiont phylogenies mirror host phylogenies; Fahrenholz 1913, Eichler 1948).

Although cospeciation between symbionts and their hosts has been inferred in some systems (Hafner and Nadler 1988, Clayton and Johnson 2003, Light and Hafner 2007), the evolutionary processes related to host–symbiont associations may also be nuanced and quite complex (Paterson and Banks 2001, Marussich and Machado 2007, Hendricks et al. 2013, Fraija-Fernández et al. 2016, Doña et al. 2017c, Klimov et al. 2017). In addition to cospeciation, various other events and processes may occur over evolutionary time, including speciation within a host species (i.e. intra-host speciation) or 'duplication' (Page 1994), symbiont extinction or 'loss' (Paterson and Gray 1997), failure of symbionts to diverge (resulting in the presence of the same symbiont on related species of hosts; Johnson et al. 2003), and host switching (a symbiont successfully colonizing a new host species, resulting in unrelated hosts sharing the same symbiont species; Page 2003). In most cases of permanent obligate symbiont–host systems, despite the simple expectation of cospeciation, the processes that have led to current associations remain virtually unknown (de Vienne et al. 2013).

One common symbiotic system is that of feather mites (Acariformes: Astigmata: Analgoidea, Pterolichoidea) and their avian hosts. Feather mites are obligate, ectosymbiotic arthropods that spend their entire life cycle on their host, where they appear to feed mainly on oils, waxes, and fatty acids secreted by the bird's uropygial gland together with the particulate material trapped in the secretions (Proctor 2003). Although our understanding of the life history of feather mites is minimal, they differ from most free-living Astigmata by lacking a dedicated nymphal dispersal stage. Females of some feather mite lineages inhabiting the skin engage in phoresy on more vagile bird-associates, such as hippoboscid flies (Philips and Fain 1991, Proctor and Jones 2004). Vane-dwelling feather mites are thought to be transmitted between host individuals only by direct contact, typically vertically from parent to offspring at the nest (Proctor 2003, Doña et al. 2017a). Rare cases of feather mite horizontal transmission have been documented (Hernandes et al. 2014), and experiments have shown that individual feather mites have the ability to move to heterospecifics when given the opportunity (Dubinin 1951, Bridge 2002). Despite these uncommon events, their permanent obligate nature, limited dispersal abilities, and vertical mode of transmission all contribute to the remarkably specific associations feather mites have with their hosts (Peterson 1975, Atyeo and Gaud 1979, Dabert and Mironov 1999, Doña et al. 2017b), suggesting they should follow strict cospeciation.

Very little is currently known about the evolutionary history of feather mites, including what processes have resulted in current associations between feather mites and their hosts. The few studies that have investigated the cophylogenetic patterns of feather mites and hosts provide support for cospeciation on a broad scale (bird orders and mite families; Dabert and Mironov 1999, Dabert 2005). Although, these previous studies have mainly used cladograms based on morphological characters; rigorous cophylogenetic tests using molecular data have only recently been implemented. At least one study has found similar patterns of cospeciation between feather mites and hosts (Štefka et al. 2011), but these hosts (Galápagos mockingbirds; Mimidae: *Mimus*) were geographically isolated. However, a recent study (Doña et al. 2017c) explored feather mites on European passerines using mitochondrial molecular data and rigorous cophylogenetic tests. They found high incongruence between feather mite and host phylogenies, which strongly supports host-shift speciation, rather than strict cospeciation, as an explanation for the evolutionary relationships between feather mites and hosts (Doña et al. 2017c). Furthermore, another recent, nearly global analysis (Klimov et al. 2017) found that even though feather mite and host (Passeriformes) phylogenies were incongruent (in some cases due to host switches), several ancient synchronous codispersals of mites and birds (for example, from the Old World to the New World) could still be inferred using a double dating approach, where divergence times on both host and mite phylogenies are compared. Nevertheless, studies of feather mites and one fine-scale group (family) of hosts are much rarer, limiting conclusions that can be drawn about cospeciation at different scales.

New World warblers (family Parulidae) are an ideal host group for assessing the level of cospeciation between feather mites and birds for a number of reasons. First, New World warblers went through a recent (compared to the evolutionary history of all extant passerines; Barker et al. 2004) and extensive radiation (Lovette and Bermingham 1999). The resulting assemblage consists of > 100 species with diverse distributions and ecologies (Lovette et al. 2010). This relatively rapid diversification and resulting species assemblage would lead us to

expect that the associated feather mites also became rapidly reproductively isolated (again due to their obligate nature and limited dispersal capabilities), but nothing is known in this regard. Secondly, recent genetic studies of warblers have produced a robust reconstructed phylogeny (Lovette et al. 2010) that provides a useful comparison to feather mite phylogenies. Finally, although extensive databases regarding feather mites and their hosts exist (PBK, HCP, Doña et al. 2016), virtually nothing is currently known about feather mites that inhabit many of these host species, from their identities, to the evolutionary relationships between these two groups, to the processes that may have led to the current associations between warblers and their feather mites.

In this study, we collected feather mites from New World warblers, used molecular techniques, and conducted cophylogenetic analyses to explore the evolutionary relationships between these mites and hosts. The specific objectives of this study were to: 1) build phylogenies for two feather mite lineages that inhabit 13 members of the host family Parulidae (and one other closely related migratory species) using mitochondrial DNA (mtDNA); 2) assess the cophylogenetic signals between warbler hosts and their feather mite symbionts using both distance-based and event-based cophylogenetic analyses; and 3) infer evolutionary and ecological processes that may have led to the current mite–warbler associations that we document. Our null hypothesis is that there is strict cospeciation between the highly specialized, obligate, vertically-transmitted feather mites and their warbler hosts.

Material and methods

Taxonomic and gene sampling

We sampled a total of 60 feather mites from 14 host species. All sampled host species are Neotropical–Nearctic migrants that breed in eastern North America and overwinter across Central and South America. However, they vary widely in their specific geographic distributions and ecological niches, which are life history traits that may affect the ability of mites to inhabit host species, as well as the likelihood of host switching. Live hosts were captured through mist netting in a variety of study locations during the breeding and migratory periods in Arkansas, Missouri, Pennsylvania and Tennessee, USA. Carcasses came mostly from migrating individuals that died from window-collisions in Arkansas or Illinois, USA. From each live bird, we inspected individual feathers of the primary wing tract (nine feathers on each wing) and the rectrix feather tract (12 feathers total) for mites and collected up to one feather from each tract that harbored mites; from each carcass, we inspected both feather tracts and collected all feathers that had mites. After collection, each feather (or feather tract from carcasses) was kept in an individually labeled envelope to prevent cross-contamination. Individual mites were later removed and preserved in 95% ethyl alcohol at –20°C until DNA extraction. Detailed information of specimens used in this study is listed in Supplementary material Appendix 1 Table A1.

We obtained genetic data for host species from the Open Tree of Life (Hinchliff et al. 2015) as deposited by Lovette et al. (2010) using package *rotl* ver. 3.0.1 (Michonneau et al. 2016) in R ver. 3.3.1 (<www.r-project.org>). The yellow-breasted chat *Icteria virens*, a previously 'non-core' parulid (Lovette et al. 2010), but now in its own family, Icteriidae (Barker et al. 2013, 2015, Chesser et al. 2017), was used as the host outgroup to root the tree. We focused our sampling of feather mites to two genera from the superfamily Analgoidea: *Amerodectes* and *Proctophyllodes* (both in family Proctophyllodidae). Sequences for feather mites came from the mtDNA cytochrome *c* oxidase subunit I (COI); this gene is useful for distinguishing and identifying feather mite species (Doña et al. 2015). *Gabucinia* sp. (Pterolichoidea: Gabuciniidae) from the San Blas jay, *Cyanocorax sanblasianus*, was used as a distant outgroup for initial exploratory analyses of feather mites (GenBank accession no.: KU203072).

DNA extraction, amplification and sequencing

Mitochondrial DNA was isolated from individual mite specimens using the QIAamp DNA Micro Kit (Qiagen) following the manufacturer's protocol for tissue samples with several modifications: 1) while in a drop of buffer ATL, a small hole was pierced into the mite using a sterile mounting needle before transferring to 180 µl of buffer ATL; 2) proteinase K was added but not pulse-vortexed as mites can easily get stuck on the tube wall and cannot be dislodged by centrifugation; 3) samples were incubated (lysed) for 24 h; 4) after adding buffer AL, samples were inverted instead of pulse-vortexed and were placed on a heat block (70°C) for 5 min; 5) no carrier RNA was added; 6) chilled ethanol was used; 7) DNA bound to the membrane was eluted to 22 µl of buffer AE and incubated at room temperature for 5 min; 8) the final centrifugation step was extended to 2 min at 14 000 rpm. After extraction, DNA was stored at –20°C. Cuticles of mite specimens were mounted on slides in PVA as primary vouchers after DNA extraction. Feather mite specimens and DNA vouchers are deposited at Arkansas State Univ. in Jonesboro, AR, USA.

A portion of the COI gene (1190 bp) was amplified using a nested PCR, which is useful for low concentrations of template DNA, as is the case when using a single mite specimen. For the first reaction, the 20 µl PCR mix contained 2.0 µl of 10× PCR buffer (1.0X), 1.4 µl of 50 mM $MgSO_4$ (3.5 mM), 1.4 µl of dNTPs (10 mM each; 0.7 mM), 0.8 µl of each 10 µM forward and reverse primer, 0.12 µl (0.6 U) of Platinum *Taq* Polymerase, and typically 1 µl of DNA template (this was increased to 3 µl when bands were absent or faint during gel electrophoresis) per sample. Distilled water was used to make up the remaining volume. For the first reaction, COX1_16F (TGANTWTTTTCHACWAAYCAYAA) and COX1_1324R (CDGWRTAHCGDCGDGGTAT) degenerate primers were used. The following thermocycling profile was used: initial denaturation of 2 min at 94°C, followed by 10 cycles of 30 s at 94°C, 1 min at 40°C, 1 min 40 s at 72°C, then 25 cycles of 30 s at 94°C, 35 s at 48°C, 2 min at 72°C, with a final extension of 7 min at 72°C.

For the second (nested) reaction, the 20 µl PCR mix was the same as above except it contained 0.08 µl (0.4 U) of Platinum *Taq* Polymerase, and typically 0.4 µl or rarely 0.55 µl of PCR product from the first reaction per sample. For the nested reaction, COX1_25Fshort_T (TCHACWAAYCAYAARRAYA) and COX1_1282R_T (CCWVYTARDCCTARRAARTGTTG) degenerate primers were used. In these primers, M13 forward (M13FORW; TGTAAAACGACGGCCAGT) and reverse (M13REV; CAGGAAACAGCTATGACC) tails were included for uniform downstream sequencing. The following thermocycling profile was used: initial denaturation of 2 min at 94°C, followed by 20 cycles of 30 s at 94°C, 35 s at 49°C, 2 min at 72°C, then 18 cycles of 30 s at 94°C, 35 s at 52°C, 2 min at 72°C, with a final extension of 7 min at 72°C. This protocol is similar to that described in Klimov and OConnor (2008). All reagents were from Invitrogen Corporation (USA). PCR products from the nested reaction were visualized on 1.5% agarose gels, purified using QIAquick Gel Extraction Kit (Qiagen), and sequenced in both directions by the Univ. of Michigan DNA Sequencing Core on an Applied Biosystems 3730 XL DNA Analyzer. Forward and reverse sequences were assembled in MacVector 15.1.4 (Int. Biotechnologies, New Haven, CT, USA).

