Parasitoids of Diptera of forensic interest collected in Goiás, Brazil

Carlos Henrique Marchiori

1 Instituto Federal Goiano

Abstract

The aim of this study was to conduct a survey of parasitoids of Diptera of forensic interest collected from cattle dung, buffalo dung, human feces, bovine liver, chicken manure, pig carcasses and fish in the state of Goiás, Brazil, between May 1998 and February 2013. The pupae were obtained using the flotation method. They were individually placed in gelatin capsules until the emergence of adult dipterous insects or their parasitoids. Musca domestica L., 1758 (Diptera: Muscidae) was the host species with the highest number of species of parasitoids. The most common parasitoid collected from the dipterous was Pachycrepoideus vindemmiae (Rondani, 1875) (Hymenoptera: Pteromalidae), followed by Nasonia vitripennis (Walker, 1836) (Hymenoptera: Pteromalidae).

Key words: Arthropoda, Insecta, Hymenoptera, Entomology, substrates.


INTRODUCTION

Forensic Entomology - the study of insects and other arthropods associated with various criminal issues - serves as an auxiliary tool, for example, in investigating crimes against victims of violent death (Pujol-Luz, et al, 2008).

The development of forensic entomology in Brazil has been facilitated by the solid Brazilian tradition of studies on insects of the orders Diptera and Coleoptera. Other groups of insects are also relevant to Forensic Entomology, but dipterous and beetles are the most important, especially in cases involving death.

Because of the importance of dipterous and beetles within medicine, public health,
veterinary science and agriculture, they have been studied extensively by professionals in various fields of knowledge. In addition, Brazilian taxonomic collections contain a reasonable representation of the diversity of insects in this region. There are obviously still important gaps, especially the scarcity of information on the biology, ecology and geographical distribution of scavenger species (Pujol-Luz, et al., 2008).

The aim of this study was to conduct a survey of parasitoids of Diptera of forensic interest collected in the cerrado region in the state of Goiás, Brazil.

MATERIALS AND METHODS

Experiment with Cattle Dung

Every fortnight, 10 plates of fecal cake (of approximately 3 kg each) were produced from fresh bovine feces that were collected immediately after defecation in pastures of Brachiaria brizantha (Hochst ex. A. Rich) and in corrals. The material was collected in plastic buckets and was homogenized. It was then placed in 10 round plastic supports of 20 cm in diameter, with a hole to allow rainwater to drain away. This methodology was used for precise determination of the time between the emission of the fecal cake and its collection.

The feces remained exposed (five in the pastures and five in the corrals) for 15 days. After this period, the feces were taken to the laboratory for extraction of pupae by means of the flotation method. The pupae were removed with the aid of a sieve; they were counted and individually stored in gelatin capsules (number 00) until the dipterous and/or parasitoids emerged. The parasitoids and dipterous that emerged were identified with the aid of a stereoscopic microscope and were conserved in 70% alcohol.

Experiment with Human Feces, Cattle Kidneys, Cattle Liver and Fish

The dipterous were collected by using traps, made of dark cans measuring 19 cm in height and 9 cm in diameter, with two openings resembling blinders, located in the lowest third of the can, to allow dipterous to enter. The top of the can was connected to a nylon funnel that was open at both ends, with the base pointing down. This was wrapped in plastic bags, so that when they were removed, the dipterous and parasitoids could be collected. The following items were used as baits: human feces, cattle kidneys, cattle liver, fish and fruit, which were placed inside the cans, over a layer of earth. Four traps were used and they were hung on trees at a height of one meter above the ground, two meters apart from each other. The insects collected were taken to the laboratory, sacrificed with ethyl ether and kept in 70% alcohol for further identification. To obtain the
parasitoids, the contents of the traps were placed in plastic containers with a layer of sand for use as a substrate for transformation of the larvae into pupae. This sand was sifted after being in the fields for 15 days and the pupae were extracted from it and were individually placed in gelatin capsules (number 00) in order to obtain the dipterous and/or parasitoids.

Experiment with Ptifall.

Each trap consisted of a plastic receptacle (basin) of 15 cm in diameter by 10 cm in height. Each receptacle was buried in earth, such that its upper extremity was at the ground surface level. One liter of water, 20 ml of detergent and 2 ml of formol were placed in each receptacle. A 200 ml pot was attached to the basin by means of a thin wire that went across it close to its edge, so as to keep the pot hanging and centralized in the basin. The bait, consisting of human feces, was placed in this pot. This trap was protected by another plastic receptacle of the same measurements (15 x 10 cm), which functioned as a cover.

This contained four diametrically opposite holes of around 5 cm in diameter and 7 cm in height, and was supported on a metal wire suspended 10 cm from the group. Six traps were used, with separations of two meters between each other, placed randomly. The bait was replaced every 15 days. The pupae that were found in the bait were separated out by means of the flotation method. These were then individually packed in gelatin capsules until the parasitoids/ and/or dipterous emerged.

