A small parasitoid of fire ants, *Pseudacteon obtusitus* (Diptera: Phoridae): native range ecology and laboratory rearing

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Abstract

The high diversity within a guild of *Pseudacteon* Coquillet (Diptera: Phoridae) parasitoid flies hosted by the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), may be based on several niche axes, including host size specialization, host location cues, climatic responses, and tolerances. The recently described species, *Pseudacteon obtusitus* Plowes, Folgarait, and Gilbert (Diptera: Phoridae), is one of the smallest species in this group (with a thorax width of only 0.3 mm), and appears to locate ant hosts on foraging trails as well as at nest disturbances. Here we examine the ecology, phenology, and development of this parasitoid at sites in Corrientes, Argentina. *Pseudacteon obtusitus* has 2 seasons of peak abundance, and exhibits low tolerance for cold temperatures or extended cold periods. It preys on the smallest ant workers (typically 0.6 mm head width) independent of the ant size offered, and has a male biased sex ratio (mean 2.78:1). Developmental times ranged from 35 d at 27 °C to 44 d at 22 °C. This species holds interest for inclusion among the multi-species assemblage of parasitoid flies considered for biological control of *S. invicta* in its invasive range in North America.

Key Words: Argentina; fly endoparasitoid; temperature; Phoridae; *Solenopsis invicta*

Resumen

La gran diversidad dentro del gremio de moscas parasitoides del género *Pseudacteon* Coquillet (Diptera: Phoridae), específicas de las hormigas de fuego, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), puede estar basada en varios ejes de nicho incluyendo una especialización por el tamaño del huésped, claves de localización del huésped y respuestas climáticas o tolerancias. La especie recientemente descripta, *Pseudacteon obtusitus* Plowes, Folgarait, Gilbert (Diptera: Phoridae), es una de las más pequeñas de este grupo con un ancho de tórax de 0.3 mm y parece localizar a las hormigas huésped en los caminos de forrajeo así como en nidos disturbados. En este trabajo se examina la ecología, fenología y desarrollo de esta especie en sitios de Corrientes, Argentina. *Pseudacteon obtusitus* tiene 2 picos de abundancia y posee poca tolerancia a las temperaturas bajas o períodos largos de frío. Esta especie ataca a las obreras más pequeñas (típicamente de 0.6 mm de ancho de cabeza) independientemente del tamaño de huésped ofrecido, y tiene una relación de sexos sesgada hacia los machos (promedio 2.78:1). Los tiempos de desarrollo varían de 35 días a 27 °C hasta 44 días a 28 °C. Esta especie es interesante para incluirla en el ensamble multi-específico de moscas parasitoides consideradas para el control biológico de *Solenopsis invicta* en su rango de invasión en América del Norte.

Palabras Clave: Argentina; fórido; mosca endoparasitoide; *Solenopsis invicta*; temperatura

The parasitoid dipteran genus *Pseudacteon* Coquillet (Diptera: Phoridae) comprises over 70 species (Chen & Fadamiro 2018), of which 23 are hosted by fire ants of the *Solenopsis saevissima* F. Smith (Hymenoptera: Formicidae) group that includes *S. invicta* Buren (Hymenoptera: Formicidae), the red imported fire ant (Pitts et al. 2018). Like other members of its genus, *Pseudacteon obtusitus* Plowes, Folgarait, and Gilbert (Diptera: Phoridae) oviposit into the thorax of host ant workers leading to decapitation and mortality of individual workers (Porter 1998). However, important indirect effects of parasitism that potentially impact colony growth rates are disruption of colony activities, such as foraging or nest defense when flies are present during such events (Feener & Brown 1992; Folgarait & Gilbert 1999; Mehdiahabi & Gilbert 2002). Given the diversity of *Pseudacteon* species hosted by *S. invicta*, it is important to understand the niche differences

and potential impacts of each parasitoid on its hosts, especially given the efforts to introduce *Pseudacteon* flies to the USA for experimental biological control of *S. invicta.*

