Ecological factors driving the feather mite associations in tropical avian hosts

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Published in:
Journal of Avian Biology

DOI:
10.1111/jav.02951

Publication date:
2022

Document version
Publisher's PDF, also known as Version of record

Document license:
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Citation for published version (APA):
Bodawatta, K. H., Shriner, I., Pigott, S., Koane, B., Vinagre-Izquierdo, C., Rios, R. S., Jønsson, K. A., & Tori, W. P. (2022). Ecological factors driving the feather mite associations in tropical avian hosts. Journal of Avian Biology, 2022(6), [e02951]. https://doi.org/10.1111/jav.02951
Birds host a diversity of ectosymbionts including feather-dwelling arthropods such as feather mites and lice that they have co-evolved and speciated with. Among these ectosymbionts, feather mites have evolved more mutualistic to commensal associations with birds than other groups. However, our understanding of the biological and ecological drivers that shape the associations between avian hosts and feather mites in tropical communities is poor. Thus, to help fill this knowledge gap we investigated the factors that govern feather mite abundances at host community, host species and individual levels in bird communities from different elevations on the tropical island of New Guinea. We examined the effects of abiotic factors, such as temperature and precipitation, the influence of host species, feeding guilds, bill morphology, body region, body conditions and infections with haemosporidian blood parasites on feather mite abundance. We found that feather mites were very prevalent among New Guinean birds and that mite abundance was not significantly different between elevations. Bird species with curved bills experienced significantly lower number of mites compared to species with straight bills. Feather mite abundance was significantly higher on flight feathers than on the rest of the body and mite abundance was not strongly associated with the body condition of individuals in most host species, except for a significant negative relationships in three species. Moreover, we did not find an association between feather mite abundance and blood parasite infections, potentially indicating a non-synergistic association of these two symbionts. Overall, our study demonstrates that tropical avian-feather mite associations are driven by different biotic and abiotic factors at host community, species and individual levels, highlighting the importance of examining these associations at both broad and fine scales to thoroughly understand the evolution of these symbioses.

Keywords: avian malaria, feather ectosymbionts, haemosporidian parasites, New Guinea, scale mass index
Introduction

Avian hosts harbour a multitude of feather symbionts, varying from microbes (Javurková et al. 2019) to macro symbionts, such as feather mites and feather lice (Barbosa et al. 2002, Proctor et al. 2003). Associations between hosts and these symbionts vary from parasitic (Barbosa et al. 2002), commensal (Galván et al. 2012) to mutualistic (Soler et al. 2012, Doña et al. 2019, Bodawatta et al. 2020a), where mutualistic symbionts aid hosts with controlling antagonists such as feather degrading bacteria and fungal pathogens (Soler et al. 2012, Doña et al. 2019, Bodawatta et al. 2020a). The factors affecting the communities of these feather symbionts and their interactions with hosts can vary depending on the host species, ecology, behaviour, body region they inhabit (Choe and Kim 1988, Johnson et al. 2012, Javurková et al. 2019) and the type of interactions between symbionts and hosts (e.g. mutualistic, commensal or parasitic) (Harbison and Clayton 2011, Meléndez et al. 2014, Bodawatta et al. 2020b). Thus, understanding the drivers of these interactions is important to untangle the spatial distribution and long and short-term stability of these symbioses.

Among feather symbionts, feather mites (subclass: Acari, suborder: Astigmata) represent a diverse clade of arachnids (Proctor and Owens 2000, Doña et al. 2016) that have evolved more commensal to mutualistic associations with their avian hosts than other ectosymbionts (Blanco et al. 2003, Galván et al. 2012, Doña et al. 2019). However, there is some evidence for negative impacts of high mite densities on bird fitness (Galván and Sanz 2006, Galván et al. 2008), but mite densities appear not to influence the host immune system (Lindstrom et al. 2004). Feather mites harbour many morphological adaptations to live in the feather environment (Proctor 2003) and there is evidence for both co-evolution (Proctor and Owens 2000, Proctor 2003, Doña et al. 2017b) and host switching (Doña et al. 2017a, b) between avian hosts and their mite symbionts. Contrary to feather lice (which feed on the feathers themselves), feather mites tend to feed on dead skin and uropygial gland secretions from the hosts (Proctor 2003), and recent work demonstrated that mites clean feathers by feeding on bacteria and fungi (Doña et al. 2019). Although feather mites have been studied globally (Díaz-Real et al. 2014, Doña et al. 2016, 2017a), most of our understanding on factors governing bird-mite interactions stems from studies in temperate and subtropical regions (Díaz-Real et al. 2014, Meléndez et al. 2014). Thus, there is a knowledge gap in our understanding of these interactions in diverse avian communities in tropical regions. The magnitude of the influence of different abiotic and biotic factors governing feather mite diversities vary significantly between temperate and tropical regions, where abiotic factors strongly influence mite diversities in temperate regions, while host communities are more important in tropical regions (Gusmão et al. 2020). These differences highlight the importance of investigating host-mite associations in the tropics to better understand the drivers of these interactions in diverse communities.

