**Unexpected bird–feather mite associations revealed by DNA metabarcoding uncovers a dynamic ecoevolutionary scenario**

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1Department of Evolutionary

**Abstract**

The high relevance of host‐switching for the diversification of highly host‐specific symbionts (i.e., those commonly inhabiting a single host species) demands a better understanding of host-switching dynamics at an ecological scale. Here, we used DNA metabarcoding to study feather mites on passerine birds in Spain, sequencing mtDNA (COI) for 25,540 individual mites (representing 64 species) from 1,130 birds (repre‐ senting 71 species). Surprisingly, 1,228 (4.8%) mites from 84 (7.4%) birds were found on host species that were not the expected to be a host according to a recent bird– feather mite associations catalog. Unexpected associations were widespread across studied mite (40.6%) and bird (43.7%) species and showed smaller average infrapopu‐ lation sizes than typical associations. Unexpected mite species colonized hosts being distantly related to the set of their usual hosts, but with similar body size. The net‐ work of bird–mite associations was modular (i.e., some groups of bird and mite spe‐ cies tended to be more associated with each other than with the others), with 75.9% of the unexpected associations appearing within the module of the typical hosts of the mite species. Lastly, 68.4% of mite species found on unexpected hosts showed signatures of genetic differentiation, and we found evidence for reproduction or the potential for it in many of the unexpected associations. Results show host coloniza‐ tion as a common phenomenon even for these putatively highly host‐specific symbi‐ onts. Thus, host‐switching by feather mites, rather than a rare phenomenon, appears as a relatively frequent phenomenon shaped by ecological filters such as host mor‐ phology and is revealed as a fundamental component for a dynamic coevolutionary and codiversification scenario.

**K E Y WO R D S**

Acariformes, Analgoidea, Aves, coevolution, diversification, horizontal transmission,

Sarcoptiformes

Ecology, Estación Biológica de Doñana (EBD‐CSIC), Sevilla, Spain

2Department of Conservation

Biology, Estación Biológica de Doñana (EBD‐ CSIC), Sevilla, Spain

3Zoological Institute, Russian Academy of Sciences, Universitetskaya Embankment 1, Saint Petersburg, Russia

4Centro de Investigaciones sobre Desertificación (CSIC‐UV‐GV), Carretera Moncada‐Náquera, Valencia, Spain

**Correspondence**

Jorge Doña and Roger Jovani, Department of Evolutionary Ecology, Estación Biológica de Doñana (EBD‐CSIC), Sevilla, Spain.

Emails: [jorged@illinois.edu](mailto:jorged@illinois.edu); [jovani@ebd.](mailto:jovani@ebd.csic.es) [csic.es](mailto:jovani@ebd.csic.es)

**Present Address**

Jorge Doña, AllGenetics & Biology SL, Edificio CICA, Coruña, Spain and Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana‐ Champaign, Champaign, Illinois

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1. | **INTRODUCTION**

Speciation via host‐switching (symbiont speciation after success‐ ful colonization of a new host species) is becoming acknowledged

as one of the primary drivers for the adaptive radiation and diver‐ sification of symbionts (Bourguignon et al., 2018; Clayton, Bush, & Johnson, 2016; de Vienne et al., 2013; Nylin et al., 2017; Ricklefs, Fallon, & Bermingham, 2004). This conception, in contrast to strict

cospeciation, makes symbionts more active agents of their evolu‐ tion. Mainly by cophylogenetic studies, we now know that the rele‐ vance of host‐switching versus other processes such as cospeciation for the diversification of symbionts varies among groups (Clayton et al., 2016; de Vienne et al., 2013 and references therein). Evidence suggests that factors such as symbiont dispersal or parasite ecomor‐ phology are related to speciation by host switch (Sweet, Bush, et al., 2018a; Sweet, Chesser, & Johnson, 2017), but we are still far from understanding both the genesis of these macroevolutionary pat‐ terns at an ecological and microevolutionary scale and which factors influence it.

The process of speciation by host‐switching requires firstly some symbionts arriving to a new host (Clayton et al., 2016). These sym‐ biont individuals are known as stragglers (Ròzsa 1993), a term which refers explicitly to individual symbionts that ended up on a different (new) host species. Stragglers rarely survive nor reproduce on the new host, but if they do, and if they eventually succeed spreading within the new host species, they are cataloged as host switches (Clayton et al., 2016; Rivera-Parra, Levin, Johnson, & Parker, 2017; Ròzsa 1993). This process may lead to genetic differentiation and finally to an event of host‐switching speciation.

The existence of stragglers has been known for a long time, even long before the relevance of host‐switching for symbionts specia‐ tion was revealed (Choudhury, Moore, & Marques, 2002; Horak et al., 2006; Kellogg, 1896; Ròzsa 1993; Shepherd & Edmonds, 1976). Rivera‐Parra et al. (2017) provide a nice recent example. Studying feather lice from Galapagos Islands, they found stragglers in ca. 5% of the individual hosts examined while concluding that most stragglers would likely fail to colonize new hosts due to different ecological filters (see also Whiteman, Santiago-Alarcon, Johnson, & Parker, 2004). However, stragglers are still poorly documented in the literature as their observation is especially challenging because of their presumed scarcity and mostly ephemeral nature, especially for highly host‐specific symbionts, which are unable to survive for long when not on or in their host species. In addition, according to these difficulties, stragglers are challenging to distinguish from methodological artefacts (e.g., sample contamination when collect‐ ing ectosymbionts from museum host specimens or from living hosts held together before sampling; Ròzsa 1993), thus likely being under‐ represented in the literature.

Moreover, even for putatively highly host‐specific symbionts such as feather mites on birds, they have been often described as multihost (or oligoxenous) symbionts (Dabert, Solarczyk, Badek, & Dabert, 2005; Doña, Proctor, Mironov, Serrano, & Jovani, 2018), and there are some evidence supporting that straggling and even‐ tual host‐switching to a new host may be a common phenomenon (Doña, Sweet, et al., 2017; Doña, Proctor, Mironov, et al., 2018; Gaud, 1992; Klimov, Mironov, & OConnor BM, 2017; Matthews et al., 2018). However, we are still far from quantifying the relevance of these processes and understanding the mechanisms governing them. In part, the study of stragglers and host switches at these scales has been hampered by the lack of appropriate methods to study this phenomenon. Here, we used DNA metabarcoding of

feather mites to discover unexpected associations according to a recent comprehensive bird–feather mite associations catalog (Doña, Proctor, Mironov, Serrano, & Jovani, 2016) and study their ecological and genetic features to gain insight on host‐switching dynamics at an ecological or microevolutionary scale.

Feather mites (Acariformes: Astigmata: Analgoidea and

Pterolichoidea) are permanent and putatively highly host‐specific ectosymbionts of birds (Dabert & Mironov, 1999; Dubinin, 1951; Gaud & Atyeo, 1996; Proctor, 2003; Proctor & Owens, 2000). Most species inhabit only one or a few, usually closely related, bird species (Doña, Proctor, Mironov, et al., 2018). Moreover, feather mites show specific adaptations to live on their hosts (Dabert & Mironov, 1999; Proctor, 2003): morphological fit to feather microstructure, micro‐ site preferences within host feathers, fine‐tuned distributions along entire bird wings, and behaviours to avoid feathers close to being moulted (Fernández‐González, Pérez‐Rodríguez, Hera, Proctor, & Pérez-Tris, 2015; Jovani & Serrano, 2001, 2004; Stefan et al., 2015). Feather mites lack specific life‐history stages for transmission and except some members of the family Epidermoptidae and the genus *Strelkoviacarus* Dubinin, 1953 (Analgidae) are not known to disperse by phoresis on parasitic insects associated with birds, such as hip‐ poboscid flies (Dabert & Mironov, 1999; Doña, Potti, et al., 2017; Jovani, Tella, Sol, & Ventura, 2001; Proctor, 2003). Current knowl‐ edge suggests that their primary mode of transmission is vertical from parents to offspring in the nest (Doña, Potti, et al., 2017). In addition, they likely maintain a mutualistic relationship with birds in which they feed upon fungi and bacteria and likely on the uropygial gland oil that birds smear on the plumage (Doña, Proctor, Serrano, et al., 2018). Thus, such host‐specific symbionts have all the ingre‐ dients to be diversifying mainly by cospeciation. Interestingly, and contrary to this expectation, there is also evidence of horizontal transfer within and between bird species (Dubinin, 1951; Gaud, 1992; Jovani & Blanco, 2000), and recent studies have inferred that host‐switching with subsequent speciation is the primary process driving their evolutionary diversification (Doña, Sweet, et al., 2017; Doña, Proctor, Mironov, et al., 2018; Klimov et al., 2017; Matthews et al., 2018). These results suggest that host‐switching, despite its apparent difficulty for feather mites, has left macroevolutionary fingerprints along millions of years (Doña, Proctor, Mironov, et al., 2018; Doña, Sweet, et al., 2017). Our specific aims here were (a) to quantify the extent of unexpected associations in feather mites; (b) to study their performance (abundance) and genetic differentiation in the atypical hosts; and (c) to gain insight on the host–symbiont and mite infracommunity‐level interactions that govern host‐switching.

