

1 **Parasite-associated histopathological lesions in free-ranging birds from Costa Rica**

2 Jessica Carballo-Vargas¹, Mario Romero-Vega^{1,2}, Alejandro Alfaro-Alarcon^{1,3}.

3 ¹Department of Pathology, School of Veterinary Medicine, National University, Costa Rica. 4104

4 ²Institute of Pathology, University of Veterinary Medicine Hannover Foundation, Hannover,
5 Germany.

6 ³Berlin Institute of Health, Institute of Virology, Charité-Universitätsmedizin, Berlin, Germany

7 **ABSTRACT**

8 Bird species can serve as both definitive and intermediate hosts for a wide variety of parasites, some
9 of which are important for veterinary and public health. These parasites can elicit different
10 physiological responses in the host, from completely asymptomatic infections to severe complications
11 that impair the animal's health and lead to death. To further study this aspect, we sampled free-ranging
12 birds, of which 53.41% were parasitized, with a prevalence of 34.09% for Coccidia, 23.86% for
13 Trematoda, 14.77% for Nematoda, 9.09% for Cestoda, and 5.68% for Parasitic cysts (Protozoan), while
14 only 20.45% of birds presented histopathological lesions concurrent with adjacent parasite presence.
15 Both inflammation presence and severity are more significant in birds infected with Trematoda.

16 **1. Introduction**

17 The gastrointestinal parasites most commonly reported in Costa Rican wild birds include *Eimeria* and
18 *Isospora* from the subclass Coccidia. Among Nematoda, the suborder Strongylida and the genera
19 *Acuaria*, *Capillaria*, *Heterakis*, and *Ascaridia* are prevalent (Gómez-Cortés & Jiménez-Rocha, 2019;
20 González Rojas, 2016). Within Cestoda, *Choanotaenia* spp. has been observed in the Clay-colored
21 Thrush (*Turdus grayi*), and other unidentified Cestoda species have been documented across various
22 bird families in Costa Rica (Pérez-Gómez et al., 2018; Sibaja Morales, 2006). In the ventriculus and
23 proventriculus, the Nematoda species *Dispharynx nasuta* has been described in Passeriformes of Costa
24 Rica and Psittacidae in Argentina and Mexico (Gornatti Churria et al., 2011; Hernandez-Urraca et al.,

25 2022; Zhang et al., 2004). Regarding parasites in other organs, *Paratanaisia bragai* is the most
26 frequently reported parasite in the kidneys of Costa Rican birds (Alfaro-Alarcón et al., 2015), while
27 *Sarcocystis* is the most common parasite found in the muscles of New World birds (Llano et al., 2022).
28 In the respiratory system, the Trematoda species *Bothriogaster variolaris* has been described in raptors
29 in Ecuador (Díaz et al., 2022), while *Circumvitellatrema momota*, *Morishitium*, *Psophiatrema greineri*,
30 and *Szidatitrema yamagutii* have been found in the air sacs of Passeriformes in the United States
31 (Greiner et al., 2023). Regarding filarioid nematodes, aortic and brachiocephalic filariasis has been
32 reported in Mexican rhamphastids (Sanchez-Godoy et al., 2020), and microfilariae have been found in
33 numerous avian species, including Passeriformes and Columbidae in Costa Rica (González Rojas,
34 2016).

35 Most studies lack information on the histopathological effects of these parasites on their avian hosts,
36 and most focus on a single species or family of birds, with varying results. A study conducted in
37 Californian hummingbirds found a prevalence of intestinal cestodiasis of 20% with no associated
38 lesions (Magagna et al., 2019), while another research on black-necked swans in Chile found mild
39 gastrointestinal inflammation that could be linked to parasites in only a fraction of cases (Oyarzún-Ruiz
40 et al., 2019). Additionally, studies evaluating *Paratanaisia bragai* in kidneys of wild birds report clinical
41 outcomes ranging from insignificant to fatal (Alfaro-Alarcón et al., 2015; De Santi et al., 2018).

42 Regarding parasite identification in tissue samples, it is accomplished by evaluating the morphological
43 structures of the metazoan parasites in the tissue sections, such as the body cavity, digestive tract,
44 cuticle, muscle type and reproductive tract, as well as unique features that only certain parasite groups
45 possess, for instance, calcareous corpuscles in Cestoda (Gardiner & Poynton, 1999). Histopathological
46 identification allows identification to the phylum or even order, family or class of the parasite.
47 However, unlike molecular techniques, morphological evaluation allows for comparison between
48 parasite presence and associated histopathological changes (Abdul-Aziz et al., 2016).

