

PARASITISM BY BOTFLIES *PHILORNIS* SP.
ON EUROPEAN STARLINGS *STURNUS VULGARIS*,
AN EXOTIC BIRD IN ARGENTINA

PARASITISMO DE ESTORNINOS PINTOS *STURNUS VULGARIS*,
UN AVE EXÓTICA EN ARGENTINA,
POR MOSCARDONES *PHILORNIS* SP.

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SUMMARY.— We studied the parasitism of the exotic European starling *Sturnus vulgaris* by native botflies *Philornis* spp. in Argentina. We installed thirty nest boxes in the northeastern Buenos Aires province in the 2010-2011 breeding season. In the first brood, subcutaneous larvae of *Philornis* (Muscidae) parasitised 34 nestlings (89.4%) of 11 clutches (91.6%) and only three nestlings fledged. In the second brood, *Philornis* parasitised 15 (48.3%) nestlings of seven clutches (70%) and all the nestlings died. Compared to the mortality of other Neotropical birds parasitised by *Philornis*, the mortality in European starling nests is the highest found in the region. Our results show that the studied population of European starling suffers a high level of parasitism by *Philornis*, although this factor was not directly associated with the high mortality of starling nestlings. The fact that most non-parasitised nestlings also died indicates that other factors are also affecting nestling survival. Experiments that allow us to isolate the effect of *Philornis* from other variables would be needed to evaluate the impact of botfly larvae on starling nestlings.

Key words: Argentina, botfly, ectoparasitism, nestling mortality, reproductive success.

RESUMEN.— Estudiamos el parasitismo de una especie de mosca nativa del género *Philornis* sobre el estornino pinto *Sturnus vulgaris* en Argentina, que es un ave introducida recientemente en este país. Utilizamos 30 cajas-nido en el noreste de la provincia de Buenos Aires en el período de reproducción 2010-2011. En la primera nidada fueron parasitados 34 pollos (89,4%) de 11 nidos (91,6%) por larvas

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subcutáneas de *Philornis* (Muscidae) y sólo tres pollos sobrevivieron. En la segunda nidada fueron parasitados por *Philornis* 15 pollos (48,3%) de 7 nidos (70%) y todos murieron. Si comparamos la mortalidad de otras aves neotropicales parasitadas por *Philornis*, la mortalidad de los pollos de estornino pinto es la mayor encontrada en la región. Nuestros resultados muestran que la población de estornino pinto estudiada sufre un alto nivel de parasitismo por *Philornis* pero este factor no estuvo directamente asociado a la alta mortalidad de pollos de estornino. El hecho de que la mayoría de los pollos parasitados y no parasitados murieran indica que otros factores están afectando la supervivencia de los pollos. Serían necesarios experimentos que permitan aislar el efecto de *Philornis* del resto de las variables para poder evaluar el impacto de las larvas sobre los pollos de estornino.

Palabras clave: Argentina, ectoparasitismo, éxito de la reproducción, mortalidad de volantones, moscardón.

INTRODUCTION

Exotic species that colonise a new place are especially susceptible to the effects of enemies such as parasites that they meet for the first time (Blackburn *et al.*, 2009). The newcomers' success and impact on the receipt community are highly affected by the direct and indirect effect of parasites (Tompkins *et al.*, 2011). If they avoid the unfamiliar parasites, the probability of population growth from the initial low numbers increases (Blackburn *et al.*, 2009). In particular, parasites that affect reproduction or mortality have a high potential to influence population levels (Newton, 1998). Møller and Cassey (2004) found that introduced bird species with stronger T-cell responses were more successful in becoming established. Such non-specific immunological characteristics allow individuals to defend themselves against new acquired parasites, allowing a higher survival and fecundity of exotic populations (Blackburn *et al.*, 2009). Conversely, it has also been shown that introduced parasites can significantly affect native bird populations (Fessl and Tebich, 2002).