1. | **MATERIAL S AND METHODS**

# | Sampling and DNA metabarcoding pipeline

We sampled feather mites during 2010–2015 from live passer‐ ine birds captured with mist nets in different localities in Spain (Supporting information Table S1). We collected the feather mites found in primary, secondary and tertial feathers from the right wing

of each bird using a cotton swab impregnated with ethanol and pre‐

served mites at −20°C in tubes with 96% ethanol.

We took particular attention to our sampling protocol to avoid the risk of artificial mite cross‐contaminations between bird species (i.e., methodological artefacts rather than true unexpected mites). A previous study did not find feather mites detached from birds in cloth bags used to transport them from the mist net to the field sta‐ tion (Fernández‐González, 2013). So, for 491 birds (of those from which we succeed sequencing their mites), we used “normal” field procedures. That is, we extracted birds from the mist net with bare hands, placed them in standard bird banding cloth bags and then handled them again with bare hands when sampling their mites using disposable cotton swabs (because of the obvious risk of cross‐con‐ tamination by reusing them). Moreover, to test whether the prev‐ alence found with this protocol came from cross‐contamination when using bare hands or even reused cloth bags, we also applied a “refined” protocol to 639 birds where (a) we used single‐use latex gloves for extracting each bird from the mist net. (b) A single-use paper envelope to carry the bird until the field workstation (some meters away) and store it till processing. (c) A new pair of disposable latex gloves for handling the bird during feather mite sampling using disposable cotton swabs. We found that the prevalence of unex‐ pected mites did not differ between both protocols (“normal”: 7.1% (35 out of 491) of samples with unexpected mites, versus 7.7% (49 out of 639) in “refined” samples; χ2 = 0.04; *df* = 1; *p* = 0.8). We also explored potential tagging errors (i.e., sticker tags which may have been mistakenly pasted to a different sample) by retrospectively checking whether natural hosts of stragglers were handled up to two birds before or after the focal bird with stragglers (i.e., birds po‐ tentially overlapping in time during sampling and thus susceptible of potential tagging interchanges). We found that in 70.5% of the cases, unexpected mites were unequivocally found even when a potential tagging error was highly unlikely (note that this does not mean that tagging errors are the cause of the remaining 29.5%). Overall, this shows that our field procedures were not introducing false‐positive bird–mite associations, and therefore, we used samples from both protocols for downstream analyses.

Mites from each sample, representing a bird's mite infracommu‐ nity (i.e., each field microtube with feather mites from each bird), were counted under the stereomicroscope; that is, we counted the total number of feather mites from each individual bird, not the num‐ ber of mites per mite species from each bird as identification of lar‐ vae, nymphs and some adult females by morphology is unfeasible. Then, we analysed each sample following the DNA metabarcoding pipeline for feather mites described in Vizcaíno et al. (2018). Briefly, each bird's mite infracommunity was placed into one well of a 96‐ well plate and filled with 96% ethanol, leaving two empty wells for a DNA-negative extraction control and a PCR-negative control. Then, DNA was isolated using the HotSHOT method (Truett et al., 2000). DNA-sequencing libraries were prepared by amplifying a region of the mitochondrial COI gene (Doña, Diaz-Real et al., 2015; Doña, Moreno-García, Criscione, Serrano, & Jovani, 2015; Doña, Proctor, Serrano, et al., 2018), and by adding the Illumina‐specific sequencing

primers, indices and adaptors in a two‐step PCR. Finally, libraries were pooled together and analysed in a total of eight MiSeq 300PE runs (miseq reagent kit v3). Wet-labwork was carried out at Al l Genetics & Biology, SL (A Coruña, Spain) and sequencing at Macrogen (Seoul, Korea). Note that all libraries were pooled, that is, irrespectively of whether they were successfully amplified or not as most of the DNA quantifications were out of range for quantification but still poten‐ tially with enough DNA for high-throughput sequencing. Obtained reads were quality‐checked and quality‐trimmed. Specifically, the forward (R1) and reverse (R2) fastq reads of each MiSeq run were quality‐checked with fastqc (Andrews, 2010). And they were then imported into geneious v.8.1.7 (Kearse et al., 2012) for visual inspec‐ tion and quality‐trimming. We trimmed a region of variable length at the 3′ end of each file, according to the average Phred score (minimum quality score of 28) of each MiSeq run. The Python script (MMIS; Vizcaíno et al., 2018) was then used to automatize sequence concatenation, OTU picking and to eliminate mistagging events (i.e., a recently described sequencing artefact that results in 1% to 10% of reads misassigned to the wrong sample; Esling, Lejzerowicz, & Pawlowski, 2015; Sinha, Stanley, & Gulati, 2017; Owens, Todesco, Drummond, Yeaman, & Rieseberg, 2018). Moreover, only OTUs with more than 100 identical reads were kept. We also checked whether representative sequences contained stop codons. Overall, this sequencing‐bioinformatic approach allowed us to get more than 300–400 bp of COI sequenced (after quality‐trimming), a sequence length over the 200 bp minibarcode known to give similar results in species identification than the total length barcode (Doña, Diaz‐Real et al., 2015).

We did not find any evidence of cross‐contamination in our blanks when visualizing PCR gels (i.e., no band was visualized). Despite this, we sequenced each of the blanks. We did not find any read in 54 out of 55 blanks, and the one with reads contained a small number of reads (155) from *Pteronysoides parinus*. In particular, for this mite species, we only retrieved reads (16,746) in another sam‐ ple from the same plate (i.e., among the 384 samples multiplexed in the same sequencing run). However, in that sample, this mite spe‐ cies was expected (i.e., these mites were collected from a *Cyanistes caeruleus* individual, a typical host for that mite species; Doña et al., 2016). On the other hand, these samples were distant wells (D1 vs. H12), suggesting that this not be a case of contamination due to pi‐ petting. Thus, this case may be the single case in which we noticed our bioinformatic filter was not able to remove artefactual reads due to mistagging (see Vizcaíno et al., 2018 for more details). Mistagging is still relatively poorly understood (e.g., Costello et al., 2018), and further advances may be used to refine our analyses and identify our blank contamination as such. In any case, we are confident that this blank contamination do not compromise the validity of our study because the number of reads in the unexpected associations was much larger than the 155 reads retrieved in the contaminated blank (mean = 3,972; range = 106–33,102); 1 out of 55 (1.8%) potential contaminations is much lower than the 7.8% of unexpected asso‐ ciations we have found (see Results); and more importantly, S.M. a posteriori checked the actual presence of unexpected mites with a

morphological examination of the mites, finding them in 70.5% of

the instances (see below).

* 1. | **Data analyses**

Unless otherwise stated, all analyses were carried out in the R en‐ vironment (R Core Team, 2015). We labelled a bird–feather mite association as “unexpected” when this was not reported with con‐ fidence in the global catalog of bird–feather mite associations (i.e., data quality = 2 in Doña et al., 2016, hereafter “typical association”). This database reviews all available information on bird–feather mite associations from the literature, and S.M. taxonomically curated it carefully.

Samples containing representative sequences unclassified at the species level or containing unexpected mites were further analysed by S.M. based on morphological characters of the exoskeletons, thanks to the fact that our DNA extraction protocol preserves this material (Doña, Diaz-Real et al., 2015); that is, the same mite individ‐ uals were used for molecular and later on morphological analyses. In doing so, we registered the proportions of juveniles and adults for each infracommunity containing unexpected mites, although larvae and nymphs could not be assigned to any mite species. For adults, we also determined the numbers of males and females. Morphological identification revealed ten mite species which could not be associated with particular species through metabarcoding, and species from the *pinnatus* species complex were identified at the species level as they cannot be identified as different species using the COI region (Doña, Diaz-Real et al., 2015). Among these molec‐ ularly unidentified mites, we found five (a 7.2% of the total of mite species sampled in this study) putative new species belonging to five genera (*Alaudicola*, *Mesalgoides*, *Proctophyllodes*, *Scutulanyssus*, and *Trouessartia*) which were excluded for downstream analyses because of the impossibility of treating them as unexpected as‐ sociations. Among the samples containing molecularly identified unexpected associations, 70.5% (43 out of 61) were also validated morphologically. From the 18 nonvalidated, eight (44%) contained nymphal stages in which species‐level identification by morphology was not possible (Supporting information Table S2). In other words, the classical taxonomic analysis of the samples corroborated that the existence of unexpected bird–mite associations was not due to artefacts from the molecular analyses. Also, note that there were seven additional samples for which species‐level identification ac‐ cording to morphological characters was inconclusive (Supporting information Table S2).

