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Philornis downsi (Diptera: Muscidae), an Avian Nest Parasite Invasive to the Galápagos Islands, in Mainland Ecuador

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ABSTRACT *Philornis downsi* (Dodge and Aitken) is a bird-parasitic muscid fly native to mainland South America that recently invaded the Galápagos Islands where it is parasitizing Darwin's finches and other land birds. This parasite was previously known only from Argentina, Brazil, and Trinidad and Tobago. The first report of *P. downsi* from mainland Ecuador is provided, supporting the hypothesis that the invasion route of *P. downsi* from its native range to the Galápagos Islands includes mainland Ecuador. Four different morphologies of pupal exuviae, which belong to different fly species, were uncovered. Dipteran puparia were found in 20% of naturally occurring nests collected in 2013 and in 27% of nests in 2014 at the two sites in western Ecuador. *P. downsi* accounted for 74% and 40% of the puparia in 2013 and 2014, respectively. Two new bird host species for the genus *Philornis* were recorded: the streak-headed woodcreeper (*Lepidocolaptes souleyetii*) and the fasciated wren (*Campylorhynchus fasciatus*). The levels of nest parasitism found were much lower than levels reported for the Galápagos Islands. Finally, parasitism of *P. downsi* by a parasitoid in the genus *Brachymeria* (Hymenoptera, Chalcididae) is documented. Overall, 9% of fly puparia collected in 2013, and 19% of those collected in 2014, had parasitoid emergence holes.

KEY WORDS bird nest box, *Brachymeria*, Ecuador, Galápagos Islands, parasitoid

Introduced parasite species can drive changes in community structure and lead to loss of biodiversity (Mack et al. 2000, McGeoch et al. 2010), and this is particularly true on islands (Groombridge 1992, Wikelski et al. 2004). One such invasive parasite is *Philornis downsi* (Dodge and Aitken), a bird-parasitic muscid fly that is native to the Caribbean and mainland South America and was accidentally introduced to the Galápagos Islands. *P. downsi* females lay eggs inside active bird nests, and the resulting larvae feed on nestlings (Fessl et al. 2006a). Adult *Philornis* flies are nonparasitic and feed on decaying organic matter, fruit, or flowers (Teixeira 1999). And while the larvae of most *Philornis* species are subcutaneous feeders, *P. downsi* larvae are free-living, with semihematophagous feeding behavior. The first-instar larvae feed inside the nares of the nestlings, while the second and third instars feed externally by rasping the skin and ingesting the serous fluid and

blood (Teixeira 1999, O'Connor et al. 2010a). This species is very abundant in the Galápagos Islands, and mortality of infected nestlings is high (Fessl et al. 2006b, Huber 2008, Koop et al. 2011, Knutie et al. 2014, Kleindorfer et al. 2014), leading to concerns about population declines and potential extinction of some species of Darwin's finches (Fessl et al. 2010, O'Connor et al. 2010b, Dvorak et al. 2012, Cimadom et al. 2014).

P. downsi is known to attack a number of bird species in its native range, with records from Trinidad and Tobago (Dodge and Aitken 1968), Brazil (Couri 1984, 1999; Mendonça and Couri 1999), and Argentina (Silvestri et al. 2011). The presence of *P. downsi* in the Galápagos can be traced to 1964 from museum specimens (Causton et al. 2006), but beginning in 1997, it was found parasitizing several land bird species. It is now known to attack 17 species, including ten species of Darwin's finches and four other endemic species (Fessl and Tebbich 2002, Dudaniec and Kleindorfer 2006, Causton et al. 2013, Knutie et al. 2014). The geographic origin of the Galápagos population of *P. downsi* is unknown, although it seems likely that the invasion route would include the western Ecuadorian port city of Guayaquil, which is the source of most commerce and tourism in the Galápagos Islands (Bataille et al. 2009, Bigne et al. 2012). Here, we provide the first report of *P. downsi* in mainland Ecuador and present information on its ecology, including parasitism rates and records of two new host species. We also report on the

