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Aspects of the reproductive behaviour and development of two forensically relevant species, *Blaesoxipha* (*Gigantotheca*) *stallengi* (Lahille, 1907) and *Sarcophaga* (*Liopygia*) *ruficornis* (Fabricius, 1794) (Diptera: Sarcophagidae)

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ABSTRACT

We studied aspects of the reproductive behaviour and development of two species of Sarcophagidae (Diptera) of potential forensic importance, *Blaesoxipha stallengi* (Lahille, 1907) and *Sarcophaga ruficornis* (Fabricius, 1794), which are dominant in assemblages in dry forests in Northeastern Brazil. We described the behavioural acts associated with courtship and mating and estimated the development time (from egg/larva until adult) – of both species. Description of the reproductive behaviour was based on 50 couples of each species whereas 250 larvae were used for the estimation of the developmental time. A total of 55 successful copula were observed for *B. stallengi* and 142 for *S. ruficornis*. Pre-copulatory behaviour differed between the species, as *S. ruficornis* presented a high rate of competition among male specimens. *Blaesoxipha stallengi* copulated more frequently in the morning and the mean duration of copulation was similar for both species. The species showed different reproductive strategies: *S. ruficornis* follows the typical strategy in Sarcophagidae and are viviparous (larviparity), but we report here the first documented evidence of ovoviviparity of *B. stallengi*. Sex ratio of the emerged adults did not differ ($p > 0.05$) markedly for either species. Total development time in days was similar with 22.9 for *B. stallengi* and 21.3 for *S. ruficornis*. The pronounced similarities in the morphology of both species – combined with their similar time of development – may act as confounding factors for forensic entomologists and stress out the need for an accurate taxonomical identification.

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Introduction

Reproductive strategies among species of Diptera are remarkably diverse and include oviparity, ovoviviparity, larviparity and pupiparity, among others (Meier et al., 1999). Complex behavioural traits also vary among species within a family, which is the case in Sarcophagidae. Flesh flies typically deposit first instar larvae (larviparity) on the substrate, as displayed by species of the genus *Sarcophaga* Meigen, 1826 whose substrates include faeces, decomposing fruit, carcasses and cadavers (Pape, 1996; Mulieri et al., 2010). On the other hand, oviparity can also occur, especially in the case of parasitoids such as those of the speciose genus *Blaesox-*

ipha Loew, 1861, which show aggressive behaviour towards their invertebrate hosts (Pape, 1994).

In the past decades, the forensic potential of Sarcophagidae has been strengthened by their frequent register on decomposing carcasses and human cadavers, especially in the Neotropical region (Mello-Patiu et al., 2014; Vasconcelos et al., 2014, 2016, 2019; Vairo et al., 2017). Several species of Sarcophagidae are also of medical and veterinary importance as causal agents of myiases and vectors of pathogens affecting men and domestic animals (Greenberg, 1973) and are of agricultural relevance due to their role as parasitoids of acridid grasshoppers and tenebrionid beetles (Pape, 1996).

