

UNIVERSIDADE FEDERAL DO PARANÁ

GABRIEL MASSACCESI DE LA TORRE

**EFFECTS OF HEMOPARASITES AND BODY CONDITION IN PLUMAGE  
COLORATION OF *TURDUS ALBICOLLIS***

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GABRIEL MASSACCESI DE LA TORRE

**EFFECTS OF HEMOPARASITES AND BODY CONDITION IN PLUMAGE  
COLORATION OF *TURDUS ALBICOLLIS***

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre, no Curso de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, da Universidade Federal do Paraná.

Orientadora: Profa. Dra. Lilian Tonelli Manica  
Coorientador: Dr. André de Camargo Guaraldo

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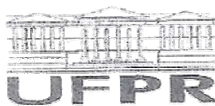
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Curitiba, 22 de Fevereiro de 2018.

LILIAN TONELLI MANICA

Presidente da Banca Examinadora (UFPR)

KARLA MAGALHÃES CAMPIÃO

Avaliador Interno (UFPR)

MARCOS ROBALINHO LIMA

Avaliador Externo (UEL)



Centro Politécnico - Setor de Ciências Biológicas - Curitiba - Paraná - Brasil  
CEP 81531980 - Tel: (41) 33611595 - E-mail: ecologia@ufpr.br

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## RESUMO

A hipótese de “seleção mediada por parasito” postula que parasitos reduzem a atratividade de indivíduos e prejudicam seu sucesso reprodutivo. Esta hipótese foi alvo de diversos estudos, porém os resultados ainda são divergentes em relação ao comportamento e morfologia das aves. O sabiá-coleira é uma espécie abundante na Floresta Atlântica, exibe garganta branca com listras negras, colar branco e peito cinza, e apresenta alta prevalência de hemoparasito, o que o torna um bom modelo para avaliar o efeito do parasitismo em caracteres secundários, neste caso, a coloração da plumagem. O objetivo deste estudo é identificar os hemoparasitos que ocorrem na população de sabiá-coleira, assim como avaliar o efeito da infecção nos caracteres sexuais secundários. Propusemos que a prevalência de hemoparasito: está relacionada com a idade; apresenta relação com a condição de saúde dos indivíduos; e influencia negativamente características potencialmente atrativas para parceiros reprodutivos. Realizamos o estudo no Módulo Rio Cachoeira, Antonina, PR. em campanhas trimestrais. Capturamos as aves com redes de neblina, marcamos os indivíduos com anilhas, identificamos sexo e idade quando possível, e medimos o comprimento do tarso, massa corporal e músculo torácico. Coletamos sangue por punção da veia braquial para fazer lâminas de esfregaços e análise molecular. Os esfregaços foram observados em microscopia para identificação de parasitos e contagem de heterofilos e linfócitos enquanto o restante do sangue foi utilizado para realizar PCR aninhada para detecção e identificação das linhagens de hemosporídeos. Medimos o brilho das penas por meio de fotografia digital de cada indivíduo, juntamente com uma escala de cinza cujos valores de refletância são conhecidos. Relacionamos a prevalência e intensidade de infecção de parasitos com a condição de saúde e coloração da plumagem de cada indivíduo utilizando modelos lineares generalizados. A intensidade de infecção de hemosporídeos e microfilária foram maiores em indivíduos com menor índice de massa corporal. Também encontramos relação positiva entre a coloração da garganta e prevalência de hemosporídeos, mas não encontramos relação entre microfilária, condição de saúde e coloração da plumagem do sabiá-coleira. Estes resultados sugerem que hemoparasitos afetam a saúde do sabiá-coleira de acordo com a intensidade de infecção já que apresentam condições morfológicas e imunológicas semelhantes entre infectado e não-infectado tanto na juventude quanto na fase adulta, e que a coloração mais evidente da garganta de indivíduos infectados pode ser um sinal de resistência aos hemosporídeos. Nosso estudo evidencia que hemoparasitos afetam a coloração da plumagem de forma contrária a hipótese da “seleção mediada por parasito” e a intensidade de infecção de parasitos apresenta relação negativa com a condição corporal. O mesmo se aplica para microfilária, pesquisas testando como a coloração da plumagem age na atratividade sexual de sabiá-coleira poderiam nos trazer maior entendimento sobre o papel deste caractere secundário na escolha de parceiros.

**Palavras-chave:** Sabiá-coleira. Hipótese de Hamilton & Zuk. Hemoparasito.

## ABSTRACT

The “parasite-mediated selection” proposes that parasites reduce individual attractiveness affecting reproductive success. This hypothesis was the focus of many studies however the results are still diverging regarding bird behavior and morphology. The White-necked Thrush is a wide spread Atlantic Forest bird, it exhibits white throat with black stripes, white collar and gray chest, and presents a high prevalence of hemoparasites, features that make it a good model to evaluate the effects of parasites in sexual secondary characters, in this case, plumage coloration. Our objectives are: identify the hemoparasites of a White-necked Thrush population and test the “parasite-mediated selection” hypothesis. We believe the hemoparasite prevalence affects individuals health condition and has a negative influence on potential secondary sexual characters. Mist-nets were used to capture and band individual birds. Birds were identified regarding their age, whenever it was possible, and tarsus length, body mass and pectoral muscle were measured. Blood slides were prepared and observed in microscope to detect and count hemoparasites. We also counted white blood cells, while the remaining blood was used to perform a nested-PCR to detect and identify haemosporidians. We calculated individuals feather brightness of throat, collar and chest using digital photography with a gray standard scale placed in the same surface as the bird. We used generalized linear model to relate health condition and hemoparasite occurrence, and also to test the effect of hemoparasite prevalence and health condition on plumage coloration. The prevalence of haemosporidians affected positively throat brightness, and intensity of infection of haemosporidians and microfilaria was higher in hosts with lower body mass condition. Nevertheless, we did not find any relationship between microfilaria occurrence, intensity of haemosporidian infection and plumage coloration of White-necked Thrush. Hemoparasites might affect White-necked Thrush health according to the intensity of infection, since body and immunological condition were not affected by prevalence. Moreover, the higher throat brightness on infected individuals might be a signal of resistance to haemosporidian and that intensity of hemoparasites infection is related with body condition. The same applies to microfilariae. Our study highlights that hemoparasites affect the plumage coloration in other direction than expected by “parasite-mediated selection”. We hope that our findings encourage others researches to investigate what is the value of the plumage coloration in communication in the White-necked Thrush and to understand the role of haemosporidians in individuals’ interactions.