The European starling *Sturnus vulgaris* is native to Europe, Asia and North Africa. It is included in the list of the 100 worst global invaders (Lowe *et al.*, 2004). It has become successfully established in New Zealand, Australia, South Africa, the United States,

Canada, Mexico and some Pacific and Caribbean islands (Feare, 1984). In Argentina, the first report of European starlings dates from 1987 in Buenos Aires city (Pérez, 1988). Thereafter it has been recorded in seven provinces: Buenos Aires (Peris *et al.*, 2005), Entre Ríos (Peris *et al.*, 2005; Jensen, 2008), Santa Fé (Peris *et al.*, 2005), Córdoba (Klavins and Álvarez, 2012), Mendoza (Zanotti, 2013), San Juan (Lucero, 2013) and Tucumán (Ortiz *et al.*, 2013). Thus far there have been no records of the starling in the southern part of the country.

In the original range of the European starlings, chicks are parasitised by the larvae of three Palearctic blowfly species (Diptera: Calliphoridae): *Protocalliphora azurea* (Hicks, 1971), *Protocalliphora falcozi* and *Trypocalliphora lindneri compacta* in a mixed infestation pattern (Grunin, 1966). We have not been able to find data in the literature on the mortality of chicks infested with these parasitic species. Liker *et al.* (2001) found that in Hungary the bird fly *Carnus hemapterus* (Diptera: Carnidae) parasitised starlings intensively, with a prevalence of 69% among nestlings, but parasite abundance did not correlate with the mortality or growth rate of nestlings.

After the introduction of the European starlings in North America in 1890 (Colautti *et al.*, 2005) some Nearctic species of *Protocalliphora* were found to parasitise their

nestlings, with little impact on breeding success (fledging survival: 89-94%) (Collins and De Vos, 1966). In Guelph, Ontario, the average egg-to-fledgling ratio was 78.6% (Collins and De Vos, 1966) and similarly, Mumby (1979) reported 75-80% fledgling survival in parasitised nests.

Botflies of the genus *Philornis* Meinert, 1890 (Diptera: Muscidae) have a Neotropical distribution, except for a few records in the United States, considered as Neotropical immigrants (Di Iorio and Turienzo, 2009). In the Neotropical Region, 23 species of *Philornis* are obligate subcutaneous parasites of birds, whose larvae feed on nestling tissues and blood cells (Teixeira, 1999). These parasites affect reproductive success, causing high nestling mortality (Arendt, 1985b) and delaying their growth and development (Arendt, 1985a), lowering their red blood cell counts and causing physical deformation (Dudaniec and Kleindorfer, 2006). A total of 146 bird species have been recorded as botfly hosts (Teixeira, 1999).

In Argentina, Quiroga and Rebores (2012) found lethal and sublethal effects of *Philornis seguyi* on nestlings of the house wren *Troglodytes aedon* (54% of the nestlings in parasitised nests died). Segura and Rebores (2011) observed similar effects on nestlings of red-crested cardinals *Paroaria coronata* (no nestlings fledged in 17% of parasitised nests) and Rabuffetti and Rebores (2007) showed that parasitism by *Philornis* produces a decrease in the reproductive success of chalk-browed mockingbirds *Mimus saturninus*, and also in the shiny cowbird *Molothrus bonariensis* (69% and over 80% nestling mortality in infested nests respectively).

Although starlings have been present in Argentina since 1987, it was not known whether or to what extent they are parasitised by botflies. The aim of this study was to analyse for the first time the impact of *Philornis* sp. on the reproductive success

of starlings in Buenos Aires province, Argentina, and to compare the intensity of the parasitism with that suffered by native bird species.