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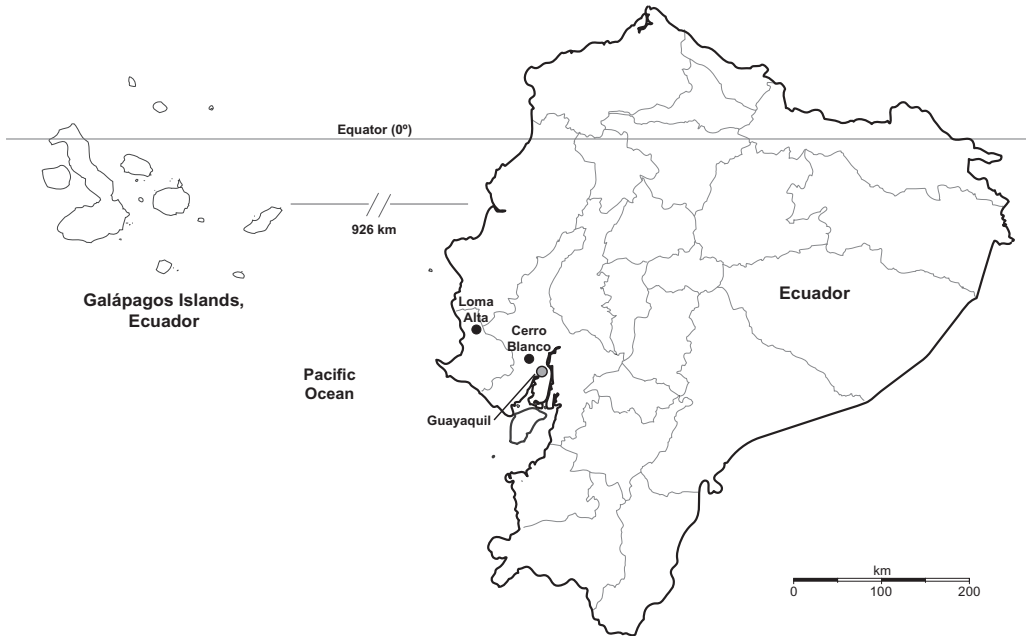


Fig. 1. Map showing the two field collection sites in Ecuador. Site 1: Bosque Protector Cerro Blanco, a tropical dry forest. Site 2: Reserva Ecológica Loma Alta, transitional dry and premontane cloud forest. The gray circle marks the position of Guayaquil.

presence of parasitoids emerging from *P. downsi* pupae. Our study supports the hypothesis that the Galápagos population of *P. downsi* originated in Ecuador, and suggests a potential biological control strategy to protect endemic land birds from this invasive parasite.

Materials and Methods

Wild Nests. We collected a total of 167 naturally occurring, previously used bird nests at two sites in western mainland Ecuador during 2013 and 2014. The first field site was the Bosque Protector Cerro Blanco, a tropical dry forest remnant located just outside of Guayaquil on the southeast edge of the Chongón-Colonche mountains ($2^{\circ} 11'13''$ S, $80^{\circ} 01'03''$ W; Fig. 1). The second site was the Reserva Ecológica Loma Alta ($1^{\circ} 54'00''$ S, $80^{\circ} 39'28''$ W; Fig. 1), part of a transitional dry and premontane cloud forest that lies between the Chocó and Tumbes ecoregions in the Chongón-Colonche mountains (Amador and Martínez Rivera 2011). The two sites are ~ 75 km apart. In both years, collections were made during the dry season after active nesting was completed for the year. In August 2013, we examined 24 nests at Cerro Blanco and 81 nests at Loma Alta while in September 2014, we surveyed 49 nests at Cerro Blanco and 13 nests at Loma Alta. None of the nests contained eggs or nestlings, and we did not identify the bird species associated with most nests. However, some nests had characteristic shapes that made identification possible, such as those made by the fasciated wren (*Campylorhynchus fasciatus*) and the yellow-rumped cacique (*Cacicus cela*).