49 Here, we present a histopathological description of the main lesions associated with parasite
50 infestation in wild birds of Costa Rica.

51 **2. Materials and Methods**

52 **2.1. Sample collection**

53 The sampling was carried out in the canton of Cuajiniquil, Guanacaste, and Talamanca, Limón as part
54 of a broader study on arboviral surveillance published elsewhere (Barrantes Murillo et al., 2022). The
55 criteria for selecting both areas were their differences in environmental factors, such as forest type,
56 land use, and annual rainfall. In each county, eight private properties were chosen, each having both
57 poultry and forest patches. Three mist nets were set up on each property: one near the gardens and
58 poultry, and two in the forest patch area. Bird capture was conducted during two periods: from 6:00
59 am to 10:00 am and from 3:00 pm to 6:00 pm. Captured birds were identified to species level and then
60 euthanized using an overdose of ketamine and xylazine and subsequently preserved in 10% buffered
61 formalin. Collection permits were issued by the National System of Conservation Areas (SINAC): R-
62 SINAC-PNI-ACLAC-054-2018 and ACT-PIM-070-17.

63 **2.2. Sample processing**

64 Birds were necropsied in the Pathology Laboratory at the School of Veterinary Medicine, National
65 University of Costa Rica, and samples were collected for histopathological analysis. The organs
66 analyzed included the brain, cerebellum, lungs, heart, esophagus, proventriculus, ventricle, small
67 intestine, pancreas, large intestine, liver, gallbladder, spleen, kidneys, ovaries or testes, and skeletal
68 muscle. Sagittal sections were prepared from organs embedded in paraffin, stained with hematoxylin
69 and eosin (H&E), and examined under a light microscope. All parasites were measured and
70 photographed using the program Euromex ImageFocusAlpha, version x64, 1.3.7.15674.2019 1008.

71 **2.3. Histopathological analysis**

72 All tissue samples were observed for presence of histopathological changes. Inflammation was
73 determined by observing inflammatory infiltration, as well as associated tissular changes, such as
74 oedema, hyperemia, hemorrhage, fibrosis and/or necrosis (Ackermann, 2017).

75 Parasite presence was also evaluated and classified according to guidelines by Gardiner et al. (1998)
76 and Gardiner & Poynton (1999), in Metazoan or Protozoan parasites. Subsequently, Metazoan
77 parasites were classified by phylum as: Nematoda, Cestoda, Trematoda, Acanthocephala,
78 Pentastomida or Arthropoda. Characteristic features of each parasite were documented and, when
79 possible, classified to the most specific taxonomic category. The evaluated features of the parasites
80 included body cavity, cuticle, hypodermis, musculature, reproductive tract and digestive tract.

81 **2.4. Statistical analysis**

82 Parasite presence and inflammation presence were classified as 0 (Absent) or 1 (Present), and
83 inflammation severity was registered as 0 (none), 1 (mild), 2 (moderate) or 3 (severe). Because every
84 individual specimen had multiple tissues analyzed, data were analyzed at bird level instead of tissue
85 level to avoid pseudoreplication.

86 Additionally, to evaluate sampling representation, a Z-Score was calculated for each avian species
87 sampled in the study.

88 To assess the relationship between parasite presence and inflammation presence in birds, a Pearson
89 Correlation Coefficient (r) was used, while a Chi-Square Test of Independence was performed to
90 evaluate if each parasite type was independently associated with inflammation, with Holm-Bonferroni
91 correction to reduce the risk of false positives due to the multiple comparisons. For statistically
92 significant results, Cramer's V was calculated for measuring strength of association. Given that each
93 bird could present more than one type of parasite, a Wilcoxon rank-sum test with Cliff's delta and Holm
94 correction were utilized to compare inflammation severity between birds with and without parasites,
95 for each parasite type.

96 Inflammation severity between different organs was compared by each parasite type using a Kruskal-
97 Wallis test, and when significant, Dunn's post hoc tests with Holm correction were used. Because
98 Trematoda infections were the only ones that presented statistically significant differences between
99 inflammation severity in different organs, and most cases were in the intestines, a Wilcoxon rank-sum

100 test and Cliff's delta were calculated to compare lesion severity in intestines versus other organs
101 infected with Trematoda.

