Annals of the Entomological Society of America, 110(3), 2017, 317–328
doi: 10.1093/aesa/saw102
Advance Access Publication Date: 17 February 2017
Research article

Research

Life Cycle and Host Specificity of the Parasitoid *Conura annulifera* (Hymenoptera: Chalcididae), a Potential Biological Control Agent of *Philornis downsi* (Diptera: Muscidae) in the Galápagos Islands

Mariana Bulgarella,[1,2,3] Martín A. Quiroga,[4] Rebecca A. Boulton,[1] Ismael E. Ramírez,[1] Roger D. Moon,[1] Charlotte E. Causton,[5] and George E. Heimpel[1]

[1]Department of Entomology, University of Minnesota, 1980 Folwell Ave., St. Paul, MN 55108 (lophonetta@gmail.com; rboulton@umn.edu; ramir238@umn.edu; rdmoon@umn.edu; heimp001@umn.edu), [2]Current address: School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, 6140, New Zealand, [3]Corresponding author, e-mail: lophonetta@gmail.com, [4]Laboratorio de Ecología de Enfermedades. Instituto de Ciencias Veterinarias del Litoral (ICiVet Litoral-CONICET-UNL), RP Kreder 2805, Esperanza, Santa Fe, 3080, Argentina (mquiroga06@hotmail.com), and [5]Charles Darwin Foundation for the Galápagos Islands, Puerto Ayora, Santa Cruz Island, Galápagos Islands, Ecuador (causton@rockbug.net)

Subject Editor: Karen Sime

Received 17 October 2016; Editorial decision 20 December 2016

Abstract

The neotropical parasitoid *Conura annulifera* (Walker) (Hymenoptera: Chalcididae) is known to parasitize bird-parasitic flies in the genus *Philornis* (Diptera: Muscidae) including *P. downsi* (Dodge and Aitken), a species that has invaded the Galápagos islands and is negatively impacting populations of Darwin's finches. We report here some aspects of the life history, field ecology, and host specificity of *C. annulifera*. We collected puparia of four *Philornis* species in 13 bird nests during 2015 and 2016 in western mainland Ecuador and found that *C. annulifera* and three other parasitoid species emerged from those puparia. This is the first record of *C. annulifera* in Ecuador. Rearing records and dissections of parasitized puparia revealed that *C. annulifera* is a solitary pupal ectoparasitoid, placing its eggs in the gap between host pupa and puparium. Laboratory studies of host specificity involving *P. downsi* and pupae from five other dipteran, three lepidopteran, and one hymenopteran species found that *C. annulifera* only produced progeny when presented with *P. downsi* pupae. Pupae of *P. downsi* that had been exposed to *C. annulifera* also failed to emerge more often than expected by chance compared with no-parasitoid controls, suggesting that the parasitoids can cause developmental mortality through means other than successful parasitism. These studies constitute the first steps in evaluating *C. annulifera* as a potential biological control agent of *P. downsi* in the Galápagos Islands.

Resumen El parasitoide neotropical *Conura annulifera* (Walker) (Hymenoptera: Chalcididae) parasita moscas parásitas de aves en el género *Philornis* (Diptera: Muscidae), incluyendo a la especie *P. downsi* (Dodge y Aitken) que ha invadido las Islas Galápagos y está afectando negativamente las poblaciones de pinzones de Darwin. Aquí describimos algunos aspectos de la historia de vida, ecología de campo y especificidad de huéspedes de *C. annulifera*. Se colectaron pupas de cuatro especies de *Philornis* en 13 nidos de aves durante 2015 y 2016 en el oeste de Ecuador continental. *Conura annulifera* y otras tres especies de parasitoides emergieron de estos puparios. Este constituye el primer registro de *C. annulifera* en Ecuador. Registros de crianza y disecciones de puparios parasitados mostraron que *C. annulifera* es un ectoparasitoide solitario de pupas, que coloca su huevo/s en el espacio que existe entre la pupa del huésped y el pupario. Estudios de especificidad de huéspedes realizados en laboratorio con pupas de *P. downsi* y de otras cinco especies de dípteros, tres especies de lepidópteros y una especie de himenóptero demostraron que *C. annulifera* solo produjo descendencia cuando se le ofrecieron pupas de *P. downsi*. Asimismo, las pupas de *P. downsi* expuestas a *C. annulifera* emergieron en menor proporción que lo esperado al azar con respecto a controles no expuestos a parasitoides, lo que sugiere que los parasitoides pueden causar mortalidad durante el desarrollo a través de otras formas que el parasitismo exitoso. Estos estudios constituyen

los primeros pasos en la evaluación de *C. annulifera* como un agente de control biológico potencial de *P. downsi* en las Islas Galápagos.

Key words: *Conura annulifera*, biological control, Galápagos Islands, *Philornis downsi*, parasitoid

The Galápagos Islands form an archipelago lying at the equator ~1,000 km west of Ecuador. Bird endemism in Galápagos is famously high, but some of these endemic bird species are threatened by human activities, including the inadvertent introduction of parasites (Parker 2009, 2017; Fessl et al. 2016). In particular, two species of Darwin's finches are critically endangered and close to extinction (Fessl et al. 2010, O'Connor et al. 2010), and a recent study predicts extinction of populations of an abundant species of Darwin's finches within the next 100 years depending on weather conditions (Koop et al. 2016). The main threat to land bird species in Galápagos is currently the invasive nest parasite *Philornis downsi* (Dodge and Aitken) (Diptera: Muscidae) which appears to be causing population declines of numerous bird species on the archipelago (Fessl et al. 2010, O'Connor et al. 2010, Dvorak et al. 2012, Cimadom et al. 2014, Kleindorfer et al. 2014, Kleindorfer and Dudaniec 2016, Koop et al. 2016).

Philornis downsi is an obligate bird-parasitic fly. Adult females lay eggs within bird nests and larvae feed on nestlings. The first-instar larvae typically feed within the nares, and second and third instars feed ectoparasitically on the developing nestlings (Fessl et al. 2006). Feeding by *P. downsi* can cause severe beak deformation and blood loss in host birds and can lead to high mortality in nestlings of various species of Darwin's finches and other passerines (Huber 2008; Galligan and Kleindorfer 2009; O'Connor et al. 2010; Koop et al. 2011, 2013, 2016; Cimadom et al. 2014; Kleindorfer et al. 2014; Knutie et al. 2014; Heimpel et al. 2017). This parasite is native to mainland South America and Trinidad where it is known as a generalist parasite of mainly passerine birds (Bulgarella and Heimpel 2015, Bulgarella et al. 2015). It was first introduced into the Galápagos Islands sometime prior to 1964, presumably through human-aided means such as transport on ships or airplanes from mainland South America (Causton et al. 2006, Kleindorfer and Sulloway 2016, Fessl et al. 2017). Management of *P. downsi* is a high priority for conservation organizations such as the Galápagos National Park Directorate and the Charles Darwin Foundation (Causton et al. 2013).

During investigations in mainland Ecuador, we discovered the parasitoid *Conura annulifera* (Walker) (Hymenoptera: Chalcididae) parasitizing *P. downsi* and *P. niger* (Dodge and Aitken). *Conura annulifera* is a little-known parasitoid that has been recorded from Mexico, Costa Rica, Panama, Trinidad, and Brazil (De Santis 1979, Delvare 1992; synonyms are *Smiera annulifera* (Walker), *Spilochalcis annulifera* (Walker), and *Spilochalcis ornitheia* (Burks)). The few host records in the literature suggest that *C. annulifera* is a specialist on flies in the genus *Philornis* with rearing records from puparia of *P. downsi* and *P. deceptivus* (Dodge and Aitken) from Trinidad (Burks 1960, Delvare 1992) and one unidentified *Philornis* species in Brazil (Couri et al. 2006). Most aspects of the life cycle and field ecology of *C. annulifera* are unknown, including the stage of host attacked, whether it is a gregarious or solitary parasitoid, and whether larvae feed ecto- or endoparasitically. And although the known host records suggest that *C. annulifera* is a *Philornis* specialist, no laboratory studies of host specificity have been conducted.

Here we report on the first laboratory studies of *C. annulifera* that describe its life history and host specificity. We also summarize field observations of its parasitism rates and sex ratio. The life cycle and host specificity of *C. annulifera* is significant in the context of using biological control against species of *Philornis* that are affecting the reproductive success of threatened bird species and is particularly relevant for the control of *P. downsi*. These studies were intended as the first steps in the consideration of this parasitoid species as a potential biological control agent of *P. downsi* in the Galápagos Islands.

Materials and Methods

Parasitism Rates in Mainland Ecuador

We collected puparia of *Philornis* spp. from naturally occurring, previously used wild bird nests in the Reserva Ecológica Loma Alta in western mainland Ecuador (1.85694° S, 80.59938° W) during May and June, 2015, and from March to May, 2016. Adult flies or parasitoids had already emerged from the viable puparia collected from these nests. Pupal characters were used to determine the species of *Philornis*, and the shape, size, and position of emergence holes were used to determine whether a fly or parasitoid had emerged (Bulgarella et al. 2015).

We also installed and monitored 46 wooden nest boxes and 24 bamboo poles with artificial nesting cavities in the lowland area of Loma Alta from January until June, 2015, and March until May, 2016. Details on the design of the wooden nest boxes can be found in Bulgarella et al. (2015). The bamboo poles consisted of 5–6-m-high bamboo canes with 10-cm-diameter circular openings in each of the horizontal sections of the cane to create between 12 and 15 artificial cavities per pole for bird nesting. Canes were strapped to trees or poles and were set at least 50 m apart from each other. Boxes and poles were checked each month to detect active nests and monitor the breeding status of the birds.

After nestlings had fledged, we collected nesting material, searched for *Philornis* puparia, and then reared them individually in 2-ml microcentrifuge tubes. Emerged adult flies were determined to species following Couri (1999), and emerged parasitoids were initially identified to family using standard entomological references. Subsampled parasitoid specimens were sent to taxonomic experts for determination of genus and species. The only parasitoid determined to species by this process was *C. annulifera*. Collection records were summarized to characterize the *Philornis* fauna at Reserva Ecológica Loma Alta, and rates of parasitism by *C. annulifera* and other unidentified parasitoids were calculated from numbers of puparia that yielded parasitoids or adult flies.

