

Life history

## First report of the *Philornis torquans* complex in adult birds in continental Ecuador: Is parasitism by *Philornis* being underestimated?

### *Primer reporte del complejo Philornis torquans en aves adultas en Ecuador continental: ¿está siendo subestimado el parasitismo por Philornis?*

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#### Abstract

Myiasis by *Philornis* is a usual phenomenon in Neotropical birds. *Philornis* larvae are hematophagous and are known to affect both nestlings and adults. Still, parasitism in adults seems opportunistic and has been poorly studied compared to parasitism in nestlings. Here, we inspected 1,429 adult and juvenile birds of 41 species from an Andean dry forest in northern Ecuador, searching for infestations by *Philornis* larvae. For the first time, we report parasitism by *Philornis* in adult birds of 6 species of passerine birds (*Troglodytes aedon*, *Conirostrum cinereum*, *Geospizopsis plebejus*, *Zonotrichia capensis*, *Pheucticus chrysogaster*, and *Spinus magellanicus*). We also report the first cases of infestation by the *Philornis torquans* complex in Ecuador and provide data on the prevalence of *Philornis* myiasis in this Andean dry forest. We extend both the host range and the geographic distribution of the *P. torquans* complex.

**Keywords:** Andean dry forest; Dipteran parasites; Host; Larvae; Myiasis

#### Resumen

La miasis por *Philornis* es un fenómeno habitual en las aves neotropicales. Las larvas de *Philornis* son hematófagas y se sabe que afectan tanto a los polluelos como a los adultos. Sin embargo, el parasitismo en adultos parece ser oportunista y ha sido poco estudiado en comparación con el parasitismo en polluelos. En este estudio inspeccionamos

1,429 aves adultas y juveniles de 41 especies de un bosque seco andino en el norte de Ecuador, en busca de infestaciones por larvas de *Philornis*. Por primera vez, reportamos parasitismo por *Philornis* en aves adultas de 6 especies de aves paseriformes (*Troglodytes aedon*, *Conirostrum cinereum*, *Geospizopsis plebejus*, *Zonotrichia capensis*, *Pheucticus chrysogaster* y *Spinus magellanicus*). También reportamos los primeros casos de infestación por el complejo *Philornis torquans* en Ecuador y proveemos datos sobre la prevalencia de miasis por *Philornis* en este bosque seco andino. Ampliamos tanto el rango de hospedadores como la distribución geográfica del complejo *P. torquans*.

*Palabras clave:* Bosque seco andino; Parásitos dípteros; Hospedador; Larvas; Miasis

## Introduction

Myiasis, or infestations by dipterous larvae that feed on vertebrate tissues (Otranto, 2001), is a usual phenomenon in birds (Sabrosky et al., 1989; Dudaniec & Kleindorfer, 2006). It is most frequently caused by hematophagous larvae that are obligate feeders on nestling birds and include members of the families Calliphoridae (*Protocalliphora*), Muscidae (*Philornis*, *Passeromyia*, *Mydaea*), and Neottiophilidae (*Neottiophilum*, *Actinoptera*), of which *Philornis* is present in the Neotropics (Little, 2008).

The genus *Philornis* encompasses near 50 species that may be host-generalist or specialists (Löwenberg-Neto, 2008). With 3 types of larval habits (coprophagous, semi hematophagous, and subcutaneous; Dudaniec & Kleindorfer, 2006), infestation by *Philornis* larvae can affect nestlings differently, even leading to death (McNew & Clayton, 2018; Hayes et al., 2019). Detrimental consequences detected in infested nestlings include reduced hemoglobin and hematocrit levels (Dudaniec et al., 2006; Manzoli et al., 2018), lower growth rates and body mass (Fessl et al., 2006; Norris et al., 2010; Segura & Reboreda, 2011; Segura & Palacio, 2021), reduced feather and tarsus length (Koop et al., 2011), and decreased future reproduction (McNew et al., 2020). Whereas nestling birds seem the primary hosts of *Philornis*, parasitism of adult birds seems to be opportunistic and is more likely to occur in adults that spend long periods in the nest (Teixeira, 1999; Dudaniec & Kleindorfer, 2006).