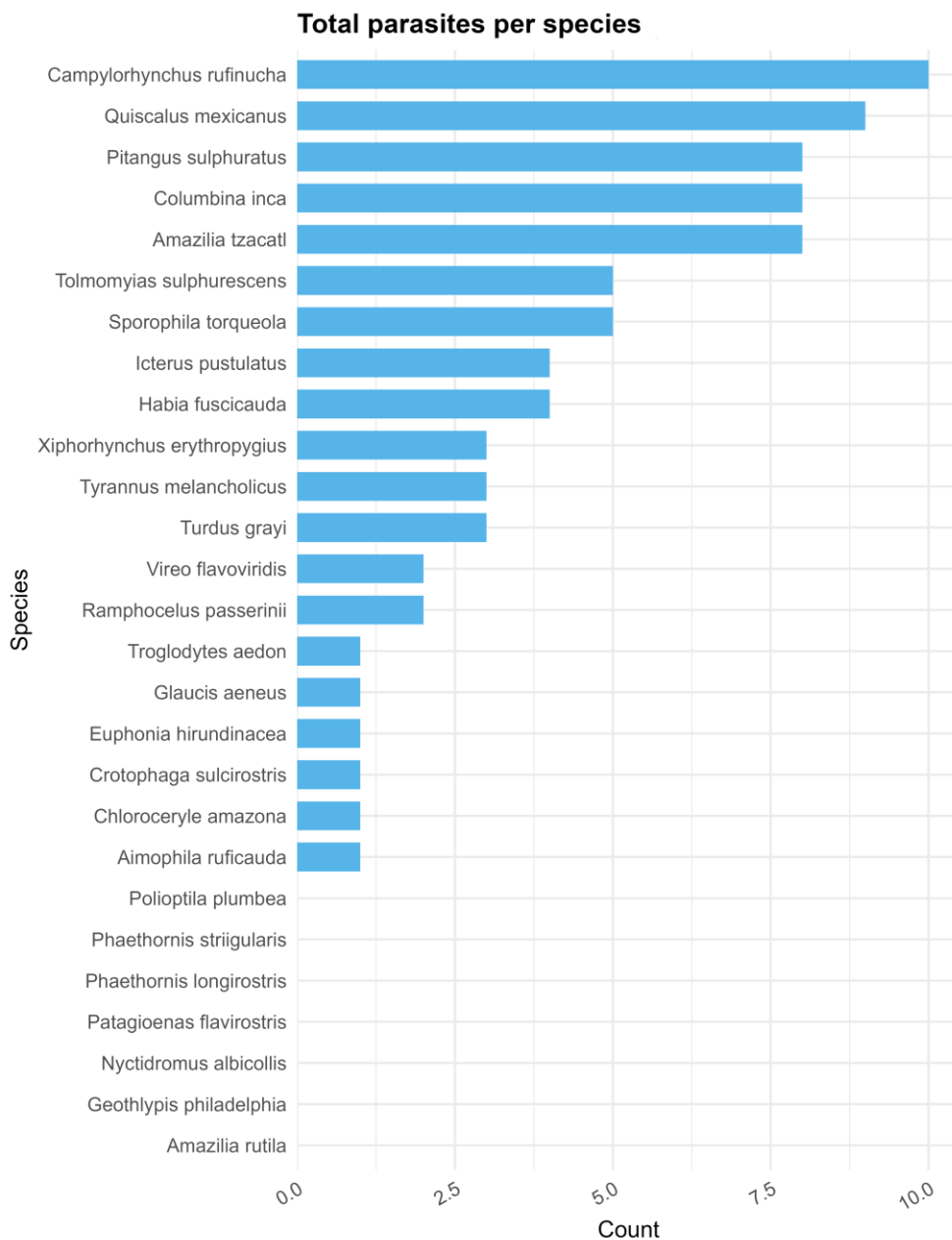
102 All statistical analyses were performed with the statistical software R version 4.5.1.