Parasitism rates were assessed by a combination of rearing records and emergence holes for *C. annulifera* and rearing records only for the other parasitoid species. *Conura annulifera* reared from unemerged *Philornis* puparia left characteristic emergence holes (see Results), and these emergence holes were attributed to *C. annulifera*. The emergence holes tended to be present on the anterior end of the puparium and so did not interfere with fly identification based on characters on the posterior end.

Insect Cultures

Philornis puparia were transported from Ecuador and reared in a quarantine laboratory at the University of Minnesota, USA. Our

captive *C. annulifera* population originated from 10 *Philornis* puparia—seven from *P. niger* that yielded six female and one male *C. annulifera*, and three from *P. downsi* that yielded two female and one male *C. annulifera* in 2015. These specimens were housed in a plexi-glass parasitoid rearing cage (29.5 by 36.5 by 35 cm) with no-see-um mesh (Quest Outfitters, FL) over two 15-cm-diameter holes, and the cage was provisioned daily with honey and cotton moistened with water. As new parasitoids emerged from our rearing operation and the studies described below, they were released into this cage, which eventually contained between five and eight females and between one and fifty males at any given time. The cage was kept in a growth chamber set for 23.5°C, 85% relative humidity, and a photoperiod of 14:10 (L:D) h provided by full-spectrum light bulbs (Sylvania, 32W, 5000K, 1800 lumens, MA).

We reared *P. downsi* pupae for laboratory studies on nestlings of the society finch, *Lonchura striata domestica* (L.) (Passeriformes: Estrildidae). Society finches breed readily in captivity and we established a colony of 30 finches held in five cages (71 by 26 by 47 cm), each with three plexi-glass nest boxes (10 by 10 by 10 cm) that we designed for nestling monitoring. Nesting material was cotton wool, cotton thread, dried fescue grass and paper towels, and the birds were fed commercial finch food mix (Kaytee Forti Diet Pro Health, WI). Bird cages were kept in a room equipped with full-spectrum light tubes (Vita-Lite 5500K, 91 CRI, NV) on a photoperiod of 12:12 (L:D) h, at a temperature of 22°C and 45% relative humidity. All bird care and husbandry protocols were approved by the Institutional Animal Care and Use Committee of the University of Minnesota (protocol 1501-32202A).

Eggs of *P. downsi* were obtained from wild captured mated female flies that were imported into quarantine from the Galápagos Islands. The flies were maintained in groups of five in mesh fly rearing cages (11 by 9 by 9 cm) that we designed, and placed into a growth chamber at the conditions described above for *C. annulifera*. Flies were fed a diet of blended papaya, egg powder, sugar, hydrolysed protein, and water (Lahuatte et al. 2016). Females deposited eggs regularly within these cages and eggs were transferred onto a moistened 6.5-cm^2 piece of paper towel within a tightly closed petri dish (Falcon Tight-fit lid dish, 9 mm high by 50 mm diameter, Corning, Inc., NY) using a small paintbrush and checked daily until larvae emerged. Recently eclosed *P. downsi* larvae were transferred to individual nestlings, with up to five larvae per nestling. Seven days after larvae were introduced into a nest, we disassembled the nest, retrieved fully developed larvae, and placed them in individual glass fly rearing vials (4.5 cm tall, 2.5 cm diameter) for pupation, which typically occurred the following day under our rearing conditions.

Conura annulifera, like other *Conura* spp., are very long-lived in the laboratory (e.g., Miall et al. (2014) and see Results), and we found that 16-wk-old *C. annulifera* successfully parasitized *P. downsi*. Thus, given the relatively low numbers of *C. annulifera* females available, we used a broad range of ages of females for the experiments described below.

Host Stage Preference, Life History, and Behavior

To determine if *C. annulifera* is a larval–pupal or pupal parasitoid (koinobiont or idiobiont, respectively; Godfray 1994), either larvae or pupae of *P. downsi* were placed onto the floor of a cage containing a group of eight *C. annulifera* females that were between two and 16 wk old along with a variable number of males (see above) in open-topped plastic petri dishes (9 mm high by 50 mm diameter) for 24 h. In all, 20 fully engorged third-instar larvae and 13 pupae that were between 2 and 6 d old were offered to the parasitoids in groups of 1–8 individual fly larvae or pupae, depending upon fly availability. The same group of eight *C. annulifera* females were used for all exposures. In addition, as experimental controls, we set up seven larvae and four pupae in an empty parasitoid rearing cage to measure natural host emergence without *C. annulifera*. After each trial, host larvae or pupae were reared individually in fly rearing vials under standard conditions until emergence.

We also observed searching and host assessment behaviors of male and female *C. annulifera* when presented with *P. downsi* larvae or pupae. Male ($n = 4$) or female ($n = 5$) parasitoids between 2 and 8 wk of age were placed individually into square plastic arenas (10 by 10 by 2 cm) that were divided into four quadrants which the parasitoid could freely move among. One fully engorged third-instar *P. downsi* larva or a 2–7-d-old pupa in a small open-topped petri dish (1 cm tall, 3.5 cm diameter) was placed in a randomly chosen quadrant of the arena. We recorded the location of the parasitoid every 30 s for 30 min and noted whether the parasitoid came into contact with the host and its behavior.

Because *P. downsi* puparia are typically enclosed in a dried frothy cocoon (Fig. 1) that incorporates nesting material (Couri 1999), we conducted an additional experiment to determine if cocoons affected stinging ability of *C. annulifera*. In choice tests, we presented 10 puparia that were covered in nesting material (cotton, thread, paper towel, etc.) and 10 others whose cover had been removed (Fig. 1).

Sex Ratio

We found that the sex ratio of field-collected *C. annulifera* was female-biased while laboratory-reared *C. annulifera* were male-biased (see Results), so we assessed the hypothesis that *C. annulifera* from smaller host individuals are more likely to be males (Godfray 1994). To do this, we measured the length (l) and width (w) of a subsample of seven female and 27 male *P. downsi* puparia from which a *C. annulifera* emerged and compared sizes of puparia yielding male or female parasitoids using a two-sample t-test.

Host Specificity

Conura annulifera females were presented with pupae of *P. downsi* and five other species of Diptera, three species of Lepidoptera, and one species of Hymenoptera in no-choice trials. Diptera were in the families Muscidae (*Musca domestica* L., *M. autumnalis* DeGeer, and *Stomoxys calcitrans* (L.)), Sarcophagidae (*Sarcophaga bullata* (Parker)), and Calliphoridae (*Calliphora vicina* Robineau-Desvoidy). Lepidoptera were in the families Tortricidae (*Epiphyas postvittana* (Walker)), Sphingidae (*Manduca sexta* (L.)), and Pyralidae (*Plodia interpunctella* (Hübner)). The single Hymenopteran species was in the family Braconidae (*Habrobracon hebetor* (Say)). These species were chosen to test a broad phylogenetic range of nontarget species that include taxa that are known to be attacked by other *Conura* species (Arthur 1958, Hansen 1980, Lee and Heimpel 2005, Marcicano et al. 2007, Miall et al. 2014, Weis et al. 2017). Specimens of *S. bullata* and *C. vicina* were purchased from commercial suppliers (Carolina Biological, NC, and NAS Best Bait Inc., OH, respectively), whereas pupae of the remaining candidate hosts came from cultures maintained at the University of Minnesota.

Pupae of *P. downsi* and nontarget species were presented to parasitoids in single-species groups within single open 50-mm-diameter petri dishes on the floor of the parasitoid rearing cages. Between five and eight *C. annulifera* females aged two to 16 wk were used for all trials (the same females were exposed to pupae more than once).