Despite their importance, applied use of data on sarcophagids is hindered by limited information on aspects of their reproduction and, more critically, life cycle. In forensic investigations, the estimation of minimum post-mortem interval (minPMI) requires
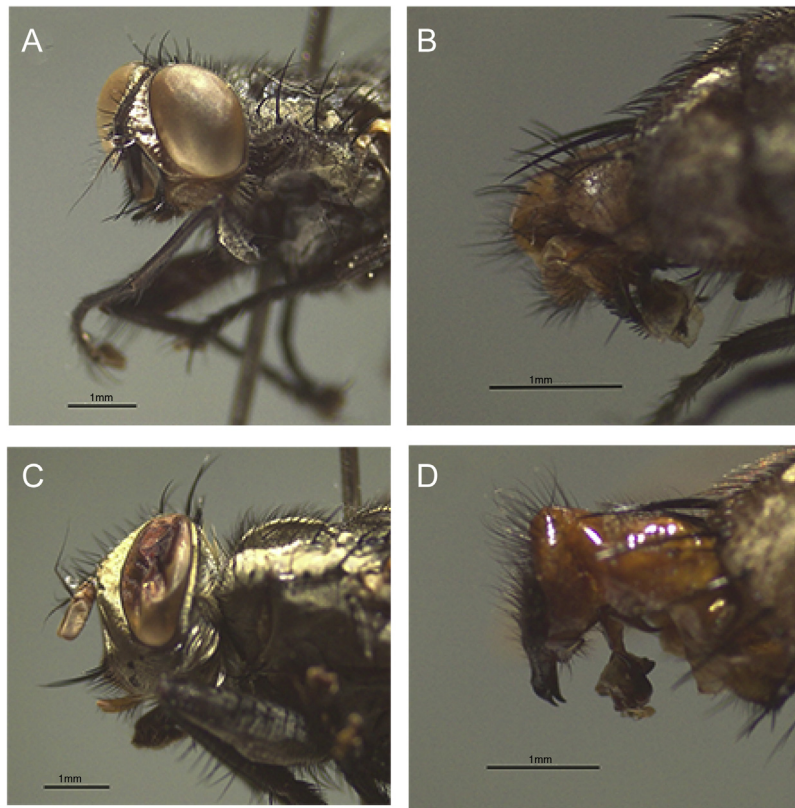
Figure 1. Characteristics used in the identification of the specimens. A and B – *Blaesoxipha stallengi* (Lahille), C and D – *Sarcophaga ruficornis* (Fabricius).

quantitative data on the duration of each developmental stage. In addition, information on reproductive behaviour is crucial in order to understand the differential competitive abilities of species in the colonization of ephemeral resources – which will determine the composition and temporal structure of necrophagous assemblages. Furthermore, bionomical data are needed to elucidate processes of biological invasions and on the assessment of environmental conservation status – as demonstrated by the use of sarcophagids as biological indicators (Barbosa et al., 2017).

Recent studies performed in fragments of the seasonally dry forest (Caatinga) in Brazil have revealed the dominance of *B. stallengi* (Lahille, 1907) and *Sarcophaga ruficornis* (Fabricius, 1794) in rat carcasses (Oliveira and Vasconcelos, 2018), which poses interesting questions regarding their biotic potential and adaptation to xeric environments. Detailed description of the behaviour and life cycle of both species is scant, particularly for *B. stallengi*. Thus, the objective of this study was to investigate aspects of the reproductive behaviour and development of *B. stallengi* and *S. ruficornis* under laboratory conditions. Specifically, we aimed to: a) describe the behavioural acts associated with courtship and mating and b) estimate the development time from egg/larva until adult.

Materials and methods

Insect identification and establishment of colonies

The adults were identified using the taxonomic keys of Carvalho and Melo-Patiu (2008) and Mulieri et al. (2010), and the morphological details used in the identification of the specimens are shown in Fig. 1. The specimens studied were deposited in the entomological collection of the Universidade Federal de Pernambuco (UFPE).

The colonies of *B. stallengi* and *S. ruficornis* used in this study were stablished from larvae collected in traps containing decomposing bovine liver as baits, exposed in a fragment of dry forest in the municipality of Afogados da Ingazeira, Northeastern Brazil (7°45′52″S; 37°35′46″W), three months prior to the experiments. Insects were reared under controlled temperature ($25 \pm 2\,^{\circ}$C) and relative humidity ($50 \pm 10\%$) and under a light:dark cycle of 12 h:12 h. Flies that emerged from field-collected larvae were maintained in wooden cages (40 cm × 60 cm × 40 cm) covered with fine mesh. A solution of water and sucrose and a mixture of minced beef and sardine (50:50) was offered to adults as source of food and site for egg/larva laying, all of which were changed daily. First instar larvae resulting from reproduction in the cages were transferred to fresh containers (400 mL) and also fed on a beef:sardine mixture. After pupariation, pupae of both species were transferred to plastic containers (100 mL) in groups of ten and monitored until the emergence of adults.