Phylogenetic and morphological analyses

Sequences were aligned and viewed using Mesquite ver. 3.2 (Maddison and Maddison 2017). All sequences were checked for potential contaminants using the NCBI BLAST database (Altschul et al. 1997). Sequences are deposited in GenBank under accession no. KY491577-KY491636 (Supplementary material Appendix 1 Table A1).

We first conducted an exploratory analysis using RAxML ver. 8.2.9 (Stamatakis 2014) under a GTR+I+Γ model of nucleotide evolution to identify taxonomic placement for our feather mite lineages using 137 feather mite COI sequences obtained from GenBank. We then separated our sequences (and all further analyses) based on feather mite genus: *Amerodectes* and *Proctophyllodes*. Genetic distances were calculated using the Kimura 2-Parameter (K2P) model (Kimura 1980) in 'ape' ver. 4.0 (Paradis et al. 2004) to conduct an exploratory, threshold-based species delimitation with a cut-off value of 4.0% (Smith et al. 2007), which is more conservative than standard species thresholds using COI (Hebert et al. 2003). An even more conservative cut-off value of 3.4% has been found to be best in delimiting feather mites specifically (Doña et al. 2015), which we also followed. Additional genetic distances were calculated in 'ape' and RAxML under the best-ranked model of nucleotide evolution, which was selected based on the lowest corrected Akaike information criterion (AIC_c) value using jModelTest ver. 2.1.10 (Guindon and Gascuel 2003, Darriba et al. 2015). We used a GTR+I+Γ model of nucleotide evolution for *Amerodectes*, and we used TrN (TN93)+Γ for *Proctophyllodes*. Interspecific and intraspecific genetic distances for *Amerodectes* and *Proctophyllodes* are in Table 1 and 2, respectively.

Maximum likelihood (ML) trees were created in R using the packages 'ape' and 'phangorn' ver. 2.1.1 (Schliep 2011) under the best-ranked model for each genus. Support values for ML trees were estimated with 100 bootstrap replicates. Bayesian trees were created in *BEAST ver. 2.4.4 (Bouckaert et al. 2014) using a relaxed lognormal molecular clock and Yule speciation priors under the best-ranked model for each genus. Although *BEAST typically uses multilocus data to infer a species tree, we used a single locus because inference is still possible using one gene (Heled and Drummond 2010, McCormack et al. 2011) and experimental simulations have shown *BEAST to be comparable to other methods to infer species trees, even when using a single locus (Ogilvie et al. 2016). *Amerodectes* Bayesian trees were estimated from multiple separate Markov chain Monte Carlo (MCMC) analyses, which were run for over 1.7 billion generations with parameters sampled every 5000 steps, for a total of over 356 000 post-burn-in generations. Burn-in percentages (between 10–60%) were selected individually for each independent run in Tracer ver. 1.6.0 (Rambaut et al. 2014). Species and gene trees produced by *BEAST had similar, but

Table 1. Genetic distances for *Amerodectes*. The values in the bold diagonal are the intraspecific K2P distances. Below this bold diagonal are the interspecific K2P distances, and above the diagonal are the genetic distances under the best model (GTR+I+Γ).

		Amerodectes sp1	*Amerodectes* sp2	*Amerodectes* sp3	*Amerodectes* sp4	*Amerodectes hribari*	*Amerodectes seiurus*
Amerodectes sp1	Min	**0.004**	0.249	0.147	0.145	0.167	0.136
	Max	**0.023**	0.293	0.167	0.156	0.190	0.167
Amerodectes sp2	Min	0.170	**0.002**	0.259	0.240	0.246	0.205
	Max	0.184	**0.018**	0.280	0.255	0.261	0.243
Amerodectes sp3	Min	0.128	0.166	**0.004**	0.082	0.089	0.086
	Max	0.139	0.175	**0.016**	0.088	0.096	0.098
Amerodectes sp4	Min	0.129	0.162	0.092	**0.000**	0.092	0.066
	Max	0.134	0.169	0.097	**0.000**	0.096	0.074
Amerodectes hribari	Min	0.136	0.163	0.093	0.094	**0.008**	0.091
	Max	0.148	0.171	0.099	0.098	**0.008**	0.098
Amerodectes seiurus	Min	0.121	0.148	0.092	0.077	0.093	**0.002**
	Max	0.138	0.164	0.104	0.084	0.101	**0.012**

Table 2. Genetic distances for *Proctophyllodes*. The values in the bold diagonal are the intraspecific K2P distances. Below this bold diagonal are the interspecific K2P distances, and above the diagonal are the genetic distances under the best model (TrN+Γ).

		Proctophyllodes quadratus	*Proctophyllodes "basal quadratus"*
Proctophyllodes quadratus	Min	**0.014**	0.113
	Max	**0.029**	0.130
Proctophyllodes "basal quadratus"	Min	0.101	**0.007**
	Max	0.115	**0.011**

slightly different topologies. *Proctophyllodes* Bayesian trees were also estimated from multiple separate MCMC analyses, which were run for over 840 million generations with parameters sampled every 5000 steps, totaling over 168 000 post-burn-in (all 10%) generations. LogCombiner ver. 2.1.3 (part of BEAST 2.1.3 package) was used to combine independent runs, and Tracer was used to assess chain convergence. Effective sample size values of each parameter exceeded 200. Maximum clade credibility trees with median node heights were calculated in TreeAnnotator ver. 2.1.2 (part of BEAST 2.1.3 package) and visualized in FigTree ver. 1.4.3 (Rambaut 2016).

For morphological identification and to confirm phylogenetic species delimitation, we chose representative individual mites from each host species from each clade on the trees produced. In addition to the primary voucher specimen, we slide mounted additional mites (in general, males) from the corresponding host feather samples for further morphological identification. Morphological identification was conducted by Drs. Sergey Mironov (Zoological Inst., Russian Academy of Sciences) and HCP. Samples that could not be morphologically identified to an already known species group (for example, due to lack of males, which are diagnostically important) were designated a unique lineage name (in Arabic numerals), based both on morphology and phylogenetic placement. Because species separation was straightforward (i.e. clusters with morphological differences separated by long branches), we did not use more complicated species delimitation analyses or methodologies (Zhang et al. 2013, Yang 2015).

Investigating cophylogenetic signal

We used both morphological and molecular evidence to identify unique feather mite lineages in order to avoid including duplicate taxa in cophylogenetic analyses, and haplotypes were grouped within parents. For *Amerodectes* cophylogenetic analyses, we used both the Bayesian species tree and gene tree to compare inferences because topologies were slightly different; the ML gene tree was topologically identical to the Bayesian species tree. For *Proctophyllodes*, we used only the Bayesian gene tree, as there were only two species groups and we could not perform cophylogenetic analyses with only the two species. We pruned mite gene trees so that no two sister tips were from the same host species. We excluded outgroups in these analyses. We performed various tests to assess cophylogenetic signal in our system. Distance-based methods included ParaFit (Legendre et al. 2002) and PACo (Balbuena et al. 2013) in R using the packages 'ape' and 'vegan' ver. 2.4.1 (Oksanen et al. 2016). TreeFitter ver. 1.0 (Ronquist 2002) and Jane ver. 4 (Conow et al. 2010) were used as event-based approaches.

ParaFit takes the host phylogeny, symbiont phylogeny, a host–symbiont association matrix, and then transposes these all into a fourth-corner matrix (Legendre et al. 1997) to derive a global host–symbiont statistic (Legendre et al. 2002). Each phylogeny is described by a pairwise patristic distance matrix, which is then transformed into a matrix of principal coordinates (PCo). ParaFit tests a null hypothesis that the two taxa are randomly associated and that their evolution has been independent of one another ($\alpha = 0.05$). To assess how individual links contribute to the overall fit (congruence) of the phylogenies and associations, ParaFit also produces two statistics for individual links (ParaFitLink1 and 2: 'F1' and 'F2'); F1 is more commonly used and more appropriate for multi-host symbionts (Legendre et al. 2002). Significant links indicate that if the link is removed, the overall congruence of the system decreases. We ran ParaFit 100 times with 999 permutations within each run with a 'cailliez' correction (the alternative 'lingoes' correction produced comparable results) for negative eigenvalues. The permutations within each ParaFit run randomize each host–symbiont association (therefore testing the null hypothesis of random host–symbiont associations). We corrected the 100 F1 individual link p-values using the Benjamini–Hochberg correction for false discovery rate (Benjamini and Hochberg 1995), as suggested by Sweet and Johnson (2016).

PACo has a similar conceptual framework as ParaFit, using both phylogenies (also as pairwise patristic distances transformed into PCo matrices) and a host–symbiont association matrix (Balbuena et al. 2013). Then the symbiont phylogeny is transformed by a Procrustean superimposition upon the host phylogeny, which scales it to fit the host phylogeny (by minimizing the differences with the host PCo matrix). The Procrustean superimposition yields a residual sum of squares (m^2_{XY}), which is inversely proportional to the topological congruence of the host and symbiont. In order to obtain a null distribution to compare m^2_{XY} with, hosts are randomly assigned to symbionts by permutation (100 000 with a 'cailliez' correction for negative eigenvalues) in the host–symbiont association matrix. The statistic represents whether host speciation drives speciation of the symbionts (as opposed to ParaFit which tests whether the host and parasite associations are random), which is an appropriate expectation for obligate, vertically transmitted symbionts that may track their hosts' speciation processes ($\alpha = 0.05$). PACo also applies a jackknife procedure that estimates m^2_{XY} (and 95% confidence intervals) for each individual association that contributes to the overall congruence between the phylogenies. Lower individual m^2_{XY} values indicate a smaller contribution to the overall m^2_{XY}, thus a stronger cophylogenetic link.

Both ParaFit and PACo illustrate the overall congruence between host and symbiont topologies, as well as the contribution of each individual host–symbiont link to the overall congruence. However, these distance-based methods do not infer evolutionary events that may have occurred. Therefore, event-based methods are also informative for reconstructing the evolutionary history between taxa.

TreeFitter is a parsimony-based method that explores different evolutionary event (i.e. cospeciation, intra-host speciation, extinction, and host switching) cost combinations, and attempts to minimize the overall (global) cost of the coevolutionary history between the host and symbiont. For each combination of cost structures, TreeFitter produces a *p* value that indicates whether the fit of those cost combinations is greater or less than expected by chance. To determine the cost structures that best describe our feather mite data, we ran exploratory analyses of all cost combinations for every 0.5 increment between 0 and 10. We then tuned our cost structures separately based on these exploratory results for all trees. However, TreeFitter does not appropriately account for multihost symbionts.

