# Brood parasitic nestlings benefit from unusual host defenses against botfly larvae (*Philornis* spp.)

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## Abstract

Brood parasitic birds lay their eggs into the nests of other birds, abandoning parental care of their nestlings to the unsuspecting hosts. Parasite and host nestlings may themselves be parasitized by botfly larvae (*Philornis*: Muscidae), which burrow under the nestlings' skin and can seriously affect growth and survival. Here, we provide the first direct evidence that adult baywings (*Agelaioides badius*), the primary host of the specialist brood parasitic screaming cowbird (*Molothrus rufoaxillaris*), regularly remove botfly larvae from their own and parasitic nestlings by pulling them out of the nestlings' skin. This is the only bird species known to remove botfly larvae. By combining nestling cross-fostering with video recording of baywing nests, we show that due to prompt removal, infection with botfly larvae had negligible effects on nestling growth and survival despite high prevalence. Our results provide the first direct observations for larva removal behavior in botfly hosts. Screaming cowbirds may benefit from using baywings as its main host, as larva removal by adult baywings reduces the costs of botfly parasitism.

## Significance statement

Infection by botfly larvae of the genus *Philornis* (Muscidae) causes nestling mortality in many Neotropical birds. Despite the lethal effects, most *Philornis* hosts studied so far lack specific defenses against these larvae. The grayish baywing (*Agelaioides badius*), primary host of the brood parasitic screaming cowbird (*Molothrus rufoaxillaris*), is the only species that, based on indirect evidence, would be able to remove *Philornis* larvae from infected nestlings. We provide the first direct evidence that adult baywings do indeed remove botfly larvae from their own nestlings as well as from parasitic cowbird nestlings and that this unusual defense may increase the survival of own and screaming cowbird nestlings at infected nests.

## Introduction

Interspecific brood parasites lay their eggs in nests of other species (hosts), which care for the parasitic offspring. This

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reproductive strategy has evolved in insects, fishes, and birds (Davies 2000; Thorogood et al. 2019). Avian brood parasites comprise about 100 species (i.e., 1% of extant species of birds) and has evolved independently at least in seven phylogenetically independent groups distributed worldwide (Sorenson and Payne 2002). One of these groups is the monophyletic genus *Molothrus* (family Icteridae), commonly known as cowbirds, that comprises five parasitic species (Lanyon 1992). The screaming cowbird (*M. rufoaxillaris*) is the most specialized cowbird species, almost exclusively parasitizing a single host, the grayish baywing (*Agelaioides badius*; hereafter baywing), throughout most of its distribution in southern South America (Ortega 1998). Why screaming cowbirds show such strong host preference is not yet well understood, although it has been proposed that host specificity can be favored if preferred hosts provide higher quality parental care that enhances the parasite's reproductive success (De Mársico and Reboreda 2008). Following this idea, parental

behaviors that reduce the burden of ectoparasites in nestlings could influence host choice by brood parasites since ectoparasites can be especially harmful for young animals (Lehmann 1993).

Botflies in the genus *Philornis* (Diptera: Muscidae) are widespread and often lethal ectoparasites of Neotropical nestlings (Carvalho et al. 2005). In most *Philornis* species, larvae live subcutaneously, feeding on blood cells (Young 1993). Botfly infection causes anemia, reduced growth, and often results in nestling mortality when it is severe or begins at an early age of chicks (Arendt 1985; Dudaniec and Kleindorfer 2006; Fessl et al. 2006; Rabuffetti and Reboreda 2007; Segura and Reboreda 2011; Quiroga and Reboreda 2012). Despite these sublethal and lethal effects, most botfly hosts appear to lack specific defenses against these larvae. Baywings, however, are thought to be a rare exception. In an early report, Fraga (1984) proposed that adult baywings were able to remove botfly larvae from nestlings based on his observations of scars that appeared at larval penetration points soon after infection occurred. Interestingly, these scars appeared on both baywing and cowbird nestlings, suggesting that larva removal benefited host as well as parasites. However, Fraga (1984) was unable to observe the proposed behavior directly or to rule out the alternative hypotheses that nestlings themselves removed the larvae from their own skin or from their nest-mates.

A previous study showed that screaming cowbird nestlings were significantly more likely to die from botfly infection when they were experimentally translocated to nests of an alternative host species that lacks defenses against botflies (the house wren, *Troglodytes aedon*), compared with those reared in baywing nests (De Mársico and Reboreda 2008). Hence, removal of larvae seems critical to the survival of brood parasitic nestlings at nests infected with botflies. The aims of this study are (i) to provide direct evidences of larva removal behavior, (ii) to determine who removes the larvae (adult baywings or nestlings themselves), (iii) to determine whether larva removal is directed indiscriminately to host and parasitic nestlings, and (iv) to assess the effect of botfly ectoparasitism and host removal behavior on the growth and survival of screaming cowbird nestlings.