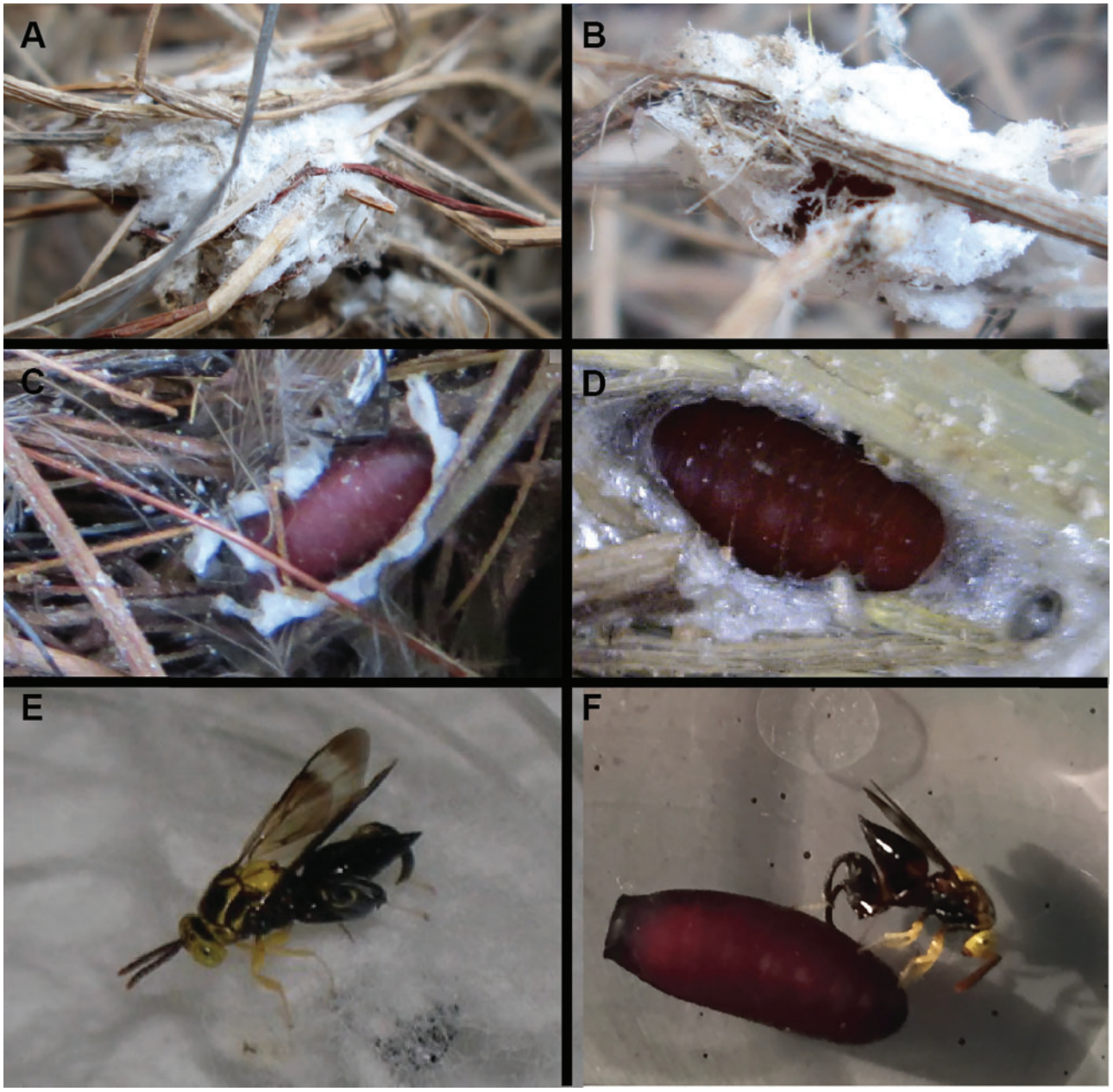

Fig. 1. (**A**, **B**) *Philornis* puparia enclosed in a cocoon with nest debris collected from a wild nest of an unknown bird species in mainland Ecuador. (**C**) *P. downsi* puparium in a wild nest partially covered in cocoon. (**D**) *P. downsi* puparium harvested from a society finch nest in captivity. (**E**) *C. annulifera* parasitizing *P. downsi* puparium covered in nesting material. (**F**) *C. annulifera* parasitizing *P. downsi* puparium cleared of nesting material. Photos: A, B: G. A. Brito Vera; C, D, E: M. Bulgarella; F: R. Boulton.

Trials consisted of 24-h exposures to the test pupae for the first two months of trials, and this was later extended to 48 h for subsequent trials since no parasitoids emerged from the nontarget hosts in the initial 24-h trials (see Results). All pupae were between 2 and 6 d old. The number of trials run per test species varied according to pupal availability. Trials from July 2015 until mid-September 2015 were conducted with the eight field-collected *C. annulifera* females and trials from October 2015 until May 2016 were carried out with the five females that emerged in the laboratory. These same parasitoid individuals were used for both target and nontarget pupae testing. The order in which each of the species was presented to *C. annulifera* was determined by the availability of *P. downsi* puparia and the other hosts and presentations of *P. downsi* were interspersed among trials with the other candidate hosts to ensure that parasitoids were capable of parasitizing hosts. With the exception of *M. sexta*, pupae were also reared in the absence of parasitoids serving as non-exposed controls. After exposure to parasitoids, test pupae were transferred from the test cages to rearing vials and incubated under standard conditions for 5 wk to allow any surviving hosts or progeny of *C. annulifera* to emerge. The same process was used for control pupae. Development time and sex of each *C. annulifera* that emerged were recorded.

Table 1. Numbers of *Philornis* puparia collected from 13 bird nests in Reserva Ecológica Loma Alta in 2015 and 2016, by species of bird and species of *Philornis*

Bird host species	Nest type	*Philornis niger*			*Philornis downsi*			*Philornis falsificus*			*Philornis* sp.		
		Fly	Parasitoid	Other	Fly	Parasitoid	Other	Fly	Parasitoid	Other	Fly	Parasitoid	Other
2015													
Chaetocercus berlepschi	Wild	0	0	0	0	0	0	0	0	0	5	1	0
Polioptila plumbea	Wild	0	0	0	2	2	1	0	0	0	0	0	0
Setophaga pitiayumi	Cane	44	1	3	26	0	0	0	0	0	0	0	0
Troglodytes aedon	Box	31	7	4	1	1	0	0	0	0	0	0	0
Troglodytes aedon	Box	74	18	4	5	0	0	0	0	0	0	0	0
Troglodytes aedon	Cane	2	8	0	0	0	0	0	0	0	0	0	0
Troglodytes aedon	Cane	9	1	0	1	0	0	1	0	0	0	0	0
Unknown Emberizidae	Box	65	9	0	56	2	0	0	0	0	0	0	0
2016													
Troglodytes aedon	Wild	5	0	0	31	0	0	0	0	0	0	0	0
Troglodytes aedon	Box	40	2	0	3	0	0	0	0	0	0	0	0
Troglodytes aedon	Cane	19	11	0	2	0	0	0	0	0	0	0	0
Troglodytes aedon	Cane	26	0	0	4	0	0	0	0	0	0	0	0
Troglodytes aedon	Cane	14	0	0	0	0	0	0	0	0	0	0	0

Data are the numbers of puparia that either yielded adult flies (either before collection or after lab incubation), parasitoids (all species combined; see Table 2 for breakout of parasitoid species), or unidentifiable material ("Other").

Data Analyses

To establish whether *C. annulifera* is a larval or pupal parasitoid, we determined if the proportion of *P. downsi* that yielded parasitoids was influenced by host stage as the main effect in a general linear model (GLM) with quasibinomial errors. From the behavioral observations, we tested whether wasps were more likely to be found in the arena quadrant containing a *P. downsi* larva or a pupa than expected by chance using a χ^2 test. We also tested whether *C. annulifera* spent a greater proportion of time in contact with larvae or pupae of *P. downsi* using host stage as main effect in a GLM with quasibinomial errors.

To evaluate if female *C. annulifera* emerge from different-sized puparia than males do, we estimated puparial volume as a cylinder with volume $= \pi r^2 l$ where r is the radius and l the length of the pupae for all females and a subset of males. Since differences between male and female puparia appeared to be small and measurement error may have been high, we measured puparia three times and assessed the intraclass correlation coefficient (ICC) for the length and diameter of pupae. The ICC provides the percentage of individual variation due to sampling error, and as it exhibited an acceptable ICC (see Results), we proceeded with a two-sample *t*-test of estimated pupal volume.

To determine whether *C. annulifera* displayed host specificity toward *P. downsi*, we assessed whether the proportion of candidate hosts yielding *C. annulifera* differed significantly according to whether the host was the target (*P. downsi*) or nontarget (all nine other species) using a binomial GLM. Even if *C. annulifera* did not successfully parasitize nontarget species, they could have reduced eclosion of nontarget hosts in other ways, for instance by stinging or host feeding on the pupae. To evaluate host mortality, we used a two-way GLM with a binomial error structure and a logit link function. The outcome variable was the proportion of pupae that successfully emerged, which was coded using the 'cbind' command in R v.3.3.1 (R Development Core Team 2016) to account for differences in the numbers of pupae that were used in each trial. The predictor variables were treatment (i.e., exposed to *C. annulifera* versus control), species of candidate host, and interaction. A significant interaction would suggest that exposure to *C. annulifera* did not affect all species consistently. All reported summary statistics are means ± SE in original scale, unless otherwise specified.

Results

Parasitism Rates in Mainland Ecuador

We obtained a total of 384 *Philornis* puparia in eight of 10 active wild and artificially housed bird nests at Reserva Ecológica Loma Alta during 2015. Bird species were House Wren, *Troglodytes aedon* (Vieillot); Tropical Gnatcatcher, *Polioptila plumbea* (Gmelin); Esmeraldas Woodstar, *Chaetocercus berlepschi* (Simon); Tropical Parula, *Setophaga pitiayumi* (Vieillot); and one unknown Emberizidae species (Table 1). One of the nests not containing *Philornis* during 2015 was constructed by a house wren and the other by a streaked flycatcher, *Myiodynastes maculatus* (Statius Muller), which is known as a host of a *Philornis* sp. in Argentina (Salvador and Bodrati 2013). In 2016, 157 *Philornis* puparia were recovered from five out of eight active nests, all of which were occupied by house wrens. Two of the three nests not containing *Philornis* during 2016 were occupied by house wrens and the other by the saffron finch, *Sicalis flaveola* (L.). This latter species is known as a host of *P. downsi* in wild nests in Ecuador (Bulgarella et al. 2015) and in Argentina (Silvestri et al. 2011), but was not recorded as a host of any *Philornis* species in artificial nest boxes in Argentina (Quiroga et al. 2012).

We used adult and pupal characters to determine that four *Philornis* species were present in our samples. Two species were determined from adults by M.B. using Skidmore (1985) and Couri (1999) as *P. downsi* and *P. falsificus* (Dodge and Aitken). These two species could be easily identified from the pupal stage based upon characteristic spiracular slits on the posterior end of the puparium. These identifications were later corroborated by Dr. B. J. Sinclair from adults that emerged from incubated puparia. The third *Philornis* species was determined to be *P. niger* (Dodge and Aitken) from emerged adults by M.A.Q. using the key in Couri (1999). Specimens of a fourth species were only available as empty puparia. They did not match available descriptions in Skidmore (1985) and Couri (1999) but were identified as *Philornis* based upon the

Table 2. Proportions of *P. niger* and *P. downsi* puparia parasitized by four parasitoid species in Reserva Ecológica Loma Alta in 2015 and 2016

	Philornis niger		*Philornis downsi*	
	2015 ($n = 280$)	2016 ($n = 117$)	2015 ($n = 97$)	2016 ($n = 40$)
Conura annulifera	0.114	0.043	0.031	0.000
Trichopria sp.	0.036	0.017	0.021	0.000
Spalangia sp.	0.000	0.034	0.010	0.000
Exoristobia sp.	0.004	0.000	0.000	0.000
Total	0.154	0.0940	0.062	0.000

spiracular slits (Dr. B. J. Sinclair, personal communication). The most abundant species was *P. niger* with an average of 36.1 ± 8.6 puparia per nest over both years ($n = 11$ nests with *P. niger*), followed by *P. downsi* (13.7 ± 6.0; $n = 10$ nests with *P. downsi*), *Philornis* sp. (six puparia in one nest) and *P. falsificus* (one puparium in one nest). Of the 13 nests with *Philornis* spp. over the two years, four contained a single species of *Philornis*, eight contained two *Philornis* species, and one contained three *Philornis* species (Table 1).