Jane ver. 4 uses heuristics to map the biological events that could possibly contribute to the phylogenetic congruence of the two taxa (i.e. cospeciation, intra-host speciation with and without host switching, extinction, and failure to diverge) of the symbiont phylogeny onto the host phylogeny, and aims to minimize the overall cost with given a priori event costs. The number of 'generations' (iterations) was set to 100, with a 'population size' (number of different solutions in each iteration) of 100. We initially set event costs to the defaults: 0 for cospeciation, 1 for intra-host speciation, 1 for extinction, and 1 for failure to diverge and 2 for intra-host speciation with host switch. The event costs chosen strongly influence the outcome of the analysis (Merkle et al. 2010), but choosing biologically meaningful costs can be difficult a priori (de Vienne et al. 2013). Therefore, in further runs, we increased the cost of cospeciation from 0 to 10 in increments of 1 in order to manually prohibit the program from falsely maximizing cospeciation in our system, while leaving the other event costs unchanged. We chose to change the cost of cospeciation because it may erroneously be assigned in the most parsimonious reconciliation due to its zero cost in the default cost structure. We randomized the mite tree 999 times to generate a null distribution, and compared these resulting costs to our original costs; significance indicates some level of congruence between the two trees. However, multihost symbionts are also problematic for Jane ver. 4, as once the program assigns a tip as a failure to diverge, the tip is not allowed to switch hosts (Charleston and Libeskind-Hadas 2014), which may not be the most likely scenario for feather mite diversification on hosts.

Statistical analyses

Based on the mite–host associations we uncovered, we ran Pearson's χ^2-test on contingency tables including host nesting ecology (ground nesters, cavity nesters, or shrub/canopy nesters) and associated *Amerodectes* feather mite species to test for a preliminary relationship. These analyses were conducted in R.

Data deposition

The data are deposited under GenBank accession numbers KY491577-KY491636.

Results

Feather mite phylogenetics

Both morphological and molecular data from the mtDNA COI gene indicated the presence of two genera of feather mites across 14 hosts: *Proctophyllodes* and *Amerodectes* (Fig. 1). Within *Amerodectes*, we found six feather mite lineages on 12 host species. Two have been described before: *Amerodectes hribari* and *A. seiurus* Mironov and Chandler 2017. The other four are undescribed and are herein called *Amerodectes* sp1, sp2, sp3 and sp4 (Fig. 2). We found three *Proctophyllodes* lineages, two of which (*P. quadratus* Atyeo et Braasch, 1966, and *P.* "*basal quadratus*") were associated with six species of the unambiguous (core) Parulidae (Fig. 3). The third, *P.* aff. *trisetosus*, was found on the yellow-breasted chat (now in Icteriidae; Chesser et al. 2017). Our morphological analyses provided similar results, except for one potential additional species (*P. breviquadratus* Atyeo et Braasch, 1966) nested within the *P. quadratus* COI lineage (S. V. Mironov pers. comm. and H. C. Proctor unpubl.), which are denoted with an asterisk (*) in Fig. 3. However, this inconsistency does not affect our overall inferences.

Feather mite and parulid cophylogenetic signal

Our cophylogenetic analyses between feather mites and their parulid hosts indicated varying degrees of phylogenetic congruence. For species-based *Amerodectes* trees, ParaFit indicated nonrandom associations between feather mites and hosts (ParaFit global = 9000.73; $p = 0.004$). ParaFit's corrected F1 statistic detected eight individual host-mite links that significantly contributed to the global statistic, and one additional link that fluctuated between significant and non-significant (Fig. 4). However, results from PACo indicated that host speciation is not driving feather mite speciation in this system (PACo global $p = 0.08$). TreeFitter found no combinations of event costs that described the associations between *Amerodectes* species and hosts as different from random (all $p > 0.18$). Similarly, Jane ver. 4 did not recover significant phylogenetic congruence between our species trees ($p = 0.07$) under default event costs (Table 3). However, under a tuned analysis with a cospeciation cost of 9 (rendering it a rare event), and the remaining costs left at the default, the overall cost of 28 was less than expected by chance ($p = 0.05$); Jane ver. 4 recovered no cospeciation events with this tuned cost structure (Table 3, Supplementary material Appendix 2 Fig. A1).

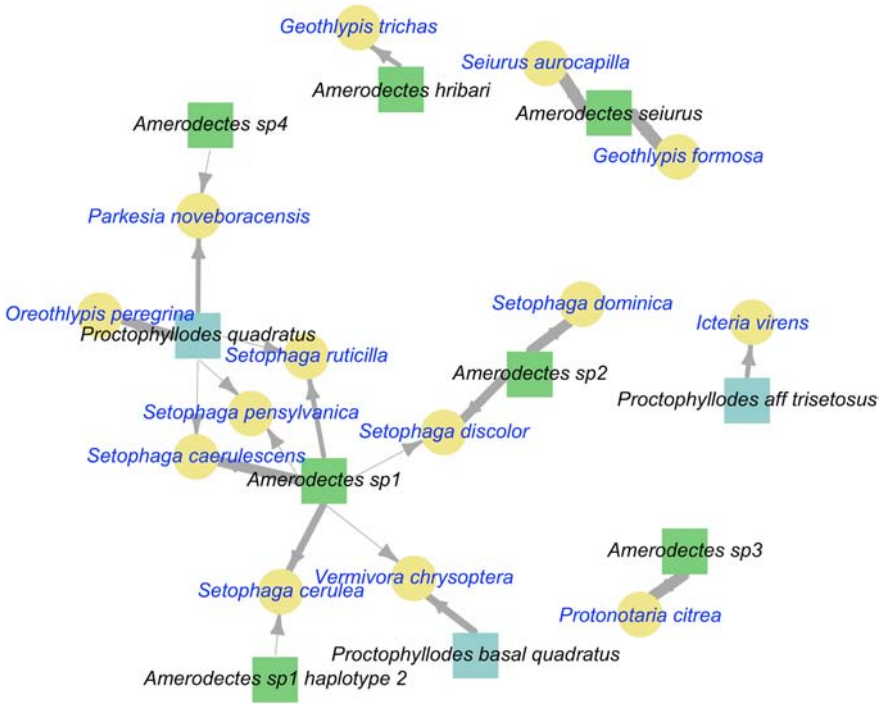

Figure 1. Network analysis of all host–mite relationships in our system. Tan circles with blue text are hosts; squares with black text are feather mites (blue = *Proctophyllodes*, green = *Amerodectes*). The thickness of the arrows indicates the number of associations between the host and the feather mite lineage in our dataset.

For the *Amerodectes* gene tree, the ParaFit global statistic also suggested nonrandom associations (ParaFit global = 106821.2; p = 0.001) with 31 out of 33 individual links being significant (Fig. 5). PACo also uncovered a global $p < 0.001$, but all of the individual links had broad 95% confidence intervals, which makes evaluating their true contribution to the overall global fit challenging. Similar to the *Amerodectes* species tree, TreeFitter found no combinations of event costs that described the *Amerodectes* gene tree association as different from random (all $p > 0.14$). Jane ver. 4 also did not recover significant phylogenetic congruence under default costs (p = 0.29; Table 3). However, under a tuned analysis with a cospeciation cost of 10 (rendering it a rare event), and the remaining costs left at the default, the overall cost of 53 was less than expected by chance (p = 0.02); again, Jane ver. 4 recovered no cospeciation events with this tuned cost structure (Table 3, Supplementary material Appendix 2 Fig. A2).

For the *Proctophyllodes* gene tree, both ParaFit and PACo suggested random associations with hosts (ParaFit global = 821.05; p = 0.11; PACo global p = 0.29). All links between hosts and *Proctophyllodes* mites were non-significant based on individual ParaFit F1 statistics (Fig. 6; all corrected $p > 0.12$). Neither the default event costs nor any tuned costs recovered significant phylogenetic congruence between the host and *Proctophyllodes* gene trees according to TreeFitter or Jane ver. 4 (all $p > 0.18$; Table 3; the lowest p-value was with a tuned analysis with a cospeciation cost of 9 and the remaining costs left at default).

Statistical analyses

Amerodectes mite diversification was strongly associated with host nesting ecology (Fig. 7; $\chi^2_{10} = 22.286$, p = 0.014; after 10 000 bootstraps, p = 0.008).

Discussion

Our assessment of the coevolutionary history of this system refutes the null hypothesis of strict cospeciation between feather mite and warbler phylogenies, with distance- and event-based cophylogenetic analyses supporting, at most, low levels of cospeciation. The structure of the current mite-warbler associations suggests host switching has been relatively common, and we infer the evolutionary and ecological implications of these results below.

Feather mite phylogenetics

Based on both mtDNA from the COI gene and morphological evidence, we documented six lineages of *Amerodectes* and three lineages of *Proctophyllodes* on 13 species of Parulidae and one species of Icteriidae. Previously, only three *Amerodectes* species and seven *Proctophyllodes* species, including *Proctophyllodes quadratus*, have been recorded from this host family (Doña et al. 2016). These previous records included 29 parulid species, six of which we also sampled for this study. With the additional species

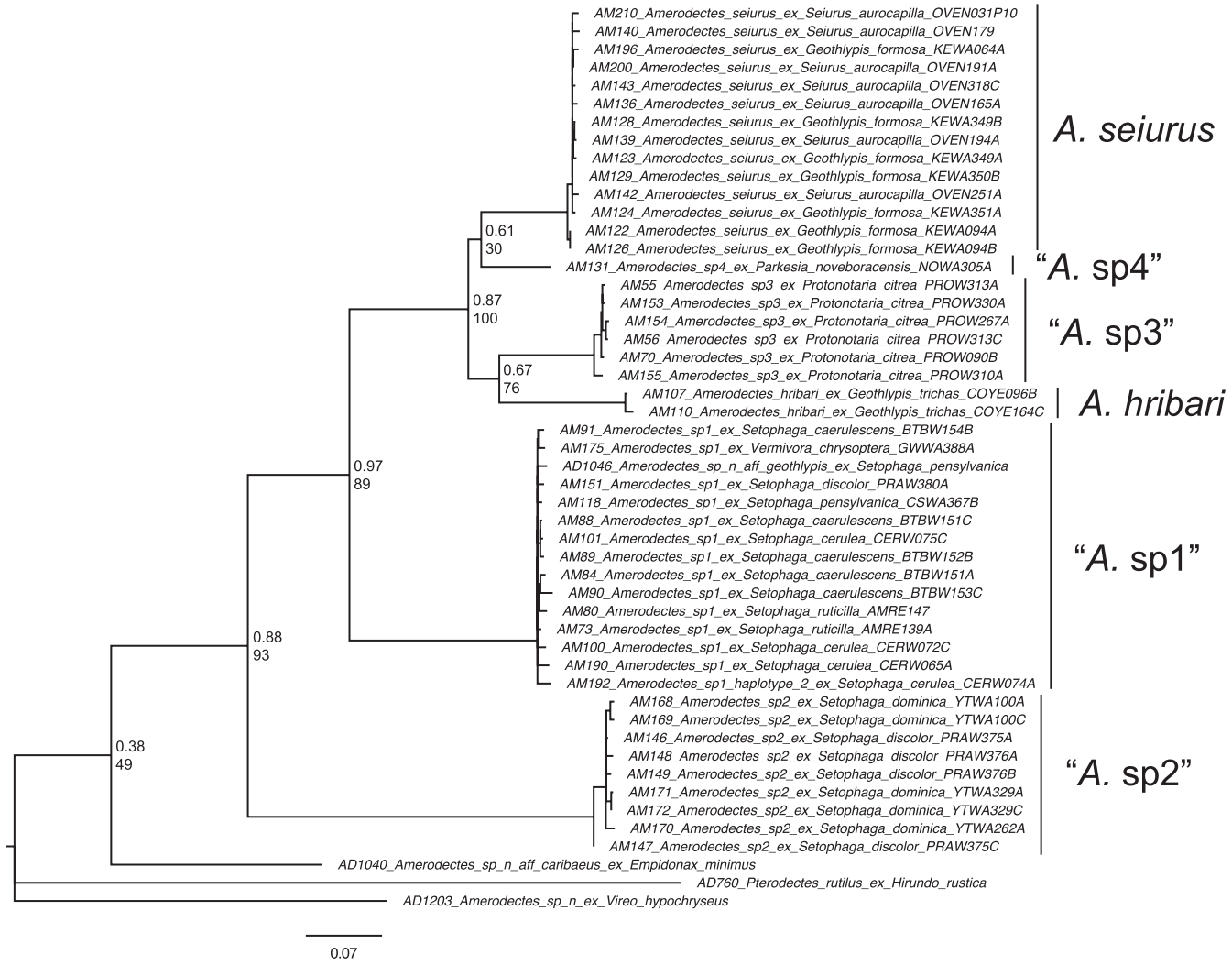

Figure 2. Phylogenetic relationships of *Amerodectes* feather mites from parulid hosts inferred from the mitochondrial COI sequences. Bayesian posterior probabilities and maximum likelihood bootstrap support values for major clades (with names on right) are indicated above and below each node, respectively. Scale bar indicates nucleotide substitutions per site.

