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Exploring the *adjustment to parasite pressure hypothesis*: differences in uropygial gland volume and haemosporidian infection in palearctic and neotropical birds

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Abstract

Parasites are globally widespread pathogenic organisms, which impose important selective forces upon their hosts. Thus, in accordance with the *Adjustment to parasite pressure* hypothesis, it is expected that defenses among hosts vary relative to the selective pressure imposed by parasites. According to the latitudinal gradient in diversity, species richness and abundance of parasites peak near the equator. The uropygial gland is an important defensive exocrine gland against pathogens in birds. Size of the uropygial gland has been proposed to vary among species of birds because of divergent selection by pathogens on their hosts. Therefore, we should expect that bird species from the tropics should have relatively larger uropygial glands for their body size than species from higher latitudes. However, this hypothesis has not yet been explored. Here, we analyze the size of the uropygial gland of 1719 individual birds belonging to 36 bird species from 3 Neotropical (Peru) and 3 temperate areas (Spain). Relative uropygial gland volume was 12.52% larger in bird species from the tropics than from temperate areas. This finding is consistent with the relative size of this defensive organ being driven by selective pressures imposed by parasites. We also explored the potential role of this gland as a means of avoiding haemosporidian infection, showing that species with large uropygial glands for their body size tend to have lower mean prevalence of haemosporidian infection, regardless of their geographical origin. This result provides additional support for the assumption that secretions from the uropygial gland reduce the likelihood of becoming infected with haemosporidians.

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Key words: adjustment to parasite pressure hypothesis, malaria, Neotropical region, preen oil, temperate region

Parasites impose important selective forces upon their hosts (Schmid-Hempel 2011). Thus, they reduce fecundity (Abbate et al. 2015), decrease reproductive success (Merino et al. 2000; Marzal et al. 2005; Møller et al. 2009) and increase mortality (Møller et al. 2009; Martínez-de la Puente et al. 2010a). These organisms are abundant and ubiquitous (Schmid-Hempel 2011), varying in species richness and/or abundance across host species and latitudes (Møller et al. 2009; García-Longoria et al. 2019). One of the most recognized ecological patterns in many different organisms is the latitudinal diversity gradient (LDG), which implies an increase in species richness or biodiversity from the poles to the tropics (Pianka 1966; Hillebrand 2004; Møller et al. 2009; Morand et al. 2015). Although inverse latitudinal gradients in species diversity have also been observed (Kindlmann et al. 2007), terrestrial biodiversity tends to be the highest near the equator. This also seems to be the case for pathogens and parasites, which usually are more diverse and abundant in the tropics and the subtropics (Møller 1998; Møller et al. 2006). For example, Salkeld et al. (2008) compared the blood parasite abundance in 7 populations of the eastern water skink *Eulamprus quoyii* over a geographical area including both temperate and tropical regions, showing that parasite load was higher in populations in the tropics. Moreover, pathogenic fungi affecting terrestrial mammals and birds are particularly diverse and abundant in the tropics and subtropics (Mueller et al. 2004). Similarly, mosquito vectors transmitting arbovirus provoking dengue, Chikungunya, or yellow fever are more abundant in regions around the equator and the tropics (Kraemer et al. 2015). Furthermore, Nunn et al. (2005) analyzed data including 330 parasite species reported from 119 primate hosts to survey latitudinal gradients in the diversity of micro- and macro-parasites per primate host species, showing that species richness increased closer to the equator for protozoan parasites, especially for protozoa that are transmitted by arthropod vectors (e.g., *Leishmania*, *Plasmodium*, or *Trypanosoma*). Different patterns have also been found in some recent studies, showing no evidences of latitudinal gradient in prevalence and/or diversity for *Plasmodium* and *Haemoproteus* parasites (Clark 2018; Williamson et al. 2019; Cuevas et al. 2020), or even an inverse latitudinal gradient in infection probability for *Leucocytozoon* parasites in Neotropical birds (Fecchio et al. 2020).

With the aim to avoid infection by parasites or counteract their detrimental effects, animals have evolved a wide range of defensive barriers and mechanisms, including recognition and avoidance of infected individuals (Hart 1994), behavioral mechanisms to remove ectoparasites (Moore 2002; Hart 2011), and innate and adaptive immune responses to destroy pathogens (Wakelin 1996; Møller et al. 2009). Because pathogens affect host fitness, it is expected that host investment in immune defenses should be linked to variation in exposure to parasite diversity and abundance (Morand and Krasnov 2010; Morand et al. 2015). Thus, the "adjustment to parasite pressure" hypothesis predicts that host species living in areas with high diversity and abundance of parasites should invest more in immune function (Møller and Erritzøe 2002; Hasselquist 2007). For example, several studies of birds have shown that cell-mediated immune response is positively related to parasitism (e.g., parasite prevalence and parasite load) at both interspecific (Møller and Rózsa 2005) and intraspecific levels (Navarro et al. 2003). This suggests that hosts invest in immune function according to parasite pressure.