Solenopsis invicta was introduced accidentally into the USA, Australia, China, Taiwan, and Japan (Ascunce et al. 2011) and are recognized as one of the most damaging exotic pests worldwide (GISP 2001). After recognizing the ineffectiveness of area-wide pesticide applications (Summerlin et al. 1977), biological control agents such as *Pseudacteon* parasitoids have been studied with the goal of experimental introductions to control fire ants (Gilbert & Patrock 2002). Many *Pseudacteon* fly species have been found to exhibit differences in host location cues, phenology, host size preferences, and microhabitat ecology (Chen & Fadamiro 2018). Because biological control programs often are characterized by the use of several species with complementary effects on

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the target, several *Pseudacteon* species have been experimentally introduced to the USA. Among these introductions, *Pseudacteon tricuspis* Borgmeier, *Pseudacteon litoralis* Borgmeier, *Pseudacteon obtusus* Borgmeier, and *Pseudacteon nocens* Borgmeier are phorids that mainly attack large sized workers, whereas *P. obtusitus*, *Pseudacteon curvatus* Borgmeier, and *Pseudacteon cultellatus* Borgmeier (all Diptera: Phoridae) attack smaller ants (Chen & Fadamiro 2018).

Long distance host location cues used by *Pseudacteon* are likely to be ant kairomones and odors associated with colony activities, such as foraging or nest defense (Mathis & Philpott 2012). For purposes of exercising control via indirect effects, *Pseudacteon* species that disrupt foraging workers are of greatest interest. Orr et al. (1997) categorized the behavioral spectrum of several fly species that specialize parasitizing ants at the time of nest disturbance or when workers are foraging. In Argentina, notable nest disturbance specialists are *P. tricuspis*, *P. litoralis*, and *P. nocens*, whereas foraging trails often attract *P. obtusus* and *P. obtusitus* (Folgarait et al. 2007a).

Pseudacteon obtusitus was described recently as being distinct from *P. obtusus*, a similar but larger species, and both species may be sympatric locally (Plowes et al. 2015). Prior to the formal description, the existence of the smaller taxon was documented in the field (e.g., Folgarait et al. 2005a), in the laboratory (Porter & Gilbert 2004), and by molecular means (Kronforst et al. 2007). This species was usually treated separately in field studies (e.g., Folgarait et al. 2007a, b; Feener et al. 2008), and especially in host-specificity trials needed for biological control assessments (Morrison & Gilbert 1999). Here, we report on the seasonal phenology, development time at different temperatures, host body size, and oviposition preference of *P. obtusitus*.

Material and Methods

SEASONAL ABUNDANCE

Adult *P. obtusitus* used in this study were collected each mo from Corrientes Province, Argentina, using aspirators from Jul 2001 to Jul 2003, then occasionally during 2004 from roadside vegetation and sown pastures around Mercedes (29.2000°S, 58.0800°W). During 2017 we collected phorids each mo throughout the yr (except in Jul and Aug due to seasonal low parasitoid abundance) from natural grasslands and vegetation around city squares from the San Roque area (28.5700°S, 58.7100°W) in Corrientes Province. Sampling of *P. obtusitus* was standardized by person and h in order to compare abundance across time periods and mo.

We obtained daily climatological data for 2001 to 2003 from the National Institute of Agronomical Technology Meteorological Station (INTA) of Mercedes, and for 2017 from INTA at Sombrerito. From those, we calculated annual and monthly climatic variables for each sampling d. Variables maintained in the analyses were annual–average daily maximum temperature (TMPA), annual–average daily minimum temperature (TmPA), monthly–average daily maximum temperature (TMPM), monthly average minimum temperature (TmPM), annual–average precipitation (PPA), monthly average precipitation (PPM), annual–average daily relative humidity (HRPA), monthly–average daily relative humidity (HRPM), annual–average number of cold h (HFPA), and monthly–average number of cold h (HFPM). Cold h are defined by the National Meteorological Service as periods when the temperature falls below 7 °C. From data gathered in 2017, we selected those d and mo for which we sampled *P. obtusitus* in the morning (until 12:00 P.M.), early afternoon (12:00–3:00 P.M.), and in the late afternoon (after 3:00 P.M.), then calculated the abundance of *P. obtusitus* in each category on a monthly basis.