Three of the four *Philornis* species at Loma Alta were collectively parasitized by four different parasitoid species (Table 1). One of these was identified as *Conura annulifera* by Dr. M. T. Tavares, and this represents the first record of this species for Ecuador. The second was an unidentified species in the genus *Trichopria* (Hymenoptera: Diapriidae), the third was an unidentified species in the genus *Spalangia* (Hymenoptera: Pteromalidae), and the fourth was an unidentified species in the genus *Exoristobia* (Hymenoptera: Encyrtidae). All cases of parasitism by *C. annulifera* and *Spalangia* sp. yielded a single parasitoid adult suggesting solitary development (see below) while both the *Trichopria* and the *Exoristobia* species exhibited gregarious development. No puparia yielded more than one parasitoid species.

Ten individual *C. annulifera* were reared from field-collected puparia in 2015 comprising eight females and two males, and five in 2016 comprising four females and one male. In addition, 22 puparia exhibited emergence holes resembling those made by *C. annulifera* over the two years. While a single specimen of a different chalcidid, *Brachymeria* sp. was reared from *Philornis* at Loma Alta 2013 and this species produces a similar emergence hole (Bulgarella et al. 2015), all chalcidids reared from *Philornis* spp. over both years of our study were *C. annulifera*. Parasitism rates for all four parasitoid species on *P. downsi* and *P. niger* in 2015 and 2016 are shown in Table 2. *Conura annulifera* exhibited the highest parasitism rate and this was higher on the more abundant host, *P. niger*.

Stage Preference, Life History, and Behavior

Our stage-preference study indicated that *C. annulifera* is an idiobiont pupal parasitoid since it both attacks the pupal stage and emerges from it. None of the 20 *P. downsi* larvae offered to *C. annulifera* yielded parasitoids, and only one of these larvae failed to develop to adulthood. In contrast, 67% of 13 pupae yielded parasitoids, 11% yielded flies, and 22% yielded nothing. The apparent association between parasitism and host stage was significantly different from random expectations (quasibinomial GLM $F_{1,12} = 23.63$, $P < 0.001$).

Behavioral observations showed that *C. annulifera* females were more frequently found in the quadrant of the experimental arena containing *P. downsi* pupae than would be expected by chance

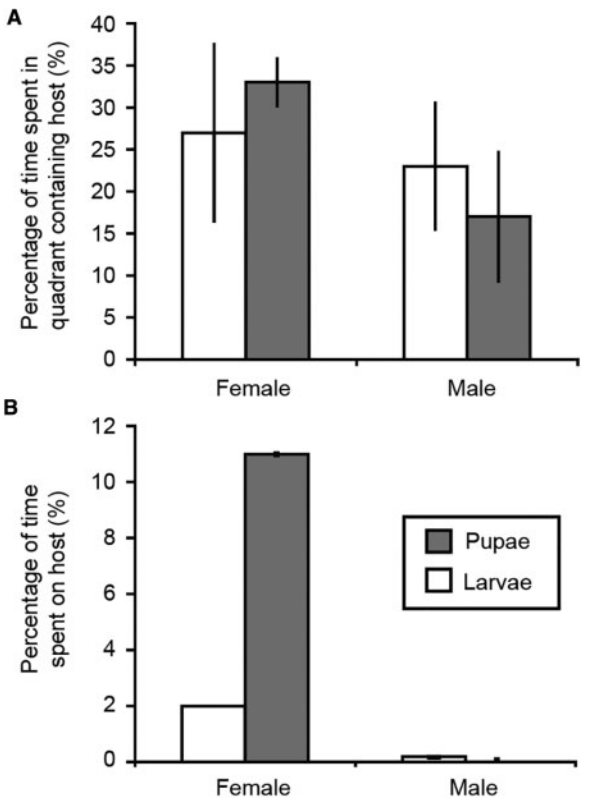

Fig. 2. (**A**) Percentage of observation times that male and female *C. annulifera* spent in arena quadrant containing a *P. downsi* pupa or larva. (**B**) Percentage of time that male and female *C. annulifera* were in contact with larvae or pupae of *P. downsi*. Error bars, SEM.

($\chi^2 = 12.03$, df = 1, $P = 0.0005$), but not in the quadrant containing *P. downsi* larvae ($\chi^2 = 0.81$, df = 1, $P = 0.37$; Fig. 2A). Female *C. annulifera* also spent significantly more time in contact with *P. downsi* pupae (195 ± 116 s) than with larvae (42 ± 18 s; quasibinomial GLM $F_{1,28} = 6.07$, $P = 0.02$; Fig. 2B). Locations of male *C. annulifera* were independent of the presence of *P. downsi* larvae ($\chi^2 = 0.53$, df = 1, $P = 0.47$), but they occurred less frequently than expected where pupae were present ($\chi^2 = 6.02$, df = 1, $P = 0.01$; Fig. 2A). And in contrast with females, males rarely interacted with *P. downsi* larvae (mean = 8 s) and never interacted with pupae (effect of sex GLM: $F_{1,28} = 10.1$, $P = 0.003$).

Encounters by female *C. annulifera* with *P. downsi* puparia involved host stinging and oviposition. Females typically walked on puparia, moved their antennae and then pierced hosts with their ovipositors and presumably deposited eggs (see Fig. 1F), although we were unable to confirm oviposition directly through observation as can be done with some other parasitoids (Ode and Strand 1995, Ode and Rosenheim 1998). Females punctured host puparia repeatedly within bouts of oviposition (i.e., without leaving the host), and between bouts after leaving and returning to puparia over successive days, suggesting that superparasitism may occur. Individual stinging bouts lasted an average of 6.1 ± 0.56 min (range 4–10 min, $n = 12$). Female *C. annulifera* displayed no host-feeding behavior (*sensu* Jervis and Kidd 1986).

The presence of cocoons and nesting material did not affect the ability of *C. annulifera* to parasitize host puparia (78% with cocoons, 61% without, $F_{1,19} = 0.46$, $P = 0.51$).

We dissected one 9–10-d-old *P. downsi* puparium that had been stung by *C. annulifera* when it was 8–9 d old and discovered a single

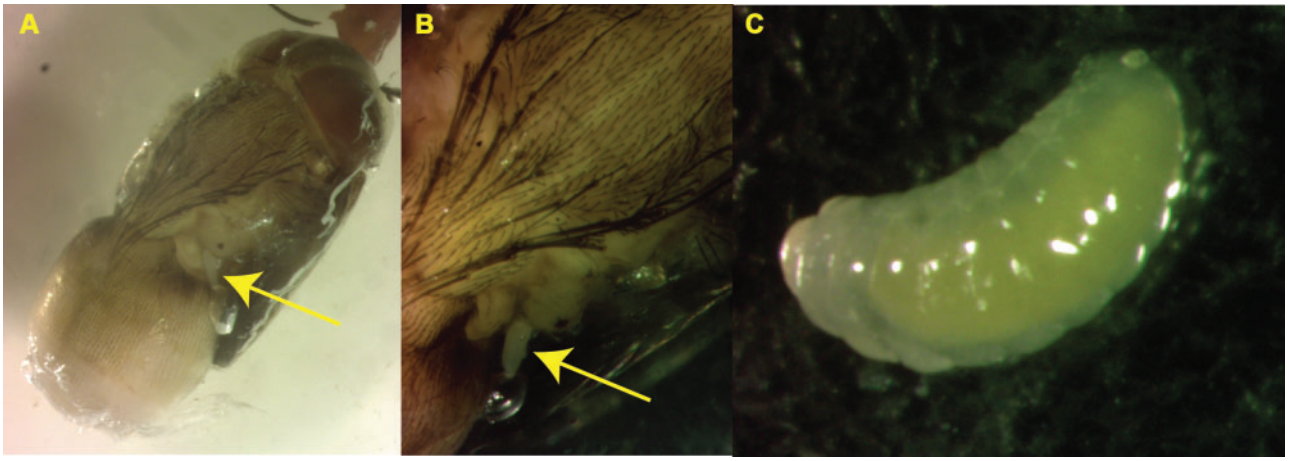

Fig. 3. (**A**) *Conura annulifera* egg that was laid on exterior of a *P. downsi* pupa (arrow). (**B**) Close up of same. (**C**) *Conura annulifera* larva removed from exterior of a *P. downsi* pupa within the puparium. Photos: G. E. Heimpel.

egg on the lateral side of the pupa, in the space between the pupa's thorax and abdomen (Fig. 3A, B). This demonstrates that *C. annulifera* is an ectoparasitoid. We dissected a second *P. downsi* puparium 7 d after being stung by *C. annulifera* at the age of 6 d and observed the parasitoid larva (Fig. 3C) in the same position as the egg in the earlier dissection.

Males of *C. annulifera* emerged slightly earlier than females did. At our rearing temperature of 23.5°C, the average male emerged 24.04 ± 0.23 d after eggs were laid (range: 20–29 d, $n = 70$), whereas females emerged 26.71 ± 0.88 d after eggs were laid (range 23–31 d, $n = 7$). This difference was marginally significant using a Mann–Whitney *U* Test ($W = 282.2$, df = 68, $P = 0.046$). In all cases, only one parasitoid emerged per puparium, confirming that *C. annulifera* is a solitary species.

Females reared from wild-collected *Philornis* pupae lived an average of 122 ± 4.2 d (range 108–151, $n = 8$), whereas females reared from pupae that developed in the laboratory averaged 207 ± 3.0 d (range 188–235, $n = 5$).

Adult *C. annulifera* emerged from *Philornis* puparia, generally at their anterior ends. Exit holes were round-to-oblong, with a mean diameter of 2.25 ± 0.05 mm ($n = 34$; Fig. 4A). Exuviae from the parasitoids' last molts, as well as the remains of *Philornis* pupae from which they fed, were evident within puparia (Fig. 4B). We observed three instances of copula by individuals less than two days old, and pairs remained *in copula* for approximately 15 minutes (Fig. 4C).