The uropygial gland (also called preen gland) is a holocrine gland exclusive to birds, and it has been suggested, among other functions, to be an important defensive mechanism against pathogens influencing survival (Magallanes et al. 2017; Moreno-Rueda 2017). The uropygial gland secretes waxes and other compounds (e.g., lipids, alcohols, terpenes, and fatty acids), which are spread over the plumage of hosts with the bill during preening for plumage protection (Jacob and Ziswiler 1982; Whittaker et al. 2011; Campagna et al. 2012; Soini et al. 2013). Although its function is still debated (see review in Moreno-Rueda 2017), uropygial secretions have been proposed to have antimicrobial and antifungal properties, thus acting as a defensive barrier of skin and plumage. For example, uropygial secretions can provide defense against feather-degrading bacteria in spotless starlings *Sturnus unicolor* (Rodríguez-Ruano et al. 2015) and house sparrows *Passer domesticus* (Moreno-Rueda 2014; Fülöp et al. 2016). It can also prevent infection by other potentially pathogenic bacteria such as *Pseudomonas*, *Staphylococcus*, and *Salmonella* (Czirják et al. 2013). Moreover, uropygial secretions have been experimentally shown to inhibit fungal growth (Bandyopadhyay and Bhattacharyya 1999). Because the size of the uropygial gland and the volume of its secretions vary considerably among species (Johnston 1988; Vincze et al. 2013), it has been proposed that this variation may have evolved as a consequence of divergent selection by pathogens on their hosts (Møller et al. 2009; Pap et al. 2013). However, this hypothesis has so far been poorly explored, thus deserving further investigation.

Avian malaria and related haemosporidian parasites (genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) are diverse and widespread causing negative effects on their avian hosts, such as decreased body condition (Valkiūnas 2005), reduced reproductive success (Asghar et al. 2015; Marzal et al. 2005), and increased mortality (Martínez-de la Puente et al. 2010b; Asghar et al. 2015; Marzal et al. 2016). Avian haemosporidian parasites require dipteran vectors in order to complete their complex life-cycles. Vectors differ among haemosporidian genera: (Para) *Haemoproteus* is transmitted by biting midges and hippoboscid flies (Valkiūnas 2005), *Leucocytozoon* by simuliid flies (Valkiūnas 2005), and *Plasmodium* by several species of mosquito (Valkiūnas 2005; Marzal 2012).

Although some studies suggested that uropygial secretions might attract haemosporidian vectors (Fallis and Smith 1964; Russell and Hunter 2005), recent studies have pointed out that uropygial secretions may prevent birds from acquiring *Plasmodium* and *Haemoproteus* infection (Magallanes et al. 2016; Marzal et al. 2018), suggesting that these secretions could act a physical barrier impairing the movement of vectors across plumage (Clayton et al. 2010) or acting as an insecticide by blocking the spiracles of vectors (Moyer et al. 2003). However, the suggested function of uropygial secretions in the fight against malarial infection is still poorly studied and limited to investigations in 1 bird species (house sparrows) (Magallanes et al. 2016). Hence, the potential anti-malarial role should be studied in more bird-malaria systems.

Here, we first tested for differences in uropygial gland size (UGS) in different bird species from Neotropical and temperate zones. If the uropygial gland has evolved as a defensive mechanism in the

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fight against pathogen exposure, then we should expect that bird species from the Neotropics should have larger uropygial glands than those from the temperate zone. We also explore the potential role of the uropygial gland in preventing *Plasmodium* and *Haemoproteus* infection. If the uropygial gland prevents birds from acquiring these haemosporidian infections, we should expect species with larger uropygial glands to have lower prevalence of malaria infection.

Material and Methods

Study sites and sample collection

Our study was conducted at 6 locations from 2 different biogeographical zones during 2014–2017, Neotropics [Peru: Pantanos de Villa (P. Villa) (12°12′S, 76°59′W), Tarapoto (6°29′S, 76°22′W), and Iquitos (3°44′S, 73°15′W)] and the temperate zone [Spain: Badajoz (38°53′N, 6°58′W), Cáceres (39°28′N, 6°22′W), and Seville (37°22′N, 5°59′W)].

