*Oecologia Australis*
24(3):748-753, 2020
https://doi.org/10.4257/oeco.2020.2403.19

# FIRST REPORT OF MYIASIS CAUSED BY *Philornis* (DIPTERA: MUSCIDAE) IN *Cacicus solitarius* (PASSERIFORME: ICTERIDAE) IN CENTRAL ARGENTINA.

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**Abstract:** In January 2016, one *Cacicus solitarius* bird nestling was found parasitized by subcutaneous larvae in a protected forest of central Argentina. Some larvae were removed from the nestling and reared under laboratory conditions. Using morphological characteristics, the adult flies that emerged were identified as belonging to the *Philornis torquans* complex. Molecular analysis of the specimens showed identical ITS2 sequences from those previously reported for *Philornis* flies from Misiones province, in northeastern Argentina. This study presents the first documented association between *Philornis* and *C. solitarius* and the first record of the "*Philornis* sp. genotype Misiones" in central Argentina.

**Keywords:** Avian host; ectoparasite; host-parasite association; parasitic fly; *Philornis torquans* complex.

*Philonis* Meinert, 1890 (Diptera: Muscidae) is the only known genus of the Muscidae family that includes species whose larvae parasitize bird nestlings in the Neotropical region (Couri & Carvalho 2003). Most parasitic *Philornis* species have larvae that cause subcutaneous myiasis in bird hosts (Teixeira 1999, Dudaniec & Kleindorfer 2006, Common *et al.* 2019). After penetrating the host integument, the larvae develop between the dermis and the body musculature (Spalding *et al.* 2002), where they feed on tissue debris, blood and serous fluids (Dudaniec & Kleindorfer 2006).

Myiasis caused by subcutaneous larvae of *Philornis* spp. has been associated with negative impacts on nestling fitness such as reduced growth, decreased corporal condition and increased mortality (Arendt 1985a, 1985b, Delannoy & Cruz 1991, Antoniazzi *et al.* 2011, Quiroga *et al.* 2012). This negative impact on the reproductive success of birds has recently stimulated interest in understanding the *Philornis*-host relationship, which has led to reports of new host species for *Philornis* (Bermudez *et al.* 2010, Luz *et al.* 2010a, Luz *et al.* 2010b, Herrera & Bermúdez 2012, Couri *et al.* 2018). To date, more than 200 species belonging to 32 bird families have been documented as hosts of subcutaneous *Philornis* species (Teixeira 1999, Salvador & Bodrati 2013). In Argentina, Salvador and Bodrati (2013) reported *Philornis* infestations in at least 80 species, most of which are Passeriformes with altricial development. Here we report a new association, parasitism by subcutaneous *Philornis* of a nestling of *Cacicus*

*solitarius* Vieillot, 1816 (Passeriforme: Icteridae) in a native forest of Santa Fe Province, Argentina.

As part of a study on the ecology of *Philornis* that has been going on since 2004, we have been monitoring the bird community that breeds at "Méd. Vet. Martin R. de la Peña" natural reserve (31º 23' S; 60º 55' W), Las Colonias department, Santa Fe province (procedures approved by the Ethics and Biosafety Committee of the Facultad de Ciencias Veterinarias - Universidad Nacional del Litoral, protocol number 327/16). This protected area is located on the banks of the Salado River, 7 km away from Esperanza city, and constitutes a small remnant of the original forest of the biogeographic province "El Espinal" (Bertonatti & Corcuera 2000, Morello 2012), most of which has now been highly modified by agriculture and cattle farming. The area consists of a 47 ha patch of forest, dominated by medium-sized tree species such as *Geoffroea decorticans* (Gillies ex Hook. & Arn.) Burkart, *Aspidosperma quebracho blanco* Schlecht, *Vachellia caven* (Molina) Seigler & Ebinger, *Prosopis alba* Griseb and *Gleditsia triacanthos* L., and a low-flood sector, where the predominant vegetation is *Schoenoplectus californicus* (C.A. Mey.) Soják and *Spartina spartinae* (Trin.) Merr. ex Hitchc. (de la Peña 2016).