## Methods

Fieldwork was conducted at "El Destino" Reserve, Province of Buenos Aires, Argentina (35° 08′ S, 57° 23′ W), during the breeding seasons of 2012–2013 and 2013–2014 (November–February). The study area comprises flooding grasslands with patches of woodland dominated by tala (*Celtis ehrenbergiana*) and coronillo (*Scutia buxifolia*). Baywings and screaming cowbirds are year-round residents in the area. Baywings breed in closed nests built by other species, mainly

furnariids, and in secondary cavities located in trees. We searched for and monitored baywing nests throughout the breeding seasons. Nearly all baywing nests are parasitized by screaming cowbirds (approximately 90-100% of nests) and, less frequently, by the host-generalist shiny cowbird (*M. bonariensis*) (15–25% of nests) (De Mársico and Reboreda 2010).

At each of six baywing nests, we experimentally placed one nestling infected with botfly larvae (3 shiny cowbird nestlings, 2 screaming cowbird nestlings, and 1 baywing nestling) and used video recording to determine whether larvae were removed, and if so, by whom. Screaming cowbird and baywing nestlings were experimentally infected by moving them for 24 h to nearby nests of two other common *Philornis* hosts, the house wren (*Troglodytes aedon*) and the chalk-browed mockingbird (*Mimus saturninus*), to allow them to be exposed to botflies. After this manipulation, the nestlings were returned to their original baywing nests. Infected shiny cowbird chicks were obtained from *Mimus saturninus* nests that had been naturally parasitized by shiny cowbirds. The six experimental baywing nests were filmed continuously for 24 h using infrared microcameras (Handykam, UK) connected to digital video recorders (Lawmate PV-500, USA) to record the behaviors. Larvae on each nestling were counted before and after video recordings.

All baywing nests were visited daily during the nestling stage to inspect host and parasitic nestlings for the presence of larvae or scars (presumably removal scars) on their skin (Fig. 1a, b). We estimated the prevalence of *Philornis* as the percentage of nests that were infected with botfly larvae at 3 days post-hatching, since infection typically occurs within the first few days after hatching. During the 2013–2014 breeding season, we estimated the intensity of *Philornis* ectoparasitism by counting the number of larvae and scars on bawying, shiny, and screaming cowbird nestlings. Scars are typically easy to detect and last for at least 2 days (C. Ursino personal observation). To avoid overestimation, we noted in each counting the anatomical location of every larva and scar. It was not possible to record data blind because our study involved focal animals in the field.

Screaming cowbird nestlings were weighed (± 0.5 g) and measured for tarsus length (± 0.1 mm) daily or every other day to compare growth rates between non-infected nestlings and infected nestlings that had the larvae removed. Growth rate was estimated as the slope of the linear regression of nestling mass or tarsal length as a function of age (in days) between days 3 and 8 post-hatching, during which period nestling growth is approximately linear (De Mársico et al. 2010). We compared the growth rates of parasitized and non-parasitized nestlings with non-parametric Mann-Whitney test. Nestling survival was estimated as the number of screaming cowbird nestlings that survived to the age of 8 days at successful nests. We used a Spearman correlation to examine whether there was
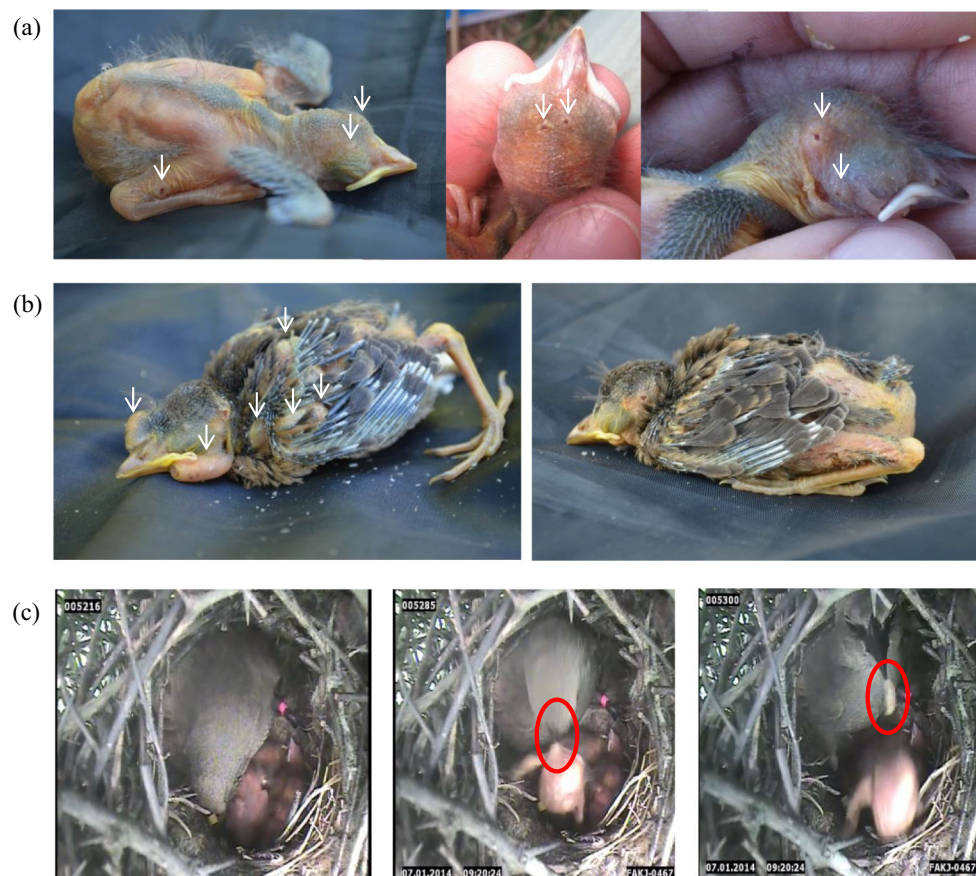
**Fig. 1** (**a**) Scars left by baywings on nestlings after removing *Philornis* larvae (left and center: screaming cowbird; right: baywing). (**b**) Infected shiny cowbird nestling before (left) and 24 h after (right) being experimentally cross-fostered to a baywing nest. (**c**) Larva removal sequence observed in video recordings