Here, we examined the influence of multiple abiotic and biotic factors on mite abundances (i.e. the number of mites a host harbours) at host community, species and individual levels in tropical New Guinean understorey birds from three different elevations. We first investigated the effect of abiotic factors such as elevation, temperature and precipitation on community-level mite abundances. We predicted average mite abundances to be higher in communities that occur in the warm lowlands compared to those that occur in the colder highlands (Meléndez et al. 2014). Second, we explored the influence of ecological and anatomical factors of different species, such as feeding guild, and bill morphology on feather mite abundances. Bill morphology (i.e. bill overhang and bill overlap) has been shown to play crucial roles for the abundance of other feather symbionts (e.g. lice) (Moyer et al. 2002, Clayton et al. 2005, Freed et al. 2008), indicating that characteristics of the bill may also influence feather mite associations. Finally, to decipher the factors that govern individual-level host-mite interactions, where we examined the effect of host body regions (spatial distribution of mites), body conditions and infections by other parasites (haemosporidian blood parasites). We predicted that flight feathers would harbour a higher number of feather mites than other feather types similar to what was found in temperate bird species (Choe and Kim 1988, Jovani and Blanco 2000). We also predicted that birds with better body condition should harbour more mites, as is the case for many temperate bird species (Jovani and Blanco 2000, Galván et al. 2012). To investigate whether feather mite abundances are influenced by parasitic infections of hosts (Magallanes et al. 2016), we examined the associations between feather mites and haemosporidian blood parasites. There is evidence that when hosts have multiple infections, the burden of one symbiont can be enhanced (synergistic interactions) or be suppressed (antagonistic interactions) by the second symbiont (Bordes and Morand 2011). Moreover, hosts with haemosporidian infections have bigger uropygial glands (Magallanes et al. 2016), and uropygial gland size tends to positively correlate with feather mite abundance, potentially due to increased food resources (Soler et al. 2012). Thus, we predicted that individuals with blood parasite infections would harbour more feather mites as a result of the host response to blood parasite presence.

Material and methods

Study sites and sample collection

The study was conducted at three sampling localities along a north-facing elevational gradient at Mt Wilhelm (Madang Province, Papua New Guinea) between July and August 2019, where we captured understorey birds at 2200 m a.s.l. (22 to 24 July), 1700 m a.s.l. (25 to 30 July) and 200 m a.s.l. (1 to 6 August) during the late dry season. Environmental data (monthly precipitation and temperature) from each locality were extracted from Giovanni – NASA (Rodell et al.
2004, Beaudoin and Rodell 2019). GeoTIFF files were uploaded to QGIS ver. 3.14.0-Pi in R ver. 4.0 (<www.r-project.org>). To extract values for each locality, one vector file with the coordinates for each locality was created and values were extracted with the Point Sampling Tool Plugin ver. 0.5.3 (Jurgiel 2020).

Birds were captured using mist nets, which were open from dawn to dusk. Upon the identification of bird species, we collected allometric measurements (e.g. wing chord, exposed culmen length and body mass) (Supporting information). For each individual, we collected ~20 μl blood for haemoparasitoid screening using a 0.4 × 12 mm syringe and a brachial venipuncture (except for a few small bird species for which blood samples were not collected). Blood samples were stored in ethanol (70%) at ambient temperatures until samples were moved to the field station and thereon stored at −20°C until DNA was extracted. Due to the lack of blood samples from many individuals, we were unable to determine sexes of some individuals, hindering the ability to investigate the effect of sex on mite abundance. However, several studies have found no effect of host sex on mite abundances (Blanco et al. 1997, Galván and Sanz 2006, Pap et al. 2010), indicating that this factor is not important as a driver of this symbiosis.

**Ectosymbiont identification**

Birds were examined in the field and ectosymbionts were counted through careful inspection of feathers. Ectosymbionts on the head, breast and back were found by blowing lightly through feathers, while wing and tail feathers were spread and viewed against light. All the ectosymbionts present in feathers were counted in specific locations of the host body (head, back, belly, wings or tail). In the field, we categorized ectosymbionts into morphospecies and voucher samples for each morphospecies were collected and preserved in ethanol. Collected samples were then re-examined using an Olympus SZX7 stereomicroscope and identified to major taxonomic groups (e.g. feather lice, feather mites and ticks).

