1 **Life-stage and sex influence *Philornis* ectoparasitism in a Neotropical woodpecker (*Melanerpes***

2 ***striatus*) with essential male parental care**

3 Running title: *Philornis* on Hispaniolan Woodpeckers

4

5 Joshua B. LaPergola [1,2]

6 [1] Department of Neurobiology and Behavior, Cornell University, Ithaca, NY; Bird Population Studies

7 program, Cornell Lab of Ornithology, Ithaca, NY, USA

8 [2] Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA

9 Corresponding author: jl101@princeton.edu; jlapblca@gmail.com

10 https://orcid.org/0000-0002-2575-0751

11

43 **Life-stage and sex influence *Philornis* ectoparasitism in a Neotropical woodpecker (*Melanerpes***

44 ***striatus*) with essential male parental care**

45

46 **Abstract**

47 The nestlings of many Neotropical bird species suffer from *Philornis* (Diptera: Muscidae) ectoparasitism.

48 While nestlings are typically considered the intended targets, recent work indicates that *Philornis* infest

49 adult birds more frequently than previously appreciated, yet few studies have concurrently surveyed

50 nestlings and adults for *Philornis* in the same population. Over six field seasons (2012–2017), I

51 documented the presence of current or recent subcutaneous *Philornis* infestations on adult and nestling

52 Hispaniolan Woodpeckers *Melanerpes striatus* from the same population. I tested the following three

53 non-mutually exclusive hypotheses regarding occurrence of *Philornis* on adult birds: (1) nestlings are

54 more vulnerable to *Philornis* parasitism than adults, (2) nesting is associated with *Philornis* parasitism in

55 adults, and (3) *Philornis* parasitism is associated with incubation and brooding investment. While nestling

56 and adult woodpeckers exhibited similar prevalence of parasitism, parasitized nestlings hosted on average

57 3.5 times more *Philornis* wounds (larvae plus empty wounds) than parasitized adults. Nesting *per se* was

58 not significantly associated with parasitism among adults, as breeding and non-breeding adults showed

59 similar prevalence and intensity. However, adult males, which perform overnight incubation and

60 brooding, were significantly more likely to be parasitized than adult females. This last result supports the

61 hypothesis that incubation and brooding investment increase the risk of *Philornis* parasitism for adults,

62 but this conclusion is complicated by the lack of an association between parasitism and nesting status.

63 Together, these results raise questions about the degree of host life-stage specialization and whether adult

64 parasitism is incidental or part of an alternative parasitic strategy for *Philornis*.

65

66 **Resumen.** Los pichones de muchas especies de aves Neotropicales sufren de ectoparasitismo por

67 *Philornis* (Diptera: Muscidae). Mientras que los pichones se consideran típicamente los objetivos

68 previstos, trabajos recientes indican que *Philornis* infestan aves adultas con más frecuencia de lo que se

69   pensaba anteriormente, sin embargo, pocos estudios han examinado simultáneamente pichones y adultos

70   para *Philornis* en la misma población. Durante seis temporadas de campo (2012–2017), documenté la

71   presencia de infestaciones subcutáneas recientes o actuales de *Philornis* en adultos y pichones de la

72   misma población del pájaro carpintero *Melanerpes striatus*. Probé las siguientes tres hipótesis no

73   mutuamente excluyentes con respecto a la aparición de *Philornis* en aves adultas: (1) los pichones son

74   más vulnerables al parasitismo de *Philornis* que los adultos, (2) la anidación está asociada con el

75   parasitismo de *Philornis* en adultos, y (3) el parasitismo de *Philornis* es asociado con la incubación y la

76   inversión de crianza. Mientras que los pichones y los adultos exhibieron una prevalencia similar de

77   parasitismo, los pichones parasitados hospedaron en promedio 3.5 veces más heridas de *Philornis* (larvas

78   más heridas vacías) que los adultos parasitados. La nidificación per se no se asoció significativamente con

79   el parasitismo entre los adultos, ya que los adultos reproductores y no reproductores mostraron una

80   prevalencia e intensidad similares. Sin embargo, los machos adultos, que realizan incubación y

81   empollando durante la noche, tenían una probabilidad significativamente mayor de ser parasitados que las

82   hembras adultas. Este último resultado apoya la hipótesis de que la inversión de incubación y de

83   empollando aumentan el riesgo de parasitismo por *Philornis* en adultos, pero esta conclusión se complica

84   por la falta de una asociación entre el parasitismo y el estado de anidación. Juntos, estos resultados

85   plantean preguntas sobre el grado de especialización de la etapa de vida del hospedador y si el parasitismo

86   de adultos es incidental o parte de una estrategia parasitaria alternativa para *Philornis*.

87

88   **Keywords**

89   Botflies, Adult birds, Myiasis, Caribbean, Dominican Republic, Picidae

4

90   **INTRODUCTION**

91   Nestlings of many bird species suffer from myiasis, "the infestation of healthy or necrotic tissue…by

92   dipteran larvae" (Little 2009 p. 546:546), and in the Neotropics, *Philornis* (Diptera: Muscidae) botflies

93   are the primary cause of healthy tissue myiasis (Teixeira 1999, Dudaniec & Kleindorfer 2006). The larvae

94   of at least 23 *Philornis* species are subcutaneous, blood-feeding parasites (Common *et al.* 2019). Botfly

95   effects on nestlings can be severe (reviewed in Dudaniec & Kleindorfer 2006), in some cases reducing

96   survival (Delanoy & Cruz 1991, Rabuffetti & Reboreda 2007, Hayes *et al.* 2019). Indeed, native and

97   introduced *Philornis* have been implicated in the decline of several island endemic birds, most notably in

98   the Galápagos where introduced *P. downsi* have impacted many endemic species (Fessl *et al.* 2006,

99   Kleindorfer & Dudaniec 2016, Leuba *et al.* 2020). Yet the extent of *Philornis* infestation's ecological

100   impacts remains poorly known, especially in these botflies' native ranges. Addressing these gaps will be

101   important for not only advancing basic ornithology but also for understanding whether to account for and

102   how to control *Philornis* in conservation and management efforts.

103   One aspect of *Philornis* parasitism that requires deeper exploration is the degree of host life stage

104   specialization. The prevailing wisdom has been that *Philornis* target altricial and semi-altricial nestlings

105   while the occasional observations of larvae on adult birds represent opportunistic or misdirected

106   infestation (Teixeira 1999). Some researchers have even posited that *Philornis* cannot successfully pupate

107   once host birds have fledged (Arendt 1985a). Understanding the degree to which *Philornis* parasitizes

108   nestlings and adults has important ramifications for bird populations since nestling parasitism directly

109   impacts reproductive success while adult parasitism could impact survival and reproductive success. In a

110   recent review of published records and analysis of new data from adult capture records from three

111   Caribbean islands, Quiroga et al. (2020) reported adult parasitism for 15 bird species representing 12

112   families and four orders. While these results indicate that adult parasitism by *Philornis* might be more

113   than opportunistic, much remains unknown, and more precise estimates of adult infestation prevalence are

114   needed to clarify this relationship.

115   My objective here is to expand on the findings of Quiroga et al. (2020) by utilizing a species

116   well-suited for investigating *Philornis* parasitism: the Hispaniolan Woodpecker *Melanerpes striatus*. This

117   woodpecker is one of the most abundant birds on Hispaniola, common from sea-level to 2,400 m asl in a

118   wide range of habitats (Latta *et al.* 2006), providing ample sampling opportunities. Additionally, the first

119   *Philornis* species (*P. pici*, reported as *Aricia pici*) was described from a subcutaneous larva collected from

120   an adult Hispaniolan Woodpecker (Macquart 1853). Despite the Hispaniolan Woodpecker's high

121   abundance, Quiroga et al. (2020) reported only two new records of *Philornis* infestation on adults: one

122   each from the Cordillera Central (prevalence = 20%, $N = 5$ individuals; H.M. Garrod pers. comm.) and

123   Punta Cana (prevalence = 7%, $N = 14$ individuals; L. Soares and S.C. Latta pers. comm.). Furthermore,

124   the parasite negatively impacts the reproductive success of at least one other Hispaniolan endemic, the

125   critically endangered Ridgway's Hawk *Buteo ridgwayi* (Hayes *et al.* 2019). Yet the woodpecker's

126   continued abundance in spite of *Philornis* and anthropogenic pressures (Mitchell & Bruggers 1985)

127   suggests it could be an excellent model system to advance *Philornis* biology. To that end, I test three

128   hypotheses (Table 1) regarding *Philornis* infestation prevalence and intensity on adult birds.

129   First, I test two predictions of the hypothesis (H1) that nestlings are more vulnerable than adults

130   to *Philornis* parasitism (Teixeira 1999). This hypothesis predicts that (P1.1) *Philornis* prevalence (the

131   proportion of birds infested) should be higher for nestlings than for adults. Assuming nestlings are easier

132   targets for infestation, this hypothesis also predicts (P1.2) that nestlings should have higher intensity

133   (number of larvae per infested individual) *Philornis* infestations compared with adult birds. Due to their

134   mobility, adult woodpeckers should provide not only fewer opportunities for larval deposition by adult

135   flies across adults, but also fewer opportunities for repeat deposition on individual adults.