we examined, 36 host species in Parulidae have now been sampled for feather mites to some extent. Combining these previous efforts with our results, and assuming the lineages we uncovered are true species, this family harbors at least nine *Amerodectes* species and eight *Proctophyllodes* species. Because we only sampled 14 species (and a subset of each species), the diversity of these mite genera on parulids is still an underestimate and will increase with increased sampling effort. Despite these limitations related to sampling, when comparing the diversity of these two mite genera on parulids to those from the closely related Icteridae (from which 32 host species have been sampled to some extent), *Proctophyllodes* diversity is slightly greater in icterids (12 mite species; Atyeo and Braasch 1966, Doña et al. 2016) while *Amerodectes* diversity is greater in parulids (with only three species described in icterids; Valim and Hernandes 2010, Doña et al. 2016).

Feather mite and parulid cophylogenetic signal

We observed varying degrees of cophylogenetic signal between *Amerodectes* and *Proctophyllodes* feather mites and their associated parulid hosts using a variety of methods. Contrary to our null hypothesis, none of these methods provided support for perfectly congruent phylogenies (strict cospeciation) and also contradict some previous feather mite–host studies (Dabert and Mironov 1999, Dabert 2005, Štefka et al. 2011), but corroborate with others somewhat (Dabert 2014, Dabert et al. 2015, Doña et al. 2017c, Klimov et al. 2017). There are a number of potential explanations for these differing inferences related to the life history and evolution of the host species, and the taxonomic scale at which cospeciation was tested.

Host life history and evolutionary processes may play a role in the variation of results across studies. For example,

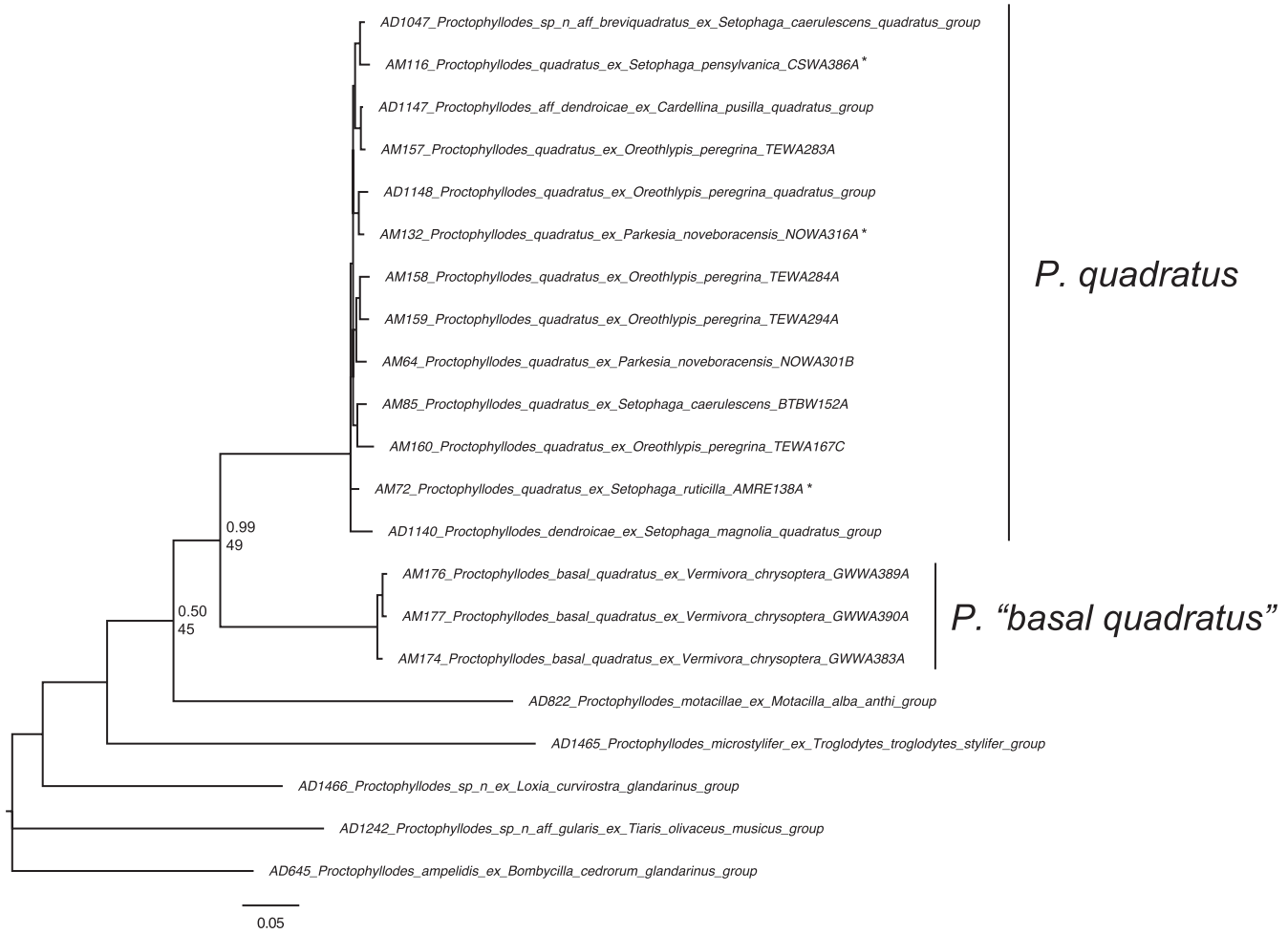

Figure 3. Phylogenetic relationships of *Proctophyllodes* feather mites from parulid (i.e. excluding *Icteria virens*) hosts inferred from the mitochondrial COI sequences. Bayesian posterior probabilities and maximum likelihood bootstrap support values for major clades (with names on right) are indicated above and below each node, respectively. Tip labels followed by an asterisk (*) denote that the morphology of that individual mite is more consistent with *Proctophyllodes breviquadratus*. Scale bar indicates nucleotide substitutions per site.

although Štefka et al. (2011) found evidence for cospeciation between four species of Galápagos mockingbirds and their feather mites, these hosts are completely isolated on various Galápagos islands, which greatly reduces the opportunities for dispersal across host species. Here geography alone can be responsible for both bird and mite divergences. This is a very different scenario than is seen with the Neotropical-Nearctic migratory parulids we studied. These species have varying degrees of overlapping distributions and ecologies on both the wintering and breeding grounds. Similarly, two migratory skua sister species (Stercorariidae: *Stercorarius*) shared some of the same feather mites, due to geographic overlap and potential physical contact during some part of their annual cycle (Dabert et al. 2015). Thus, our system represents a more complex, and possibly more common, arena for cospeciation (or lack thereof) between feather mites and their hosts.

Not only is our system more complex, but we also assessed cospeciation at a taxonomically finer scale (one host family at the species-level) than have most other studies. With one exception (Štefka et al. 2011), the scale at which most other studies have explicitly tested for cospeciation in feather mite systems has been quite broad (i.e. the order- or family-level of birds or mites). For example, Dabert and Mironov (1999) inferred cospeciation between species of feather mites in a single genus (*Zachvatkinia*) and two orders of hosts (Procellariiformes and Charadriiformes). Ehrnsberger et al. (2001) also found that at the family-level, the evolution of the feather mite family Freyanidae was a result of cospeciation with their avian hosts. Klimov et al. (2017) and Doña et al. (2017c) both investigated feather mite coevolution at the species-level across many host species. Despite both studies finding no evidence for strict cospeciation, they almost exclusively uncovered one-to-one host–mite associations, which differs from our results (multiple host species sharing the same mite and multiple mites sharing the same host). These differing results may again be explained by differences in taxonomic scale; our study was finely-focused on closely related species within one host family, while the other stud-