When a wild nest was found, we disassembled it in the field, searched for fly pupal cases (puparia), and counted the total number per nest. The puparia were then brought to the laboratory where they were examined and photographed under a stereomicroscope at $8\text{--}35\times$. We compared the posterior ends and the shape of the anal spiracles of the puparia to illustrations in Dodge and Aitken (1968), Skidmore (1985), and Couri (1999). We also looked for the presence of emergence holes on the remains of the puparia. The emergence of an adult *Philornis* fly leaves the entire anterior end of the puparium either missing or broken through. On the other hand, some puparia showed no sign of fly emergence but instead exhibited much smaller holes positioned anywhere on the puparium (see Results). These were considered emergence holes of parasitoid wasps (Petersen and Meyer 1985), and they were noted as well. If puparia did not have any emergence hole, we opened them and searched for the presence of either dead flies or parasitoids. Adult parasitoids were in the family Chalcididae and identified to genus following Bouček (1992). We used these collections to determine the overall incidence of dipteran nest parasites in all nests found, the proportion of dipteran nest parasites that were identified as *P. downsi*, as well as the proportion of fly puparia with parasitoid emergence holes.

Artificial Nest Boxes. We installed 158 nest boxes at Cerro Blanco and 50 nest boxes at Loma Alta in November and December 2013, respectively. Nest boxes were made of 50% pine and 50% mixed soft wood boards 2 cm thick and measured 25.4 by 16.5 by 17.8 cm³ (height, width, depth). Nest boxes were set

Table 1. Incidence of dipteran puparia collected from wild bird nests at Cerro Blanco and Loma Alta, Ecuador, in 2013 and 2014

Site/Year	Number of nests with dipteran puparia	Number of nests with no dipteran puparia ^a	Total number of nests	Proportion of nests with dipteran puparia
2013				
Cerro Blanco	8	16	24	0.33
Loma Alta	13	68	81	0.16
Total	21	84	105	0.20
2014				
Cerro Blanco	16	33	49	0.33
Loma Alta	1	12	13	0.08
Total	17	45	62	0.27

^a Nests that clearly looked unused were excluded from this analysis.

on trees by attaching wire to nails driven into the trees at a height of 1.5–1.7 m and separated by at least 60 m. Each box had an entrance hole of 3.8 cm in diameter and a lateral opening for nest inspection (Quiroga and Reboreda 2012). During the 2014 breeding season (January–May), we checked the boxes weekly (either by eye or by inserting a wireless endoscopic snake camera into the nest) to detect active nests and monitor the breeding biology of the birds. After the bird breeding activity finished (nestlings fledged and the nest box was abandoned), we collected the nesting material and searched for fly pupae. If found, these pupae were collected and held individually in 2-ml microcentrifuge tubes until emergence. Once flies emerged as adults, we determined the *Philornis* species following Couri (1999) using a stereomicroscope at 8–35 \times .

Genetics. We extracted, amplified, and sequenced a 497 bp locus of ITS2 rDNA from larvae and adult flies collected during March 2014 in Cerro Blanco, mainland Ecuador ($n=8$), and in Los Gemelos, Santa Cruz, Galápagos Islands ($n=2$), following the same protocols as described in Monje et al. (2013). Sequences from opposite strands were reconciled and verified for accuracy using Sequencher v.5.2.4 (Gene Codes, Ann Arbor, MI). One previously published ITS2 rDNA sequence was available for *P. downsi* from Argentina (Silvestri et al. 2011). We aligned the sequences by eye using the software SeAl v2.0a11 (Rambaut 2007). Sequences are archived in GenBank (accession numbers KP730049–KP730058). We also downloaded all available ITS2 rDNA sequences for the genus *Philornis* archived in GenBank and aligned them to our sequences using the Geneious v8.0.2 alignment algorithm (Biomatters, New Zealand) with the default parameter settings. We then conducted a maximum likelihood analysis as implemented in the PhyML (Guindon and Gascuel 2003) plug-in for Geneious. Statistical support for clades was evaluated by 2,000 replicates nonparametric bootstrapping (Felsenstein 1985).

