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Original Article

First list of parasitoids in flies of medical, veterinary, and forensic importance in Rio Grande do Sul – Brazil

Primeira lista de parasitoides em moscas de importância médica, veterinária e forense no Rio Grande do Sul - Brasil

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Abstract

Necrophagous flies are of great importance for human and animal health. In places where their development occurs, parasitoids can be used as a tool to control these dipterans. In Brazil, the fauna of these parasitoids has been investigated in some regions. However, in Rio Grande do Sul, it is known the occurrence of only one species. Thus, this study aimed to create the first list of parasitoids in flies of medical and veterinary importance in Southern Brazil. Collections took place in the municipality of Pelotas (31º 42' S; 52º 18' W). Three replicates consisting of a 500 g bovine liver chunk placed in a tray were exposed to open air for 20 days. Then pupae were individualized and observed until their emergence. We identified 4,882 adult flies of Calliphoridae, Fanniidae, Muscidae, and Sarcophagidae. 4,040 parasitoids emerged, belonging to eight species, of which *Nasonia vitripennis, Spalangia cameroni, Spalangia chontalensis*, and *Tachinephagus zealandicus* are new records in Rio Grande do Sul state. Also, three undescribed species of the genus *Aphaereta* were collected. In spite of being the first attempt to inventory the fauna of hymenopteran parasitoids, this study may help in the development of management programs of these dipterans in the region.

Keywords: biological control, Diptera, forensic entomology, Hymenoptera.

Resumo

Moscas necrófagas possuem grande importância para a sanidade humana e animal. Onde estas se desenvolvem ocorrem parasitoides que podem ser utilizados como ferramentas para o controle desses dípteros. No Brasil, a fauna desses parasitoides vem sendo investigada em algumas regiões e no Rio Grande do Sul é conhecida a ocorrência de apenas uma espécie. Portanto, o objetivo deste estudo foi criar a primeira lista de espécies de parasitoides de moscas de importância médica e veterinária ocorrentes no extremo sul do Brasil. As coletas foram realizadas no município de Pelotas (31º 42' S; 52º 18' O). Foram montadas três réplicas de bandejas contendo, cada uma, 500 g de fígado bovino, expostas ao ar livre durante 20 dias. Na sequência, as pupas foram individualizadas e observadas até a emergência. Foram identificados 4.882 adultos pertencentes às famílias Calliphoridae, Fanniidae, Muscidae e Sarcophagidae. Emergiram 4.040 parasitoides, pertencentes a oito espécies, das quais *Nasonia vitripennis, Spalangia cameroni, Spalangia chontalensis* e *Tachinephagus zealandicus* são novas ocorrências para o Rio Grande do Sul. Foram coletadas três espécies ainda não descritas do gênero *Aphaereta*. Apesar de ainda ser um primeiro esforço de inventariar a fauna de himenópteros parasitoides, este estudo pode auxiliar no desenvolvimento de programas de manejos desses dípteros na região.

Palavras-chave: controle biológico, Diptera, entomologia forense, Hymenoptera.

1. Introduction

Necrophagous dipterans are important for acting as a mechanical vector of bacteria, protozoa, helminths, and viruses to human and domestic animals (Marcondes and Thyssen, 2017), as well as for some species being able to cause myiasis (Guimarães and Papavero, 1999). Also, they are the first insects to reach the carcass, appearing in the first few hours after the death. Due to that and other aspects, they have been used as important forensic indicators (Battán Horenstein et al., 2010; Oliveira-Costa et al., 2014).

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In places where these dipterous larvae occur, we are able to find, naturally, a rich parasitoid fauna of Braconidae, Ichneumonidae, Pteromalidae, Figitidae, Eulophidae, Chalcididae, and Diapriidae (Hymenoptera) (Sereno et al., 2016). The immature phase of these wasps uses an insect (host) as a resource, always resulting in the death of the parasitized insect. That can be caused by a toxin injected by the female wasp during oviposition, by poisoning through salivary secretion or by feeding (Quicke, 1997; Asgari and Rivers, 2011). These hymenopterans use larvae, prepupae, or pupae of flies to develop, and because of this; they are found next to the sites that the flies occur (Rivers, 2016).