103 3. Results

104 Birds from the orders Passeriformes (n=57), Apodiformes (n=17), Columbiformes (n=11),

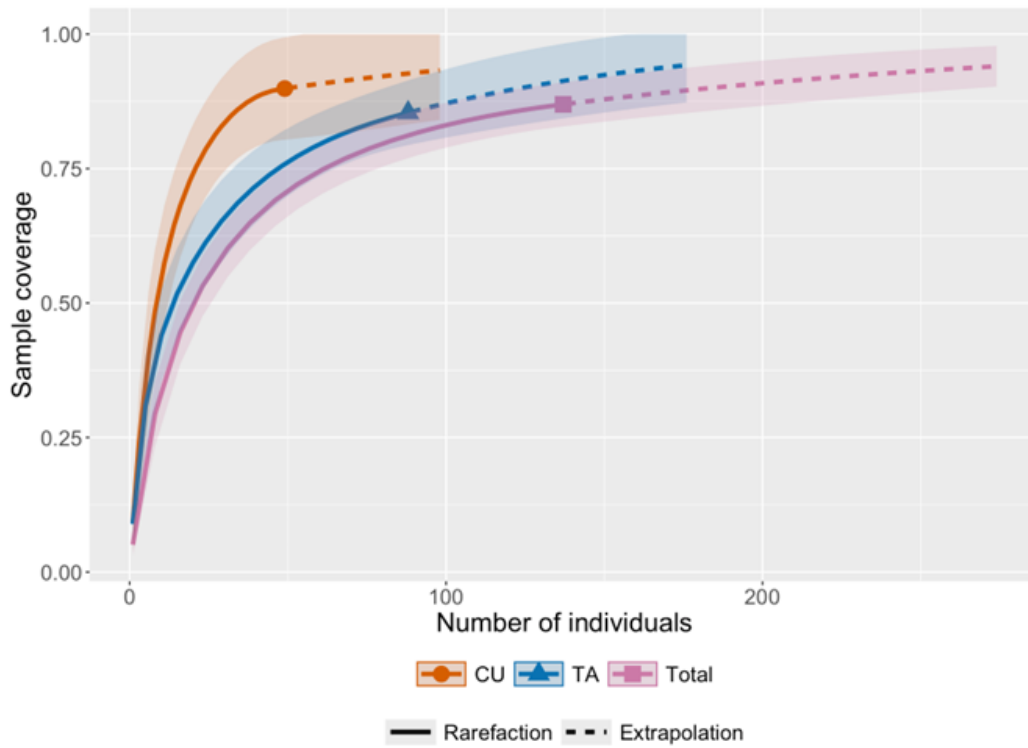
105 Caprimulgiformes (n=1), Cuculiformes (n=1), and Coraciiformes (n=1) were collected and euthanized.

106 The study included 27 unique avian species, as observed in Figure 1.



108 **Figure 1.** All avian species included in the study along with the number of parasites recorded for each
109 species.

110 In Figure 2, the sample coverage curve is shown, comparing the total bird diversity in the area to the
111 species sampled in the study.



112

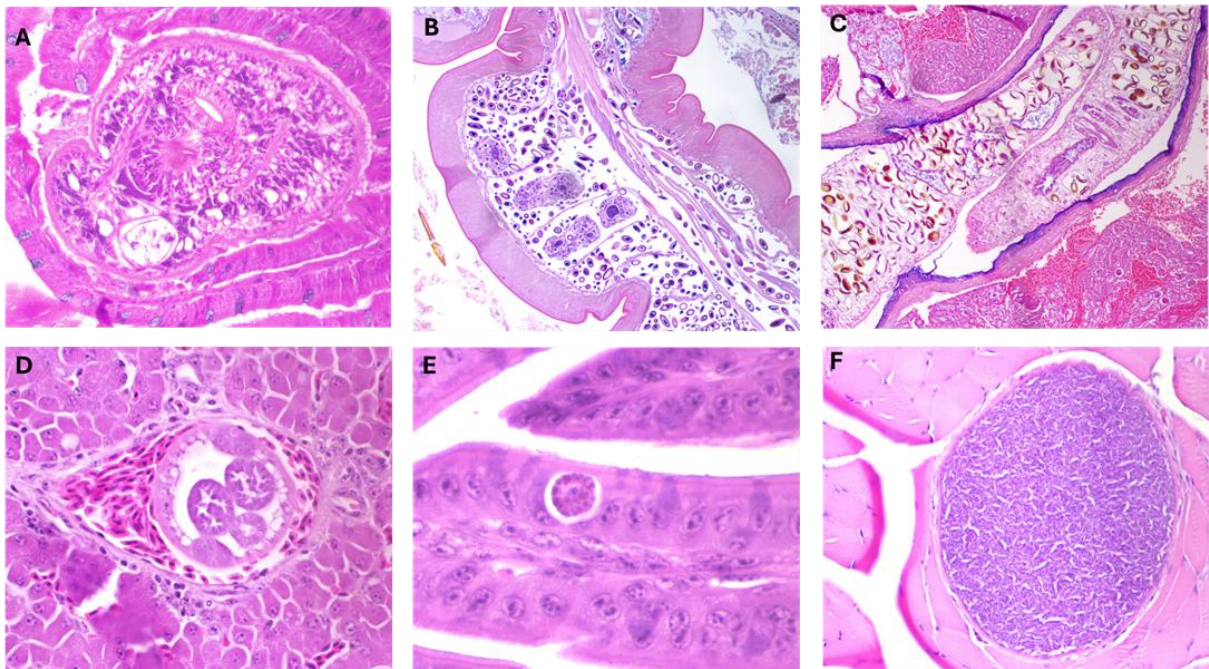
113 **Figure 2.** Species accumulation curve. Abbreviations: (CU) Cuajiniquil; (TA) Talamanca.