Results

Wild Nests. We found evidence of dipteran puparia in 20% of the nests collected in 2013 and in 27% of the nests from the 2014 breeding season (Table 1). The puparia collected from nests at Cerro Blanco and

Loma Alta showed morphological differences, suggesting that there were at least four different species associated with birds (Fig. 2). One of these pupal case morphotypes matched the descriptions and illustrations of Skidmore (1985) and Couri (1999) for *P. downsi* (Fig. 2A), and this conclusion was corroborated by examination of adult morphology and DNA (see below, Fig. 3). Of the other three puparial morphotypes, one resembled *Philornis trinitensis* (Dodge and Aitken) (Skidmore 1985; Fig. 2B) and the other two are members of the family Sarcophagidae (Dr. B.J. Sinclair, personal communication; Fig. 2C, D). As we did not obtain adult specimens from these puparial morphotypes (see below), the latter three identifications are tentative. However, we used the puparial characters to estimate the proportion of pupae that were *P. downsi* (Table 2). At Cerro Blanco, *P. downsi* accounted for 97% of the fly puparia found in 2013 ($n=134$) and 35% ($n=155$) in 2014 (Table 2). At Loma Alta, *P. downsi* accounted for 40% of the pupae found in 2013 ($n=88$) and 100% ($n=13$) of the pupae collected in 2014 (Table 2).

We found a parasitoid wasp inside a *P. downsi* puparium collected at Loma Alta in 2013. This wasp was keyed to the genus *Brachymeria* (Hymenoptera: Chalcididae). The overall incidence of parasitism on dipteran puparia, as measured using presumed parasitoid emergence holes coupled with absence of fly emergence holes, was 9% in 2013 and 19% in 2014 (Table 2; Fig. 4). Of the 20 puparia with parasitoid emergence holes from 2013, 17 were *P. downsi* puparia and three were of the morphotype shown in Fig. 2B (possibly *P. trinitensis*). Of the 32 puparia with emergence holes from 2014, nine were *P. downsi*, one was of the morphotype shown in Fig. 2B, and 22 were the sarcophagid morphotypes shown in either Fig. 2C or 2D.

Artificial Nest Boxes. Four of the nest boxes at Cerro Blanco and none of the nest boxes at Loma Alta became occupied and produced eggs and nestlings. Of these four nests at Cerro Blanco, two were parasitized by *P. downsi* (nest 1: house wren, *Troglodytes aedon*, eight *P. downsi* pupae, Fig. 5; nest 2: streak-headed woodcreeper, *Lepidocolaptes souleyetii*, 18 *P. downsi* pupae). We observed free-living *Philornis* larvae in both of the parasitized nest boxes. None of the *Philornis* reared from these nests showed signs of parasitism