The taxonomic identity of many morphologically different feather mites and one tick were further determined through sequencing of the cytochrome c oxidase subunit 1 gene (CO1), using the mite specific primer pair bcdF05 (5′-TTTTCTACHAAYCATAAGATATTGC-3′) and bcdR04 (5′-TATAAACYTCDDGAGTGNCCAAAAA-3′) (Dabert et al. 2008). DNA was extracted from mites (1–5 individuals per each morphotype) using the Qiagen DNeasy blood and tissue kit, following the manufacturer’s guidelines. PCRs were conducted in 25 μl reactions with 12.5 μl of each of the primers and 5 μl DNA template using the PCR conditions described in Dabert et al. (2008). Successfully amplified samples were sequenced using the bcdF05 primer under a Sanger platform at Eurofins genomics (Copenhagen, Denmark). Sequences were blasted against the NCBI database using BLASTn.

We acknowledge that due to only genotyping morphologically different mites, we are not able to identify all mites into biological species level, hindering our ability to conduct any co-speciation/evolution analyses.

**Detection of haemoparasitoid blood parasite prevalence and lineage identification**

We extracted DNA from blood samples of bird species with at least six captured individuals to examine haemoparasitoid blood parasite prevalence. DNA was extracted from a sub-sample of blood (~10 μl) using the Qiagen DNeasy blood and tissue kit, similar to mites, but with an extended incubation period (~14 hrs). Haemoparasitoid screening was done using a well-established nested PCR protocol (Hellgren et al. 2004), following a few modifications in the PCR master mixes and conditions (below). *Leucocytozoon* infections were not assessed as they are rare among New Guinean birds (Bodawatta et al. 2020b), therefore, we screened for only *Haemoproteus* and *Plasmodium* infections.

The first round of PCRs was conducted in triplicates per sample using primer pairs HaemNF1 and HaemNR3 (Hellgren et al. 2004). PCRs were run with a negative control for every 16 samples and if it showed signs of amplification, it was run again. PCRs were conducted with 25 μl per sample (12.5 μl RedTaq polymerase, 1 μl of each primer, 8.5 μl autoclaved Milli-Q® water and 2 μl DNA template), under an initial denaturation step of 3 min at 94°C, followed by 20 cycles of denaturing (30 s at 94°C), annealing (30 s at 50°C) and elongation (45 s at 72°C) and a final step of 10 min at 72°C. Products from the initial PCRs were diluted by adding 5× Milli-Q® water and 8 μl of this was used as the template for the second PCRs. The second PCRs were conducted in 14 μl volumes using primer pair HaemF and HaemR2 (5 μl commercial multiplex master mix, 0.5 μl of each primer and 8 μl diluted first PCR product). PCR conditions for the second PCRs were the same except for 35 cycles instead of 20 cycles. PCR products were visualized using agarose gel (2%) electrophoresis and positively amplified samples were cleaned using Exosap and sequenced for the forward strain (HaemF) at Eurofins Genomics (Copenhagen, Denmark) under a Sanger platform. Sequences were edited and aligned using Geneious Prime 2019.2.3 and haplotypes were identified using the MalAvi database (Bensch et al. 2009) and the malaviR ver. 0.2.0 package (Ellis et al. 2020). Sequences with >98% similarity to known sequences in the MalAvi database were considered the same haplotypes. Sequences that had double base calling in the electropherogram were considered as co-infections or mixed infections (Valkiūnas et al. 2006, Bensch and Hellgren 2020).

**Feeding guild determination**

Feeding guilds of bird species were identified by reviewing the literature (Hoyo et al. 1992, Tvardikova 2013, Freeman and Freeman 2014, Pratt and Beehler 2014, Sam et al. 2017) and through personal field observations by the authors. In the few cases of conflicting information, we assigned the guild by giving more weight to sources that were consistent and by
using knowledge about the natural history of the species. The guild categories we used are: 1) nectarivorous, 2) frugivorous, 3) insectivorous (insects and other invertebrates), 4) insectivorous–frugivorous, 5) insectivorous–nectarivorous, 6) granivorous, 7) carnivorous (vertebrates) and 8) omnivorous (vertebrates and notable amounts of fruit or seeds).