114 3.1. Parasites

115 On histopathologic analysis, out of the total eighty-eight birds, forty-seven were parasitized (53.41%).
116 Coccidia was the most common parasite, found in a total of thirty birds (34.09%), followed closely by
117 Trematoda in twenty-one birds (23.86%), then Nematoda in thirteen birds (14.77%), Cestoda in eight
118 (9.09%), and Parasitic cysts (Protozoan) in the muscles of five birds (5.68%). No parasites from the
119 phyla Acanthocephala, Pentastomida nor Arthropoda were observed.

120 Overall, twenty birds (21.59%) exhibited multiparasitism. The gastrointestinal tract showed the highest
121 number of parasites and related lesions. Coccidia was the most common parasite in the intestines
122 (34.09%), followed by Trematoda (19.32%) and Cestoda (9.09%), as shown in Figures 4.E., 4.A., and

123 4.B., respectively. Nematoda (1.14%) was the least common intestinal parasite. Conversely, in the
124 ventriculus, the most prevalent parasites were Nematoda (11.36%), followed by Trematoda (1.14%).
125 Trematoda was also found in the gallbladder of one animal (1.14%).
126 Regarding other organs, Nematoda were also found in blood vessels within the liver in one specimen
127 (1.14%), which is illustrated in Figure 3D, as well as a cardiac chamber in another bird (1.14%). In
128 addition, microfilariae were observed in the lungs of one animal (1.14%), as well as Trematoda in the
129 lungs of another sample (1.14%). Moreover, Trematoda were found in the kidneys of two different
130 birds (2.27%), as seen in Figure 3C. Protozoan parasitic cysts compatible with *Sarcocystis* spp. or
131 *Toxoplasma* spp. were found in both skeletal muscles and myocardium of five different animals
132 (5.68%), as observed in Figure 3F.



133

134 **Figure 3.** Histological sections of various organs, evidencing the presence of parasites. (A) Trematode
135 in the villi of the small intestine; (B) Cestoda in the lumen of the small intestine; (C) Gravid trematode
136 in dilated renal collecting ducts; (D) Nematode in a hepatic blood vessel; (E) Coccidia suggestive of
137 *Eimeria* spp. in the small intestine; (F) Protozoan parasitic cyst in skeletal muscle compatible with
138 *Sarcocystis* spp. or *Toxoplasma* spp.

139 All Trematoda observed were consistent with the class Digenea. With the case of Nematoda, all
140 specimens were consistent with the order Spirurida, with 3 of these cases possibly suggesting a
141 parasite member of the superfamily Filarioidea. Regarding Cestoda, 7 parasites were compatible with
142 the order Pseudophyllidea and 1 with the order Cyclophyllidea.

143 No parasites from the phyla Acanthocephala, Pentastomida or Arthropoda were observed. The
144 detailed characteristics of each parasite is presented in detail in Table S1 (Supplementary material).

145 **3.2. Histopathological lesions**

146 In total, eighteen birds (20.45%) presented different degrees of histopathological lesions concurrent
147 with adjacent parasite presence. The most affected structures were the intestines (12.5%), followed
148 by the skeletal muscles (2.27%), the myocardium (2.27%) and the gallbladder (1.14 %). Lesions were
149 also observed in the gizzards (2.27%) and kidneys (2.27%).

