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Short Communication

# *Philornis* sp. bot fly larvae in free living scarlet macaw nestlings and a new technique for their extraction

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ABSTRACT

Bot fly larvae (*Philornis* genus) are obligate subcutaneous blood-feeding parasites of Neotropical birds including psittacines. We analyze twelve years of data on scarlet macaw (*Ara macao*) nestlings in natural and artificial nests in the lowland forests of southeastern Peru and report prevalence and intensity of *Philornis* parasitism. Bot fly prevalence was 28.9% while mean intensity was 5.0 larvae per infected chick. Prevalence in natural nests (11%, $N = 90$ nestlings) was lower than in wooden nest-boxes (39%, $N = 57$) and PVC boxes (39%, $N = 109$). We describe a new technique of removing *Philornis* larvae using a reverse syringe design snake bite extractor. We compare this new technique to two other methods for removing bots from macaw chicks and find the new method the most suitable.



## 1. Introduction

The parasitic fly genus *Philornis* (Meinert, 1890, Diptera, Muscidae) comprises 51 species (Carvalho et al., 1993; Skidmore, 1985) and has a mainly Neotropical distribution (Carvalho and Couri, 2002). Their larvae are obligate subcutaneous blood-feeding parasites of nestlings of a wide range of avian hosts (Allgayer et al., 2009; Arendt, 2000; Couri, 1999). Larval development is rapid taking 4–6 days in furuncles with their caudal spiracles extending through the dermal openings of their avian hosts (Uhazy and Arendt, 1986). *Philornis* infestations can increase bird mortality, decrease reproductive success, and affect nest site selection (Loye and Carroll, 1998). They may even increase extinction risk for some avian hosts (Fessl and Tebbich, 2002; Snyder et al., 1987). *Philornis* infestations have been noted repeatedly on parrot nestlings including macaws (Berkunsky et al., 2005; Nycander et al., 1995; Renton, 2002).

The Tambopata Macaw Project has been studying the breeding ecology and natural history of large macaws (*Ara* spp.) in natural and artificial nests in the southern Peruvian Amazon for over 20 years (Brightsmith et al., 2008; Brightsmith, 2005; Nycander et al., 1995). During nest inspections researchers found that scarlet macaw (*Ara macao*) nestlings heavily infested by bot fly larvae showed reduced survival (Nycander et al., 1995). Motivated by this observation, researchers at the site have opportunistically removed parasitic larvae to improve chick growth and fledging.

This situation gave rise to the following questions which guide the present study: (i) what are the overall rates of infestation, (ii) do different nest types affect levels of infestation, and (iii) what is the most suitable method of parasite removal in this particular host-parasite system?

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## 2. Materials and methods

The study was conducted in the forests surrounding the Tambopata Research Center (TRC) in south-eastern Peru (13° 8.070′ S, 69° 36.640′ W), in the Department of Madre de Dios, in the Tambopata National Reserve. The center is located in tropical moist forest near the boundary with subtropical wet forest (Tosi, 1960) at 350 m elevation with an average annual rainfall of 3236 mm (Brightsmith, 2004). At this site scarlet macaws nest in natural hollows (Brightsmith, 2005; Renton and Brightsmith, 2009) and in artificial wooden and PVC nest-boxes installed on emergent and isolated trees (Nycander et al., 1995).

We studied scarlet macaw nests in natural hollows, artificial PVC nests and wooden nest-boxes from November 2000 to March 2011 (12 breeding seasons). Nests were located within a 2.2 km radius of TRC. To determine the growth and health status of nestlings, we climbed to the nests using single-rope ascending techniques (Perry, 1978; Perry and Williams, 1981). We removed the chicks and lowered them to the ground in plastic buckets (Nycander et al., 1995). Once on the ground, each chick was checked visually for signs of bot flies and the number of bot flies was recorded. Chicks were also weighed and measured as part of ongoing studies (Vigo et al., 2011). On average, each of the 256 chicks involved in the study was handled 29.8 ± 1.7 SE times during the ±86 day period of nestling development. These visits lasted about 30–50 min. The anatomic location of bot fly infestations was recorded in 89 cases.