MATERIAL AND METHODS

Starlings engage in breeding behaviour from September to December; nestlings are present in October, and fledglings are observed from December, with maximum numbers in January (Peris *et al.*, 2005; Rebolo Ifran and Fiorini, 2010). Starling nests in Argentina have been found in hollow trees, inside nests of the rufous hornero *Furnarius rufus*, in woodpecker holes and in artificial cavities (Peris *et al.*, 2005; Rizzo, 2010; Turienzo and Di Iorio, 2010).

We installed 30 nest boxes in the Estación de Cría de Animales Silvestres (ECAS), 36° 19' S, 58° 13' W, 14 km north of La Plata city, Buenos Aires province, Argentina, on 10 July 2010. The ECAS is located on 230 hectares with pastures and woods of native and exotic trees. We distributed the nest boxes randomly on trees 2.0-3.6 m above the ground. The nest boxes were of 31×20 cm cross-section and 23 cm deep, with an entrance hole diameter of 4.5 cm. Starlings were the only species that occupied them. They used 22 nest boxes, 14 for a first brood (the first egg was laid on 28 September 2010 and the last on 16 October 2010), and 15 for a second brood (the first egg was laid on 27 October 2010 and the last on 26 November 2010). This period overlaps with that of other hole-nesting birds in the area. Seven of the nests used for a second brood were previously used earlier in the season. Nest boxes were checked at 1-4 day intervals during the nestling period and the number of larvae per nestling was recorded when we detected parasitism by *Philornis*.

To test the effect of the number of larvae and the brood (first or second) on nestling

survival (1-0), we performed a generalised linear mixed model (GLMM) with binomial error distribution and logit-link function, using the brood and the number of larvae as predictor variables and nestling survival as the response variable. Considering the non-independence of nestlings in the same nest we incorporated the nest as a random factor. To simplify models we used the backward stepwise method. Statistical analyses were carried out using R software, version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria).

To count the number of puparia per parasitised nest at the end of the breeding season (intensity of parasitism), we extracted the nest material from the nest boxes that were used only once, and dissected it to look for puparia. All puparia in the nests were already empty by the time of collection. The intensity of parasitism in starling nests was statistically compared with previously published data (Turienzo and Di Iorio, 2008, 2010) to which we added new additional information (Supplementary Electronic Material) of puparia in natural nests of native species: the firewood-gatherer *Anumbius annumbi*, rufous hornero, house sparrow *Passer domesticus* and house wren. In all cases, each nest content analysed corresponded to a one-clutch nest. Avian species nomenclature follows Remsen *et al.* (2014).

We collected 70 botfly larvae from seven starling nests during the 2011 breeding season. Larvae of the species involved live subcutaneously feeding on the red blood cells of nestlings for 5-8 days. They emerge at third instar to pupate in the nest material, so adult botflies can be obtained by collecting pupae from nests (Uhazy and Arendt, 1986). Emerged botflies were identified by Luciano Patitucci (Entomology Division, Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”), using the abbreviated key of Couri *et al.* (2009). Both *P. seguyi* and *P. torquans* were present and equally abundant

but it is not known whether some nestlings were parasitised by both species in a mixed infestation.

RESULTS

Larval parasitism in first clutches

A total of 38 nestlings hatched in 12 of the 14 nest boxes in which starlings laid eggs. *Philornis* larvae parasitised 34 nestlings (89.5%) of 11 clutches (91.7%). Of the parasitised nestlings, only three (8.8% of 34) from two nest boxes fledged and 31 from 11 nest boxes died (91.2% of 34) (table 1). A maximum of three, seven and nine larvae were observed in each of the nestlings that fledged. The four unparasitised nestlings died 3-5 days after hatching.

We found the maximum amount of larvae in chicks from 4 to 13 days old. Larvae to-

TABLE 1

Data of nests and nestlings of first and second broods.