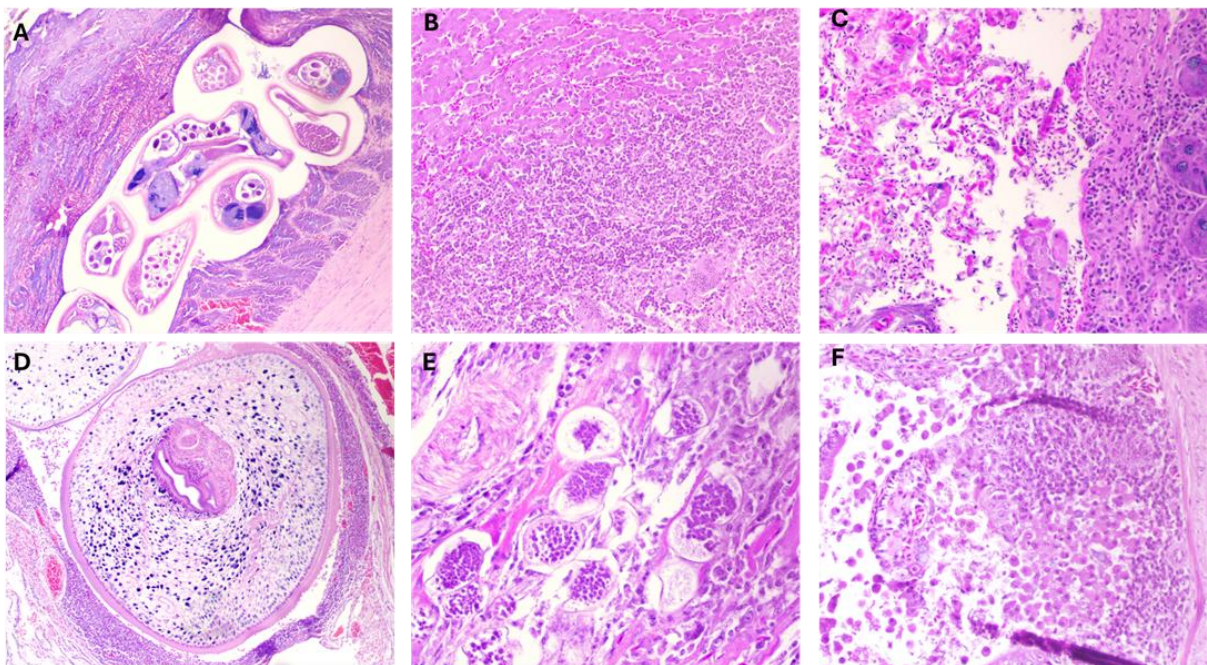
150 In the gastrointestinal tract, enteritis was the most common lesion observed, characterized by a mixed
151 inflammatory infiltrate mainly consisting of macrophages and heterophils, with occasional lymphocyte
152 infiltration and hemorrhages in some specimens, as shown in Figure 4C and 4F. A total of 8 animals
153 exhibited enteritis: 2 with only Trematoda, 3 with only Coccidia, 2 with both, and 1 with only Cestoda.
154 The severity varied regardless of the parasite type. Additionally, heterophilic ventriculitis was
155 identified in 2 animals: one case was exclusively linked to the presence of nematodes in the koilin layer,
156 as depicted in Figure 4A, while in the other case, the inflammation extended into the muscular layer.
157 In this case, although no parasites were visible in the gizzard, Trematoda were found in the small
158 intestine.

159 In the hepatobiliary system, one bird with histiocytic enteritis associated with Coccidia also presented
160 histiocytic hepatitis featuring multiple multinucleated giant cells scattered with other macrophages,
161 as observed in Figure 4B. Ziehl-Neelsen stain was performed in this specimen to detect the presence
162 of *Mycobacterium* spp., however, it was negative. Additionally, another bird infected with Trematoda
163 in the gallbladder presented bile duct hypertrophy.

164 Regarding the muscles, two cases of histiocytic myocarditis were observed, and two cases of histiocytic
165 myositis and muscle degeneration in skeletal muscle were noted. In all four instances, the presence of
166 cysts filled with Protozoa compatible with *Sarcocystis* spp. was directly associated with the cysts, as
167 shown in Figure 4E.

168 In the urinary system, 2 birds presented gravid Trematoda within the renal collecting ducts, which were
169 dilated and presented moderate surrounding fibrosis, and adjacent renal tubules were necrotic, as
170 observed in Figure 3C. Additionally, multifocal hemorrhages were present in the interstitial tissue of
171 the kidney. However, no notable inflammatory infiltrate was observed.