We estimated the intensity or infrapopulation size (i.e., number of individual mites) of each feather mite species found within each bird's mite infracommunity by multiplying the proportion of reads retrieved from each mite species by the total number of feather mites counted in each infracommunity and then rounding to the nearest integer. Differences in the number of primer mismatches in the primer annealing regions may potentially bias some of these estimations, especially in those species which were not studied in Vizcaíno et al. (2018). However, we have shown elsewhere that this

yields a reasonable estimate of the number of individual mites (Diaz‐

Real, Diaz-Real et al., 2015; Vizcaíno et al., 2018).

For each feather mite species, we calculated when possible (see below) the genetic distances between unexpected mites and mites inhabiting typical hosts (according to Doña et al., 2016; hereafter nonunexpected mites) with the *dist.dna* function (“raw” model) from APE (Paradis, Claude, & Strimmer, 2004). First, we aligned represen‐ tative DNA sequences from unexpected and nonunexpected mites of each mite species (only in this analysis we did not use sequences with stop codons) with muscle v3.8.31 using default parameters (maximum number of iterations, 2) (Edgar, 2004). Then, alignments were trimmed to discard those columns which contained a signif‐ icant proportion of gaps (i.e., to discard those sequences having different length consequence of having being sequenced and bio‐ informatically processed separately and potential low‐frequency indels) using the function *msaTrim* with default parameters (fraction of gaps tolerated at the ends of the alignment, 0.5; fraction of gaps tolerated inside the alignment, 0.9) from microseq v1.2.2 (Snipen & Hovde Liland 2018). Also, we explored the distribution of haplotypes of unexpected mites by building haplotype networks with the *haplo‐ type* and *haplonet* (using raw genetic distances) functions from pegas v0.10 (Paradis, 2010). Three species containing unexpected associ‐ ations were identified only in the morphological assessments and therefore were not included in this analysis. For three other species, we obtained sequences from the unexpected associations but not from the typical hosts, so they were not included in this analysis ei‐ ther. Finally, *P. pinnatus* was also excluded from the genetic analysis as this a controversial taxonomic group whose species delimitation using COI is problematic (Doña, Diaz-Real et al., 2015).

Host phylogenetic information was obtained from BirdTree (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012; [http://birdtree.org](http://birdtree.org/)). We downloaded 1,000 trees from the Ericson backbone tree and then summarized them by computing a single 50% majority-rule con‐ sensus tree using sumtree v 4.1.0 in dendropy v4.1.0 (Sukumaran & Holder, 2010, 2015), following Rubolini, Liker, Garamszegi, Møller, and Saino (2015). We found phylogenetic information for all the bird species we studied. Following Doña, Sweet, et al. (2017), Avibase information (accessed on March 2016; Lepage et al., 2014) was used to match avian taxonomy in Doña et al. (2016) with that of Jetz et al. (2012).

Following Doña, Proctor, Mironov, et al. (2018), we estimated the probability density function of the phylogenetic distances be‐ tween host species sharing a mite species to study host phylogenetic specificity of unexpected and typical feather mites. To do so, we cal‐ culated the phylogenetic distance (as in Doña, Proctor, Mironov, et al., 2018) between each bird species pair sharing a mite species and calculated the proportion of bird pairs falling within ten phylogenetic distance bins (i.e., host range was split into 10 equal sized bins).

To understand whether host morphology imposes an eco‐ logical constraint to establishing onto a new host, we explored the relationships between the phylogenetic distance between typical and unexpected hosts and their differences in body size. Bird body mass is evolutionary conserved, so that closely related

species tend to have similar body sizes (Smith & Lyons, 2013). In addition, host body size is correlated with morphological variables of feathers that may constrain feather mite successful establish‐ ment, such as the interbarb distance of feathers (Pap et al., 2017). If unexpected hosts are phylogenetically distant from the typical hosts but with a similar body size, this would suggest that body size imposes a constraint to host‐switching (Clayton et al., 2016; Smith & Lyons, 2013). For this purpose, we calculated the body mass differences and phylogenetic distances between all pairs of hosts in which a particular mite species was found (Doña et al., 2016). The phylogenetic distance was measured as the sum of branch lengths from the most recent common ancestor to the two tips (species) of the bird phylogenetic tree with the function *cophenetic.phylo* from ape v5.1 (Paradis et al., 2004). We measured body mass distance (i.e., the difference in the mean body mass for the two species) as the difference between the maximum (i.e., the heavier bird species) and the minimum (i.e., the lighter bird species) body mass of each pairwise comparison. We obtained body mass information from Dunning (2008). Body mass and phylogenetic differences were analysed using generalized linear mixed models (GLMM) (GLMER function from package lme4 1.1–12; Bates et al ., 2015). We ran a Gaussian GLMM considering body mass distance as the response variable, the type of association (i.e., unexpected or typical) as the predictor variable, the phylogenetic distance as a fixed factor, and mite species as the random term. We confirmed assumptions underlying GLMMs by exploring regression residuals for normality against a Q‐Qplot.

To further explore the ecology of the unexpected associations from a nonpairwise point of view, we first identified groups of birds and feather mites that tended to associate more among each other than with other species in the network of associations (i.e., mod‐ ules), using the simulated annealing method implemented in the *net‐ carto* function with default parameters (iteration factor = 1; cooling factor = 0.995, bipartite = False) from rnetcarto v0.2.4 (Doulcier & Stouffer, 2015; Guimera & Amaral, 2005a, 2005b). The adjacency matrix (i.e., a presence–absence matrix with hosts in columns and mites in rows) was built with all the bird–mite associations found in our DNA metabarcoding study (i.e., the unexpected and the typi‐ cal). To evaluate whether hosts included in each module were more closely related than expected by chance (i.e., phylogenetic signal of hosts included in each module), we calculated the D‐statistic using *phylo.d* function from caper v0.5.2 (Fritz & Purvis, 2010; Orme et al., 2013). The network was plotted using the *plotweb* function from bipartite v2.08 (Dormann, Gruber, & Fruend, 2008).

1. | **RESULTS**

We collected mite infracommunities from 3,477 individual birds, from which we successfully built 3,090 libraries. Mainly because of DNA isolation failures, we eventually obtained sequences from 1,130 mite infracommunities (25,540 individual mites; 50 mite species identified by DNA metabarcoding, plus 14 mite species

identified only by morphological characters; see Materials and meth‐ ods) from 71 bird species.

We found unexpected mites in 84 bird individuals (1,228 indi‐ vidual mites; Supporting information Table S4), that is, 7.4% of the infracommunities and 4.8% of the individual mites studied. The presence of unexpected mites was not taxonomically restricted, but involved 43.7% (*N* = 31) of birds and 40.6% (*N* = 26) of mite species; 25.9% (14 out of 54) of the unexpected bird–mite associations were found in more than one bird individual (Table 1). Also, we found lar‐ val or nymphal stages in 30.9% (*N* = 22) of the mite infracommunities where unexpected mites were present and exoskeletons preserved for morphological analyses (*N* = 71), and in a 45.1% (*N* = 32), we found both males and females, thus suggesting reproduction (note that feather mites do not have dispersal stages) or potential for re‐ production on that bird, respectively (Supporting information Table S2). The potential for reproduction may be even higher in those birds with females, as potential inseminated females alone have the po‐ tential for reproduction.

Excluding unexpected mites, most birds (94.6%; *N* = 1,017) bore one mite species, 5.2% (*N* = 52) had two, and only 0.2% (*N* = 3) had three mite species. However, in 70.2% (*N* = 59) of the birds with un‐ expected mites, these were the only mite species. In the remaining

29.8% (*N* = 25), unexpected mites shared the host with a typical mite species. Thus, unexpected mites were found coinhabiting a bird with another mite species more frequently than for typical mite species (i.e., 5.2% vs. 29.8%; χ2 = 19.24; *df* 1; *p* < 0.001).

Overall, the average infrapopulation size (i.e., all the mites of a particular mite species occurring in an individual host) estimated for unexpected mites was smaller than that for typical species (Wilcoxon rank sum test; *W* = 43,042, *p* < 0.001). However, in some samples, some unexpected mites reached similar average intensities to typical mites (Figure 1). Among unexpected mites, mite infracommunities with reproductive stages (i.e., adult males and females; see above) showed higher intensity values (Wilcoxon rank sum test; *W* = 152.5, *p* < 0.001).