Experiment with Buffalo Dung

Every fortnight, 10 plates of fecal cake (of approximately 3 kg each) were produced from fresh bovine feces that were collected immediately after defecation in pastures of Brachiaria brizantha (Hochst ex. A. Rich) and in corrals. The material was collected in plastic buckets and was homogenized. It was then placed in 10 round plastic supports of 20 cm in diameter, with a hole to allow rainwater to drain away.

This methodology was used for precise determination of the time between the emission of the fecal cake and its collection. The feces remained exposed (five in the pastures and five in the corrals) for 15 days. After this period, the feces were taken to the laboratory for extraction of pupae by means of the flotation method. The pupae were removed with the aid of a sieve; they were counted and individually stored in gelatin capsules (number 00) until the dipterous and/or parasitoids emerged. The parasitoids and dipterous that emerged were identified with the aid of a stereoscopic microscope and were conserved.
in 70% alcohol.

Experiment with Manure Chicken
The feces collected originated from 40 "Hyline" chickens that were reared in a cage system. The feces that accumulated under the cages varied in terms of moisture content, ranging from pasty to firm. Fresh feces (pasty), which were collected immediately after emission, were placed in five basins of 30 cm in diameter and 12 cm in height, and were left in an appropriate environment, in a dry location, for 15 days. For extraction of the pupae using the floatation method, the routine procedures for this type of experiment were modified. The parasitoids and dipterous that emerged were identified with the aid of a stereoscopic microscope.

Experiment with Pig Carcasses
In a natural area, two pig carcasses (Sus scrofa) weighing approximately 10 kg each were used as bait. The pigs were killed mechanically with a blow to the head and were immediately placed inside metal frame cages to exclude large vertebrate scavengers. Underneath the cages, metal trays with sawdust were placed to collect the pupae. The pupae were extracted by means of flotation in water. The pupae were individually placed in gelatin capsule.

The experiments were conducted in May 1998 and February 2013.

RESULTS
Studies on the Diptera and their parasitoids in south of Goiás were selected according to the type (specie) of host in which natural enemies were found.

1 - *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae).
*Hemencyrtus* sp. (Hymenoptera: Encyrtidae), *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) and *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae) (Marchiori et al., 2002a; Marchiori et al., 2003a; Marchiori et al., 2004a; Marchiori, 2005a).

2 - *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae).
*Brachymeria podagrica* (Fabricius, 1789) (Hymenoptera: Chalcididae), *N. vitripennis* and *P. vindemmiae* (Marchiori et al., 2000a; Marchiori et al., 2003a; Marchiori et al., 2004a; Marchiori, 2005a).

3 - *Cyrtoneurina pararescita* Couri, 1995 (Diptera: Muscidae).
Paraganaspis egeria Diaz, Gallardo & Walsh, 1996 (Hymenoptera: Figitidae), *P. vindemmiae*, Spalangia cameroni/Perkins, 1910 (Hymenoptera: Pteromalidae), *Spalangia endius* Walker, 1839 (Hymenoptera: Pteromalidae), *Spalangia nigra* Latrielle, 1805 (Hymenoptera: Pteromalidae), *Spalangia nigroaenea* Curtis, 1839 (Hymenoptera: Pteromalidae) and *Spalangia* sp. (Hymenoptera: Pteromalidae) (Marchiori et al., 1999; Marchiori et al., 2000a; Marchiori et al., 2001b; Marchiori et al., 2002b; Marchiori et al., 2003b; Marchiori et al., 2005b).

4 - *Euboettcheria collusor* (Curran et Walley, 1934) (Diptera: Sarcophagidae).
*Hemencyrtus* sp. (Marchiori et al., 2003a; Marchiori et al., 2004a).

5 - *Fannia pusio* (Wiedemann, 1810) (Diptera: Fanniidae).
*Eurytoma* (Hymenoptera: Eurytomidae), *P. vindemmiae, P. egeria, Spalangia drosophilae* Ashmead, 1887 (Hymenoptera: Pteromalidae), *S. endius* and *S. nigra* (Marchiori et al., 2003a;c; Marchiori et al., 2004a; Marchiori et al., 2005c; Marchiori, 2007a).

6 - *Hemilucilia flavifacies* Engel, 1931 (Diptera: Calliphoridae).
*B. podagrica* and *Hemencyrtus herbertti* Ashmead, 1900 (Hymenoptera: Encyrtidae) (Marchiori et al., 2003b; Marchiori et al., 2004).

7 - *Musca domestica* L., 1758 (Diptera: Muscidae).
*Hemencyrtus* sp., *H. herbertti*, *Muscidifurax raptor* Girault & Sanders, 1910 (Hymenoptera: Pteromalidae) *N. vitripennis, P. vindemmiae, Spalangia* sp. (Hymenoptera: Pteromalidae), *S. cameroni, S. endius, S. nigra* and *S. nigroaenea* (Marchiori et al., 2001b; Marchiori et al., 2002a;b;c;d; Marchiori et al., 2003a;c; Marchiori, 2004; Marchiori et al., 2004a; Marchiori et al., 2005d; Marchiori et al., 2006a).