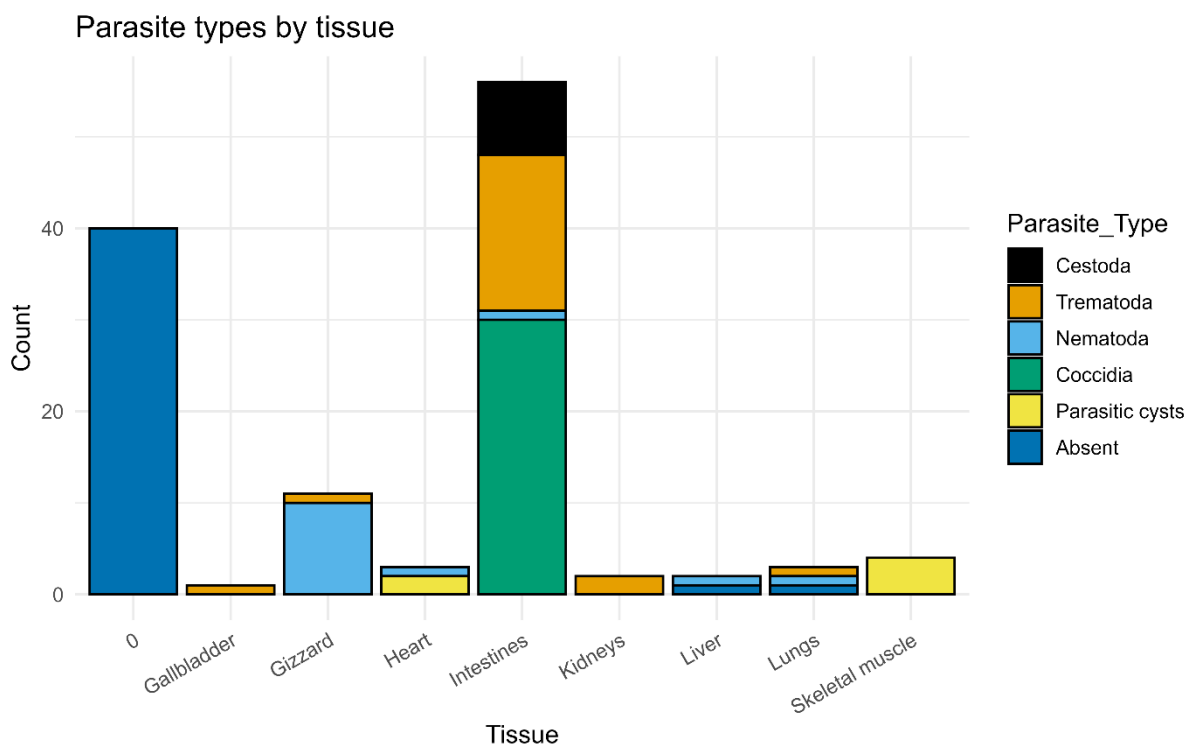
172 Lastly, severe lymphohistiocytic inflammation with hyperemia and mild fibrosis was observed
173 surrounding a trematode in the bronchus of a *Quiscalus mexicanus*, as observed in Figure 4D.



174
175 **Figure 4.** Histological sections of various organs evidencing the different lesions associated with the
176 presence of parasites. (A) Nematodes in the mucosa of the gizzard, accompanied by an inflammatory
177 infiltrate of mostly heterophils and macrophages, and fragmentation of the coilin layer in an *Amazilia*
178 *tzacatl*; (B) Severe histiocytic hepatitis with considerable inflammatory infiltrate composed mainly of
179 macrophages in a *Columbina inca*; (C) Hemorrhagic enteritis with ulcers and necrosis in a specimen
180 with intestinal Trematodes in an *Aimophila ruficauda*; (D) Trematode in the bronchus with associated

181 lymphohistiocytic inflammation of a *Quiscalus mexicanus*; (E) Protozoan cysts in the myocardium of an
 182 *Icterus pustulatus*, with associated inflammatory infiltrate of mainly macrophages, along with necrosis
 183 of the muscle cells; (F) Necrosis and enteritis predominated by heterophils and macrophages in a
 184 *Columbina inca* with Coccidia.

185 A detailed summary of the parasites detected, the corresponding lesions observed, and the bird
 186 species in which they were found can be found in the supplementary material, in Table S1. Parasite
 187 types and associated tissues can be observed in a graphic corresponding to Figure 5.



189
 190 **Figure 5.** Parasite types by tissue location.

191 **3.3. Statistical analysis**

192 On average, 3 birds were sampled per species (\bar{x} = 3.259). However, *Sporophila morelleti* (n=13) and
 193 *Columbina inca* (n=10) were the most overrepresented species in the study, with a Z-Score of 3.21 and
 194 2.22 respectively, while other species were only represented by 1 individual.

195 The species with the most parasites is *Campylorhynchus rufinucha*, with a total of 10 individuals
196 parasitized, followed by *Quiscalus mexicanus* with 9, then *Columbina inca*, *Pitangus sulphuratus* and
197 *Amazilia tzacatl* with 8 each.