Two separate studies were performed to integrate information on behaviour and development of both species. Data on mating behaviour, instar determination, pupation, and emergence of adults were collected twice a day, in the morning (9:00–11:00) and in the afternoon (14:00–16:00).

Mating behaviour

The description of the reproductive behaviour was based on 50 pairs of each species, maintained in the experimental cages described above. We recorded behavioural acts related to precopulation, copulation and strategies of offspring deposition. An ethogram was built to depict the behavioural repertoire and confirmed by photography. Filming was performed using a digital camera (Sony HDR-CX130) fixed to one side of the cage. For each species, we quantified the time elapsed from ovi/larviposition until sexual maturation and the duration of copulation. We focused on three phases:
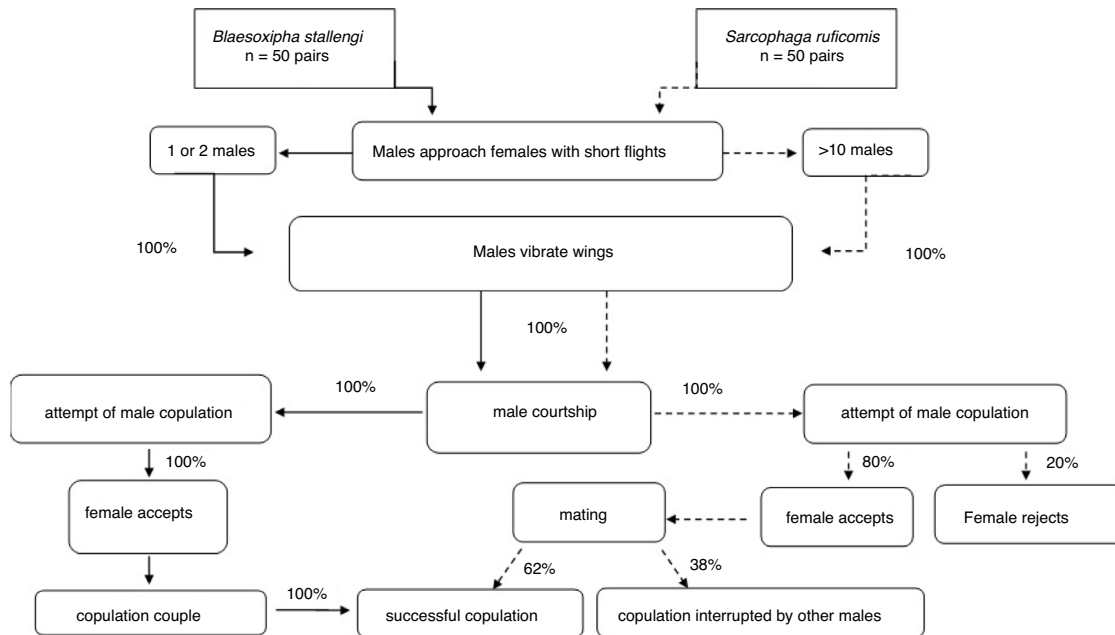
Figure 2. Summary of the observed reproductive behaviour for *Blaesoxipha stallengi* (Lahille) and *Sarcophaga ruficornis* (Fabricius) in the laboratory.

1. Pre-copulation: involved pre-nuptial behavioural acts, including courtship, observed from the moment the male approached the female (<5 cm distance) until the insertion of the aedeagus in the female genitalia. This observation had no limit of time and focused on a single female at a time.
2. Copulation: comprised the acts associated with the insertion of the aedeagus in the female until the pair separated spontaneously. A single female was observed continuously until the end of copulation.
3. Post-copulation: behaviour exhibited by male and female following separation of the pair (e.g., feeding, flying, landing and oviposition), observed continuously for 15 min.