**Key-words:** White-necked Thrush. Hamilton & Zuk Hypothesis. Hemoparasite.

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## 1 INTRODUCTION

Host-parasite relation benefits one of the organisms and harms the other (Pianka, 1994). Hosts develop responses to combat pathogens using resources that could be allocated to other activities essential to life, such as foraging, predation avoidance, and reproduction (Anderson & May, 1979; Lochmiller & Deerenberg, 2000). Pathogens can influence the individual fitness (Hayworth et al., 1987; Foreyt, 2001; Valkiunas, 2005), impact population demography (van Ripper et al., 1986; Tompkins & Begon, 1999) and play an important role at the community and ecosystem levels (Ricklefs & Renner, 2012; Wood & Johnson, 2015).

The negative consequences of parasites upon their hosts (Zuk, 1991; Hill & Brawner III, 1998; Costa & Macedo, 2005; Fülöp et al., 2016) drove Hamilton and Zuk (1982) to propose the "parasite-mediated selection" hypothesis. According to this hypothesis, individuals that are resistant to parasites would present more striking secondary sexual characteristics to indicate their healthier condition. To preserve themselves and offspring against parasitism, females may use these indicators to choose a partner, thus ensuring descendants with better fitness through inheritance of "good genes" (Hamilton & Zuk, 1982).

Birds are the most used study-group to test the influences of parasites on attractiveness (Hamilton & Poulin, 1997; Balenger & Zuk, 2014) because they host innumerable parasites (Hadley, 1916; Boyd, 1951; Valkiunas, 1997; Bentz et al., 2006; Belo et al., 2011) and have complex vocalizations, displays, and plumage coloration (Zuk, 1991). Despite parasite-mediated hypothesis has been broadly tested on birds, the results are still divergent, showing null (Gibson, 1990; Read & Weary, 1990, Hamilton & Poulin, 1997, Edler et al., 2004; Mougeot et al., 2009), positive (Korpimäki et al., 1995, Henschen et al., 2017) or negative effects of parasites on secondary sexual characters (Clayton, 1990; Møller, 1990; Aguilar et al., 2008; Bosholn et al., 2016).

Haemosporidians are the commonest birds' hemoparasites (Sloss et al., 1999, Foreyt, 2001), responsible for causing avian malaria. Their virulence varies according to the species, however they are considered mostly as "moderated virulent" at the acute phase (Valkiunas, 2005). Their infection can affect host physiological functions, such as thermoregulation (Hayworth et al., 1987), fat storage (Valkiunas, 1997) and

telomere shortening (Ashgard et al., 2016), as well as behavior (e.g. escape and foraging; Valkiunas, 2005).

Regarding the effect of parasites on bird plumage attractiveness, most studies are focused on carotenoid-based coloration (eg. Hill, 1990; Baeta et al., 2008; Dias et al., 2015). However, literature emphasizes that melanin-based coloration can also be condition-dependent (Jawor & Breitwisch, 2003; Griffith et al., 2006; Béziers et al., 2017), as well as related to mating and reproductive success (Bókoni et al., 2003; Tarof et al., 2005 and Kingma et al., 2008, Almasi et al., 2013). Thus, the quality of melanin coloration in plumage can also be considered an honest signal (Jawor & Breitwisch, 2003) and a potential trait to test for the effects of parasites in birds' conspicuousness. Here we studied the hemoparasite diversity and tested their effects on the White-necked Thrush (*Turdus albicollis*), a Brazilian Atlantic Forest understory bird (BirdLife, 2015). This is a social monogamous Thrush (Snow & Snow, 1963) – but that also present extra-pair paternity (Biagolini-Jr et al., 2016) – with a breeding season lasting at least four months (Snow & Snow, 1963; Biagolini-Jr et al., 2016). It has a contrasting black and white stripes in the throat, a white collar and grayish chest (Ridgely et al., 2015), a contrasting plumage coloration in relation to its habitat (Snow & Snow, 1963). We focused on the contrast between the melanin-based colored plumage (such as the black and gray) and white, assuming it is an important signal of the individuals' condition at same time that influences its competitiveness and sexual attractiveness (Hauser, 1997; Roulin, 1999; Roulin et al., 2001).

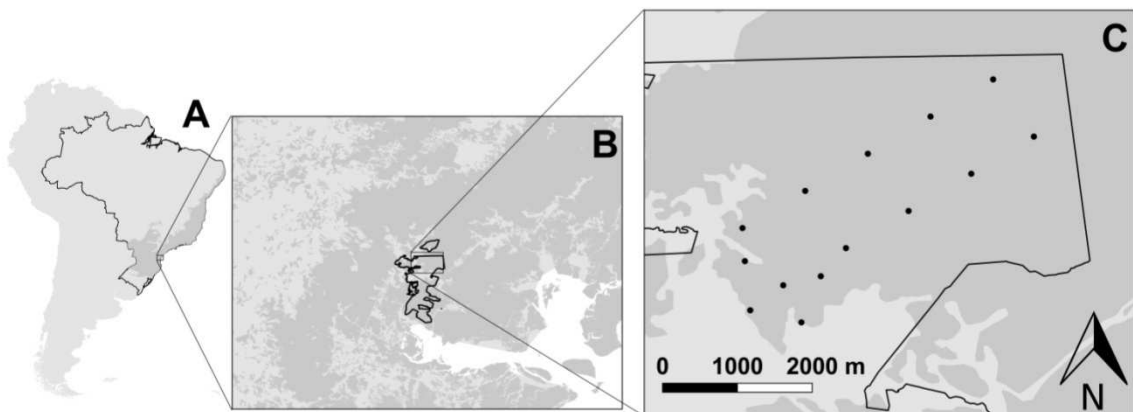
Despite the high bird diversity in Brazil (Piacentini et al., 2015), only few studies of bird-parasite interaction were carried out, mostly related to the understanding of abundance, distribution, and parasite prevalences (e.g. Marini et al., 1996; Lima et al., 2010; Fecchio et al., 2011; Daud et al., 2015; Ferreira-Junior et al., 2017). Nonetheless, investigations of parasite effects on sexual characters in the Brazilian Atlantic Forest are still scarce (Costa & Macedo, 2005; Aguilar et al., 2008; Dias et al., 2015; Bosholn et al., 2016). In this study, we identified the hemoparasites of a White-necked Thrush population and tested the hypothesis that infection would negatively affect the plumage coloration, morphological traits and immunological condition. We expected that infected individuals would have relatively reduced throat and collar brightness, lower body mass relative to the skeletal size (hereafter, body mass index), and a higher immunological stress response.