Scale-mass index (SMI) for body condition and bill morphology

To determine the effect of mites on body condition of the bird species, we calculated the SMI for each species using exposed culmen length and body mass (Peig and Green 2009). We utilize the formula $SMI = M_i [L_{ai} L_{bi}]^{bSMA}$, where $M_i$ is the body mass and $L_i$ is the culmen length of the individual. $L_{ai}$ represents the arithmetic mean of the culmen length of the species and the scaling exponent $bSMA$ is the slope of the ordinary least squares (OLS) regression of the natural log of $M_i$ and $L_i$ divided by the Pearson’s correlation coefficient $r$ (Peig and Green 2009). To account for individual size variation and because feather mites were more prevalent on wings, we only analysed feather mites counted on the wing, controlled by wing length (feather mites per mm of wing chord). We excluded $Tocorhynchus poliopterus$ from the analyses because only one individual out of 12 had ectosymbionts on the wings.

To investigate if bill serrations (comb-like or saw-like edges on the upper mandible), bill curvature and overhang (upper mandible extending beyond the lower mandible at the bill tip) had an impact on mite abundances, we examined the bills of alcohol bird specimens housed at the Natural History Museum of Denmark (Univ. of Copenhagen, Denmark). Bills were categorized according to the shape (curved or straight), presence of serrations (serrated or non-serrated) and presence of bill overhang (overhang or not). The bill shape was determined by examining the convex nature of the lower side of the upper mandible (e.g. if the lower side was convex, then the bill was categorized as curved).

Generating a host phylogeny

We generated a host phylogeny using a concatenated alignment of three mitochondrial (NADH dehydrogenase 2: ND2, NADH dehydrogenase 3: ND3 and Cytochrome b: cytb) and three nuclear genes (Ornithine decarboxylase: ODC, Myoglobin 2: Myo2, glyceraldehyde-3-phosphate dehydrogenase: GAPDH) sourced from GenBank (Supporting information). We applied the General Time Reversible nucleotide substitution model to the concatenated data and ran the analysis for 100 million generations using a relaxed uncorrelated lognormal distribution for the molecular clock model, and assuming a birth–death speciation process as a tree prior using BEAST ver. 1.8.4 (Drummond et al. 2012). Tracer ver. 1.6 (Rambaut et al. 2014) was utilized for convergence diagnostics and TreeAnnotator ver. 1.8.3 (Drummond et al. 2012) was used to summarize the final output tree as a maximum clade credibility (MCC) tree with the first 10 million generation discarded as burn-in.

Statistical analyses and data processing

We assessed the influence of sampling locality (i.e. three elevations) on mite abundance on the full dataset, based on a generalized linear model (GLM) with a negative binomial distribution and log link function using the glm.nb function in the MASS package (Venables and Ripley 2002). We included body mass as a covariate in the model to control for differences in size across individuals and tested for an interaction between locality and body mass to evaluate if differences in size across individuals influenced mite abundance equally among localities (Venables and Ripley 2002). The monthly temperature, and precipitation vary with the elevation of the sampling locality, where both temperature and precipitation are negatively associated with elevation (Table 1). Thus, we utilized sampling locality as an independent categorical variable, the log transformed body mass as continuous independent variable and total feather mites per individual as the dependent variable in the model. If a significant interaction was found, differences in the relationship between total

Table 1. Difference in total number of bird species richness, number of unique species and overall feather mite abundance and prevalence among different field sites along the Mt Wilhelm elevational gradient. Confidence intervals (CI) at 95% are given for bird species richness and feather mite prevalence and N/A indicates non-calculated CIs.

| Diversity, feather mite and environmental metrics | 2200 m a.s.l. | 1700 m a.s.l. | 200 m a.s.l. |
|---------------------------------------------------|-------------|-------------|-------------|
| Total number of individuals captured              | 36          | 105         | 69          |
| Unique species                                    | 3           | 18          | 16          |
| Observed species richness                         | 16          | 31          | 17          |
| Rarefied species richness at 36 individuals (95% CI) | 16 (N/A)    | 18.87 (15–22) | 12.56 (9–15) |
| Simpson’s diversity index                         | 8.76        | 16.29       | 5.36        |
| Number of families captured                       | 10          | 15          | 10          |
| Number of orders captured                         | 2           | 3           | 2           |
| Feather mite prevalence (95% confidence intervals) | 77% (CI=64–91%) | 78.09% (CI=70–86%) | 60.87% (CI=49–72%) |
| Feather mite abundance (± SD)                     | 51.89 ± 70.38 | 92.24 ± 186.55 | 105.86 ± 400.17 |
| Average body mass of birds (g ± SD)               | 19.5 ± 20.5  | 23.1 ± 19.7  | 28.9 ± 29.5  |
| Proportion of curve-billed individuals            | 17.14%       | 17.14%       | 65.22%       |
| Average precipitation in sampling month (mm day−1) | 2.45         | 2.45         | 6.3          |
| Average temperature in sampling month (°C)        | 13.7         | 13.7         | 24.1         |
feather mites and body mass among localities were assessed based on pairwise comparisons of the slopes estimates at each locality using a Tukey method with adjusted p-values calculated with the lstrends function from the emmeans package (Lenth 2020).