[*Datos de nidos y polluelos de primeras y segundas nidadas.*]

	1 st brood	2 nd brood
Nests with nestlings	12	10
Nests with parasitised nestlings	11	5
Total nestlings	38	31
Parasitised nestlings	34	15
Dead nestlings in parasitised nests	31	15
Fledged nestlings	3	0

talled 236 larvae in all nestlings, a mean of 21 ± 3.17 larvae per nest (range = 2-37) (fig. 1a) and a mean of 6.9 ± 4.02 larvae per nestling per nest (range = 1-16, $N = 34$ nestlings) (fig. 1b). We found a positive correlation between the number of larvae per nestling and nestling age (Spearman's correlation: $r_s = 0.41$, $N = 73$, $P < 0.001$).

Larval parasitism in second clutches

A total of 31 nestlings hatched in 10 of the 15 nest boxes in which starlings laid eggs. *Philornis* larvae parasitised 15 of 31 (48.3%) nestlings from five nest boxes (50%). All the parasitised and non-parasitised nestlings died (100%) (table 1). The maximum amount of larvae was found in chicks from 4 to 10 days old with a total of 365 larvae in all the nestlings, a mean of 73 ± 31.95 larvae per nest (range = 7-195) (fig. 1a) and a mean of 24.3 ± 4.44 larvae per nestling per nest (range = 7-63, $N = 15$ nestlings) (fig. 1b).

We found a significant positive correlation between the number of larvae and nestling age ($r_s = 0.78$, $N = 65$, $P < 0.001$). In this second clutch, parasitised chicks lived a maximum of 10 days.

The mean number of larvae per nestling in the second clutches was significantly higher than in the first clutches (Mann-Whitney U test: $U = 63.5$, $N_1 = 34$, $N_2 = 15$, $P < 0.001$).

Nestling survival was not affected by the brood nor by the number of larvae that parasitised the nestling (GLMM: intercept: estimate = -6.94 , $P = 0.03$; brood (second brood): estimate = -16.6 , $P = 0.68$, number of larvae: estimate = -0.17 , $P = 0.47$).

Puparia per nest box

We counted puparia in nests extracted from nest boxes that had held broods with parasitised nestlings ($N = 9$). We found a total of 465 puparia (51.7 ± 17.22 puparia per nest box, range = 3-207).

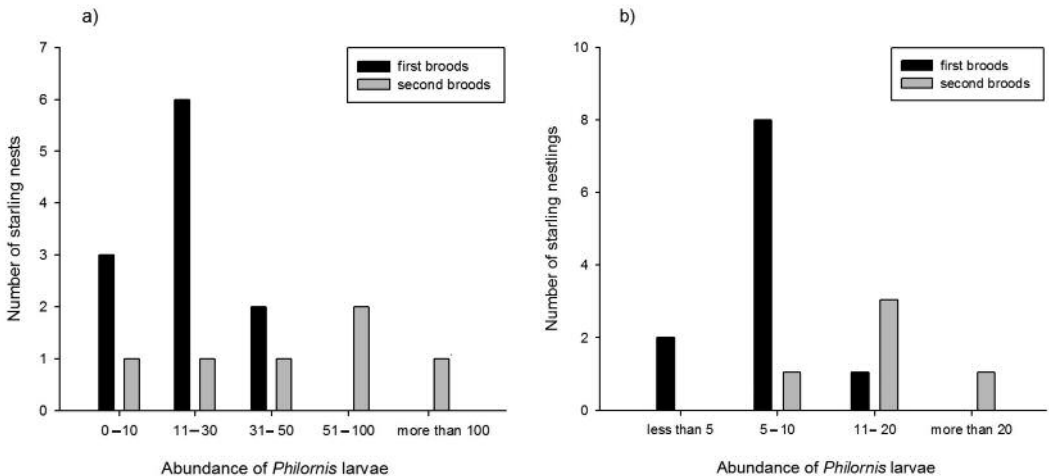


FIG. 1.—Frequency distribution of the number of larvae of *Philornis* in first and second broods. a) per nest, b) per nestling.

[Distribución de frecuencias del número de larvas de *Philornis* en primeras y segundas nidadas. a) por nido, b) por polluelo.]