The impact of parasitism by *Philornis* on nestlings is of particular concern in most already threatened bird species (Bulgarella et al., 2019). In the Galapagos islands, the invasive *Philornis downsi* is known to severely affect all 14 Darwin's finch species (Kleindorfer & Dudaniec, 2016). In continental Ecuador, *Philornis falsificus* and *P. obscurus* were found infesting nestlings in Guayas province, while *P. grandis* was recorded in nests from Pastaza and Napo provinces, in the Amazon basin (records compiled in Löwenberg-Neto & De Carvalho, 2013). In 2015, Bulgarella et al. found *P. downsi* larvae, along with pupal cases from other unidentified *Philornis* species in nests from Santa Elena and Guayas provinces. Finally, a

*Philornis* larvae was found parasitizing a nestling of the Choco Screech-Owl (*Megascops centralis*) in Santa Elena province (Reyes & Astudillo-Sánchez, 2017). Thus, while over 145 studies have been carried on *P. downsi* (Quiroga et al., 2020), only 4 studies have addressed the presence of *Philornis* species in continental Ecuador. This situation reveals the notorious lack of information on parasitism by *Philornis* in continental Ecuador, where many bird species are of conservation concern (BirdLife International, 2021).

Our study is set at Bosque Protector Jerusalem (BPJ), the only track of protected Andean dry forest in the inter-Andean valleys of northern Ecuador. Between 2012 and 2014, we monitored the avian community at BPJ and surroundings, to generate a baseline for avian host-parasite dynamics in this poorly studied ecosystem. Here, we captured, inspected, and released adult birds with the next objectives: 1) detect parasitism by *Philornis* spp. in adult birds, 2) provide data on the prevalence of *Philornis* myiasis in adult birds, and 3) increase knowledge on the distribution and host range of the *Philornis* spp.

## Materials and methods

Fieldwork was conducted at Bosque Protector Jerusalem (~10 km north of Quito; 00°00'05" N, 78°21'18" W; 2,300 m asl), a 1,110 ha protected dry forest, in the dry Andean valley of Guayllabamba, northern Ecuador. This area is located on a seasonal ecosystem, where the dry season extends from May to August (average annual rainfall of 125 mm), and the wet season extends from September to April (average annual rainfall of 360 mm) (Carvajal-Campos, 2009). Average annual temperature under shade is 19 °C (data for 2012-2013 provided by Instituto Nacional de Meteorología e Hidrología, INAMHI).

From December 2012 to June 2013, and February to August 2014, we placed 7 mist-nets (4 shelves; 12 m × 2.5 m; 36 mm mesh) for 72 h per month, for a total sampling effort of 1,008 h (or 72 h/net). We carefully inspected the body surface of captured birds for parasitism by *Philornis* spp. When found, subcutaneous larvae were removed from birds' bodies and stored in 70% ethanol. Bird taxonomy used in this study follows that of Remsen et al. (2021).

In October 2020, the collected larvae were molecularly identified by sequencing the second internal transcribed spacer region (ITS2) of the rRNA gene, a barcode for insects (García-Robledo et al., 2013). Genomic DNA was extracted from the whole larvae (1 per host) using a guanidine isothiocyanate protein precipitation, followed by isopropanol DNA precipitation protocol (Peñañiel et al., 2019). We amplified a portion of ITS2 using primers ITS2-LEcEn-F and ITS2-LEcEn-R (Monje et al., 2013). Amplification products were visualized in 2% agarose gel, and unincorporated primers and dNTPs were degraded using ExoSAP-IT PCR Product Cleanup Reagent (Applied Biosystems). Purified amplicons were sequenced with big-dye chemistry and PCR primers, using capillary electrophoresis in an ABI3730xl sequencer. Chromatograms were inspected in Geneious® 11.1.5 (Biomatters Ltd., Kearse et al., 2012). For some samples, we obtained good quality sequence readings for the forward DNA chain but not for the reverse; thus, we re-sequenced the forward chain to minimize the chance of sequencing errors.