Precise information on the species of these families that occur in such different ecosystems is of the utmost importance for both medical and forensic entomology (Voss et al., 2009). According to Rivers (2016), these insects can widen the PMI window to include the time after necrophagous flies cease feeding, reveal if the corpse was moved to another location prior to discovery, indicate the period of the year that death occurred and help to find concealed bodies. The information about the natural occurrence of these hymenopterans is also important to develop control strategies of these dipterans in a given region because parasitoids in high abundance are probably the most promising to rearing and mass release (Geden and Hogsette, 2006).

The knowledge about the fauna of parasitoids of necrophagous flies in Brazil is still incipient and is focused on Southeast and Mid-West regions of the country (Paiva et al., 2018; Silveira et al., 1989). The few reports on the occurrence of these insects in Southern Brazil are concentrated in Paraná (Silveira et al., 1989; Gibson, 2009) and Santa Catarina (Gibson, 2009) states. In Rio Grande do Sul, there is just one report on the occurrence of *Spalangia endius* Walker, 1839 (Hymenoptera: Pteromalidae) (Brandão et al., 2011). Thus, this study aimed to report the occurrence of parasitoids in flies of medical, veterinary, and forensic importance in Southern Brazil.

2. Material and Methods

The study was carried out in the municipality of Pelotas, Southern Brazil. The average temperature ranges from 12.3 ºC in July to 23.2 ºC in January and average annual precipitation is 1,367 mm, with well-distributed rain throughout the year (Estação Agroclimatológica de Pelotas, 2020). The collection took place in the Instituto Federal Sul-Rio-Grandense, Câmpus Pelotas - Visconde da Graça (31º 42' 48.13" S; 52º 18' 53.48" W). The *campus* area is located in the city outskirts and has rural characteristics. The area is about 200 ha, where about 200 animals such as equines and bovines are raised. Also, there are some agricultural school buildings.

In the forenamed area, three collection points were chosen, with at least 150 m between them. A tray (44 x 28.5 x 8.2 cm) with the bottom covered with 3 cm of moistened sawdust was placed at each collection point. A 500 g piece of raw bovine liver was placed over the sawdust. Trays were monthly exposed for 20 days between September 2017 and January 2018. After the exposure period, the trays were taken to the laboratory, where pupae were manually collected, placed individually in gelatin capsules (number 00), and kept at room temperature. Pupae were daily observed until the emergence of flies or parasitoids. If pupae have not emerged after 60 days from the collection, they were dissected to look for traces of parasitism.

Fanniidae, Muscidae, and Calliphoridae were identified with a dichotomic key proposed by, respectively, Wendt and Carvalho (2007), Carvalho (2002), and Carvalho and Mello-Patiu (2008). Parasitoid genera of Encyrtidae and Pteromalidae were identified with the dichotomic keys made by, respectively, Noyes et al. (1997) and Bouček and Heydon (1997). Parasitoid species of *Tachinaephagus*, *Nasonia*, and *Spalangia* were identified with dichotomic keys proposed by, respectively, Subba Rao (1978), Darling and Werren (1990), and Gibson (2009). Individuals of Braconidae were sent to Drª. Maria Angélica Penteado-Dias.

We evaluated the number of pupae collected per month, the number of adult flies or parasitoids that emerged from each collection and the number of parasitized pupae per collection (with adult emergence or parasitism signs). We also recorded the parasitism rate (relation between the number of parasitized pupae and the total number of pupae) and the parasitism viability (relation between the number of pupae that emerged parasitoids and the number of parasitized pupae).

3. Results

A total of 5,899 dipterous pupae were collected, of which 4,882 have originated adult flies. A number of 426 pupae were parasitized and the parasitism rate ranged from 2.41 to 19.27% throughout the months. Of those parasitized pupae, 309 pupae had emergency of parasitoids and the parasitism viability ranged from 35.84% in December to 84.48% in September (see Table 1).