Sex Ratio

The sex ratio (proportion males) of *C. annulifera* reared from *Philornis* pupae collected in the field was female-biased (three males, 12 females, see above). In contrast, the sex ratio of *C. annulifera* reared from *P. downsi* pupae in the laboratory was extremely male-biased at 0.91 (70 males and seven females). Measurements of length and diameter of the puparia were significantly repeatable (length: ICC = 0.76 ± 0.13 95% CI, diameter: ICC = 0.72 ± 0.15 95% CI) and estimated volumes of pupae yielding male *C. annulifera* (87.5 ± 8.1 mm^3, $n = 27$) versus females (90.8 ± 15.9 mm^3, $n = 7$) were not significantly different ($t_{32} = 0.18$, $P = 0.86$).

Host Specificity

Conura annulifera reproduced only on puparia of *P. downsi*, and not on any of the other nine species of candidate hosts (*P. downsi* versus others: quasibinomial GLM: $F_{1,85} = 293.5$, $P < 0.0001$, Table 3). Furthermore, *C. annulifera* did not cause significant mortality to any of the species other than *P. downsi*. Host emergence rates varied significantly among the nine nontarget species (quasibinomial $F_{8,\ 37} = 183.54$, $P < 0.0001$), but there was no main effect of exposure (exposed versus controls: $F_{1,45} = 0.36$, $P = 0.55$), and there was no interaction between exposure and host species ($F_{7,\ 39} = 2.48$ $P = 0.93$; Fig. 5).

Pupae of *P. downsi* that had been presented to *C. annulifera*, but that did not yield parasitoid adults, had a reduced rate of survival compared to fly pupae that were not exposed to parasitoids (quasibinomial GLM $F_{1,38} = 6.60$, $P = 0.01$). This could have been due to probing of pupae by *C. annulifera* that did not result in oviposition or to incomplete development of *C. annulifera* immature stages that also resulted in death of the host. Host feeding is another potential cause, but as noted above, host feeding was not observed during our trials. Survival to adulthood by larval *P. downsi* that were exposed to *C. annulifera* was not significantly different to control un-exposed larvae (quasibinomial GLM $F_{1,6} = 0.88$, $P = 0.38$).

Discussion

The present research has established that *C. annulifera* parasitizes two species of *Philornis* flies in western Ecuador. One of the flies is *P. downsi*, the target of a management program currently being developed in the Galápagos Islands to protect endemic finches. Host specificity trials involving *P. downsi* and available nontarget hosts, including other cyclorrhaphan Diptera, Lepidoptera, and Hymenoptera, showed that only *P. downsi* was attacked. This parasitoid is thus a promising candidate for introduction into the Galápagos Islands to achieve biological control of the introduced *P. downsi*. However, while our studies suggest that *C. annulifera* is a specialist on *Philornis* spp., more information is needed to confirm this hypothesis. In particular, additional host-specificity experiments with insect species endemic to the Galápagos Islands will be needed before a release there can be fully contemplated. Short of this though, the hypothesis that *C. annulifera* is a *Philornis* specialist can be addressed by investigating the scientific literature and by considering the life cycle of the parasitoid. We consider these lines of reasoning in turn below.

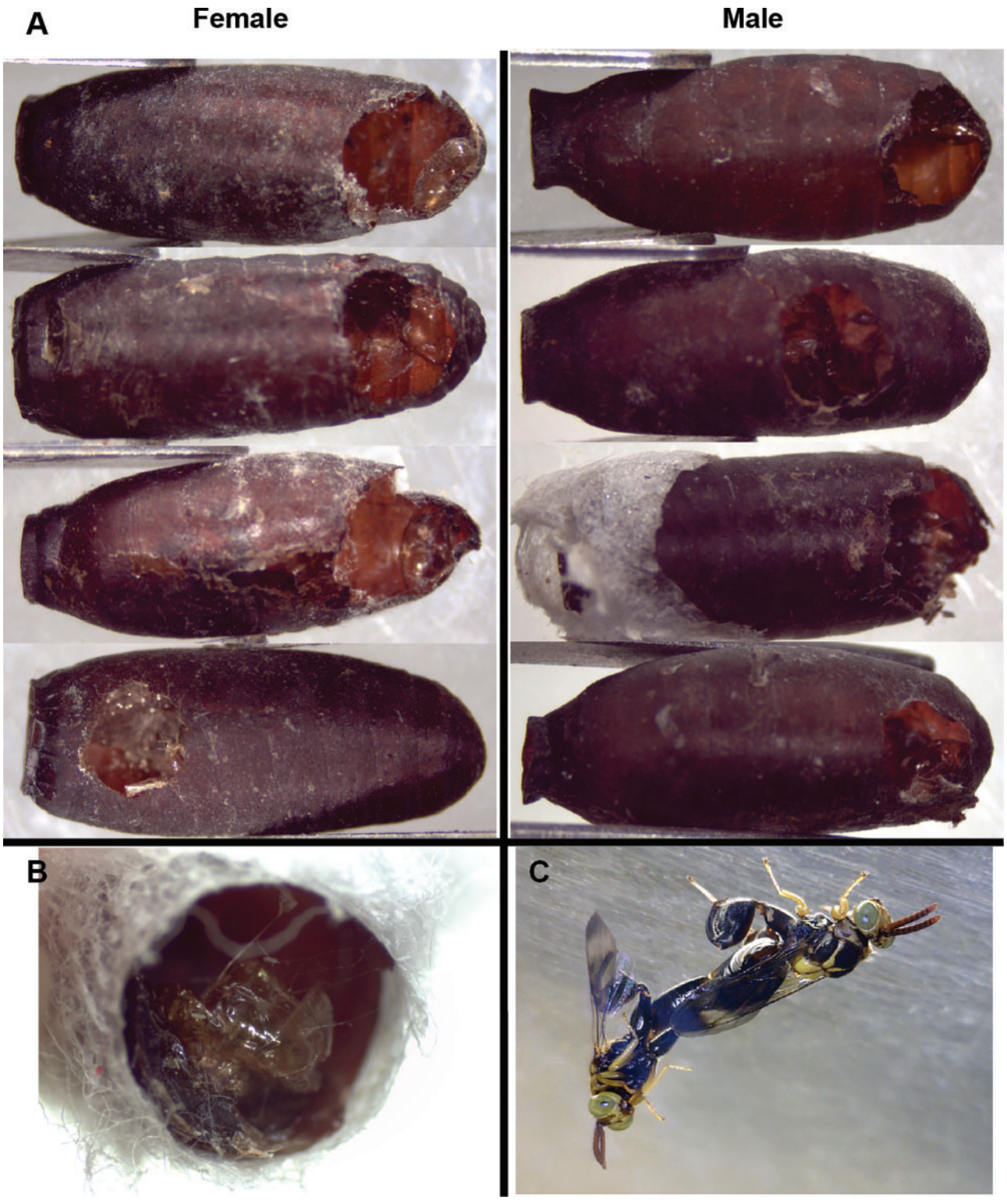

Fig. 4. (**A**) Exit holes in *P. downsi* puparia that yielded female and male *C. annulifera*. (**B**) Exuvium shed by a *C. annulifera* wasp after emergence from a *P. downsi* puparium. (**C**) Male and female *C. annulifera* in copula. Photos: A, B: M. Bulgarella, C: P. Lahuatte.

Conura annulifera has previously been reported from Mexico, Costa Rica, Panama, Trinidad, and Brazil (De Santis 1979, Delvare 1992). The present study extends its known geographic range to coastal Ecuador, which has a climate similar to that of the Galápagos Islands. The previously known host range of *C. annulifera* includes *P. downsi* and *P. deceptivus* in Trinidad (Burks 1960, Delvare 1992) and an unidentified *Philornis* species in Brazil (Couri et al. 2006). The present study adds *P. niger* to *C. annulifera*'s known host range. While such a limited number of rearing records may be seen as weak evidence for host specificity, numerous other studies have reared parasitoids from dipteran puparia in the neotropics, and none of these have yielded *C. annulifera*. A review of 18

Table 3. Numbers of candidate host species presented to female *C. annulifera* in the host specificity experiment, and proportions of puparia that yielded adult *C. annulifera*, adult candidate hosts, or did not emerge (see also Fig. 5)

Test species (Order Family)	No. of pupae presented to *C. annulifera*	Prop. *C. annulifera* emerged	Prop. candidate hosts emerged	Prop. with no emergence
Philornis downsi (Diptera: Muscidae)	164	0.47	0.30	0.23
Musca domestica (Diptera: Muscidae)	67	0.00	0.97	0.03
Musca autumnalis (Diptera: Muscidae)	40	0.00	0.67	0.33
Stomoxys calcitrans (Diptera: Muscidae)	70	0.00	0.36	0.64
Sarcophaga bullata (Diptera: Sarcophagidae)	59	0.00	0.97	0.03
Calliphora vicina (Diptera: Calliphoridae)	83	0.00	0.25	0.75
Epiphyas postvittana (Lepidoptera: Tortricidae)	48	0.00	0.94	0.06
Manduca sexta (Lepidoptera: Sphingidae)	24	0.00	1.00	0.00
Plodia interpunctella (Lepidoptera: Pyralidae)	20	0.00	0.70	0.30
Habrobracon hebetor (Hymenoptera: Braconidae)	41	0.00	0.51	0.49