A total of 1,719 adult birds belonging to 36 different species (N_{Peru}=16; N_{Spain}=20) were analyzed (Table 1). These species belong to 19 different families (Peru=9; N_{Spain}=12) with two families captured in both biogeographical zones. We captured with mist-nets and individually identified with colored or numbered metal rings. Because uropygial gland volume may differ between adult and juvenile birds (Salibian and Montalti 2009), we only included adult birds in this study. From each individual, we measured body mass (BM) with a digital scale to the nearest 0.1 g, and we also recorded length, height, and width of the uropygial gland with a digital calliper with a precision of 0.01 mm. Uropygial gland volume was estimated as the product of the 3 dimensions of the uropygial gland (Galván and Sanz 2006), which is positively related to the volume of secretions (Martín-Vivaldi et al. 2009; Møller et al. 2009; Pap et al. 2010). Because the uropygial gland is soft tissue (Martín-Vivaldi et al. 2009; Møller et al. 2009), we measured the 3 dimensions of uropygial glands 3 times to calculate average measures (Møller et al. 2009; Moreno-Rueda 2010, 2015). We used BM to estimate the percentage of the UGS in relation to BM of birds (100× UGS/BM) (Johnston 1988; Montalti and Salibián 2000). Finally, we took 1 microcapillary tube (50 µL) of blood from the brachial vein from each individual and stored it in 500 µL of SET buffer for DNA analyses.

Molecular detection of haemosporidian infection

With the aim of determining presence or absence of haemosporidian infection, blood samples from each bird were analyzed using molecular methods described in Waldenström et al. (2004). Blood samples were extracted using GeneJET^{TM} Genomic DNA Purification Kit (Thermo Scientific Inc., reference #K0722). Diluted genomic DNA (25 ng/μL) was used as a template in a nested polymerase chain reaction (nested-PCR). We used specific primer HaemoNF and HaemoNR2 for the first PCR, and HaemoF and HaemoR2 for the second PRC, to detect avian malaria parasites from the genera *Haemoproteus* and *Plasmodium* following Waldenström et al. (2004). All PCR experiments contained one negative control for every 8 samples. We evaluated the amplification using 2.5 µL of final PRC product on a 2% agarose gel. In the very few cases of negative controls showing signs of amplification (never more than faint bands in agarose gels), the whole PCR-batch was run again. In addition, all positive samples were sequenced to confirm that positive samples were true. Information on haemosporidian lineages will be added in further studies exploring lineage diversity.

Statistical procedures

We only included in the analyses those bird species from which we had a minimum sample size of 5 individuals. We used Shapiro–Wilk test to establish the normality of the residuals of the model and of the variable. We used 1-way ANOVA to test for significant differences in mean haemosporidian prevalence between tropical and temperate bird species. We analyzed the relationship between the mean value of relative UGS per species as response variable, and mean prevalence of haemosporidian infection per species, biogeographical area (temperate versus tropical), and their interaction as predictors.

Because UGS may vary among bird species (Vincze et al. 2013), we controlled for similarity due to common phylogenetic descent in our analyses. With this aim, we included a phylogenetic tree in our analyses with all the bird species analyzed. The phylogeny relationships were established by using birdtree.org web (Jetz et al. 2012), a useful and well-known tool for avian comparative studies (Rubolini et al. 2015). Birdtree.org distributes samples from a Bayesian Markov chain Monte Carlo chain (not bootstrap trees). Thus, using birdtree.org we created 1,000 unrooted phylogenetic trees based on the real comparative dataset that it poses (genetic information for more than 150 clades) and relaxed-clock trees that this tool generates for each clade (for more details see Jetz et al. 2012; Supplementary Section 1.1.1). Once these 1,000 trees were created we used the software Geneious (Kearse et al. 2012) for creating a consensus tree. A maximum-likelihood method and 1,000 bootstraps were used in order to create our consensus tree (final tree) (Figure A1). This final bird tree was edited by using the software R studio (R Core Computing Team 2017) and the libraries ggplot (Wilkinson 2011) and ggtree (Yu et al. 2017).