Three different methods of killing or removing the parasitic larvae were used over the course of the study. From 2000 to 2007 all bot fly larvae were treated with Negasunt® powder. The powder was placed liberally on the swollen area caused by the larvae. Normally only a single treatment was needed as the larvae were dead and swelling reduced by the next nest inspection 1–3 days later (DJB pers. obs.). In 2007 researchers attempted to remove the dead bot fly larvae using hemostats the day after treatment with Negasunt® powder. From 2007 to 2010 bot fly larvae were removed by holding an alcohol soaked swab against the skin over the larvae for about 30 s to prevent the larva from breathing and forcing it to the surface. The swab was then removed and the veterinarian removed the larva with a hemostat. Sometimes after removing the larva an anti-parasite aerosol (Curabichera Spray) was applied. This technique required speed and experience and was often unsuccessful in the case of small larvae located deep in the skin.

Starting in 2010 we began to remove bot fly larvae using the Sawyer Extractor™ Pump Kit (a reverse syringe design device designed to extract snake venom). Larvae were removed by (1) cleaning the area around the bot with an alcohol soaked swab, (2) placing the head of the extractor over the larva, and (3) depressing the plunger of the extractor to start the suction. Usually within a few seconds small larvae were sucked completely out of the bird. Larger larvae only partially emerged from the wound but were easily grasped and removed with a hemostat. After bot fly removal the area was cleaned with an alcohol swab and covered with an antiseptic cream.

To quantify levels of infestations, we calculated prevalence as the percent of all chicks which had ≥1 larvae with 95% exact confidence intervals (CI). We also calculated the mean and median number of larvae per chick with ≥1 larvae (heretofore intensities). As parasites typically show an aggregated distribution across host individuals (Crofton, 1971), we presented bias-corrected and accelerated bootstrap confidence limits (CI) around the mean and median intensities. We used Fisher's exact test and Mood's median test to compare prevalences and median intensities and present 2-sided exact p-values in each case. Index of discrepancy (Poulin, 1993) was used to quantify skewness of parasite distribution. For statistical analysis Quantitative Parasitology 3.0 was used (Rozsa et al., 2000).

The analyses discussed above included multiple chicks hatched and raised in the same nest. This means that our results may be influenced by pseudoreplication (treating each chick as statistically independent instead of the more conservative method of treating each different nest as statistically independent). To eliminate the effects of this pseudoreplication, we pooled all chicks hatched in the same nest through all years so that we created one prevalence (±SE), mean, and median intensity of its chick pool per nest. These fully independent parameters were compared across nest types using Kruskal–Wallis Tests using GenStat 13.2. Pearson chi-square test was used to compare observed and expected bot fly infestations in nests with multiple chicks.

We tested the effects of bot fly infestation on nestling growth using growth data from 45 scarlet macaw chicks studied from 2000–2008 as presented in Vigo et al. (2011). For each chick we determined the number of bot flies recorded during the following time periods: 0–33 days (the period of fast weight gain), 34–63 days (the period of slow weight gain) and 64 days to fledging (the period of weight loss). We used linear mixed models (LMM) of GenStat 13.2 to determine whether numbers of bot fly larvae in each of the above mentioned phases influence the (a) asymptotic size and (b) maximum growth rate and (c) age of maximum growth rate for the three biometric variables weight, wing, culmen, and tarsus.

## 3. Results

We monitored 19 natural tree cavities, 10 wooden and 19 PVC pipe boxes occupied by scarlet macaws and an average of 16.6 (±1.2 SE, range: 10–25) nesting events (laid at least 1 egg) per breeding season. We examined a total of 256 nestlings, 21.3 (±2 SE) nestlings per breeding season (range: 10–33 chicks). In total, 372 bot flies were registered during the 12 years of the study. Bot fly larvae prevalence was 28.9% (CI: 23.4–34.9%), mean intensity was 5.03 larvae per infected chick (CI: 3.54–7.81) and median intensity was 2 (CI: 1–2) botflies per infected chick. The index of discrepancy was 0.89 indicating a rather high level of skewness, close to the theoretical maximum of 1. Larvae were most frequently located on the wings (36% of 89 reports), in open internal cavities such as ears (10%) or nares (7%), on the feet (9%), the face (7%) or the rump (7%). Other body parts affected less frequently were the

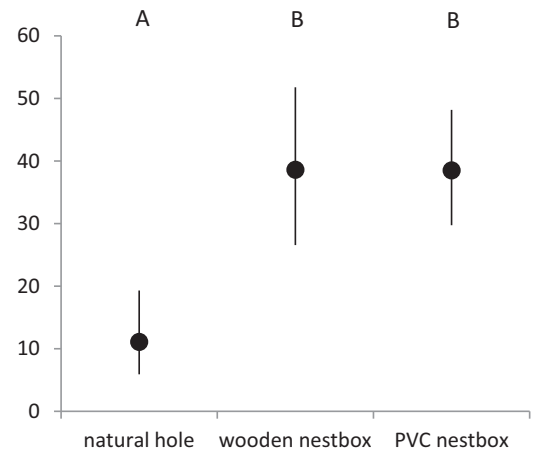


**Fig. 1.** The prevalence of *Philornis* infestations of scarlet macaw chicks in natural cavities (11%, $N = 90$ nestlings monitored), artificial wooden nest boxes (39%, $N = 57$), and PVC nest boxes (39%, $N = 109$) in southeastern Peru. Vertical lines represent 95% confidence intervals around means (black dots). Columns labeled with different letters differ significantly (Fisher's exact test; $p < 0.001$).