136   Second, I test two predictions of the hypothesis (H2) that nesting behavior itself is associated with

137   *Philornis* parasitism of adults. If *Philornis* is more prevalent and intense on nestlings than adults (Arendt

138   1985a), parasitism of adults might be an opportunistic direct result of nesting activity. This hypothesis

139   thus predicts that *Philornis* (P2.1) prevalence and (P2.2) intensity should be higher for nesting birds than

140   birds not nesting.

141   Lastly, I test four predictions of the hypothesis (H3a,b) that adult *Philornis* parasitism is

142   associated with incubation and brooding investment (Teixeira 1999). While nesting itself might increase

143   exposure to *Philornis*, intersexual differences in breeding behavior might result in females and males

144   experiencing different levels of parasitism. Both female and male Hispaniolan Woodpeckers develop

145   brood patches and share approximately equivalent diurnal incubation and brooding (unpubl. data). If

146   incubation and brooding behavior increase exposure (H3a), *Philornis* (P3.1a) prevalence and (P3.2a)

147   intensity should be similar in female and male Hispaniolan Woodpeckers. Like most woodpecker species

148   (Winkler *et al.* 1995), though, male Hispaniolan Woodpeckers perform all overnight incubation of eggs

149   and brooding of nestlings (pers. obs.), a form of essential parental care. This male-biased nocturnal

150   incubation and brooding behavior might be important because adults of at least some *Philornis* species

151   will visit nests at night (O'Connor et al. 2010) and in the late afternoon and dusk (Pike et al. 2021). If

152   overnight incubation and brooding increase exposure (H3b), *Philornis* (P3.1b) prevalence and (P3.2b)

153   intensity should be higher for nesting males than nesting females.

154   Tests of these hypotheses and predictions (Table 1), which require data from both nestlings and

155   adults from the same population, have only been reported for the Caribbean endemic Pearly-eyed

156   Thrasher *Margarops fuscatus*. Both Pearly-eyed Thrashers and Hispaniolan Woodpeckers nest in cavities,

157   a life-history trait that could impact parasitism exposure (Nilsson 1986), so one might predict similar

158   patterns of *Philornis* prevalence and intensity in both species. In support of H1, nestling Pearly-eyed

159   Thrashers exhibited a far higher prevalence (96%) and intensity (mean = 37 larvae/nestling) of *P.*

160   *deceptivus* compared with adult prevalence (31%) and intensity (mean = 3.1 larvae/adult) on Puerto Rico

161   (Arendt 1985a). To the best of my knowledge, H2 has not been directly tested in Pearly-eyed Thrashers

162   and has only indirect support from immunological data in the Galápagos endemic Medium Ground Ginch

163   *Geospiza fortis*, which showed higher *Philornis*-specific antibody levels during nesting than pre-nesting

164   (Huber *et al.* 2010). Pearly-eyed Thrasher data support H3a since *Philornis* prevalence among nesting

165   females, which perform all incubation and brooding, was ~3.5 times higher than for nesting males

166   (Arendt 1985a). Indirect evidence supporting H3a was also found in the medium ground finch: nesting

167   females, who brood nestlings, had higher *Philornis*-specific antibody levels than nesting males (Huber *et*

7

168   *al.* 2010). However, no studies have investigated *Philornis* in a species where males perform essential

169   incubation and brooding.

170

171   **METHODS**

172   ***Field methods*.** I studied Hispaniolan Woodpeckers in the community of Piedra Blanca, 3 km east of

173   Jarabacoa (19.1193ºN, 70.5819ºW; 550–700 m asl), La Vega, Dominican Republic, between April 2012

174   and July 2017. The site (~84 ha) comprised several private properties on a landscape of pine (*Pinus*

175   *occidentalis* and *P. caribaea*) and broadleaf wet forest fragments immersed in a matrix of cattle pastures

176   with isolated or clustered royal palms *Roystonea hispaniolana*, small fragments of secondary vegetation,

177   and "living tree" (predominantly *Gliricidia sepium*) fences. This region experiences a mild, dry winter

178   (January–March), followed by a short wet spring season (April–May), a long, dry summer season (June -

179   September), and a short, wet fall season (October–December) coinciding with the latter half of the

180   Atlantic hurricane season (Climate-data.org 2021). For the remainder of the Methods, I use "we" in lieu

181   of "I" to describe most activities because they involved a team of tireless volunteer field assistants.

182   We evaluated *Philornis* infestation status on nestling and adult woodpeckers at trees monitored

183   for nesting activity, which we selected based on the presence of cavities and nesting activity (e.g., cavity

184   excavation, adults entering/exiting cavities, etc.). To determine nesting activity, we inspected cavities

185   using a penlight and small inspection mirror (1–2" diameter) while climbing or with a wireless camera

186   attached to a 15.2 m telescoping pole that broadcasted images to a portable digital television (Huebner &

187   Hurteau 2007, Waldstein 2012). Once we detected a nesting attempt (i.e., ≥1 eggs), we typically checked

188   the clutch every 3–5 days and, when possible, daily if we did not know the clutch completion date.

189   Incubation typically lasted 11 days (range = 9–14 days). The nestling sampling protocol differed slightly

190   in timing across years, but in general, sampling involved collecting morphometric measurements and

191   inspecting the entire body surface for the presence/absence of *Philornis*, including counting the number of

192   active and empty wounds (Fig. 1). We considered a wound active if it contained ≥1 subcutaneous

193   *Philornis* larvae, and, in cases where ≥2 larvae inhabited the same wound (see Fig. 1a for example of two

194   sets of posterior spiracles of larvae visible in a single wound), we recorded the total number of detectable

195   larvae. Empty *Philornis* wounds resembled active wounds in appearance, except that empty wounds

196   tended to look less swollen (Fig. 1b), lacked detectable larvae, and retained an opening where a larva had

197   resided. For all years when we did not know the nest's hatch date (e.g., nest was found with nestlings), we

198   sampled and banded nestlings as soon as they were large enough to carry four bands—two colour bands

199   on one leg and one colour band and one metal band on the other leg. For nests with known hatch dates

200   from 2013–2015, we sampled and fully banded nestlings when they were ~14 days old and resampled at

201   ~21 days old. For nests with known hatch dates in 2016 and 2017, we sampled and metal banded

202   nestlings at ~7 days old, resampled at ~14 days old, and resampled and added three colour bands at ~21

203   days old.

204   For adult sampling, we captured birds via two approaches: (1) ambushing adults in nest cavities

205   and (2) an elevated, dual-tower mist-net system (LaPergola & Kenyon in prep.). Ambushing involved

206   setting up ambush traps as in Stanback and Koenig (1994) to allow pre-dawn capture of roosting birds.

207   See Garrod and LaPergola (2018) for more details on implementation. To reduce nest abandonment, we

208   used the ambush method ≥7 days before egg-laying or ≥22 days post-hatch. The mist-net tower system

209   involved erecting two 15.2 m tower poles supported with guy lines (ropes) and, using pulleys and ropes,

210   raising two stacked 12 m mist-nets in front of nesting trees. This method reduced disturbance at nests,

211   enabled capture of woodpeckers using trees too unstable to climb, increased sampling efficacy before

212   nesting, and increased sampling of non-nesting birds. As with nestlings, each adult received a unique

213   four-band combination and was inspected for the absence or presence of *Philornis*. When present, we

214   counted the number of active and empty *Philornis* wounds. We also recorded sex of adults based on

215   crown colour, which is black for females and red for males.

216   We determined the nesting and breeder status of captured birds by monitoring nesting attempts

217   and identifying attendant birds via focal nest watches. Nest watches involved 2 or 3 hour sessions in

218   which an observer sat 15–20 m from a nest tree in a burlap blind, trained a 15X or 20X spotting scope on

219   a focal cavity entrance for a nest, and recorded the identities (i.e., band combinations) and behaviors of

220   woodpeckers that visited the nest. For testing the hypothesis that *Philornis* parasitism in adults is

221   associated with nesting, I coded adults as belonging to one of two categories: nesting or not nesting. I

222   counted an adult as nesting if it met two criteria: (1) we observed the bird incubating at or provisioning ≥1

223   nest within the year of capture, and (2) we captured the bird after the earliest possible clutch initiation

224   date for its earliest possible nesting attempt within the year of capture. I counted banded birds as not

225   nesting if they met one of the following criteria: (1) we captured the bird early in the field season before

226   most nests were initiated (between January and before early April), or (2) the bird was not associated with

227   a nesting attempt prior to the date of capture in the same calendar year.

228   Although we did not attempt to identify larvae to species, *Philornis pici* is the only *Philornis*

229   species currently known to infest birds on Hispaniola, and, as mentioned earlier, was first described from

230   the Hispaniolan Woodpecker (Macquart 1853). Elsewhere in the Dominican Republic, researchers have

231   confirmed this species to parasitize Ridgway's Hawk (Hayes *et al.* 2019, Quiroga *et al.* 2020). However,

232   *P. porteri* has also been identified parasitizing Ridgway's Hawk (M.A. Quiroga pers. comm.). The

233   distribution of *P. porteri* on Hispaniola is currently unknown, but it is possible that the *Philornis* detected

234   in the present study could be *P. pici*, *P. porteri*, or both.