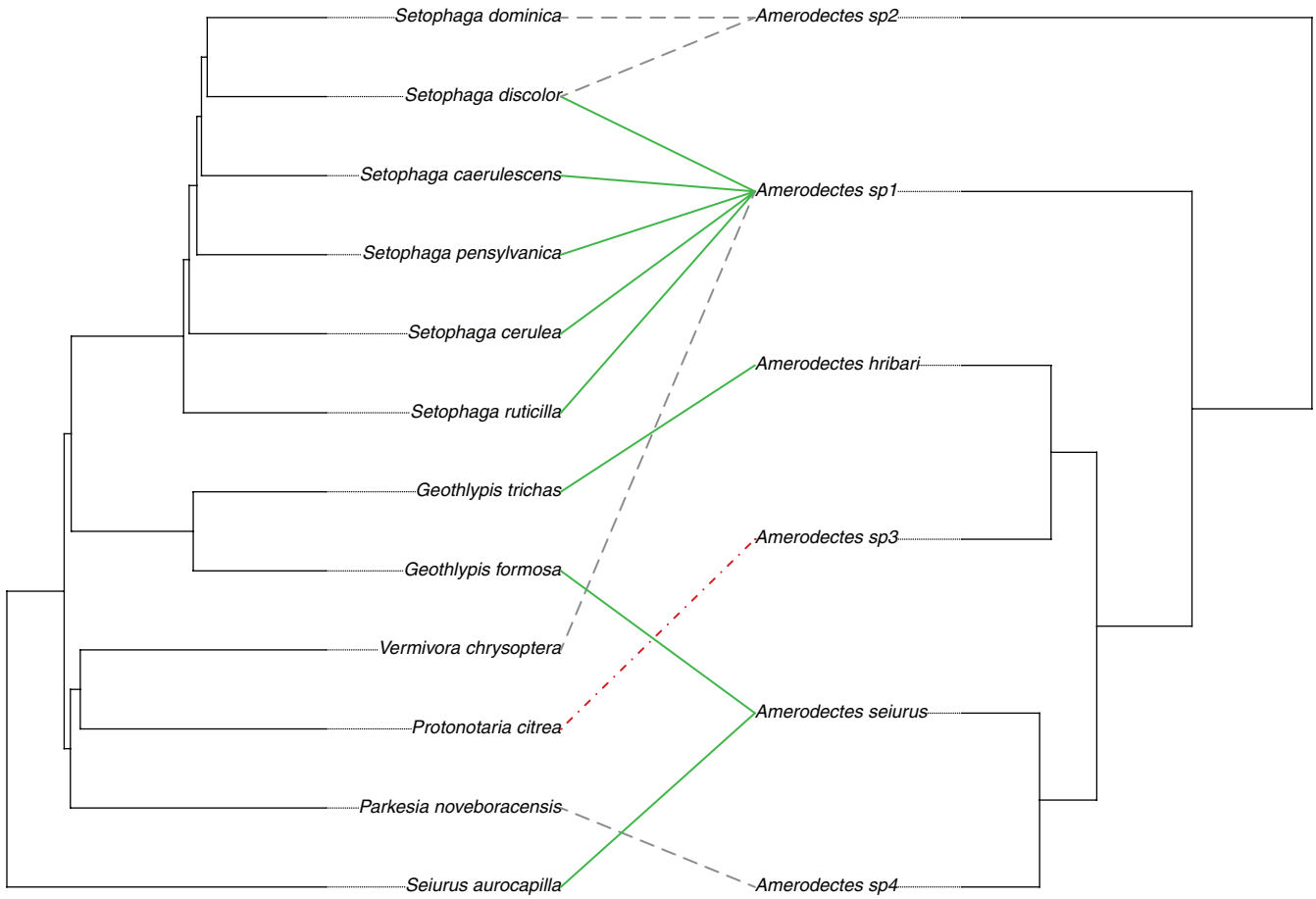

Figure 4. Tanglegram of *Amerodectes* feather mites (on right; species tree) and their parulid hosts (on left). Line styles and colors indicate level of significance of each host–mite link estimated by the ParaFitLink1 (F1) after 100 ParaFit runs and correcting each p-value using the Benjamini–Hochberg correction for false discovery rate: solid green lines indicate a significant host–mite link, dot-dashed red lines indicate a mix of significant and non-significant results over the 100 ParaFit runs, and long-dashed gray lines indicate non-significant links.

ies rarely included more than two or three species within the same genus (many of our hosts were strongly phylogenetically clustered; Doña et al. 2017b), so the chance of finding symbionts that share host species was reduced in these other cases. In total, it is now clear that congruent (or incongruent) phylogenies at broader taxonomic levels does not imply the same pattern at finer scales or vice versa (Demastes and Hafner 1993, Johnson et al. 2011), so our study contributes to a better understanding of cospeciation between feather mites and their hosts at a finely-focused taxonomic scale.

Influence of host ecology on feather mite diversification

Despite variability in the inferred evolutionary processes that have led to current host–mite associations, it seems qualitatively clear that host switching is more prevalent than has

Table 3. Summary of results from Jane ver. 4 for all analyses of feather mites and their parulid hosts, including both the default costs and the tuned costs. Solutions that were significant ($\alpha = 0.05$) are in bold.

	Cospeciation	Intra-host speciation	Intra-host speciation and host-switching	Extinction	Failure to diverge	p-value
Default costs						
Amerodectes (species)	1	2	2	12	7	0.07
Amerodectes (gene)	4	10	18	2	0	0.29
Proctophyllodes (gene)	3	1	4	2	0	0.81
Tuned costs						
Amerodectes (species)	0	2	3	13	7	**0.05**
Amerodectes (gene)	0	11	21	0	0	**0.02**
Proctophyllodes (gene)	0	3	5	0	0	0.18

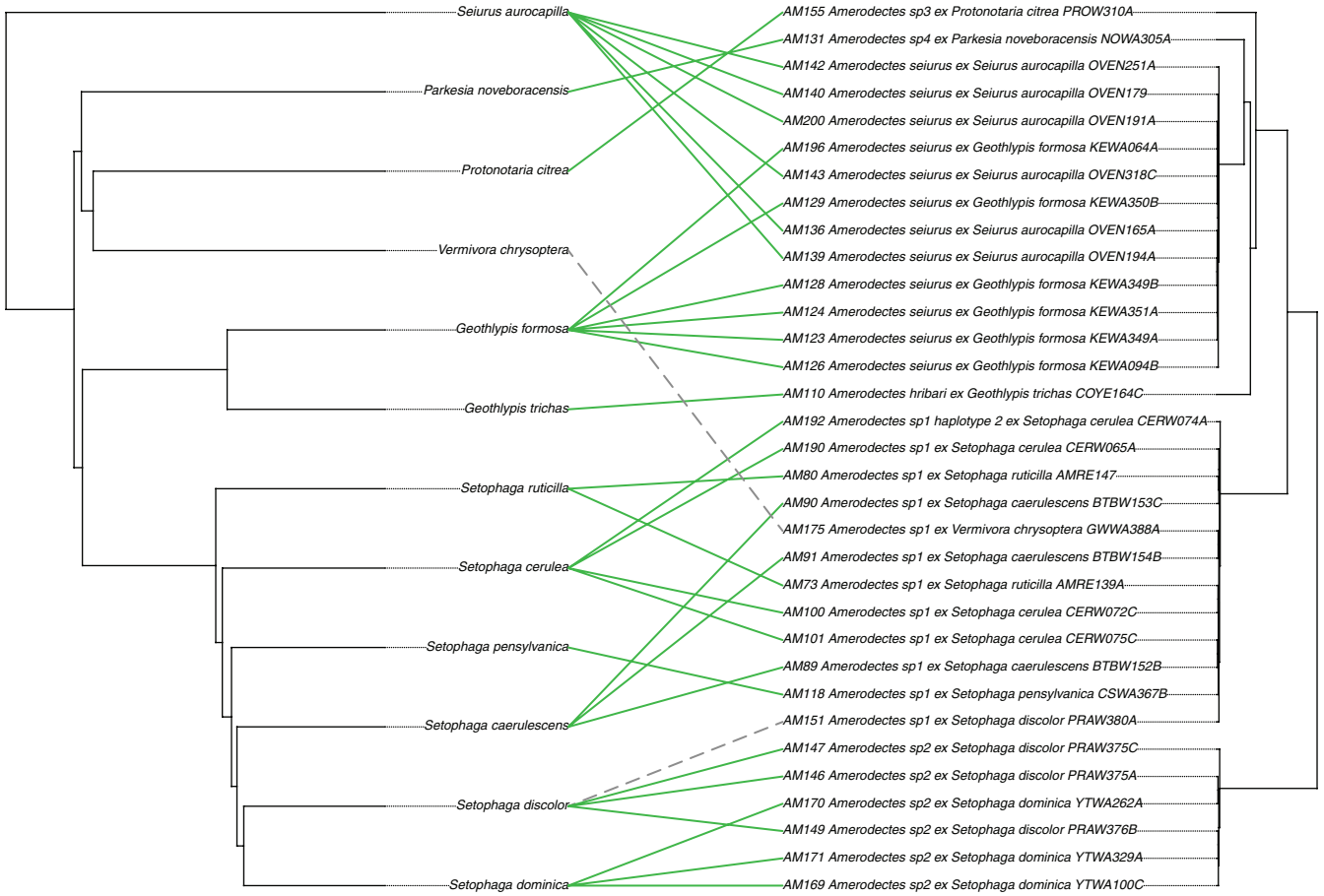

Figure 5. Tanglegram of *Amerodectes* feather mites (on right; gene tree) and their parulid hosts (on left). Line styles and colors indicate level of significance of each host-mite link estimated by the ParaFitLink1 (F1) after 100 ParaFit runs and correcting each p-value using the Benjamini–Hochberg correction for false discovery rate: solid green lines indicate a significant host–mite link and long-dashed gray lines indicate non-significant links.

been previously assumed. However, much of the life history of feather mites is still unknown, and it may be that longer distance dispersal and/or horizontal transmission across species (host switching) may be more common than previously thought, as was supported in Doña et al. (2017c) with feather mites of European passerines, and was supported in some instances for Passeriformes in Klimov et al. (2017). An individual symbiont successfully colonizing a new host, which may ultimately lead to an entire symbiont species switching to that new host, is not simple for most symbionts (e.g. the Stockholm paradigm; Hoberg and Brooks 2015), and can only occur when there is: 1) an opportunity, 2) the ability to survive on a new host (compatibility), and 3) an ability to maintain a symbiotic relationship with the new host (Araujo et al. 2015).

Our results offer a chance to consider the factors that may allow for a symbiont species to meet these three requirements for host switching. Non-mutually-exclusive factors could include ecological or geographic similarities during any stage of their annual cycle, or behavioral traits that bring them into contact (possibly in conjunction with evolutionary relatedness). Species that share ecological niche space or habitat affinities may be exposed to higher levels of propagule pressure due to the greater likelihood and frequency of contact between heterospecifics (similar to invasive species; Lockwood et al. 2005), leading to the successful switching of mite species from one host species to another.

Specifically, nesting or foraging ecology may be life history traits that promote host switching among feather mites on parulids. In our case, a preliminary analysis suggested there may be a strong association between host nesting ecology (ground, cavity, or shrub/canopy nesters) and *Amerodectes* mite species (Fig. 7). Two specific examples of this are *A. seiurus*, which we found on both *Seiurus aurocapilla* and *Geothlypis formosa*, two host species that are relatively distantly related within Parulidae, but both are ground-nesting/dwelling species (Porneluzi et al. 2011, McDonald 2013), and *Amerodectes* sp3, which was only found on *Protonotaria citrea*, the only cavity-nester that we sampled. Although sharing of nesting or foraging ecology may increase the likelihood of two species coming into contact (and thus increasing the opportunity to share mites), there are other ecological and behavioral traits that may influence the likelihood as much, or more so, than this feature. These include interspecific