The flies collected belong to the families Calliphoridae, Sarcophagidae, Muscidae, and Fanniidae. Calliphoridae was the family with the highest number of species collected, represented by the genera *Calliphora*, *Chrysomya*, *Lucilia*, and *Sarconesia*. Among them, *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae) was the most abundant species (2,536 specimens). However, the most frequent species was *Lucilia eximia* (Wiedemann, 1819) (Diptera: Calliphoridae), collected in all the sampled months. Sarcophagidae was the second most abundant family, but the individuals were not identified at a specific level. Muscidae was represented by *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae), *Hydrotaea aenescens* (Wiedemann, 1830) (Diptera: Muscidae), and *Muscina stabulans* (Fallen, 1817) (Diptera: Muscidae), the latter being the most abundant and frequent species of this family. Fanniidae was represented by just one genus, *Fannia* (see Table 2).

The parasitoids collected belong to the families Braconidae, Encyrtidae, and Pteromalidae. The family with the highest number of species was Pteromalidae, with four species, while Encyrtidae was represented

Table 1. Pupae, adult flies, parasitized pupae number, pupae number with parasitoid emergence, parasitism rate and viability in samples collected in bovine liver in Southern Brazil.

Months	Pupae number	Adult flies number	Parasitized pupae number	Pupae number with parasitoid emergence	Parasitism rate (%)	Parasitism viability (%)	Mean temperature (ºC)
September*	393	297	58	49	14.75	84.48	18.3
October	851	564	164	131	19.27	79.87	18.2
November	1,839	1,411	103	80	5.60	77.66	19.1
December	832	738	53	19	6.37	35.84	22.5
January	1,984	1,872	48	30	2.41	62.50	23.7
Total	5,899	4,882	426	309	-	-	-

*There was a loss of one replicate due to an attack of necrophagous vertebrates to the bait.

Table 2. Number of individuals from Diptera and Hymenoptera collected in Southern Brazil.

Diptera		Months				
Family	Genus/species	September	October	November	December	January
Calliphoridae	*Calliphora vicina*	58	61	-	-	-
	Chrysomya megacephala	-	-	-	56	155
	Chrysomya albiceps	-	386	631	503	1,016
	Lucilia eximia	72	76	632	109	393
	Sarconesia chlorogaster	27	-	-	-	-
Muscidae	*Musca domestica*	-	-	19	-	-
	Muscina stabulans	73	12	94	-	-
	Hydrotaea aenescens	09	-	-	-	-
Fanniidae	*Fannia*	13	-	17	09	-
Sarcophagidae		45	29	18	61	308
Total		297	564	1,411	738	1,872

Hymenoptera		Months									
Family	Genus/species	September		October		November		December		January	
		PP	EP	PP	EP	PP	EP	PP	EP	PP	EP
Pteromalidae	*Nasonia vitripennis*	-	-	3	35	1	5	-	-	2	16
Pteromalidae	*Spalangia cameroni*	-	-	-	-	21	21	-	-	-	-
Pteromalidae	*Spalangia chontalensis*	9	9	-	-	9	9	1	1	24	24
Pteromalidae	*Spalangia endius*	13	13	1	1	30	30	-	-	-	-
Encyrtidae	*Tachinaephagus zealandicus*	15	204	124	3,126	15	204	18	263	3	29
Braconidae	*Aphaereta* sp nov. 1	10	10	3	3	3	3	-	-	1	1
Braconidae	*Aphaereta* sp nov. 2	2	15	-	-	-	-	-	-	-	-
Braconidae	*Aphaereta* sp nov. 3	-	-	-	-	1	18	-	-	-	-
Total		49	251	131	3,165	80	290	19	264	30	70

PP – parasitized pupae number; EP – emerged parasitoid number.

only by *Tachinaephagus zealandicus* Ashmead, 1904 (Hymenoptera: Encyrtidae). Notwithstanding, Encyrtidae was the family with the highest number of individuals and was collected in all months. The number of collected species varied within the months, of which November had the highest richness – seven species – and December, the lowest richness – two species (see Table 2).

