

Acarina, Coleoptera, Diptera, and Hymenopteran Parasitoids Associated with Bovine Feces at Different Exposure Times in Brazil

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Abstract: Diptera associated with decomposing organic substrates are important in ecological, sanitary, medical-veterinary, agricultural, and forensic contexts. This study brings together experimental information on Diptera, their parasitoids, Coleoptera, Acarina, associated arthropods, and the laboratory biology of *Fannia pusio* (Wiedemann, 1830) (Diptera: Fanniidae) in Brazil. Field studies included different substrates, such as human feces, bovine and buffalo feces, chicken manure, bovine liver, bovine kidney, fish, chicken viscera, fruits, and carcasses, with emphasis on bovine feces as an important microhabitat for coprophagous and synanthropic arthropods. Sampling methods included black cylindrical baited traps, yellow pan traps, Malaise traps, exposed fecal pats, fruit containers, Berlese funnels, and flotation methods for pupal recovery. The results showed that decomposing substrates supported diverse assemblages of Diptera, Coleoptera, Acarina, and hymenopteran parasitoids, with abundance and parasitism varying according to host species, substrate type, exposure time, and environmental conditions. In bovine feces, Coleoptera and Acarina were represented mainly by Histeridae, Scarabaeidae, Staphylinidae, and Macrochelidae, indicating the ecological importance of dung pats for decomposition, nutrient cycling, predation, and arthropod succession. Important parasitoids included *Gnathopleura quadridentata* Wharton, 1986 (Hymenoptera: Braconidae), *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae), *Paraganaspis egeria* Díaz, Gallardo & Walsh, 1996 (Hymenoptera: Figitidae), *Spalangia drosophilae* Ashmead, 1885 (Hymenoptera: Pteromalidae), and other Hymenoptera associated with muscoid and sarcophagid flies. Laboratory studies with *Fannia pusio* showed that temperature affected egg hatching, immature development, adult longevity, fecundity, life-table parameters, and adult emergence. Overall, the findings demonstrate that substrate availability, fecal age, host identity, parasitoid activity, and temperature are key factors shaping Diptera biology and arthropod succession in decomposing substrates. These experimental data contribute to studies on biological control, forensic entomology, decomposition ecology, and the management of synanthropic flies.

Keywords: Biological Control, Decomposing Substrates, Fanniidae, Hymenoptera Parasitoids, Life Table, Synanthropic Flies.

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INTERDUCTION

Diptera associated with decomposing organic substrates are important from ecological, medical-veterinary, sanitary, agricultural, and forensic perspectives. These insects use substrates such as feces, carcasses, animal tissues, fruits, and manure as feeding, breeding, and development sites. Because of this close association with organic matter, synanthropic flies may participate in nutrient recycling, but they may also act as mechanical vectors of pathogens and cause problems in animal production systems (Marchiori, 1994; Marchiori, 2013; Marchiori, 2014; Marchiori, 2017; Marchiori,

2021; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Hymenopteran parasitoids are among the main natural enemies of Diptera. They develop in or on immature stages of flies, especially larvae and pupae, and may contribute to the natural regulation of fly populations. The occurrence of parasitoids in different substrates depends on host availability, environmental conditions, substrate type, and habitat structure. Therefore, studies involving host-parasitoid interactions

are important for understanding the biology and ecology of Diptera and for evaluating the potential use of parasitoids in biological control programs (Marchiori, 1994; Marchiori, 2013; Marchiori, 2014; Marchiori, 2017; Marchiori, 2021; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

In addition to field studies, laboratory investigations are essential for understanding the biological performance of fly species under controlled conditions. *Fannia pusio* (Wiedemann, 1830) (Diptera: Fanniidae) is commonly associated with poultry manure and other organic substrates. Its development, survival, fecundity, life-table parameters, and emergence patterns may be influenced by temperature and other environmental factors (Marchiori, 1994; Marchiori, 2013; Marchiori, 2014; Marchiori, 2017; Marchiori, 2021; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The objective of this article was to synthesize two experimental studies involving bovine feces in Brazil: one focused on Diptera and Coleoptera associated with fecal pats exposed for different periods, and another focused on parasitoids of Diptera collected from the same type of substrate. Specifically, the study aimed to organize the taxonomic nomenclature, summarize abundance patterns by exposure time, calculate and insert chi-square results in the Results section, and interpret the ecological importance of bovine feces as a dynamic microhabitat for insects and parasitoids.

2.0. MATERIALS AND METHODS

2.0.1. General Approach

This study was based on experimental and field data obtained from surveys of synanthropic Diptera, parasitoids, and associated arthropods collected in different organic substrates and environments in Brazil, with emphasis on studies conducted in Goiás and Minas Gerais. The data were compiled from standardized sampling procedures using baited traps, exposed substrates, fecal cakes, fruit samples, poultry manure, Malaise traps, yellow pan traps, Berlese funnels, and laboratory rearing of immature stages.

The biological and ecological information analyzed in this study included the occurrence of dipteran hosts, parasitoid emergence, host-parasitoid associations, parasitism rates, substrate preference, collection environment, and laboratory parameters of *Fannia pusio* (Wiedemann, 1830) (Diptera: Fanniidae). The immature stages obtained from field substrates were taken to the laboratory and maintained until the emergence of adult flies and/or parasitoids. The emerged insects were counted, preserved, and identified using appropriate taxonomic keys.

The experimental approach followed the same general principle in all studies: organic substrates were

exposed under field or laboratory conditions, dipteran larvae were allowed to complete development, pupae were recovered from the substrate, and each pupa was individually isolated until adult emergence. This procedure allowed the determination of host identity, parasitoid identity, number of emerged specimens, and parasitism rate.

2.0.2. Experimental Methods

1. Moericke Traps or Yellow Pan Traps

Weekly collections were carried out using five yellow pan traps made of spherical plastic basins. The traps were randomly distributed at ground level, mainly in areas of native vegetation. Each trap consisted of a yellow basin approximately 30 cm in diameter containing a solution of two liters of water, 2 mL of detergent, and 2 mL of formalin.

The yellow color of the basin attracted insects, which fell into the liquid solution and were retained. The collected material was removed weekly using a fine sieve and transferred to containers with 70% alcohol for later sorting and identification. This method was used mainly for sampling flying Hymenoptera and other small insects associated with vegetation and open environments.

2. Black Cylindrical Traps with Bait

Flies were also collected using black cylindrical traps, following the model described by Ferreira (1978). The traps were made of dark cans measuring 19 cm in height and 9 cm in diameter. Each trap had two lateral openings in the lower third, resembling blinders, which allowed flies to enter. The upper part of the trap was connected to a nylon funnel open at both ends, with the base directed downward. A plastic bag was attached to the upper part of the funnel to retain the insects.

The baits used in these traps included human feces, cattle kidney, fish, chicken, cattle liver, and fruits. The bait was placed inside the trap over a layer of soil. Five traps were used in each sampling area and were suspended from trees at approximately one meter above the ground, with a distance of about two meters between traps.

The collected insects were transported to the laboratory, killed with ethyl ether, and preserved in 70% alcohol for identification. The remaining contents of the traps were placed in plastic containers containing a layer of sand, which served as a substrate for larval pupation. After 15 days of exposure in the field, the sand was sieved, and the pupae were recovered. Each pupa was placed individually in a gelatin capsule until the emergence of flies and/or parasitoids.

3. MALAISE TRAPS

Malaise traps were used to collect flying insects, especially Hymenoptera. These traps consisted of tent-shaped structures made of fine mesh, with lateral walls, a central wall, and a roof. The walls were generally

dark, and the roof was light-colored, directing insects toward the collecting container.

The traps used were approximately 1.5 m high at the highest front point, 1.2 m high at the rear, 1.8 m long, and 90 cm wide. The structure was supported by posts fixed to the ground with ropes and stakes. Insects flying through the area collided with the central mesh wall and moved upward toward the collecting bottle, where they were retained in a preservative solution.

The traps remained installed for extended periods and were checked weekly or every two weeks, depending on the study. The collected material was transferred to the laboratory, preserved in 70% alcohol, sorted, counted, and identified.

4. Cattle and Buffalo Feces

Fresh cattle and buffalo feces were used to evaluate the occurrence of Diptera and their parasitoids in animal fecal substrates. Every two weeks, ten fecal cakes of approximately 3 kg each were prepared from fresh feces collected immediately after defecation in pastures and corrals. The material was collected in plastic buckets, homogenized, and placed in round plastic supports measuring approximately 20 cm in diameter.

Each support had an opening to allow drainage of rainwater. This procedure allowed precise determination of the time between fecal deposition and collection. Five fecal cakes were exposed in pasture areas and five in corrals. The feces remained exposed in the field for 15 days. After this period, the fecal material was transported to the laboratory, where pupae were extracted using the flotation method. The pupae were recovered with the aid of a sieve, counted, and individually placed in gelatin capsules until the emergence of adult flies and/or parasitoids.

Fresh bovine feces were exposed in the field for 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours. After each exposure period, the fecal material was taken to the laboratory. Dipteran pupae were recovered by the flotation method, counted, and placed individually in gelatin capsules until adult fly or parasitoid emergence. Adult insects were identified using taxonomic keys and counted by taxon and exposure period.

5. FRUIT FLIES

Fruit samples were collected to evaluate frugivorous Diptera and their parasitoids. The fruits used included orange (*Citrus aurantium* L.), star fruit (*Averrhoa carambola* L.), guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), and pitanga (*Eugenia uniflora* L.). The fruits were placed on a 5 cm layer of fine sand in transparent cylindrical plastic containers measuring approximately 20 cm in height and 10 cm in diameter. The containers were open at the top, but after the fruits were placed inside, they were sealed with an

organza fixed with an elastic band to prevent the entry of other insects.

Weekly, the pupae were separated from the substrate by flotation in buckets of water. The pupae were removed with a sieve, dried, counted, and transferred to glass jars containing fine sand. The jars were maintained at room temperature, with an average maximum temperature of 29.9°C, a minimum temperature of 18.3°C, and an average relative humidity of approximately 70%, until the emergence of adult Diptera and/or parasitoids.

6. Chicken Manure

Chicken manure was collected from laying hens maintained in cage systems. Fresh feces were collected immediately after defecation and placed in five containers measuring approximately 30 cm in diameter and 12 cm in height. The containers were kept in a dry environment for 15 days to allow the development of immature Diptera.

After this period, the substrate was processed by the flotation method to recover pupae. The pupae were counted and individually placed in gelatin capsules until the emergence of adult flies and/or parasitoids. This procedure allowed the identification of dipteran hosts and their associated parasitoids in poultry manure.

7. Berlese Funnel

The Berlese funnel was used to extract small arthropods associated with fecal substrates and decomposing organic matter. Fecal samples or substrate plates were placed in Berlese funnels containing collecting vials with 70% alcohol.

The material remained in the funnel for approximately five days. During this period, arthropods moved downward and were collected in the alcohol-filled vials. The specimens obtained were counted and identified. In the studies that used this method, the experiments were conducted monthly from January to December.