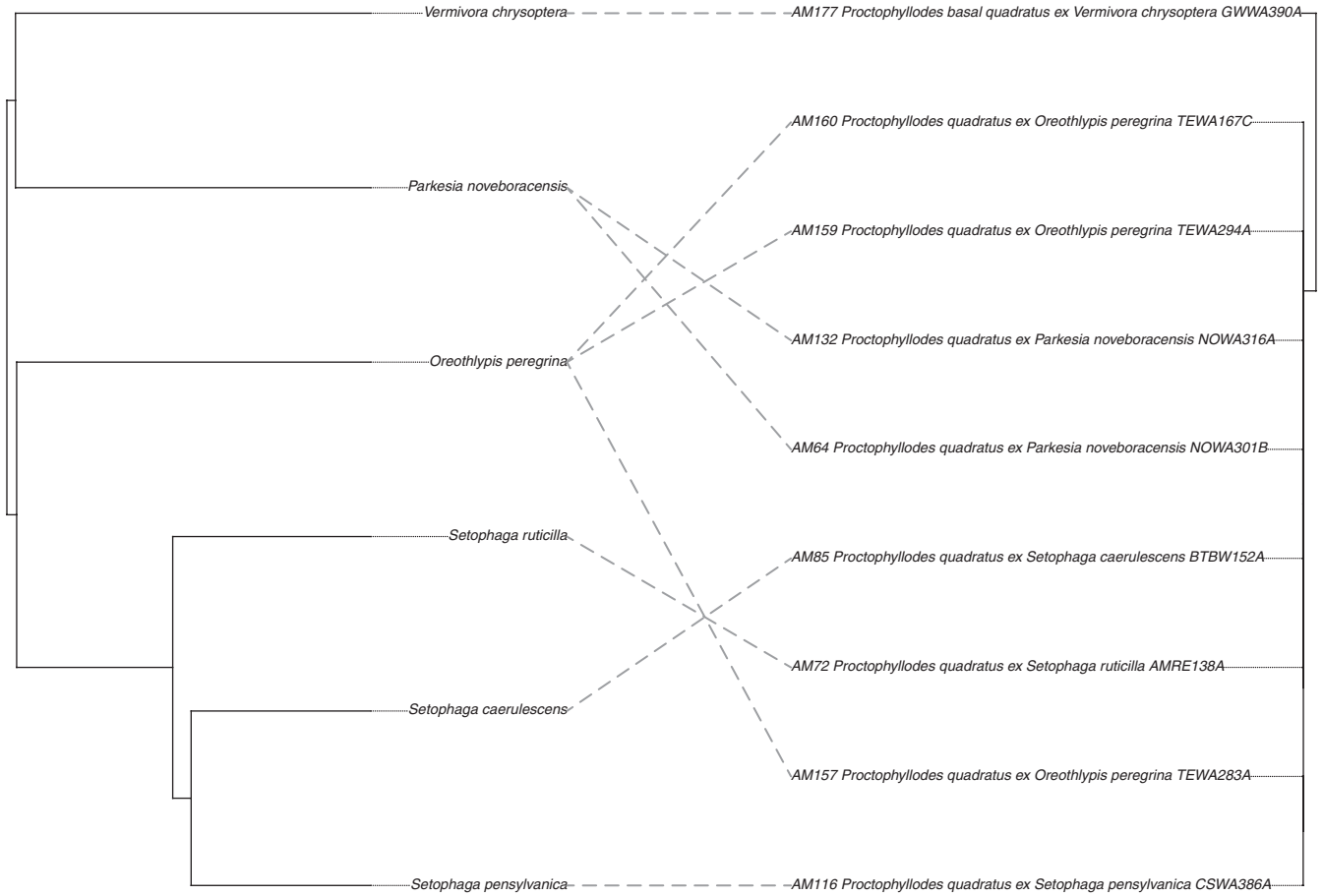

Figure 6. Tanglegram of *Proctophyllodes* feather mites (on right; gene tree) and their parulid hosts (on left). The long-dashed gray lines indicate that no host-mite links were significant according to the ParaFitLink1 (F1) estimate after 100 ParaFit runs and correcting each p-value using the Benjamini–Hochberg correction for false discovery rate.

aggression/territoriality, interspecific sociality (likely during winter or spring; Eaton 1953, Jones et al. 2000), and kleptoparasitism of nest material (as has been documented with *Setophaga virens* and *Setophaga fusca* stealing nesting material from *Setophaga cerulea*; Jones et al. 2007, T. J. Boves unpubl.).

Geography may also help explain the patterns of likely host switching that we uncovered (similar to avian lice studied in Weckstein 2004, Sweet and Johnson 2016, Catanach et al. 2017, Sweet et al. 2017). The hosts we studied vary in their geographic overlap during different stages of their annual cycle (breeding, migration, non-breeding; Stephenson and Whittle 2013). This extent of overlap, which may be correlated with the likelihood that two species would come into contact, could explain the pattern that we documented. Again, using *Seiurus aurocapilla* and *G. formosa* as an example, although they are minimally sympatric during the breeding season (mainly only in the central and southern Appalachian Mountains), they overlap more so during the winter and migration period, when they may also be less territorial and even potentially aggregate (Porneluzi et al. 2011, McDonald 2013).

Another possible factor that could help explain the patterns of host switching that we documented is brood parasitism. Many of these warblers are parasitized by the brown-headed cowbird, *Molothrus ater* (Icteridae), and although it is not thought that *M. ater* retain feather mites from foster parents (Atyeo and Gaud 1983), it may be possible that they act as a temporary carrier that could distribute symbionts to novel hosts (Krüger 2007). This premise is supported by the fact that *M. ater* are not only known to harbor nasal mites associated with closely related icterids, but also from other host families (such as Cardinalidae; Pérez 2016).

Finally, it is possible that host switching could occur during inter-species mating events (Willis et al. 2014). There are many documented cases of successful hybridization between parulid species (McCarthy 2006, Burrell et al. 2016, Toews et al. 2016) and the acts of copulation and subsequent brooding may provide enough direct contact across species for mites to transfer both horizontally and vertically. Investigation of feather mites on parulid species that are known to frequently hybridize (e.g. *Vermivora cyanoptera* and *V. chrysoptera*), and the hybrids themselves, could help elucidate

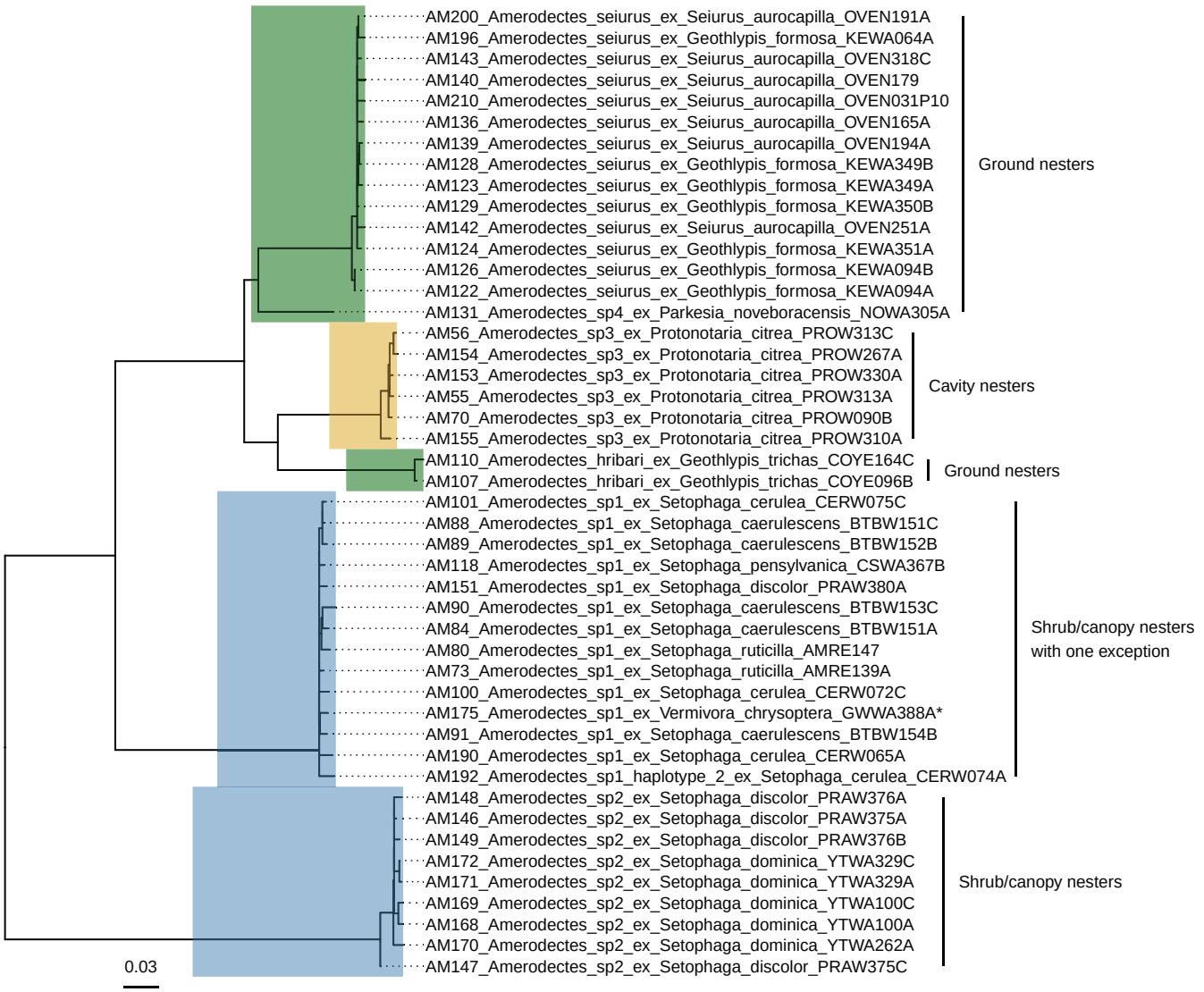

Figure 7. *Amerodectes* phylogenetic tree with host nesting ecology mapped. One ground-nesting species (*Vermivora chrysoptera*) is found in a clade with shrub/canopy nesters, and is denoted with an asterisk (*), but this did not influence the overall significant pattern.

the probability of this scenario. Importantly, due to hybridization in hosts, inferences based solely on mitochondrial genes may be problematic due to the potential of subsequent introgression (Funk and Omland 2003). Thus, incorporating nuclear genes (for both hosts and mites) would help provide insight to results obtained from mitochondrial genes alone. In general, the next step in understanding how these symbionts have been able to successfully move between, survive on, and maintain symbiotic relationships with multiple hosts would be to model the explanatory power of each of these factors in relation to the sharing of hosts that we have documented.

Another especially interesting outcome of our study is that five host species harbored *P. quadratus* (three of which, all in the genus *Setophaga*, also harbored *A.* sp1). This could imply that *P. quadratus* has only recently jumped to new hosts, and incorporating a molecular clock may be useful for addressing this question. Alternatively, the fact that so little diversification has occurred may suggest that either there has been considerable gene flow within *P. quadratus* associated with a wide diversity of parulid hosts, the mite has very large population sizes compared to its hosts, or that it has a relatively general ecological niche compared to *Amerodectes* species. Analyzing this hypothesis will require experimental testing, such as transplanting *P. quadratus* onto different hosts and assessing its ability to survive. The ability to rear feather mites in a laboratory setting would also be helpful for this type of experimental testing.

We also documented a case of synhospitality, which occurs when one host species simultaneously bears more than one symbiont species of one genus (Eichler 1966, Bochkov and Mironov 2008). Most feather mites of *Setophaga discolor* were *A.* sp2, but there was one instance of *A.* sp1 inhabiting *S. discolor*. This may have resulted from a more common host

of *A.* sp1 horizontally transferring mites to *S. discolor* (host switching), which was inferred from the *Amerodectes* gene tree analysis in Jane ver. 4.

A feather mite provides evidence for phylogenetic placement of its host

Although not directly related to our objectives, uncovering *P.* aff. *trisetosus* on *Icteria virens* is notable because: 1) feather mites from this host species have not been investigated before, and 2) placement of *I. virens* within Parulidae has been long-debated, with arguments of better placement within Icteridae (Sibley and Ahlquist 1982, Klicka et al. 2000, Lovette and Bermingham 2002, Lovette et al. 2010), or in its own family, Icteriidae (Barker et al. 2013, 2015). Recently, *I. virens* has officially been placed in its own family, Icteriidae (Chesser et al. 2017), which is more closely related to Icteridae than Parulidae (Barker et al. 2013, 2015). If a symbiont's evolution reflects the evolution of their hosts, our evidence supports the recent placement within Icteriidae because *P.* aff. *trisetosus* is more closely related to mite species found on hosts of Icteridae (such as *P. egglestoni* and *P. anthi*; Atyeo and Braasch 1966) than with any of the other parulids we studied. Of course, additional genetic data is necessary to support this conclusion, but it merits further investigation.