## 2 METHODOLOGY

### 2.1 STUDY AREA AND GENERAL PROCEDURES

The study area is located at Reserva Natural Guaricica (22J 735218E 7201341S; Figure 1), Antonina-PR (Brazil). The reserve is located in an important continuous Atlantic Forest region between São Paulo and Paraná states, and the region is one of the most conserved patches of this biome in Brazil (Ribeiro et al., 2009). It is composed by dense rainforest with humid subtropical weather (Cfa at Köppen classification), annual mean temperature of 20.5°C and annual mean rainfall of 2778mm (Vanhoni & Mendonça, 2008).

FIGURE 1 – MAP OF THE STUDY AREA. IN A, A REPRESENTATION OF SOUTH AMERICA, BRAZIL AND THE ATLANTIC FOREST BIOME (SHADED AREA); IN B, FOREST REMNANTS (SHADED AREA) AND LIMITS OF THE RESERVA NATURAL GUARICICA (RNG), AND IN C, THE 14 SAMPLING POINTS, INCLUDING PBIOMA MODULE



We conducted quarterly samples from September 2016 and July 2017 using a standardized protocol from Programa de Pesquisas em Biodiversidade (PPBioMA). We used mist-nets to capture and band individual birds, which were identified at species level. Age was identified based on plumage coloration and cranial ossification. For each individual we measured tarsus length with caliper (0.05mm) and body mass with a spring scale (0.1g). We calculated body mass index (BMI) for each individual as the residuals from the linear regression between body mass and tarsus length (Peig and Green, 2009), values above the regression curve indicating better body condition.

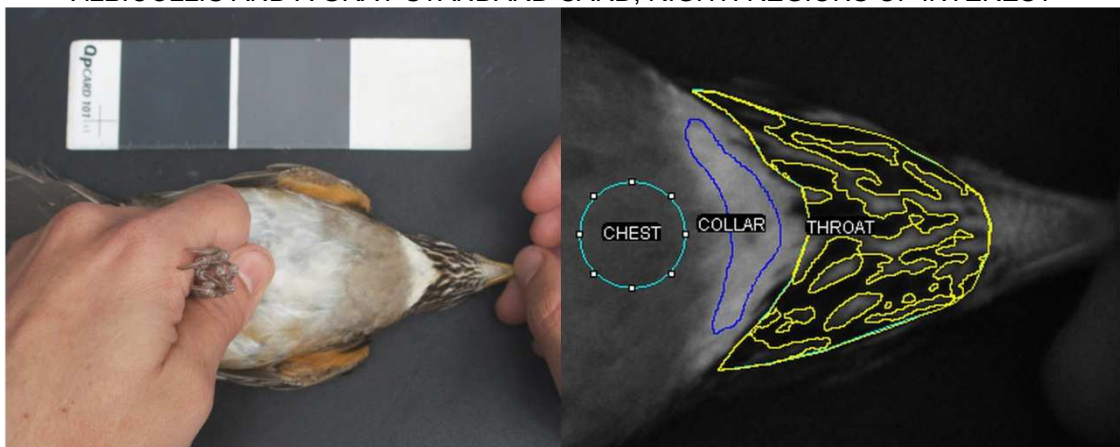
Pectoral muscle size (PM) was obtained using a 0 to 3 scale that used the prominence of the sternal keel as reference (Ritchie et al., 1994), we considered 0 for

stunted muscle (evident sterna keel) and 3 for more developed muscle (hidden sternal keel). Blood ( $\sim 30\mu\text{l}$ ) was obtained by puncturing the brachial vein with a hypodermic needle and collected using capillary tubes (Owens, 2011). Blood smears were prepared and fixed with 100% methanol in the field, and stained in lab using with GIEMSA solution (Valkiunas, 2005). The remaining blood was stored in a plastic container with Queen's Lysis Buffer solution to preserve DNA (Seutin et al., 1991).

## 2.2 PHOTOGRAPHY PROCEDURES

Photographs were taken from the ventral surface to measure feather coloration of the chest and throat (Figure 2), using a Canon EOS Rebel T3i with 18-50mm lens. We used a tripod to fix the camera face down over a horizontal platform. Photographs were taken under natural light condition with focal distance of 40cm and manually fixed configurations of aperture (f/5.0), ISO (400) and zoom (18mm). We set shutter speed to automatic and photographs were taken using a 3s timer to prevent camera movements and undesirable shadows (Stevens et al., 2007). In order to avoid low luminosity, photographs were always taken one hour after sunlight or one hour before sunset. To control for light condition, we used a gray standard card (QPCard 101, 18% of reflectance) placed in the same surface as the bird, thus enabling the comparison of images taken from different sample sites and periods (Stevens et al., 2007).

FIGURE 2 – LEFT: PHOTOGRAPH OF THE VENTRAL SURFACE OF *TURDUS ALBICOLLIS* AND A GRAY STANDARD CARD; RIGHT: REGIONS OF INTEREST



All images were made in RAW format, which is uncompressed and thus preserve color information (Stevens et al., 2007). We used MicaToolBox (Troschianko

& Stevens, 2015) to create linearized and normalized multispectral images of red (R), green (G) and blue (B) and to calculate the reflectance of each spectrum. We selected areas of the throat, the collar and the chest as regions of interest (ROIs; Figure 2). The black and white striped throat, white collar and gray chest of the White-necked Thrush have achromatic colors and thus have only brightness variation (sum of R, G and B reflectance; Kelber et al., 2003). Therefore, we considered only brightness as the color variable in this study. For each sampled bird, we estimate the throat and collar brightness. The first value was calculated as the difference in brightness between the light background and dark stripes and the latter as the difference in brightness between the white collar and gray chest. We used the default settings of the Adaptive Thresholding plug-in for multispectral images in ImageJ (Rasband, 2014), which outline automatically the dark stripes and the throat background color (Figure 2) before calculating the brightness.