The minimum, mean and maximum genetic distances between sequences from unexpected and typical mite individuals showed different patterns among mite species. First, even maximum genetic distances between unexpected and typical mite individuals of the same species were lower than the mean smallest interspecific dis‐ tances found for feather mites in Doña, Diaz-Real et al. (2015) in all cases (Figure 2). Second, in ten of the species inhabiting unexpected hosts, we found that at least some haplotypes found in unexpected mites were also found in the sequences of typical individuals (i.e., min distance = 0). However, in 68.4% (*N* = 13) of these mite species, mean or maximum genetic distances were above the median intra‐ specific distance of that mite species in typical hosts (Figure 2). Also, haplotype networks were overall more diverse in these species (i.e., with genetic distance values over the median) than in mite species with lower genetic distances, but differences in sample size may influence this (e.g., undersampled mite species may present artifi‐ cial star‐like structures; Figure 2; Supporting information Table S3). Lastly, for those mite species in which more than one typical host

**TA B L E 1** The number of mite infracommunities with unexpected mites found in each bird species. Numbers in parentheses indicate the total number of individuals sampled for that bird species

***Dolichodectes edwardsi***

***Dolichodectes hispanicus***

***Joubertophyllodes modularis***

***Monojoubertia microphylla***

***Proctophyllodes cetti***

***Proctophyllodes ciae***

***Proctophyllodes clavatus***

***Proctophyllodes cotyledon***

***Proctophyllodes doleophyes***

***Proctophyllodes lusciniae***

***Proctophyllodes mesocaulus***

***Proctophyllodes motacillae***

***Proctophyllodes musicus***

***Proctophyllodes pinnatus***

***Proctophyllodes rubeculinus***

***Proctophyllodes schwerinensis***

***Proctophyllodes stylifer***

***Proctophyllodes sylviae***

***Pteronyssoides parinus***

***Scutulanyssus obscuroides***

***Trouessartia bifurcata***

***Trouessartia inexpectata***

***Trouessartia minuscula***

***Trouessartia reguli***

***Trouessartia serrana***

***Trouessartia trouessarti***

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Acrocephalus arundinaceus* (27) |  |  |  |  |  |  |  |  |  |  |  | 1 |  | | | | | | | | | | | | | |
| *Acrocephalus melanopogon* (16) |  |  |  |  | 2 |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Acrocephalus schoenobaenus* (9) |  |  |  |  | 2 |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Acrocephalus scirpaceus* (29) | 2 |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |
| *Carduelis carduelis* (22) |  |  |  | 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Chloris chloris* (7) |  |  |  | 2 |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |
| *Carduelis citrinella* (2) |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  | 1 |  |  |  |  |  |  |  |  |  |  |
| *Cettia cetti* (39) |  |  |  |  |  |  | 1 |  |  | 1 |  |  |  |  | 5 |  | 1 |  | 1 |  |  |  |  |  |  |  |
| *Emberiza cirlus* (8) |  |  |  | 6 |  | 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Erithacus rubecula* (95) |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |
| *Estrilda troglodytes* (2) |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |
| *Ficedula hypoleuca* (50) |  |  |  | 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Galerida cristata* (3) |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Hirundo daurica* (1) |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Hirundo rupestris* (1) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |
| *Hirundo rustica* (50) |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Lanius excubitor* (2) |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  | 1 |  |  |  |  |  |  |  |  |  |  |  |
| *Locustella luscinioides* (12) |  |  |  |  | 8 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Luscinia megarhynchos* (67) |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  | 1 |
| *Luscinia svecica* (46) |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |
| *Muscicapa striata* (22) |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Oenanthe hispanica* (2) |  |  |  |  |  |  |  |  |  |  | 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Oenanthe leucura* (1) |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Oenanthe oenanthe* (7) |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |
| *Phylloscopus bonelli* (3) |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Phylloscopus collybita* (17) |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  | 1 |  |  | 1 |  |  |  | 3 |  |  |  |  |
| *Phylloscopus trochilus* (34) |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |
| *Regulus ignicapilla* (2) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |
| *Saxicola torquatus* (2) |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Sylvia melanocephala* (26) |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  | 4 |  |  |  |  |  |  |  |  |  |  |  |
| *Turdus merula* (19) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  | 2 |  |

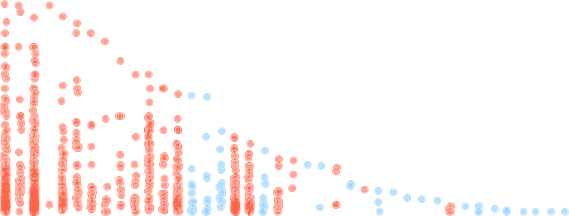
was sampled, genetic distances of unexpected infrapopulations of most mite species (seven out of eight; 87.5%) were in the range of variation of those of typical mites (Figure 2).

Unexpected mites inhabited hosts that were more distantly re‐ lated than expected according to the relatedness of usual hosts of feather mite species in this study (Figure 3; Wilcoxon rank sum test; W = 476,650; *p* < 0.001). The same result was found for the global database of bird–feather mite associations (Figure 3; Wilcoxon rank sum test; *W* = 101,250; *p* < 0.001, Doña et al., 2016). However,

unexpected mites were found on hosts which differed slightly less in body mass than typical hosts, but this difference was negligi‐ ble at large phylogenetic distances (Figure 4; GLMM, unexpected vs. typical: χ2 = 15.9, *p* < 0.0001; phylogenetic distance: χ2 = 40.0, *p* < 0.0001; unexpected vs. typical × phylogenetic distance: χ2 = 11.6, *p* = 0.0007).

The bird–feather mite network was composed of 26 modules (Figure 5), with an average (min-max) of two mite species (1–8) and three (1–11) bird species per module. All modules but one were

200



150

Intensity

100

50

0

Mite species

*Monojoubertia microphylla*

*Proctophyllodes cetti*

*Others*

*Joubertophyllodes modularis*

*Proctophyllodes lusciniae*

*Dolichodectes hispanicus*

*Trouessartia trouessarti*

*Trouessartia inexpectata*

*Proctophyllodes musicus*

*Pteronyssoides parinus*

*Proctophyllodes sylviae*

*Proctophyllodes clavatus*

*Proctophyllodes stylifer*

*Monojoubertia microphylla #*

*Proctophyllodes doleophyes*

*Proctophyllodes rubeculinus*

*Proctophyllodes rubeculinus #*

*Proctophyllodes mesocaulus*

*Proctophyllodes cotyledon*

*Proctophyllodes lusciniae #*

*Trouessartia inexpectata #*

*Proctophyllodes mesocaulus #*

of settling there; stable bird–mite associations that may have been overlooked in previous studies; and even long‐lasting bird–mite as‐ sociations that show enough genetic differentiation to suggest that they may eventually lead to an instance of host‐switching specia‐ tion. Also, those unexpected mites found in closely related hosts may even be due to a process of codivergence or failure to speciate (i.e., host divergence without symbiont speciation; Johnson, Adams, Page, & Clayton, 2003). Cophylogenetic analyses using time‐cali‐ brated trees as well as population genomic analyses (e.g., Sweet et al., 2018) would probably shed light on these aspects, and further research integrating quantitative data (e.g., prevalence, intensity) is needed to understand the performance of the same mite species in different bird hosts.

Lack of bird–feather mites phylogenetic congruence (at low taxonomic ranks) and the power of host‐switching to trigger fur‐ ther diversification have been shown elsewhere (Doña, Sweet, et al., 2017; Doña, Proctor, Mironov, et al., 2018; Matthews et al., 2018), and here, we provide evidence on how these pat‐

*Others #*

*Proctophyllodes cetti #*

*Trouessartia bifurcata*

*Trouessartia reguli*

*Proctophyllodes stylifer #*

*Proctophyllodes cotyledon #*

*Dolichodectes hispanicus #*

*Pteronyssoides parinus #*

*Joubertophyllodes modularis #*

*Trouessartia minuscula*

*Trouessartia bifurcata #*

*Proctophyllodes doleophyes #*

*Proctophyllodes musicus #*

*Proctophyllodes sylviae #*

*Proctophyllodes clavatus #*

*Trouessartia minuscula #*

*Trouessartia trouessarti #*

*Trouessartia reguli #*

**F I G U R E 1** Scatter plot showing intensity values of feather mites’ infrapopulations. Hash marks by species names and blued dots depict infrapopulations of unexpected mites while non‐hashed names and red dots depict infrapopulations of nonunexpected. “Others” *x*‐axis ticks group species only sampled either in typical

or in unexpected associations

composed of hosts more closely related than expected by chance (mean (min, max) *D* = −1.19 (−4.4, −0.34); and (mean (min, max) Pr (*D* = 1) = 0.22 (0, 0.131; Supporting information Table S3). 75.9% of the unexpected bird–mite associations were found within the mod‐ ules of the usual hosts of the same bird–mite association.