To control for the evolutionary relationship among the sampled species, we used phylogenetic generalized least square (PGLS) regression linear model as implemented in R statistical environment [see Díaz et al. (2013) and García-Longoria et al. (2014) for similar approaches], using the function (PGLS) depends on R packages *MASS* used in R-3.6.1 (R Core Computing Team 2017). In this model, the phylogenetic relationship was taken into account using the R packages *geiger* (Harmon et al. 2009) and *caper* (Orme et al. 2012). We used package *MuMIn* to select the best model. The strength and type of the phylogenetic signal in the data matrix was accounted for by adjusting branch length transformations (λ) (Freckleton et al. 2002). These transformations were optimized to find the maximum-likelihood transformation given the data and the model. All models were weighted by sample size to correct for heterogeneity in sampling effort among species (see Garamszegi and Møller 2010; Paradis 2011).

Results

We analyzed 1,719 individual samples from 36 bird species to test for differences in the size of the uropygial gland between bird species living in tropical (N = 189 individuals from 16 bird species) and temperate (N = 1530 individuals from 20 bird species) biogeographical zones (Table 1). The model residuals and variable showed a normal distribution (Shapiro–Wilk test) N = 36, W > 0.946, P > 0.05. Biogeographical origin of the bird species and mean prevalence of haemosporidian infection significantly explained variation in relative volume of the uropygial gland (Table 2). Specifically, mean corrected uropygial gland volume was on average 25% larger in bird species from tropical areas than in species from temperate areas [mean uropygial gland volume corrected for BM (standard deviation): tropical area=7.642 mm^3 (1.990); temperate

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Table 1. Mean and standard deviation (SD) uropygial gland volume (UGV) (mm^3) and mean haemosporidian prevalence of infection for bird species from temperate and tropical biogeographical zones

Bird species	Family	Mean UGV (SD)	Mean prevalence	Biogeographical zone	Location	Sample size
Chloroceryle aenea	Cerylidae	112.663 (21.39)	0	Tropical	Iquitos	9
Glaucis hirsutus	Trochilidae	56.341 (17.799)	0	Tropical	Iquitos	15
Amazilia amazilia	Trochilidae	31.621 (9.166)	0	Tropical	P. Villa	5
Amazilia fimbriata	Trochilidae	54.159 (35.203)	0.111	Tropical	Iquitos	10
Amazilia lactea	Trochilidae	34.866 (14.004)	0.143	Tropical	Tarapoto	9
Vireo olivaceus	Vireonidae	74.05 (7.714)	0.857	Tropical	Tarapoto	7
Miliaria calandra	Emberizidae	372.701 (49.06)	0	Temperate	Badajoz	6
Zonotrichia capensis	Emberizidae	193.805 (42.984)	0	Tropical	Tarapoto	8
Sporophila castaneiventris	Thraupidae	58.234 (12.355)	0	Tropical	Tarapoto	8
Oryzoborus angolensis	Thraupidae	67.173 (15.686)	0.238	Tropical	Iquitos	30
Ramphocelus carbo	Thraupidae	123.334 (32.42)	0.5	Tropical	Iquitos	8
Volatinia jacarina	Thraupidae	84.211 (16.071)	0.167	Tropical	P. Villa	7
Thraupis episcopus	Thraupidae	204.893 (46.653)	0.118	Tropical	P. Villa and Tarapoto	19
Coereba flaveola	Coerebidae	74.996 (12.36)	0.25	Tropical	P. Villa	9
Fringilla coelebs	Fringillidae	144.616 (25.417)	0.25	Temperate	Badajoz	5
Carduelis carduelis	Fringillidae	109.226 (76.478)	0.167	Temperate	Badajoz	12
Serinus serinus	Fringillidae	73.378 (19.582)	0.143	Temperate	Badajoz	12
Passer domesticus	Passeridae	165.887 (41.326)	0.245	Temperate	Badajoz and Caceres	352
Passer hispaniolensis	Passeridae	181.596 (40.605)	0.038	Temperate	Badajoz	29
Sturnus unicolor	Sturnidae	358.911 (78.193)	0.025	Temperate	Badajoz	40
Erithacus rubecula	Muscicapidae	65.58 (13.488)	0.1	Temperate	Badajoz	23
Luscinia megarhynchos	Muscicapidae	105.885 (24.722)	0.214	Temperate	Badajoz	14
Turdus ignobilis	Turdidae	318.261 (52.983)	0.133	Tropical	Tarapoto	18
Turdus merula	Turdidae	359.297 (59.853)	0.417	Temperate	Badajoz	13
Sylvia atricapilla	Sylviidae	103.201 (26.078)	0.079	Temperate	Badajoz	43
Sylvia melanocephala	Sylviidae	67.382 (13.078)	0.24	Temperate	Badajoz	28
Riparia riparia	Hirundinidae	93.831 (15.877)	0	Temperate	Badajoz	40
Delichon urbicum	Hirundinidae	58.245 (15.636)	0.152	Temperate	Badajoz	683
Hirundo rustica	Hirundinidae	98.68 (21.527)	0.096	Temperate	Badajoz and Sevilla	166
Aegithalos caudatus	Aegithalidae	38.521 (6.83)	0	Temperate	Badajoz	16
Phylloscopus collybita	Phylloscopidae	40.315 (6.407)	0	Temperate	Badajoz	8
Cettia cetti	Cettiidae	118.775 (22.185)	0	Temperate	Badajoz	8
Cyanistes caeruleus	Paridae	55.536 (10.11)	0.143	Temperate	Badajoz	10
Parus major	Paridae	110.312 (30.755)	0.353	Temperate	Badajoz	22
Tachuris rubrigastra	Tyrannidae	62.711 (10.893)	0	Tropical	P. Villa	12
Phleocryptes melanops	Furnariidae	149.826 (27.82)	0.267	Tropical	P. Villa	15