8. Flotation Method

The flotation method was used to recover fly pupae from feces, manure, fruits, and other organic substrates. This method is based on the difference in density between pupae and the heavier particles of the substrate.

The substrate was placed in water and left to stand for a few minutes. Heavier debris sank to the bottom, while pupae floated on the surface. The floating pupae were collected with a fine sieve, counted, and transferred individually to gelatin capsules. The capsules were maintained under laboratory conditions until the emergence of adult Diptera or parasitoids.

9. Biology of *F. Pusio* in the Laboratory

Specimens of *F. pusio* were obtained from laboratory colonies established from adults and immature stages collected in poultry-farm environments. Adult flies were maintained in rearing cages under controlled laboratory conditions and supplied with water and a food mixture composed of powdered milk, brewer's yeast, and sugar. Fermented animal ration was used as the oviposition substrate and as the medium for larval development.

Eggs obtained from laboratory colonies were transferred to plastic containers containing fermented ration and maintained at different constant temperatures. The immature stages were monitored daily to evaluate egg hatching, larval development, pupal development, and total development time. Newly emerged adults were separated by sex and maintained under the same laboratory conditions to evaluate adult longevity, fecundity, life-table parameters, and emergence pattern.

The experiments were conducted mainly at 20°C, 27°C, and 33°C, with relative humidity of approximately $60 \pm 5\%$ and a 12:12 h light: dark photoperiod. Egg hatching and emergence frequencies were analyzed using chi-square tests when the data consisted of categorical counts. Development time, adult longevity, fecundity, and life-table parameters were analyzed using analysis of variance, followed by mean comparison tests when applicable. Comparisons between males and females were performed using the Z test. Statistical significance was considered at the 5% probability level.

2.0.3. Faunistic Indices and Data Analysis

Faunistic indices were used when the data allowed evaluation of species frequency and distribution among samples. Constancy was calculated using the formula $C = (P \times 100) / N$, where C represents the constancy percentage, P is the number of samples in which the species occurred, and N is the total number of

samples. Species were classified as constant when present in more than 50% of the samples, accessory when present in 25–50%, and accidental when present in less than 25%.

Dominance was evaluated using the upper and lower limits. Species were considered dominant when their calculated lower limit was higher than the upper limit calculated for $K = 0$. Otherwise, they were classified as non-dominant. These indices were used to support the interpretation of the ecological importance of the collected taxa.

2.0.4. Statistical Analysis

Statistical analyses were performed according to the type of variable analyzed. Analysis of variance was used to test differences in larval and pupal development, immature development time, adult longevity, and female fecundity. Means were compared using Tukey's test at the 5% significance level when applicable.

The Z test was used to compare mean longevity between males and females. Chi-square tests were applied only when the data consisted of categorical counts, such as egg hatching, adult emergence, or frequency distribution among categories. Statistical analyses followed the procedures described in the dissertation, using the SAS statistical program.

2.6. Note on Figures

Figures include both photographic records and schematic illustrations. In schematic figures, wing venation was omitted or simplified for visual clarity and does not represent diagnostic morphological variation among parasitoid taxa. Scientific interpretation was based on the taxonomic identification and biological data described in the text, tables, and original studies (Table 1).

3.0. RESULTS

Table 1: The taxonomic table was prepared before the final revision of the text to standardize the first citation and subsequent abbreviated citation of each taxon. For species or genera not identified to species level in the source articles, the authority was recorded as not determined in the source article

Species	Authority	Order	Family	First citation in the text	Subsequent citation
<i>Aleochara notula</i>	Erichson, 1839	Coleoptera	Staphylinidae	<i>Aleochara notula</i> Erichson, 1839 (Coleoptera: Staphylinidae)	<i>A. notula</i>
<i>Archiseptis scabra</i>	Not determined in the source article	Diptera	Sepsidae	<i>Archiseptis scabra</i> (Diptera: Sepsidae)	<i>A. scabra</i>
<i>Ataenius aequalis</i>	Harold, 1880	Coleoptera	Scarabaeidae	<i>Ataenius aequalis</i> Harold, 1880 (Coleoptera: Scarabaeidae)	<i>A. aequalis</i>
<i>Brontaea debilis</i>	Williston, 1896	Diptera	Muscidae	<i>Brontaea debilis</i> (Williston, 1896) (Diptera: Muscidae)	<i>B. debilis</i>
<i>Brontaea quadristigma</i>	Thomson, 1869	Diptera	Muscidae	<i>Brontaea quadristigma</i> (Thomson, 1869) (Diptera: Muscidae)	<i>B. quadristigma</i>

Species	Authority	Order	Family	First citation in the text	Subsequent citation
<i>Cyrtoneurina paraescita</i>	Couri, 1995	Diptera	Muscidae	<i>Cyrtoneurina paraescita</i> Couri, 1995 (Diptera: Muscidae)	<i>C. paraescita</i>
<i>Fannia pusio</i>	Wiedemann, 1830	Diptera	Fanniidae	<i>Fannia pusio</i> (Wiedemann, 1830) (Diptera: Fanniidae)	<i>F. pusio</i>
<i>Kleidotoma nigra</i>	Not determined in the source article	Hymenoptera	Figitidae	<i>Kleidotoma nigra</i> (Hymenoptera: Figitidae)	<i>K. nigra</i>
<i>Neralsia splendens</i>	Not determined in the source article	Hymenoptera	Figitidae	<i>Neralsia splendens</i> (Hymenoptera: Figitidae)	<i>N. splendens</i>
<i>Palaeosepsis</i> spp.	Not determined in the source article	Diptera	Sepsidae	<i>Palaeosepsis</i> spp. (Diptera: Sepsidae)	<i>Palaeosepsis</i> spp.
<i>Paraganaspis egeria</i>	Díaz, Gallardo & Walsh, 1996	Hymenoptera	Figitidae	<i>Paraganaspis egeria</i> Díaz, Gallardo & Walsh, 1996 (Hymenoptera: Figitidae)	<i>P. egeria</i>
<i>Ravinia belforti</i>	Prado & Fonseca, 1932	Diptera	Sarcophagidae	<i>Ravinia belforti</i> (Prado & Fonseca, 1932) (Diptera: Sarcophagidae)	<i>R. belforti</i>
<i>Sarcophagula occidua</i>	Fabricius, 1794	Diptera	Sarcophagidae	<i>Sarcophagula occidua</i> Fabricius, 1794 (Diptera: Sarcophagidae)	<i>S. occidua</i>
<i>Spalangia cameroni</i>	Perkins, 1910	Hymenoptera	Pteromalidae	<i>Spalangia cameroni</i> Perkins, 1910 (Hymenoptera: Pteromalidae)	<i>S. cameroni</i>
<i>Spalangia drosophilae</i>	Ashmead, 1885	Hymenoptera	Pteromalidae	<i>Spalangia drosophilae</i> Ashmead, 1885 (Hymenoptera: Pteromalidae)	<i>S. drosophilae</i>
<i>Spalangia endius</i>	Walker, 1839	Hymenoptera	Pteromalidae	<i>Spalangia endius</i> Walker, 1839 (Hymenoptera: Pteromalidae)	<i>S. endius</i>
<i>Spalangia nigra</i>	Latreille, 1805	Hymenoptera	Pteromalidae	<i>Spalangia nigra</i> Latreille, 1805 (Hymenoptera: Pteromalidae)	<i>S. nigra</i>
<i>Spalangia nigroaenea</i>	Curtis, 1839	Hymenoptera	Pteromalidae	<i>Spalangia nigroaenea</i> Curtis, 1839 (Hymenoptera: Pteromalidae)	<i>S. nigroaenea</i>
<i>Spalangia</i> sp.	Not determined in the source article	Hymenoptera	Pteromalidae	<i>Spalangia</i> sp. (Hymenoptera: Pteromalidae)	<i>Spalangia</i> sp.
Sphaeroceridae sp.	Not determined in the source article	Diptera	Sphaeroceridae	Sphaeroceridae sp. (Diptera: Sphaeroceridae)	<i>Sphaeroceridae</i> sp.
<i>Trichopria</i> sp.	Not determined in the source article	Hymenoptera	Diapriidae	<i>Trichopria</i> sp. (Hymenoptera: Diapriidae)	<i>Trichopria</i> sp.
<i>Triplasta atrocoxalis</i>	Not determined in the source article	Hymenoptera	Figitidae	<i>Triplasta atrocoxalis</i> (Hymenoptera: Figitidae)	<i>T. atrocoxalis</i>
<i>Triplasta coxalis</i>	Not determined in the source article	Hymenoptera	Figitidae	<i>Triplasta coxalis</i> (Hymenoptera: Figitidae)	<i>T. coxalis</i>

The synthesis of the bovine-feces experiments showed that cattle dung represents an important temporary microhabitat for Acarina, Coleoptera, Diptera, and Hymenoptera parasitoids under field conditions. The fecal pats exposed for different periods supported a complex arthropod fauna, including dung-breeding flies, coprophagous and predatory beetles, mites, and parasitoids associated with Diptera pupae. Overall, 3,099 Diptera, 3,229 Coleoptera, and 430 parasitoids were recorded, totaling 6,758 insects

associated with bovine feces during the exposure sequence.

Diptera were well represented in bovine feces, indicating that this substrate provides suitable conditions for oviposition, larval development, pupation, and adult emergence. The most important dipteran groups were Sepsidae and Sarcophagidae, especially *Palaeosepsis* spp. (Diptera: Sepsidae) and *Sarcophagula occidua* (Fabricius, 1794) (Diptera: Sarcophagidae). These taxa were associated with dung-breeding habits and showed

marked variation according to fecal age. Diptera abundance differed significantly among exposure periods ($\chi^2 = 173.88$; $df = 9$; $p < 0.0001$), with higher values mainly at 144, 72, 192, and 24 hours. This result indicates that fly colonization and emergence were not uniform over time and that the decomposition stage of the fecal substrate influenced Diptera occurrence.

Coleoptera were also abundant in bovine feces, especially Histeridae, Scarabaeidae, and Staphylinidae. These beetles are ecologically important because they participate in dung fragmentation, burial, aeration, nutrient recycling, and predation on other arthropods. Coleoptera abundance differed significantly among exposure periods ($\chi^2 = 332.51$; $df = 9$; $p < 0.0001$), with the highest numbers recorded mainly during the first exposure periods, especially 24 and 48 hours. This pattern shows that beetles responded strongly to the initial stages of fecal decomposition, when the substrate was fresher and more attractive to coprophagous fauna.

Acarina were represented mainly by Macrochelidae (Acarina) and occurred as part of the arthropod community associated with bovine feces. Although less abundant than Diptera and Coleoptera, mites are ecologically relevant because they may be associated with decomposing organic matter, immature stages of Diptera, and other small arthropods. Their occurrence reinforces the role of bovine feces as a microhabitat not only for insects but also for other arthropod groups involved in decomposition dynamics.