1. | **DISCUSSION**

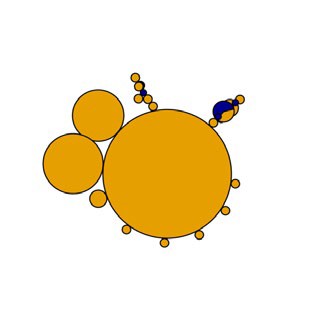
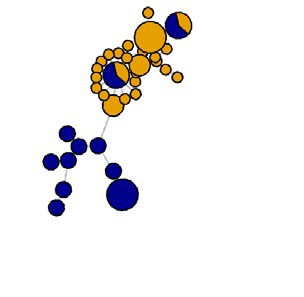
Contrary to what was expected for these purportedly highly host‐specific symbionts, we found a dynamic association scenario evidenced by a higher‐than‐expected frequency of unexpected as‐ sociations (7.4% of the infracommunities and 4.8% of the individual mites). A rough calculation of unexpected feather mites in European passerines shows the relevance of our result. A conservative estima‐ tion of population size for European passerine species is of ca. 109 bird individuals (BirdLife International, 2017). This number, jointly with a conservative mean individual bird feather mite abundance of 10 mites per bird (Diaz‐Real et al., 2014), leads to 1010 feather mites living in European passerines. Therefore, the prevalence of unexpected mites reported here yields a minimum of 108 individual birds with unexpected mites and 108 unexpected feather mites only for European passerines, which gives an idea of the potential rel‐ evance of unexpected associations for ecological and evolutionary processes.

Our results show that within these unexpected associations, there is a continuum of circumstances: mites recently “landed” on a new host species (stragglers) but with presumably low prospects

terns emerge from processes occurring at ecological and micro‐ evolutionary scales. Perhaps more importantly, a highly dynamic ecoevolutionary scenario where macroevolutionary patterns are only one of its outcomes is depicted, demanding to focus on the dynamics of these unexpected associations. In fact, we found a host–symbiont scenario in highly host‐specific symbionts com‐ patible with a geographic mosaic of coevolution in which network modules may be informative of the coevolutionary and codiversi‐ fication dynamics (Thompson 1994, 2005; Poulin, 2010; Clayton et al., 2016; Ivens, Beeren, Blüthgen, & Kronauer, 2016; Pinheiro et al., 2016). The dynamics of the coevolutionary scenario of puta‐ tively highly host‐specific symbionts, such as feather mites, could be analogous to that of a geographic mosaic of coevolution found in other systems in which populations are more connected (i.e., as the gene flow between symbiont populations inhabiting different hosts may be higher than previously thought), providing new av‐ enues of research. In doing so, questions such as to what extent these dynamics are generalizable to other feather mite groups and what factors are in play should be addressed. For instance, feather mites of passerine birds may present a higher rate of straggling and host‐switching than those of other bird groups (e.g., because potential donor–recipient hosts are morphologically more similar between them). Also, the dynamics of major host switches at this ecological scale could provide further valuable information (Doña, Proctor, Mironov, et al., 2018; Klimov et al., 2017). Moreover, habitat sharing may play an important role in straggling and host‐ switching dynamics and can vary through the year with different ecological processes (e.g., bird migration).

Also, our results provide important hints about the role of strag‐

gling and host‐switching in the coevolutionary dynamics of bird feather mites. Interestingly, we found that unexpected associations reached, on average, lower infrapopulation sizes likely as a result of the lack of specialization on these hosts (Figure 1). Moreover, these associations were found in hosts from the same network module (which were composed by closely related birds). However,



**F I G U R E 2** Boxplots showing the genetic distances of unexpected infrapopulations when compared to mites inhabiting typical hosts. Dashed grey line shows reference interspecific threshold for feather mites from Doña et al. (2015). Boxplots colours depict different statistics descriptors: orange (maximum), blue (mean), green (minimum) and grey, which depict intraspecific genetic distances for each mite species. Asterisks on the top of grey boxplots indicate mite species for which more than one typical (i.e., nonunexpected) host was sampled. Example haplotype networks showing contrasting diversity patterns belong to *Proctophyllodes cetti* (left) and *Proctophyllodes rubeculinus* (right). In yellow are depicted haplotypes of symbionts inhabiting typical hosts, and in blue, haplotypes of symbionts inhabiting unexpected hosts. Circle size is proportional to haplotype frequency



specifically, these hosts were more distantly related to the typical hosts than expected according to the phylogenetic host specificity of typical bird–feather mite associations (Figure 3). And this degree of relatedness was partially overlapping with the longest phyloge‐ netic distances reported for typical associations in Doña, Proctor, Mironov, et al. (2018) (Figure 3). Finally, these unexpected associa‐ tions were found in hosts with phylogenetic distances much shorter than potential associations with other bird species found in the same localities (e.g., mite species coming from non‐passerine birds of the study localities would have introduced hosts in the analysis which would have shown phylogenetic distances above 100 in Figure 3). First, this supports that feather mites present a high phylogenetic host specificity (Doña, Proctor, Mironov, et al., 2018) not because of a lack of transmission opportunities, but likely because of strong ecological filters. Also, this shows that while most stragglers would probably not persist much time in their new hosts, some may succeed (and in fact, we have found mixed evidence of potential early‐stage



host‐switching, and even of genetic differentiation). However, if they succeed, the comparison with typical associations strongly suggests that many of them would speciate due to host‐switching, thus reduc‐ ing the host range of the (parent) mite species again, although gene flow with the source host may persist and therefore influence the speciation process.

As already mentioned, our results advance in our understanding

of the ecological filters encountered by mites once they reach a new host. The most plausible are those imposed by host morphology or other host traits with a strong phylogenetic signal, as evidenced by the short phylogenetic distances between hosts occupied by feather mite species in their natural host range. Feather mites may be only able to settle at least temporarily in those hosts that are morpholog‐ ically similar to their typical hosts (Figure 3), so morphological traits related to body mass are potential candidates. Among them, wing flight feather traits such as interbarb distance would merit further study. Also, our results suggest that some of these filters may be not

0.6

0.4

Probability

0.2

Global database

Mite species from this study, only unexpected associations Mite species from this study, without unexpected associations Phylogenetic potential

200

150

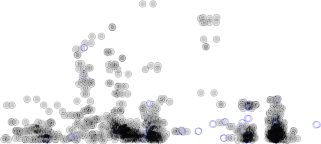
Body mass distance

100

50

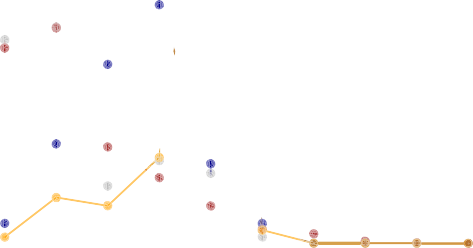
0

0 40 80 120



Phylogenetic distance

0.0



20

40

60

80

100

120

140

160

180

Phylogenetic distance between bird species sharing mite species

**F I G U R E 3** The probability that a pair of bird species sharing a feather mite species has a particular phylogenetic distance. Each line depicts probabilities of different mite subsets. Phylogenetic potential shows pairwise genetic distances between all hosts. Error bars represent confidence intervals (*α* = 0.05). Note that we only included mite species inhabiting more than one host

related to host morphology. The fact that unexpected mites coex‐ isted with another (typical) mite species in the same host infracom‐ munity more frequently than typical mite species (which usually do not coexist with congeneric species in the same host infracommu‐ nity) suggests that interspecific competition may preclude host range expansion (Johnson et al., 2009; Fernández-González et al., 2015; Doña, Potti, et al., 2017). Indeed, feather mite species from the same genera rarely coinhabit the same host, likely as a consequence of interspecific competition (Doña et al., 2016). On the other hand, de‐ spite out of the scope of this study, *Trouessartia* and *Proctophyllodes* mite species have been found coinhabiting (but to some extent com‐ peting for space) the same hosts in higher prevalences than found here (Fernández-González et al., 2015). Our lower prevalence may be due to differences in prevalence among host populations (e.g., as found between sedentary and migratory blackcaps, Fernández‐ González et al., 2015) or due to detection problems intrinsic to our molecular approach. In this sense, Vizcaíno et al. (2018) showed that this methodology is prone to false negatives. So, while making our study conservative because among the false negatives there should be other unexpected bird–mite associations, it should be refined be‐ fore using it for comprehensive catalogs of feather mite diversity.