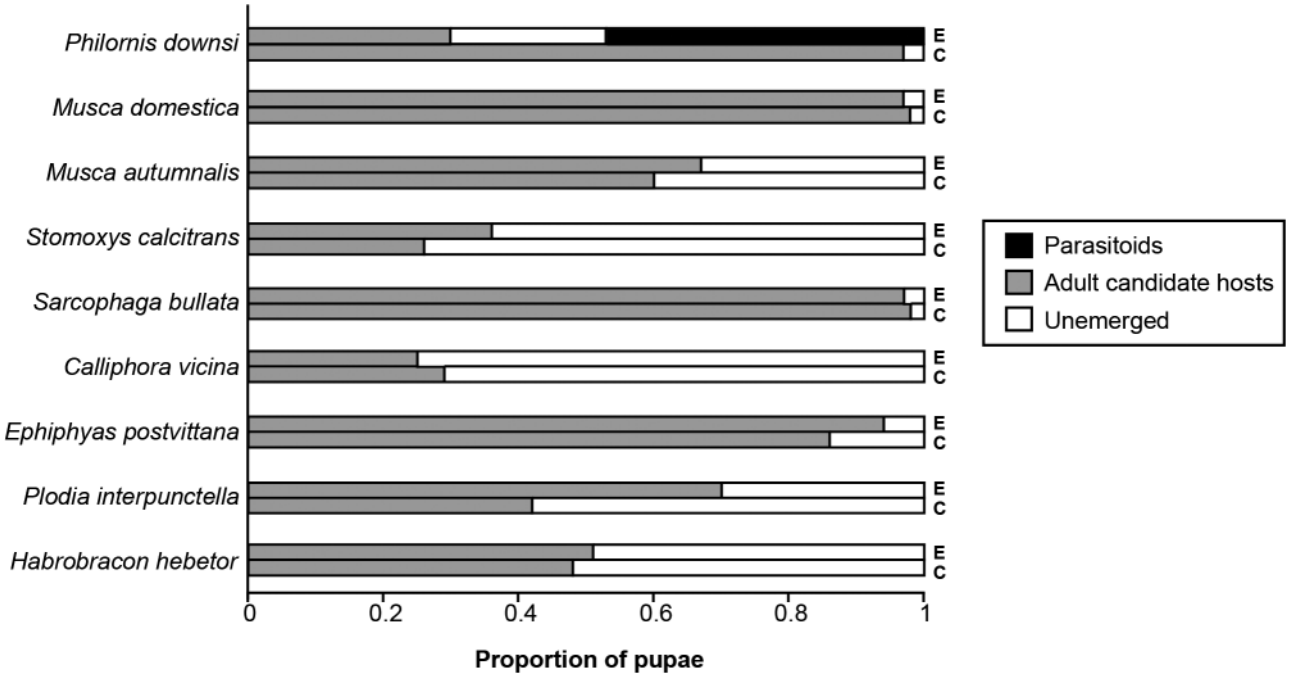

Fig. 5. Proportions of pupae presented to *C. annulifera* that yielded parasitoids, adults of candidate hosts, or nothing. E, experimental (presented to *C. annulifera*); C, control pupae (no parasitoids).

studies in Argentina, Brazil, Mexico, the Dominican Republic, and Peru revealed no evidence of *C. annulifera* being reared from at least 24 species of tephritid, muscid and sarcophagid Diptera (Diaz et al. 1996, Mendes and Linhares 1999, Ovruski et al. 2005, Marchiori et al. 2002, Aguiar-Menezes et al. 2004, Garcia and Corseuil, 2004, Geden et al. 2006, Hernández-Ortiz et al. 2006, Marchiori 2006, Marchiori and Silva Filho 2007, Loera-Gallardo et al. 2008, Romero et al. 2010, Battán Horenstein and Salvo 2012, Ávila-Rodríguez et al. 2015, Taveras and Hansson 2015, Montoya et al. 2016). These studies did, however, yield 31 other pupal parasitoid species, including the well-known dipteran generalists *Nasonia vitripennis* (Walker), *Muscidifurax raptor* (Girault and Sanders), *Spalangia endius* (Walker) (all Pteromalidae), and *Brachymeria podagrica* (F.) (Chalcididae). This shows that the methods used in these studies would likely have been sufficient to detect *C. annulifera*, had it been present. *C. annulifera* was also not reported by Ovruski et al. (2000) in a meta-analysis of 75 parasitoid species of tephritid flies occurring across 17 neotropical countries. Taken together, these studies suggest that while there have been multiple opportunities to document attacks of various non-*Philornis* dipteran puparia by *C. annulifera* within the neotropical region, no cases have been reported. This is consistent with the hypothesis of *Philornis* specificity.

Our laboratory studies have established that *C. annulifera* is a solitary, idiobiont pupal parasitoid whose larvae feed ectoparasitically within the pupa-puparial gap of their cyclorrhaphan hosts. Unlike pupae of other holometabolous insects, cyclorrhaphan Diptera have a gap between the soft body of the pupa and the hard puparium (Whitten 1957). Parasitoid eggs in this gap are protected from desiccation and unfavorable climatic conditions, and yet are not exposed to their host's immune response, as occurs with endoparasitoids (Bouletreau 1986, Pennacchio and Strand 2006, Geden and Moon 2009). All known pupal ectoparasitoids of cyclorrhaphan

Diptera oviposit in their hosts' pupa-puparial gap (13 species in six genera in Pteromalidae, Chalcididae and Ichneumonidae; Richardson 1913, Johnston and Tiegs 1921, Crandell 1939, Dresner 1954, Monteith 1956, Whiting 1967, Gerling and Legner 1968, Wylie 1971, Arellano and Rueda 1988, Harvey and Gols 1998, Wang and Messing 2004, Tormos et al. 2009). We only found a single report of a pupal ectoparasitoid attacking a host outside of Cyclorrhapha (a lepidopteran by *N. vitripennis*; Noyes 2007) but a more in-depth study by Peters (2010) has cast doubt on the validity of that report. Taken together, these observations suggest that the host range of ectoparasitoids that attack the pupae of cyclorrhaphan Diptera does not extend beyond the Cyclorrhapha (Ueno 2015).

Our results are relevant to efforts to manage populations of *P. downsi* in the Galápagos Islands, where it has invaded and is causing great harm to populations of endemic bird species. The Galápagos National Park Directorate has identified reduction of *P. downsi* populations as a major conservation goal, and biological control via importation of a specialized parasitoid is being considered as a potential control strategy to achieve this goal (Causton et al. 2013, Fessl et al. 2017). Our investigations have revealed that at least three parasitoid species attack *P. downsi* in mainland Ecuador including *C. annulifera*, the only species so far identified to the species level. The present studies are consistent with the hypothesis that *C. annulifera* is a specialist parasitoid of flies in the genus *Philornis*. Because there are no native or endemic *Philornis* species in Galápagos (Sinclair 2009), *C. annulifera* could be a potentially safe candidate for introduction into Galápagos against *P. downsi*. In addition its presence there could be beneficial in the case of further *Philornis* introductions (Fessl et al. 2017). However, 18 species of other Cyclorrhapha are endemic to Galápagos (Sinclair 2009), so additional studies of nontarget effects will be needed to more fully assess the ecological risk of such an introduction. Risks of biological control introductions must also be weighed against risks and costs of other potential control tactics, which in the case of *P. downsi* includes recurring use of insecticides (Knutie et al. 2014), trapping with lures (Cha et al. 2016), and the sterile insect technique (Lahuatte et al. 2016).

Although importation biological control has been used mostly in agricultural and forest settings, it has been increasingly seen as a viable conservation tactic to limit the damaging effects of invasive species in natural settings (van Driesche et al. 2010). Indeed, one of the most successful of these interventions took place in Galápagos where the specialist lady beetle *Rodolia cardinalis* (Mulsant) was introduced to control the cottony cushion scale, *Icerya purchasi* (Maskell), which was posing a severe threat to native and endemic plant species there (Causton 2009). That project showed that importation biological control can be an effective and safe conservation tool in Galápagos (Causton et al. 2004, Lincango et al. 2011, Calderón Alvarez et al. 2012, Hoddle et al. 2013).

Acknowledgments

We thank Mauricio Torres for checking nest boxes and helping to collect puparia; Dr. Jaime Buestán Aucancela for help obtaining permits; Gabriel Brito Vera for field assistance and for providing the wild nest photographs; Paola Lahuatte and Andrea Cahuana for providing *P. downsi* females from Galápagos; Kristina Friesen and Dennis Berkebile at the Agroecosystem Management Research Unit, USDA Agricultural Research Service, Lincoln, NE, USA, for providing stable fly eggs; Amy Morey, Anthony Auletta, Jonathan Dregni for providing pupae of nontarget species; and Paola Lahuatte, Roxanne Sage, Carleigh Windhorst, Ashley Sampedro and Justine Bleick for help with bird husbandry and care. Stephanie Dahl and Nik Prenevost at the Minnesota Department of Agriculture quarantine facility helped with laboratory logistics. We also thank Drs. Marcelo T. Tavares from the Universidade Federal do Espírito Santo, Brazil, and Bradley J. Sinclair from the Canadian National Collection of Insects and Ottawa Plant Laboratory, Canada, for helping to identify *C. annulifera* and *Philornis* spp., respectively. Milan Plećaš, Joe Kaser, Jonathan Dregni, J. J. Weis, Hannah Gray and two anonymous reviewers provided helpful comments on an earlier version of the manuscript. We thank the Ecuadoran Ministry of the Environment for issuing permits to work in Loma Alta and to export samples. Permission to conduct this study was granted by the Galápagos National Park Directorate (Project: PC-10-15 & 18-16: Control of the Invasive Parasite, *Philornis downsi* and its Impact on Biodiversity). We are grateful to the Galápagos Conservancy, International Community Foundation (with a grant awarded by The Leona M. and Harry B. Helmsley Charitable Trust), the National Geographic Society award no. C257-13), and the University of Minnesota Institute on the Environment for funding. This is contribution number 2147 of the Charles Darwin Foundation for the Galápagos Islands.

References Cited

Aguiar-Menezes, E. L., R. J. Nascimento, and E. B. Menezes. 2004. Diversity of fly species (Diptera: Tephritoidea) from *Passiflora* spp. and their hymenopterous parasitoids in two municipalities of the Southeastern Brazil. Neotrop. Entomol. 33: 113–116.

Arellano, G. M., and L. M. Rueda. 1988. Biological study of the house fly pupal parasitoid, *Spalangia endius* (Walker) (Hymenoptera: Pteromalidae). Philipp. Entomol. 7: 329–360.

Arthur, A. P. 1958. Development, behaviour, and descriptions of immature stages of *Spilochalcis side* (Walk.) (Hymenoptera: Chalcididae). Can. Entomol. 90: 590–595.

Ávila-Rodríguez, V., R. Martínez-López, U. Nava-Camberos, and M. M. Sosa-Rubio. 2015. Parasitoides de pupas (Hymenoptera: Pteromalidae) de moscas (Diptera: Muscidae) en establos lecheros de Jalisco, México. Southwest. Entomol. 40: 141–150.

Battán Horenstein, M., and A. Salvo. 2012. Community dynamics of carrion flies and their parasitoids in experimental carcasses in central Argentina. J. Insect Sci. 12: 8.

Bouletreau, M. 1986. The genetic and coevolutionary interactions between parasitoids and their hosts. *In* J. K. Waage and D. J. Greathead (eds.), Insect Parasitoids. Academic Press, London, United Kingdom.