Time of development

For the study of the developmental time, 250 larvae of each species were observed from egg (or first instar when applicable) until pupation. Larvae were transferred using a soft brush from the substrate to 200 mL cages containing 250 g of the beef/sardine mixture, where they were maintained in groups to mimic larval aggregation found in nature. Instar was determined twice a day until pupariation. Pupae were transferred to individual plastic containers and monitored until the emergence of the adult.

For each species, we quantified the mean time of development from egg/larvae to adult, the mean duration of each larval instar and of the pupal stage, and the sex and longevity of the emerged adults. Statistical analyses were performed using the Program Biostat 5.0®, with a 5% significance level. We used a Kruskal–Wallis test to compare means related to: duration of the mating process; time of development and adult longevity. The sex ratio of the adults was compared by applying a Chi-Square test.

Results

Reproductive behaviour

Throughout the experiment, we observed 55 copulations for *B. stallengi*. In the case of *S. ruficornis*, 87 (38%) out of 229 mating attempts were interrupted by competing males, that is, males that approached the couple forcing its separation before copulation, and thus 142 mating events were considered successful.

Pre-copulation acts included part of the courtship dynamics and involved sound signals, mechanical stimulation and a variety of body movements (Fig. 2). In both species, males approached females and performed repeated, short and rapid flights associated with strong wing vibration and intense sound. Immediately following the approach of the male (ca. 15 cm from the female), attempts at copulation took place, in which the male performed short-distance flights around the female until landing on her dorsum. For *B. stallengi*, no rejection behaviour by the female and no aggressive male-male competition were observed. All females were courted by a single male per event. Every attempt to mate was successful and the pair remained in copula until separation.

On the other hand, pre-copulatory behaviour of *S. ruficornis* differed markedly, with evident pre-copulatory selection and male-male competition. Males approached females faster and more aggressively than *B. stallengi* males. Successful copula in *S. ruficornis* only occurred after several failed courting attempts by different males. Females often showed non-receptivity behaviour, refusing the male's attempt to mate in two ways: (1) taking away – female attempted to escape the male's overture by flying off to the vicinity of the male, or to the other side of the cage, and (2) repelling movements – when a male successfully mounted a female, she insistently fluttered her wings and kicked the male using here forelegs. This behaviour was effective to reject the male before the insertion of the aedeagus.

In both species, the copulating male remained behind and close to the dorsal surface of the female, while it curled its abdomen downward. The front legs tended to stay up or on the mesonotum of the female. At the same time, females grasped the everted cerci of the mounting male with their hindlegs tarsi, intermittently. During copulation, the male's body remained at an angle of 45° in relation to the horizontal position of female's body. Females performed slight lateral movements, with different intensities (Video 1).

In *S. ruficornis*, male-male aggressive behaviour was recorded and several attempts were performed by other males to disturb the mating pair (Video 2), which included: (1) another male flying

Table 1
Behavioural acts associated with mating in two species of Sarcophagidae.

Behavioural acts in courtship and mating	*Blaesoxipha stallengi*	*Sarcophaga ruficornis*
Courtship		
Abundance of males around the female (15 cm radius) in attempt courtship	Low (1–2 males)	High (>10 males)
Interference of males (e.g., attempt to prevent approach of competitors towards female)	Absent	Present
Initial/preliminary rejection of males by the female, by pushing away males with hind legs	Absent	Present
Sound production (intense wing vibration) by males	Present	Present
Female accepts male by allowing physical contact	Immediate/easy	Hesitant/difficult
Mating		
Female opens wings prior to mating	Present	Present
Body position of the male-female association	At a 45° angle	At a 45° angle
Post-abdomen stimulus (male and female) during mating	Present	Present
Movements of the body (latero-lateral) during mating	Present	Present
Intense competition between males during mating	Absent	Present
Mating disruption by competitor males	Absent	Present
Mean duration of mating (min)	64.2 ± 8	69.8 ± 5
Retraction of aedeagus for copulation completion.	Rapid	Rapid
Post-copulation		
Male grooming (legs and aedeagus)	Present	Present
Displacement of the female to the food after mating	Present/immediate	Present/immediate
Reproductive strategy	Oviposition	Larviposition

Table 2
Preferential time for mating and ovi/larviposition of two forensically relevant species, *Blaesoxipha stallengi* (Lahille) and *Sarcophaga ruficornis* (Fabricius).