head, chin, neck, legs, and upper chest (24%). Bot fly infestations occurred from the second day to the 86th day of nestlings' age with a peak time of infestation in the first month. Bot fly infestations were not randomly distributed among chicks. In the 44 cases where there were multiple chicks in bot fly infested nests multiple chicks were infested in 50% of the cases. The probability that in nests with multiple chicks more than one chick has bot fly infestation was significantly higher than expected (chi-square = 12.5, 2 d.f., $P = 0.002$).

Larval prevalence in natural nests (11%, CI: 6–19%, $N = 90$ nestlings monitored) was significantly lower than in wooden nest-boxes (39%, CI: 27–52%, $N = 57$) and PVC boxes (39%, CI: 30–48%, $N = 109$, Fisher's exact test: $p < 0.001$; Fig. 1). Mean and median parasitism intensities did not differ significantly across different nest types (Bootstrap 2-sample $t$-test: $p_{(\text{natural vs. wooden})} = 0.219$, $p_{(\text{natural vs. PVC})} = 0.431$, $p_{(\text{PVC vs. wooden})} = 0.147$; Mood's median test for the 3 nest types: $p = 0.125$).

When data from each nest were pooled across years, the mean of prevalence in natural nests (13%, ±5.5 SE, $N = 17$ nests monitored) was significantly lower than in wooden nest-boxes (46%, ±8.7 SE, $N = 8$) and PVC boxes (27%, ±6.4 SE, $N = 12$; Kruskal–Wallis statistic = 9.5, $p < 0.009$). Mean and median intensities for nestlings did not differ significantly among nest types (Kruskal–Wallis statistics <1.9, $p > 0.39$ for all three comparisons).

Over the study period we killed or removed larvae from nestlings 188 times including repeated treatments of reinfected chicks. We attempted to remove larvae using Negasunt® Powder and hemostats ($N = 27$ cases), alcohol and hemostat ($N = 49$) and Sawyer Extractor™ ($N = 112$). The bot fly larvae were successfully removed from nestlings in 33% with the Negasunt® method, 80% with the alcohol and hemostat method, and 100% with the Sawyer Extractor™ method. The efficiency of the Sawyer Extractor™ method was significantly higher than the two other methods (Fisher's exact test $p < 0.001$).

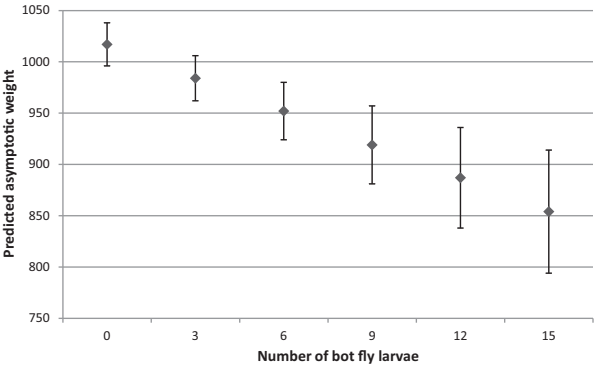


**Fig. 2.** The predictions of linear mixed model (LMM) for the effects of bot fly number for asymptotic weight (±SE) of scarlet macaw nestlings during 0–63 days.

Asymptotic tarsus length was negatively correlated with the number of bot flies during the fast growth phase (0–33 days) (LMM $_{\text{bot flies 0–33 days}}$: $\chi^2_1 = 7.81$, $P = 0.008$). Asymptotic body mass was negatively correlated with the number of bot flies during the fast growth phase (LMM $_{\text{bot flies 0–33 days}}$: $\chi^2_1 = 6.64$, $P = 0.014$) and during the 0–63 day phase as well (LMM $_{\text{bot flies 0–63 days}}$: $\chi^2_1 = 6.59$, $P = 0.015$). Higher bot fly number also predicted lower weight of nestlings in these phases (LMM predictions; Fig. 2).

