Host use by *Philornis* sp. in a passerine community in central Argentina

Uso de hospedadores por *Philornis* sp. en una comunidad de aves paseriformes de la parte central de Argentina

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Abstract. We studied host use by parasitic botflies (*Philornis* sp.) in a passerine community in central Argentina and analyzed characteristics of nests and hosts associated with botfly parasitism. We conducted a four-year field study as well as a bibliographical survey where we determined: presence of botfly parasitism, type of nest, presence of green material and small sticks in the nest, average height of the nest, date of last nesting attempt during the breeding season and egg volume (as a surrogate of species body mass). Our field study of 3 birds species showed that botflies parasitized *Troglodytes aedon* (25% of nests), but not *Sicalis flaveola* and *Tachycineta leucorroha* in spite of nesting in similar boxes, at the same place and during the same time of the year. However *T. aedon* built nests using dry material while *S. flaveola* and *T. leucorroha* used green material.The analysis of published data (35 species considered) showed a negative association between botfly parasitism and presence of green material in the nest, and a positive association between botfly parasitism and presence of small sticks in the nest and date of the last nesting attempt during the breeding season. Our results indicate that the materials used to build the nest and the extent of the breeding season are factors that influence host use by botflies in central Argentina.

Key words: birds, central Argentina, host use, miasis, ectoparasites.

Resumen. Analizamos el uso de hospedadores de moscas parásitas del género *Philornis* en una comunidad de aves paseriformes en la región centro de Argentina, así como las características de nidos y hospedadores asociadas con el parasitismo de *Philornis*. Se realizó un estudio de campo de 4 años así como una revisión bibliográfica donde determinamos: presencia de parasitismo de *Philornis*, tipo de nido, presencia de material verde y pequeñas ramas en el nido, altura promedio del nido, fecha del último intento de nidificación y volumen del huevo (como un estimador de la masa corporal de las especies). Los datos de nuestro estudio de campo mostraron que *Philornis* parasitó a *Troglodytes aedon* (25% de los nidos), pero no nidos de *Sicalis flaveola* y *Tachycineta leucorroha*, a pesar de estar nidificando en cajas nidos en un mismo sitio y época del año. Sin embargo, *T. aedon* utilizó material seco para construir el nido, mientras que S. *flaveola* y *T. leucorroha* lo hicieron con material verde. El análisis de la información bibliográfica (35 especies consideradas) mostró una asociación negativa entre el parasitismo de *Philornis* y la presencia de material verde en el nido y una asociación positiva entre el parasitismo de *Philornis* y la presencia de pequeñas ramas en los nidos y la fecha del último intento de nidificación en la temporada reproductiva. Nuestros resultados indican que los materiales usados para construir los nidos y la duración de la temporada reproductiva son factores que afectan el uso de hospedadores por parte de *Philornis* en la región central de Argentina.

Palabras clave: aves, región central de Argentina, uso de hospedador, miasis, ectoparásitos.

Introduction

Nests of alticial birds are microhabitats inhabited by a wide variety of invertebrates (Hicks, 1971; Szabó et al., 2002; Turienzo and Di Iorio, 2007) that find there a source of energetic resources and protection (Majka et al., 2006). Some of these species are external parasites like feather lice (Phthiraptera, Clayton et al., 1999, 2008), mites (Parasitiformes and Acariformes, Proctor and Owens, 2000), fleas (Ceratophyllidae, Tripet and Richner, 1999) and bugs (Hemiptera, Brown and Brown, 2004). Other species, like flies of the genus *Philornis* (Diptera) are typically subcutaneous parasites (i.e. Texeira, 1999; Spalding et al., 2002).