Subsequent analyses were limited to groups with sample sizes ≥ 6 (e.g. foraging guilds, bird species). To examine if feather mite abundance differed among body regions (i.e. head, back, breast/belly, wings or tail), we used a generalized linear mixed model (GLMM) with a negative binomial distribution, a log link function and bird ID (unique individual code) as a random effect. Post-hoc comparisons were conducted using estimated marginal means (EMMs) adjusted using the Tukey method (Lenth 2020).

We examined if feather mite abundances differed among 11 bird species using generalized linear models (GLMs). We further explored the effect of foraging guilds and characteristics of bill morphology on average mite abundance and prevalence (the proportion of individuals with mites within species). We used phylogenetic generalized linear models (PGLS, correcting for phylogenetic relationship) in the caper package (Orme et al. 2018) with lambda set to maximum likelihood. Separate models were conducted for bill morphology and feeding guilds. Furthermore, we utilized GLMs with gaussian distribution with a log link function to examine whether feather mites per mm of wing length had an association with host body conditions (measured as SMI). We conducted these analyses only for individual bird species (as opposed to pulling species together and running an overall community analysis), due to the association between SMI and body mass of different species, where larger species inherently have larger SMIs compared to smaller species. We selected data points that were greater than a mean + two standard deviations as outliers and removed them from these analyses.

Associations between haemosporidian infections (presence/absence of infections) and mite abundances were assessed using a binomial GLM and significance was tested using anova function in R. We based the analysis on the presence/absence of *Haemoproteus* infections (we found no *Plasmodium* infections in this study) as the dependent variable to test for the association with mite abundance, bird species and SMI in the same model. Of blood parasite infected individuals, we further investigated the association between individuals with coinfections (more than one type of haemosporidian lineages) and both feather mites and SMIs. All statistical analyses were conducted in R ver. 4.0 (<www.r-project.org>). To generate figures, we used the ggplot2 (Wickham 2016) and viridis (Garnier 2018) packages.

**Results**

**Feather mites are ubiquitous across tropical birds**

We captured a total of 252 birds, forty-two of which were recaptures (16.67%). Species were dominated by the order Passeriformes (201 individuals), representing 16 families (Supporting information). Based on initial morphological assessment in the field, we identified three main categories of ectosymbionts: feather mites (subclass: Acari) (Fig. 1A), ticks (order: Ixodida) (Fig. 1B) and chewing lice (order: Phthiraptera) (Fig. 1C). Molecular barcoding results indicated that all the feather mites belong to the superfamily Analogoea (families: Proctophylloidae and Analogidae) and ticks belong to the family Ixodidae (order: Ixodida). Of the ectosymbiont categories feather mites colonized 152 out of 210 (72%) individuals to varying degrees (Fig. 1D). Six individuals were found to have ticks, and only one host had a louse. We were unable to identify certain ectosymbionts on eight individuals (Fig. 1D).

**Influence of host body mass on mite abundance varies between different elevations**

Despite average mite abundance being higher in the lowland community (Table 1), abundance did not vary significantly across localities along the elevational gradient ($\chi^2 = 3.05, p = 0.2179$). We did, however, find significant differences in log transformed body mass across elevations ($\chi^2 = 23.23, p < 0.0001$), as well as a significant interaction between locality and body mass ($\chi^2 = 12.64, p = 0.002$). This indicates that associations between host body mass and feather mite abundances vary between elevations (Supporting information). Comparisons of the relationship between mite abundance and body mass among elevations showed that the slopes for 1700 m a.s.l. and 200 m a.s.l. were not significantly different (diff $= -0.80, z = -1.69, p = 0.2087$), but the slopes of these localities differed significantly from 2200 m a.s.l. (diff $= 1.45, z = 2.52, p = 0.0314$ and diff $= 2.25, z = 3.76, p = 0.0005,$ respectively) (Supporting information). Therefore, birds at mid and low elevations increase their mite abundance with an increase in body mass at an equal rate (slope $= 0.90, CI = 0.29–1.52$; slope $= 1.70, CI = 1.01–2.39$; respectively) and at the high elevation body mass shows no effect on mite abundance (slope $= -0.55, CI = -1.50$ to 0.40; Supporting information).