To assess the identity of our larvae, we compared the sequences obtained to those available on GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) using BLAST (Basic Local Alignment Search Tool; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>). To corroborate the identity of samples from a phylogenetic perspective, we performed a maximum likelihood (ML) analysis, as follows. We assembled a matrix with our novel sequences and those of *Philornis* (*P. torquans*, *P. seguyi*, *Philornis* sp. “Misiones”, *P. pici*, *P. porteri*, and *P. downsi*) available on GenBank. All sequences from the same species and those obtained by us were aligned separately in Mafft (Katoh et al., 2019), using Auto alignment. Based on these preliminary alignments, we retained only the unique sequences (in terms of both nucleotide sequences and insertions/deletions). Given the presence of several ambiguous indels in ITS2 and the potential of these indels to generate very different tree topologies, we assembled 2 alignment matrices: one that contained the sequences more similar to our novel sequences (plus outgroup) and one that included sequences from all the species. Final alignments were also obtained with Mafft Auto feature. Using PartitionFinder 2 (Lanfear et al., 2016), we estimated the best-fit model of molecular evolution for both data matrices, which was the TIM + G model. For each dataset, we conducted a ML phylogenetic reconstruction as implemented in Garli 2.0 (Zwickl, 2006), using nst = 6 and gamma distribution (4 gamma categories); all other settings were left at default values. We ran 20 search replicates to obtain the ML tree, followed by 1,000 bootstrap replicates (1 search replicate each) to assess the nodal support for each clade.

## Results

We sampled a total of 1,429 birds from 41 species. Among these, 15 individuals (14 adults and 1 immature) belonging to 6 species were infested by subcutaneous *Philornis* larvae. Parasitized species were: *Troglodytes aedon* (Troglodytidae), *Conirostrum cinereum* (Thraupidae), *Geospizopsis plebejus* (Thraupidae), *Zonotrichia capensis* (Passerellidae), *Pheucticus chrysogaster* (Cardinalidae), and *Spinus magellanicus* (Fringillidae) (Table 1; Fig. 1). The prevalence of myiasis by *Philornis* per bird species ranged from 0.9% (*G. plebejus*) to 6.9% (*T. aedon*), and intensity of parasitism ranged from 1 to 12 larvae (Table 1). Birds' abdomen was the area where more larvae were found (Table 1). The complete list of species and individuals examined in this study is provided in Appendix 1. Other individuals for which we found traces (i.e., rounded wounds in the skin) of likely myiasis are summarized in Appendix 2.

We extracted, amplified, and sequenced DNA from larvae found in 14 individuals. However, the DNA in the larvae of 8 individuals was degraded because their preserving ethanol evaporated during at least part of the storage period. Thus, we were able to obtain ITS2 sequences for larvae found on 6 individuals: 1 *T. aedon*, 1 *G. plebejus*, and 4 *Z. capensis* (GenBank accession numbers MW853826- MW853831). From the sequences of these 6 larvae, we obtained 2 unique haplotypes, A and B (different by 1 indel; Table 1) identified by BLAST as belonging to *P. torquans*. Also, phylogenetically, they were placed within the *P. torquans* clade (Fig. 2A, B), which was sister to the only available haplotype of *P. seguyi* in the reduced-dataset tree (Fig. 2A). In that same tree, *Philornis* sp. “Misiones” showed as sister to *P. seguyi* + *P. torquans*. We also found a few mostly unambiguous indels (Fig. 3) that may prove phylogenetically informative in the future, when more extensive haplotype sampling is available. The sequences obtained in this study allowed us to identify the sampled larvae as part of the *P. torquans* complex that includes both *P. torquans* and *P. seguyi* (Quiroga et al., 2016; see Discussion).

## Discussion

We report, for the first time in continental Ecuador, parasitism by *Philornis* in adult birds of 6 species of passerine birds (*T. aedon*, *C. cinereum*, *G. plebejus*, *Z. capensis*, *P. chrysogaster*, and *S. magellanicus*). There are records *Philornis* myiasis in nestlings of *T. aedon* (Young, 1993; Quiroga & Rebores, 2012), *Z. capensis*, and *S. magellanicus* (Salvador & Bodrati, 2013) in other latitudes (Costa Rica, Argentina), but never in adult birds.

Table 1

Birds showing *Philornis* myiasis at Bosque Protector Jerusalem. Specific ID is only provided for larvae identified molecularly.