8 - *Oxysarcodexia thornax* (Walker, 1849) (Diptera: Sarcophagidae).
*B. podagrica, Gnathopleura quadridentata* Wharton, 1986 (Hymenoptera: Braconidae), *Hemencyrtus* sp., *N. vitripennis, Neralsa sp.* (Hymenoptera, Figitidae), *P. vindemmiae, S. endius, S. drosophilae, S. nigra*, Tachinobia sp. and *Trybliographa* sp. (Marchiori et al., 2000b; Marchiori et al., 2001b; Marchiori, et al., 2003a; Marchiori, 2004; Marchiori et al., 2005d; Marchiori, et al., 2006a; Marchiori, 2007b; Marchiori et al., 2007).

9 - *Ophyra aenascens* (Wiedemann, 1830) (Diptera: Muscidae).
*Tachinobia* sp. (Hymenoptera: Eulophidae) (Marchiori, 2003c; Marchiori, 2006).
10 - *Ophyra* sp. (Diptera: Muscidae).
*B. podagrica* and *P. vindemmiae* (Marchiori, 2001; Marchiori et al., 2002a).

11 - *Palaeosepsis* spp. (Diptera: Sepsidae).
*Kleidotoma nigra* (Hartig, 1840) (Hymenoptera: Figitidae), *Muscidifurax* sp.
(Hymenoptera: Pteromalidae), *P. egeria, S. cameroni, S. drosophilae, S. endius, S. nigra, S. nigroaenea, Spalangia sp., Trichopria sp.* (Hymenoptera: Diapriidae), *Triplasta atrocoxalis* (Ashmead, 1895) (Hymenoptera: Figitidae) and *Triplasta coxalis* (Ashmead, 1895) (Hymenoptera: Figitidae) (Marchiori et al., 2000a; c; d; e; Marchiori et al., 2001c; Marchiori et al., 2005d; Marchiori et al., 2007; Marchiori, 2014).

12 - *Peckia chrysostoma* (Wiedemann, 1830) (Diptera: Sarcophagidae).
*Aphaereta* sp. (Hymenoptera: Braconidae), *Brachymeria* sp. (Hymenoptera: Chalcididae), *B. podagrica, Hemencyrtus sp., G. quadridentata, N. vitripennis, P. vindemmiae, S. drosophilae* and *S. endius* (Marchiori et al., 2000d; Marchiori et al., 2002c; Marchiori, 2003a; Marchiori et al., 2005d; Marchiori, 2007b).

*P. vindemmiae, S. cameroni, S. nigra and S. nigroaenea* (Marchiori et al., 2004; Marchiori et al.; 2006b).

14 - *Sarcodexia lambens* (Walker, 1861) (Diptera: Sarcophagidae).
*Aphaereta sp., B. podagrica, G. quadridentata, N. vitripennis, P. vindemmiae and S. endius* (Marchiori et al., 2002d; Marchiori, 2003a;d; Marchiori et al., 2004b; Marchiori, 2007b).

*Hemencyrtus sp.* and *N. vitripennis* (Marchiori, 2003; Marchiori et al., 2004b).

**DISCUSSION**

Insects mostly involved in the forensic investigations are true flies or Diptera. The predominant species in this order are Calliphoridae (blow flies), Sacrophagidae (flesh flies) and Muscidae (house flies). Calliphoridae (blow flies), Sacrophagidae (flesh flies) may arrive within minutes following death. Muscidae (house flies) delay colonization until the body reaches bloat stages of decomposition (Joseph, et al., 2011). Similar families were found in this study.

*Musca domestica* was the host species with the highest number of species of parasitoids.
because of its synanthropic nature, abundance in urban districts, ability to develop in various types of substrates and high reproductive power

As could be seen, the most common parasitoid collected from the dipterous was *P. vindemmiae*, followed by *N. vitripennis*.

*Pachycrepoideus vindemmiae* is considered to be a solitary parasitoid that controls a great number of Diptera in the families Anthomyiidae, Calliphoridae, Muscidae, Sarcophagidae, Tachinidae and Tephritidae. This species presents diversified (cosmopolitan) distribution and it has been found in North America and Africa (Rueda & Axtell, 1985; Marchiori et al., 2003a).

*Nasonia vitripennis* behaves as gregarious parasitoid is ectoparasitoid in pupae of several species of Diptera families, particularly Calliphoridae, Muscidae, Sarcophagidae and Tachinidae (Rivers & Denlinger, 1995; Schurmanm et al., 2014). It is a polyphagous insect parasite over 68 species of Diptera.

Studies on the biology, life cycle and ecology of carrion insects are still at an early stage, due to the complexity and high cost of such studies and the time taken for results to be obtained. Taxonomic knowledge of dipterous and carrion beetles is essential for Forensic Entomology, but not enough. Estimation of the range of death, for example, also depends on ecological and biological information, especially with regard to post-embryonic development (Pujol-Luz, et al., 2008).

**CONCLUSION**

*Musca domestica* was the host species with the highest number of species of parasitoids. The most common parasitoid collected from the flies was *P. vindemmiae*, followed by *N. vitripennis*.

**REFERENCES**


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