235

236   ***Statistical analyses.*** See Table 1 for a summary of the hypotheses and their predictions. For testing the

237   hypothesis that nestlings are more vulnerable to *Philornis* parasitism than adults (H1), I tested the two

238   predictions with separate generalized linear mixed-effects models (GLMMs). For the prediction that the

239   probability of being parasitized is higher for nestlings than for adult birds (P1.1), I used a GLMM with a

240   binomial fit to test for an association between infestation status and age coded as a categorical fixed effect

241   (adult vs. nestling). Infestation status was treated as a binary response (0 = no evidence of *Philornis*, 1 =

242   presence of ≥1 *Philornis* larvae, empty wounds, or both) in this model. For the prediction that nestlings

243   host greater numbers of *Philornis* wounds than adults (P1.2), I used a GLMM with a negative binomial

244   distribution to test for an association between the total number of *Philornis* wounds (summing the

245   numbers of empty and active wounds, or total number of larvae) and age. Because many birds were never

10

246   observed with infestations, including all sampled individuals would lead to zero-inflation for the total

247   number of *Philornis* wounds; consequently, I used a manual hurdle model approach, including only

248   infested birds in this model.

249   To test the predictions of the hypotheses that (H2) nesting and (H3a, H3b) incubation and

250   brooding investment are associated with *Philornis* parasitism in adults, I used four GLMMs to test for

251   associations of adult infestation status with nesting status, sex, and the interaction of nesting status and

252   sex. I coded both nesting status (nesting vs. not nesting) and sex (female vs. male) as categorical fixed

253   effects for all four models. To test predictions regarding prevalence (P2.1, P3.1a, and P3.2a), I used

254   GLMMs with a binomial fit: infestation status was treated as a binary response as with the analysis

255   comparing nestlings and adults. For the first GLMM, I included all adults of known nesting status,

256   retaining birds known not to have bred within the year of capture. For the second GLMM, I retained only

257   birds known to have bred within the year of capture to exclude any effects of unanticipated differences

258   between breeders and non-breeders. For testing the predictions regarding intensity as they relate to

259   nesting status and sex (P2.2, P3.2a, and P3.2b), I used a GLMM with a Poisson distribution to test for an

260   association of the total number of *Philornis* wounds (summing the numbers of active and empty wounds,

261   or total number of larvae) with nesting status, sex, and the interaction effect of nesting status and sex. As

262   with the nestling-adult comparison, many observations involved no infestation and would lead to zero-

263   inflation for the total number of *Philornis* wounds; consequently, I used a manual hurdle model approach,

264   including only infested adults in this model. Additionally, I only ran this model with the dataset that

265   included all adults of known nesting status, including birds known not to have bred within the year of

266   capture.

267   For all models, I also included capture date as a continuous fixed effect based on the following.

268   The capture date range was fairly large (range = 168 d, 28-Feb–4-Aug), which included the end of the

269   winter dry season, the short wet spring season, and the long dry summer season. Furthermore, previous

270   studies have documented a positive association between the probability of adults and nests having

271   *Philornis* and the timing of breeding (Arendt 1985a, Rabuffetti & Reboreda 2007). For all analyses, I

11

272   scaled capture date in day of year format via Z-transformation by subtracting the mean capture date and

273   dividing by the standard deviation.

274   For all models except for those testing predictions regarding intensity only in adults (P2.2, P3.2a,

275   and P3.2b), I included the following as random effects: the tree where a bird was captured or, for known

276   breeders, where it bred in the year of capture (Tree ID); year of capture; and individual ID. I included

277   Tree ID as a random effect because the Hispaniolan Woodpecker is unique among the Picidae, being one

278   of only three known woodpecker species to exhibit facultative colonial nesting. Within the same

279   population, Hispaniolan Woodpeckers pairs can nest singly or in clusters, with two or more pairs nesting

280   concurrently in separate cavities on the same tree (Short 1974, Winkler *et al.* 1995, LaPergola 2018).

281   Additionally, I wanted to account for the non-independence of nestlings from the same brood and thus the

282   same parents, but using a nest ID random effect would have precluded using adults without nests. Using

283   Tree ID as a random effect is thus a more conservative approach to account for non-independence,

284   especially for nestlings. I included year as a random effect in all analyses because I was not confident that

285   interannual variation was sampled adequately enough to interpret the fixed effects of year (Bennington &

286   Thayne 1994). Lastly, I included individual ID because some individuals were captured multiple times.

287   For testing predictions regarding intensity only in adults (P2.2, P3.2a, and P3.2b), I included only year as

288   a random effect because including Tree ID and individual ID led to failed model convergence.

289   I conducted all statistical analyses in RStudio v. 1.1.463 using R v. 3.6.3 (R Core Team 2020).

290   For fitting GLMMs, I used the *glmer* (binomial and Poisson fits) and *glmer.nb* (negative binomial)

291   functions in the *lme4* package (Bates *et al.* 2015). For models where interaction terms were not

292   significant, I report only the results of the additive models. All means are reported ± the standard error of

293   the mean, and all confidence intervals for count data are 95% and were calculated via the Wald Method.

294

295   **RESULTS**

296   **Summary of *Philornis* parasitism prevalence**

297 Over six years, I obtained 218 adult records representing 184 unique individuals (83 females and 101

298 males), which included 26 individuals (eight females and 18 males) recaptured once and four individuals

299 (one female and three males) recaptured twice. Of all adult records, 40 (18%; CI = 14–24%) included

300 individuals with evidence of *Philornis* parasitism. Of all individuals (*n* = 184), 36 (20%; CI = 14–26%)

301 had evidence of *Philornis* parasitism, which included 24 (67%; CI = 50–80%; *n* = 36 individuals) with

302 *Philornis* empty wounds, nine (25%; CI = 14–41%) with active wounds, and three (8%; CI = 2–23%)

303 with both empty and active wounds. Of all adults with more than one capture (*n* = 26), 11 individuals

304 exhibited changed infestation status (Table S1). These records included four individuals recaptured within

305 the same year, of which two had active wounds on the second capture but no wounds on the first, one had

306 old wounds on the first capture but not the second, and one individual had old wounds on the first capture

307 but no visible wounds on the second capture 82 days later.

308 Across six years, I collected 554 nestling records representing 381 individuals from 127 nesting

309 attempts. These figures amounted to a mean of 4.4 ± 2.4 records per nesting attempt (range = 1–10

310 records per nesting attempt) and a mean of 3.0 ± 1.0 nestlings per nesting attempt (range = 1–5 nestlings

311 per nesting attempt). Of all nestling records, 123 (22%; CI = 19–26%) showed evidence of *Philornis*

312 parasitism, and of all nestlings observed, 107 (28%; CI = 24–33%) exhibited evidence of *Philornis*

313 parasitism on at least one sampling event. Of the nestling individuals with evidence of *Philornis*, most

314 (73%; CI = 64–80%) involved active wounds (45 observations with only active wounds and 33

315 observations with both active and old wounds), while fewer observations involved only old wounds

316 (19%; CI = 12–27%; for 8% of nestling observations, the wound status was not recorded). Infested

317 nestlings came from 43 (34%; CI = 26–43%) of all monitored nesting attempts.

318

319 **H1: Comparison of adults and nestlings**

320 Using the full set of adult and nestling capture records, age and scaled day of year captured alone were

321 not significant predictors of the presence/absence of *Philornis* parasitism (Table 2a). However, there was

322 a significant interaction effect of age and scaled day of year captured, such that the probability of

13

323   exhibiting *Philornis* parasitism increased with the scaled day of year for nestlings but not for adults (Fig.

324   2A). In contrast to presence/absence, age alone was significantly associated with the total number of

325   *Philornis* wounds (empty plus active wounds) (Table 2b). Infested nestlings had an average of 7.1 ± 0.5

326   *Philornis* wounds (range = 1–39 *Philornis* wounds; $n = 123$ nestling records) while infested adults had an

327   average of only 2.0 ± 0.2 wounds (range = 1–5 *Philornis* wounds; $n = 40$ adult records; Fig. 2b).

328

329   **H2 and H3: Nesting status and sex**

330   When restricting the analyses to adults of known nesting status, there was no significant association

331   between *Philornis* infestation and whether an adult was currently nesting (Table 3). This result was true

332   for both the analysis including all adults of known nesting status, i.e., retaining birds known not to have

333   bred within the capture year (Table 3a), and for the analysis restricted to only birds that nested within the

334   capture year (Table 3b). Additionally, the scaled day of year was not significantly associated with

335   infestation status. There was no significant interaction between sex and nesting status, but adult sex was

336   significantly associated with infestation in both analyses. For all adults of known nesting status, 9% of

337   females (CI = 4–17%; $n = 82$ observations) and 27% of males (CI = 20–36%; $n = 111$ observations)

338   showed signs of current or past infestation (Figure 3). These proportions remained similar for the subset

339   that included only birds that nested within the capture year (8% of females: CI = 4–18%, $n = 60$

340   observations; 26% of males: CI = 17–37%, $n = 74$ observations).