### 2.3 MICROSCOPY PROCEDURE

Light microscopy was used to detect and count hemoparasites and leukocytes from blood slides. In each slide, we examined 100 fields at 400X magnification to detect extracellular parasites, and 200 fields at 1000X magnification to detect intracellular parasites, such as *Haemoproteus* and *Plasmodium*, following Valkiunas (2005). Leukocytes were counted in 100 fields at 1000X magnification. We only sampled areas in the slide where cells formed a monolayer. We used leukocytes count to calculate heterophil/lymphocyte ratio (H/L). This ratio is a reliable measure of stress condition, and animals under higher stress will present higher ratios (Davis et al., 2008). We calculated the prevalence of each hemoparasite group using the ratio between numbers of infected samples and the number of analyzed samples. Infection intensity for each parasite group was calculated as the proportion of the number parasites observed per the number of fields analyzed. We present results as the maximum and minimum values among individuals sampled, mean and standard deviation.

### 2.4 MOLECULAR PROCEDURE

We used molecular procedures to increase the detectability of haemosporidians and to identify lineages. We extracted DNA from bird blood

samples using protocols of the Puregene®DNA Isolation Kit. We amplified a DNA fragment with 478bp located in cytochrome *b* of the haemosporidian using a nested Polymerase Chain Reaction (nested PCR; Hellgren et al., 2004). We used HaemNF and HaemR3 as primers for the first reaction and, for the nested reaction, primers HaemF and HaemR2 (Bensch et al., 2000).

In the first reaction, we prepared a working volume of 25µl with 2µl (50-100ng) of extracted DNA and in the second reaction we included 1µl of amplified DNA as template. In both reactions, we used 1× buffer, 4 mM of MgCl<sub>2</sub>, 0.3 mM of each dNTP, 1 unit of Taq (Phoneutria, Belo Horizonte, Brazil), 0.4 mM of each primer, and nuclease-free water in 25µl reaction volumes. In each plate, we included one positive control containing *Plasmodium gallinaceum* DNA to certify successful DNA amplification and one negative control (ultrapure water) to identify potential contamination during the process. Reactions were performed in a SimpliAmpli Thermal Cycler by Applied Biosystem. The first reaction was programmed to denature samples for 3 min at 94°C, followed by 25 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 45 s, with a terminal step of 72°C for 10 min. The first reaction included 25 cycles and the second, 35 cycles. We purified the positive nested PCRs with Polyethylene Glycol 8000 (Fallon et al., 2003) and sequenced it bi-directionally using dye-terminator fluorescent labeling in an ABI Prism 3100 sequencer (Applied Biosystems, Foster City, United States).

## 2.5 PHYLOGENETIC ANALYSIS

We aligned and edited the DNA sequences with ChromasPro (Technelysium Pty Ltd. Helensvale, Australia), and compared with lineages available in Genbank and MalAvi databases (Bensch et al., 2009). Different cytochrome *b* lineages were defined by at least one nucleotide variation among sequences.

We used MRBayes 3.2.2. (Ronquist & Huelsenbeck, 2003) to construct a Bayesian phylogenetic tree under the GTR+I+G model of nucleotide evolution, as suggested by ModelTest (Posada & Crandall, 1998), with *Leucocytozoon caulleryi* as the outgroup. We simultaneously ran two Markov chains for 5 million generations, sampling every 1000 generations. We used the first 1250 trees as burn-in and used the remaining trees to calculate the posterior probability of each estimated node of the consensus tree.

## 2.6 STATISTICAL ANALYSIS

We ran three generalized linear models (GLM) to analyze the effect of morphological and stress condition on the occurrence of the hemoparasites. We considered occurrence (0 or 1) and infection intensity (count) of hemoparasites as dependent variables and age, BMI, PM, H/L and coinfection as independent variables.

In the first model, we used binomial distribution with log link function and considered as dependent variable the results of haemosporidian occurrence based on data from nested-PCR analysis. To perform the second model, we opted to use zero-inflated mixture model with Poisson distribution and considered the results of haemosporidians occurrence and intensity of infection as dependent variable. We excluded PM and coinfection of the haemosporidian intensity of infection' analysis due to the low number of samples. The third model was related to extracellular parasites occurrence and infection intensity detected through microscopy analysis using zero-inflated mixture model with negative binomial distribution. The zero-inflated mixture model allows analyzing count data with excess of zeros values and overdispersion and provides two outputs as result: one based on presence and absence (logistic regression) and another with count data solely considering the positive values (Lim et al., 2014).

We ran two linear regressions for each feather brightness variable (throat and collar brightness) to associate with BMI, PM, H/L and the presence of hemoparasites. For these analyses, we only used adults' data because juveniles present different plumage coloration (Perrins, 2003; Sigrist, 2013) and thus may not provide comparable data. We used a backward stepwise model selection process based on a hypothesis testing approach ( $F$  test and  $P$ -values) for selecting the best model. In each step, we generated models dropping one variable at a time, and compared  $F$  statistics of the full and reduced models. We then followed the selection procedure using the model with the best fit as new reference, until reaching a final model retaining only the most important variables. This selection method proved being capable to identify which variables are important to yield a predictor model (Murtaugh, 2009). To understand how the intensity of infection of hemoparasites affect the plumage coloration, we tested the relationship between the positive

samples of hemoparasites detected on microscopy analysis and the plumage coloration results using Pearson's Correlation Test.