Species	Field number	Age	Date	Observations	Prevalence	ID (haplotype)
<i>Troglodytes aedon</i>	HFC-745	Adult	27 Apr 2013	1 in chest	6.9% (2/29)	<i>P. torquans</i> complex (A)
	HFC-859	Immature	7 Jun 2013	1 in abdomen		<i>Philornis</i> sp.
<i>Conirostrum cinereum</i>	HFC-121	Adult	16 Dec 2012	1 in abdomen, bird showing incubation patch	3.6% (1/28)	<i>Philornis</i> sp.
<i>Geospizopsis plebejus</i>	HFC-386	Adult	2 Feb 2013	1 in forehead; bird first captured on 2 Dec 2012 with no trace of infestation	0.9% (1/107)	<i>Philornis</i> sp.
	HFC-1076 <sup>1</sup>	Adult	19 Mar 2014	1 in chest, 5 in abdomen		<i>P. torquans</i> complex (A)
<i>Zonotrichia capensis</i>	HFC-188	Adult	27 Dec 2012	1 in abdomen, bird showing incubation patch	2.6% (8/303)	<i>Philornis</i> sp.
	HFC-444	Adult	10 Feb 2013	2 in abdomen; bird first captured on 12 Jan 2013 with no trace of infestation, showing incubation patch		<i>Philornis</i> sp.
	HFC-698	Adult	14 Apr 13	12 in abdomen		<i>P. torquans</i> complex (A)
	HFC-736	Adult	21 Apr 2013	4 in abdomen		<i>P. torquans</i> complex (A)
	HFC-904	Adult	19 Feb 2014	4 in abdomen		<i>P. torquans</i> complex (B)
	HFC-1018	Adult	11 Mar 2014	Near cloaca		<i>Philornis</i> sp.
	No number	Adult	4 May 2014	Larvae above cloaca; traces of former incubation patch		<i>Philornis</i> sp.
<i>Pheucticus chrysogaster</i>	HFC-1392	Adult	6 Jun 2014	in abdomen	5.3% (1/19)	<i>P. torquans</i> complex (A)
	HFC-248	Adult	11 Jan 2013	1 in abdomen		<i>Philornis</i> sp.
<i>Spinus magellanicus</i>	HFC-226	Adult	10 Jan 2013	4 in abdomen	1.2% (1/82)	<i>Philornis</i> sp.

<sup>1</sup> Captured 3.5 km outside Bosque Protector Jerusalem. Not included in the calculation of prevalence.

Although *Philornis* infecting non-nestlings is a rare event, there are at least 15 additional bird species for which adult infestation, by at least 5 species of *Philornis*, have been documented (Huber et al., 2010; Quiroga et al., 2020). The infestation prevalences found herein are close to the ranges summarized in Quiroga et al. (2020), when considering species with 19 or more individuals analyzed (i.e., 0.2-8.75%), except for the high prevalence shown by *Margarops fuscatus* (31%; Arendt, 1985).

Although parasitism by *Philornis* in adults may be more common than previously thought (Quiroga et al., 2020), nestlings seem to be more susceptible than mature birds, as they stay for a longer time in the nest and are less mobile, and then more vulnerable to infestation (Herrera

& Bermúdez, 2012). In our study, at least 4 individuals showed myiasis in the abdomen with current or traces of former incubation patch and in the forehead and near the cloaca, areas that are usually less protected by contour feathers. These surfaces may be more easily reached by *Philornis* larvae that emerge from eggs laid in nest material (Quiroga et al., 2020).

*Philornis torquans* was formerly reported in Argentina and Brazil (Nielsen, 1912; Löwenberg-Neto & De Carvalho, 2013), but now it is considered a complex of cryptic species from southern South America named the *P. torquans* complex (Quiroga et al., 2016). Here, we report the *P. torquans* complex in Ecuador for the first time, broadening its distribution range. This complex has been