341   Infested female and male adults had similar numbers of *Philornis* wounds. Infested females had a

342   mean of 2.0 ± 0.7 wounds (range = 1–5; $n = 7$ observations), and infested males had a mean of 2.0 ± 0.2

343   (range = 1–5; $n = 29$ observations). None of the fixed effects were significant in the model (Table 4).

344   The 12 adults with active infestations were mostly (58%) known breeders (Table S2). Two of the

345   three females with active infestations also had infested nestlings at the time of capture. Of the nine males

346   with active infestations, four had infested nestlings at the time of capture and one male had fledged two

347   young (one infested and one not) one month prior to capture. The remaining individuals of uncertain

14

348   breeding status (one female and four males) were all caught within the known breeding season at the site

349   (Fig. S1); the earliest capture was on 8 March and the latest capture 20 July.

350

351   **DISCUSSION**

352   Most previous work on *Philornis* myiasis has understandably focused on these parasites' impacts on

353   nestling birds (Arendt 1985b, Dudaniec & Kleindorfer 2006, Hayes *et al.* 2019) since this life stage has

354   long been considered the primary target of parasitism (Teixeira 1999). The present study is one of very

355   few that has concurrently documented *Philornis* prevalence and intensity on nestlings and adults from the

356   same population (see also Arendt 1985a). Intriguingly, adult Hispaniolan Woodpeckers were just as likely

357   to exhibit evidence of *Philornis* as nestlings, although nestlings experienced greater intensity of

358   infestation. For adults, while nesting status itself was not a significant predictor of being infested, males,

359   which invest more in overall incubation and brooding, were significantly more likely to have *Philornis*

360   infestations than females.

361

362   **Nestlings vs. adults**

363   The present study's results falsify the first prediction of the hypothesis that nestlings are more vulnerable

364   to *Philornis* parasitism but support the second prediction that nestlings experience more intense

365   infestations. While adult and nestling Hispaniolan Woodpeckers did not differ in the probability of being

366   parasitized (Table 2a), the probability of being parasitized for nestlings did increase with passage of the

367   breeding season yet remained more or less static for adults across the breeding season (Fig. 2a).

368   Furthermore, when infested, nestlings bore greater numbers of *Philornis* wounds than did adult birds

369   (Table 2b; Fig. 2b.). This difference in intensity is likely due to the increased accessibility of nestlings to

370   *Philornis* flies in contrast to lower accessibility of adults. Of the two results and corresponding

371   predictions, the contrast in *Philornis* intensity supports the hypothesis that nestlings are indeed more

372   vulnerable to parasitism. However, the similarity in prevalence suggests a complementary hypothesis that

373   adult *Philornis* are equally likely to find nestling and adult Hispaniolan Woodpeckers but that nestlings

15

374   are less resistant to infestation. Unfortunately, we currently lack the necessary *Philornis* natural history

375   data to evaluate this possibility. If *Philornis* females oviposit directly in woodpecker nests, this behavior

376   would help explain higher intensity of *Philornis* on nestling woodpeckers since adults would have even

377   lower overall exposure to infestation. *Philornis downsi* oviposits in the nest material (Lahuatte et al.

378   2016), and at least one subcutaneous species, *P. torquans*, will oviposit on inanimate surfaces in captivity

379   (Patitucci et al. 2017, Saravia-Pietropaolo *et al.* 2018). It is therefore plausible, though yet to be

380   confirmed, that *P. pici* and *P. porteri*, the two species most likely parasitizing Hispaniolan Woodpeckers,

381   oviposit directly in the nest.

382   Regardless of the manner of egg/larval deposition, there are at least three non-mutually exclusive

383   mechanistic hypotheses for lower parasite intensity on adults. First, the adults' well-developed plumage

384   might reduce accessibility by presenting a physical barrier to burrowing larvae (Oniki 1983). This might

385   be especially relevant for Hispaniolan Woodpeckers as they hatch naked and remain so until 7–8 d post-

386   hatch when their pin feathers typically begin erupting, and while pin break begins around 14 d post-hatch,

387   these feathers fail to cover most of the body other than the feather tracts until about 21 d post-hatch

388   (unpubl. data). Thus the young have little to no physical barrier against *Philornis* other than a brooding

389   adult for roughly their first three weeks. Hispaniolan Woodpecker nestlings also remain in the nest 29–38

390   d post-hatch (unpubl. data), providing additional exposure time, albeit with an increasing amount of

391   feather coverage. A non-mutually exclusive alternative hypothesis is that the greater mobility of adults

392   reduces their accessibility to *Philornis* (Teixeira 1999). When not actively attending a nest, adults are

393   literally moving targets for flies, covering areas of 1.7–4.2 km^2 while foraging (Mitchell & Bruggers

394   1985), whereas nestlings remain relatively stationary, confined to the same nest cavity until fledging. A

395   third hypothesis is that the immune memory of adult birds might make them better able to resist

396   infestation. This immune defense hypothesis is plausible given that mother, but not nestling, Medium

397   Ground Finches had elevated levels of *P. downsi*-binding antibody when exposed to the parasite (Koop et

398   al. 2013). Whether any of these mechanisms can explain differences in *Philornis* infestation intensity for

399   Hispaniolan Woodpeckers remains to be examined.

16

400   The patterns of *Philornis* prevalence and intensity reported here for Hispaniolan Woodpeckers

401   contrast somewhat with those from the only other study (see Arendt 1985a) comparing nestlings and

402   adults in the same population. Whereas *Philornis* prevalence among nestling Pearly-eyed Thrashers was

403   much higher than for adults (96% vs. 31%, respectively; Arendt 1985a), prevalence was only non-

404   significantly higher for nestling woodpeckers than for adults (28% vs. 20%). This contrast might be

405   explained by two inter-related factors that differ between the Pearly-eyed Thrasher study and the present

406   study: habitat and climate. The thrasher study took place in a tropical rainforest, with annual rainfall

407   averaging 4460 mm (Arendt 1985a), while the present study occurred in more open, drier habitat, with

408   annual rainfall of 1723 mm (Climate-data.org 2021). Rainfall is a significant predictor of *Philornis*

409   infestation, showing a positive correlation with intensity (Antoniazzi *et al.* 2011, Manzoli *et al.* 2013),

410   and moisture and humidity predict the geographic distribution of at least one *Philornis* species (Cuervo *et*

411   *al.* 2021). The greater canopy cover and humidity of the rainforest might have promoted larger

412   populations of adult *Philornis* than those in the drier habitat of the Dominican Republic, and these

413   hypothetical larger fly populations might have more fully exploited the vulnerable nestling thrashers

414   while adults could effectively avoid or prevent parasitism. Alternatively, the drier habitat on the

415   Dominican Republic might have reduced access for adult *Philornis* because they would have needed to

416   cross open (i.e., no canopy cover) habitat to reach woodpecker nests. In other words, the Hispaniolan

417   Woodpecker's habitat structure provides a barrier for adult *Philornis* so they are prevented from fully

418   exploiting the vulnerable nestling woodpeckers. The pattern of intensity differences was similar, though:

419   both nestling thrashers and woodpeckers had greater intensity of *Philornis* than adult birds, and this aligns

420   with the second prediction of the hypothesis that nestlings in both species are more vulnerable to

421   *Philornis* parasitism.

422   One limitation of the present study was that the precise timing of active infestation for adults was

423   often unknown, especially relative to timing of nesting. This issue arose because most evidence of

424   *Philornis* on adult Hispaniolan Woodpeckers was in the form of empty wounds rather than active wounds

425   containing larvae (33% of adult records involved wounds containing ≥ 1 larvae). In contrast, most

17

426   nestling observations involved active wounds. This difference is due in part to the sampling effort relative

427   to the age of target birds. Nestling Hispaniolan Woodpeckers were sampled at known ages and within 25

428   days of hatching so the period of exposure was limited, increasing the probability of detecting

429   subcutaneous *Philornis* larvae, which can remain attached for 5–8 days (Arendt 1985a, Young 1993). The

430   exposure period for adult birds bearing empty wounds, however, was presumably all the days they lived

431   prior to the date of capture, decreasing the probability that we would detect their wounds when they

432   contained larvae. This limitation is important for two reasons regarding timing. First, while it might be

433   most parsimonious to assume all adult Hispaniolan Woodpeckers with empty *Philornis* wounds were

434   infested as adults, we do not know the maximum number of days empty wounds persist after larval

435   detachment from woodpeckers. This uncertainty means that some adults bearing empty wounds might

436   have been infested as nestlings though this seems unlikely. Quiroga et al. (2020, p. 2) posited that all

437   adults in their sample were likely parasitized as adults because "scars [i.e., empty wounds] usually heal

438   ca. one week after larvae detach from the host…". There are few published accounts of the time it takes

439   for an empty wound from a subcutaneous *Philornis* infestation to heal completely and leave no visible

440   trace, but scars left by subcutaneous *Philornis* after removal from nestling hosts of three species

441   (Baywings *Agelaioides badius*; Screaming Cowbirds *Molothrus rufoaxillaris*; and Shiny Cowbirds *M.*

442   *bonariensis*) lasted at least two days (Ursino et al. 2019). Second, uncertainty of adult exposure

443   potentially reduces the accuracy of nestling-adult seasonality comparisons.