The genus *Philornis* (hereafter botflies) comprises approximately 50 species with neotropical distribution (Dodge and Aitken, 1968). This group has special interest

because their larvae parasitize nestlings establishing different types of associations (coprophagous, semihaematophagous or subcutaneous) and reduce markedly chick survival (i.e. Couri and Carvalho, 2003; Dudaniec and Kleindorfer, 2006). Previous studies on botflies have mostly focused on the impact they produce on host growth and survival (see Dudaniec and Kleindorfer, 2006), and on the influence of some environmental conditions on the frequency of parasitism (Delannoy and Cruz, 1991; Arendt, 2000; Antoniazzi et al., 2010), but to our knowledge, no previous studies have analyzed the pattern of host use by botflies in a passerine community.

Botfly parasitism may be influenced by host characteristics. For example, it has been reported that frequency of botfly parasitism increases as the breeding season advances (Arendt, 1985a, 1985b; Young, 1993; Rabuffetti and Reboreda, 2007) and therefore, early breeder species would have a higher probability to scape parasitism. Similarly, the materials used to build the nest, the type of nest (i.e. open or closed), or its location could also influence the probability of botfly parasitism. Clark (1990) stated that the presence of secondary compounds in green material (leaves) used to build the nest might reduce the probability of ectoparasites (Nest Protection Hypothesis) and some hosts reduce the load of ectoparasites (i.e. blowflies, Ontiveros et al., 2007 or fleas, Shutler and Campbell, 2006) by adding nest material with secondary volatile compounds. Host body mass may also be important for explaining patterns of botfly parasitism as larger hosts can support higher parasite loads (Dudaniec and Kleindorfer, 2006; Dudaniec et al., 2007) and therefore could be preferred by botflies.

The objective of this study is to describe host use by botflies in a passerine community of central Argentina and analyze characteristics of hosts and nests associated with botfly parasitism. Considering the previous information on the interactions between botflies and their hosts we expect a negative association between host use and presence of green material in the nest and a positive association between host use and 1) date of last nesting attempt during the breeding season and 2) body mass of the host.

Materials and methods

Field study. We collected data on botfly parasitism on 3 potential hosts that nest in cavities (House Wren, *Troglodytes aedon*, Saffron Finch, *Sicalis flaveola*, and White-rumped Swallow, *Tachycineta leucorrhoa*) at 2 sites near the city of Santa Fe (Argentina), during the breeding seasons (October – February) 2004-2005, 2005-2006, 2006-2007 and 2007-2008. Site A was located on the campus of University of Litoral (31°38' S, 60°40' W) and site B at a private cattle ranch about 10 km away from Site A (31°38' S, 60°35' W).

Study sites were seasonally flooded marsh/woodland areas located at the Paraná River floodplain and surrounded by many watercourses like Setubal lagoon and Colastiné River. Sites included environmental units such as aquatic vegetation, forest, beach and gallery forest where *Salix humboltiana*, *Acacia caven*, *Tessaria integrifolia*, *Azola* sp., *Salvinia* sp. and *Pistia stratiotes* were strongly represented. Mean monthly temperatures for the studied years were 27.6°C in January (mid-summer) and 13.9°C in July (mid-winter). Average annual rainfall at this site was 1083 ± 54 mm (mean ± SE for the period 1989-2008).

To facilitate data collection we placed 60 and 56 nest boxes at sites A and B, respectively. Boxes were on poles at a height of 1.6 m and at least 20 m apart. Their external measurements were 25.4 x 16.5 x 17.8 cm (height, width, depth) and had a 3.8 cm (in diameter) entrance hole and a lateral opening. We checked nest boxes daily during laying and near the time of hatching, and every 2-3 days during incubation and after hatching. We checked nests until chicks were 12 days of age (House Wren and Saffron Finch) and 15 days of age (White-rumped Swallow). At that time, we stopped physically checking the nests to avoid premature fledging. Each nestling was carefully examined looking for botfly larvae and the day each nest had its first chick infested was registered. We also recorded the material used to build each nest.