Species		Morning	Afternoon	χ^2	d.f.	*p*
B. stallengi	Mating (*n* = 55)	92.7%	7.3%	71.23	1	<0.001
	Oviposition (*n* = 30)	90.0%	10.0%	62.41	1	<0.001
S. ruficornis	Mating (*n* = 229)	40.2%	59.8%	3.84	1	0.05
	Larviposition (*n* = 75)	77.3%	22.7%	28.73	1	<0.001

around and close to the mating pair, (2) another male trying to engage copula with a mating male, and (3) other males crawling constantly around the mating pair. When the interruption process was successful, the female usually did not engage in a second copula with the aggressing male.

Mating behaviour are summarized in Table 1. *B. stallengi* preferred to copulate in the morning (92.7% of the cases; *p* < 0.001), whereas *S. ruficornis* copulated slightly more frequently in the afternoon – 59.8% of the successful mating (*p* = 0.05). Mean duration of copulation was 64.2 min for *B. stallengi* and 69.8 min for *S. ruficornis* and this difference was not significant (H = 0.013; d.f. = 1; *p* = 0.987).

In both species, the female took the initiative in ending copulation, by kicking the male with the hindlegs until separation, simultaneously to a rotatory movement (360°) to help disengaging. Near the copulation ending, males cleaned the front legs and genitalia and females walked towards the available food.

The species differed in offspring deposition: *B. stallengi* is ovoviviparous and *S. ruficornis* is viviparous, laying first instar larvae. Females of *S. ruficornis* deposited the majority of larvae (ca. 90%) in protected parts of the substrate, such as crevices and orifices whereas *B. stallengi* typically laid their eggs in arrangements (packets) on the surface of the substrate. Eggs of *B. stallengi* were whitish and cylindrical, and laid in packs of 5–8 eggs scattered on the substrate. Both species demonstrated a significantly more frequent ovi/larviposition in the morning (Table 2) which reached 90% in the case of *B. stallengi*.

Time of development

Of the 250 larvae of each species tested, 180 *B. stallengi* and 200 *S. ruficornis* completed the life cycle until adult. The mean duration was based on the data of surviving adults. For *B. stallengi*, the total development time, from egg to the emergence of adult, was 22.9 ± 0.8 days. Adult mean longevity was 15.7 ± 0.8 days. The sex ratio for the emerged adults was 0.68 female:male (Table 3). For *S.*

ruficornis, larviposition occurred 2.5 days after the copula and took place in both morning and afternoon. Total development time, from 1st instar larva to the emergence of adult, was 21.3 ± 0.7 days. Mean longevity of adults was 16.3 ± 0.7 days. Sex ratio of emerged adults was 1.00 female:male (Table 3).

The mean time until emergence varied slightly between males and females of *B. stallengi*, as females tended to emerge one day earlier. The time of development and the longevity for both species did not differ significantly ($H_{\text{life cycle}}$ = 2.001; d.f. = 1; *p* = 0.157; $H_{\text{longevity}}$ = 1.370; d.f. = 1; *p* = 0.241) and are shown in Table 3.

Discussion

This study reveals similarities (e.g., preferential time for ovi/larviposition) in the reproductive behaviour of the flesh flies *B. stallengi* and *S. ruficornis*. Some peculiarities, however, distinguish their pre-copulatory behaviour (Fig. 2), such as interruptions caused by male competition in the case of *S. ruficornis* and differences in the laying strategy – eggs for *B. stallengi* and larvae for *S. ruficornis*. Males take the initiative for mating without noticeable signs from the female that lead to encouragement and produce sound by vibrating the wings fiercely during courtship, a behaviour previously described for *Sarcophaga* species (Thomas, 1950).