PARASITOID DEVELOPMENT

In 2003, field caught *P. obtusus* flies were placed in separate plastic vials (10 × 40 mm) and transported at approximately 10 °C in a cooler to Buenos Aires, Argentina, on the same or following d. Upon arrival, or no later than 72 h after field collection, oviposition attacks were conducted in flight attack boxes (see details in Folgarait et al. 2002). The *S. invicta* ants used in these attack boxes were from nests dug from Corrientes Province, where the ants were extracted by dripping water into the nest material in buckets. These ants came from healthy, monogyne colonies kept for less than 3 mo in the laboratory. We sieved the *S. invicta* workers (ZONYTEST sieves, Rey & Ronzoni S.R.L., Buenos Aires, Argentina) in order to offer these different size-classes to *P. obtusitus*: (1) large ants (those could not pass through sieve #18), (2) medium (not passing through sieve #20), and (3) small ants (passing through sieve #20). For each attack box, we offered either small class ants (0.8 g of *S. invicta*), or a mixture of size classes of *S. invicta* ants (0.8 g which included 0.20 g of large class, 0.22 g of medium class, and 0.38 g of small class ants). Fly oviposition continued in attack boxes for 3 to 5 h or until parasitoids died.

In 2017, flies were collected and used immediately in oviposition attacks in the field. We used oviposition boxes made from plastic trays (30 × 17 × 13 cm) with walls covered with Fluon© (Daikin America Inc., Orangeburg, New York, USA) to prevent ants from escaping. Trays were kept open only if *P. obtusitus* was present in the field, or otherwise covered. Trays were shaded to maintain temperatures below 30 °C. In each tray, we used 20 mL of *S. invicta* workers of different sizes randomly chosen, which were obtained from the area. Each tray had pieces of humid plaster, a tube with water, and another with sugar water. Tests usually ran for 3 to 5 hours, but on occasion were left overnight if flies were still active.

Attacked ants were maintained in the laboratory following the methods of Folgarait et al. (2002). Dead workers were recovered from the trays every other d for 45 d, then examined for the appearance of fly pupae in their heads. Each pupa was placed in an individual plastic cup and all cups within a large plastic container (27 × 11.5 × 11.5 cm) with moistened plaster until fly emergence. At the time each fly pupa first was observed, we measured the head width (across the maximum width) of the ant. After adult emergence, we determined the sex and measured the thorax width. All measurements were made using a micrometer equipped a stereoscopic microscope (Nikon 102 and SMZ 745, Nikon Instruments Inc., Melville, New York, USA). Larval developmental times were estimated as the number of d since the d of the attack to the d we first recorded the pupae. Pupal developmental time was calculated as time from appearance of a pupa to adult fly eclosion. Parasitized ants were maintained in the laboratory during 2003 and 2004 at 22 °C and 80% RH, conditions at which the flies were reared. During 2017, parasitized ants were maintained at 27 °C and 75% RH, ants with fly larvae at 27 °C and 95% RH, and pupae at 22 °C and 95% RH. Ants were maintained at 12:12 h (L:D) photoperiod, except during summer when we used a 14:10 h or winter when we used a 10:14 h (L:D) photoperiod.

STATISTICS

Step-wise regression analysis was conducted on climatic variables associated with mean monthly abundance of adult *P. obtusitus* to determine best-fit models with significant predictors of seasonal fly presence. Ant head and fly thorax size distributions were subjected separately to Kolmogorov-Smirnov non-parametric tests when sample sizes allowed comparisons (Statistix 1998).

For the laboratory data from 2003 to 2004, there were no significant differences between the source ants being smaller or of mixed

sizes, and therefore the data were pooled for the following analyses. Comparisons of developmental time and thorax size, as well as rearing temperature and ant head size, used non-parametric statistics (Kruskal-Wallis for 3 or more variables, and Mann-Whitney for 2 variables). Multiple comparisons were adjusted using Bonferroni's method, α = 0.05 (Siegel 1974). Frequency of viable and non-viable fly pupae (i.e., presence or absence of respiratory horns) was compared using X^2 Fisher Exact Test using the Yates correction when expected frequencies were smaller than 5 (Siegel 1974). Differences in all analyses were considered significant $P = 0.05$.