444   The difference between adults and nestling Hispaniolan Woodpeckers with respect to the

445   seasonality of prevalence begs further consideration. The lack of an effect of day of capture on prevalence

446   in adults might be related to the abovementioned limitation: i.e., sampling date relative to the day(s) of

447   active infestation. Because the majority of nestling observations involved active wounds while most adult

448   records only involved empty wounds, the day of capture for adults was a less reliable indicator of the

449   timing of infestation for them. In other words, it could be that adults showed the same type of seasonality

450   in infestation as nestlings, with the probability of being infested increasing as the season progressed, but

451   the sampling effort precluded detecting such a pattern. If the difference in seasonality between adults and

452   nestlings was a real pattern, though, Hispaniolan Woodpeckers would differ from Pearly-eyed Thrashers,

453   in which adults showed increasing prevalence of *Philornis* as the season progressed but prevalence among

454   nestlings was high throughout the nesting season (Arendt 1985a). To more fully understand the

455   seasonality of *Philornis* infestation will require data on the seasonality of emergence and population

456   dynamics of adult flies (e.g., Causton et al. 2019). Unfortunately, there are no published data on the

457   seasonality of *Philornis* emergence for Hispaniola.

458

459   **Nesting status, sex, and brooding/incubation investment**

460   The nesting status of Hispaniolan Woodpecker adults was not significantly associated with prevalence nor

461   intensity of *Philornis* parasitism (Tables 3 & 4), refuting the two predictions of the hypothesis that such

462   parasitism is associated with nesting. To the best of my knowledge, these results represent the first direct

463   test of this hypothesis. The lack of an effect of nesting status in the present study could be an artifact of

464   the sampling period, which mostly comprised the nesting season. However, the inclusion of adults known

465   to not be actively nesting at the time of capture should lessen the impact of such an artifact. Another

466   possible limitation was the uncertainty around the time when an adult was first infested because it makes

467   it harder to discern the amount of overlap between infestation and nesting. It will be crucial to more

468   precisely define the window of infestation for sampled adults to accurately compare prevalence and

469   intensity among nesting and non-nesting birds in future studies. One could achieve increased accuracy

470   here by sampling more birds in the non-breeding season and capturing more adults when they have chicks

471   of known age. However, it is worth considering whether the observed pattern is not an artifact, i.e.,

472   nesting and non-nesting Hispaniolan Woodpeckers are equally likely to be parasitized. If *Philornis*

473   typically finds hosts by searching for nest-related cues (e.g., olfactory), adult Hispaniolan Woodpeckers

474   might be parasitized outside the context of actively breeding if they spend time in nest cavities for other

475   activities. For example, Hispaniolan Woodpeckers roost in previously used nest cavities (pers. obs.). If

476   the cues adult *Philornis* use to find nestlings remain detectable, opportunistic parasitism of adult

477   woodpeckers could occur. Such a scenario might apply in the non-breeding season or even within the

19

478 breeding season prior to active nesting. One could test this idea experimentally by setting un-baited traps

479 for adult *Philornis* in old or recently used cavities. Another possible reason that nesting status might be

480 less relevant for Hispaniolan Woodpeckers concerns their habit of colonial nesting. For example, adults

481 lacking active nests might still be subjected to parasitism when one or more other colony members are

482 nesting and thus attracting adult *Philornis*. This hypothesis and the impacts of colonial nesting on

483 *Philornis* parasitism more broadly warrant further study since group-living can either increase (Brown &

484 Brown 1986) or decrease (Mooring & Hart 1992) the risk of parasitism. Local heterospecific nesting

485 density was associated with increased intensity of the invasive *P. downsi* (Kleindorfer & Dudaniec 2009),

486 indicating that this hypothesis is well worth investigating in the native ranges of *Philornis* (e.g., see

487 Antoniazzi et al. 2011).

488   The combined results of adult Hispaniolan Woodpeckers being parasitized regardless of nesting

489 status and nestlings and adults exhibiting similar prevalence suggest that parasitism of adult woodpeckers

490 might be part of a mixed strategy by *Philornis* in which they target adult birds. As suggested by Quiroga

491 et al. (2020), such a strategy might allow flies to reproduce when nestlings are unavailable or in short

492 supply. In the present study, the Hispaniolan Woodpecker population had a defined breeding season,

493 beginning in early March, peaking in May, and tapering off in August (LaPergola 2018) so nestling

494 woodpeckers are unavailable for approximately half the year and only abundant for roughly three months.

495 Some other local species that might host *Philornis* (e.g., *Crotophaga ani*, *Columbina passerina*, *Zenaida*

496 *aurita*, *Z. asiatica*, and *Coereba flaveola*) have been suggested to breed year-round (Latta *et al.* 2006), but

497 the extent to which they do so in addition to whether they are parasitized at the study site remains

498 unknown. Additionally, capture records of Hispaniolan Woodpeckers from the site are unavailable from

499 most of the non-breeding season, especially September through December. Fully testing this hypothesis

500 that *Philornis* target adult birds when nestlings are unavailable or scarce requires year-round monitoring

501 for infestation of both adults and nestlings and data on the availability and abundance of host nestlings.

502   Despite the non-significant effect of nesting status, there was some support for the hypothesis that

503 *Philornis* parasitism is associated with incubation and brooding investment in Hispaniolan Woodpeckers.

20

504 Although the sexes did not differ in intensity of infestation, males were 3.4 times more likely than

505 females to host *Philornis*. This result mirrors the pattern observed in Pearly-eyed Thrashers, where

506 females, who are the sole incubators/brooders, were 3.5 times more likely to host *Philornis* than males

507 (Arendt 1985a). Since female Hispaniolan Woodpeckers perform only diurnal incubation/brooding while

508 males perform diurnal *and* nocturnal incubation/brooding, males might experience increased *Philornis*

509 exposure at night. Unfortunately, almost nothing is known about the temporal activity patterns of

510 *Philornis* on Hispaniola nor for most other *Philornis* with subcutaneous larvae. In the Galápagos, adult *P.*

511 *downsi* enter host nests to oviposit when the parent birds are absent during the day when nestlings are

512 young and at night when nestlings are older (O'Connor *et al.* 2010), and peak nest visitation rates of adult

513 flies occurs in the late afternoon and dusk in the nestling phase (Pike et al. 2021). But *P. downsi* larvae

514 are free-living and hematophagous and eggs are oviposited in the nest. An important assumption of the

515 hypothesis that nocturnal incubation increases exposure thus needs testing. Additionally, the lack of an

516 interaction effect of nesting status and sex suggests alternative hypotheses warrant testing.

517   Three major sets of alternative explanations for higher prevalence of *Philornis* among adult male

518 Hispaniolan Woodpeckers are sexual dimorphisms in behavior, morphology, and immunology (Zuk &

519 McKean 1996). One behavioral difference could be that males experience greater exposure by spending

520 more time in a particular site or habitat (e.g., Tinsley 1989). For example, male Hispaniolan Woodpeckers

521 might spend more time than females in cavities overall, even when not tending a nest with eggs or young.

522 This could be the case if males played a larger role in defending cavities from competitors and were thus

523 more likely to encounter *Philornis* searching for nestlings. Another behavioral difference might be that

524 males simply invest less in anti-parasitic behaviors like preening and grooming such that they are less

525 likely than females to remove *Philornis*. At present, it is unknown whether male and female Hispaniolan

526 Woodpeckers differ in preening behavior. In at least some bird species, though, males tend to spend more

527 time grooming rather than less (Cotgreave & Clayton 1994, Oswald *et al.* 2019). With respect to

528 morphology, male Hispaniolan Woodpeckers have bills that are on average 25% longer than those of

529 females (unpubl. data; see also Selander 1966), and it could be that their longer bills reduce their

21

530   effectiveness at removing *Philornis* eggs or larvae. Male Hispaniolan Woodpeckers are also larger in

531   other dimensions of size, including weight, and it could be that their larger size increases the probability

532   that they miss a parasite during preening. Lastly, with regards to immunology, male Hispaniolan

533   Woodpeckers might be more tolerant and/or less resistant to *Philornis* infestation. Widespread evidence

534   exists for sex differences in immunocompetence (e.g., Kelly et al. 2018), but to the best of my knowledge,

535   this possibility remains unstudied with respect to *Philornis*. These alternative behavioral, morphological,

536   and immunological explanations clearly warrant future study.

537

538   **Future considerations and implications for *Philornis* biology**

539   Inter-population comparisons of *Philornis* parasitism in Hispaniolan Woodpeckers could be a fruitful

540   course of future research since this woodpecker occupies a range of habitats and elevations. The *Philornis*

541   prevalence on adult woodpeckers documented in the present study was the same as that reported for the

542   species at nearby Rancho Baiguate (H.M. Garrod pers. comm.; Quiroga *et al.* 2020) but higher than that

543   reported from coastal, low elevation Punta Cana (7%; L. Soares and S.C. Latta pers. comm.; Quiroga *et*

544   *al.* 2020). Whether these differences correspond to *Philornis* population sizes differing according to

545   habitat or climatic conditions could be explored with the Hispaniolan Woodpecker.