Note: Study location and sample size for each bird species is shown.

area=5.941 mm^3 (1.313)] (Table 2 and Figures 1 and A2). Moreover, species with larger uropygial glands tended to have lower mean prevalence of haemosporidian infection within each geographical region (Table 2 and Figure 2).

The value of lambda (λ) in our statistical analyses was 0.220, thus indicating that our results were not influenced by phylogenetic relationships among bird species analyzed in this study.

We found no significant difference in mean haemosporidian prevalence between tropical (mean haemosporidian prevalence=17.39%) and temperate bird species (13.30%, ANOVA: $F_{1,35}=0.472$, $P > 0.05$).

Table 2. Results for a phylogenetically generalized least squares (PGLS) regression for the effect of biogeographical area (temperate versus Tropical), mean prevalence of haemosporidian infection, and their interaction on the relative uropygial gland volume corrected for BM

Factor	Value	SE	*t*-value	*P*-value
Biogeographical area	1.202	0.553	2.173	**0.037***
Prevalence infection	−2.456	1.211	−2.027	**0.050***
Biogeographical area * Prevalence infection	0.138	3.477	−0.039	0.968
R^2 (%)	29.34 (25.06)			

Notes: PGLS model outputs refer to linear estimates (value) and their standard errors, and the associated *P*-values in phylogenetic analyses weighted by sample size. λ=0.220, residual SE =0.451, df=36. Significant code indicate: * $P \leq 0.05$.

Discussion

Parasites exert direct selection on host immune defenses (Schmid-Hempel 2011). Thus, different components of immunity should be influenced by parasite diversity and complexity. Following this idea, Alcaide et al. (2010) found a larger number of alleles and more divergent major histocompatibility complex (MHC) class I and class II haplotypes in Eurasian kestrels *Falco tinnunculus* than in the phylogenetically related lesser kestrel *Falco naumanni*, as expected from the higher pathogen diversity, richness, and prevalence in Eurasian than in lesser kestrels. This conclusion should be made with care since the study was based on a comparison of just two species. The "adjustment to parasite pressure" hypothesis predicts a higher investment in immune function in species living in parasite-rich areas

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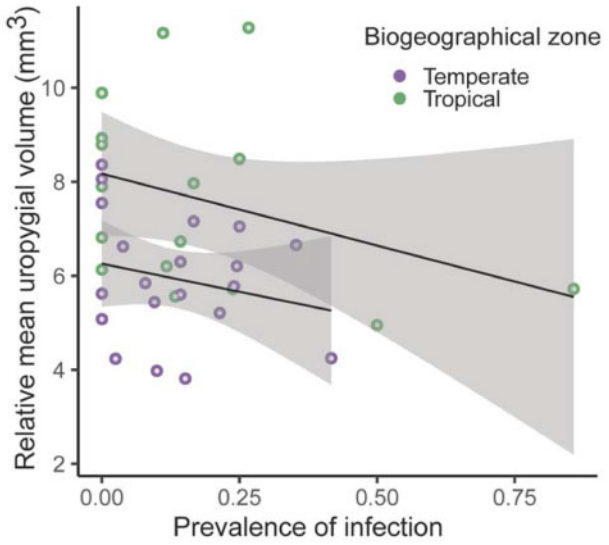


Figure 1. Scatterplot showing the relationship between mean prevalence of haemosporidian infection per species and mean value of relative uropygial gland size (UGS) per species (mm^3) (estimated as the percentage of the UGS in relation to BM) in temperate (purple circles, $N = 20$) and tropical species (green circles, $N = 16$). The lines are the linear regression lines, and the bands show 95% confidence intervals. Standard deviations are described in Table 1.