Overall, these findings show that the host range of many of the stud‐ ied feather mite species is larger than previously thought. This highlights the need of future studies aimed to understand mite transmission, with

**F I G U R E 4** Scatter plot showing the differences in body mass between hosts sharing a mite species, accounting for the

phylogenetic relatedness. Black circles depict pairwise comparisons between typical hosts, and blue circles depict comparisons including unexpected hosts. Points are horizontally jittered

(two points) to improve visibility

the potential of discovering unexpected ways of horizontal transfer be‐ tween bird species. Most importantly, our study urgently encourages routinely integrating unexpected associations in host‐symbiont studies and catalogs rather dropping them out as methodological contamina‐ tions and to study them as essential components to understand the link between the ecology and the macroevolution of host‐symbiont systems.

### ACKNOWLEDGEMENTS

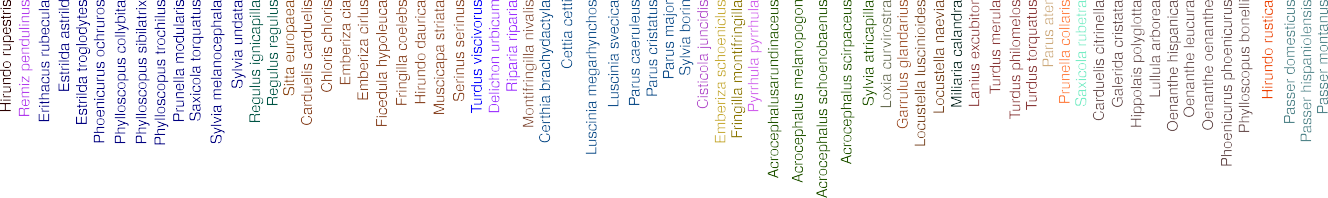
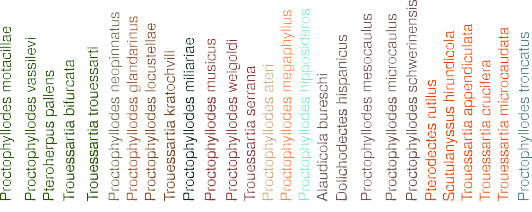
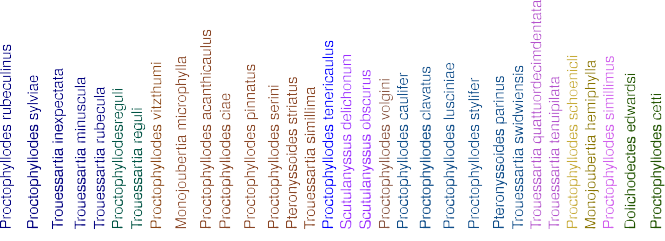
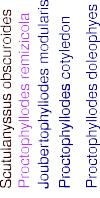
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### AUTHOR CONTRIBUTIONS

J.D., D.S., and R.J. conceived the study. J.D., D.S., and R.J. designed the study. J.D. collected the samples with the help of R.J. and D.S.

J.D. analysed the data with the support of A.M., S.M, D.S. and R.J.

S.M did the morphological identifications. J.D. wrote the manuscript with the help of all authors.



**F I G U R E 5** Feather mites and birds’ ecological network. Colour labels depict module composition (mites above, host birds below). Link colours represent feather mite module composition. Thicker dashed black lines represent unexpected associations found in the same module, and thicker dashed grey lines represent unexpected associations found outside the module

### DATA ACCESSIBILIT Y

We deposited HiSeq raw data and the processed representa‐ tive sequences files in Figshare ([https://doi.org/10.6084/](https://doi.org/10.6084/m9.figshare.6384095) [m9.figshare.6384095](https://doi.org/10.6084/m9.figshare.6384095); private link for review: [https://figshare.](https://figshare.com/s/f040ec6720733f7c742b) [com/s/f040ec6720733f7c742b](https://figshare.com/s/f040ec6720733f7c742b)).

### REFERENCE S

Andrews, S. (2010). fastqc: A quality control tool for high throughput se‐ quence data. Retrieved from [http://www.bioinformatics.babraham.](http://www.bioinformatics.babraham.ac.uk/projects/fastqc) [ac.uk/projects/fastqc](http://www.bioinformatics.babraham.ac.uk/projects/fastqc)

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., ... Green, P. (2015). Package "lme4" (Version 1.1-12). Retrieved from [https://cran.r‐project.org/web/packages/lme4/](https://cran.r-project.org/web/packages/lme4/)

BirdLife International (2017). *European birds of conservation concern: Populations, trends and national responsibilities*. Cambridge: BirdLife International.

Bourguignon, T., Lo, N., Dietrich, C., Šobotník, J., Sidek, S., Roisin, Y., … Evans, T. A. (2018). Rampant host switching shaped the termite gut microbiome. *Current Biology*, *28*, 649–654. [https://doi.org/10.1016/j.](https://doi.org/10.1016/j.cub.2018.01.035) [cub.2018.01.035](https://doi.org/10.1016/j.cub.2018.01.035)

Choudhury, A., Moore, B. R., & Marques, F. L. (2002). Vernon Kellogg, host‐switching, and cospeciation: Rescuing straggled ideas. *Journal of Parasitology*, *88*, 1045–1048. https://doi.org/10.1645/0022-3395( 2002)088[1045:VKHSAC]2.0.CO;2

Clayton, D. H., Bush, S. E., & Johnson, K. P. (2016). *Coevolution of life on hosts: Integrating ecology and history*. Chicago, IL: University of Chicago Press.

Costello, M., Fleharty, M., Abreu, J., Farjoun, Y., Ferriera, S., Holmes, L., … Vicente, G. (2018). Characterization and remediation of sam‐ ple index swaps by non‐redundant dual indexing on massively par‐ allel sequencing platforms. *BMC Genomics*, *19*, 332. [https://doi.](https://doi.org/10.1186/s12864-018-4703-0) [org/10.1186/s12864‐018‐4703‐0](https://doi.org/10.1186/s12864-018-4703-0)

Dabert, J., & Mironov, S. V. (1999). Origin and evolution of feather mites (Astigmata). *Experimental and Applied Acarology*, *23*, 437–454. https://doi.org/10.1023/A:1006180705101.

Dabert, M., Solarczyk, P., Badek, A., & Dabert, J. (2005). Taxonomic sta‐ tus of the oligoxenous feather mite species: Are we dealing with spe‐ cies in statu nascendi? *Phytophaga*, *14*, 425–431.

de Vienne, D. M., Refrégier, G., López-Villavicencio, M., Tellier, A.,

Hood, M. E., & Giraud, T. (2013). Cospeciation vs host‐shift

speciation: Methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist*, *198*, 347–385. [https://](https://doi.org/10.1111/nph.12150) [doi.org/10.1111/nph.12150](https://doi.org/10.1111/nph.12150)

Diaz-Real, J., Serrano, D., Piriz, A., & Jovani, R. (2015). NGS metabarcod‐ ing proves successful for quantitative assessment of symbiont abun‐ dance: The case of feather mites on birds. *Experimental and Applied Acarology*, *67*, 209–218.

Diaz‐Real, J., Serrano, D., Pérez‐Tris, J., Fernández‐González, S., Bermejo, A., Calleja, J. A., … Jovani, R. (2014). Repeatability of feather mite prevalence and intensity in passerine birds. *PLoS ONE*, *9*, e107341. <https://doi.org/10.1371/journal.pone.0107341>.

Doña, J., Diaz‐Real, J., Mironov, S., Bazaga, P., Serrano, D., & Jovani, R. (2015). DNA barcoding and mini-barcoding as a powerful tool for feather mite studies. *Molecular Ecology Resources*, *15*, 1216–1225.

Doña, J., Moreno‐García, M., Criscione, C. D., Serrano, D., & Jovani, R. (2015). Species mtDNA genetic diversity explained by infrapop‐ ulation size in a host‐symbiont system. *Ecology and Evolution*, *5*, 5801–5809.

Doña, J., Potti, J., De La Hera, I., Blanco, G., Frias, O., & Jovani, R. (2017). Vertical transmission in feather mites: Insights into its adaptive value. *Ecological Entomology*, *42*, 492–499.