Before generating the models, we identified the distribution of each dependent variable and tested for collinearity between independent variables. Graphics are often the most informative method to identify outliers and to check the assumptions of multiple linear regressions and GLMs (Quinn & Keough, 2002; Zuur et al., 2009). Thus, we used traditional graphical techniques (Q-Q plot, fitted values by raw residuals and Cook's distance) to check for residuals distribution and influential observations. All statistical analysis was performed in R Core Team (2017).

### 3 RESULTS

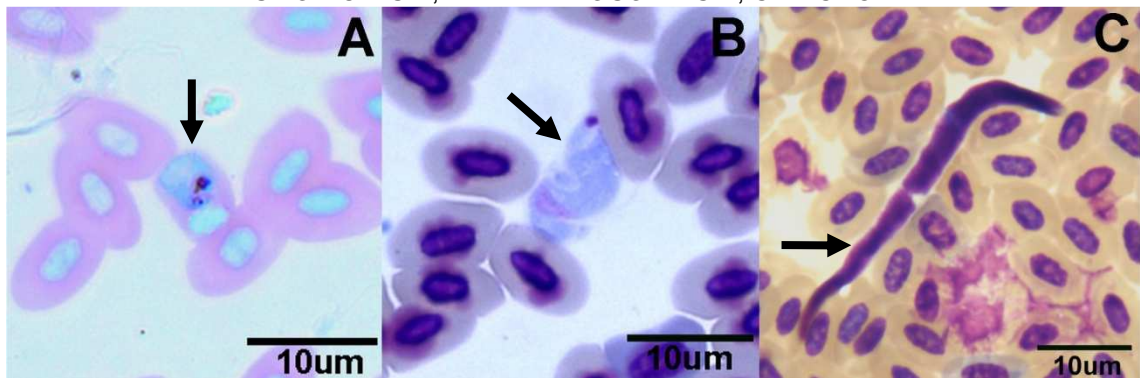
We captured 54 *Turdus albicollis* individuals, of which 43 adults and 11 juveniles. We analyzed 45 blood smear slides (37 adults and 8 juveniles) and amplified 53 DNA samples (42 adults and 11 juveniles). Haemosporidian prevalence was similar between adults and juveniles (Table 1). All positive blood smear slides (n = 11) were also positive in PCR analyses, and we only identified *Plasmodium* sp. in both methods. Microfilaria prevalence was higher among adults than juveniles (Table 1). *Trypanosoma* infected only two adults. Nine adults and one juvenile presented co-infection of microfilaria and *Plasmodium* (Table 1), but haemosporidian and microfilaria prevalence were independent.

TABLE 1 – HEMOPARASITES PREVALENCE IN ADULTS (N=37) AND JUVENILES (N=8) FROM THE STUDIED POPULATION OF *TURDUS ALBICOLLIS* (N=45). \*N=42 ADULTS AND 11 JUVENILES SAMPLED. WE PRESENT THE NUMBER OF INFECTED INDIVIDUALS AND; PERCENTAGE (IN PARENTHESIS)

Hemoparasite	Adults	Juveniles	Total
<i>Plasmodium</i> *	17 (40.4%)	4 (36.4%)	21 (39.6%)
Microfilaria	21 (56.7%)	1(12.5%)	22 (48.8%)
<i>Trypanosoma</i>	2(5.4%)	0(0%)	2 (4.4%)
<i>Plasmodium</i> + Microfilaria	8 (21.6%)	1 (12.5%)	9 (20%)
<i>Plasmodium</i> + Microfilaria + <i>Trypanosoma</i>	1 (2.7%)	0 (0%)	0 (2.2%)
Microfilaria + <i>Trypanosoma</i>	1 (2.7%)	0 (0%)	0 (2.2%)

The *Plasmodium* infection intensity in adults varied from 0.5 to 9.0% ( $\bar{x} = 3.3 \pm 1.6\%$ ,  $n = 9$ ), and the two positive juveniles on microscopic analyses had infection intensity of 6.0 and 7.5%, respectively. Microfilaria infection intensity in adults varied from 1.0% to 30.0% ( $\bar{x} = 11.3 \pm 7.9\%$ ,  $n = 21$ ) and the only juvenile infected had intensity of 1.0%. *Trypanosoma* was observed in two adults, with infection intensity of 2.0 and 3.0% (Figure 3).

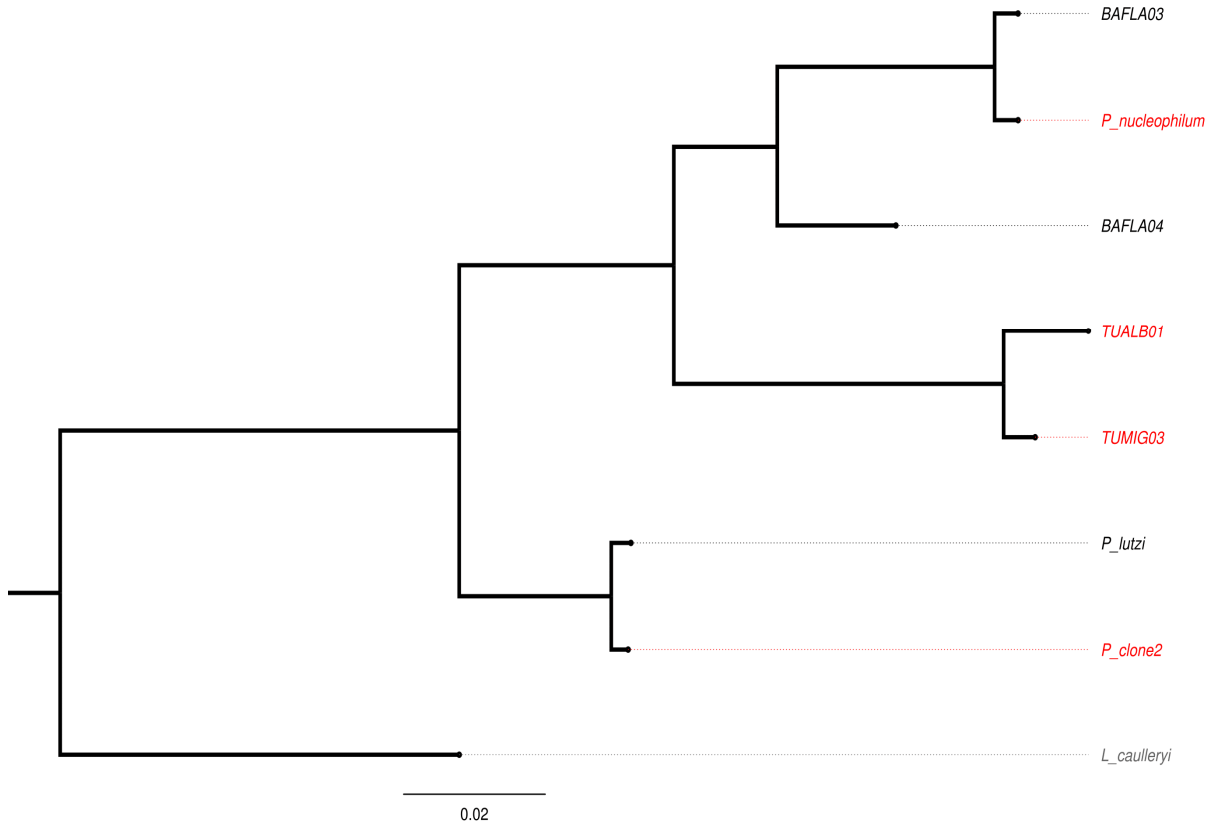
FIGURE 3 – HEMOPARASITES IN BLOOD SMEAR SLIDES OF *TURDUS ALBICOLLIS* SAMPLES. A: *PLASMODIUM* SP.; B: *TRYPANOSOMA* SP.; C: MICROFILARIA