For the first time in Rio Grande do Sul, the occurrence of *Nasonia vitripennis* (Walker 1836) (Hymenoptera: Pteromalidae), *Spalangia cameroni* Perkins, 1910 (Hymenoptera: Pteromalidae), *Spalangia chontalensis*

Cameron, 1884 (Hymenoptera: Pteromalidae), and *T. zealandicus* (see Table 2) was reported. Furthermore, three undescribed species of *Aphaereta* were found.

4. Discussion

The dipterans reported in this study are commonly found in Southern Brazil, in livestock farming areas (Vianna et al., 1998; Bicho et al., 2004; Azevedo and Krüger, 2013) as well as in decomposing carcasses (Souza et al., 2008; Anjos et al., 2010).

Calliphoridae family has dominated the necrophagous dipteran community. This taxon is commonly found in faunal succession in forensic studies using human corpses (Carvalho et al., 2000) or animal models (Carvalho et al., 2000; Battán-Horenstein et al., 2012). The most abundant species, *C. albiceps* and *L. eximia*, are both indicated as potential tools to estimate *post mortem* interval in forensic entomology (Vasconcelos and Araujo, 2012; Sanford et al., 2014). Besides, they are important species that can cause myiasis (Sanford et al., 2014) and act as a mechanical vector for human pathogens (Greenberg, 1971).

The parasitoids identified in this study have already been reported in Brazil. However, only *S. endius* was recorded in Rio Grande do Sul (Brandão et al., 2011). The most southern state where *S. cameroni* and *S. chontalensis* had been found so far was Santa Catarina, while *N. vitripennis* and *T. zealandicus* were reported in São Paulo and Paraná, respectively (De Santis, 1980; Silveira et al., 1989; Lecheta and Luz, 2015). Thus, our study extends the occurrence area of these four species until the southernmost state of the country.

The high abundance and frequency of *T. zealandicus*, plus its ability to infest important fly species, such as *M. domestica* (Legner, 1967), *Cochliomyia hominivorax* Coquerel, 1858 (Diptera: Calliphoridae) (Silveira et al., 1989), *Stomoxys calcitrans* (Linnaeus, 1758) (Diptera: Muscidae) (Costa, 1989), *Chrysomya putoria* (Wiedemann, 1830) (Diptera: Calliphoridae) (Monteiro and Prado, 2000), *Sarconesia chlorogaster* Wiedemann, 1830 (Diptera: Calliphoridae) (Lecheta and Luz, 2015) and *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) (Moretti and Ribeiro, 2006), make this species a potential biological control agent of these dipterans in Southern Brazil.

The constancy of *S. endius*, *S. cameroni*, and *N. vitripennis* populations throughout the months in Rio Grande do Sul, in addition to the fact that these species have already been commercialized in the United States and Canada (Lebeck and Leppla, 2020), shows that they are potential biological control agents of flies for integrated pest management programs in the area of this study.

Although there is a lack of knowledge about the biology, ecology, and behavior of *S. chontalensis*, this species seems to be important in the population dynamics of necrophagous flies in Southern South America, because it has already been reported in Argentina, Paraguay, and Uruguay (Gibson, 2009) and it was the solitary parasitoid most frequently collected throughout the months in this study.

Among the collected parasitoids, only *N. vitripennis* and *T. zealandicus* have already been reported in a forensic case study (Turchetto and Vanin, 2004). According to Rivers (2016), the potential use of parasitoids of necrophagous flies as forensics evidence is still not very explored due to the lack of knowledge about the biology, ecology, and behavior of these species. The occurrence of *N. vitripennis*, *S. cameroni*, *S. chontalensis*, and *S. endius* in just some months can be important from the forensic point of view because seasonality is especially useful to determine if a corpse was removed from the crime scene (Rivers, 2016).

In spite of being performed in just one area, this study was able to report three species from the genus *Aphaereta* that have not been described yet. Besides, four new occurrences of parasitoids of necrophagous flies in Southern Brazil were reported, and the diversity of this group in this area is likely to be even bigger. Thus, it is of utmost importance that other studies are carried out in this region, once they are important insects for biological control and forensic entomology.

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