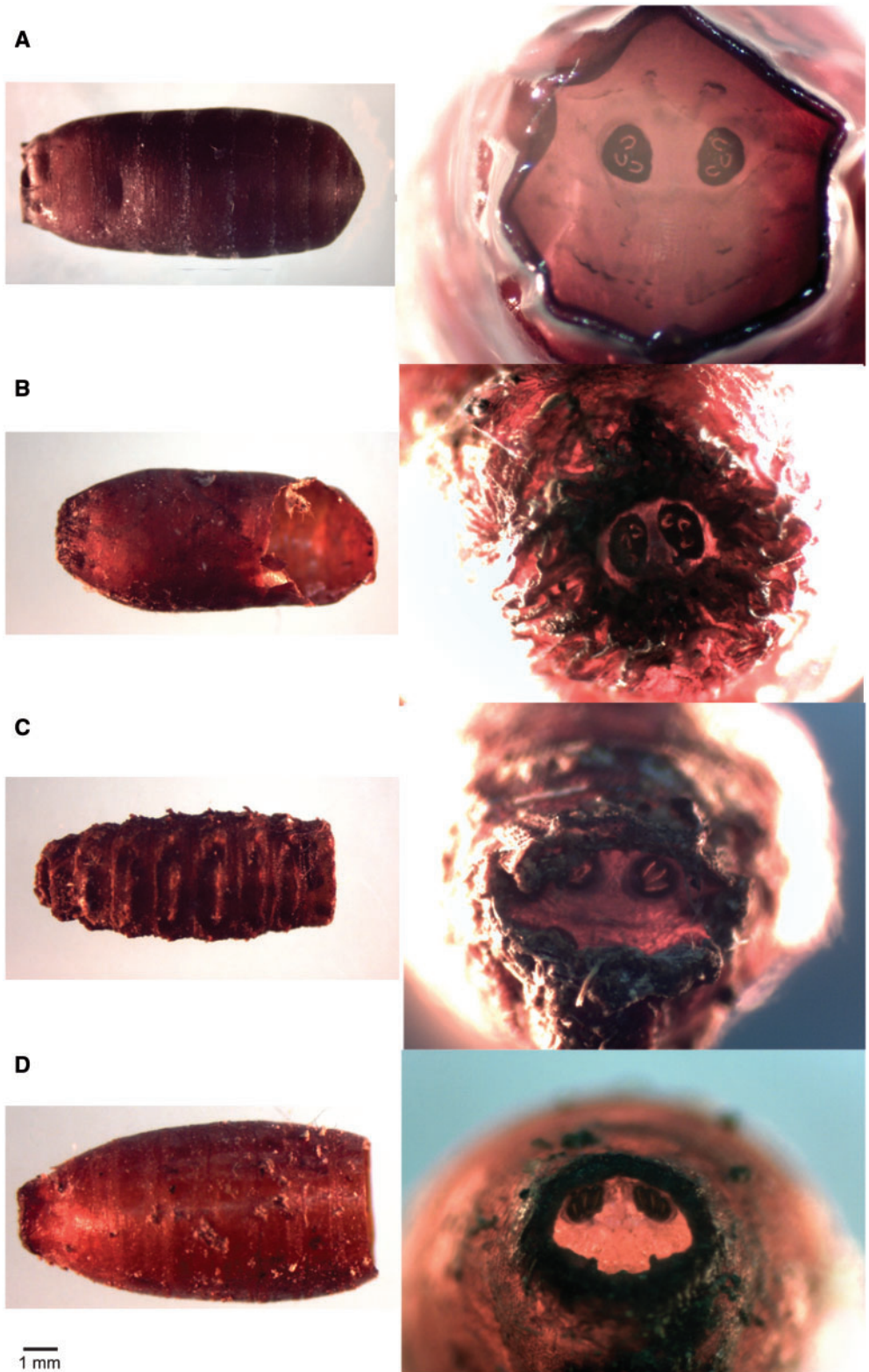


Fig. 2. Photographs of the four morphologically distinct dipteran puparia found in wild bird nests on mainland Ecuador. The left column shows the lateral view of each puparium and the right column, the posterior end of the pupa with the anal spiracles. (A) *Philornis downsi*, (B) Similar to *Philornis trinitensis*, (C, D) Unidentified Sarcophagidae.