Bulgarella, M., and G. E. Heimpel. 2015. Host range and community structure of avian nest parasites in the genus *Philornis* (Diptera: Muscidae) on the island of Trinidad. Ecol. Evol. 5: 3695–3703.

Bulgarella, M., M. A. Quiroga, G. A. Brito Vera, J. S. Dregni, F. Cunninghame, D. A. Mosquera Muñoz, L. D. Monje, C. E. Causton, and G. E. Heimpel. 2015. *Philornis downsi*, an avian nest parasite invasive to the Galápagos Islands, in mainland Ecuador. Ann. Entomol. Soc. Am. 108: 242–250.

Burks, B. D. 1960. A *Spilochalcis* parasitic on flies that infest bird nests (Hymenoptera, Chalcididae). Acta Hymenop. 1: 95–97.

Calderón Alvarez, C., C. E. Causton, M. S. Hoddle, C. D. Hoddle, R. van Driesche, and E. J. Stanek III. 2012. Monitoring the effects of *Rodolia cardinalis* on *Icerya purchasi* populations on the Galapagos Islands. BioControl 57: 167–179.

Causton, C. E. 2009. Success in biological control: the scale and the ladybird, pp. 184–190. *In* R De Roy (ed.), Galapagos: Preserving Darwin's Legacy. David Bateman, Auckland, New Zealand.

Causton, C. E., M. P. Lincango, and T. Poulsom. 2004. Feeding range studies of *Rodolia cardinalis* (Mulsant), a candidate biological control agent of *Icerya purchasi* Maskell in the Galapagos islands. Biol. Control 29: 315–325.

Causton, C. E., S. B. Peck, B. J. Sinclair, L. Roque-Albelo, C. J. Hodgson, and B. Landry. 2006. Alien insects: Threats and implications for conservation of Galapagos Islands. Ann. Entomol. Soc. Am. 99: 121–143.

Causton, C. E., F. Cunninghame, and W. Tapia. 2013. Management of the avian parasite *Philornis downsi* in the Galapagos Islands: A collaborative and strategic action plan. Puerta Ayora, Galápagos, Ecuador.

Cha, D. H., A. E. Mieles, P. Lahuatte, A. Cahuana, M. P. Lincango, C. E. Causton, S. Tebbich, A. Cimadom, and S. A. Teale. 2016. Identification and optimization of microbial attractants for *Philornis downsi*, an invasive fly parasitic on birds. J. Chem. Ecol. 42: 1101–1111.

Cimadom, A., A. Ulloa, P. Meidl, M. Zöttl, E. Zöttl, B. Fessl, E. Nemeth, M. Dvorak, F. Cunninghame, and S. Tebbich. 2014. Invasive parasites, habitat change and heavy rainfall reduce breeding success in Darwin's finches. PLoS ONE 9: e10751.

Couri, M. S. 1999. Myiasis caused by obligatory parasites. Ia. *Philornis* Meinert (Muscidae), pp. 44–70. *In* J. H. Guimaraes and N. Papavero (eds.), Myiasis in Man and Animals in the Neotropical Region. Editora Pleiade, São Paulo, Brazil.

Couri, M. S., M. T. Tavares, and R. R. Stenzel. 2006. Parasitoidism of chalcidid wasps (Hymenoptera, Chalcididae) on *Philornis* sp. (Diptera, Muscidae). Braz. J. Biol. 66: 553–557.

Crandell, H. A. 1939. The biology of *Pachycrepoideus dubius* Ashmead (Hymenoptera), a pteromalid parasite of *Piophila casei* Linne. Ann. Entomol. Soc. Am. 32: 632–654.

Delvare, G. 1992. A reclassification of the Chalcidini with a checklist of the New World species. Mem. A. Entomol. Inst. 53: 315.

De Santis, L. 1979. Catálogo de los Himénopteros Calcidoideos de América al sur de los Estados Unidos. Publicación Especial de la Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, La Plata, Buenos Aires, Argentina.

Diaz, N. B., F. E. Gallardo, and G. C. Walsh. 1996. *Paraganaspis egeria*, a new genus and species of Eucoilidae (Hymenoptera: Cynipoidea). Ann. Entomol. Soc. Am. 89: 497–500.

Dresner, E. 1954. Observations on the biology and habits of pupal parasites of the oriental fruit fly. Proc. Hawaii. Entomol. Soc. 15: 299–311.

Dvorak, M., B. Fessl, E. Nemeth, S. Kleindorfer, and S. Tebbich. 2012. Distribution and abundance of Darwin's finches and other land birds on Santa Cruz Island, Galápagos: Evidence for declining populations. Oryx 46: 78–86.

Fessl, B., S. Kleindorfer, and S. Tebbich. 2006. An experimental study on the effects of an introduced parasite in Darwin's finches. Biol. Conserv. 27: 55–61.

Fessl, B., H. G. Young, R. P. Young, J. Rodríguez-Matamoros, M. Dvorak, and S. Tebbich. 2010. How to save the rarest Darwin's finch from extinction: The mangrove finch on Isabela Island. Phil. Trans. R. Soc. Lond. B Biol. Sci. 365: 1019–1030.

Fessl, B., D. Anchundia, J. Carrión, A. Cimadom, J. Cotín, F. Cunninghame, M. Dvorak, D. Mosquera, E. Nemeth, C. Sevilla, et al. 2016. Galapagos landbirds (passerines, cuckoos, and doves): Status, threats, and knowledge gaps. *In* Galápagos Report 2015-2016. GNPS, GCREG, CDF and GC. Puerto Ayora, Galápagos, Ecuador.

Fessl, B., G. E. Heimpel, and C. E. Causton. 2017. Invasion of an avian nest parasite, *Philornis downsi*, to the Galapagos Islands: colonization history, adaptations to novel ecosystems, and conservation challenges. *In* P.G. Parker. (ed.), Disease Ecology: Galapagos Birds and their Parasites. Springer, Dordrecht, The Netherlands.

Galligan, T. H., and S. Kleindorfer. 2009. Naris and beak malformation caused by the parasitic fly, *Philornis downsi* (Diptera: Muscidae), in Darwin's small ground finch, *Geospiza fuliginosa* (Passeriformes: Emberizidae). Biol. J. Linn. Soc. 98: 577–585.

Garcia, F.R.M., and E. Corseuil. 2004. Native hymenopteran parasitoids associated with fruit flies (Diptera: Tephritidae) in Santa Catarina State, Brazil. Fla. Entomol. 87: 517–521.

Geden, C. J., and R. D. Moon. 2009. Host ranges of gregarious muscoid fly parasitoids: *Muscidifurax raptorellus* (Hymenoptera: Pteromalidae), *Tachinaephagus zealandicus* (Hymenoptera: Encyrtidae), and *Trichopria nigra* (Hymenoptera: Diapriidae). Environ. Entomol. 38: 700–707.

Geden, C. J., R. D. Moon, and J. F. Butler. 2006. Host ranges of six solitary filth fly parasitoids (Hymenoptera: Pteromalidae, Chalcididae) from Florida, Eurasia, Morocco, and Brazil. Environ. Entomol. 35: 405–412.

Gerling, D., and E. F. Legner. 1968. Developmental history and reproduction of *Spalangia cameroni*, parasite of synanthropic flies. Ann. Entomol. Soc. Am. 61: 1436–1443.

Godfray, H.C.J. 1994. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, NJ.

Hansen, J. D. 1980. The life history and behavior of *Spilochalcis albifrons* (Hymenoptera: Chalcididae), a parasite of the larch casebearer, *Coleophora laricella* (Lepidoptera: Coleophoridae). J. Kansas Entomol. Soc. 53: 553–566.

Harvey, J. A., and G.J.Z. Gols. 1998. The influence of host quality on progeny and sex allocation in the pupal ectoparasitoid, *Muscidifurax raptorellus* (Hymenoptera: Pteromalidae). Bull. Entomol. Res. 88: 299–304.

Heimpel, G. E., A. Hillstrom, D. Freund, S. A. Knutie, and D. H. Clayton. 2017. Invasive parasites and the fate of Darwin's finches in the Galapagos Islands: The case of the vegetarian finch. Wilson J. Ornithol. (in press).

Hernández-Ortiz, V., H. Delfín-González, A. Escalante-Tio, and P. Manrique-Saide. 2006. Hymenopteran parasitoids of *Anastrepha* fruit flies (Diptera: Tephritidae) reared from different hosts in Yucatan, Mexico. Fla. Entomol. 89: 508–515.

Hoddle, M. S., C. Crespo Ramirez, C. D. Hoddle, J. Loayza, M. P. Lincango, R. G. Van Driesche, and C. E. Causton. 2013. Post release evaluation of *Rodolia cardinalis* (Coleoptera: Coccinellidae) for control of *Icerya purchasi* (Hemiptera: Monophlebidae) in the Galapagos Islands. Biol. Control 67: 262–274.

Huber, S. K. 2008. Effects of the introduced parasite *Philornis downsi* on nestling growth and mortality in the medium ground finch (*Geospiza fortis*). Biol. Conserv. 41: 601–609.

Jervis, M. A., and N.A.C. Kidd. 1986. Host-feeding strategies in Hymenopteran parasitoids. Biol. Rev. 61: 395–434.

Johnston, T. H., and O. W. Tiegs. 1921. On the biology and economic significance of the chalcid parasites of Australian sheep maggot flies. Proc. R. Soc. Queensl. 33: 99–128.

Kleindorfer, S., and R. Y. Dudaniec. 2016. Host-parasite ecology, behavior and genetics: a review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. BMC Zool. 1: 1–19.

Kleindorfer, S., and F. J. Sulloway. 2016. Naris deformation in Darwin's finches: Experimental and historical evidence for a post-1960's arrival of the parasite *Philornis downsi*. Glob. Ecol. Conserv. 7: 122–131.

Kleindorfer, S., K. J. Peters, G. Custance, R. Y. Dudaniec, and J. A. O'Connor. 2014. Changes in *Philornis* infestation behavior threaten Darwin's finch survival. Curr. Zool. 60: 542–550.

Knutie, S. A., S. M. McNew, A. W. Bartlow, D. A. Vargas, and D. H. Clayton. 2014. Darwin's finches combat introduced nest parasites with fumigated cotton. Curr. Biol. 24: R355–R356.