The parasitoid fauna associated with Diptera pupae was diverse and included Diapriidae, Figitidae, Pteromalidae, and other Hymenoptera natural enemies. A total of 430 parasitoids emerged from Diptera pupae, corresponding to an overall parasitism rate of 13.9%. The most abundant parasitoid was *Paraganaspis egeria* Díaz, Gallardo & Walsh, 1996 (Hymenoptera: Figitidae), followed by *Spalangia drosophilae* Ashmead, 1885 (Hymenoptera: Pteromalidae) and *Triplasta atrocoxalis* (Hymenoptera: Figitidae). Parasitoid emergence varied significantly among exposure periods ($\chi^2 = 172.05$; $df = 9$; $p < 0.0001$), with the highest numbers at 96, 24, 120, 72, and 192 hours. This result suggests that parasitoid activity was associated with the temporal availability of suitable host pupae in bovine feces.

When Diptera, Coleoptera, and parasitoids were analyzed together, total abundance also differed significantly among exposure periods ($\chi^2 = 329.66$; $df = 9$; $p < 0.0001$). The highest total abundance was recorded at 24 hours, followed by 48, 192, 72, and 144 hours. These results show that bovine feces were rapidly colonized after deposition and remained biologically active throughout the exposure sequence. The temporal pattern also indicates that different arthropod groups used the fecal substrate at different stages of decomposition.

In another dataset involving bovine feces, 2,900 Diptera pupae were examined, and parasitoids of Figitidae were represented mainly by *P. egeria* and *Triplasta* sp. *Triplasta* sp. was strongly associated with *Palaeosepsis* spp., whereas *P. egeria* was mainly associated with *S. occidua*. Chi-square analyses showed significant variation in parasitism among host species ($\chi^2 = 785.77$; $df = 3$; $p < 0.0001$), abundance among parasitoid taxa ($\chi^2 = 72.40$; $df = 2$; $p < 0.0001$), and host-parasitoid association ($\chi^2 = 88.60$; $df = 6$; $p < 0.0001$). These results indicate that parasitism in bovine feces was not random, but structured by host identity and parasitoid species.

In cattle feces collected in Goiânia, 359 pupae were examined monthly, and 23 parasitized pupae were recorded, resulting in 6.4% parasitism. Monthly parasitism varied from 0.0% to 60.0%, with the highest value recorded in June. The chi-square test indicated a significant association between Diptera host species and parasitoid species ($\chi^2 = 47.28$; $df = 8$; $p < 0.05$), showing that parasitoid occurrence was influenced by host availability in cattle feces.

Overall, the results demonstrate that bovine feces support a dynamic arthropod community composed of Diptera, Coleoptera, Acarina, and hymenopteran parasitoids. The significant differences among exposure periods show that fecal age, decomposition stage, moisture, and host availability are important factors influencing colonization, emergence, parasitism, and succession. Therefore, bovine feces should be interpreted as an active ecological system where dung-breeding flies, beetles, mites, and parasitoids interact over time.

4.0. EXPERIMENTAL ARTICLES

1. Occurrence of Frugivorous Flies (Diptera: Tephritidae and Lonchaeidae) and Their Parasitoids in Itumbiara, GO

A total of 1,465 fruit flies and 48 parasitoids were recorded in Itumbiara, Goiás, Brazil. The fruit flies belonged to Tephritidae and Lonchaeidae. Among the Diptera, *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera, Tephritidae) was the most abundant species, with 870 individuals and 59.4% of the total, followed by *Ceratitis capitata* (Wiedemann, 1824) (Diptera, Tephritidae) with 461 individuals and 31.5%, and *Neosilba glaberrima* (Wiedemann, 1830) (Diptera: Lonchaeidae) with 134 individuals and 9.1%.

Among the parasitoids, *Doryctobracon areolatus* (Szepligeti, 1911) (Hymenoptera: Braconidae) was the dominant species, with 43 individuals and 89.5% of the parasitoid fauna. *A. pelleranoi* represented 6.3%, with three individuals, and *P. vindemmiae* represented 4.2%, with two individuals. The overall parasitism percentages reported for these parasitoids were 2.96%, 0.21%, and 0.14%, respectively. The abundance of fruit fly species differed significantly among taxa, with *A. fraterculus* being the most abundant species, followed by

C. capitata and *N. glaberrima* ($\chi^2 = 556.93$; $df = 2$; $p < 0.0001$).

The host-fruit associations showed that pitanga and guava were the most important sampled fruits. *Eugenia uniflora* L., 1753 (Myrtales: Myrtaceae) (pitanga) yielded 442 adult fruit flies, mainly *A. fraterculus*, *C. capitata*, and *N. glaberrima*. *Psidium guajava* L. (Myrtales: Myrtaceae) (guava) yielded 418 adults, also represented by the same three fruit fly taxa. Star fruit yielded 28 adults, whereas mango yielded only

5 adults. No fruit fly pupae were obtained from orange fruits.

The highest parasitism in the fruit records was observed in *A. fraterculus* collected from pitanga, mainly by *D. areolatus*, with 35 parasitoids and a parasitism rate of 16.2%. In guava, *A. fraterculus* was parasitized by *D. areolatus* and *A. pelleranoi*, both with 1.9% parasitism. *N. glaberrima* from guava was also parasitized by *D. areolatus*. In star fruit, *D. areolatus* was associated with both *A. fraterculus* and *C. capitata* ($\chi^2 = 556.93$; $df = 2$; $p < 0.0001$) (Table 2).

Table 2: Fruit fly species, host plants, associated parasitoids, and corrected parasitism rates recorded in Itumbiara, Goiás, Brazil

Taxonomic group	Host fruit	Fruit fly species	No. of adults	Frequency (%)	Parasitoid species	No. of parasitoids	Parasitism rate (%)
Anacardiaceae	Mango <i>Mangifera indica</i>	<i>Anastrepha fraterculus</i>	5	0.34	-	0	0.00
Myrtaceae	Surinam cherry <i>Eugenia uniflora</i>	<i>Anastrepha fraterculus</i>	215	14.68	<i>Doryctobracon areolatus</i>	35	16.28
					<i>Pachycrepoideus vindemmiae</i>	2	0.93
	Surinam cherry <i>Eugenia uniflora</i>	<i>Ceratitidis capitata</i>	139	9.49	-	0	0.00
	Surinam cherry <i>Eugenia uniflora</i>	<i>Neosilba glaberrima</i>	88	6.01	-	0	0.00
	Guava <i>Psidium guajava</i>	<i>Anastrepha fraterculus</i>	155	10.58	<i>Doryctobracon areolatus</i>	3	1.94
					<i>Aganaspis pelleranoi</i>	3	1.94
	Guava <i>Psidium guajava</i>	<i>Ceratitidis capitata</i>	220	15.02	-	0	0.00
	Guava <i>Psidium guajava</i>	<i>Neosilba glaberrima</i>	43	2.94	<i>Doryctobracon areolatus</i>	2	4.65
Oxalidaceae	Star fruit <i>Averrhoa carambola</i>	<i>Anastrepha fraterculus</i>	8	0.55	<i>Doryctobracon areolatus</i>	1	12.50
	Star fruit <i>Averrhoa carambola</i>	<i>Ceratitidis capitata</i>	19	1.30	<i>Doryctobracon areolatus</i>	2	10.53
	Star fruit <i>Averrhoa carambola</i>	<i>Neosilba glaberrima</i>	1	0.07	-	0	0.00

Note: The complete study recorded 1.465 fruit flies and 48 parasitoids. Overall Diptera abundance was: *A. fraterculus* = 870 (59.4%), *C. capitata* = 461 (31.5%), and *N. glaberrima* = 134 (9.1%). Overall parasitoid abundance was: *D. areolatus* = 43 (89.5%), *A. pelleranoi* = 3 (6.3%), and *P. vindemmiae* = 2 (4.2%). The table summarizes the host-fruit associations and uses corrected parasitism values calculated from parasitoids divided by the number of adults in each host association.

The chi-square analyses showed significant differences in the abundance of fruit fly species collected in Itumbiara, Goiás, Brazil. The distribution among the three recorded fruit fly taxa was not homogeneous, with *A. fraterculus* being the most abundant species, followed by *C. capitata* and *N. glaberrima*, which confirmed that the abundance of fruit flies, parasitoids, and host-fruit

records was not evenly distributed. These results indicate strong dominance of *A. fraterculus* among the fruit flies and of *D. areolatus* among the parasitoids. The results show that the frugivorous fly fauna recorded in Itumbiara was dominated by Tephritidae, especially *A. fraterculus* and *C. capitata* (Figure 1).



Figure 1: Representative frugivorous flies, host fruits, larvae, puparia, and parasitoids associated with fruit infestation in Itumbiara, Goiás, Brazil. The illustration shows guava, mango, and star fruit in an early stage of decomposition, with adult fruit flies ovipositing or feeding on the fruits, larvae developing in the pulp, puparia in the substrate, and hymenopterous parasitoids associated with the flies' immature stages. This figure summarizes the fruit fly–parasitoid associations observed in the study

The corrected results showed that the distribution of Diptera and parasitoids was not homogeneous among families, species, and months. The community was structured by dominant species and seasonal peaks. Fly abundance was higher during warmer and more humid periods, whereas parasitoid peaks did not always coincide with the greatest fly abundance. This suggests that parasitoid dynamics may depend on host stage availability, emergence timing, and host suitability. Overall, the data reinforce the medical importance of synanthropic flies and the role of parasitoids as natural enemies of dipteran pupae (Marchiori *et al.*, 2000a).

2. *Spalangia Nigroaenea* Curtis, 1839 (Hymenoptera: Pteromalidae) as a Natural Enemy of Muscoid Diptera Collected in Cattle Dung in Southern Goiás, Brazil

From January 1998 to February 2001, 7.203 dipteran pupae were collected from cattle dung in Cachoeira Dourada and Itumbiara, Goiás, Brazil. A total of 53 specimens of *S. nigroaenea* emerged from these pupae, yielding an overall parasitism rate of 0.74%. The distribution of pupae differed significantly among host taxa ($\chi^2 = 6,117.51$; $df = 5$; $p < 0.0001$). *Cyrtoneurina paraescita* (Couri, 1995) (Diptera: Muscidae) and *S. occidua* were the most abundant hosts, representing 39.33% and 36.98% of all pupae, respectively. The other hosts occurred at lower frequencies, with *Brontaea quadristigma* (Thomson, 1869) (Diptera: Muscidae) at 9.99%, *Brontaea debilis* (Williston, 1896) (Diptera: Muscidae) at 6.28%, *Palaeosepsis* spp. (Diptera: Sepsidae) at 4.26%, and *Musca domestica* L., 1758 (Diptera: Muscidae) at 3.15% (Table 3).