At our study site the House Wren and Saffron Finch began laying during early October and continued until early-mid February. Clutch size was 3-5 eggs (modal size= 4) and eggs were incubated for 13-14 days. Nestlings fledged when they were 14-15 days of age (Quiroga, 2009). White-rumped Swallows began laying during late September and continued until early December. Clutch size was 3-6 eggs (modal size= 5) and eggs were incubated for 13-14 days. Nestlings fledged when they were 20-22 days of age (2004-2006: Lorenzón 2010; 2007-2008: Quiroga unpublished data).

Bibliographical review. Based on results observed from our field data we decided to test if the observed pattern (see results) was consistent at a community level. We then collected published information of host use by botflies through a bibliographical survey of studies on the breeding biology of passerine species of central Argentina. We did not include our own field data here since it was collected form nest boxes instead of wild nests (as provided by bibliographical data). Because most studies did not identify the parasite to the species level and did not provide data on intensity of parasitism, for our analysis we considered hosts as parasitized by botflies (i.e. flies of the genus *Philornis*) and did not include intensity of parasitism as a variable. The species of *Philornis* reported for this region are *P. seguyi* and *P. torquans* (Couri et al., 2009). Data on host use were

obtained from 3 studies conducted in Santa Fe Province (De la Peña et al., 2003; De la Peña 2005, 2010). We obtained additional data from another study conducted by Nores (1995), in Cordoba Province.

Statistical analysis. For our analysis we only included data of species with 5 or more nests with chicks (n= 35 species, Appendix 1). For each species we collected the following information: 1), presence of botflyparasitism (0/1); 2), type of nest (open or closed, we included dome like and cavity nests in the latter category); 3), presence of green material (0/1) and small sticks (0/1) in the nest; 4), average height of the nest; 5), date of the last nesting attempt during the breeding season, and 6), volume of host eggs (as a surrogate of host body mass). Egg volumes were calculated based on average values of egg length and width reported by De la Peña (2005, 2010) using Hoyt's (1979) formula:

$$volume= 0.0051 \times length \times width^2$$

We used contingency tests to analyze the association between botfly parasitism and presence of green material and small sticks in the nests and type of nest. To analyze the association between botfly parasitism (dichotomous variable) and other continuous variables we performed a logistic regression with presence of botfly parasitism (0/1) as dependent variable and: 1), date of the last nesting attempt during the breeding season (day 0= September 15th); 2), nest height, and 3), egg volume as independent variables. All tests were 2 tailed, and differences were considered significant at $p < 0.05$. Reported values are means ± SE.

Results

We surveyed 157 House Wren, 62 Saffron Finch and 97 White-rumped Swallow nests. Frequency of parasitism in the House Wren was 25% and did not differ significantly among years (goodness of fit: G_3= 5.9, p= 0.18) or sites (goodness of fit: G_3= 3.37, p= 0.07). Botflies parasitized 147 House Wren chicks in 39 nests. We did not detect any evidence of parasitism in the Saffron Finch and the White-rumped Swallow and no other parasites were found on nestlings or nests of the 3 studied species. The House Wren used dry material (small sticks) to build their nests while the Saffron Finch and the White-rumped Swallow built their nests with green material (swallows also added feathers). Egg morphology and breeding season span of the studied species are shown in Table 1.

Nest boxes were occasionally used by House Sparrows (*Passer domesticus*). However as a consequence of the low number of cases (n= 8) and nest architecture (dome shaped) we decided not to check nestlings in order to avoid destroying the nests.

Table 1. Egg morphology and breeding season span (years 2004 to 2008 combined) of 3 species nesting in nest boxes in central Argentina. Reported values are means ± SE

Species	EL	EW	EM	EV	FNA	LNA
T. aedon	17.02 ± 0.04	12.93 ± 0.02	1.58 ± 0.04	1.46 ± 0.07	Sept 9th	Feb 15th
S. flaveola	19.08 ± 0.1	13.86 ± 0.05	1.97 ± 0.02	1.871 ± 0.17	Nov 15th	Feb 14th
T. leucorroha	20.25 ± 0.99	14.13 ± 0.56	2.17 ± 0.21	2.04 ± 0.14	Sept 26th	Dec 5th

EL= egg length (mm). EW= egg width (mm). EM= egg mass (g). EV= egg volume (cm³). FNA= date of first nesting attempt. LNA= date of the last nesting attempt.