546   Though the difference in nestling and adult *Philornis* infestation intensity is suggestive of a

547   preference for nestlings by botflies, more work is needed to robustly test this hypothesis. In studies of

548   choice and decision-making, confirming the presence of a positive association provides support for a

549   preference hypothesis, but a more discriminating test involves an experimental choice assay (Dougherty

550   2020). Similar approaches reveal host preferences in insects (e.g., Linn *et al.* 2003). In this case,

551   presenting adult *Philornis* with a choice test between depositing eggs on a nestling or adult bird would be

552   most revealing. Additionally, it is often assumed that parasitizing nestlings yields a higher fitness payoff,

553   but this hypothesis, as far as I know, remains untested.

554   Given the observed prevalence and intensity of *Philornis* on both nestlings and adults, the

555   Hispaniolan Woodpecker would make an excellent model system to study this parasite's biology. For

556   example, it would be revealing to conduct *Philornis* exclusion experiments to better understand how non-

557   nesting use of cavities impacts parasitism outside the breeding season or even for non-breeders during the

558   nesting season. The woodpecker's abundance would also facilitate testing alternative *Philornis*

559   management programs before using them with species of conservation concern.

560

561   **REFERENCES**

562   **Antoniazzi, L.R., Manzoli, D.E., Rohrmann, D., Saravia, M.J., Silvestri, L. & Beldomenico, P.M.**

563   2011. Climate variability affects the impact of parasitic flies on Argentinean forest birds. *J. Zool.*

564   **283**: 126–134.

565   **Arendt, W.J.** 1985a. *Philornis* ectoparasitism of Pearly-eyed Thrashers. II. Effects on adults and

566   reproduction. *Auk* **102**: 281–292.

567   **Arendt, W.J.** 1985b. *Philornis* ectoparasitism of Pearly-eyed Thrashers. I. Impact on growth and

568   development of nestlings. *Auk* **102**: 270–280.

569   **Bates, D., Mächler, M., Bolker, B. & Walker, S.** 2015. Fitting linear mixed-effects models using lme4.

570   *J. Stat. Softw.* **67**: 1–48.

571   **Bennington, C.C. & Thayne, W. V**. 1994. Use and misuse of mixed model analysis of variance in

572   ecological studies. *Ecology* **75**: 717–722.

573   **Brown, C.R. & Brown, M.B.** 1986. Ectoparasitism as a cost of coloniality in Cliff Swallows (*Hirundo*

574   *pyrrhonota*). *Ecology* **67**: 1206–1218.

575   **Causton, C.E., Moon, R.D., Cimadom, A., Boulton, R.A., Cedeño, D., Lincango, M.P., Tebbich, S.**

576   **& Ulloa, A.** 2019. Population dynamics of an invasive bird parasite, *Philornis downsi* (Diptera:

577   Muscidae), in the Galapagos Islands. *PLoS One* **14**: e0224125.

578   **Climate-data.org**. 2021. *Climate Jarabacoa (Dominican Republic)*. Available at: https://en.climate-

579   data.org/north-america/dominican-republic/la-vega/jarabacoa-766532/ (Accessed on 10 December

580   2021).

581   **Common, L.K., Dudaniec, R.Y., Colombelli-Négrel, D. & Kleindorfer, S.** 2019. Taxonomic shifts in

582   *Philornis* larval behaviour and rapid changes in *Philornis downsi* Dodge & Aitken (Diptera:

583   Muscidae): an invasive avian parasite on the Galápagos Islands. In Sarwar, M. (ed): *Life Cycle and*

584   *Development of Diptera*: 1–22. London: IntechOpen.

585   **Cotgreave, P. & Clayton, D.H.** 1994. Comparative analysis of time spent grooming by birds in relation

586   to parasite load. *Behaviour* **131**: 171–187.

587   **Cuervo, P.F., Percara, A., Monje, L., Beldomenico, P.M. & Quiroga, M.A.** 2021. Environmental

588   variables determining the distribution of an avian parasite: the case of the *Philornis torquans*

589   complex in South America. *Med. Vet. Entomol.* **35**: 284–292.

590   **Delanoy, C.A. & Cruz, A.** 1991. *Philornis* parasitism and nestling survival of the Puerto Rican Sharp-

591   Shinned Hawk. In Loye, J.E. & Zuk, M. (eds) *Bird-parasite Interactions*: 93–103. Oxford: Oxford

592   University Press.

593   **Dougherty, L.R.** 2020. Designing mate choice experiments. *Biol. Rev.* **95**: 759–781.

594   **Dudaniec, R.Y. & Kleindorfer, S.** 2006. Effects of the parasitic flies of the genus *Philornis* (Diptera:

595   Muscidae) on birds. *Emu* **106**: 13–20.

596   **Fessl, B., Sinclair, B.J. & Kleindorfer, S.** 2006. The life-cycle of *Philornis downsi* (Diptera: Muscidae)

597   parasitizing Darwin's finches and its impacts on nestling survival. *Parasitology* **133**: 739–747..

598   **Garrod, H.M. & LaPergola, J.B.** 2018. Overlap in molt and breeding phenology in the Hispaniolan

599   Woodpecker (*Melanerpes striatus*). *Ornitol. Neotrop.* **68(Special Is)**: 29–36.

600   **Hayes, C.D., Hayes, T.I., McClure, C.J.W., Quiroga, M., Thorstrom, R.K. & Anderson, D.L.** 2019.

601   Native parasitic nest fly impacts reproductive success of an island-endemic host. *Anim. Conserv.* **22**:

602   157–164.

603   **Huber, S.K., Owen, J.P., Koop, J.A.H., King, M.O., Grant, P.R., Grant, B.R. & Clayton, D.H.** 2010.

604   Ecoimmunity in Darwin's finches: Invasive parasites trigger acquired immunity in the medium

605   ground finch (*Geospiza fortis*). *PLoS One* **5**.

606   **Huebner, D.P. & Hurteau, S.R.** 2007. An economical wireless cavity-nest viewer. *J. F. Ornithol.* **78**:

607   87–92.

608   **Kelly, C.D., Stoehr, A.M., Nunn, C., Smyth, K.N. & Prokop, Z.M.** 2018. Sexual dimorphism in

609   immunity across animals: a meta☐analysis. *Ecol. Lett.* **21**: 1885–1894.

610   **Kleindorfer, S. & Dudaniec, R.Y.** 2009. Love thy neighbour? Social nesting pattern, host mass and nest

611   size affect ectoparasite intensity in Darwin's tree finches. *Behav. Ecol. Sociobiol.* **63**: 731–739.

612   **Kleindorfer, S. & Dudaniec, R.Y.** 2016. Host-parasite ecology, behavior and genetics: a review of the

613   introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. *BMC Zool.* **1**: 1.

614   **Koop, J.A.H., Owen, J.P., Knutie, S.A., Aguilar, M.A. & Clayton, D.H.** 2013. Experimental

615   demonstration of a parasite-induced immune response in wild birds: Darwin's finches and

616   introduced nest flies. *Ecol. Evol.* **3**: 2514–2523.

617   **Lahuatte, P.F., Lincango, M.P., Heimpel, G.E. & Causton, C.E.** 2016. Rearing larvae of the avian nest

618   parasite, *Philornis downsi* (Diptera: Muscidae), on chicken blood-based diets. *J. Insect Sci.* **16**: 1–7.

619   **LaPergola, J.B.** 2018. *Behavioral ecology of coloniality in the Hispaniolan Woodpecker*. Dissertation,

620   Cornell University, Ithaca, New York, U.S.A.

621   **Latta, S., Rimmer, C., Allan, K., Wiley, J., Raffaele, H., McFarland, K. & Fernandez, E.** 2006. *Birds*

622   *of the Dominican Republic and Haiti*. Princeton: Princeton University Press.

623   **Leuba, C., Tebbich, S., Nemeth, E., Anchundia, D., Heyer, E., Mosquera, D.A., Richner, H., Rojas**

624   **Allieri, M.L., Sevilla, C. & Fessl, B.** 2020. Effect of an introduced parasite in natural and

625   anthropogenic habitats on the breeding success of the endemic little vermilion flycatcher

626   *Pyrocephalus nanus* in the Galápagos. *J. Avian Biol.* **51**: 1–13.

627   **Linn, C., Feder, J.L., Nojima, S., Dambroski, H.R., Berlocher, S.H. & Roelofs, W.** 2003. Fruit odor

628   discrimination and sympatric host race formation in *Rhagoletis*. *Proc. Natl. Acad. Sci. U. S. A.* **100**:

629   11490–11493.

630   **Little, S.E.** 2009. Myiasis in Wild Birds. In Atkinson, C.T., Thomas, N.J. & Hunter, D.B. (eds) *Parasitic*

631   *Diseases of Wild Birds*: 546–556. Oxford: Wiley-Blackwell.

632   **Macquart, J.** 1853. Notice sur une nouvelle espéce d'aricie, diptère de la tribu des anthomyzides. *Ann. la*

633   *Société Entomol. Fr.* **Series 3,**: 567–661.

634 **Manzoli, D.E., Antoniazzi, L.R., Saravia, M.J., Silvestri, L., Rorhmann, D. & Beldomenico, P.M.**

635 2013. Multi-level determinants of parasitic fly infection in forest passerines. *PLoS One* **8**: e67104.