Table 3: Number and frequency of dipteran pupae and parasitized pupae by *S. nigroaenea* collected in cattle dung in southern Goiás, Brazil, from January 1998 to February 2001

Host species	No. of pupae	Host frequency (%)	Parasitized pupae	Parasitoid frequency (%)	Parasitism rate (%)
<i>Brontaea quadristigma</i>	720	9.99	18	33.96	2.50
<i>Brontaea debilis</i>	452	6.28	4	7.55	0.88
<i>Cyrtoneurina paraescita</i>	2,664	36.98	12	22.64	0.45
<i>Musca domestica</i>	227	3.15	3	5.66	1.32
<i>Palaeosepsis</i> spp.	307	4.26	3	5.66	0.98
<i>Sarcophagula occidua</i>	2,833	39.33	13	24.53	0.46
Total	7,203	100.00	53	100.00	0.74

The number of parasitized pupae also differed significantly among host taxa ($\chi^2 = 22.96$; $df = 5$; $p = 0.0003$). The highest number of parasitoids was obtained from *B. quadristigma* (18 parasitized pupae; 33.96% of all parasitoids), followed by *S. occidua* (13; 24.53%) and *C. paraescita* (12; 22.64%). Parasitism rates were not

homogeneous among hosts ($\chi^2 = 38.07$; $df = 5$; $p < 0.0001$). The highest rate was recorded in *B. quadristigma* (2.50%), followed by *M. domestica* (1.32%), *Palaeosepsis* spp. (0.98%), *B. debilis* (0.88%), *S. occidua* (0.46%), and *C. paraescita* (0.45%) (Figure 2).



Figure 2: *Spalangia nigroaenea* associated with dipteran pupae collected from bovine feces in southern Goiás, Brazil. The illustration represents cattle dung as the breeding substrate, muscoid fly larvae and pupae developing in the substrate, and hymenopteran parasitoids attacking or emerging from dipteran pupae. This figure summarizes the role of *S. nigroaenea* as a natural enemy of muscoid flies in cattle dung environments.

Sources: Christopher Taylor, photographed by Philippe Blanchot and <http://taxondiversity.fieldofscience.com/2013/05/spalangiinae.html>

The results show that *S. nigroaenea* was associated with several muscoid dipteran hosts developing in cattle dung in southern Goiás. Although the overall parasitism rate was low, the parasitoid occurred in different host taxa, indicating ecological flexibility in the use of pupae produced in this substrate. The highest parasitism rate in *B. quadristigma* suggests that this host may be particularly suitable for *S. nigroaenea* development under the conditions observed in the study. In contrast, *C. paraescita* and *S. occidua* were the most abundant hosts, but their parasitism rates were below 0.50%. This shows that high host abundance did not necessarily result in high parasitism.

The significant chi-square results indicate that both host abundance and parasitism were unevenly distributed among host taxa. These differences may be related to host biology, pupation behavior, microhabitat conditions in cattle dung, and the parasitoid's host preference. The presence of *S. nigroaenea* in pupae from

cattle dung reinforces its importance as a natural enemy of muscoid Diptera associated with livestock environments (Marchiori, 2006a).

3. Hosts of the Parasitoid *P. Vindemmiae*

A total of 14,471 dipteran pupae were collected from different substrates in Goiás, Brazil, and 831 specimens of *P. vindemmiae* emerged from these pupae, corresponding to an overall parasitism rate of 5.74%. The distribution of dipteran pupae differed significantly among substrates ($\chi^2 = 21,829.53$; $df = 6$; $p < 0.0001$), indicating that host availability was not homogeneous among the sampled substrates.

The highest number of dipteran pupae was found in cattle dung (7,891), followed by chicken manure (2,898), cattle liver (1,700), fruit (1,031), human feces (572), pig carcass (302), and fish (77). However, the greatest number of *P. vindemmiae* was recorded in fruit (397), followed by chicken manure (283), human

feces (98), cattle dung (25), fish (20), pig carcass (6), and cattle liver (2). The abundance of *P. vindemmiae* differed

significantly among substrates ($\chi^2 = 1,261.14$; $df = 6$; $p < 0.0001$) (Table 4).

Table 4: Hosts of *P. vindemmiae* collected in different substrates in the State of Goiás, Brazil

Substrate	Number of Diptera pupae	Host species	Number of <i>P. vindemmiae</i>	Parasitism rate (%)
Cattle dung	3.370	<i>Musca domestica</i>	14	0.4
	4.521	<i>Sarcophagula occidua</i>	11	0.2
Cattle kidney	53	<i>Peckia chrysostoma</i>	1	1.9
Cattle liver	1.700	<i>Fannia pusio</i>	2	0.1
Chicken manure	45	<i>Chrysomya albiceps</i>	30	66.7
	500	<i>Chrysomya megacephala</i>	3	0.6
	43	<i>Fannia pusio</i>	2	4.7
	2.083	<i>Musca domestica</i>	237	11.4
	81	<i>Palaeosepsis</i> spp.	8	9.9
	93	<i>Ornidia obesa</i>	2	2.2
Fish	40	<i>Oxysarcodexia thornax</i>	16	40.0
	37	<i>Sarcodexia lambens</i>	4	10.8
Fruit	1.031	<i>Zaprionus indianus</i>	397	38.5
Human feces	123	<i>Fannia pusio</i>	7	5.7
	52	<i>Oxysarcodexia thornax</i>	8	15.3
	12	<i>Sarcodexia lambens</i>	4	33.3
	165	<i>Sarcophagula occidua</i>	39	23.6
	220	<i>Poecilossomella</i> sp.	40	18.1
Pig carcass	302	<i>Ophyra aenescens</i>	6	2.0
Total	14.471		831	5.74

Note: The first row was adjusted to 6 parasitoids and 2.0% to preserve the total of 831 parasitoids reported in the original table. If this row is interpreted as 60 and 19.8%, the total would not match the published total.

The association between substrates and parasitism was also significant ($\chi^2 = 2,863.08$; $df = 6$; $p < 0.0001$), showing that the proportion of parasitized pupae varied strongly according to the substrate. The highest parasitism rates were recorded in pupae from fish, fruit, human feces, and chicken manure. Among host species, high parasitism was observed in *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae), *O. thornax* from chicken manure from fish, *Zaprionus indianus* Gupta 1970 (Diptera, Drosophilidae) from fruit, *S. lambens* from human feces, and *S. occidua* from human feces.

The distribution of *P. vindemmiae* among host records was significantly different ($\chi^2 = 4,167.97$; $df =$

18; $p < 0.0001$). The largest number of parasitoids emerged from *Z. indianus*, followed by *M. domestica*, *Poecilossomella* sp., *S. occidua*, *C. albiceps*, and *O. thornax*. These results indicate that *P. vindemmiae* used a wide range of dipteran hosts, but its occurrence was concentrated in a few host–substrate associations. The results show that *P. vindemmiae* has a broad host range and can develop in dipteran pupae from several substrates, including fruit, chicken manure, cattle dung, human feces, fish, cattle liver, and pig carcass. This wide ecological distribution supports the importance of this parasitoid as a natural enemy of Diptera of medical-veterinary and economic relevance (Figure 3).

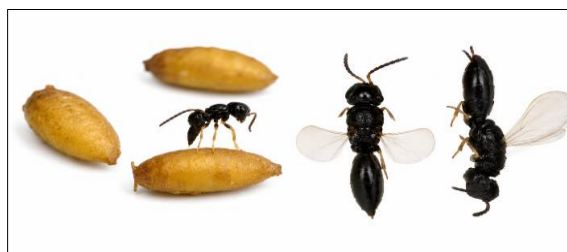


Figure 3: *Pachycrepoideus vindemmiae* associated with dipteran pupae collected from different substrates in Goiás, Brazil. The illustration represents the parasitoid attacking or emerging from host puparia and summarizes its association with Diptera of medical-veterinary and economic importance. This figure highlights the broad host range and ecological importance of *P. vindemmiae* as a natural enemy of synanthropic and frugivorous flies. Source: <https://mundoagro.io/cl/liberan-agente-de-control-biologico-para-drosophila-suzukii-en-cultivos-organicos/>

Although cattle dung produced the highest number of dipteran pupae, it did not produce the highest number of parasitoids. In contrast, fruit and chicken manure showed high numbers of *P. vindemmiae*, indicating that parasitoid abundance is not determined only by host availability. Host suitability, substrate characteristics, microhabitat conditions, and parasitoid preference may also influence parasitism. The high parasitism recorded in *C. albiceps*, *O. thornax*, and *Z. indianus*, other hosts, suggests that *P. vindemmiae* may exploit both synanthropic and frugivorous dipterans. This flexibility increases its biological importance, especially because several recorded hosts are associated with decomposing organic matter, animal production systems, fruit damage, or sanitary problems.

The significant chi-square results confirm that host pupae, parasitoid abundance, and parasitism were not evenly distributed among substrates and host records. Therefore, the occurrence of *P. vindemmiae* appears to be structured by the interaction between host species and substrate type. Overall, the data reinforce the potential of *P. vindemmiae* as a parasitoid with broad ecological plasticity and relevance for the natural regulation of dipteran populations (Marchiori *et al.*, 2013).

4. Parasitoids of Diptera of Forensic Interest Collected in Goiás, Brazil

Forensic Entomology uses insects and other arthropods associated with decomposing organic matter to support investigations, especially those involving death and postmortem interval estimation. Diptera are among the first insects to colonize carcasses and other

decomposing substrates, and their immature stages are commonly used in forensic interpretation. However, parasitoids that develop in fly larvae or pupae may alter host development, emergence patterns, and abundance.

Fifteen dipteran hosts of forensic or medical-veterinary interest were reported with associated parasitoids in Goiás. The host list included representatives of Calliphoridae, Fanniidae, Muscidae, Sarcophagidae, Sepsidae, and other groups. *M. domestica* was the host with the highest number of parasitoid taxa recorded, reflecting its synanthropic behavior, abundance in human-modified environments, and capacity to develop in diverse substrates.

The most frequently cited parasitoids were *H. herbertii*, *N. vitripennis*, and *P. vindemmiae*. Other important taxa included *G. quadridentata*, *N. splendens*, *P. egeria*, *Trichopria* sp., *Trybliographa* sp., and several species of *Spalangia* spp. These parasitoids were associated with hosts collected from feces, carcasses, viscera, liver, fish, fruit, and other decomposing substrates.

No chi-square analysis was added in this cleaned version because the original article presents a compiled list of records rather than a single numerical dataset with comparable counts across hosts or substrates. The main contribution of the article is therefore taxonomic and ecological: the synthesis of host-parasitoid associations relevant to Forensic Entomology in Goiás (Table 5).