We also analyzed botfly parasitism (whether bird species were parasitized or not) in 35 passerines species belonging to 10 families (Appendix 1), where 11 of them (31%) were parasitized by botflies, 30 (86%) had open nests and 23 (66%) used green material to build the nest. Nest height ranged from 45 to 490 cm, date of the last nesting attempt varied between November 6 and March 14 and egg volume varied between 1.12 and 6.32 cm³.

The proportion of species parasitized with botflies was lower for species with green material in the nest that in those without green material (with: 4/23, without: 7/12, Contingency test, χ^2= 6.13, p= 0.01, n= 35). On the contrary, the proportion of species parasitized with botflies was higher in the species with small sticks in the nest than those without small sticks (with: 7/11, without: 4/24, Contingency test, χ^2= 7.72, p= 0.005, n= 35). There was no association between botfly parasitism and type of nest (open nests 9/30, closed nests 2/5, Contingency test, χ^2= 0.33, p= 0.85, n= 35). In addition, there was a positive association between botfly parasitism and date of the last nesting attempt during the breeding season (Logistic regression, χ^2= 4.34, p= 0.04, n= 35 [Fig. 1]), but there was no association between botfly parasitism and nest height (Logistic regression, χ^2= 0.35, p= 0.54, n= 35). With regard to botfly parasitism and host egg volume there was a tendency (although nonsignificant) towards a positive association (Logistic regression, χ^2= 3.2, p= 0.07, n= 35).

Discussion

Our field data indicated that despite nesting at the same location, at the same time and in the same type of nest-boxes, botflies parasitized House Wrens, but did not parasitize Saffron Finches and White-rumped Swallows. The main difference between these 3 species is that the
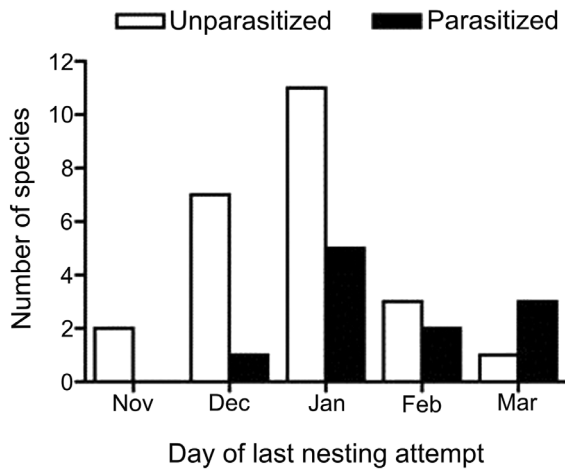
Figure 1. Number of passerine species of a region of central Argentina unparasitized and parasitized by botflies (*Philornis* spp.) according to the date of the last nesting attempt in the breeding season (n= 35).

Saffron Finch and the White-rumped Swallow used green material to build their nests, while the House Wren used small dry sticks. We considered this may be the reason why the first 2 species were not parasitized. Egg morphology and breeding season span data collected from our nest box study was similar to that provided by the bibliographical survey. Prevalence of parasitism in House Wrens (16.7%) was slightly higher than that observed by Antoniazzi et al. (2010) for this species in a nearby area and similar to other species (*Pitangus sulphuratus:* 25%, *Paroaria coronata:* 20% and *Phacellodomus sibilatrix:* 23%). Studies by Young (1993) of the House Wren in a different geographical area (Costa Rica) and parasitized by another botfly species (*P. carinatus*), mention an average prevalence of parasitism of 22.1% (La Lucha: 27.3%, San Luis: 30.6 and Monteverde: 8.4), values which are similar to those reported in our study.

As observed in our field study, the analysis of host use by botflies in 35 passerine species of central Argentina also showed a negative association between botfly parasitism and presence of green material in the nest, and a positive association between botfly parasitism and presence of small sticks in the nest. These associations were not independent, as most nests that contained green material did not contain small sticks and viceversa.