Conclusions

In conclusion, by using distance- and event-based cophylogenetic analyses, we found that the symbiosis between *Amerodectes* and *Proctophyllodes* feather mites and 13 of their Parulidae hosts has a faint cophylogenetic signal, but no evidence for strict cospeciation, contrary to what we hypothesized. The observed patterns are likely due to host switching between hosts that are closely linked evolutionarily, ecologically, behaviorally and/or geographically. Future investigations that model how ecological traits may explain the associations between feather mites and their hosts will be informative in further untangling the complex evolutionary ecology of this host–symbiont system. Similar studies involving obligate symbionts are needed so that we can better understand how these associations may differ over time or space and across host–symbiont systems.

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Conflicts of interest – The authors declare they have no conflict of interest.
Permits – Capturing and handling of birds was conducted under United States Geological Survey federal banding permits no. 23877, no. 23277 and no. 23340, and was conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Arkansas State Univ. (no. 63-8363) and Indiana Univ. of Pennsylvania (no. 14-1314). Carcasses collected were salvaged under state Scientific Permit no. NH11.0313 (Illinois Dept of Natural Resources) and federal Salvage Permit no. MB08907A-0 (US Fish and Wildlife Service).

References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. – Nucleic Acids Res. 25: 3389–3402.

Araujo, S. B. L., Braga, M. P., Brooks, D. R., Agosta, S. J., Hoberg, E. P., Von Hartenthal, F. W. and Boeger, W. A. 2015. Understanding host-switching by ecological fitting. – PLoS One 10: e0139225.

Atyeo, W. T. and Braasch, N. L. 1966. The feather mite genus *Proctophyllodes* (Sarcoptiformes: Proctophyllodidae). – Bull. Univ. Nebraska State Mus. 5: 1–354.

Atyeo, W. T. and Gaud, J. 1979. Feather mites and their hosts. – Recent Adv. Acarol. 2: 355–361.

Atyeo, W. T. and Gaud, J. 1983. Feather mites of obligate brood parasites. – J. Parasitol. 69: 455–458.

Balbuena, J. A., Míguez-Lozano, R. and Blasco-Costa, I. 2013. PACo: a novel procrustes application to cophylogenetic analysis. – PLoS One 8: e61048.

Barker, F. K., Cibois, A., Schikler, P., Feinstein, J. and Cracraft, J. 2004. Phylogeny and diversification of the largest avian radiation. – Proc. Natl Acad. Sci. USA 101: 11040–11045.

Barker, F. K., Burns, K. J., Klicka, J., Lanyon, S. M. and Lovette, I. J. 2013. Going to extremes: contrasting rates of diversification in a recent radiation of new world passerine birds. – Syst. Biol. 62: 298–320.

Barker, F. K., Burns, K. J., Klicka, J., Lanyon, S. M. and Lovette, I. J. 2015. New insights into New World biogeography: an integrated view from the phylogeny of blackbirds, cardinals, sparrows, tanagers, warblers and allies. – Auk 132: 333–348.

Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. – J. R. Stat. Soc. B 57: 289–300.

Bochkov, A. V. and Mironov, S. V. 2008. The phenomenon of synhospitality in acariform mites (Acari: Acariformes) – the permanent parasites of vertebrates. – Parazitologiia 42: 81–100, in Russian with English summary.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., Suchard, M. A., Rambaut, A. and Drummond, A. J. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. – PLoS Comput. Biol. 10: 1–6.

Bridge, E. S. 2002. Host specificity and colonization by *Zachvatkinia caspica*, an analgoid feather mite of Caspian terns. – Can. J. Zool. 80: 2200–2206.

Burrell, K. G. D., Skevington, J. H., Kelso, S., Burrell, M. V. A., Leclair, D. L. and Mackenzie, S. A. 2016. A previously undocumented hybrid New World warbler (*Setophaga pensylvanica* × *S. magnolia*) captured at Long Point, Ontario. – Wilson J. Ornithol. 128: 624–628.

Catanach, T. A., Valim, M. P., Weckstein, J. D. and Johnson, K. P. 2017. Cophylogenetic analysis of lice in the *Colpocephalum* complex (Phthiraptera: Amblycera). – Zool. Scripta doi:10.1111/zsc.12262.

Charleston, M. and Libeskind-Hadas, R. 2014. Event-based cophylogenetic comparative analysis. – In: Garamszegi, L. Z. (ed.), Modern phylogenetic comparative methods and their application in evolutionary biology. Springer, pp. 465–480.

Chesser, R. T., Burns, K. J., Cicero, C., Dunn, J. L., Kratter, A. W., Lovette, I. J., Rasmussen, P. C., Remsen, J. V., Rising, J. D., Stotz, D. F. and Winker, K. 2017. Fifty-eighth supplement to the American Ornithological Society's check-list of North American birds. – Auk 134: 751–773.

Clayton, D. H. and Johnson, K. P. 2003. Linking coevolutionary history to ecological process: doves and lice. – Evolution 57: 2335–2341.

Clayton, D. H., Bush, S. E. and Johnson, K. P. 2004. Ecology of congruence: past meets present. – Syst. Biol. 53: 165–173.

Clayton, D. H., Bush, S. E. and Johnson, K. P. 2016. Coevolution of life of hosts: integrating ecology and history. – Univ. of Chicago Press.

Conow, C., Fielder, D., Ovadia, Y. and Libeskind-Hadas, R. 2010. Jane: a new tool for the cophylogeny reconstruction problem. – Algorithms Mol. Biol. 5: 16.

Dabert, J. 2005. Feather mites (Astigmata; Pterolichoidea, Analgoidea) and birds as models for cophylogenetic studies. – Phytophaga 14: 409–424.

Dabert, J. 2014. A new feather mite species of the genus Neumannella Trouessart, 1916 (Analgoidea, Dermoglyphidae) from the red-winged tinamou *Rhynchotus rufescens* (Temminck, 1815) (Aves, Tinamiformes) with remarks to the evolution of host–parasite associations of the genus. – Acta Parasitol. 59: 197–205.

Dabert, J. and Mironov, S. V. 1999. Origin and evolution of feather mites (Astigmata). – Exp. Appl. Acarol. 23: 437–454.

Dabert, M., Coulson, S. J., Gwiazdowicz, D. J., Moe, B., Hanssen, S. A., Biersma, E. M., Pilskog, H. E. and Dabert, J. 2015. Differences in speciation progress in feather mites (Analgoidea) inhabiting the same host: the case of *Zachvatkinia* and *Alloptes* living on arctic and long-tailed skuas. – Exp. Appl. Acarol. 65: 163–179.

Darriba, D., Taboada, G. L., Doallo, R. and Posada, D. 2015. jModelTest 2: more models, new heuristics and high-performance computing. – Nat. Methods 9: 6–9.

de Vienne, D. M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M. E. and Giraud, T. 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. – New Phytol. 198: 347–385.

Demastes, J. W. and Hafner, M. S. 1993. Cospeciation of pocket gophers (*Geomys*) and their chewing lice (*Geomydoecus*). – J. Mammal. 74: 521–530.

Doña, J., Diaz-Real, J., Mironov, S., Bazaga, P., Serrano, D. and Jovani, R. 2015. DNA barcoding and minibarcoding as a powerful tool for feather mite studies. – Mol. Ecol. Resour. 15: 1216–1225.

Doña, J., Proctor, H., Mironov, S., Serrano, D. and Jovani, R. 2016. Global associations between birds and vane-dwelling feather mites. – Ecology 97: 3242.

Doña, J., Potti, J., De La Hera, I., Blanco, G., Frías, O. and Jovani, R. 2017a. Vertical transmission in feather mites: insights into its adaptive value. – Ecol. Entomol. 42: 492–499.

Doña, J., Proctor, H., Mironov, S., Serrano, D., and Jovani, R. 2017b. Host specificity, infrequent major host switching and the diversification of highly host-specific symbionts: the case of vane-dwelling feather mites. – Global Ecol. Biogeogr. doi:10.1111/geb.12680.

Doña, J., Sweet, A. D., Johnson, K. P., Serrano, D., Mironov, S., and Jovani, R. 2017c. Cophylogenetic analyses reveal extensive host-shift speciation in a highly specialized and host-specific symbiont system. – Mol. Phylogenet. Evol. 115: 190–196.

Dubinin, V. B. 1951. Feather mites (Analgesoidea). Part I. Introduction to the study. – Fauna USSR Arachnol. 6: 1–363.

Eaton, S. W. 1953. Wood warblers wintering in Cuba. – Wilson Bull. 65: 169–174.

Ehrnsberger, R., Mironov, S. V and Dabert, J. 2001. A preliminary analysis of phylogenetic relationships of the feather mite family Freyanidae Dubinin, 1953 (Acari: Astigmata). – Biol. Bull. Poznań 38: 181–202.

Eichler, W. 1948. XLI.— Some rules in Ectoparasitism. – Ann. Mag. Nat. Hist. Ser. 12 1: 588–598.

Eichler, W. 1966. Two new evolutionary terms for speciation in parasitic animals. – Syst. Zool. 15: 216–218.

Fahrenholz, H. 1913. Ectoparasiten und abstammungslehre. – Zool. Anz. 41: 371–374.

Fraija-Fernández, N., Aznar, F. J., Fernández, A., Raga, J. A. and Fernández, M. 2016. Evolutionary relationships between digeneans of the family Brachycladiidae Odhner, 1905 and their marine mammal hosts: a cophylogenetic study. – Parasitol. Int. 65: 209–217.

Funk, D. J. and Omland, K. E. 2003. Species-level paraphyly and polyphyly: frequency, causes and consequences, with insight from animal mitochondrial DNA. – Annu. Rev. Ecol. Evol. Syst. 34: 397–423.

Guindon, S. and Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. – Syst. Biol. 52: 696–704.

Hafner, M. S. and Nadler, S. A. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. – Nature 332: 258–259.

Hebert, P. D. N., Cywinska, A., Ball, S. L. and DeWaard, J. R. 2003. Biological identifications through DNA barcodes. – Proc. R. Soc. B 270: 313–321.

Heled, J. and Drummond, A. J. 2010. Bayesian inference of species trees from multilocus data. – Mol. Biol. Evol. 27: 570–580.

Hendricks, S. A., Flannery, M. E. and Spicer, G. S. 2013. Cophylogeny of quill mites from the genus *Syringophilopsis* (Acari: Syringophilidae) and their North American passerine hosts. – J. Parasitol. 99: 827–834.

Hernandes, F. A., Pedroso, L. G. A. and Mironov, S. V 2014. From cuckoos to chickens: a caught-in-the-act case of host shift in feather mites (Arachnida: Acari: Psoroptoididae). – Parasitol. Res. 113: 4355–4361.

Hinchliff, C. E., Smith, S. A., Allman, J. F., Burleigh, J. G., Chaudhary, R., Coghill, L. M., Crandall, K. A., Deng, J., Drew, B. T., Gazis, R., Gude, K., Hibbett, D. S., Katz, L. A., Laughinghouse, H. D., McTavish, E. J., Midford, P. E., Owen, C. L., Ree, R. H., Rees, J. A., Soltis, D. E., Williams, T. and Cranston, K. A. 2015. Synthesis of phylogeny and taxonomy into a comprehensive tree of life. – Proc. Natl Acad. Sci. USA 112: 12764–12769.