### 3.1 PHYLOGENETIC ANALYSIS

We were able to sequence 12 samples of haemosporidian lineages, to identify a total of four different parasite lineages infecting *T. albicollis* and deposited one new parasite lineages in GenBank. Only parasites of the *Plasmodium* genera were detected and the sequencing results revealed that eight individuals were infected by the subgenus *Haemoamoeba* sp. (P\_clone2), two individuals hosted TUMIG03 lineage (lineage from *Turdus migratorius*), one presented *P. nucleophilum*, and the new lineage TUALB01 were found in one *T. albicollis* individual (Figure 4).

FIGURE 4 – BAYESIAN PHYLOGENETIC TREE SHOWING THE RELATIONSHIPS BETWEEN *PLASMODIUM* SPP. DETECTED IN *TURDUS ALBICOLLIS*. RED: DETECTED LINEAGES; BLACK: COMPARATIVE LINEAGES FOUND IN BRAZILIAN SAVANNA; GRAY: OUTGROUP, *LEUCOCYTOZON CAULLERYI*; *PLASMODIUM LUTZI* (P\_lutzi), BAFLA03 AND BAFLA04 WERE FOUND ON BIRDS AT BRAZILIAN SAVANNA.



### 3.2 CORRELATES OF HEMOPARASITE INFECTION WITH BODY MASS INDEX AND STRESS CONDITION

The body mass index was negatively related to intensity of microfilaria infection (Table 2), however none of independent variables affected the prevalence of microfilaria.

TABLE 2 – RESULTS OF THE ZERO INFLATED NEGATIVE BINOMIAL REGRESSION OF MICROFILARIA OCCURRENCE (BINOMIAL) AND COUNT (TRUNCATED NEGATIVE BINOMIAL) ON BLOOD SMEAR SAMPLES OF *TURDUS ALBICOLLIS* IN RELATION TO AGE, MORPHOLOGICAL TRAITS, STRESS CONDITION AND COINFECTION WITH *PLASMODIUM*. DF: DEGREES OF FREEDOM FROM EACH VARIABLE  $\chi^2$ : QUI SQUARE TEST; *P*: P-VALUE; H/L RATIO: HETEOPHIL/LYMPHOCITE RATIO

Model coefficients	Binomial				Truncated Negative Binomial		
	DF	Likelihood Ratio Test	$\chi^2$	<i>P</i>	Likelihood Ratio Test	$\chi^2$	<i>P</i>
Age	1	-82.55	1.14	0.29	-81.98	<0.01	0.99
Pectoral Muscle Size	3	-82.62	1.28	0.74	-83.91	3.86	0.28
H/L ratio	1	-82.30	0.65	0.42	-82.91	1.86	0.17
Body Mass Index	1	-82.01	0.07	0.79	<b>-86.19</b>	<b>8.43</b>	<b>&lt;0.01</b>
Coinfection	1	-81.99	0.03	0.86	-82.94	1.93	0.17

*Plasmodium* infection intensity was related to body mass index and age (Table 3). Juveniles have higher infection intensity than adults, and infection increased with lower body mass index. The other independent variables did not present any relation to parasite occurrence ( $p > 0.05$ ; Table 3).

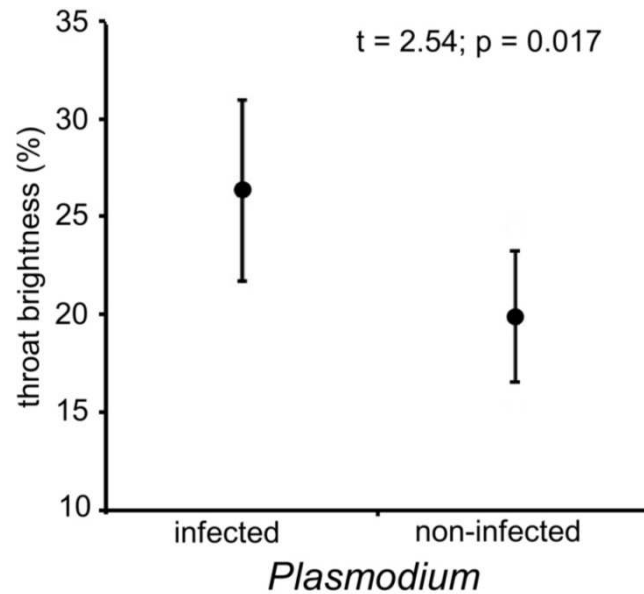
TABLE 3 – RESULTS OF THE ZERO INFLATED POISSON REGRESSION FOR *PLASMODIUM* OCCURRENCE (BINOMIAL) AND COUNT (TRUNCATED POISSON) ON BLOOD SMEAR SAMPLES OF *TURDUS ALBICOLLIS* IN RELATION TO AGE, MORPHOLOGICAL TRAITS AND STRESS CONDITION. Df: DEGREES OF FREEDOM FROM EACH VARIABLE  $\chi^2$ : QUI SQUARE TEST; *P*: P-VALUE; H/L RATIO: HETEOPHIL/LYMPHOCITE RATIO