an association between number of baywing nests that were available for *Philornis* parasitism and number of nests infected throughout the breeding season (dilution effect).

## Results

**Experimental infections** We obtained direct observations of larva removal behavior in all of the six nests video recorded. Video recordings revealed that adult baywings removed larvae from experimentally infected nestlings by using the bill like a pair of tweezers to hold the larva by the spiracle and pull it from the nestling's skin (Fig. 1, ESM 1). Baywings began to preen their own and cowbird nestlings immediately after experimental introduction to the nest, turning the nestlings around to inspect and remove the larvae (ESM 1). They inspected the nest lining material as well. In all cases, most larvae (95–100%) disappeared within 24 h following manipulation, concomitant with the appearance of removal scars on nestlings.

**Description of natural botfly infection** Prevalence of *Philornis* larvae in baywing nests was 47.8% in 2012–2013 ($n = 23$) and

62.9% in 2013–2014 ($n = 27$), and it did not vary between years (Fisher's exact test $\chi^2_1 = 1.93$, $p = 0.27$). The earliest infection each season occurred within 4 days of the earliest hatching date (Dec. 8 and 17 for 2012–2013 and 2013–2014, respectively). The number of infected nests during the season was positively associated with the total number of nests with nestlings (Spearman correlation, $r = 0.75$, $n = 14$, $p = 0.002$). Intensity of botfly parasitism (mean ± SD) was $5.7 \pm 3.6$ larvae per nestling for baywings (32 nestlings in 14 nests), $7.3 \pm 5.1$ for screaming cowbirds (4 nestlings in 4 nests), and 5 for shiny cowbird (1 nestling). One hundred percent of the botflies were removed from infected nestlings ($n = 49$) and in 98% of these cases, the removal was at a very early stage of larvae' development (< 1 mm), disappearing within 1–2 days and leaving a characteristic scar on nestling skin (Fig. 1a, b). Only one baywing nestling had more developed larvae (3 of ∼ 5 mm).

**Nestling growth and survival** Growth rates did not differ between non-infected screaming cowbird nestlings and those that had the larvae removed (Table 1). We found no difference in fledging success between parasitized and non-parasitized nestlings (100% survival to the age of 8–9 days in both groups; $n = 6$ and 7 nestlings, respectively).

## Discussion

We present the first direct evidence for a *Philornis* host of active removal of botfly larvae from beneath nestlings' skin. To the best of our knowledge, no other species has been recorded to exhibit such adaptation against botfly parasitism, despite the detrimental effects of *Philornis* larvae on nestling growth and survival in a wide range of hosts. This finding adds to the body of knowledge on avian adaptations for reducing the impact of harmful ectoparasites (reviewed in Clayton et al. 2010). Interestingly, the mechanism of larva removal used by baywings has something in common with parasite resistance behaviors recorded in Darwin's finches parasitized by the invasive *Philornis downsi* (McNew and Clayton 2017). *P. downsi* larvae do not live subcutaneously, but emerge from the nest base at night to feed on nestlings and brooding adults. A study involving video recording of nests of three common finch species (*Geospiza fuliginosa*, *G. fortis*, and *Camarynchus parvulus*) showed a female finch using her bill to probe nest material and preen nestlings, possibly as a way to remove larvae from the nest (O'connor et al. 2010). However, how widespread this behavior is across non-native *P. downsii* host species is not well known (Kleindorfer and Dudaniec 2016; McNew and Clayton 2017).