Results

SEASONAL ABUNDANCE

Pseudacteon obtusitus fly abundance during 2001 to 2003 exhibited 2 seasonal peaks, 1 at the end of the summer until mid-fall (Feb to May) and another during the spring (Sep to Nov), the first peak being more pronounced (Fig. 1). This pattern was encountered again in 2017, in the same province, although not the same area, with a more pronounced peak in the spring. This species had little to no activity during the winter (Jun to Aug) for any sampling yr.

From Aug 2001 to Jul 2002, 3 climatic variables significantly explained 60.6% (adjusted $R^2 = 0.6$) of the abundance of adult *P. obtusitus*. Fly abundance increased 1.5-fold for each 1 °C increase in annual–average daily maximum temperature. However, abundance decreased 1.6-fold for each 1 °C decrease in average–annual minimum daily temperature, and by 4-fold for each 1 h increase in annual–average daily number of cold h (Table 1).
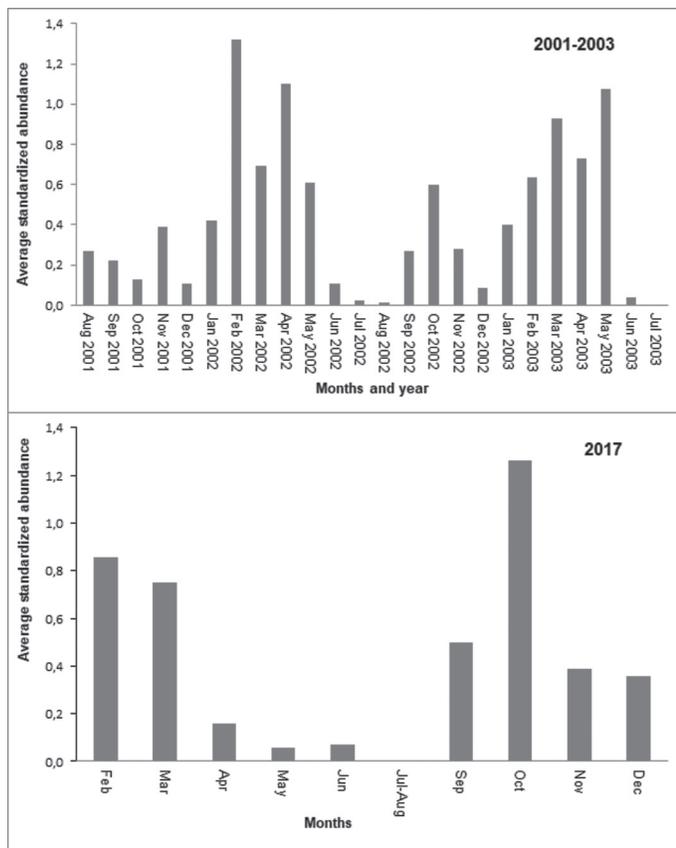

Fig. 1. Monthly phenology of *Pseudacteon obtusitus* across mo and yr at Corrientes Province, Argentina.

Table 1. Linear regression models for averaged monthly data for yr 2001 to 2002 (A), 2002 to 2003 (B), and yr 2017 (C). TMPA = annual average daily maximum temperature, TmPA = annual average daily minimum temperature, HFPA = annual average daily number of cold hours, HRPA = annual average daily relative humidity, TmPM = monthly average daily minimum temperature.

(A)

SOURCE	DF	SS	MS	F	P
REGRESSION	3	1.336	0.445	6.63	0.014
RESIDUAL	8	0.537	0.006		
TOTAL	11	1.498			

Predictor variables	Coefficient	SE	Student's t	P	VIF
CONTANT	−14.094	10.189	−1.38	0.2040	
TMPA	1.471	0.489	3.01	0.0169	2.2
TmPA	−1.642	0.448	−3.67	0.0063	2.3
HFPA	−4.134	1.390	−2.97	0.0178	1.2

(B)

SOURCE	DF	SS	MS	F	P
REGRESSION	1	0.778	0.778	10.8	0.0082
RESIDUAL	10	0.720	0.072		
TOTAL	11	1.498			

Predictor variables	Coefficient	SE	Student's t	P	VIF
CONSTANT	21.505	6.416	3.35	0.0073	
TmPA	−1.615	0.491	−3.29	0.0082	1

(C)