636 **Mitchell, B. & Bruggers, R.L.** 1985. Aspects of woodpecker damage to cacao in the Dominican

637 Republic. *Trop. Pest Manag.* **31**: 148–152.

638 **Mooring, M.S. & Hart, B.L.** 1992. Animal grouping for protection from parasites: selfish herd and

639 encounter-dilution effects. *Behaviour* **123**: 173–193.

640 **Nilsson, S.G.** 1986. Evolution of hole-nesting in birds: on balancing selection pressures. *Auk* **103**: 432–

641 435.

642 **Kelly, C.D., Stoehr, A.M., Nunn, C., Smyth, K.N. & Prokop, Z.M. 2018.** Sexual dimorphism in

643 immunity across animals: a meta□analysis. *Ecol. Lett.* **21**: 1885–1894.

644 **O'Connor, J.A., Robertson, J. & Kleindorfer, S.** 2010. Video analysis of host-parasite interactions in

645 nests of Darwins finches. *Oryx* **44**: 588–594.

646 **Oniki, Y.** 1983. Notes on fly (Musicdae) parasitism of nestlings of South American birds. *Le Gerfaut* **73**:

647 281–286.

648 **Oswald, K.N., Smit, B., Lee, A.T.K. & Cunningham, S.J.** 2019. Behaviour of an alpine range-restricted

649 species is described by interactions between microsite use and temperature. *Anim. Behav.* **157**: 177–

650 187.

651 **Patitucci, L.D., Quiroga, M., Couri, M.S. & Saravia-Pietropaolo, M.J.** 2017. Oviposition in the bird

652 parasitic fly *Philornis torquans* (Nielsen, 1913) (Diptera: Muscidae) and eggs' adaptations to dry

653 environments. *Zool. Anz.* **267**: 15–20.

654 **Pike, C.L., Ramirez, I.E., Anchundia, D.J., Fessl, B., Heimpel, G.E. & Causton, C.E.** 2021. Behavior

655 of the avian parasite *Philornis downsi* (Diptera: Muscidae) in and near host nests in the Galapagos

656 Islands. *J. Insect Behav.* **34**: 296–311.

657 **Quiroga, M.A., Hayes, T.I., Hayes, C.D., Garrod, H., Soares, L., Knutie, S.A., Latta, S.C. &**

658 **Anderson, D.L.** 2020. More than just nestlings: incidence of subcutaneous *Philornis* (Diptera:

659 Muscidae) nest flies in adult birds. *Parasitol. Res.* **119**: 2337–2342.

26

660   **R Core Team**. 2020. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation

661   for Statistical Computing.

662   **Rabuffetti, F.L. & Reboreda, J.C.** 2007. Early infestation by bot flies (*Philornis seguyi*) decreases chick

663   survival and nesting success in chalk-browed mockingbirds (*Mimus saturninus*). *Auk* **124**: 898–906.

664   **Saravia-Pietropaolo, M.J., Arce, S.I., Manzoli, D.E., Quiroga, M. & Beldomenico, P.M.** 2018.

665   Aspects of the life cycle of the avian parasite *Philornis torquans* (Diptera: Muscidae) under

666   laboratory rearing conditions. *Can. Entomol.* **150**: 317–325.

667   **Selander, R.K.** 1966. Sexual dimorphism and differential niche utilization in birds. *Condor* **68**: 113–151.

668   **Short, L.L.** 1974. Habits of three endemic West Indian woodpeckers (Aves, Picidae). *Am. Museum Novit.*

669   **2549**: 1–44.

670   **Stanback, M.T. & Koenig, W.D.** 1994. Techniques for capturing birds inside natural cavities. *J. F.*

671   *Ornithol.* **65**: 70–75.

672   **Teixeira, D.M.** 1999. Myiasis caused by obligatory parasites. Ib. General observations on the biology of

673   the species of the genus Philornis Meinert, 1890 (Diptera, Muscidae). In Guimarães, J.H. &

674   Papavero, N. (eds) *Myiasis in Man and Animals in the Neotropical Region. Bibliographic Database*:

675   71–96. Sao Paulo: Editora Pleiade.

676   **Tinsley, R.C.** 1989. The effects of host sex on transmission success. *Parasitol. Today* **5**: 190–195.

677   **Ursino, C.A., De Mársico, M.C. & Reboreda, J.C.** 2019. Brood parasitic nestlings benefit from unusual

678   host defenses against botfly larvae (*Philornis* spp.). *Behav. Ecol. Sociobiol.* **73**.

679   **Waldstein, A.L.** 2012. An inexpensive camera system for monitoring cavity nests. *J. F. Ornithol.* **83**:

680   302–305.

681   **Winkler, H., Christie, D.A. & Nurney, D.** 1995. *Woodpeckers: an Identification Gguide to the*

682   *Woodpeckers of the World*. Boston: Houghton Mifflin.

683   **Young, B.E.** 1993. Effects of the parasitic botfly *Philornis carinatus* on nestling House Wrens,

684   *Troglodytes aedon*, in Costa Rica. *Oecologia* **93**: 256–262.

685   **Zuk, M. & McKean, K.A.** 1996. Sex differences in parasite infections: patterns and processes. *Int. J.*

686   *Parasitol.* **26**: 1009–1024.

687

688   **SUPPORTING INFORMATION**

689   Additional Supporting information may be found in the online version of this article:

690   **Figure S1.** *Hispaniolan Woodpecker hatch date histogram with earliest and latest adult capture dates.*

691   **Table S1.** *Individual adult Hispaniolan Woodpeckers recapture records.*

692   **Table S2.** *Individual adult Hispaniolan Woodpeckers with active* Philornis *infestations.*

693

694

Table 1. Summary of hypotheses and predictions regarding *Philornis* infestation status.

Hypothesis	Prediction
H1: Nestlings are more vulnerable than adults to *Philornis* parasitism.	P1.1: *Philornis* prevalence is higher for nestlings than for adults. P1.2: Infested nestlings host more *Philornis* larvae than adults.
H2: Nesting is associated with *Philornis* parasitism of adults.	P2.1: *Philornis* prevalence is higher in nesting birds than birds not nesting. P2.2: Nesting birds host more *Philornis* larvae than birds not nesting.
H3a: *Philornis* parasitism is associated with diurnal incubation and brooding investment.	P3.1a: *Philornis* prevalence is equal for nesting males and females, who share daytime incubation and brooding. P3.2a: Infested males and females host similar numbers of *Philornis* larvae.
H3b: *Philornis* parasitism is associated with total incubation and brooding investment.	P3.1b: *Philornis* prevalence is higher for males, the sex that conducts nocturnal incubation and brooding. P3.2b: Infested males host more *Philornis* larvae.

695

Table 2. Results of two generalized linear mixed-effects models testing for an association of *Philornis* parasitism with age in Hispaniolan Woodpeckers. Model (a) included the binary response of *Philornis* parasitism (yes/no) and fixed effects of age (adult vs. nestling), scaled date of capture (DOY scaled), and their interaction, and was fit with a binomial distribution. Model (b) tested for an association of total number of *Philornis* wounds on infested birds only with age, DOY scaled, and their interaction, and was fit with a negative binomial distribution. Random effects for both models were individual identity (a: n = 559 individuals; b: n = 143 individuals), year of capture (n = 6 years in both a and b), and tree ID where captured or bred (a: n = 41 trees; b: n = 25 trees).

Model and factors	Estimate ± S.E.	z-value	*P*
a. *Philornis* parasitism (yes/no)			
Intercept	-3.092 ± 0.971	-3.184	0.00145 **
Age (nestling)	-0.689 ± 0.365	-1.888	0.059
DOY Scaled	0.170 ± 0.205	0.829	0.407
*Age (nestling) * DOY Scaled*	*2.383 ± 0.438*	*5.443*	*5.25e-08 ***
b. Total *Philornis* wounds			
Intercept	0.598 ± 0.192	3.113	0.00185 **
Age (nestling)	*0.935 ± 0.191*	*4.895*	*9.82e-07 ***
DOY scaled	-0.004 ± 0.085	-0.047	0.962

696

697

Table 3. Results of two generalized linear mixed-effects models testing for an association of the binary response of *Philornis* parasitism (yes/no) of adult Hispaniolan Woodpeckers with sex (female or male), nesting status (actively nesting or not nesting at time of capture), the interaction of sex and nesting status, and the scaled day of the year captured (DOY scaled). Random effects were individual identity (a: $n = 163$ individuals; b: $n = 113$ individuals), tree ID where an individual was captured or bred (a: $n = 30$ trees; b: $n = 27$ trees), and year of capture ($n = 6$ years in both a and b). Model (a) included the full set of individuals with known nesting status within a year, including individuals that never bred (non-breeders) within the capture year ($n = 193$ observations). Model (b) included only individuals that were known to have bred within the capture year ($n = 134$ observations).