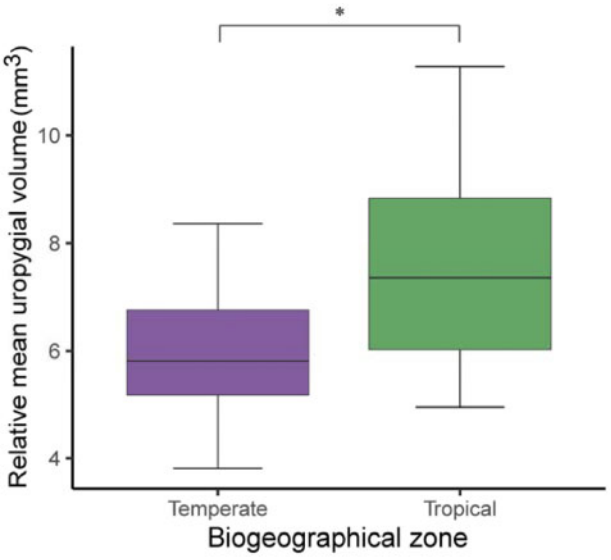


Figure 2. Box plots showing relative volume of uropygial gland (mm^3) (estimated as the percentage of the uropygial gland volume corrected for BM) for tropical species ($N = 16$) and temperate species ($N = 20$). Values are medians, upper and lower quartiles, and extreme observations.

(Møller 1998; Hasselquist 2007). Because parasite abundance and richness are normally greater in tropical and subtropical areas than at higher latitudes (Nunn et al. 2005; Merino et al. 2008; Salkeld et al. 2008), it should be expected that host species living near the equator should invest more in defenses against pathogens than host species living in temperate zones. Our results are consistent with this prediction, since bird species from tropical areas had relatively larger uropygial glands than those from temperate areas. These outcomes are similar to those reported by previous studies. For example, Møller (1998) conducted a pairwise comparative analysis of host investment in anti-parasite defense in bird species from tropical and temperate zones, showing that the circulating concentration of leukocytes in the blood was consistently higher, and the relative size of the spleen for a given body size was significantly larger in tropical when compared with that of closely related non-tropical species. Moreover, Hasselquist (2007) reported in passerine birds that species breeding closer to the equator showed enhanced humoral immunity, but not cell-mediated immune response. More recently, Marzal et al. (2018) have shown that house sparrows living in a Neotropical area had larger uropygial glands and higher antibacterial activity in their secretions than sparrows from the temperate zone despite both host populations originating from novel environments. Again, this latter conclusion should be made cautiously since it is based on a single species. These studies suggest that host species facing high pathogen exposure are likely to experience selection for high investment and maintenance of defensive mechanisms, when compared with host species facing low parasite diversity. However, some recent studies have found no evidences of latitudinal gradient, or even an inverse latitudinal gradient, in parasite richness and abundance of haemosporidian parasites (Clark 2018; Williamson et al. 2019; Cuevas et al. 2020; Fecchio et al. 2020). These contrasting results suggest a between species variation in such latitudinal pattern, probably due to differences in specific local biotic and abiotic conditions driving parasite distribution (Santiago-Alarcón et al. 2012; Ellis et al. 2017; Fecchio et al. 2019).

Alternatively, larger uropygial glands from Neotropical bird species could also be the result of phenotypic plasticity to higher pathogen pressure at lower latitudes. Previous studies have shown that the size of uropygial gland is dynamic and can increase its volume in response to parasitic infection risk (Vincze et al. 2013; Jacob et al. 2014). Thus, Neotropical bird species could modify their investment in size of the uropygial gland in response to increased parasite pressure. In agreement with this idea, Jacob et al. (2014) experimentally showed that the volume of the uropygial gland of male great tits *Parus major* increased when exposed to higher bacterial densities on feathers. Furthermore, Giraudeau et al. (2017) studied house finches *Haemorhous mexicanus* along a gradient of urbanization, showing a higher abundance of feather-degrading bacteria on the plumage of urban birds. They also reported an increase in size of the uropygial gland along the same urban gradient, suggesting that birds exposed to higher abundance of microbes coat their feathers with more uropygial secretions.