**Bird species with curved bills harbour lower mite abundances**

We found a significant difference in feather mite abundance among the 11 species ($n \geq 6$) ($\chi^2 = 52.811, p < 0.0001$) and a significant relationship between feather mite abundance and body mass ($\chi^2 = 4.482, p = 0.03425$). However, there was no significant interaction between host body mass and species ($\chi^2 = 9.48, p = 0.4872$). Post hoc analyses revealed that species from the genus *Toxorhamphus* tend to have significantly lower feather mite abundances than other species (Fig. 2, Supporting information).

There was a significant effect of bill curvature on both average mite abundance (PGLS: $F = 10.48, p = 0.0143$) and prevalence (PGLS: $F = 8.332, p = 0.0234$), where species with curved bills experienced lower mite abundance.
and prevalence (Fig. 2). We did not find a strong effect of bill serration on average mite abundance (PGLS: $F = 2.743$, $p = 0.1417$) nor prevalence (PGLS: $F = 2.256$, $p = 0.1768$). Bill overhang did not reveal a significant effect on mite abundance (PGLS: $F = 0.3093$, $p = 0.5954$), but was significantly associated with prevalence (PGLS: $F = 14.48$, $p = 0.0067$), where species with bill overhang experienced higher prevalences (average ± SD: 86.8% ± 11.5) compared to species without bill overhang (58.5% ± 9.1). There was no effect of the host feeding guild on both average mite abundance (PGLS: $F = 1.938$, $p = 0.2235$) and prevalence (PGLS: $F = 1.118$, $p = 0.4292$), once controlled for host phylogeny.

Figure 1. Images of main ectosymbiont groups identified through morphology (A: feather mite – 0.06 mm, B: tick – 3.717 mm and C: chewing louse – 0.1378 mm). (D) Pie chart representing the break-down of different ectosymbionts found in individual birds from all localities. (E) Total feather mite abundance on different body parts of birds. Different letters on bars represent significant differences between body regions based on estimated marginal means (EMMs) adjusted to Tukey post-hoc tests with a $p < 0.05$. Bird illustration is acquired with permission from © Lynx edicions.
Figure 2. Phylogenetic tree (posterior probability values > 0.95 are indicated with ‡ signs), bill morphology and the average feather mite abundances (± SD) of 11 species with ≥ 6 sampled individuals. Bill serrations are indicated with asterisks while the curve-billed species are underlined. Numbers within parenthesis indicate the number of sampled individuals. Colours on the tips of the dendrogram indicate the feeding guild of the species, while elevational distribution of each species is indicated next to the species name (H: highland, L: lowland).
Spatially structured feather mite abundances did associate with host body conditions

In general, feather mite abundances differed significantly according to the body region, with the highest abundance in flight feathers ($\chi^2 = 266.72, p < 0.0001$). Post-hoc comparisons (EMMs) revealed that all body regions differed from one another except the tail, which was not different from the head (Fig. 1E and Supporting information). The strength of the relationship between body condition of individual bird species (SMI) and wing mite abundances corrected by wing chord varied among species (Fig. 3, Supporting information) and only three species had a significant negative relationship between SMI and number of feather mites per mm of wing [$Melanocharis versteri$ (GLM: $\chi^2 = 6.87, p = 0.0087$); $Melanocharis striativentris$ (GLM: $\chi^2 = 8.571, p = 0.0034$); $Peneothello cyanus$ (GLM: $\chi^2 = 4.324, p = 0.0376$)] (Fig. 3; Supporting information).

No association between mite abundance and haemosporidian blood parasite infections

We investigated haemosporidian blood parasite infections in eight bird species. We were unable to examine these infections in $Sericornis perspicillatus$, $S. nouhuysi$ and $Meliphaga analoga$ due to the low number of blood samples collected (<4). Overall, 67 out of 81 (82.7%) tested individuals were infected with haemosporidian blood parasites (Supporting information). All infections were caused by the genus $Haemoproteus$. Of the infected individuals, 25 demonstrated co-infections. All the $Haemoproteus$ lineages demonstrated $>98\%$ match to known lineages from the MalAvi database resulting in 20 unique lineages (Supporting information). There was an effect of host species on $Haemoproteus$ infections (GLM: $\chi^2 = 15.52, df = 7, p = 0.0299$). Most bird species experienced high levels of blood parasite prevalence, with $T. novaeguineae$ being infected the least (56.52%, Supporting information). Post-hoc tests revealed that $Haemoproteus$ prevalence of $T. novaeguineae$ differed significantly from $M. versteri$ ($p = 0.0007$), $M. striativentris$ ($p = 0.0007$) and $P. cyanus$ ($p = 0.0007$). There was no significant association, however, between $Haemoproteus$ prevalence and mite abundance (GLM: $\chi^2 = 2.927, df = 1, p = 0.0871$; Supporting information) nor with SMI (GLM: $\chi^2 = 0.0983, df = 1, p = 0.7539$). We also did not observe an effect of coinfections (compared to single infections) on mite abundance (GLM: $\chi^2 = 2.566, df = 1, p = 0.1092$) or individual SMIs (GLM: $\chi^2 = 1.779, df = 1, p = 0.1823$).