Why has larva removal behavior evolved in baywings and not in other *Philornis* hosts? Baywings breed later than most other sympatric passerines and its breeding season overlaps completely with the reproductive season of botflies. Hence, virtually all nests are susceptible to botfly attacks and the prevalence of larvae is higher than that reported for other common hosts (Rabuffetti and Reboreda 2007; Segura and Reboreda 2011). In addition, the number of parasitized nests increased with the number of active nests with nestlings, indicating that increased nest availability did not dilute the risk of *Philornis* attacks. Given these conditions, *Philornis* ectoparasitism may impose a strong selective pressure on baywing populations, favoring behavioral defenses such as larva removal (Peng et al. 1987). Besides the prevalence of parasitism, the lack of resistance behaviors in other native and non-native hosts could also be the result of evolved tolerance towards *Philornis* larvae. For instance, nestlings of Galapagos

mockingbirds (*Mimus parvulus*) increased their begging effort when attacked by *P. downsii*, which elicited higher provisioning rates from parents and, thus, helped them to compensate the costs of infection (Knutie et al. 2016). Some studies suggest that tolerance to *Philornis* may be widespread among native hosts (McNew and Clayton 2017).

Baywings removed *Philornis* larvae indiscriminately from their own and brood parasitic nestlings, as previously suspected based on removal scars (Fraga 1984) and directly observed in this study. As far as we know, this is the first well-documented example of heterospecific ectoparasite cleaning in birds (Bush and Clayton 2018). All nestlings in the brood were preened exhaustively throughout the day, presumably by the breeding female, which would allow her to detect and remove the larvae at a very early developmental stage. As a result, *Philornis* larvae had negligible effects on nestling growth and survival of parasitic nestlings. Unfortunately, a direct test of the fitness effects of larva removal on nestling success was unfeasible because baywings show no plasticity in this behavior (i.e., nestlings always had the larva removed). Previous studies have reported lethal effects of botfly larvae on screaming and shiny cowbird nestlings in nests of other host species lacking larva removal behavior (Rabuffetti and Reboreda 2007; De Mársico and Reboreda 2008). Therefore, our results indirectly support that botfly removal behavior by baywings can improve the survival of parasitic cowbird nestlings at infected nests.

It has also been proposed that larva removal could have favored the specialization of brood-parasitic screaming cowbirds on baywing hosts (Fraga 1984). If this is correct, the survival of screaming cowbird nestlings should be higher in baywing nests than in nests of other potential hosts attacked by *Philornis* spp. Consistent with this hypothesis, screaming cowbird nestlings experimentally cross-fostered into nests of two unused, but suitable hosts, chalk-browed mockingbirds and southern house wrens, suffered higher mortality rates when infected with larvae (100%, $n = 6$ and 60%, $n = 10$, respectively) (De Mársico and Reboreda 2008; MCDM unpublished data) than in baywing nests (0%, $n = 11$; this study). Whether alternative screaming cowbird hosts like the brown-and-yellow marshbird *Pseudoleistes virescens* or the chopi blackbird *Gnorimopsar chopi* (Mermoz and Fernández 2003; Di Giacomo and Reboreda 2015) also provide protection against *Philornis* parasitism remains to be determined. Our results indicate that larva removal by baywings may provide fitness benefits to screaming cowbird nestlings, which may favor its use as hosts. Future comparative studies that examine the association between host use and botfly parasitism are needed to determine the importance of baywings' larva removal behavior in driving host specialization by screaming cowbirds.

**Table 1**  Comparison of growth estimates of screaming cowbird nestlings between nests infected and non-infected with *Philornis* larvae. Values are mean ± SE. Sample sizes are in parentheses

| Growth variables | Infected nests ($n = 4$) | Non-infected nests ($n = 7$) | Mann-Whitney $U$ test |
|---|---|---|---|
| Mass growth rate (g/day) | $4.7 \pm 0.2$ | $4.5 \pm 0.3$ | $w = 11, p = 0.54$ |
| Tarsus growth rate (mm/day) | $2.4 \pm 0.2$ | $2.2 \pm 0.2$ | $w = 11, p = 0.54$ |

**Data availability** The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

## Compliance with ethical standards

**Conflict of interest** The authors declare they have no conflict of interest.

**Ethical approval** Research presented in the manuscript was conducted in accordance with the Argentine law. The study was conducted under permit (202/12) from the local authority (OPDS, Province of Buenos Aires, Argentina). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. As the experimental protocols involving handling of birds were of minimal impact, the University of Buenos Aires committee for animal care and use did not intervene. No nestlings died or were injured during manipulations, and experimental infection had negligible effects on nestling growth and survival.

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