On further consideration, because humidity increases growth and activity of feather-degrading bacteria (Burtt and Ichida 2004), the larger sizes of uropygial glands of species living close to the equator could also benefit plumage maintenance in these moist environments. Therefore, individuals with larger uropygial glands should be favored at lower and more humid latitudes, and/or bird species inhabiting humid habitats could increase investment in the production of uropygial secretions to combat feather-degrading bacteria and preserve plumage function. Given that our tropical sampling sites may largely differ in humidity and precipitation, future studies exploring variation in uropygial gland volume across humidity and precipitation gradient would gain insights into this question.

Two recent studies have shown that the size of the uropygial gland varied with haemosporidian infection in house sparrows, suggesting that uropygial glands may be involved in defensive mechanisms against malarial infections (Magallanes et al. 2016; Marzal et al. 2018). Here, we analyzed blood samples from 1,719 individuals searching for haemosporidian infection in 36 bird species,

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showing that individuals with larger uropygial gland volumes have lower prevalence of malaria infection, regardless of geographical zone. This outcome is consistent with our previous findings about malaria infection and uropygial gland volume in house sparrows, providing additional support consistent with the hypothesis that uropygial secretions may interact with haemosporidian vectors and hence minimize the likelihood of becoming infected. We propose different mechanisms to explain these results. First, the antimicrobial properties of uropygial secretions may prevent haemosporidian infection. Bacteria from skin and plumage are responsible for the production of odors and chemical attractants for haemosporidian vectors like *Culex* spp. and simulids (Fallis and Smith 1964; Syed and Leal 2009). Thus, removal of bacteria and fungi from feathers and skin by antimicrobial activity of uropygial secretions could decrease vector attraction and thus minimize the likelihood of becoming infected with haemosporidians. Second, uropygial secretions may reduce the mobility of vectors on bird feathers and skin by acting as physical barriers (Clayton et al. 2010), thus avoiding mosquito bites. Third, uropygial secretions could act as an insecticide and affect ectoparasites by covering the surface of the vector or blocking their spiracles (Moyer et al. 2003). Finally, several components of uropygial secretions may include chemicals with arthropod-repellent properties, as shown for some bird species (Dumbacher and Pruett-Jones 1996). Further experimental studies are desirable to demonstrate whether uropygial secretions may have properties that decrease or remove the risk of malarial infection.

Given that inconclusive results mentioned above regarding latitudinal gradient and abundance and diversity of bird haemosporidian (e.g., Nunn et al. 2005; Clark 2018; Fecchio et al. 2020), we cannot rule out the possibility that the observed association between UGS and haemosporidian infection prevalence could be an exaptation linked to the selection of uropygial gland for anti-bacterial activity. Further studies exploring the functionality and selective forces acting on uropygial gland would provide insight into the evolution of this defensive trait.

To summarize, we have shown variation in relative size of uropygial glands in birds related to latitudinal distribution and malaria infection. Although this pattern is consistent with selection driven to parasites in this defensive trait, other abiotic (e.g., humidity) and biotic factors (e.g., feather-degrading bacteria) may also influence the evolution of uropygial secretion. Further experimental studies manipulating parasite loads on plumage of birds from different latitudes would provide insights into the evolutionary mechanisms involved in host immunity. Finally, empirical evidence would be desirable to examine the potential role of uropygial secretions for avoidance of malaria infections.

Ethics Statement

All experiments comply with the current laws of Spain and Peru (200-2016-SERFOR/DGGSPFFS), where the experiments were performed.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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Appendix