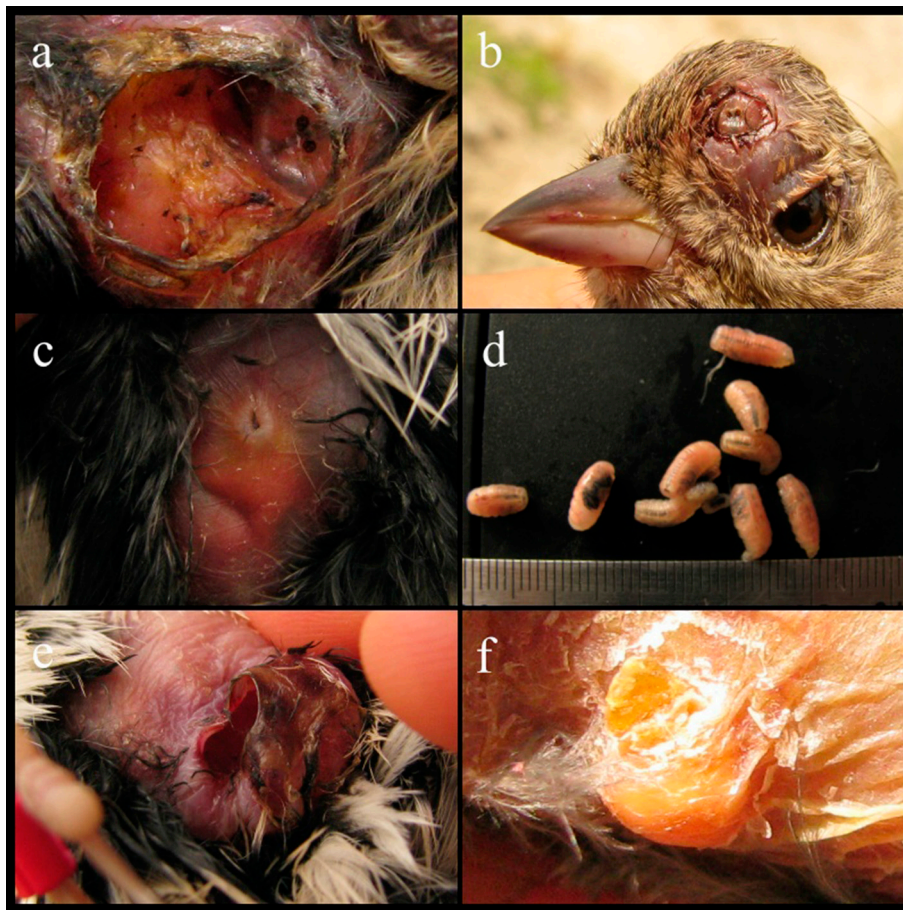


Figure 1. *Philornis torquans* larvae parasitizing: a) abdomen of *Conirostrum cinereum* HFC-121, larval orifice that still harbors a larva; b) *Geospizopsis plebejus* HFC-386; c) abdomen of *Zonotrichia capensis* HFC-188; d) larvae extracted from abdomen of *Z. capensis* HFC-698 and e) orifice after extraction, f) dead larvae exposed on the belly of *Pheucticus chrysogaster* HFC-248.

previously found affecting 34 species of birds, including *T. aedon* and *Z. capensis* (Cuervo et al., 2020). Thus, our findings expand the host range of the *P. torquans* complex by including a new host: *G. plebejus*. Furthermore, 3 other bird species —*C. cinereum*, *P. chrysogaster*, and *S. magellanicus*— also presented myiasis by *Philornis* in the same locality. The presence of the *P. torquans* complex along the Central and Northern Andes was predicted by the abiotic niche model of Cuervo et al. (2020). While this model was developed to predict the distribution of the *P. torquans* complex in southern South America, the results suggested (with some uncertainty) that this complex of cryptic species may be present in our study area. The finding of the *P. torquans* complex in the Andean dry forest of northern Ecuador represents the first independent confirmation of this model. This result is also consistent with those of Percara et al. (in press) who

reported the *P. torquans* complex covering heterogeneous habitats and environmental conditions (average mean temperature 10-28 °C, mean rainfall 100-2,000 mm/year), thus encompassing the environmental conditions present at BPJ.