Koop, J.A.H., S. K. Huber, S. M. Laverty, and D. H. Clayton. 2011. Experimental demonstration of the fitness consequences of an introduced parasite of Darwin's finches. PLoS ONE 6: e19706.

Koop, J.A.H., J. P. Owen, S. A. Knutie, M. A. Aguilar, and D. H. Clayton. 2013. Experimental demonstration of a parasite-induced immune response in wild birds: Darwin's finches and introduced nest flies. Ecol. Evol. 3: 2514–2523.

Koop, J.A.H., P. S. Kim, S. A. Knutie, F. Adler, and D. H. Clayton. 2016. Introduced parasitic fly may lead to local extinction of Darwin's finch populations. J. Appl. Ecol. 53: 511–518.

Lahuatte, P., M. P. Lincango, G. E. Heimpel, and C. E. Causton. 2016. Rearing larvae of the avian nest parasite, *Philornis downsi* (Diptera: Muscidae), on chicken blood-based diets. J. Insect Sci. 16: 1–7.

Lee, J. C., and G. E. Heimpel. 2005. Impact of flowering buckwheat on lepidopteran cabbage pests and their parasitoids at two spatial scales. Biol. Control 34: 290–301.

Lincango, M. P., C. E. Causton, C. C. Alvarez, and G. Jimenez-Uzcategui. 2011. Evaluating the safety of *Rodolia cardinalis* to two species of Galapagos finch; *Camarhynchus parvulus* and *Geospiza fuliginosa*. Biol. Control 56: 145–149.

Loera-Gallardo, J., J. F. Luna-Salas, and G.A.P. Gibson. 2008. First report of pupal parasitoids of filth-breeding flies (Diptera) from bovine manure in northeastern Mexico. Can. Entomol. 140: 682–689.

Marchiori, C. H. 2006. Microimenóteros de *Musca domestica* L. (Diptera: Muscidae) coletados em diferentes substrates em Itumbiara, Goiás. Arq. Bras. Med. Vet. Zootec. 58: 447–449.

Marchiori, C. H., E. R. Caldas, and K.G.S. Dias. 2002. Parasitóides de Diptera coletados em fezes bovinas em vários tempos de exposição em Itumbiara, Goiás, Brasil. Arq. Inst. Biol. Sao Paolo. 69: 37–42.

Marchiori, C. G., and O. M. Silva Filho. 2007. New host for the parasitoid *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) in Brazil. Arq. Bras. Med. Vet. Zootec. 59: 271–272.

Marcicano, M.D.L., I.M.M. Lima, M. T. Tavares, and M. N. Casagrande. 2007. Parasitism of *Brassolis sophorae laurentii* Stichel (Lepidoptera: Nymphalidae, Brassolinae) pupae by *Conura morleyi* (Ashmead) (Hymenoptera: Chalcididae, Chalcidini), in the State of Alagoas, Brazil. Netrop. Entomol. 36: 629–631.

Mendes, J., and A. X. Linhares. 1999. Diapause, pupation sites and parasitism of the horn fly, *Haematobia irritans*, in south-eastern Brazil. Med. Vet. Entomol. 13: 185–190.

Miall, J. H., P. K. Abram, N. Cappuccino, and P. G. Mason. 2014. Potential impact of the native hyperparasitoid *Conura albifrons* (Hymenoptera: Chalcididae) on the exotic biological control agent *Diadromus pulchellus* (Hymenoptera: Ichneumonidae). Biocontrol Sci. Technol. 24: 611–624.

Monteith, A. E. 1956. *Phygadeuon trichops* Thoms. (Hymenoptera: Ichneumonidae), an occasional parasite of *Hylemya* spp. (Diptera: Anthomyiidae). Can. Entomol. 88: 69–73.

Montoya, P., A. Ayala, P. López, J. Cancino, H. Cabrera, J. Cruz, M. M. Martinez, I. Figueroa, and P. Lideo. 2016. Natural parasitism in fruit fly (Diptera: Tephritidae) populations in disturbed areas adjacent to commercial mango orchards in Chiapas and Veracruz, Mexico. Environ. Entomol. 45: 328–337.

Noyes, J. S. 2007. Universal Chalcidoidea Database. World Wide Web electronic publication. (www.nhm.ac. uk/research-curation/projects/chalcidoids/index) (accessed 30 June 2016).

O'Connor, J. A., F. J. Sulloway, J. Robertson, and S. Kleindorfer. 2010. *Philornis downsi* parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch (*Camarhynchus pauper*). Biodiv. Conserv. 19: 853–866.

Ode, P. J., and J. A. Rosenheim. 1998. Sex allocation and the evolutionary transition between solitary and gregarious parasitoid development. Am. Nat. 152: 757–761.

Ode, P. J., and M. R. Strand. 1995. Progeny and sex allocation decisions of the polyembryonic wasp *Copidosoma floridanum*. J. Anim. Ecol. 64: 213–224.

Ovruski, S., M. Aluja, J. Sivinski, and R. A. Wharton. 2000. Hymenopteran parasitoids of fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: Diversity, distribution, taxonomic status and their use in fruit fly biological control. Integrated Pest Manag. Rev. 5: 81–107.

Ovruski, S., R. A. Wharton, P. Scliserman, and M. Aluja. 2005. Abundance of *Anastrepha fraterculus* (Diptera: Tephritidae) and its associated native parasitoids (Hymenoptera) in "Feral" Guava growing in the endangered northernmost Yungas forests of Argentina with an update on the taxonomic status of opine parasitoids previously reported in this country. Environ. Entomol. 34: 807–818.

Parker, P. 2009. Parasites and pathogens: threats to native birds, pp. 177–83. *In* T. De Roy (ed.), Galapagos: Preserving Darwin's Legacy. David Bateman, Auckland, New Zealand.

Parker, P. 2017. Disease Ecology: Galapagos Birds and their Parasites. Springer, Dordrecht, The Netherlands.

Pennacchio, F., and M. R. Strand. 2006. Evolution of developmental strategies in parasitic Hymenoptera. Ann. Rev. Entomol. 51: 233–258.

Peters, R. S. 2010. Host range and offspring quantities in natural populations of *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Chalcidoidea: Pteromalidae). J. Hymenop. Res. 19: 128–138.

Quiroga, M. A., J. C. Reboreda, and A. H. Beltzer. 2012. Host use by *Philornis* sp. in a passerine community in central Argentina. Rev. Mex. Biodivers. 83: 110–116.

R Development Core Team 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (http://www.R-project.org/)

Richardson, C. H. 1913. Studies on the habits and development of a hymenopterous parasite, *Spalangia muscidarum* Richardson. J. Morphol. 24: 513–557.

Romero, A., J. A. Hogsette, and A. Coronado. 2010. Distribution and abundance of natural parasitoid (Hymenoptera: Pteromalidae) populations of house flies and stable flies (Diptera: Muscidae) at the University of Florida Dairy Research Unit. Neotrop. Entomol. 39: 424–428.

Salvador, S. A., and A. Bodrati. 2013. Aves víctimas de parasitismo de moscas del género *Philornis* en Argentina. Nuestras Aves. 58: 16–21.

Silvestri, L., L. R. Antoniazzi, M. S. Couri, L. D. Monje, and P. M. Beldomenico. 2011. First record of the avian ectoparasite *Philornis downsi* Dodge & Aitken, 1968 (Diptera: Muscidae) in Argentina. Syst. Parasitol. 80: 137–140.

Sinclair, B. J. 2009. Dipteran biodiversity of the Galápagos, pp. 98–120. *In* T. Pape, D. Bickel, and R. Meier (eds.), Dipteran diversity: Status, challenges and tools. Koninklijke Brill NV, Leiden, The Netherlands.

Skidmore, P. 1985. Genus *Philornis* Meinert (Neomusca Malloch). *In* The Biology of the Muscidae of the World. Dr. W. Junk Publishers, The Netherlands.

Taveras, R., and C. Hansson. 2015. *Pediobius cajanus* sp. N. (Hymenoptera, Eulophidae), an important natural enemy of the Asian fly (*Melanagromyza obtuse* (Malloch)) (Diptera, Agromyzidae) in the Dominican Republic. J. Hymenop. Res. 45: 41–54.

Tormos, J., F. Beitia, E. A. Böckmann, J. D. Asís, and S. Fernández. 2009. The preimaginal phases and development of *Pachycrepoideus vindemmiae* (Hymenoptera, Pteromalidae) on mediterranean fruit fly, *Ceratitis capitata* (Diptera, Tephritidae). Microsc. Microanal. 15: 422–434.

Ueno, T. 2015. Unexpected use of pupal stage of a lepidopteran host by the ectoparasitoid wasp Agrothereutes lanceolatus (Hymenoptera: Ichneumonidae). Entomol. Ornithol. Herpetol. 4: 140.

van Driesche, R. G., R. I. Carruthers, T. Center, M. S. Hoddle, J. Hough-Goldstein, L. Morin, L. Smith, D. L. Wagner, B. Blossey, V. Brancatini, et al. 2010. Classical biological control for the protection of natural ecosystems. Biol. Control. 54: S2–S33.

Wang, X. G., and R. H. Messing. 2004. Two different life-history strategies determine the competitive outcome between *Dirhinus giffardii* (Chalcididae) and *Pachycrepoideus vindemmiae* (Pteromalidae), ectoparasitoids of cyclorrhaphous Diptera. B. Entomol. Res. 94: 473–480.

Weis, J. J., H. Gray, and G. E. Heimpel. 2017. High hyperparasitism of *Cotesia rubecula* (Hymenoptera: Braconidae) in Minnesota and Massachusetts. J. Kansas Entomol. Soc. (in press).

Whiting, A. R. 1967. The biology of the parasitic wasp *Mormoniella vitripennis* [= *Nasonia brevicornis*] (Walker). Q. Rev. Biol. 42: 333–406.

Whitten, M. J. 1957. The supposed pre-pupa in cyclorrhaphous Diptera. Q. J. Microsc. Sci. 98: 241–250.

Wylie, H. G. 1971. Oviposition restraint of *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae) on parasitized housefly pupae. Can. Entomol. 103: 1537–1544.