Model coefficients	Binomial				Truncated Poisson		
	DF	Likelihood Ratio Test	$\chi^2$	<i>P</i>	Likelihood Ratio Test	$\chi^2$	<i>P</i>
Age	1	-37.52	0.26	0.61	<b>-43.42</b>	<b>12.06</b>	<b>&lt;0.01</b>
H/L ratio	1	-37.40	0.02	0.88	-37.94	1.09	0.30
Body Mass Index	1	-37.46	0.14	0.71	<b>-39.57</b>	<b>4.36</b>	<b>0.03</b>

### 3.3 EFFECTS ON PLUMAGE COLORATION

Throat brightness was related to *Plasmodium* infection ( $r^2 = 0.16$ ,  $F_{1,39} = 7.25$ ,  $p = 0.01$ ), but not to microfilaria infection, BMI or H/L (Supplementary Material). Throat brightness was higher in infected than in non-infected individuals (Figure 5). However, the collar brightness did not depend on infection by parasites, neither did morphology and stress condition (all variables with  $p > 0.05$  in all selection steps; Supplementary Material).

FIGURE 5 - MEAN AND CONFIDENCE INTERVAL OF THROAT BRIGHTNESS ON INFECTED (N=16) AND NON-INFECTED (N=25) INDIVIDUALS



Considering only the positive samples of parasites detected in blood smears, the plumage coloration did not relate to intensity of microfilaria infection (throat brightness:  $r = 0.01$ ,  $df = 19$ ,  $p = 0.98$ ; collar brightness:  $r = 0.01$ ,  $df = 19$ ,  $p = 0.98$ ), neither to intensity of *Plasmodium* infection (throat brightness:  $r = -0.1$ ,  $df = 6$ ,  $p = 0.82$ ; collar brightness:  $r = -0.16$ ,  $df = 6$ ,  $p = 0.7$ ).

#### 4 DISCUSSION

Our results revealed that the plumage coloration of the White-necked Thrush is related to parasite infection, such that the throat brightness was higher on individuals infected by *Plasmodium* than in non-infected. However, the collar brightness, the morphological and immunological variables did not differ between these individuals. Our study also revealed a relationship between infection intensity and body mass index of both parasites, *Plasmodium* and microfilaria. In our samples, *T. albicollis* was particularly susceptible to *P. relictum*, which occurred in four individuals from the 12 identified haemosporidians lineages.

The subgenus *Haemoamoeba* can be found in all continents except Antarctica, it presents several lineages and hosts a wide range of bird species (Valkiunas, 2005). Thrushes usually are chronic infected by this haemosporidian and may act as reservoirs (Tompkins & Gleeson, 2006), which contribute to the maintenance of *Haemoamoeba* sp. in nature. The high prevalence of *Haemoamoeba* sp. in our

phylogenetic analysis indicates that White-necked Thrush might be a reservoir of this hemoparasite as well.

We found a negative relationship between parasite infection intensity and body mass index, and that juveniles presented higher intensity of *Plasmodium* infection, but we did not find a relationship between prevalence and age, coinfection, and health condition. During the first haemosporidian infection, the host presents higher parasitemias and increased loss on body and immunological condition (Cellier-Holzem et al., 2010; Atkinson et al., 2001). Juveniles were born a few months before our sampling, therefore it is likely that it was their first infection by *Plasmodium*, resulting in higher infection intensity. Birds are commonly chronically infected in natural condition and may maintain infection at low intensities (Bennet et al., 1993; Valkiunas, 2005) without pathogenicity (Atkinson & van Ripper, 1991). However, under stress condition, the chronic infection may relapse and affect hosts' parasitemia and fitness (Weatherhead & Bennet, 1991). Our result of reduced body mass in individuals with higher infection intensity indicates that parasites are costly to the host depending on the parasitemia but not on the occurrence of the parasite, a statement supported by the similar health condition found in the infected and non-infected groups (Merino et al., 2000; Westerdahl et al., 2013). The studied White-necked Thrush population might represent chronic infected hosts since they had high prevalence, relatively low *Plasmodium* infection intensity, and no apparent difference in body or immunological condition between infected and non-infected individuals.

Our results on plumage coloration did not support Hamilton & Zuk hypothesis (1982), which predicts that sexual characters are positively related to parasite resistance. In contrast, we observed that the difference in brightness between the white background and the black stripes in the *T. albicollis* plumage is more apparent in individuals infected by *Plasmodium*. This result might indicate that some individuals of White-necked Thrush could bear the infection and more extravagant plumage coloration could signal increased resistance (Westerdahl, 2007; Heschen et al., 2017). Haemosporidians are known to present a moderate virulence in acute phase (Valkiunas 2005) – especially *Plasmodium* (Atkinson & van Ripper, 1991) – and demonstrated to be lethal to passerines in experimental (Atkinson et al., 2001; Williams 2005; Cellier-Holzem et al., 2010) and natural conditions (van Ripper et al., 1986). They are also responsible for the reduced breeding success (Korpimäki et al., 1995; Merino et al., 2000; Asghar et al., 2011; Asghar et al., 2015), body condition

(Cellier-Holzem et al., 2010; DeGroot & Rodewald, 2010) and immunological condition (Palinaskas et al., 2008). The virulence of *Plasmodium* could be severe to White-necked Thrush as well, and individuals with more contrasted throat brightness might be the ones that had overcome the acute phase and survived, but unable to clear the infection (i.e. *selective mortality*; see Henschen et al., 2017). Hence, considering that Hamilton & Zuk hypothesis predicts a positive correlation between resistance and ornamentation, an interaction between host and lethal parasites will favour individuals that survive after infection, and striking plumage coloration would be an honest signal of its immunological capacity.