In a recent study, Antoniazzi et al. (2010) also described botfly parasitism in a bird community of central Argentina (31°23' S, 60°55' W). These authors reported 12 species with 5 or more nest records that were parasitized by botflies, 10 of which coincided with those reported in our study.

Our results were consistent with the hypothesis of a repellent effect of green material on ectoparasites (Nest Protection Hypothesis, Clark, 1990), but also with the hypothesis of an attractive effect on botflies of the dry material. In regard to the former hypothesis, several studies have noted that some secondary metabolites present in plants may act as toxins for arthropods (Lozano, 1998; Petit et al., 2002; Dawson, 2004; Shutler and Campbell, 2006). Moreover, experimental work demonstrated that the addition of plants containing secondary volatile compounds to the nest resulted in a marked reduction on the load of mites (Clark and Mason, 1988) and fleas (Shutler and Campbell, 2006). The effect of some secondary compounds has been also tested in laboratory studies, which show that the growth and development of mites are effectively reduced by exposing them to plant species present in nest material (Clark and Mason, 1985). However, because our study is correlational and the presence of green and dry material in the nest are negatively associated, we cannot rule out the hypothesis of an attractive effect of the dry material on botflies.

We also found that species that nested late in the breeding season have a higher probability of botfly parasitism. The same pattern has been found within species (i.e. increase in the frequency of parasitism with time of breeding, Arendt, 1985a, 1985b; Young, 1993; Dudaniec et al., 2007; Rabuffetti and Reboreda, 2007). This association could be the result of seasonal variation in food resources available for adult botflies, or variation in ambient temperature or rainfall (Arendt 1985b, 2000; Delannoy and Cruz, 1991). Alternatively, the increase in frequency of parasitism with time of breeding could be the result of an increase in botfly population. Because hosts are not available during winter it is likely, as observed in other dipterans (i.e. Krafsur et al.,1985; Schmidtmann and Pickens, 1986; Danks, 2006), that botflies have overwintering pupae. This would allow the survival of a few individuals from the end of one breeding season to the beginning of the following one. At that time, a few adults will start to reproduce and, as new adults emerge, the size of botfly population and the frequency of botfly parasitism will increase.

To summarize, our results indicate that the materials used to build the nest and the extent of the breeding season are factors that influence host use by botflies. Further studies on the mechanisms involved in host selection may help us to better understand the population dynamics of these parasites and to predict the impact they may produce on preferred host species. It is also desirable for future studies to consider intensity of infestation (and not just presence/absence and prevalence of parasitism) since this may highly influence chick survival.

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Appendix 1. List of species unparasitized and parasitized by botflies (*Philornis* sp.) in a passerine community of central Argentina, and species and nests characteristics. Data were obtained from: a), De la Peña et al. (2003); b), De la Peña (2005); c), De la Peña (2010), and d), Nores (1995). # nests: number of nests surveyed. Par: absence (0) or presence (1) of *Philornis* parasitism. Type of nest: open (0) or closed (1) nests. Nest height: average nest height (m). Date: date of the last nesting attempt in the breeding season.