The complexity of pre-copulatory behaviour in Sarcophagidae encompasses aggression between competing males (Moore et al., 2014; Shropshire et al., 2015) and elaborate courtship displays (Spofford and Kurczewski, 1985). The intense and aggressive male-male competition and the rejection of mating attempts by females suggest that *S. ruficornis* shows strong pre-copulatory sexual selection. This strategy is characterized by females being highly selective to specific physical and behavioural male traits prior to mating, and a second mating is usually rare or inexistent (Kvarnemo. and Simmons, 2013). On the other hand, *B. stallengi* showed no apparent pre-copulatory selection, given the absence of male competitors in

Table 3
Duration of immature stages, sex ratio and longevity of the adult of *Blaesoxipha stallengi* (Lahille) and *Sarcophaga ruficornis* (Fabricius) under laboratory conditions (25 ± 5 °C, 50 ± 10% RH with 12 h of photophase).

Duration of each phase, in hours (mean ± SD)	Blaesoxipha stallengi	Sarcophaga ruficornis
Egg	18.2 ± 3.7	–
1st instar larva	24 (n = 250)	24 (n = 250)
2nd instar larva	24 (n = 250)	43 ± 9.3 (n = 250)
3rd instar larva	82.2 ± 17 (n = 200)	72.0 ± 2 (n = 220)
Pupa	403.5 ± 20 (n = 200)	372.5 ± 12.8 (n = 220)
Total time of development (from egg/1st instar to adult)	551.0 ± 27.6 (n = 180)	512.1 ± 15.3 (n = 200)
Sex ratio (female/male + female)	0.68	1
Longevity of the adult (h)	378.0 ± 19.0	391.7 ± 17.2

the vicinity of the available female, the female's acceptance of each courting male and the lack of mating disruption by another male (Thornhill and Alcock, 1983).

A common ecological factor shared by *S. ruficornis* and *B. stallengi* is the unpredictability and scarcity of the resource (e.g., carcass or cadaver). The brief period of availability of the carcass imposes a narrow window of opportunity for mating events, offspring deposition and larval development. In this situation, mating strategies may evolve differently. For example, in species depending of a time-limited resource, such as a rotting carcass, females may be less selective for available males, mating as soon as possible to avoid the risk to remain unmated at the end of the resource, so that the progeny can utilize the decomposing resource while it remains suitable. This explains why females with limited time to copulate are usually less discriminating for mates (Moore and Moore, 2001). On the other hand, a strong pre-copulatory sexual selection can also be associated with an ephemeral resource. Females must be more selective for high-quality males to ensure a greater survivorship of the offspring in a food resource exposed to intense competition, which is the case of carrion.

xxxxxxxxxxxxxxThe precopulatory behaviour shown in *S. ruficornis* may be a result of a female sexual mating system such as monandry (Thornhill and Alcock, 1983), or a consequence of ecological factors such as a biased operational sex ratio (Weir et al., 2011; Painting et al., 2014). On the other hand, if the sex ratio tends to be female biased, as observed for *B. stallengi*, we expect that the number of available females overcoming the number of males may reduce male-male competition, resulting in low or absence of pre-copulatory sexual selection (Emlen and Oring, 1977; Weir et al., 2011).

For the highly competitive *S. ruficornis*, when copulation is interrupted early, females tend to mate again. A successful copula requires a minimum time for the male to completely inseminate the female (Thornhill, 1980), and when the mating pair is interrupted, females still tend to be receptive for a second male, if the interrupted copulation did not allow for the transfer of a satisfactory amount of sperm (Gilchrist and Partridge, 2000).