Figure 3. Species-level relationship between the body condition (SMI) and feather mites on wings (mites per mm of wing). Analyses were only conducted on species with 6 or more sampled individuals (except for $T. poliopterus$, which had one individual with parasites on wings). The grey area around the regression line represents the standard error around the model. Significant associations are indicated with red asterisks. Bird illustrations are acquired with permission from © Lynx edicions.
Discussion

Community-level mite abundances are governed by host community compositions

We did not observe a significant difference in mite abundances between elevations (Table 1), which contradicts previous findings for feather mite associations in temperate elevational gradients, where mite abundances were higher in warmer and drier low elevations (Meléndez et al. 2014). This indicates a reduced importance of these abiotic variables on shaping community-level mite abundances in the tropics compared to the temperate regions. Our results align with previous findings that suggest a decreased importance of abiotic factors for feather mite richness in tropical regions (Gusmão et al. 2020). Additionally, we found varying relationships between host body mass and mite abundances at different elevations (i.e. positive relationship at low and mid elevations and no relationship at high elevations, Supporting information), suggesting that bird community composition could be an important driver of host-symbiont associations as seen for other symbiont groups (e.g. haemosporidian parasites) in the tropics (Bodawatta et al. 2020b). We observed on average more large birds in low and mid elevations compared to high elevations (Table 1), indicating that community-level mite abundances are likely governed by the size distribution of the birds in a particular community.

Host bill morphology impacts mite abundances

Of the explored bill characteristics, only curvature negatively influenced both mite abundance and prevalence. Interestingly, even though we did not observe a significant effect of feeding guild, three out of four bird species with curved bills are categorized as insectivorous–nectarivores (Fig. 2). This might indicate that the curved bills associated with host feeding guild lead to improved removal of feather mites.

Despite the increased time allocation by hosts on preening flight feathers (Delius 1988, Chang et al. 2019), these feathers had the highest abundance of mites. The adaptations that feather mites harbour to anchor (e.g. small body size, specialized clasp ing structures on their legs) to the feathers (Proctor 2003) suggest that preening alone cannot remove feather mites (Choe and Kim 1988, Dowling et al. 2001). Curved bills, however, might disrupt the attachment of feather mites and remove them as a side-effect of preening. We did not observe an effect of bill overhang on mite abundance, which did not align with the negative associations observed in previous studies on bird-feather lice interactions (Clayton and Walther 2003, Freed et al. 2008). Instead, we found increased feather mite prevalence in species with a bill overhang, further contradicting findings from feather lice studies. Overall, this indicates that birds may not actively be trying to remove feather mites, which are more commensal to mutualistic symbionts, compared to parasitic feather lice.

Feather type, but not host body condition or co-infections, influence individual-level host-mite associations

Our results concur with previous studies that found high feather mite abundance on wing feathers, compared to other feather types (Blanco et al. 1997, Jovani and Blanco 2000). There have been multiple nonexclusive explanations proposed for this pattern. For example, it has been suggested that wing feathers provide a larger surface area compared to other feather types (Choe and Kim 1988). In addition, the structural properties of feathers [(e.g. quantity of keratin invested, shaft diameter, barb and barbule density, feather flexibility (Pap et al. 2015, Chang et al. 2019)] vary significantly among feather types (Sternheim 2000). Wing feathers are exposed to strong aerodynamic forces during flight and are optimized for lift, stiffness, aerodynamics and damage resistance (Sullivan et al. 2016). Wing feathers may thus be the feather type that is best suited for mites to hold on to with their modified clasp ing structures on the legs (Proctor 2003). This pattern may also be a result of food availability for feather mites on wing feathers compared to other body regions. Contrary to other groups of bird ecosymbionts, the diet of feather mites consists of uropygial gland secretions, dead skin, bacteria and fungi (Proctor 2003, Doña et al. 2019). There is also a positive association between the uropygial gland size and feather mite abundances in many bird species, indicating that uropygial secretions are important for maintaining these host–symbiont associations (Galván et al. 2008, Soler et al. 2012). Birds utilize uropygial gland secretions during preening and tend to allocate a higher proportion of time on preening their wings (Delius 1988), thereby increasing the amount of uropygial secretions that is applied. The wing feather environment may also harbour higher densities of bacteria and fungi (Labrador et al. 2021), which feather mites feed on (Doña et al. 2019). Thus, flight feathers may provide a habitat rich with food resources and with an advantageous microhabitat for mites to colonize. We also note that the visibility of feather mites is greater on flight feathers compared to other body feathers, that could have influenced our observations.