Model and factors	Estimate ± S.E.	z-value	*P*
a. *Philornis* parasitism (yes/no) on breeders and non-breeders			
Intercept	-3.116 ± 0.874	-3.565	0.0004
Sex (male)	*1.674 ± 0.483*	*3.468*	*0.0005***
Nesting Status (not nesting)	0.312 ± 0.651	0.480	0.632
DOY Scaled	0.091 ± 0.307	0.297	0.767
b. *Philornis* parasitism (yes/no) on breeders only			
Intercept	- 3.391 ± 0.961	-3.529	0.0004
Sex (male)	*1.697 ± 0.592*	*2.869*	*0.0041***
Nesting Status (not nesting)	1.221 ± 0.898	1.360	0.174
DOY Scaled	0.475 ± 0.384	1.238	0.216

31

698

Table 4. Results of a generalized linear mixed-effect model with a Poisson distribution testing for an association between the total number of *Philornis* wounds on adult Hispaniolan Woodpeckers and sex (female or male), nesting status (actively nesting or not nesting at time of capture), the interaction of sex and nesting status, and scaled day of year captured (DOY scaled), including year as a random effect ($n = 4$ years). This analysis used birds of known nesting status ($n = 36$ observations), including birds that bred and those that did not within the year of capture.

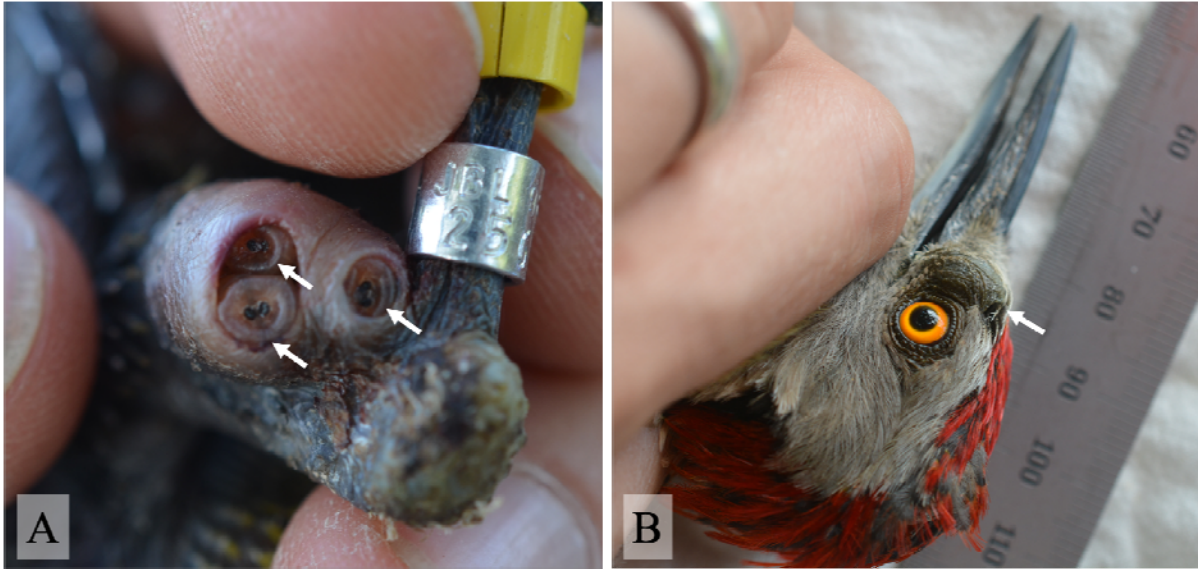| Factors	Estimate ± S.E.	z-value	*P*
Intercept	0.601 ± 0.357	1.684	0.0921 .
Sex (male)	0.045 ± 0.230	0.150	0.8804
Nesting Status (not nesting)	0.100 ± 0.454	0.220	0.8256
DOY Scaled	-0.126 ± 0.232	-0.543	0.5874

699   **Figure legends**

700   **Figure 1.** Example of active and empty wounds associated with *Philornis* parasitism in Hispaniolan

701   Woodpeckers. (A) Active wounds containing three *Philornis* larvae (indicated by white arrows) on a

702   nestling woodpecker's leg, where the posterior spiracles of larvae are visible. Two larvae inhabit one

703   wound while one larva inhabits an adjacent wound. (B) Empty *Philornis* wound on an adult male

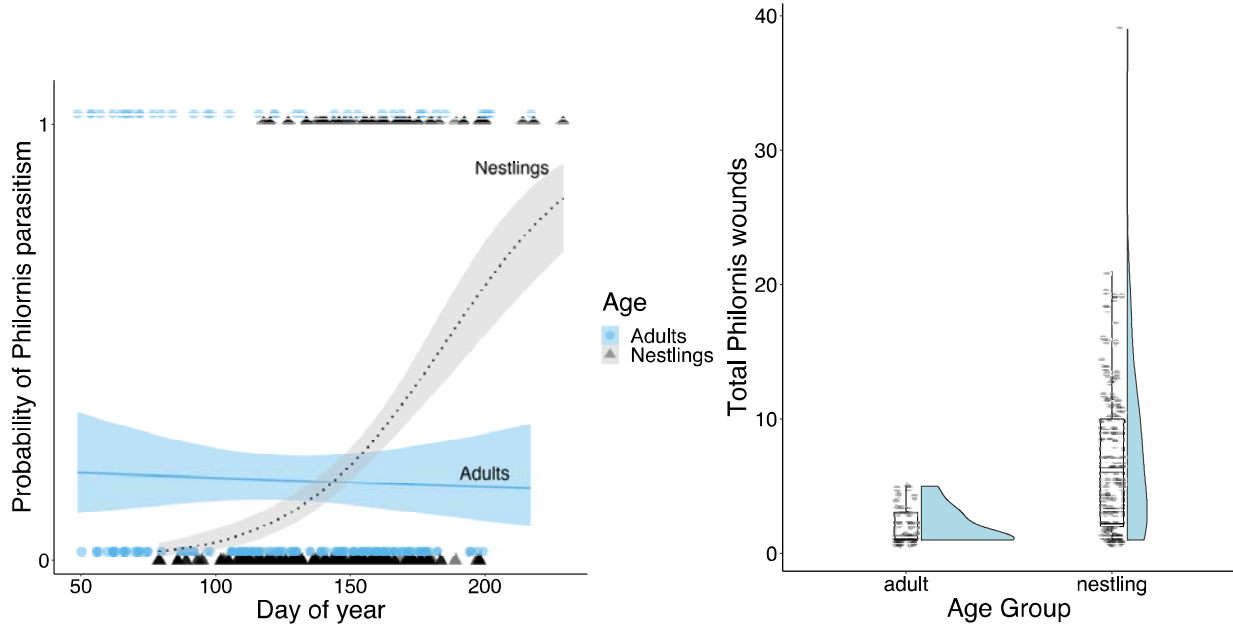704   woodpecker's face. All photos by author.

705

706   **Figure 2.** Probability and intensity of *Philornis* parasitism on adult and nestling Hispaniolan

707   Woodpeckers. (A) Probability of *Philornis* parasitism plotted as raw data (adults = blue circles, $n = 218$

708   observations; nestlings = gray triangles, $n = 554$ observations) and model predictions from a generalized

709   linear mixed model testing for an association with age, day of year captured, and their interaction. The

710   blue solid line and black dashed line represent model predictions for adults and nestlings, respectively.

711   Raw data were artificially vertically separated to improve visibility of points. (B) Raincloud plot

712   comparing adults ($n = 40$ observations) and nestlings ($n = 123$ observations) for the total number of

713   *Philornis* wounds observed on infested individuals (i.e., only non-zero values for the total number of

714   *Philornis* wounds). Sample sizes indicate the number of observations.

715

716   **Figure 3.** Proportion of female and male adult Hispaniolan Woodpeckers with ≥ 1 *Philornis* parasite.

717   Male woodpeckers were significantly more likely to be infested (Table 3). Error bars represent 95%

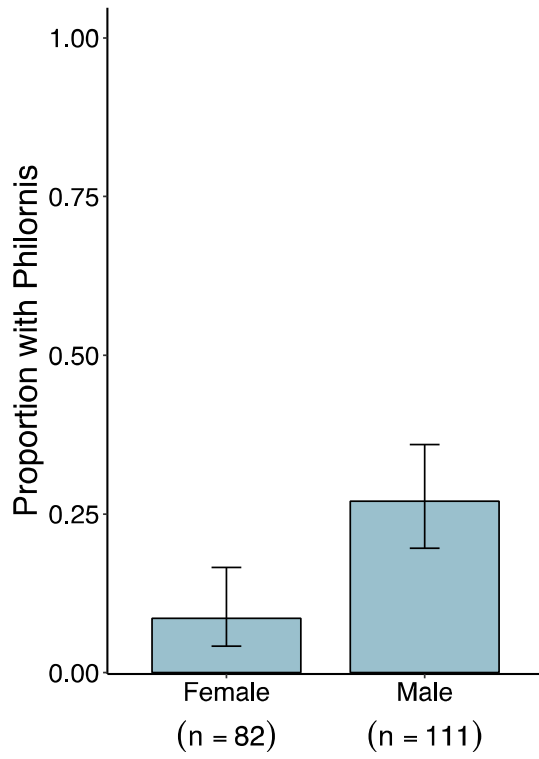718   confidence intervals. Sample sizes indicate the number of observations.

719

720

721

722

723