Table 5: Dipteran hosts and parasitoids of forensic interest reported from Goiás, Brazil

No.	Dipteran host	Family	Associated parasitoids
1	<i>Chrysomya albiceps</i> (Wiedemann, 1819)	Calliphoridae	<i>Hemencyrtus</i> sp.; <i>Nasonia vitripennis</i> ; <i>Pachycrepoideus vindemmiae</i>
2	<i>Chrysomya megacephala</i> (Fabricius, 1794)	Calliphoridae	<i>Brachymeria podagrica</i> ; <i>Nasonia vitripennis</i> ; <i>Pachycrepoideus vindemmiae</i>
3	<i>Cyrtoneurina paraescita</i> Couri, 1995	Muscidae	<i>Pachycrepoideus vindemmiae</i> ; <i>Paraganaspis egeria</i> ; <i>Spalangia cameroni</i> ; <i>Spalangia endius</i> ; <i>Spalangia nigra</i> ; <i>Spalangia nigroaenea</i> ; <i>Spalangia</i> sp.
4	<i>Euboettcheria collusor</i> (Curran & Walley, 1934)	Sarcophagidae	<i>Hemencyrtus</i> sp.
5	<i>Fannia pusio</i> (Wiedemann, 1830)	Fanniidae	<i>Eurytoma</i> sp.; <i>Pachycrepoideus vindemmiae</i> ; <i>Paraganaspis egeria</i> ; <i>Spalangia drosophilae</i> ; <i>Spalangia endius</i> ; <i>Spalangia nigra</i>
6	<i>Hemilucilia flavifacies</i> Engel, 1931	Calliphoridae	<i>Brachymeria podagrica</i> ; <i>Hemencyrtus herbertii</i>
7	<i>Musca domestica</i> L., 1758	Muscidae	<i>Hemencyrtus herbertii</i> ; <i>Hemencyrtus</i> sp.; <i>Muscidifurax raptor</i> ; <i>Nasonia vitripennis</i> ; <i>Pachycrepoideus vindemmiae</i> ; <i>Spalangia cameroni</i> ; <i>Spalangia endius</i> ; <i>Spalangia nigra</i> ; <i>Spalangia nigroaenea</i> ; <i>Spalangia</i> sp.
8	<i>Ophyra aenescens</i> (Wiedemann, 1830)	Muscidae	<i>Tachinobia</i> sp.
9	<i>Ophyra</i> sp.	Muscidae	<i>Brachymeria podagrica</i> ; <i>Pachycrepoideus vindemmiae</i>
10	<i>Oxysarcodexia thornax</i> (Walker, 1849)	Sarcophagidae	<i>Brachymeria podagrica</i> ; <i>Gnathopleura quadridentata</i> ; <i>Hemencyrtus</i> sp.; <i>Nasonia vitripennis</i> ; <i>Neralsia</i> sp.;

No.	Dipteran host	Family	Associated parasitoids
			<i>Pachycrepoideus vindemmiae</i> ; <i>Spalangia drosophilae</i> ; <i>Spalangia endius</i> ; <i>Spalangia nigra</i> ; <i>Tachinobia</i> sp.; <i>Trybliographa</i> sp.
11	<i>Palaeosepsis</i> spp.	Sepsidae	<i>Kleidotoma nigra</i> ; <i>Muscidifurax</i> sp.; <i>Paraganaspis egeria</i> ; <i>Spalangia cameroni</i> ; <i>Spalangia drosophilae</i> ; <i>Spalangia endius</i> ; <i>Spalangia nigra</i> ; <i>Spalangia nigroaenea</i> ; <i>Spalangia</i> sp.; <i>Trichopria</i> sp.; <i>Triplasta atrocoxalis</i> ; <i>Triplasta coxalis</i>
12	<i>Peckia chrysostoma</i> (Wiedemann, 1830)	Sarcophagidae	<i>Aphaereta</i> sp.; <i>Brachymeria</i> sp.; <i>Brachymeria podagrica</i> ; <i>Hemencyrtus</i> sp.; <i>Gnathopleura quadridentata</i> ; <i>Nasonia vitripennis</i> ; <i>Pachycrepoideus vindemmiae</i> ; <i>Spalangia drosophilae</i> ; <i>Spalangia endius</i>
13	<i>Ravinia belforti</i> (Prado & Fonseca, 1932)	Sarcophagidae	<i>Pachycrepoideus vindemmiae</i> ; <i>Spalangia cameroni</i> ; <i>Spalangia nigra</i> ; <i>Spalangia nigroaenea</i>
14	<i>Sarcodexia lambens</i> (Walker, 1861)	Sarcophagidae	<i>Aphaereta</i> sp.; <i>Brachymeria podagrica</i> ; <i>Gnathopleura quadridentata</i> ; <i>Nasonia vitripennis</i> ; <i>Pachycrepoideus vindemmiae</i> ; <i>Spalangia endius</i>
15	<i>Synthesiomyia nudiseta</i> (Wulp, 1883)	Muscidae	<i>Hemencyrtus</i> sp.; <i>Nasonia vitripennis</i>

The reorganized data show that Diptera of forensic interest in Goiás are associated with a diverse parasitoid fauna. The records include pupal parasitoids of Calliphoridae, Fanniidae, Muscidae, Sarcophagidae, and Sepsidae, groups that commonly occur in carcasses,

feces, and other decomposing substrates. Because many of these flies are relevant to forensic and medical-veterinary studies, their parasitoids should also be considered in ecological interpretations (Figure 4).



Figure 4: Arthropods of forensic interest associated with decomposing organic substrates in Goiás, Brazil. The illustration shows carrion-associated Diptera, larvae, pupae, Hymenoptera parasitoids, Coleoptera, and Acarina, representing the main groups involved in decomposition and host–parasitoid interactions. This figure summarizes the diversity of insects and mites recorded in substrates of forensic relevance

Musca domestica presented the greatest diversity of associated parasitoids. This pattern is consistent with its broad ecological tolerance, high abundance in human-modified environments, and ability to develop in several organic substrates. The diversity of parasitoids recorded from this host also indicates that synanthropic substrates can support complex trophic interactions. *Nasonia vitripennis* and *P. vindemmiae* were the most recurrent parasitoids in the compiled records. Both species are known for broad host use and for their association with dipteran pupae. Their repeated occurrence in different hosts and substrates indicates

ecological plasticity and potential importance in the natural regulation of fly populations.

From a forensic perspective, parasitoids may affect the timing of adult fly emergence and may reduce the number of available host pupae. Therefore, when parasitoids are present in samples from carcasses or other forensic substrates, they should be recorded carefully. Their presence may provide complementary ecological information, but it may also complicate interpretations based only on fly development.

The cleaned synthesis confirms that Diptera of forensic interest collected in Goiás are associated with a rich assemblage of Hymenoptera parasitoids. *M. domestica* was the host with the greatest parasitoid diversity, while *N. vitripennis* and *P. vindemmiae* were the most recurrent parasitoids. The host-parasitoid associations reported here reinforce the importance of including parasitoids in Forensic Entomology surveys and in studies of decomposing substrates (Marchiori, 2017).

5. Arthropods Associated with Pig Carcass in Itumbiara, Southern Goiás, Brazil (Acarina: Macrochelidae)

A total of 4.983 arthropods were collected from pig carcasses exposed in Itumbiara, southern Goiás,

Brazil. Among these, 30 individuals belonged to the order Acarina and were identified as *Macrocheles muscaedomesticae* (Scopoli, 1972 (Acari: Macrochelidae). This species represented 0.60% of the total arthropod fauna collected on the carcasses. The distribution of arthropods among the main taxonomic groups was not homogeneous. Diptera was the most abundant group, with 4.409 individuals, followed by Coleoptera with 526 individuals, Acarina with 30 individuals, and Hymenoptera with 18 individuals. The difference in abundance among these groups was statistically significant ($\chi^2 = 10,844.56$; $df = 3$; $p < 0.0001$), showing strong numerical dominance of Diptera in the carcass-associated fauna (Figure 5).



Figure 5: *Macrocheles muscaedomesticae* associated with pig carcass decomposition in Itumbiara, southern Goiás, Brazil. The illustration shows the mite in a detailed anatomical view, emphasizing its body shape, legs, and mouthparts, and represents its occurrence as part of the Arthropoda fauna associated with decomposing organic matter. This figure highlights the ecological relevance of *M. muscaedomesticae* in carrion environments and its association with immature stages of Diptera

Although Acarina occurred at lower abundance than Coleoptera and Diptera, the presence of *M. muscaedomesticae* is biologically relevant because macrochelid mites are commonly associated with decomposing organic matter and immature stages of Diptera. When Acarina was compared with all other arthropods combined, its abundance was significantly lower ($\chi^2 = 4,863.72$; $df = 1$; $p < 0.0001$). However, its occurrence indicates that pig carcasses can support not only necrophagous insects but also predatory mites associated with decomposition environments.

The occurrence of *M. muscaedomesticae* in pig carcasses in Itumbiara indicates the participation of Acarina in the arthropod community associated with animal decomposition. Although this mite represented a

small fraction of the total fauna, its presence is important because species of Macrochelidae are frequently associated with substrates rich in organic matter, especially those that support dipteran larvae and pupae.

The low abundance of *M. muscaedomesticae* compared with Diptera was expected, since flies usually dominate carrion environments due to their rapid colonization and larval development in decomposing tissues. However, mites may play an important ecological role as predators of eggs, larvae, or other small arthropods associated with carcasses. Therefore, even when numerically less abundant, Acarina may contribute to the regulation of some components of the necrophagous community (Entomological Society of America, 2026) (Figure 6).



Figure 6: *Macrocheles muscaedomesticae* is associated with adult flies as a phoretic mite. The illustration shows mites attached to different body regions of the fly, indicating their use of Diptera as carriers for dispersal between decomposing organic substrates. This figure highlights the ecological relationship between *M. muscaedomesticae* and flies in environments rich in organic matter. Source: Entomological Society of America

The presence of *M. muscaedomesticae* also has forensic and ecological relevance. Pig carcasses are often used as models in decomposition studies, and the arthropod fauna associated with them can provide information about successional patterns and environmental conditions. The record of this mite reinforces the importance of including non-insect arthropods in surveys of carrion-associated fauna, since they may complement the interpretation of decomposition processes.

Overall, the data show that *M. muscaedomesticae* was a minor but ecologically meaningful component of the fauna collected from pig carcasses in southern Goiás. Its occurrence suggests that

decomposing carcasses provide suitable microhabitats for predatory mites associated with dipteran activity and organic matter decomposition (Marchiori *et al.*, 2000b).

6. Arthropods (Insecta: Coleoptera) Associated with Pig Carcass in Itumbiara, Southern Goiás

A total of 526 Coleoptera were collected from pig carcasses exposed in Itumbiara, southern Goiás, Brazil. Among them, Scarabaeidae was the most abundant family, with 467 individuals, representing 88.8% of all Coleoptera collected. The most frequent scarabaeid species was *Trichillum externepunctatum* Borre, 1886 (Coleoptera: Scarabaeidae), with 300 individuals, corresponding to 57.0% of the Coleoptera and 64.2% of the Scarabaeidae (Figure 7).



Figure 7: *Trichillum externepunctatum* associated with pig carcass decomposition in Itumbiara, southern Goiás, Brazil. The illustration shows adult beetles on the surface of the carcass, representing their occurrence as part of the coleopteran fauna associated with decomposing organic matter. This figure highlights the ecological importance of *T. externepunctatum* among Scarabaeidae collected from pig carcasses

The abundance of Scarabaeidae species differed significantly, with clear dominance of *T. externepunctatum* over the other recorded taxa ($\chi^2 = 1048.94$; df = 6; $p < 0.0001$). This result indicates that the scarabaeid assemblage associated with pig carcasses was not homogeneous and was strongly structured by the predominance of this species.