Between 2007 and 2016, we identified four nests of *C. solitarius* in the study site using a field guides for birds (de la Peña 2005, 2013). Since most of them were built in the upper stratum of the forest, above 4 m of height, only two nests could be examined. One of these was parasitized, a nest that was found on 29 January 2016 at 3.5 m of height (31º 22' 57.36" S; 60º 55' 12.2" W). The nest contained a single featherless nestling that was monitored weekly to assess fledging success. At each visit, the nestling was removed from the nest to record morphometric data and examine it for ectoparasites. On the first visit, three small subcutaneous larvae (< 4 mm) were found on the chest and wings of the nestling. A week later, the nestling harbored 26 larvae of various sizes distributed in different body regions, such as head, neck, chest, legs and wings. Also, several myiasis scars were observed in the skin of the nestling. During this inspection, 11 fully developed larvae were carefully removed from the nestling with fine tweezers and taken to the laboratory. On 12 February 2016, on the third visit, the nestling was dead. Because the carcass was in an advanced state of decomposition it was left where it was found; samples for histopathological studies were not taken.

The larvae collected from the nestling were kept in plastic containers lined with tissue paper until pupation and were examined daily to record adult emergence. Adults flies were identified using taxonomic keys and descriptions provided by Couri (1999) and Couri *et al.* (2009). Because the use of morphological characters to distinguish *Philornis* species may sometimes lead to species misidentification (Monje *et al.* 2013, Quiroga *et al.* 2016), we used the second internal transcribed spacer region (ITS2) of the rRNA gene as a molecular marker to identify adult specimens. Genomic DNA from each specimen was extracted using the AccuPrep® Genomic DNA Extraction Kit (Bioneer, USA) following the manufacturer's protocol. One ITS2 fragment was amplified using the primers described by Monje *et al.* (2013). The PCR product was separated by electrophoresis in a 1.5 % agarose gel stained with GelRedTM (Biotium, USA) and examined by UV transillumination. The PCR product was purified and sequenced directly in forward direction using the amplifying primer. Sequence was aligned with those previously reported for *Philornis* and an outgroup sequence of *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) (GenBank accession number: EU306667), using the ClustalW sequence alignment tool. Phylogenetic analysis was performed with the Maximum-likelihood (ML) method. The best fitting substitution model was determined with the Akaike Information Criterion, using the ML model test. Support for the topology was tested by bootstrapping over 1,000 replications, including gaps. The sequence alignment and the analyses mentioned above were carried out with the program Mega version 7.0 (Tamura *et al.* 2011).

Of the 11 larvae that were collected, seven completed metamorphosis and reached the adult stage, five were males and two were females. Flies were identified using taxonomic keys as belonging to the *P. torquans* complex (Figure 1). The best fitting model for the sequence retrieved was the Tamura 3-parameter model with uniform rates among sites. For a detailed description of the best model and the different nucleotide substitution models generated, see Supplementary Material Table S1. The phylogenetic ML tree inferred showed

that the new sequence was grouped in the same cluster as the ITS2 sequences previously reported as "*Philornis* sp. genotype Misiones" (Quiroga *et al.* 2016) (Figure 2). The divergence between the new sequence and other known *Philornis*' ITS2 sequences, "*Philornis* sp. genotype Misiones" (KJ187043) (Quiroga *et al.* 2016), "*Philornis* sp. genotype Magdalena" (KC585557) (Monje *et al.* 2013), "*Philornis* sp. genotype Central Argentina" (KC485565) (Monje *et al.* 2013) and "*P. downsi*" (KP730051) (Silvestri *et al.* 2011), was 1.49 %, 6.81 %, 9.31 % and 23.84 %, respectively. The new sequence was deposited in GenBank (GenBank accession numbers MN386054).

The present contribution represents the first record of myiasis caused by *Philornis* in *C. solitarius* and the first report of the *Philornis-Cacicus* association for Santa Fe Province. There are previous records of *Philornis* parasitism in two other *Cacicus* species, but these records were from northeastern Argentina, in the Provinces of Misiones (*C. haemorrhous*, Fraga 2011, Quiroga *et al.* 2016) and Chaco (*C. chrysopterus*, Salvador & Bodrati 2013). Although these two *Cacicus* species occur in Santa Fe province, their distributions are very restricted (Fandiño & Giraudo 2010). It is noteworthy that *C. solitarius* is the only species of the genus found in the area of the reserve "Méd. Vet. Martin R. de la Peña", and its occurrence there is sporadic (de la Peña 2016)