Hoberg, E. P. and Brooks, D. R. 2015. Evolution in action: climate change, biodiversity dynamics and emerging infectious disease. – Phil. Trans. R. Soc. B 370: 20130553.

Johnson, K. P., Adams, R. J., Page, R. D., and Clayton, D. H. 2003. When do parasites fail to speciate in response to host speciation? – Syst. Biol. 51: 37–47.

Johnson, K. P., Weckstein, J. D., Meyer, M. J. and Clayton, D. H. 2011. There and back again: switching between host orders by avian body lice (Ischnocera: Goniodidae). – Biol. J. Linn. Soc. 102: 614–625.

Jones, J., Perazzi, P. R., Carruthers, E. H. and Robertson, R. J. 2000. Sociality and foraging behavior of the Cerulean warbler in Venezuelan shade-coffee plantations. – Condor 102: 958–962.

Jones, K. C., Roth, K. L., Islam, K., Hamel, P. B. and Smith, C. G. 2007. Incidence of nest material kleptoparasitism involving Cerulean warblers. – Wilson J. Ornithol. 119: 271–275.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. – J. Mol. Evol. 16: 111–120.

Klassen, G. J. 1992. Coevolution: a history of the macroevolutionary approach to studying host-parasite associations. – J. Parasitol. 78: 573–587.

Klicka, J., Johnson, K. P. and Lanyon, S. M. 2000. New world nine-primaried oscine relationships: constructing a mitochondrial DNA framework. – Auk 117: 321–336.

Klimov, P. B. and O'Connor, B. M. 2008. Origin and higher-level relationships of psoroptidian mites (Acari: Astigmata: Psoroptidia): evidence from three nuclear genes. – Mol. Phylogenet. Evol. 47: 1135–1156.

Klimov, P. B., Mironov, S. V. and O'Connor, B. M. 2017. Detecting ancient codispersals and host shifts by double dating of host and parasite phylogenies: application in proctophyllodid feather mites associated with passerine birds. – Evolution 71: 2381–2397.

Krüger, O. 2007. Cuckoos, cowbirds and hosts: adaptations, trade-offs and constraints. – Phil. Trans. R. Soc. B 362: 1873–1886.

Legendre, P., Galzin, R. and Harmelin-Vivien, M. L. 1997. Relating behavior to habitat: solutions to the fourth-corner problem. – Ecology 78: 547–562.

Legendre, P., Desdevises, Y. and Bazin, E. 2002. A statistical test for host–parasite coevolution. – Syst. Biol. 51: 217–234.

Light, J. E. and Hafner, M. S. 2007. Cophylogeny and disparate rates of evolution in sympatric lineages of chewing lice on pocket gophers. – Mol. Phylogenet. Evol. 45: 997–1013.

Lockwood, J. L., Cassey, P. and Blackburn, T. 2005. The role of propagule pressure in explaining species invasions. – Trends Ecol. Evol. 20: 223–228.

Lovette, I. J. and Bermingham, E. 1999. Explosive speciation in the New World Dendroica warblers. – Proc. R. Soc. B 266: 1629–1636.

Lovette, I. J. and Bermingham, E. 2002. What is a wood-warbler? Molecular characterization of a monophyletic Parulidae. – Auk 119: 695–714.

Lovette, I. J., Pérez-Emán, J. L., Sullivan, J. P., Banks, R. C., Fiorentino, I., Córdoba-Córdoba, S., Echeverry-Galvis, M., Barker, F. K., Burns, K. J., Klicka, J., Lanyon, S. M. and Bermingham, E. 2010. A comprehensive multilocus phylogeny for the wood-warblers and a revised classification of the Parulidae (Aves). – Mol. Phylogenet. Evol. 57: 753–770.

Maddison, W. P. and Maddison, D. R. 2017. Mesquite: a modular system for evolutionary analysis, ver. 3.2. – < http://mesquiteproject.org >.

Martínez-Aquino, A. 2016. Phylogenetic framework for coevolutionary studies: a compass for exploring jungles of tangled trees. – Curr. Zool. 62: 393–403.

Marussich, W. A. and Machado, C. A. 2007. Host-specificity and coevolution among pollinating and nonpollinating New World fig wasps. – Mol. Ecol. 16: 1925–1946.

McCarthy, E. M. 2006. Handbook of avian hybrids of the World. – Oxford Univ. Press.

McCormack, J. E., Heled, J., Delaney, K. S., Peterson, A. T. and Knowles, L. L. 2011. Calibrating divergence times on species trees versus gene trees: implications for speciation history of *Aphelocoma* jays. – Evolution 65: 184–202.

McDonald, M. V. 2013. Kentucky warbler (*Oporornis formosus*). – In: Rodewald, P. G. (ed.), The birds of North America online. Cornell Laboratory of Ornithology, Ithaca, NY.

Merkle, D., Middendorf, M. and Wieseke, N. 2010. A parameter-adaptive dynamic programming approach for inferring cophylogenies. – BMC Bioinformatics 11: S60.

Michonneau, F., Brown, J. W. and Winter, D. J. 2016. rotl: an R package to interact with the Open Tree of Life data. – Methods Ecol. Evol. 7: 1476–1481.

Mironov, S. V. and Chandler, C. R. 2017. New feather mites of the genus *Amerodectes* Valim and Hernandes (Acariformes: Proctophyllodidae) from passerines (Aves: Passeriformes) in Georgia, USA. – Zootaxa 4344: 201–245.

Morelli, M. and Spicer, G. 2007. Cospeciation between the nasal mite *Ptilonyssus sairae* (Acari: Rhinonyssidae) and its bird hosts. – Syst. Appl. Acarol. 12: 179–188.

Ogilvie, H. A., Heled, J., Xie, D. and Drummond, A. J. 2016. Computational performance and statistical accuracy of *BEAST and comparisons with other methods. – Syst. Biol. 65: 381–396.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. and Wagner, H. 2016. vegan: community ecology package. – R-package ver. 2.4.1 < http://cran.r-project.org/web/packages/vegan >.

Page, R. D. M. 1994. Parallel phylogenies: reconstructing the history of host–parasite assemblages. – Cladistics 10: 155–173.

Page, R. D. M. 2003. Tangled trees: phylogeny, cospeciation and coevolution. – Univ. of Chicago Press.

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

Paterson, A. M. and Gray, R. D. 1997. Host–parasite cospeciation, host switching and missing the boat. – In: Clayton, D. H. and Moore, J. (eds), Host–parasite evolution: general principles and avian models. Oxford Univ. Press, pp. 236–250.

Paterson, A. M. and Banks, J. 2001. Analytical approaches to measuring cospeciation of host and parasites: through a looking glass, darkly. – Int. J. Parasitol. 31: 1012–1022.
Pérez, A. H. 2016. Survey of nasal mites (Acari: Rhinonyssidae, Ereynetidae, Turbinoptidae) in birds from three states in the United States. – MSc thesis, Univ. Arkansas.
Peterson, P. C. 1975. An analysis of host–parasite associations among feather mites (Acari: Analgoidea). – Misc. Publ. Entomol. Soc. Am. 9: 237–242.
Philips, J. R. and Fain, A. 1991. Acarine symbionts of louseflies (Diptera, Hippoboscidae). – Acarologia 32: 377–384.
Porneluzi, P., Van Horn, M. A. and Donovan, T. M. 2011. Ovenbird (*Seiurus aurocapilla*) – In: Rodewald, P.G. (ed.), The birds of North America online. Cornell Laboratory of Ornithology, Ithaca, NY.
Proctor, H. C. 2003. Feather mites (Acari: Astigmata): ecology, behavior, and evolution. – Annu. Rev. Entomol. 48: 185–209.
Proctor, H. C. and Jones, D. N. 2004. Geographical structuring of feather mite assemblages from the Australian brush-turkey (Aves: Megapodiidae). – J. Parasitol. 90: 60–66.
Rambaut, A. 2016. FigTree, ver. 1.4. – <http://tree.bio.ed.ac.uk/software/figtree/>.
Rambaut, A., Suchard, M. A., Xie, D. and Drummond, A. J. 2014. Tracer, ver. 1.6. – <http://beast.bio.ed.ac.uk/Tracer>.
Ronquist, F. 2002. TreeFitter, ver. 1.0. – <https://sourceforge.net/projects/treefitter/>.
Schliep, K. P. 2011. phangorn: phylogenetic analysis in R. – Bioinformatics 27: 592–593.
Sibley, C. G. and Ahlquist, J. E. 1982. The relationships of the yellow-breasted chat (*Icteria virens*) and the alleged slowdown in the rate of macromolecular evolution in birds. – Postilla 187: 1–19.
Smith, M. A., Wood, D. M., Janzen, D. H., Hallwachs, W. and Hebert, P. D. N. 2007. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. – Proc. Natl Acad. Sci. USA 104: 4967–4972.
Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. – Bioinformatics 30: 1312–1313.
Štefka, J., Hoeck, P. E. A., Keller, L. F. and Smith, V. S. 2011. A hitchhikers guide to the Galápagos: co-phylogeography of Galápagos mockingbirds and their parasites. – BMC Evol. Biol. 11: 284.
Stephenson, T. and Whittle, S. 2013. The warbler guide. – Princeton Univ. Press.
Sweet, A. D. and Johnson, K. P. 2016. Cophylogenetic analysis of New World ground-doves (Aves: Columbidae) and their parasitic wing lice (Insecta: Phthiraptera: *Columbicola*). – Mol. Phylogenet. Evol. 103: 122–132.
Sweet, A. D., Boyd, B. M., Allen, J. A., Villa, S. M., Valim, M. P., Rivera-Parra, J. L., Wilson, R. E. and Johnson, K. P. 2017. Integrating phylogenomic and population genomic patterns in avian lice provides a more complete picture of parasite evolution. – Evolution doi:10.1111/evo.13386.
Toews, D. P. L., Taylor, S. A., Vallender, R., Brelsford, A., Butcher, B. G., Messer, P. W. and Lovette, I. J. 2016. Plumage genes and little else distinguish the genomes of hybridizing warblers. – Curr. Biol. 26: 2313–2318.
Valim, M. P. and Hernandes, F. A. 2010. A systematic review of feather mites of the *Pterodectes* generic complex (Acari: Proctophyllodidae: Pterodectinae) with redescriptions of species described by Vladimír Cerný. – Acarina 18: 3–35.
Weckstein, J. 2004. Biogeography explains cophylogenetic patterns in toucan chewing lice. – Syst. Biol. 53: 154–164.
Willis, P. M., Symula, R. E. and Lovette, I. J. 2014. Ecology, song similarity and phylogeny predict natural hybridization in an avian family. – Evol. Ecol. 28: 299–322.
Yang, Z. 2015. The BPP program for species tree estimation and species delimitation. – Curr. Zool. 61: 854–865.
Zhang, J., Kapli, P., Pavlidis, P. and Stamatakis, A. 2013. A general species delimitation method with applications to phylogenetic placements. – Bioinformatics 29: 2869–2876.

Supplementary material (Appendix jav-01580 at <www.avianbiology.org/appendix/jav-01580>). Appendix 1–2.