The dominance of *T. externepunctatum* indicates that this species was one of the main coleopteran components associated with pig carcass decomposition in Itumbiara. Scarabaeidae are commonly associated with decomposing organic matter and may use carrion environments for feeding, shelter, or resource exploitation. The high abundance of *T. externepunctatum* suggests that pig carcasses provided favorable conditions for its occurrence.

Although Diptera dominated the total arthropod fauna, the high representation of Scarabaeidae among Coleoptera shows the ecological importance of beetles during carcass decomposition. The predominance of *T. externepunctatum* may be related to resource availability, decomposition stage, environmental conditions, and the ability of this species to exploit organic substrates. These results reinforce the importance of including Coleoptera in studies of carrion-associated arthropods, especially because beetles may

contribute to decomposition processes and may also have forensic relevance in studies involving animal carcasses (Marchiori *et al.*, 2000c).

7. Parasitoids of Flies Collected in Chicken Viscera in Itumbiara, Goiás, Brazil

A total of 695 dipteran pupae were collected in traps baited with chicken viscera in Itumbiara, Goiás, Brazil, from March 2001 to March 2002. The specimens belonged to Calliphoridae, Fanniidae, Muscidae, Phoridae, Sarcophagidae, and Sphaeroceridae. The distribution of dipteran pupae differed significantly among species or taxonomic groups ($\chi^2 = 852.80$; df = 7; $p < 0.0001$). *Peckia chrysostoma* was the most abundant host, with 280 pupae and 40.3% of the total, followed by *Poecilomella* sp. with 172 pupae (24.7%) and *M. domestica* with 158 pupae (22.7%).

A total of 145 parasitoids emerged from the collected pupae, corresponding to a corrected overall parasitism rate of 20.9%. Parasitoid abundance differed significantly among taxa ($\chi^2 = 117.32$; df = 3; $p < 0.0001$). *H. herbertii* was the most frequent parasitoid, with 75 individuals and 51.7% of the parasitoid fauna, followed by *B. podagrica* with 62 individuals (42.8%), *N. vitripennis* with 6 individuals (4.1%), and *P. egeria* with 2 individuals (1.4%) (Table 6).

Table 6: Abundance and frequency of Diptera and parasitoids collected with chicken viscera-baited traps in Itumbiara, Goiás, Brazil, from March 2001 to March 2002

Taxonomic group	Hosts species	Number of specimens	Frequency (%)
Diptera			
Calliphoridae	<i>Chrysomya albiceps</i>	20	2.9
Fanniidae	<i>Fannia pusio</i>	10	1.4
Muscidae	<i>Musca domestica</i>	158	22.7
Phoridae	<i>Megaselia scalaris</i>	20	2.9
Sarcophagidae	<i>Oxysarcodexia thornax</i>	19	2.7
	<i>Peckia chrysostoma</i>	280	40.4
	<i>Poecilomella</i> sp.	172	24.7
Total Diptera		695	100.0
Hymenoptera			
Chalcididae	<i>Brachymeria podagrica</i>	62	42.8
Encyrtidae	<i>Hemencyrtus herbertii</i>	75	51.7
Figitidae	<i>Paraganaspis egeria</i>	2	1.4
Pteromalidae	<i>Nasonia vitripennis</i>	6	4.1
Total parasitoids		145	100.0

The highest parasitism rate was recorded for *Hemilucilia flavifacies* (Engel, 1931) (Diptera: Calliphoridae) parasitized by *H. herbertii* (87.5%). Other important associations were *C. albiceps* with *B. podagrica* (45.0%), *P. chrysostoma* with *H. herbertii* (21.8%), *P. chrysostoma* with *B. podagrica* (18.2%), and

O. thornax with *B. podagrica* (10.5%). The distribution of parasitized pupae among host-parasitoid associations also differed significantly ($\chi^2 = 220.96$; df = 7; $p < 0.0001$). The original study reported a significant preference of parasitoids for specific fly hosts ($\chi^2 = 149.7$; df = 15; $p < 0.0001$) (Table 7).

Table 7: Parasitism rate in pupae of flies collected with chicken viscera-baited traps in Itumbiara, Goiás, Brazil, from March 2001 to March 2002

Hosts species	No. of pupae	Parasitoid species	No. of parasitoids	Parasitism rate (%)
<i>Chrysomya albiceps</i>	20	<i>Brachymeria podagrica</i>	9	45.0
<i>Fannia pusio</i>	10	<i>Paraganaspis egeria</i>	2	20.0
<i>Hemilucilia flavifacies</i>	16	<i>Hemencyrtus herbertii</i>	14	87.5
<i>Musca domestica</i>	158	<i>Nasonia vitripennis</i>	5	3.2
<i>Oxysarcodexia thornax</i>	19	<i>Brachymeria podagrica</i>	2	10.5
<i>Peckia chrysostoma</i>	280	<i>Brachymeria podagrica</i>	51	18.2
		<i>Hemencyrtus herbertii</i>	61	21.8
		<i>Nasonia vitripennis</i>	1	0.4
Total	695		145	20.0

The results show that chicken viscera were an effective substrate for attracting synanthropic flies and obtaining their parasitoids in Itumbiara. The dominance of *M. domestica*, *P. chrysostoma*, and *Poecilosomella* sp. indicates that the substrate favored species associated with decomposing organic matter and environments modified by human activity.

The parasitoid fauna was dominated by *B. podagrica* and *H. herbertii*, which together represented

most of the parasitoids obtained. The high frequency of *H. herbertii* in *H. flavifacies* suggests an important host-parasitoid association, probably influenced by host suitability and the gregarious behavior of this parasitoid. The association of *B. podagrica* with *C. albiceps*, *O. thornax*, and *P. chrysostoma* also indicates ecological flexibility and a broader host range. Although *N. vitripennis* and *P. egeria* were less frequent, their occurrence contributes to the diversity of natural enemies associated with Diptera in chicken viscera (Figure 8).



Figure 8: Synanthropic flies and parasitoids associated with chicken viscera used as bait in traps in Itumbiara, Goiás, Brazil. The illustration shows chicken viscera attracting adult Diptera, with larvae and pupae developing in the substrate and hymenopteran parasitoids associated with the flies' immature stages. This figure summarizes the host-parasitoid interactions observed in chicken viscera-baited traps

The significant chi-square results indicate that both fly hosts and parasitoids were unevenly distributed, with clear dominance patterns and specific host-parasitoid associations. Overall, the study reinforces the importance of parasitoids as natural enemies of synanthropic flies and supports the use of decomposing animal substrates as useful systems for studying biological control agents associated with Diptera (Marchiori *et al.*, 2003a).

8. Gregarious Parasitoids Collected in *C. Albiceps* in Brazil

A total of 86 pupae of *C. albiceps* were obtained using dark cylindrical traps baited with human feces in Caldas Novas, Goiás, Brazil. From these pupae, 6 were

parasitized, resulting in an overall parasitism rate of 7.0%. The study recorded two gregarious parasitoid taxa associated with *C. albiceps*: *H. herbertii* and *Trichopria* sp. Among the parasitized pupae, *Trichopria* sp. emerged from 4 pupae, whereas *H. herbertii* emerged from 2 pupae. The difference between the number of pupae parasitized by the 2 parasitoid taxa was not statistically significant ($\chi^2 = 0.67$; $df = 1$; $p = 0.4142$), indicating that, within the small number of parasitized puparia, no clear dominance was detected between the two parasitoid taxa.

However, when parasitized and non-parasitized pupae were compared, the difference was highly significant. Of the 86 puparia obtained, 6 were parasitized, and 80 were not parasitized ($\chi^2 = 63.67$; $df =$

1; $p < 0.0001$). This result indicates that most *C. albiceps* pupae completed development without parasitoid emergence under the conditions observed in the study. The use of human feces as bait was important for attracting adult *C. albiceps* to the dark traps. The record

of *H. herberti* and *Trichopria* sp. emerging from *C. albiceps* pupae indicates the presence of gregarious parasitoids associated with this calliphorid species in Brazil (Table 8).

Table 8: Gregarious parasitoids obtained from *C. albiceps* pupae collected with dark black traps baited with human feces deposited inside the cans in Caldas Novas, Goiás, Brazil

Trap/bait system	Host species	Total pupae	Parasitoid species	Parasitized pupae	Parasitoids emerged	Parasitism rate (%)	Frequency among parasitoids (%)
Dark black can trap human feces deposited inside the can	<i>Chrysomya albiceps</i>	86	<i>Trichopria</i> sp.	4	25	4.7	62.5
		8	<i>Hemencyrtus herberti</i>	2	15	2.3	37.5
Total		86	—	6	40	7.0	100.00

Note: The overall prevalence of parasitism was 7.0%, based on 6 parasitized pupae among 86 pupae. The parasitoids were gregarious, with several individuals emerging from the same host puparium.

The results show that *C. albiceps* pupae collected from human feces-baited dark traps supported the development of gregarious parasitoids. Although the overall parasitism rate was relatively low, the occurrence of *H. herberti* and *Trichopria* sp. It is relevant because it expands knowledge of natural enemies associated with this medically important fly. The low number of

parasitized pupae suggests that parasitism was limited in the sampled material. This may be related to host availability, environmental conditions, parasitoid density, or the short period of host exposure in the field. Even so, the emergence of multiple parasitoids from individual puparia confirms the gregarious behavior of the parasitoids recorded in the study (Figure 9).



Figure 9: Dark trap baited with human feces deposited on the ground, showing synanthropic flies attracted to the substrate and hymenopteran parasitoids associated with the immature stages of Diptera. The illustration represents the field collection system used to obtain fly pupae and their parasitoids and summarizes the host–parasitoid interactions associated with human feces in Brazil

Chrysomya albiceps is an important synanthropic fly because it is associated with decomposing organic matter and may act as a mechanical vector of pathogens. Therefore, records of parasitoids associated with its immature stages are relevant for understanding natural regulation mechanisms in environments influenced by human activity.

The association with human feces also underscores the sanitary importance of the system under study. Human feces can attract flies of medical relevance, and dark cylindrical traps using this substrate may be useful for detecting both fly populations and their parasitoids. Overall, the study contributes to the knowledge of gregarious parasitoids associated with *C. albiceps* in Brazil and supports the importance of

parasitoids as natural enemies of synanthropic Diptera (Marchiori *et al.*, 2004).

9. Parasitoids of *Z. indianus* Collected in Itumbiara, Goiás, Brazil. Dark Trap Baited with Cut Fruit: Mango, Guava, Banana, Pear, and Apple

A total of 963 pupae of *Z. indianus* were obtained using fruit-baited traps in an urban area and woodland in Itumbiara, Goiás, Brazil, from March to November 2001. From these pupae, 293 parasitoids emerged, corresponding to a total parasitism rate of

30.4%. The parasitoids recorded were *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillaut, 1979) (Hymenoptera Figitidae), *P. vindemmiae*, and *S. endius*. Parasitoid abundance differed significantly among species ($\chi^2 = 539.00$; $df = 2$; $p < 0.0001$). *P. vindemmiae* was clearly dominant, with 285 specimens, representing 97.3% of all parasitoids and a parasitism rate of 29.5%. In contrast, *S. endius* and *L. boulardi* were much less frequent, with 5 specimens (1.7%; 0.5% parasitism) and 3 specimens (1.0%; 0.3% parasitism), respectively (Table 9).