According to Monje *et al.* (2013), specimens identified morphologically as *P. seguyi* and *P. torquans* in localities from central Argentina are genetically indistinguishable, corresponding to the genotype "*Philornis* sp. genotype Central Argentina". As a consequence, the name "*P. torquans* complex" has been suggested for the subcutaneous *Philornis* resembling *P. seguyi* or *P. torquans* (Quiroga *et al.* 2016). The flies identified here correspond to the *P. torquans* complex but they belong to a different lineage than specimens previously reported for central Argentina (Monje *et al.* 2013, Quiroga *et al.* 2016) and were determined to belong to the "*Philornis* sp. genotype Misiones". This finding increases to two the number of documented genotypes found in the central portion of the bio-region El Espinal. This suggests that the genetic structure of the genus *Philornis* and its distribution in Argentina is more complex than thought. "*Philornis* sp.

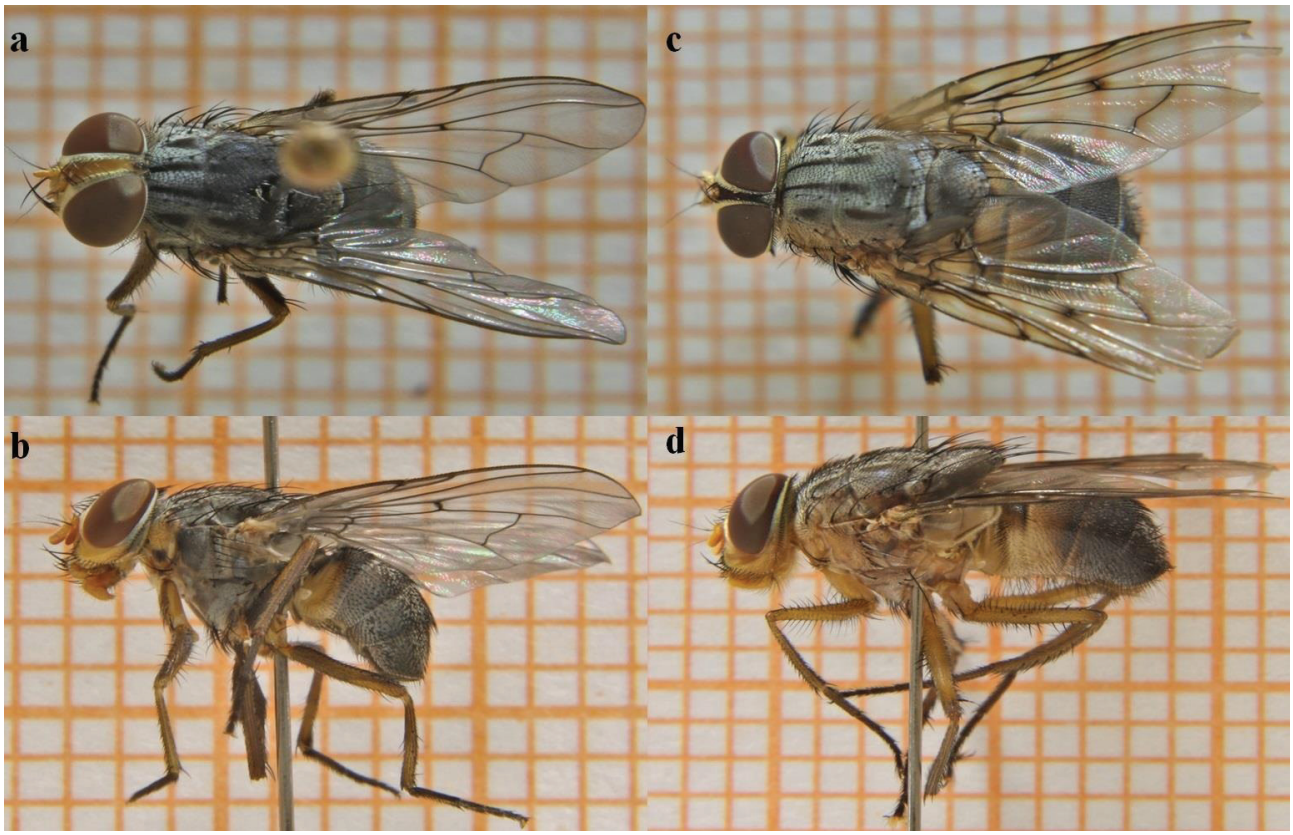


**Figure 1.** *Philornis torquans* complex adult female, dorsal (a) and lateral (b) view, and adult male, dorsal (c) and lateral (d) view.