Few field studies have focused on the prevalence of *Philornis* in both adults and nestlings. To our knowledge, the most extensive research is that of Arendt (1985) on *Margarops fuscatus*, in Puerto Rico. In that study, adult prevalence ranged 0-92%, depending on the month of the year, whereas nestling prevalence ranged 81-100% for the same site and time period. Thus, the presence of myiasis in adults at this Andean dry forest may signal a broader *Philornis* infestation in the nestlings of those species (Texeira, 1999) and, likely, the nestlings of other species. Moreover, in this seasonally dry forest, infected adults may act as reservoirs for the parasites, allowing

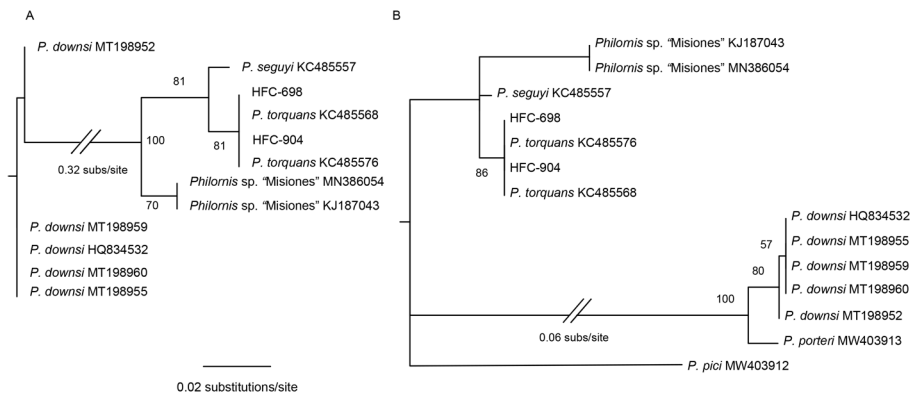


Figure 2. Phylogenetic reconstructions by maximum likelihood showing the position of our novel sequences in the context of the unique haplotypes of other *Philornis* species available on GenBank. A, Shows most closely related sequences, with *P. downsi* as outgroup; B, shows all *Philornis* included in this study.

	A		B		C		D	
	108	121	160	189	253	262	405	427
<i>P. downsi</i> MT198959	TTTA-----ATAT		TATT-----ATATTTA		TTTA--CGAA		CAAACATATATTATTTAAGAA	
<i>P. downsi</i> MT198952	TTTA-----ATAT		TATT-----ATATTTA		TTTA--CGAA		CAAACATATATTATTTAAGAA	
<i>P. downsi</i> MT198955	TTTA-----ATAT		TATT-----ATATTTA		TTTA--CGAA		CAAACATATATTATTTAAGAA	
<i>P. downsi</i> MT198960	TTTA-----ATAT		TATT-----ATATTTA		TTTA--CGAA		CAAACATATATTATTTAAGAA	
<i>P. downsi</i> HQ834532	TTTA-----ATAT		TATT-----ATATTTA		TTTA--CGAA		CAAACATATATTATTTAAGAA	
<i>P. seguyi</i> KC485557	TTTA-----ATAT		TTATTGTATATAT-----ACACACATAAT		TTTT--ACGA		TGAGAAAA-----AAAA	
<i>P. torquans</i> KC485568	TTTA-----ATAT		TTATTGTGTATATATACACACACATAAT		TTTT--ACGA		TGAGAAAA-----AAAA	
<i>P. torquans</i> KC485576	TTTA-----ATAT		TTATTGTGTATATATAC--AACACACATAAT		TTTT--ACGA		TGAGAAAA-----AAAA	
HFC-904	TTTA-----ATAT		TTATTGTGTATATATAC--AACACACATAAT		TTTT--ACGA		TGAG-----	
HFC-698	TTTA-----ATAT		TTATTGTGTATATATACA--AACACACATAAT		TTTT--ACGA		TGAG-----	
<i>P. "Misiones"</i> MN386054	TTTA--ATATATAT		TTATTG-----TAAT		TTTTACCGA		TGAGAAAA-----GAAAA	
<i>P. "Misiones"</i> KJ187043	TTTAATATATATAT		TTATTG-----TAAT		TTTTACCGA		TGAGAAAA-----GAAAA	