Comparison of numbers of puparia in nests of starlings and native species

The number of puparia per nest was significantly different between species (ANOVA: $F_{1,6} = 5.65$, $P < 0.001$) but post-hoc tests showed that significant differences were only found between other exotic species (*P. domesticus*) and two native birds (Unequal N HSD: *P. domesticus* and *T. aedon*, $P = 0.01$; *P. domesticus* and *Schoeniophylax phryganophilus*, $P < 0.01$) and between two

native species (Unequal N HSD: *A. annumbi* and *S. phryganophilus*, $P = 0.03$) (table 2). There were no differences in the number of puparia between the starling and other species.

DISCUSSION

In our study area, starlings were intensively parasitised by botflies, showing one of the highest prevalences of parasitism by

TABLE 2

Mean number (\pm SE), range and total number of *Philornis* puparia in birds' nests from Buenos Aires province, Argentina.

[Número medio (\pm SE), rango y número total de puparia de *Philornis* en nidos de aves de la provincia de Buenos Aires, Argentina.]

Species (number of examined nests)	Mean puparia per nest	Minimum-Maximum	Total puparia	Source
House sparrow (N = 34)	15.7 \pm 5.8	1-57	173	Turienzo and Di Iorio, 2010
Rufous hornero (N = 110)	29.3 \pm 3.3	1-242	3194	87 nests (Turienzo and Di Iorio, 2010) 23 nests (this study)
Chalk-browed mockingbird (N = 19)	44.2 \pm 8.0	10-85	421	This study
Firewood-gatherer (N = 60)	43.9 \pm 15.3	1-325	1098	20 nests (Turienzo and Di Iorio, 2008) 40 nests (this study)
House wren (N = 17)	103.5 \pm 24.7	44-209	621	15 nests (Turienzo and Di Iorio, 2010) 2 nests this study
Chotoy spinetail (N = 11)	123.5 \pm 32.9	7-300	1235	4 nests (Turienzo and Di Iorio, 2008) 7 nests (this study)
European starling (N = 9)	51.67 \pm 17.22	3-207	465	This study

Philornis larvae in Argentina. Previous work on native species show parasitism rates range from 11% to 58% (Nores, 1995; Rabuffetti and Reboreda, 2007; Turienzo and Di Iorio, 2008, 2010; Segura and Reboreda, 2011; Quiroga and Reboreda, 2012; Supplementary Electronic Material), except for nests of the chotoy spinetail *Schoeniophylax phryganophilus* (86% parasitised; Turienzo and Di Iorio, 2010; Supplementary Electronic Material) and nests of the rufous hornero in Campo de Mayo, in which puparia were found in all the nests analysed (Supplementary Electronic Material).

Nestling mortality

Starling nestlings in both parasitised and non-parasitised nests suffered very high mortality. Over 90 % of parasitised nests did not fledge any chicks, a very high figure compared to chick mortality due to botflies on native species (Nores, 1995; Rabuffetti and Reboreda, 2007; Turienzo and Di Iorio, 2008, 2010; Segura and Reboreda, 2011; Quiroga and Reboreda, 2012). Nores (1995) found that 30.7% of the nestlings of the brown cacholote *Pseudoseisura lophotes* and 31% of the nestlings of the firewood-gatherer died on account of botflies. The red-crested cardinal was parasitised by botflies and these were the cause of death of all the nestlings in 17% of the nests studied (Segura and Reboreda, 2011). Quiroga and Reboreda (2012) found that 54% of house wren nests parasitised by botflies suffered complete mortality. Compared to the nest mortalities summarised by Dudaniec and Kleindorfer (2006), which range from 0 to 62%, the mortality in parasitised nests of starlings in this study is by far the highest value seen in a bird in the Neotropical Region. This may be because native birds could have developed some co-evolutionary defences, such as immunological mechanisms (Dudaniec and Kleindorfer,

2006), that allow them to fledge more nestlings (Richner, 1998). Another reason could be that nests in a cluster of nest boxes may be easier for botflies to find. Nevertheless, since the non-parasitised nestlings also died it is evident that other factors such as weather conditions, starvation or other diseases are affecting chick survival. Therefore it is not possible to know if the mortality of the parasitised nestlings was solely due to parasitism by *Philornis* or to an interaction of factors. It is also likely that the high level of parasitism and the reduced survival probabilities of the brood may have led to a large reduction in parental care by parents.