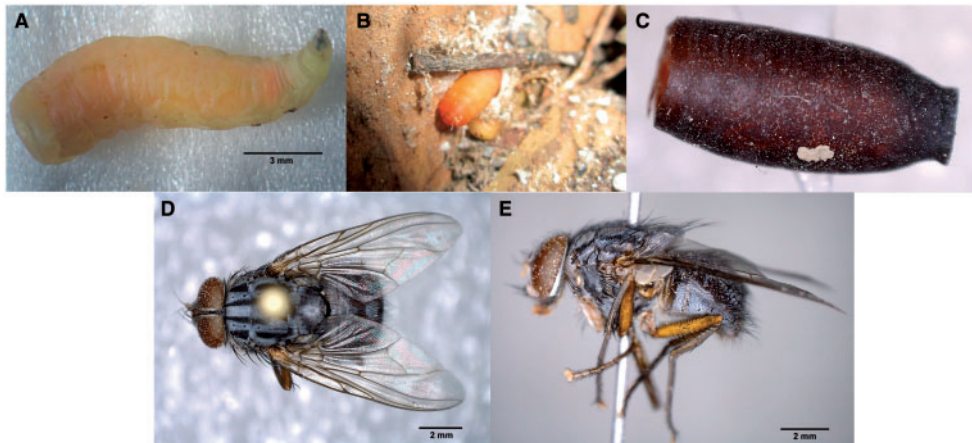


Fig. 3. Photographs of *Philornis downsi* found in a nest box at Bosque Protector Cerro Blanco during the 2014 breeding season. (A) Free-living larva removed from the nest box, (B) Two pupae in the nest box, (C) Emerged puparial case from larva in Fig. 3A, (D) Adult fly (male) emerged from pupa in Fig. 3C, and (E) Lateral view of the same adult specimen.

Table 2. Number of dipteran puparia per nest (with standard error of the mean, SEM), the proportion of pupae identified as *P. downsi*, and two measures of parasitism as determined by presumed parasitoid emergence holes

Site	Mean dipteran pupae per nest	SEM	Proportion of <i>P. downsi</i> pupae	Proportion of infested nests that had at least one puparium with parasitoid emergence holes	Proportion of puparia with parasitoid emergence holes
2013					
Cerro Blanco	16.75	7.63	0.97	0.50	0.10
Loma Alta	6.77	2.21	0.40	0.31	0.08
Total	10.57	3.28	0.74	0.38	0.09
2014					
Cerro Blanco	9.69	2.05	0.35	0.37	0.20
Loma Alta	13.00	–	1.00	0.00	0.00
Total	9.9	1.94	0.40	0.35	0.19



Fig. 4. Parasitoid emergence hole in a *Philornis downsi* puparium collected in mainland Ecuador.



Fig. 5. Nest box from Cerro Blanco showing house wren (*Troglodytes aedon*) nestlings in nest and a *Philornis downsi* pupa (lower right).

by Hymenoptera. In nest 1, five eggs were laid, and three nestlings hatched and fledged. In nest 2, three eggs were laid, two of which hatched and fledged. Regarding the other two nonparasitized nests, a *L. souleyetii* pair built nest 3, in which three eggs were laid, and one nestling fledged. Another *T. aedon* pair built nest 4, in which five eggs were laid and five nestlings fledged.

New Host Records for *Philornis*. We recorded two new host species for the genus *Philornis*: the fasciated wren (*C. fasciatus*) and the streak-headed wood-creeper (*L. souleyetii*). Fasciated wren nests with