SOURCE	DF	SS	MS	F	P
REGRESSION	3	1.144	0.381	9.13	0.0118
RESIDUAL	6	0.251	0.042		
TOTAL	9	1.000			

Predictor variable	Coefficient	SE	Student's t	P	VIF
CONSTANT	−183.277	37.17	−4.93	0.0026	0.0018
HFPA	−1.532	0.507	−3.02	0.0234	3.5000
HRPA	2.175	0.441	4.93	0.0026	2.6000
TmPM	0.228	0.053	4.27	0.0053	4.2000

During Aug 2002 to Jul 2003, only 1 variable (average–annual minimum daily temperature) was significant, and explained 50% (adjusted $R^2 = 0.52$) of the abundance of *P. obtusitus*. Like 2001, fly abundance declined 1.6-fold for each decrease of 1 °C of annual–average daily minimum temperature (Table 1).

In the 2017 study, 3 climatic variables significantly explained 70% (adjusted $R^2 = 0.73$) of the abundance of *P. obtusitus*. Again, fly abundance declined 1.5-fold for each 1 °C decrease in the annual–average daily number of cold h but increased by 2-fold with a 1% increase in annual–average daily relative humidity, and slightly with an increase of the monthly–average daily minimum temperature (Table 1).

DIEL ACTIVITY

Pseudacteon obtusitus was active mainly from 12:00 P.M. through the late afternoon before darkness (Fig. 2). Very few individuals were observed during the morning. This species was active between 14 to 34 °C, although most activity occurred above 20 °C, and primarily from 27 to 31 °C.
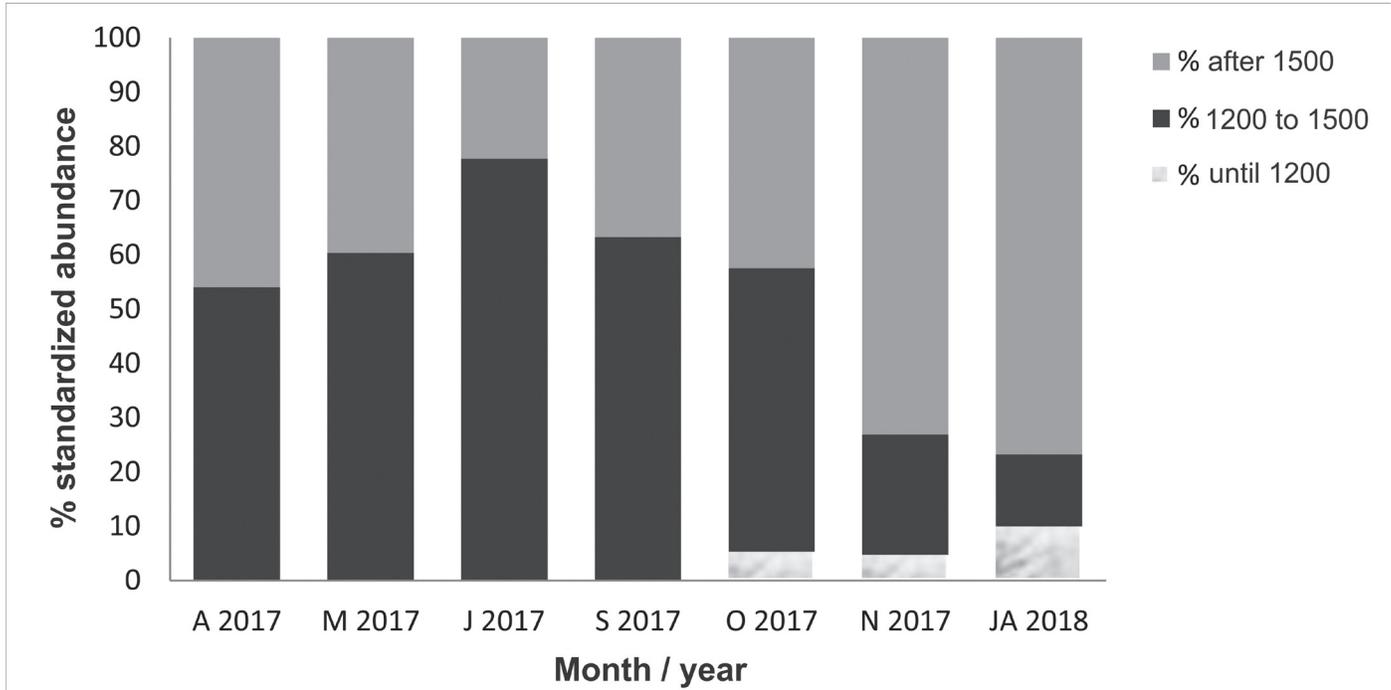
Fig. 2. Relative percent abundance of *Pseudacteon obtusitus* categorized by the time of d (morning until 12:00 P.M., 12:00 to 3:00 P.M., after 3:00 P.M.) and sample mo (Apr, May, Jun, Sep, Oct, Nov, Jan).