A total of 10 bot infested chicks died during the study, but only 3 were confirmed to have died due to the infestations: one died at age of 33 days due to a bot fly related ear infection, one died at 40 days old of infection after 26 larvae were detected all over its body, wings, head and nostrils, and one died at age 26 days after a single bot severed tendons in the leg and the bird was unable to stand. In some cases we observed the natural disappearance of *Philornis* larvae before expected emergence day. We cannot exclude the possibility that adult birds may remove some larvae from their chicks.

## 4. Discussion

Artificial nests are important tools in conservation of different parrot species. By testing different types of artificial nests compared to natural ones can result better designs for the birds. In this study we compared parasite prevalence among different nests to see whether any of the nest types results in higher bot fly infestation. Parasite prevalence was significantly lower in natural nest hollows than in either artificial wooden or PVC nests. This could be the result of the material of the nest, as usually temperature in PVC nests can raise quickly and might result in higher parasite prevalence (DJB unpubl. data). However, mean and median intensity did not differ significantly among nest types. The most extreme intensities in our study (63, 40 larvae per chick) were higher than those found for other Neotropical parrot chicks: 31 larvae for a hyacinth macaw (*Anodorhynchus hyacinthinus*) nestling (Guedes, 1993), >15 larvae per scarlet macaw nestling (Nycander et al., 1995), and >25 larvae in two blue-fronted amazon (*Amazona aestiva*) nestlings (Seixas and

Mourao, 2003). But are much lower than some reports for passerines: pearly-eyed thrashers (*Margarops fuscatus*) had a maximum of 220 larvae/nestling with an overall mean intensity of 37 (Arendt, 1985).

Bot fly larvae are subcutaneous blood-feeders whose presence may facilitate secondary bacterial infections. However we found little evidence of this as most infested chicks survived to fledging. In general higher bot fly numbers during early development correlated with smaller overall chick size (weight and tarsus). We suspect that the bot flies are causing reduced chick size, but we cannot rule out the possibility that smaller nestlings get more bot flies for some other reason related to parental care or other unmeasured variable. Our findings that direct mortality caused by bot flies was uncommon agree with those from the literature, where the clearest direct impacts on chick health are from cases where the larvae invade sensitive locations such as sensory organs, respiratory pathways, mouth, or limbs (Arendt, 2000).

Removing larvae may be helpful to the chicks, but also comes with a risk of injury, infection, impairment, and even death to the host if done incorrectly. For this reason, it is important for field personnel to use methods which maximize the benefits while minimizing risk. The Negasunt® Powder used contains 3% Coumaphos that kills the bot fly larvae in the bird, 2% Propoxur that repels other insects from the lesion and 5% Sulfanilamide anti-bacterial. We found no gross negative effects on the chicks. However, Coumaphos is classed as highly to very highly acutely toxic to birds if consumed (Abdelsalam, 1999; Abou-Donia et al., 1982; US-EPA, 1996) and may be consumed by either by the parents or chicks. Therefore, we feel that using Negasunt® Powder should be avoided in wild birds.

The 'alcohol and hemostat' method reduces the risk of toxicity to the chick but it had a lower rate of success as bots that did not come to the surface of the skin were difficult to remove. The individual level of skill and veterinary training of the person using the technique also appeared to influence success. In addition, when unsuccessful, follow up attempts to remove the larvae often required incisions to remove the living or dead larvae. As a result, we do not recommend this method for extracting bot fly larvae.

By comparison, the new extractor method described here was highly efficient (100% in this study) and relatively easy for researchers of varied levels of skill and training. The age of the youngest macaw nestling we have subjected to this method was 2 days and we performed the process without complications. However, there are two concerns. When the bot is in areas where the extractor cannot get a good seal (tip of the wing, toe, etc.) suction may not be sufficient to remove the bot. In addition, the design of the extractor we used does not allow researchers to regulate the amount of suction. As a result, one must be careful when applying this method to young chicks of small-bodied species so as not to tear the skin. For this reason researchers interested in using this technique should test it first on older individuals and monitor for bruising and skin tears before trying it on younger individuals.

Bot flies of various genera are known to infect a wide array of wild and domesticated vertebrate hosts (Angulo-Valadez et al., 2010; Cogley and Cogley, 2000; Milton, 1996) and this new extractor method should be effective on a wide range of taxa. If an extractor with variable suction levels was available, it would allow removal and collection of skin-dwelling arthropods from an even broader array of vertebrate hosts. Regardless, as presented, this technique should have broad application for veterinarians and scientists who wish to remove parasitic fly larvae quickly and easily without making incisions.

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