Figure 3. Indels found in the alignment of *P. seguyi*, *P. torquans*, *Philornis* sp. “Misiones” and our novel samples; upper numbers indicate sequence position with respect to *P. torquans* ITS2 sequence KC485568. A, 2 different indels in *Philornis* sp. “Misiones”; B, 2 synapomorphic (?) indels in *Philornis* sp. “Misiones” and others in *P. torquans* and *P. seguyi*; C, 2 synapomorphic (?) indels in *Philornis* sp. “Misiones”; D, 2 synapomorphic (?) indels in *Philornis* sp. “Misiones” and others in *P. torquans* and *P. seguyi*; sequences of HFC-698 and 904 ended at position 408.

them to maintain viable parasite populations year-round. Although our sampling of the area was designed to cover the diversity of micro habitats in this dry forest, year-round sampling, including the inspection of nests and adults, is needed to support this hypothesis.

Bird populations living in this Andean dry valley are experiencing continuous landscape transformation (Aguirre et al., 2006), the presence of avian malaria parasites in high prevalence and intensity (Cadena et al., 2019), the likely expansion of the Shiny Cowbird, *Molothrus bonariensis* (Medrano-Vizcaino et al., 2020; J.F. Freile pers. comm.), and probably, the non-documented effects of global climate change. Thus, they may be exposed to the synergistic effects of these factors and *Philornis* infestations, which are likely increasing the pressures on their reproductive effort. For these reasons, for a broader perspective on the ecology of this community and other communities along

the dry Andean valleys, future studies should focus on studying how *Philornis* infestation, habitat degradation, malaria and cowbird parasitism, climate change, and their interactions may be affecting bird populations.

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### Appendix 1. Families, species, and numbers of individuals per species examined during this study.

Columbidae: *Leptotila verreauxi* (5), *Zenaida auriculata* (1), *Columbina passerina* (180). Caprimulgidae: *Systemellura longirostris* (1). Trochilidae: *Colibri coruscans* (74), *Lesbia victoriae* (26), *Patagona gigas* (5), *Myrtis fanny* (64), *Chaetocercus mulsant* (2), *Chlorostilbon melanorhynchus* (34), *Amazilia tzacatl* (23). Picidae: *Colaptes rivolii* (8). Furnariidae: *Synallaxis azarae* (13). Tyrannidae: *Camptostoma obsoletum* (25), *Elaenia albiceps* (14), *Anairetes parulus* (4), *Myiophobus fasciatus* (2), *Pyrocephalus rubinus* (47), *Myiotheretes striaticollis* (1). Hirundinidae: *Pygochelidon cyanoleuca* (26). Troglodytidae: *Troglodytes aedon* (29). Turdidae: *Turdus fuscater* (14). Mimidae: *Mimus gilvus* (1). Fringillidae: *Spinus magellanicus* (82), *Chlorophonia cyanocephala* (16). Passerellidae: *Zonotrichia capensis* (303). Cardinalidae: *Pheucticus chrysogaster* (19). Thraupidae: *Conirostrum cinereum* (28), *Sicalis luteola* (1), *Geospizopsis plebejus* (107), *Rhopospina alaudina* (2), *Catamenia analis* (43), *Diglossa sittoides* (17), *Sporophila nigricollis* (13), *Saltator striatipectus* (55), *Tiaris olivaceus* (1), *Asemospiza obscura* (5), *Pipraeidea melanonota* (1), *Rauenia bonariensis* (25), *Stilpnia vitriolina* (103), *Thraupis episcopus* (9).

### Appendix 2. Individuals that showed traces of likely current or past myiasis (by *Philornis* or other insects).

*Pyrocephalus rubinus*: HFC-604 (29/03/2013) 1 likely larval exit orifice in venter. *Zonotrichia capensis*: HFC-1112 (4/May/2014), cavity in the pectoral area with subcutaneous infestation; HFC-1205 (6/May/2014), rugose and yellowish venter, cutaneous orifice (7 mm) above cloaca; HFC-1347 (23/May/2014), 1 likely larval exit orifice in venter; HFC-1358 (3/Jun/2014), 1 likely larval exit orifice, inflamed area around bill likely made by subcutaneous larvae. *Geospizopsis plebejus*: HFC-911 (25/Feb/2014), 2 likely larval exit orifices above cloaca.

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