The duration of copulation in both species is similar to what has been observed for other sarcophagid species (Thomas, 1950). Longer copula may arise as a means of mate guarding, when males must ensure that the transferred ejaculate will be used to fertilize the egg clutches before the female is ready to copulate with a rival male (Simmons, 2001). In *Drosophila melanogaster* Meigen, 1830, for example, a long copula allows the male to transfer chemical substances along with the seminal fluid, which increase refractory period and stimulate oviposition (Chen et al., 1988). Curiously, the period of copulation differed between the species, despite similarities in their period of activity.

According to Pape (1987), all sarcophagids deposit incubated eggs with mature first instar larvae ready to hatch, or newly-hatched first instar larvae, or, additionally, second instar larvae that have been nourished by the maternal accessory glands.

The ovoviviparity in *B. stallengi*, as opposed to the majority of sarcophagid flies, which are viviparous (Pape, 1996), has been recorded for *Sarcophaga* (*Liosarcophaga*) *aegyptica* (Salem, 1935) (Salonã Bordas et al., 2007) and *Blaesoxipha* (*Gigantotheca*) *plinthopyga* (Wiedemann, 1830) (Pimsler et al., 2014), which suggests that oviposition has been largely overlooked for the family.

Total development time did not differ much between the two species (22.9 days for *B. stallengi* and 21.3 for *S. ruficornis*), and this variation derives from the additional time of egg development in *B. stallengi*. Bionomic data for *B. stallengi* is limited to those presented in this paper, while *S. ruficornis* takes an average of 420 h to complete its life cycle at 25 °C (Nassu et al., 2014). Nevertheless, in the present study the species had a longer development time, requiring 512 h at 25 °C.

Development time for *B. stallengi* was similar to other flesh flies with ability to lay eggs under laboratory conditions, for example, *Sarcophaga* (*Liopygia*) *argyrostoma* (Robineau-Desvoidy, 1830) (Grassberger and Reiter, 2002). The life cycle of *S. argyrostoma* takes 22 days at 25 °C, similar to our data for *B. stallengi*. In both species, pupal stage has the longest duration, which corroborates previous studies (Grassberger and Reiter, 2002; Nassu et al., 2014). In fact, duration of pupal stage varied little among the species (15.5–16.8 days), and was similar to that recorded for another species associated with cadaveric colonization such as *Peckia* (*Peckia*) *chrysostoma* (Wiedemann, 1830) (Ferraz, 1992). Mean longevity also varies little - both live for ca. 16 days – and is lower than that observed for other species of Sarcophagidae (Kamal, 1958).

Eggs of *B. stallengi* resemble – and are laid in a similar way – as those of *S. aegyptica* and *B. plinthopyga* (Salonã Bordas et al., 2007; Pimsler et al., 2014), as whitish, elliptical and cylindrical egg mass of no more than ten eggs. Both *B. stallengi* and *S. ruficornis* deposit their progeny preferentially in the morning, which supports data by Oliveira and Vasconcelos (2018), who demonstrated that over 90% of the sampled flesh flies in a fragment of Caatinga were active during the morning.

Due to the absence of studies on the life cycle and reproductive behaviour of flesh flies endemic to the Brazilian Caatinga we present here the first integrated data on the reproductive behaviour and development of *B. stallengi*, a species attracted to carcasses at early and intermediate stages of decomposition (Oliveira and Vasconcelos, 2018). Data on the life cycles of species are crucial for the consolidation of forensic entomology in South America, in which several cities exhibit extremely high rates of homicide. The pronounced similarities in the morphology of both species – combined with their similar time of development – may act as confounding factors for forensic entomologists and stress out the need for an accurate taxonomical identification. Furthermore, since both species studied here develop in carrion, bionomical data are of the utmost importance to subsidize reliable estimation of the post-mortem interval.

Conflicts of interest

The authors declare no conflicts of interest.

Supplementary material

Video 1. Position of *Blaesoxipha stallengi* (Lahille) adults during copulation, with emphasis on body movements.

Video 2. Attempts of copulation disruption by competing males of *Sarcophaga ruficornis* (Fabricius).

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rbe.2019.02.006.

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