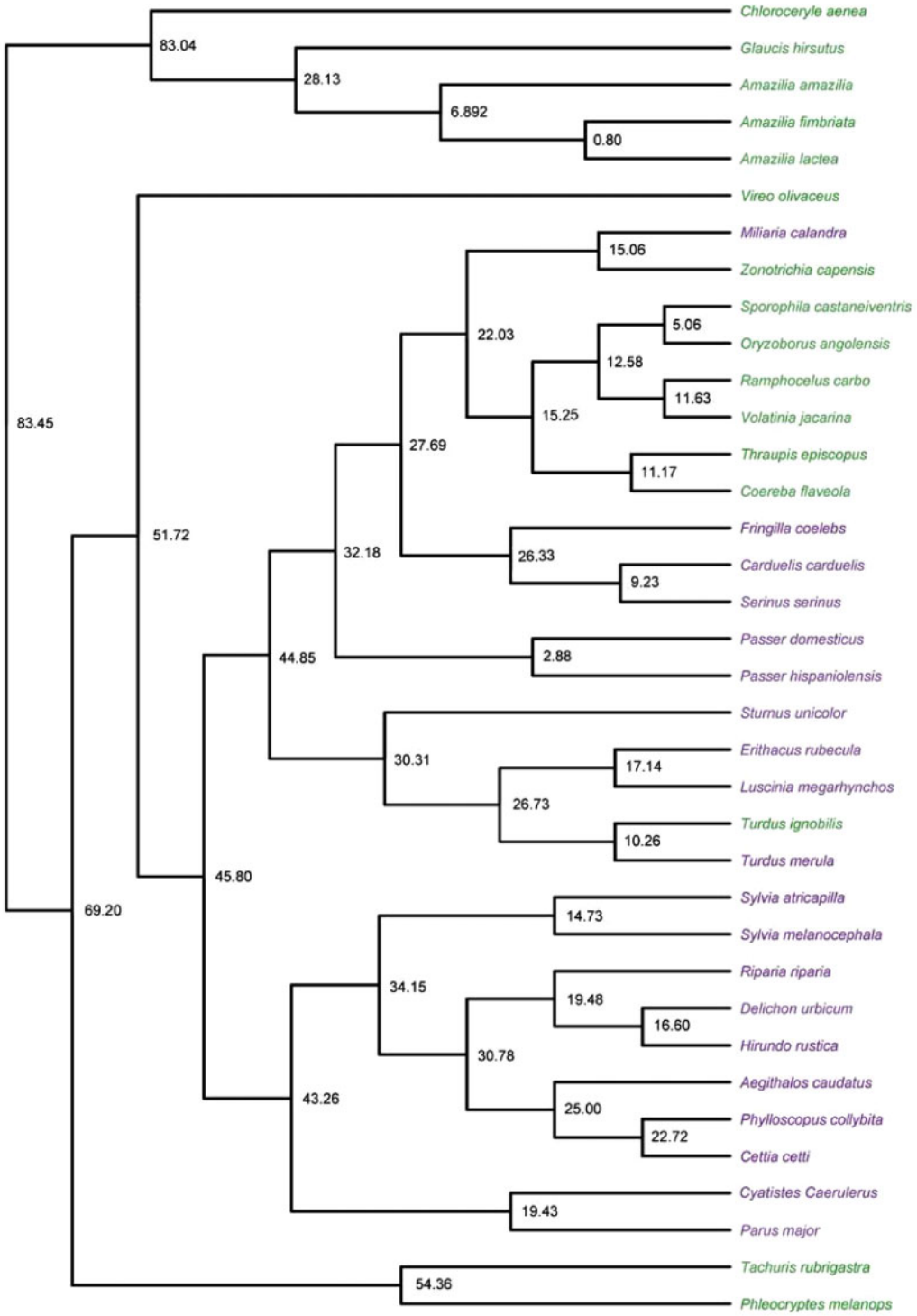


Figure A1. Phylogenetic consensus tree of all the bird species analyzed in the present study. Bird species have been colored according to the biogeographical region, green (16 tropical species) and purple (20 temperate species). Numbers indicate bootstrap values.

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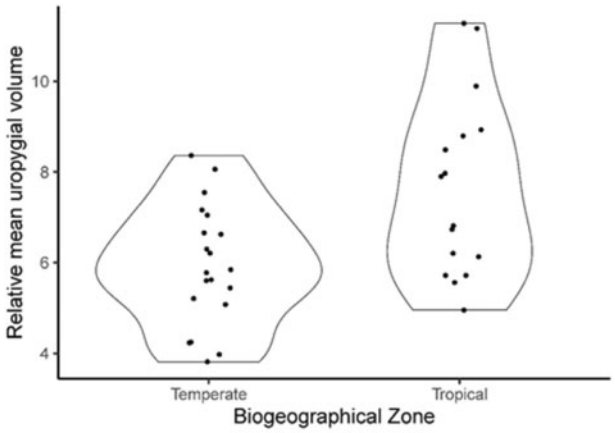


Figure A2. Jittered points plot showing the mean relative volume of uropygial gland (mm^3) for tropical species ($N = 16$) and temperate species ($N = 20$). We only included in the analyses those bird species from which we had a minimum sample size of 5 individuals. Jitters assign random values to the dots to separate them so that they are not plotted directly on top of each other.

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