Alternatively, the throat brightness in White-necked Thrush could only be a sexual cue and not necessarily a signal (Számadó, 2011) because Thrushes likely have other traits influencing mating selection such as song (Shutler, 2011) and territory quality (Hauser, 1997; Siefferman & Hill, 2005). In fact, Thrushes are songbirds with complex vocalization (Ince and Slater, 1985; Lavinia-Oblanca & Tubaro, 2012), and the White-necked Thrush has particularly longer song elements than other Neotropical Thrushes, possibly to optimize sound propagation and enhance communication efficiency in dense understory vegetation (Nemeth et al., 2006). Such acoustic signal might be costly and impose a trade-off between the investment in plumage coloration and vocalization (Badyaev et al., 2002; Shutler, 2011). Under stress conditions, such as those caused by parasitism, vocalizations then could be more prone to effects of reduced physiological investment than plumage coloration (Spencer et al., 2005).

Interestingly, we found the highest microfilaria prevalence reported for Neotropical Thrushes so far (White et al., 1978; Bennet & Lopes, 1980; Rodrigues & Mata, 2001; Valkiunas et al., 2003; Silveira et al., 2010; Sebaio et al., 2011). The epidemiology of microfilaria is still unknown for bird species from the Brazilian Cerrado biome (Silveira et al., 2010) and, to our knowledge, the Atlantic Forest as well. Although filarial nematodes are considered harmless to wild birds even in high infection intensity (Tsai et al., 1992; Campbell, 1995), we found a relationship between microfilaria infection intensity and host's body mass index. However, we lack knowledge whether individuals with reduced body mass are more prone to be infected or if filarial nematodes affect host fitness.

In conclusion, our results indicate that the melanin-based coloration of the White-necked Thrush can be used as an indicator of haemosporidian infection.

However, it remains unknown if this trait is useful as a mate choice signal that demonstrates parasite resistance or if just a cue about any other individual quality. Despite *T. albicollis* being widespread and reasonably common in the Atlantic Forest (Sick, 1998; Ridgely et al., 2015), information regarding this species is still lacking and mostly focused on vocalization (Nemeth et al., 2006; Lavinia-Oblanca & Tubaro, 2012) and ecological interactions (Storni et al., 2005; Gasperini & Pizo, 2009; Lopes et al., 2014). Considering the remarkable deficiency of sexual selection studies on Atlantic Forest birds (e.g. Auer et al., 2007; Cestari et al., 2017; Xiao et al., 2017), we hope our findings encourage other researches to investigate the value of plumage coloration in communication in the White-necked Thrush and to understand the role of haemosporidians in individuals' interactions.

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## SUPPLEMENTARY MATERIAL

TABLE S1: RESULTS OF THE STEPWISE MODEL SELECTION BASED ON *F*-TEST AND *P*-VALUE FOR THE DIFFERENCE OF BRIGHTNESS BETWEEN CHEST AND COLLAR (DBC) AND DIFFERENCE OF BRIGHTNESS IN THE THROAT (DBT) OF *TURDUS ALBICOLLIS* IN RELATION TO BODY MASS INDEX (BMI), PECTORAL MUSCLE (PM), H/L RATIO, PRESENCE OF MICROFILARIA (MICROFILARIA), AND PRESENCE OF HAEMOSPORIDIAN (HAEMOSPORIDIAN). DF: DEGREES OF FREEDOM. *ITALIC*: VARIABLE EXCLUDED ON THE NEXT MODEL SELECTION

STEP	MODEL	DF	F-VALUE	P(>F)
	<b><u>DBC</u></b>			
	<b>DBC=BMI+PM+H/L+MICROF+HAEMOSP</b>			
	<i>BMI</i>	<i>1</i>	<i>0</i>	<i>0.97</i>
	PM	3	1.02	0.4
	H/L	1	0.14	0.71
	MICROFILARIA	1	0.03	0.86
	HAEMOSPORIDIAN	1	0.96	0.34
	<b>DBC=BMI+PM+H/L+HAEMOSP</b>			
	PM	3	1.08	0.37
	H/L	1	0.15	0.7
	<i>MICROFILARIA</i>	<i>1</i>	<i>0.03</i>	<i>0.86</i>
	HAEMOSPORIDIAN	1	0.99	0.33
	<b>DBC=PM+H/L+HAEMOSP</b>			
	PM	3	1.12	0.36
	<i>H/L</i>	<i>1</i>	<i>0.15</i>	<i>0.7</i>
	HAEMOSPORIDIAN	1	1.07	0.31
	<b>DBC=PM+HAEMOSP</b>			
	<i>PM</i>	<i>3</i>	<i>1.45</i>	<i>0.25</i>
	HAEMOSPORIDIAN	1	2.11	0.16
	<b>DBC=HAEMOSP</b>			
	<i>HAEMOSPORIDIAN</i>	<i>1</i>	<i>1.55</i>	<i>0.22</i>
	<b>DBC=*</b>			
	<b><u>DBT</u></b>			
	<b>DBT=MI+PM+H/L+MICROF+HAEMOSP</b>			
	MI	1	0.04	0.84
	PM	3	1.4	0.27
	H/L	1	0.68	0.42
	<i>MICROFILARIA</i>	<i>1</i>	<i>0</i>	<i>0.99</i>
	HAEMOSPORIDIAN	1	3.44	0.07
	<b>DBT=MI+PM+H/L+HAEMOSP</b>			
	<i>MI</i>	<i>1</i>	<i>0.04</i>	<i>0.83</i>
	PM	3	1.46	0.25

H/L	1	0.71	0.41
HAEMOSPORIDIAN	1	3.61	0.07
<b>DBT=PM+H/L+HAEMOSP</b>			
<i>PM</i>	3	1.57	0.22
H/L	1	0.74	0.4
HAEMOSPORIDIAN	1	3.81	0.06
<b>DBT=PM+HAEMOSP</b>			
<i>PM</i>	3	1.49	0.24
HAEMOSPORIDIAN	1	7.23	0.01
<b>DBT=HAEMOSP*</b>			
HAEMOSPORIDIAN	1	7.26	0.01

\* Selected model