Table 9: Parasitoids associated with pupae of *Z. indianus* collected in fruit-baited traps in Itumbiara, Goiás, Brazil

Host specie	No. of pupae	Parasitoid species	No. of parasitoids	Frequency among parasitoids (%)	Parasitism rate (%)
<i>Zaprionus indianus</i>	963	<i>Pachycrepoideus vindemmiae</i>	285	97.3	29.5
		<i>Spalangia endius</i>	5	1.7	0.5
		<i>Leptopilina boulardi</i>	3	1.0	0.3
Total	963	—	293	100.0	30.4

Note: The total number of pupae was 963. The total number of parasitoids was 293, corresponding to 30.4% total parasitism. Frequencies among parasitoids were calculated using 293 as the denominator.

The predominance of *P. vindemmiae* indicates a strong association with pupae of *Z. indianus* under the sampled conditions. The high total parasitism rate was probably related to host density, availability of fruit resources, and the searching capacity of the parasitoids. The results show that *Z. indianus* supported a parasitoid complex composed of three hymenopteran species in

Itumbiara. However, this community was strongly dominated by *P. vindemmiae*, which accounted for almost all parasitoids obtained. This dominance suggests that *P. vindemmiae* was the most efficient or best-adapted parasitoid associated with *Z. indianus* pupae in the sampled environment (Figure 10).



Figure 10: Cut fruits used as bait in traps for collecting frugivorous flies and their parasitoids in Itumbiara, Goiás, Brazil. The illustration shows infested fruits with larvae and puparia of Diptera, adult flies associated with the decomposing fruit substrate, and hymenopterian parasitoids associated with the immature stages of the flies.

The trap represents the collection system used to obtain fruit-infesting Diptera and their natural enemies

The low occurrence of *L. boulardi* and *S. endius* indicates that these species were secondary components of the parasitoid fauna. Their reduced abundance may be related to lower host-searching efficiency, competition with *P. vindemmiae*, environmental conditions, or differences in host-stage preference. Even so, their presence increases the known diversity of natural enemies associated with *Z. indianus*. The total parasitism rate of 30.4% is biologically relevant because *Z. indianus* is an invasive drosophilid associated with fruits and may cause economic losses, especially in fig production. The

occurrence of parasitoids associated with its pupae suggests that natural enemies may contribute to the regulation of this fly population.

Overall, the data indicate that *P. vindemmiae* was the principal parasitoid of *Z. indianus* in Itumbiara, while *L. boulardi* and *S. endius* occurred at low frequency. These parasitoids may represent useful components for future studies on biological control of fruit-infesting Drosophilidae (Marchiori *et al.*, 2003b).

10. Coleoptera (Insecta) and Macrochelidae (Acarina) Associated with Bovine Dung in the State of Goiás: Constancy, Dominance, and Monthly Frequency

A total of 11.599 arthropods were collected from 240 bovine dung pats in Itumbiara, Goiás, Brazil. Of these, 11.114 specimens belonged to Coleoptera and 485 to Acarina. Among Coleoptera, Staphylinidae was the most abundant family, with 4.973 individuals, followed by Scarabaeidae with 4.884 individuals and Histeridae with 1.257 individuals. Acarina was represented by Macrochelidae with 485 individuals. The abundance differed significantly among the main taxonomic groups ($\chi^2 = 5,781.62$; $df = 3$; $p < 0.0001$), indicating that the arthropod community associated with

bovine dung was not homogeneously distributed among groups.

The most abundant species or taxa were *Ataenius aequalis* Harold, 1880 (Coleoptera: Scarabaeidae), with 4,001 individuals, *Oxytelus* sp. (Coleoptera: Staphylinidae), with 3.895 individuals, *Philonthus* spp. (Coleoptera: Staphylinidae), with 660 individuals, *Histeridae* sp.1, with 492 individuals, *Phelister* sp. (Coleoptera: Histeridae), with 441 individuals, *Heterothops* spp. (Coleoptera: Staphylinidae), with 415 individuals, and *Ateuchus striatulus* (Preudhomme de Borre, 1886) (Coleoptera: Scarabaeidae), with 371 individuals. The distribution among species or taxa was highly significant ($\chi^2 = 70,132.27$; $df = 28$; $p < 0.0001$), showing strong numerical dominance of a few taxa (Table 10).

Table 10: Number and percentage of Coleoptera and Acarina collected from eight-day-old bovine dung pats at the Faculty of Agronomy farm in Itumbiara, Goiás, Brazil

Taxonomic group / Species	Frequency	Frequency (%)
Total Histeridae	1.257	10.84
Coleoptera – Scarabaeidae		
<i>Aphodius</i> sp.	14	0.12
<i>Aphodius negrita</i>	4	0.03
<i>Aphodius pseudolivinus</i>	328	2.83
<i>Ataenius</i> sp.1	18	0.16
<i>Ataenius</i> sp.2	3	0.03
<i>Ataenius</i> sp.3	3	0.03
<i>Ataenius</i> sp.5	1	0.01
<i>Ataenius</i> sp.6	1	0.01
<i>Ataenius aequalis</i>	4.001	34.49
<i>Dichotomius anaglypticus</i>	18	0.16
<i>Euparia</i> sp.	45	0.39
<i>Leucothyreus</i> sp.	2	0.02
<i>Onthophagus gazella</i>	73	0.63
<i>Onthophagus hirculus</i>	2	0.02
<i>Ateuchus striatulus</i>	371	3.20
Total Scarabaeidae	4.884	42.11
Coleoptera – Staphylinidae		
<i>Aleochara notula</i>	3	0.03
<i>Heterothops</i> spp.	415	3.58
<i>Philonthus</i> spp.	660	5.69
<i>Oxytelus</i> sp.	3.895	33.58
Total Staphylinidae	4.973	42.87
Total Coleoptera	11.114	95.82
Acarina – Macrochelidae		
<i>Glyptolaspis confusa</i>	322	2.78
<i>Macrocheles</i> sp.	80	0.69
<i>Macrocheles glaber</i>	33	0.28
<i>Macrocheles merdarius</i>	6	0.05
<i>Macrocheles muscaedomesticae</i>	41	0.35
<i>Macrocheles punctoscutatus</i>	3	0.03
Total Acarina	485	4.18
Total general	11.599	100.00

Note: Percentages were calculated in relation to the total number of Coleoptera and Acarina collected ($n = 11.599$).

Among Acarina, *Glypholaspis confusa* (Foà, 1900) (Acari: Macrochelidae), was the most frequent macrochelid mite, with 322 individuals, representing 66.4% of all Acarina. It was followed by *Macrocheles* sp. (Acari: Macrochelidae) with 80 individuals, *M. muscaedomesticae* with 41, *Macrocheles glaber* (Müller 1860) (Acari: Macrochelidae) with 33, *Macrocheles merdarius* (Berlese, 1889) (Acari: Macrochelidae) with 6, and *Macrocheles punctoscutatus* (Evans & Browning, 1956) (Acari: Macrochelidae) with 3. Regarding constancy, 41.5% of the recorded taxa were classified as constant, 34.5% as accessory, and 24.1% as accidental. No species was classified as dominant according to the dominance index. Most arthropods occurred mainly during the warm and humid period, although several taxa were present throughout the year.

The results show that bovine dung in Itumbiara supported a rich community of Coleoptera and Acarina. Coleoptera was numerically dominant, especially Staphylinidae and Scarabaeidae, indicating that cattle dung provided suitable resources for coprophagous and predatory beetles.

The high abundance of *A. aequalis* and *Oxytelus* sp. suggests that these taxa were well adapted to bovine dung environments in the study area. Scarabaeidae are important in dung decomposition because they feed, tunnel, and bury organic matter, improving soil structure and reducing the availability of breeding sites for synanthropic flies. Staphylinidae, especially *Heterothops* spp., and *Philonthus* spp., may act as predators of eggs and larvae of Diptera, contributing to natural biological control (Table 11).

Table 11: Constancy and dominance of Coleoptera and Acarina collected from eight-day-old bovine dung pats at the Faculty of Agronomy farm in Itumbiara, Goiás, Brazil

Taxonomic group	Constancy C (%)	C	D
Coleoptera			
Histeridae			
<i>Acritus</i> sp.	100.0	X	ND
<i>Hister dubius</i>	25.0	X	ND
<i>Histeridae</i> sp.1	100.0	Y	ND
<i>Phelister</i> sp.	91.6	X	ND
Scarabaeidae			
<i>Aphodius</i> sp.	16.6	Z	ND
<i>Aphodius negrita</i>	25.0	Y	ND
<i>Aphodius pseudolivinus</i>	75.0	X	ND
<i>Ataenius</i> sp.1	66.6	X	ND
<i>Ataenius</i> sp.2	8.3	Z	ND
<i>Ataenius</i> sp.3	8.3	Z	ND
<i>Ataenius</i> sp.5	8.3	Z	ND
<i>Ataenius</i> sp.6	8.3	Z	ND
<i>Ataenius aequalis</i>	100.0	Y	ND
<i>Dichotomius anaglypticus</i>	50.0	X	ND
<i>Euparia</i> sp.	33.3	Y	ND
<i>Leucothyreus</i> sp.	8.3	Z	ND
<i>Onthophagus gazella</i>	75.0	X	ND
<i>Onthophagus hirculus</i>	8.3	Z	ND
<i>Ateuchus striatulus</i>	41.6	Y	ND
Staphylinidae			
<i>Aleochara notula</i>	25.0	Y	ND
<i>Heterothops</i> spp.	88.3	X	ND
<i>Philonthus</i> spp.	100.0	X	ND
<i>Oxytelus</i> sp.	100.0	X	ND
Acarina			
Macrochelidae			
<i>Glypholaspis confusa</i>	100.0	X	ND
<i>Macrocheles</i> sp.	25.0	Y	ND
<i>Macrocheles glaber</i>	75.0	X	ND
<i>Macrocheles merdarius</i>	16.6	Z	ND
<i>Macrocheles muscaedomesticae</i>	66.6	Z	ND
<i>Macrocheles punctoscutatus</i>	16.6	Z	ND

Faunistic Indices

Constancy was calculated using the formula $C = (P \times 100) / N$, where C is the constancy percentage, P is the number of samples containing the species, and N is the total number of samples. Species were classified as constant (X) when present in more than 50% of the samples, accessory (Y) when present in 25–50%, and accidental (Z) when present in less than 25%.

Histeridae were less abundant than Scarabaeidae and Staphylinidae, but they also represent important predators in dung-associated communities.