Number of larvae per nestling

The maximum number of larvae in nestlings of starlings were found in those between 4 and 13 days old (first clutches) and between 4 and 10 days old (second clutches). Similarly, in the planalto wood-creeper *Dendrocolaptes platyrostris*, the number of botflies per nestling reached a maximum when chicks were 10 days old, then declined as chicks approached fledging (Norris *et al.*, 2010). Mortality due to botflies was evident in nestlings of the brown cacholote of 6 to 12 days old and in the firewood-gatherer of 6 to 15 days old (Nores, 1995).

The mean number of larvae per nestling in native species was similar to those recorded in the first clutches of starlings. In contrast, the mean number of larvae per nestling of starlings in the second clutches was the highest reported value relative to all native birds (Nores, 1995; Turienzo and Di Iorio, 2010; Segura and Reboreda, 2011). The increase in the prevalence of parasitism by *Philornis* with the advance of the breeding season has previously been observed in native birds in Buenos Aires province (Rabuffetti and Reboreda, 2007; Segura and Reboreda,

2011). In starling second clutches, the minimum number of larvae per nestling was not different from that in other species but the maximum was higher with respect to that observed in other birds in Argentina (Nores, 1995; Turienzo and Di Iorio, 2010; Segura and Rebores, 2011).

Furthermore, the number of puparia found in starling nests was similar to that in nests of other bird species in Argentina. Counting puparia in nests is a good method for determining if nestlings have been parasitised by *Philornis* and to estimate the intensity of parasitism if the nest could not be checked during the nestling period.

Final remarks

Our results show that the studied population of an exotic bird suffers a high level of parasitism by native botflies, but our data do not allow us to test whether this was the cause of the observed high nestling mortality. Taking into account that most nestlings died, whether parasitised or unparasitised, additional factors together with the parasitism by *Philornis* could be associated with high nestling mortality, resulting in a significant reduction in starling nest productivity.

According to Dudaniec and Kleindorfer (2006), it is very difficult to determine whether chick mortality is directly caused by *Philornis* larvae or whether these are an indirect cause and the mortality is produced by opportunistic diseases in the stressed nestlings. Such levels of *Philornis* parasitism in starling chicks may have exposed them to new diseases in the area that they are colonising. On the other hand, parasitism of botflies on another exotic species, the house sparrow, shows a lower percentage of infested nests and lower mortality (Turienzo and Di Iorio, 2010). However, since this bird has been exposed to botflies since at least 1873 (Smith, 1972), it has had much longer

to develop an effective response to this parasite. Nevertheless, it remains unknown why some bird species are more infested than others nesting in the same area and having similar nests (closed or open), or why yet other species are mainly unaffected (Teixeira, 1999; Dudaniec and Kleindorfer, 2006).

These results also support the alleged unspecificity of some botfly species with respect to their bird hosts, giving support to the idea that they are generalist parasites (Teixeira, 1999).

Despite the high levels of botfly infestations and nestling mortality found in this study, experiments under controlled conditions are needed to evaluate the effects of *Philornis* on starling nestlings.

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SUPPLEMENTARY ELECTRONIC MATERIAL

Additional supporting information may be found in the on-line version of this article. See volume 62 (2) on www.ardeola.org.

Table S1: Additional nests of some native birds sampled in the province of Buenos Aires, Argentina.

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