Species	Family	# nests	Par	Type of nest	Nest height	Date	EV	GM	DM
Furnarius rufus [b,c,d]	Furnaridae	6	0	1	1.86	15-Dec	6.02	0	0
Pseudoseisura lophotes [d]	Furnaridae	67	1	0	4.9	12-Jan	6.32	0	1
Certhiaxis cinnamomea [a,b,c,d]	Furnaridae	5	1	0	1.22	16-Jan	2.2	0	1
Anumbius annumbi [d]	Furnaridae	50	1	0	2.27	2-Jan	3.88	0	1
Schoeniophylax phryganophila [a,b,c,d]	Furnaridae	5	1	0	3.38	14-Feb	2.45	0	1
Phacellodomus ruber [a,b,c,d]	Furnaridae	5	1	0	3.71	2-Jan	3.86	0	1
Phacellodomus sibilatrix [b,c,d]	Furnaridae	7	0	0	2.6	30-Dec	2.51	0	1
Phacellodomus striaticollis [b,c,d]	Furnaridae	5	0	0	1.7	30-Dec	3.16	0	1
Xolmis irupero [b,c,d]	Tyrannidae	7	0	1	2.32	27-Nov	3.59	0	1
Fluvicola albiventer	Tyrannidae	8	0	0	1.6	28-Jan	1.95	1	0
Satrapa icterophrys [b,c,d]	Tyrannidae	6	0	0	1.96	1-Dec	2.23	1	0
Pitangus sulphuratus [a,b,c,d]	Tyrannidae	5	1	0	3.56	15-Jan	5.88	1	0
Tyrannus savana	Tyrannidae	9	0	0	3.75	18-Jan	2.67	1	0
Empidonomus aurantioatrocristarus [b,c,d]	Tyrannidae	10	0	0	3.47	18-Jan	2.36	0	1
Sublegatus modestus [a,b,c,d]	Tyrannidae	5	1	0	1.54	24-Dec	1.59	1	0

Appendix 1. Continues.

Species	Family	# nests	Par	Type of nest	Nest height	Date	EV	GM	DM
Myiophobus fasciatus [b,c,d]	Tyrannidae	5	0	0	1.28	10-Feb	1.78	1	0
Pyrocephalus rubinus [b,c,d]	Tyrannidae	7	0	0	3.1	2-Jan	1.46	1	0
Elaenia spectabilis [b,c,d]	Tyrannidae	9	0	0	3.9	14-Feb	3.28	1	0
Elaenia parvirostris [b,c,d]	Tyrannidae	13	0	0	3.43	17-Feb	2.08	1	0
Tachycineta leucorroha [b,c,d]	Hirudinidae	62	0	1	2.41	16-Dec	2.1	1	0
Troglodytes aedon [b,c,d]	Troglodytidae	23	1	1	1.77	4-Mar	1.56	0	1
Mimus saturninus [a,b,c,d]	Mimidae	19	1	0	2.15	1-Feb	6.28	0	1
Turdus amaurochalinus [b,c,d]	Turdidae	5	0	0	2.98	27-Dec	5.58	1	0
Turdus rufiventris [b,c,d]	Turdidae	5	0	0	3.06	18-Jan	5.91	1	0
Polioptila dumicola [b,c,d]	Polioptilidae	29	0	0	2.19	12-Jan	1.12	1	0
Saltator aurantiirostris [b,c,d]	Cardinalidae	8	0	0	2.73	12-Jan	4.82	1	0
Paroaria coronata [a,b,c,d]	Emberizidae	14	1	0	3.56	14-Mar	3.31	1	0
Sporophila caerulescens [b,c,d]	Emberizidae	9	0	0	0.52	18-Jan	1.33	1	0
Sicalis flaveola [b,c,d]	Emberizidae	34	0	1	2.75	1-Mar	1.94	1	0
Zonotrichia capensis [b,c,d]	Emberizidae	11	0	0	1.43	22-Dec	2.23	1	0
Poospiza nigrorufa [b,c,d]	Emberizidae	6	0	0	0.45	2-Jan	2.26	1	0
Poospiza melanoleuca [b,c,d]	Emberizidae	5	0	0	1.7	27-Nov	1.75	1	0
Saltatricula multicolor [b,c,d]	Emberizidae	6	0	0	1.35	15-Jan	3.03	1	0
Icterus cayanensis [b,c,d]	Icteridae	6	0	0	3.75	25-Jan	2.99	1	0
Agelaioides badius [a,b,c,d]	Icteridae	11	1	1	2.59	10-Mar	3.61	1	0

EV= average volume of host eggs (cm3). GM= absence (0) or presence (1) of green material (leaves of different vegetal species) in the nest. DM= absence (0) or presence (1) of small sticks (dry material) in the nest.