198 Parasite presence and inflammation are moderately correlated, $r(86) = 0.45$, $p < 0.001$.

199 The Chi-Square Test of Independence evidenced that birds parasitized by Trematoda are more likely
200 to present inflammation ($\chi^2(1, N = 88) = 16.73$, $p < .0001$, *Cramer's V*: 0.44). Additionally, a Wilcoxon
201 rank-sum test indicated that birds with Trematoda presented higher inflammation severity ($W = 396$,
202 $p < .0001$, *Cliff's δ* = -0.41, medium effect). Inflammation severity between different organs was
203 statistically significant in the case of Trematoda (*Kruskal Wallis*: $\chi^2 = 10.65$, $df = 4$, $p = 0.0307$), however,
204 no significant differences were found after Holm's correction in Dunn's post hoc tests. Severity
205 comparison between intestines and other organs revealed that Trematoda presence in other organs is
206 associated with more severe inflammation compared to intestinal Trematoda (Wilcoxon rank-sum test,
207 $W = 14.5$, $p = 0.015$, *Cliff's δ* = -0.66, large effect size).

208 No other significant correlations were found for other parasite types.

209 **4. Discussion**

210 The study demonstrates that both inflammation presence and severity are more significant in birds
211 infected with Trematoda. While most Trematoda species are usually described as not being associated
212 with clinical disease in wild birds (Atkinson et al., 2008), other factors must also be considered,
213 including the location of the parasite within the host, as evidenced by the Wilcoxon rank-sum test (W
214 = 14.5, $p = 0.015$, *Cliff's δ* = -0.66, large effect size) in which inflammation was more severe in other
215 organs in comparison to the intestines. Previous studies suggest that certain species of Trematoda are
216 associated with severe tissue damage and death in the host, as in the case of *Paratanaisia bragai* in
217 the avian kidneys, where fatal cases have been reported (Alfaro-Alarcón et al., 2015), as well as death
218 in birds following air sac flukes infestation (Díaz et al., 2022), which coincide with the higher severity
219 of the inflammation observed in this case, particularly for extra-intestinal Trematoda. However,

220 reports of clinical disease in wild birds as a result of parasitosis, whether helminths or protozoa, range
221 from subclinical to severe (Alfaro-Alarcón et al., 2015; Gornatti Churria et al., 2011; Ladds, 2009;
222 Oyarzún-Ruiz et al., 2019; Ozmen et al., 2013).

223 On the other hand, all other parasite types in this study were not associated with inflammation
224 presence nor severity, which could be a result of different factors not limited to parasite type, but also
225 host susceptibility. For instance, the different prevalence of parasites in this study may be associated
226 with the diversity of bird species, feeding habits, age and habitat preference (Parsa et al., 2023), while
227 the variation in inflammation severity could be explained by a combination of factors, including each
228 individual parasite's life cycle, as well as host susceptibility, which is determined by various
229 components, including age and immunological status (Anthony et al., 2008; Vaziri et al., 2019).

230 Among the limitations of this study are the relatively small sample sizes, and the inability to identify
231 the specific taxonomy of the parasites observed. However, this is the first study in the region to
232 describe the association between histopathological findings and parasites in wild birds, which is crucial
233 for wildlife conservation programs and understanding population dynamics.

234 **5. Conclusions**

235 More than half (53.41%) of the birds exhibited parasites; however, only 20.45% showed associated
236 histopathological lesions. This highlights the complex relationship between wildlife and parasitism and
237 underscores the need for further studies to correlate the potential factors that determine the severity
238 of lesions in relation to the types of parasites and the parasitic load.

239 **Author Contributions**

240 Conceptualization, L.M.R.V. and J.C.V.; methodology, L.M.R.V.; validation, L.M.R.V. and A.A.A.; formal
241 analysis, J.C.V. and L.M.R.V.; investigation, L.M.R.V. and J.C.V.; resources, A.A.A.; data curation, X.X.;
242 writing—original draft preparation, J.C.V.; writing—review and editing, J.C.V., L.M.R.V. and A.A.A.;
243 visualization, J.C.V. and L.M.R.V.; supervision, L.M.R.V. and A.A.A.; project administration, A.A.A.;

244 funding acquisition, A.A.A. All authors have read and agreed to the published version of the
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249 **Institutional Review Board Statement**

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252 **Informed Consent Statement**

253 Not applicable.

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257 **Conflicts of Interest**

258 The authors declare no conflicts of interest.

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