The observed significant negative associations between wing feather mite density and host body condition of only three species (M. versteri, M. striativentris and P. cyanus) suggest that such associations are species-specific. Cases from the literature suggest that associations between mite abundance and body conditions or reproductive success can be both positive (Blanco et al. 1997, Jovani and Blanco 2000, Galván et al. 2012, Soler et al. 2012) and negative (Galván and Sanz 2006, Galván et al. 2012). In our study (Fig. 3), all the negative associations appear to be driven by single individuals with high mite abundances. This suggests that feather mites might be acting as conditional parasites, where moderate amounts of mites have no effect, but higher mite abundances can have significant negative impacts on the hosts (Blanco et al. 2003, Galván et al. 2008). Alternatively, the negative associations may represent an increased abundance
of food resources due to microbial feather infections. For example, if a host is suffering from a fungal infection (negatively affecting its body condition), we expect mite numbers to increase, as these symbionts feed on fungal and bacterial antagonists of feathers (Doña et al. 2019). These hypotheses should be tested through both mite abundance manipulation and fungal infection studies.

The lack of an association between feather mite abundance and haemosporidian blood parasite prevalence indicates a lack of synergy between these two symbionts. This pattern is not in agreement with previous studies on parasitic feather lice in satin bowerbirds (Borgia et al. 2004) and willow ptarmigan (Holmstad et al. 2008), which demonstrated that blood parasites and feather lice infections can be highly correlated and can act synergistically. On the contrary, our results suggest that commensal to mutualistic feather mites do not capitalize on the effect of blood parasite infections of their hosts in the tropics. Our results also demonstrated that Haemoproteus infections are not associated with host SMI, suggesting that these infections do not influence the body conditions of the hosts. The effect of avian malaria infections on host body conditions tend to vary between host species, ranging from no effect (Schoenle et al. 2017) to an effect but only during the breeding season (Ganthon and Williams 2017). However, the presence of haemosporidians alone does not indicate that infection intensities of these parasites are high (Ganthon and Williams 2017). Moreover, the virulence levels of parasite lineages can differ, and host are shown to respond toward these parasites differently based on their virulence levels (Videvall et al. 2020). Thus, future studies should investigate both parasite intensity and virulence levels of lineages to better understand the effect of haemosporidian infections on host body conditions and potential synergies with other avian symbiont groups. Interestingly, T. novaeguineae, which experienced one of the lowest abundances of feather mites also experienced the lowest prevalence of haemosporidian parasites, suggesting that this species may be a good candidate to further investigate the potential association between ectosymbionts and endoparasites.

Conclusion

Symbioses between bird hosts and their feather mites have led to coevolution (Proctor and Owens 2000, Doña et al. 2017a, b) and a multitude of morphological adaptations in mites to live in a feather environment (Proctor 2003). We found that at the host community-level, feather mite associations are not influenced by elevation-specific abiotic factors, but potentially associated with host community composition (mainly the size of the birds in a community), mirroring the patterns of feather mite richness found in the tropics (Gusmão et al. 2020). Furthermore, species with curved bills tend to harbour reduced numbers of feather mites, but whether this reduction is due to active removal or merely a side effect of an ‘anti-mite’ bill shape associated with host feeding guild is yet to be determined. At the individual level, body conditions of most species were not associated with mite abundances, however, the trends differ between species, indicating potential species-specific associations and context specific interactions between feather mites and tropical avian hosts. Overall, results from this study demonstrate that different abiotic, ecological and biological factors can act in varying degrees on shaping this avian-feather mite associations at different scales in diverse tropical ecosystems.

Acknowledgements – We thank the New Guinea Binatang Research Centre in Madang, Papua New Guinea, and all the local people who assisted us with the research project. We also thank the Biology Department at Earlham College. Special thanks to Dan Atwater and Andrea P. Loayza, for their help and guidance with statistical analyses. We also thank the two anonymous reviewers for their helpful comments on earlier drafts of the manuscript.

Funding – WPT, IS and SP thank Earlham College student-faculty research support through an anonymous donor, the Matthews Student/Faculty Research in Physics/Biological Science fund, the Scantland Family Student/Faculty Collaborative Research Fund, the James B Cope Endowed Student-Faculty Vertebrate Zoology Field Research Fund and the Alpheaus Test Research Fund. KHB and KAJ are grateful for a Carlsberg Foundation Distinguished Associate Professor Fellowship to Knud A. Jønsson (CF17-0248).

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