PARASITOID DEVELOPMENT

Between Mar 2003 and May 2004, we conducted 38 field attack tests of which 18 produced 87 pupae that generated 16 females and 39 *P. obtusitus* males. The most productive attacks occurred in Nov 2003. In 2017, we conducted 87 attacks from which 58 produced 318 pupae, where 7 females and 25 males emerged. The most productive attacks occurred in Sep and Oct of 2003. At that time, we did not find any relationship between the number of pupae produced and number of female flies in oviposition boxes, or number of ovipositional attempts.

In 2003, we recorded the presence of respiratory horns in 70 pupae. When this structure was present, we found that pupal viability significantly increased $(X^2 = 26.77; P < 0.0001)$; 70% of pupae were viable when respiratory horns were protruding from the puparia compared with 2.8% survival when absent.

In field observations of oviposition boxes during 2017, we observed that when a female fly attacked solitary or trailing ants, the ants demonstrated known behavioral postures, such as the U-posture (gaster raised, legs stilted), C-posture (gaster underneath the thorax with tip pointing the head from below), and toppled posture (bodies sideways with first 2 pairs of legs raised) (Wuellner et al. 2002). Additionally, we observed a new posture which we named the "J-posture." This behavior was displayed as the ant raised her gaster while pressing her head towards the ground. This posture is similar to that seen when ants venom flag (LeBrun et al. 2016). Apart from these individual behaviors, the most frequent reaction of the ants was to group together. On disturbed mounds in the field where baits were placed, we observed more than 1 phorid attacking simultaneously where the ants stopped performing other tasks, such as nest reconstruction.

For the experiments conducted during 2003 through 2004, flies successfully emerged from ants with head widths < 0.75 mm (Fig. 3). Male flies emerged from significantly smaller host head sizes than female flies ($U = 2.001$; $P = 0.045$; Table 2) and had significantly smaller thorax sizes ($U = 1.914$; $P < 0.056$).

In the 2017 experiments, ant head sizes from which adult flies emerged corresponded to the smaller range (< 0.75 mm) of *S. invicta* offered. On the other hand, the median and distribution of head
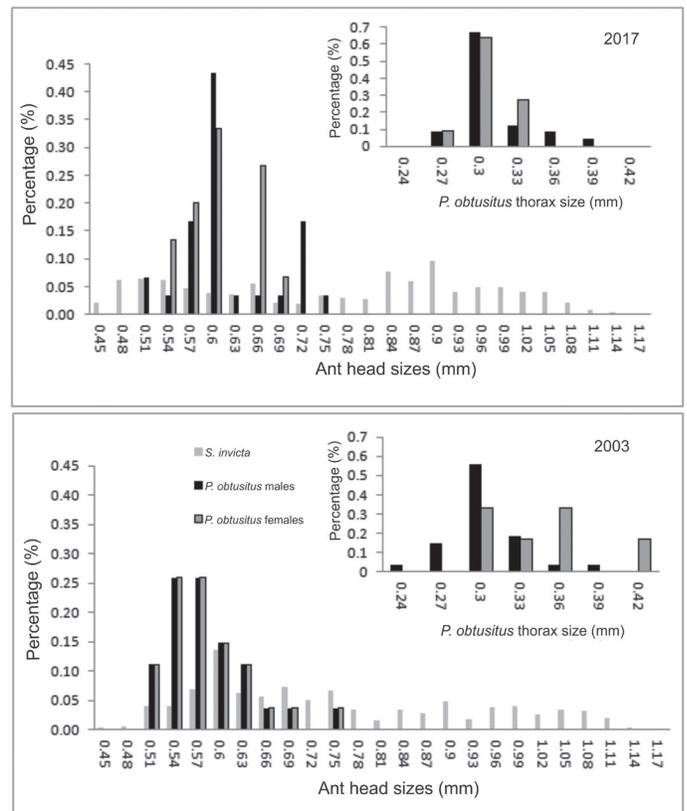