Doña, J., Proctor, H., Mironov, S., Serrano, D., & Jovani, R. (2016). Global associations between birds and vane‐dwelling feather mites. *Ecology*, *97*, 3242–3242. <https://doi.org/10.1002/ecy.1528>

Doña, J., Proctor, H., Mironov, S., Serrano, D., & Jovani, R. (2018). Host specificity, infrequent major host switching and the diversification of highly host‐specific symbionts: The case of vane‐dwelling feather mites. *Global Ecology and Biogeography*, *27*, 188–198.

Doña, J., Sweet, A. D., Johnson, K. P., Serrano, D., Mironov, S., & Jovani,

R. (2017). Cophylogenetic analyses reveal extensive host‐shift spe‐ ciation in a highly specialized and host‐specific symbiont system. *Molecular Phylogenetics and Evolution*, *115*, 190–196.

Doña, J. , Proctor, H. C. , Serrano, D. , Johnson, K. P. , Oddy‐van Oploo,

A. , Ascunce, M. S. , … Jovani, R. (2018). Feather mites play a role in cleaning host feathers: New insights from DNA metabarcoding and microscopy. *Molecular Ecology*, *27*, [https://doi.org/10.1111/](https://doi.org/10.1111/mec.14581) [mec.14581](https://doi.org/10.1111/mec.14581).

Dormann, C. F., Gruber, B., & Fruend, J. (2008). Introducing the bipartite

Package: Analysing Ecological Networks. *R News*, *8*, 8–11.

Doulcier, G., & Stouffer, D. (2015). rnetcarto: Fast network modularity and roles computation by simulated annealing. R package version 0.2,

4. Retrieved from [https://CRAN.R-project.org/package=rnetcarto](https://CRAN.R-project.org/package%3Drnetcarto) Dubinin, V. B. (1951). Feather mites (Analgesoidea). Part 1. Introduction

to their Study. *Fauna USSR*, *6*, 1–363. [in Russian].

Dunning, J. B. J. (2008). *CRC* handbook of avian body masses (2nd ed.).

Boca Raton, FL: CRC Press.

Edgar, R. C. (2004). muscle: Multiple sequence alignment with high ac‐ curacy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797. <https://doi.org/10.1093/nar/gkh340>

Esling, P., Lejzerowicz, F., & Pawlowski, J. (2015). Accurate multiplexing and filtering for high‐throughput amplicon‐sequencing. *Nucleic Acids Research*, *43*, 2513–2524. <https://doi.org/10.1093/nar/gkv107>

Fernández‐González, S. (2013). Ecology and evolutionary perspec‐ tives of feather mite coexistence on the blackcap *Sylvia atricapilla* (Doctoral dissertation). Retrieved from UCM Database. (Accession No. T35167).

Fernández-González, S., Pérez-Rodríguez, A., de la Hera, I., Proctor, H. C., & Pérez-Tris, J. (2015). Different space preferences and within- host competition promote niche partitioning between symbiotic feather mite species. *International Journal for Parasitology*, *45*, 655– 662. <https://doi.org/10.1016/j.ijpara.2015.04.003>

Fritz, S. A., & Purvis, A. (2010). Selectivity in mammalian extinction risk and threat types: A new measure of phylogenetic signal strength in binary traits. *Conservation Biology*, *24*, 1042–1051. [https://doi.](https://doi.org/10.1111/j.1523-1739.2010.01455.x) [org/10.1111/j.1523-1739.2010.01455.x](https://doi.org/10.1111/j.1523-1739.2010.01455.x)

Gaud, J. (1992). Acquisition d’hotes nouveaux par les acariens plumi‐ coles. *Bulletin De La Societe Française De Parasitologie*, *10*, 79–91.

Gaud, J., & Atyeo, W. T. (1996). Feather mites of the world (Acarina,

Astigmata): The supraspecific taxa. *Annales Zoologici Wetenschappen*, *277*, 1–193.

Guimera, R. , & Amaral, L. A. N. (2005a) Cartography of complex net‐ works: modules and universal roles. *Journal of Statistical Mechanics: Theory and Experiment* , *2*, P02001.

Guimera, R., & Amaral, L. A. N. (2005b). Functional cartography of com‐

plex metabolic networks. *Nature*, *433*, 895.

Horak, I. G., McKay, I. J., Henen, B. T., Heyne, H., Hofmeyr, M. D., & De Villiers, A. L. (2006). Parasites of domestic and wild animals in South Africa. XLVII. Ticks of tortoises and other reptiles. *Onderstepoort Journal of Veterinary Research*, *73*, 215–227. [https://doi.org/10.4102/](https://doi.org/10.4102/ojvr.v73i3.148) [ojvr.v73i3.148](https://doi.org/10.4102/ojvr.v73i3.148)

Ivens, A. B., von Beeren, C., Blüthgen, N., & Kronauer, D. J. (2016). Studying the complex communities of ants and their symbionts using ecological network analysis. *Annual Review of Entomology*, *61*, 353– 371. <https://doi.org/10.1146/annurev-ento-010715-023719>

Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K., & Mooers, A. O. (2012). The global diversity of birds in space and time. *Nature*, *491*, 444–448. <https://doi.org/10.1038/nature11631>

Johnson, K. P., Adams, R. J., Page, R. D., & Clayton, D. H. (2003). When do parasites fail to speciate in response to host speciation? *Systematic Biology*, *52*, 37–47. <https://doi.org/10.1080/10635150390132704>

Johnson, K. P. , Malenke, J. R. , & Clayton, D. H. (2009) Competition promotes the evolution of host generalists in obligate parasites. *Proceedings of the Royal Society of London B: Biological Sciences* , *276*, rspb20091174.

Jovani, R., & Blanco, G. (2000). Resemblance within flocks and individual differences in feather mite abundance on long‐tailed tits *Aegithalos caudatus*. *Écoscience*, *7*, 428–432.

Jovani, R., & Serrano, D. (2001). Feather mites (Astigmata) avoid moult‐

ing wing feathers of passerine birds. *Animal Behaviour*, *62*, 723–727. <https://doi.org/10.1006/anbe.2001.1814>

Jovani, R., & Serrano, D. (2004). Fine‐tuned distribution of feather mites (Astigmata) on the wing of birds: The case of blackcaps *Sylvia atricap‐ illa*. *Journal of Avian Biology*, *35*, 16–20.

Jovani, R., Tella, J. L., Sol, D., & Ventura, D. (2001). Are hippoboscid flies a major mode of transmission of feather mites? *Journal of Parasitology*, *87*, 1187–1189. https://doi.org/10.1645/0022-3395(2001)087[118

7:AHFAMM]2.0.CO;2

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012) Geneious Basic: An integrated and ex‐ tendable desktop software platform for the organization and analy‐ sis of sequence data. *Bioinformatics*, 28, 1647–1649. [https://doi.org/](https://doi.org/10.1093/bioinformatics/bts199) [10.1093/bioinformatics/bts199](https://doi.org/10.1093/bioinformatics/bts199)

Kellogg, V. I. (1896). New Mallophaga, 1, with special reference to a col‐ lection made from maritime birds of the Bay of Monterey, California. *Proceedings of the California Academy of Science*, *6*, 31‐168.

Klimov, P. B., Mironov, S. V., & OConnor BM,, (2017). Detecting ancient codispersals and host shifts by double dating of host and parasite phylogenies: Application in proctophyllodid feather mites asso‐ ciated with passerine birds. *Evolution*, *71*, 2381–2397. [https://doi.](https://doi.org/10.1111/evo.13309) [org/10.1111/evo.13309](https://doi.org/10.1111/evo.13309)

Lepage, D., Vaidya, G., & Guralnick, R. (2014). Avibase – a database sys‐ tem for managing and organizing taxonomic concepts. *ZooKeys*, *420*, 117.

Matthews, A. E., Klimov, P. B., Proctor, H. C., Dowling, A. P. G., Diener, L., Hager, S. B., … Boves, T. J. (2018). Cophylogenetic assessment of New World warblers (Parulidae) and their symbiotic feather mites (Proctophyllodidae). *Journal of Avian Biology*, *49*(3), jav-01580. [https://doi.org/10.1111/jav.0158](https://doi.org/10.1111/jav.01580)0.

Nylin, S., Agosta, S., Bensch, S., Boeger, W. A., Braga, M. P., Brooks, D. R.,

… Schäpers, A. (2017). Embracing colonizations: A new paradigm for

species association dynamics. *Trends in Ecology & Evolution*, *33*, 4–14.