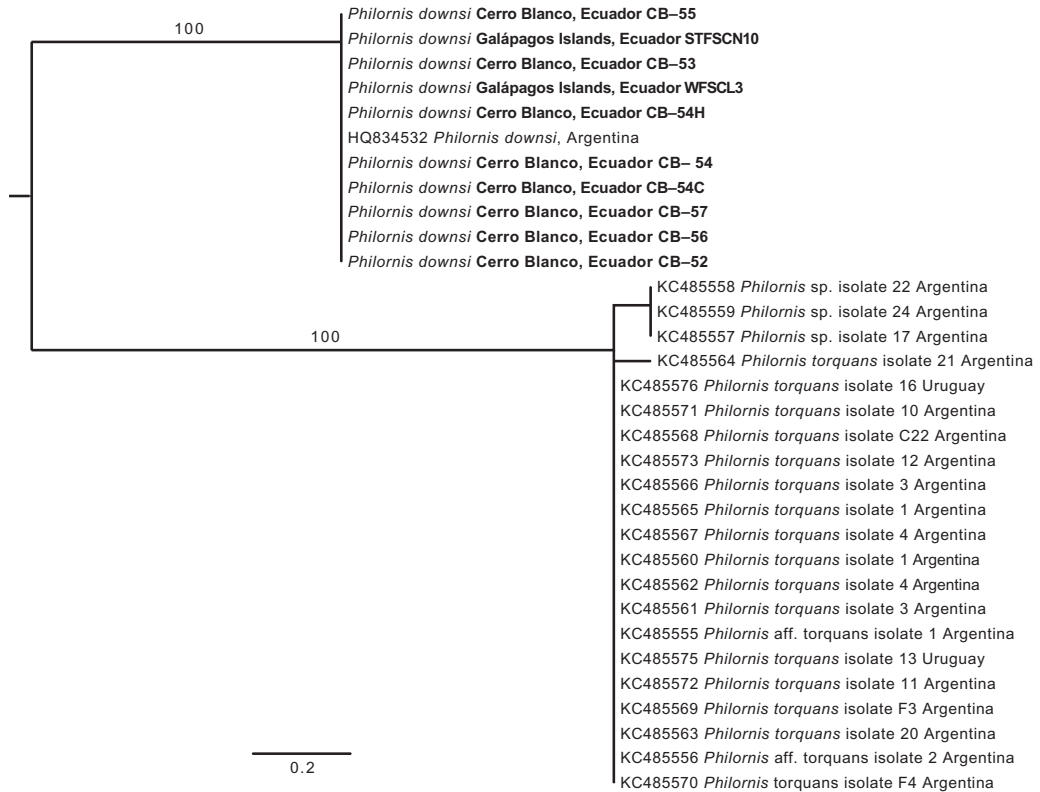


Fig. 6. Most likely phylogenetic tree for the ITS2 locus that includes all sequences available in GenBank for the genus *Philornis* showing that our samples (in bold) grouped with the sequence for *Philornis downsi* from Argentina with 100% support. The columns of the alignment with insertions and deletions are discarded when PHyML builds the tree. The number before the sequence name is the GenBank accession number. Support values above branches correspond to nonparametric bootstrapping.

P. downsi pupal cases were found in natural nests at Cerro Blanco ($n = 1$) and Loma Alta in 2013 ($n = 1$). The streak-headed woodcreeper was parasitized in a nest box set at Cerro Blanco by *P. downsi* in 2014 as noted above. In addition, *P. downsi* puparia were recovered from characteristic fallen nests of the yellow-rumped cacique (*Cacicus cela*) in one nest each at both Cerro Blanco and Loma Alta in 2013, and from a nest of the saffron finch (*Sicalis flaveola*) in Loma Alta in 2014 (M. Torres, personal communication). The remainders of the nests containing *P. downsi* puparia were built by unidentified bird species.

Genetic Comparison. DNA sequences from eight *P. downsi* collected in mainland Ecuador were very similar to the two *P. downsi* individuals from the Galápagos Islands (99.4% identical sites). At position 398, the two Galápagos individuals and two of the Cerro Blanco individuals have an A insertion, one individual from Cerro Blanco has an R (A or G) at position 416, and one individual from Cerro Blanco has an A insertion at position 424. The Argentinian *P. downsi* sequence available from GenBank had an A insertion at position 458, and the sequence was 99.2% similar to *P. downsi* from Ecuador. The maximum likelihood tree generated using all sequences for the genus *Philornis*

available, showed that all of our samples grouped with the *P. downsi* sequence from Argentina with 100% bootstrap support, whereas all other *Philornis* species grouped together with 100% support (Fig. 6).