Fig. 3. Frequency distribution of *Solenopsis invicta* head size from which *Pseudacteon obtusitus* emerged, sorted by gender and sample yr. Insert: frequency distribution of *Pseudacteon obtusitus* thoraces sorted by gender and sample yr.

Table 2. *Solenopsis invicta* worker median head sizes (mm), from which *Pseudacteon obtusitus* emerged, median thorax sizes (25 and 75% quartiles), and sampling sizes of *Pseudacteon obtusitus*, discriminated by sex (F = female; M = male) and sampling year.

Year	Statistic	F head size	M head size	F thorax size	M thorax size
2003	Median	0.64	0.57	0.31	0.30
	Quartiles	0.60–0.68	0.54–0.63	0.30–0.36	0.29–0.31
	n	12	34	12	34
2017	Median	0.60	0.60	0.30	0.30
	Quartiles	0.57–0.66	0.57–0.67	0.30–0.33	0.30–0.37
	n	15	30	11	30

sizes from which females and males emerged did not differ significantly ($P > 0.05$; Fig. 3; Table 2). The distribution of fly thorax sizes was significantly broader compared with 2003 to 2004 for males only (Kolmogorov Smirnov test, $P < 0.001$). The distribution and median thorax size did not differ significantly between genders in each case ($P > 0.05$).

In the 2003 to 2004 studies, larval fly development times were significantly shorter for females than males ($U = 2.057$; $P < 0.039$), although with the Bonferroni adjustment these differences became insignificant. Pupal and total development times did not differ significantly between gender ($U < 1.5$; $P > 0.05$). Overall development time was approximately 44.5 d (Table 3).

Similarly, we did not find significant differences in larval developmental times for males and females ($P > 0.061$) in the 2017 study. However, pupal development was significantly shorter for females than males ($P < 0.009$). Overall total development time was about 35 d for both genders, with no significant difference in total development between them ($P > 0.295$) (Table 3). Larval development was significantly shorter ($P < 0.0001$) in 2017 when the flies were reared at 27 °C (16 d) compared with 22 °C in the previous yr (23 d). Pupal developmental time was very similar ($P = 0.05$) as previous yr at 22 °C (20.5 d during 2017, and 23 d during 2003–2004).

Discussion

Our studies of *P. obtusitus* at sites in Corrientes, Argentina, revealed important insights into aspects of its ecology concerning phenology, climatic affinities, and development by gender in different host body sizes. Our phenological analysis showed that *P. obtusitus* is present throughout the yr but in greater numbers during the end of summer to mid-fall and in spring. We noted that *P. obtusitus* was affected by the number of cold h as well as minimum temperatures that significantly reduce its abundance. Because adult populations decline to minima during winter (prior to their hosts rebuilding nests the following spring), any extended winter conditions are likely to result in population bottlenecks and delayed growth of flies in the spring.

Despite the broad distributional range of this species (Patrock et al. 2009) where warmer climatic zones of its host range include mesic (this study) as well as xeric occupied habitats (Folgarait et al. 2007a), we have never collected this species as far south as Buenos Aires (34.4700°S, 58.5000°W) throughout many yr of sampling. The absence of *P. obtusitus* at southern latitudes can be explained by a low tolerance to cold. For example, in Buenos Aires there are harsher winters (from 7.5 °C to 8.9 ± 1.5 °C) while annual average minimum temperatures of 13.8 ± 0.8 °C (National Meteorological Service, historical data), either are lower or similar to conditions present during the yr of this study in Corrientes (Table 4). Therefore, we predict that *P. obtusitus* may not expand its current distribution into southern latitudes. Our studies found that *P. obtusitus* was active mainly from 12:00 P.M. through the late afternoon until sunset when temperatures were above 20 °C. Other unpublished observations (R.M. Plowes & E.G. LeBrun, personal communication 2017) revealed a common pattern of late afternoon and early evening activity at disturbed nests. These results agree with the characterization of *P. obtusitus* as a heat-dry tolerant, non-morning species, typical of a xeric and more extreme environment (Folgarait et al. 2007a). We suggest that any field evaluations of this species should be done when temperatures are above 20 °C.