<https://doi.org/10.1016/j.tree.2017.10.005>

Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., & Pearse, W. (2013). caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.2. Retrieved from [https://](https://CRAN.R-project.org/package%3Dcaper) [CRAN.R-project.org/package=caper](https://CRAN.R-project.org/package%3Dcaper)

Owens, G. L., Todesco, M., Drummond, E. B., Yeaman, S., & Rieseberg,

L. H. (2018). A novel post hoc method for detecting index switch‐ ing finds no evidence for increased switching on the Illumina HiSeq X. *Molecular Ecology Resources*, *18*, 169–175. [https://doi.](https://doi.org/10.1111/1755-0998.12713) [org/10.1111/1755-0998.12713](https://doi.org/10.1111/1755-0998.12713)

Pap, P. L., Vincze, O., Wekerle, B., Daubner, T., Vágási, C. I., Nudds, R. L., … Osváth, G. (2017). A phylogenetic comparative analysis reveals correlations between body feather structure and habitat. *Functional Ecology*, *31*, 1241–1251. <https://doi.org/10.1111/1365-2435.12820>

Paradis, E. (2010). pegas: An R package for population genetics with an

integrated–modular approach. *Bioinformatics*, *26*, 419–420. [https://](https://doi.org/10.1093/bioinformatics/btp696) [doi.org/10.1093/bioinformatics/btp696](https://doi.org/10.1093/bioinformatics/btp696)

Paradis, E., Claude, J., & Strimmer, K. (2004). ape: Analyses of phylogenet‐ ics and evolution in *R* language. *Bioinformatics*, *20*, 289–290. [https://](https://doi.org/10.1093/bioinformatics/btg412) [doi.org/10.1093/bioinformatics/btg412](https://doi.org/10.1093/bioinformatics/btg412)

Pinheiro, R. B., Félix, G. M., Chaves, A. V., Lacorte, G. A., Santos, F. R., Braga, É. M., & Mello, M. A. (2016). Trade-offs and resource breadth processes as drivers of performance and specificity in a host–parasite system: A new integrative hypothesis. *International Journal for Parasitology*, *46*, 115–121. [https://doi.org/10.1016/j.](https://doi.org/10.1016/j.ijpara.2015.10.002) [ijpara.2015.10.002](https://doi.org/10.1016/j.ijpara.2015.10.002)

Poulin, R. (2010). Network analysis shining light on parasite ecol‐ ogy and diversity. *Trends in Parasitology*, *26*, 492–498. [https://doi.](https://doi.org/10.1016/j.pt.2010.05.008) [org/10.1016/j.pt.2010.05.008](https://doi.org/10.1016/j.pt.2010.05.008)

Proctor, H. C. (2003). Feather mites (Acari: Astigmata): Ecology, behavior, and evolution. *Annual Review of Entomology*, *48*, 185–209. [https://doi.](https://doi.org/10.1146/annurev.ento.48.091801.112725) [org/10.1146/annurev.ento.48.091801.112725](https://doi.org/10.1146/annurev.ento.48.091801.112725).

Proctor, H., & Owens, I. (2000). Mites and birds: Diversity, parasitism and coevolution. *Trends in Ecology & Evolution*, *15*, 358–364. [https://doi.](https://doi.org/10.1016/S0169-5347(00)01924-8) [org/10.1016/S0169-5347(00)01924-8](https://doi.org/10.1016/S0169-5347(00)01924-8)

R Core Team (2015). *R: A language and environment for statistical com‐ puting*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from [https://www.R‐project.org/](https://www.R-project.org/)

Ricklefs, R. E., Fallon, S. M., & Bermingham, E. (2004). Evolutionary relationships, cospeciation, and host switching in avian ma‐ laria parasites. *Systematic Biology*, *53*, 111–119. [https://doi.](https://doi.org/10.1080/10635150490264987) [org/10.1080/10635150490264987](https://doi.org/10.1080/10635150490264987)

Rivera-Parra, J. L., Levin, I. I., Johnson, K. P., & Parker, P. G. (2017). Host sympatry and body size influence parasite straggling rate in a highly connected multihost, multiparasite system. *Ecology and Evolution*, *7*, 3724–3731. <https://doi.org/10.1002/ece3.2971>

Ròzsa, L. (1993). Speciation patterns of ectoparasites and “straggling”

lice. *International Journal for Parasitology*, *23*, 859–864.

Rubolini, D., Liker, A., Garamszegi, L. Z., Møller, A. P., & Saino, N. (2015). Using the BirdTree. org website to obtain robust phylogenies for avian comparative studies: A primer. *Current Zoology*, *61*, 959–965. <https://doi.org/10.1093/czoolo/61.6.959>

Shepherd, R. C., & Edmonds, J. W. (1976). Ectoparasite stragglers (Siphonaptera and Acarina) of the wild rabbit, '*Oryctolagus cuniculus'* (L.), in Victoria. *The Australian Entomologist*, *3*, 63–66.

Sinha, R. , Stanley, G. , Gulati, G. S. , Ezran, C. , Travaglini, K. J. , Wei,

E. , & Weissman, I. L. (2017). Index Switching Causes “spreading- of-signal” among Multiplexed Samples in Illumina HiSeq 4000 DNA Sequencing. *Biorxiv*. <https://doi.org/10.1101/125724>

Smith, F. A., & S. K. Lyons (Eds.) (2013). *Animal body size: Linking pat‐*

*tern and process across space, time, and taxonomic group*. Chicago, IL:

University of Chicago Press.

Snipen, L., & Hovde Liland, K. (2018). microseq: Basic Biological Sequence Handling. R package version 1.2.2. Retrieved from https://CRAN.R- project.org/package=microseq

Stefan, L. M., Gómez-Díaz, E., Elguero, E., Proctor, H. C., McCoy, K. D., & González-Solís, J. (2015). Niche partitioning of feather mites within a seabird host, *Calonectris Borealis*. *Plos One*, *10*, e0144728. [https://](https://doi.org/10.1371/journal.pone.0144728) [doi.org/10.1371/journal.pone.0144728](https://doi.org/10.1371/journal.pone.0144728)

Sukumaran, J., & Holder, M. T. (2010). dendropy: A Python library for phylogenetic computing. *Bioinformatics*, *26*, 1569–1571. [https://doi.](https://doi.org/10.1093/bioinformatics/btq228) [org/10.1093/bioinformatics/btq228](https://doi.org/10.1093/bioinformatics/btq228).

Sukumaran, J., & Holder, M. T. (2015). sumtrees: Phylogenetic tree sum‐ marization. 4.0.0. Retrieved from [https://github.com/jeetsukuma‐](https://github.com/jeetsukumaran/DendroPy) [ran/ DendroPy](https://github.com/jeetsukumaran/DendroPy)

Sweet, A. D., Boyd, B. M., Allen, J. M., Villa, S. M., Valim, M. P., Rivera- Parra, J. L., & Johnson, K. P. (2018) Integrating phylogenomic and population genomic patterns in avian lice provides a more complete picture of parasite evolution. *Evolution*, *72*, 95–112.

Sweet, A. D., Chesser, R. T., & Johnson, K. P. (2017). Comparative co‐

phylogenetics of Australian phabine pigeons and doves (Aves: Columbidae) and their feather lice (Insecta: Phthiraptera). *International Journal for Parasitology*, *47*, 347–356. [https://doi.](https://doi.org/10.1016/j.ijpara.2016.12.003) [org/10.1016/j.ijpara.2016.12.003](https://doi.org/10.1016/j.ijpara.2016.12.003)

Sweet, A. D., Bush, S. E., Gustafsson, D. R., Allen, J. M., DiBlasi, E., Skeen, H. R., … Johnson, K. P. (2018a). Host and parasite morphol‐ ogy influence congruence between host and parasite phylogenies. *International Journal for Parasitology*, *48*, 641–648.

Thompson, J. N. (1994). *The coevolutionary process*. Chicago, IL: University

of Chicago Press.

Thompson, J. N. (2005). *The geographic mosaic of coevolution*. Chicago, IL:

University of Chicago Press.

Truett, G. E., Heeger, P., Mynatt, R. L., Truett, A. A., Walker, J. A., & Warman, M. L. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *BioTechniques*, *29*, 52–54. <https://doi.org/10.2144/00291bm09>

Vizcaíno, A., Doña, J., Vierna, J., Marí-Mena, N., Esteban, R., Mironov, S., ... Jovani, R. (2018). Enabling large‐scale feather mite studies: An Illumina DNA metabarcoding pipeline. *Experimental and Applied Acarology*, *76*, 81–97.

Whiteman, N. K., Santiago-Alarcon, D., Johnson, K. P., & Parker, P. G.

(2004). Differences in straggling rates between two genera of dove lice (Insecta: Phthiraptera) reinforce population genetic and cophylo‐ genetic patterns. *International Journal for Parasitology*, *34*, 1113–1119. <https://doi.org/10.1016/j.ijpara.2004.06.003>

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