Discussion

Our data indicate that *P. downsi* occurs naturally on mainland Ecuador and is relatively abundant in the vicinity of Guayaquil. One of our field sites, Cerro Blanco, is located ~15 km from the Guayaquil airport and 20 km from the Guayaquil harbor and the other, Loma Alta, is ~75 km from Cerro Blanco. These collections therefore support the hypothesis that *P. downsi* could have been introduced to the Galápagos from mainland Ecuador. The mode of introduction could have been as adult flies on cargo ships or airplanes as documented for other insect introductions from mainland Ecuador (Causton et al. 2006, Bataille et al. 2009). The importation of poultry and pigeons constitutes the mode of introduction to the Galápagos Islands for the majority of infectious avian pathogens and parasites (Gottdenker et al. 2005, Parker et al. 2006). We deem this mode to be unlikely for *P. downsi* because it does

not attack the domestic chicken and despite its wide host range, there are no records on any Columbiformes (Teixeira 1999). The fly could have also been introduced as larvae or pupae with nest material brought from the mainland.

Levels of *P. downsi* parasitism on nests of host species in the Galápagos Islands are consistently >90% (e.g., Fessl and Tebbich 2002, Fessl et al. 2006b, Koop et al. 2011, Kleindorfer et al. 2014) compared with the 12–14% we found for mainland Ecuador. The values for western Ecuador are lower than the values obtained elsewhere on mainland South America for other *Philornis* species (Young 1993: 19.5%; Nores 1995: 16.4%; Couri et al. 2005: 36.7%; Rabuffetti and Reboreda 2007: 44.8%; Antoniazzi et al. 2010: 38%; Quiroga and Reboreda 2012: 25%). Higher levels of parasitism of birds by *P. downsi* in the Galápagos Islands than in its mainland South American range is consistent with the “enemy release hypothesis,” which holds that introduced species reach high levels of abundance because of the absence of natural enemies that suppress them in their native range (Keane and Crawley 2002, Roy et al. 2011). It is also possible that higher host resistance in the native range and higher abundance and quality of resources in the introduced range can explain the high numbers of *P. downsi* on the Galápagos Islands. While some of Darwin’s finches have developed either behavioral or immunological defenses against *P. downsi*, these are ineffective in increasing the birds’ overall reproductive success (Huber et al. 2010; O’Connor et al. 2010a, Koop et al. 2013).

We found evidence of at least one parasitoid species attacking *P. downsi* in mainland Ecuador, but the significance of this parasitoid in suppressing *P. downsi* in the mainland is unknown. The genus *Brachymeria* has been previously associated with other *Philornis* species (Couri et al. 2006), and *B. podagrica* has also been found parasitizing *P. downsi* at low levels in the Galápagos (Lincango and Causton 2008). Morphological examinations indicate, however, that the species recovered in our study is not *B. podagrica* (Dr. M. Tavares, personal communication; see also Bouček 1992). Further research on the host specificity and life history of this parasitoid is necessary before biological control releases can be contemplated. While biological control entails inherent risks, these can be minimized by using specialized agents; its use in managing invasive species of conservation concern has been growing (van Driessche et al. 2010). Biological control has been applied successfully in the Galápagos Islands to protect endemic plants from an invasive herbivorous insect (Causton 2009, Calderón Alvarez et al. 2012).

Finally, two new host bird species are presented here for the genus *Philornis*: *L. souleyetii*, a furnariid found in Central and Northern South America, and *C. fasciatus*, a common species restricted to western Ecuador and northwest Peru where it principally

inhabits arid and semiarid habitats. When Dodge and Aitken (1968) described *P. downsi*, they listed 16 species of host birds on the Island of Trinidad alone. Data from studies published since increase the number of hosts to 20 species in 13 families within the native range (Mendonça and Couri 1999, Teixeira 1999, Silvestri et al. 2011, this study), plus 17 bird species in five families in the Galápagos Islands, to a total number of 37 host birds. Continued research on the host range of *P. downsi* will provide more insights into the ecology and community structure of this parasite.

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