Pseudacteon obtusitus is the smallest fly recorded so far among *Pseudacteon* species known to attack *S. invicta*. Its average thorax size lies within the smaller range documented for other small flies such as *P. curvatus* (Chirino et al. 2009), *P. cultellatus* (Folgarait et al. 2002), and *P. nudicornis* (Orr et al. 1997). In contrast to these other small species of *Pseudacteon* where the sex ratios are typically equal (Folgarait et al. 2002; Chirino et al. 2009), the sex ratio of *P. obtusitus* is biased towards males.

Development times of *P. obtusitus* were similar to other small *Pseudacteon* (Folgarait et al. 2002; Chirino et al. 2009), where lower temperatures decreased development (Folgarait et al. 2002, 2005a, 2006). A pattern of flexibility in developmental period is important given that *P. obtusitus* and other *Pseudacteon* species disappear from their community in winter to appear again in spring (Folgarait et al. 2003, 2007b). We also found that the presence of protruding respiratory horns significantly increased fly pupae survivorship and, there-

Table 3. Median developmental times in d (25 and 75% quartiles), and sample sizes for reared larvae and pupae, and total development of *P. obtusitus* discriminated by sex (F = female; M = Male) and sampling year.

Year	Statistic	F larvae	F pupae	F total	M larvae	M pupae	M total
2003	Median	22	23	45	24	22	44
	Quartiles	20.2–23.0	22.0–25.5	42.2–47.7	21.5–26.2	19.7–24.0	42.0–48.5
	n	12	12	12	26	26	26
2017	Median	16	18	34	15	22	36
	Quartiles	16.0–17.0	14.0–20.0	30.0–40.0	13.0–17.0	19.5–25.5	33.5–40.0
	n	11	11	11	28	25	25

Table 4. Mean (± SD) *Pseudacteon obtusitus* and climatic variables that were significant in the multivariate regression analyses, discriminated by sample yr. TMPA = annual average daily maximum temperature (°C), TmPA = annual average daily minimum temperature (°C), HFPA = annual average daily number of cold h, HRPA = annual average daily relative humidity (%), TmPM = monthly average daily minimum temperature (°C).

Year	Abundance	TMPA	TmPA	HFPA	HRPA	TmPM
2001–2002						
Average	0.45	27.43	14.02	0.67	86.73	14.65
SD	0.42	0.16	0.24	0.06	3.27	3.16
2002–2003						
Average	0.43	26.35	13.09	0.95	72.74	12.92
SD	0.37	0.24	0.20	0.15	3.17	4.16
2017						
Average	0.46	27.82	16.93	0.33	82.80	17.99
SD	0.39	0.56	0.69	0.25	0.25	2.62

fore, adult emergence. This pattern also has been reported for *P. borgmeieri*, *P. cultellatus*, *P. nocens*, and *P. obtusus* where the presence of respiratory horns explained the viability of 80% of the pupae reared in the laboratory (Folgarait et al. 2005b). Those authors have shown that the presence of these respiratory structures appears independently of ant host species and size, and they have proposed it as an indicator of rearing efficiency.

In summary, *P. obtusitus* could be an important component of a multi-species assemblage of parasitoids for consideration in biological control of invasive populations of *S. invicta*. In particular, *P. obtusitus* fills the important niche axes of being a small fly, attacking small ants, and one that often locates its host during foraging activities (Feener et al. 2008). Because the majority of foraging *S. invicta* workers are small bodied, *P. obtusitus* may have an important role in disrupting these foragers from their tasks. The temperature-dependent flexibility in developmental times may be useful for rearing cultures of this fly species by speeding or delaying development according to release needs. However, rearing temperatures should not be below 16 °C (Table 4). Further work is required to better understand the mating behavior and oviposition cues to allow effective rearing of *P. obtusitus* in laboratories.

Acknowledgments

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