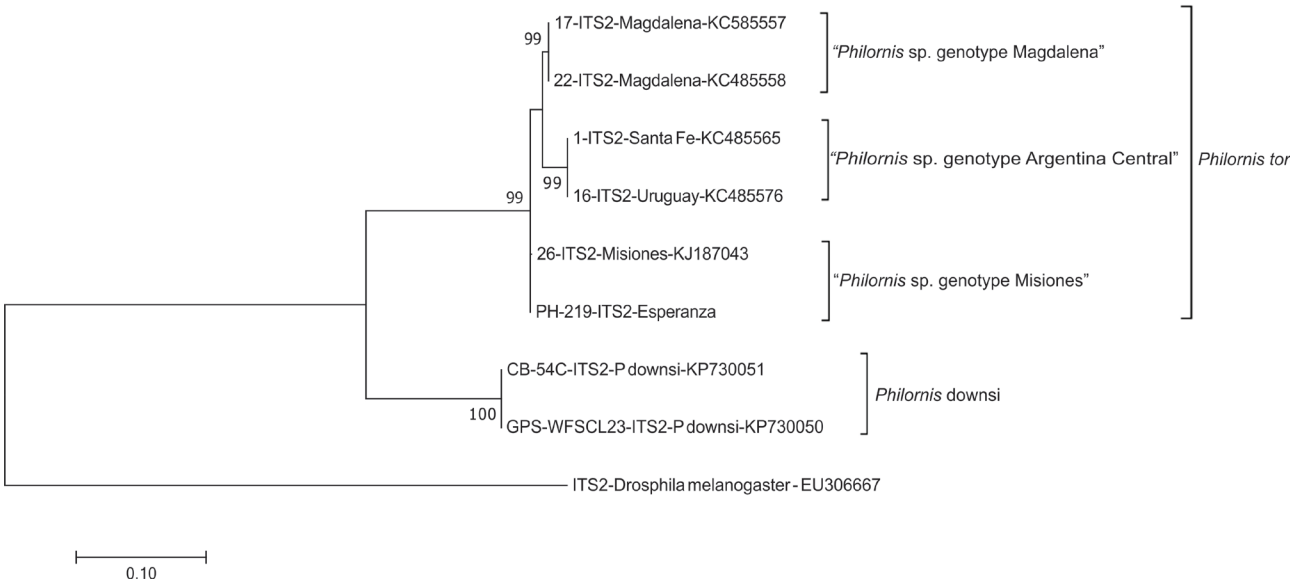


**Figure 2.** Phylogenetic analysis of ITS2 sequences from specimens of *Philornis* species present in Argentina. The tree is drawn to scale, with branch lengths indicating the number of substitutions per site. The analysis involved 9 nucleotide sequences (see Supplementary Table S2). There was a total of 444 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.* 2016). The ITS2 sequence for *D. melanogaster* (EU306667) was used as an outgroup.

genotype Misiones" occurrence is probably determined by the availability of suitable hosts, as this genotype has so far only been found on *Cacicus* spp. (Quiroga *et al.* 2016).

This new host record along with other new records published since the last list of *Philornis*-host associations was compiled (Aramburu *et al.* 2013, Domínguez *et al.* 2014, Pretelli *et al.* 2017, Sovrano *et al.* 2018) brings the number of bird species parasitized by *Philornis* species in Argentina up to 85. Further studies are needed to determine the impact of *Philornis* on *C. solitarius* populations in the region.

## ACKNOWLEDGMENTS

The authors are grateful to Ivana Monsalvo, Lucia Reynoso and Carina Sosa for field assistance. This work was funded by Consejo Nacional de Investigaciones Científicas y Técnicas (grant PIP11220100100261). All procedures conducted in this study comply with current national and provincial laws, and were approved by the Bioethics Committee of Universidad Nacional del Litoral.

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*Submitted: 12 September 2019*
*Accepted: 20 March 2020*
*Published on line: 01 April 2020*
*Associate Editor: Rosana Gentile*