Their occurrence reinforces the ecological complexity of bovine dung pats, where different groups participate in decomposition and in the regulation of dipteran populations. Among Acarina, *G. confusa* was the most frequent species, indicating that Macrochelidae were also important components of the dung fauna. Macrochelid mites are commonly associated with decomposing organic matter and may feed on eggs and immature stages of flies. Therefore, even at lower abundance than Coleoptera, these mites may contribute to the natural control of muscoid flies (Figure 11).



Figure 11: Coleoptera and Macrochelidae associated with bovine dung in Itumbiara, Goiás, Brazil. The illustration shows cattle dung as the breeding and feeding substrate, a Berlese funnel used for arthropod extraction, adult beetles representing the principal coleopteran groups, and macrochelid mites associated with dung pats. This figure summarizes the arthropod fauna collected from bovine dung and the extraction method used in the study

Overall, the significant chi-square results confirm that a few abundant taxa strongly structured the community. The presence of constant species throughout the year suggests that bovine dung serves as a stable microhabitat for arthropods that are ecologically important for decomposition and biological control (Marchiori *et al.*, 2000d).

11. Occurrence of Figitidae (Hymenoptera: Cynipoidea) in Itumbiara, Goiás, Brazil

This study recorded Figitidae associated with bovine dung and native vegetation in Itumbiara, Goiás, Brazil. In yellow traps placed in pasture and native vegetation, five figitid specimens were collected: four specimens of *Acanthaegilips brasiliensis* Ashmead, 1897 (Hymenoptera: Figitidae) and one specimen of *N. splendens*. All Figitidae obtained with yellow traps were collected in pasture, while none were recorded in native vegetation. This difference between environments was

significant ($\chi^2 = 5.00$; $df = 1$; $p = 0.0253$), indicating that pasture was the main environment associated with the Figitidae collected by this method.

The distribution between the two figitid species collected in yellow traps was not statistically significant ($\chi^2 = 1.80$; $df = 1$; $p = 0.1797$), although *A. brasiliensis* was numerically more frequent than *N. splendens*. The occurrence of *A. brasiliensis* is relevant because this species is usually associated with Neuroptera, and its capture in pasture may have been accidental.

In bovine dung, 14 specimens of *N. splendens* were obtained from 4,521 pupae of *S. occidua*, corresponding to a parasitism rate of 0.30%. The difference between parasitized and non-parasitized pupae was highly significant ($\chi^2 = 4465.17$; $df = 1$; $p < 0.0001$), showing that parasitism occurred at very low frequency in the sampled pupae. The results show that

Figitidae occurred in both pasture-associated traps and bovine dung in Itumbiara. Although the number of specimens collected in yellow traps was low, the record of *A. brasiliensis* and *N. splendens* contributes to the

knowledge of Cynipoidea in Goiás and expands information on the local occurrence of this family (Figure 12).



Figure 12: Figitidae parasitoids associated with bovine dung and pasture environments in Itumbiara, Goiás, Brazil. The illustration shows bovine feces as a substrate for dipteran pupae, yellow pan traps used for collecting small hymenopterans, and parasitoids associated with immature stages of dung-breeding flies. This figure summarizes the occurrence of Figitidae recorded in cattle dung and pasture vegetation

The capture of *A. brasiliensis* in pasture is noteworthy because this species is usually associated with Neuroptera rather than Diptera. Therefore, its occurrence in yellow traps may reflect the general attractiveness of the traps to small hymenopterans rather than a direct association with bovine dung. In contrast, *N. splendens* was directly associated with pupae of *S. occidua*, confirming its role as a parasitoid of dung-breeding Diptera.

The low parasitism rate of *N. splendens* in *S. occidua* indicates that this parasitoid was present but not abundant in the sampled material. This may be related to host availability, seasonal occurrence, environmental conditions, or the structure of the dung-associated arthropod community. Even at low frequency, the record is important because it confirms the presence of a natural enemy associated with dipteran pupae in bovine dung.

Overall, the study documents the occurrence of Figitidae in Itumbiara and reinforces the importance of surveying parasitoids in different microhabitats, including pasture vegetation and cattle dung. These records contribute to the understanding of parasitoid

diversity and host associations in Brazilian agroecosystems (Marchiori *et al.*, 2000e).

12. New occurrence of *Bentonia longicornis* Achterberg, 1992 (Hymenoptera: Braconidae, Orgilinae)

In the article, a total of 61 specimens of *B. longicornis* were collected in Itumbiara, Goiás, Brazil. The material was obtained using yellow pan traps placed in pasture and native vegetation from January to December 1998. The specimens were recorded in several months of the year, with the highest abundance observed in June, followed by August, indicating that the species was not evenly distributed throughout the sampling period. The monthly occurrence of *B. longicornis* showed clear temporal variation. Although the species was present in different months, the concentration of individuals in a few sampling periods suggests a seasonal pattern, probably related to climatic conditions and host availability. The original figure indicates that the highest records occurred during months associated with pasture and native vegetation sampling, especially in the middle of the year (Figure 13).

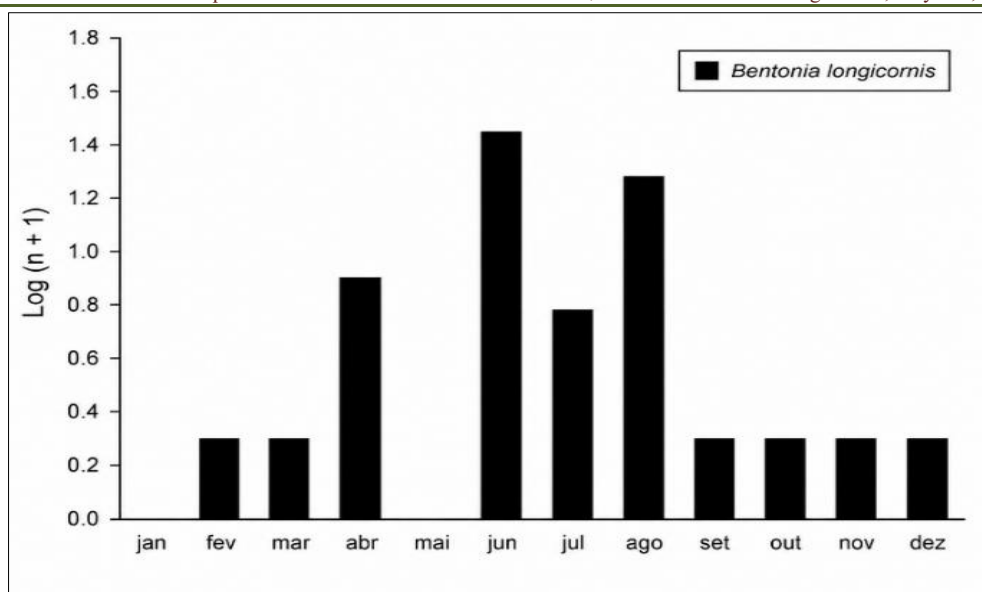


Figure 13: Monthly distribution of *B. longicornis* collected with yellow pan traps in pasture and native vegetation in Itumbiara, Goiás, Brazil, from January to December 1998. The black bars represent the monthly occurrence of the species, with higher abundance recorded mainly in June and August

The record of *B. longicornis* in Itumbiara represents an important contribution to the knowledge of Braconidae in Goiás. This occurrence extends the known geographical distribution of the species and confirms its presence in the Brazilian Cerrado region. The occurrence of *B. longicornis* in Itumbiara is relevant because

Orgilinae are endoparasitoids mainly associated with lepidopteran larvae, especially Pyralidae and Tortricidae. The presence of this parasitoid in pasture and native vegetation suggests that these environments may provide suitable hosts and ecological conditions for its establishment (Figure 14).



Figure 14: *Bentonia longicornis* associated with yellow pan trap surveys in pasture and native vegetation in Itumbiara, Goiás, Brazil. The illustration shows adult braconid parasitoids in dorsal and lateral views, highlighting the general morphology of the species recorded in the study. This figure summarizes the occurrence of *B. longicornis* as a parasitoid representative of Braconidae collected in Goiás. Source: <https://doi.org/10.11646/zootaxa.5195.5.3>

The concentration of individuals in June and August may reflect seasonal variation in host availability or favorable environmental conditions for adult activity. Since the species was collected with yellow pan traps, the data indicate adult occurrence in the field, but do not allow direct confirmation of host association in the

sampled area. The collection of *B. longicornis* in both pasture and native vegetation also suggests that mosaics of agricultural and natural environments may contribute to parasitoid diversity. Native vegetation may act as a reservoir for parasitoids, while pasture areas may provide additional resources or host insects.

Overall, this record is important because it expands the known distribution of *B. longicornis* in Brazil and reinforces the need for surveys of Braconidae in different habitats of the Cerrado. The use of yellow pan traps proved useful for detecting this parasitoid and may be applied in future studies on hymenopteran diversity and biological control potential (Marchiori *et al.*, 2000f).

13. Microhymenopterans of *M. Domestica* Collected in Different Substrates in Itumbiara, Goiás, Brazil

A total of 11,403 pupae of *M. domestica* were collected from different substrates in Itumbiara, Goiás, Brazil. From these pupae, 74 were parasitized, and 113 parasitoid specimens emerged, corresponding to an overall parasitism rate of approximately 0.7%. The distribution of *M. domestica* pupae among substrates was not homogeneous. Chicken manure was the main substrate, with the highest number of pupae collected, followed by bovine feces. The remaining substrates,

including human feces, bovine liver, chicken viscera, and food remains, showed lower numbers of pupae. Considering the substrate values visible in the table, the difference among substrates was highly significant ($\chi^2 = 17,580.24$; $df = 5$; $p < 0.0001$), indicating a strong concentration of *M. domestica* pupae in a few substrates.

When parasitized and non-parasitized pupae were compared using the total reported in the article, the difference was also highly significant ($\chi^2 = 11,108.92$; $df = 1$; $p < 0.0001$). This result indicates that most pupae were not parasitized, despite the presence of several parasitoid species associated with *M. domestica*. Among the parasitoids, *P. vindemmiae* was the most frequent species, accounting for 35.4% of the parasitoid fauna, followed by *N. vitripennis*. Chicken manure supported the highest number of collected pupae and parasitoids, probably because this substrate provided favorable conditions for larval development and pupal availability (Table 12).

Table 12: Parasitoids of *M. domestica* collected in different substrates in Itumbiara, Goiás, Brazil

Substrates	Pupae collected	Parasitoid species	No. of parasitoids	Parasitized pupae	Frequency (%)
Bovine feces	3.370	<i>Pachycrepoideus vindemmiae</i>	—	—	—
Bovine liver	48	<i>Nasonia vitripennis</i>	34	3	6.3
Chicken manure	7.779	<i>Pachycrepoideus vindemmiae</i>	17	17	0.2
		<i>Spalangia cameroni</i>	12	12	0.2
		<i>Spalangia endius</i>	8	8	0.1
		<i>Spalangia nigra</i>	1	1	0.1
		<i>Spalangia nigroaenea</i>	6	6	0.8
Chicken viscera	45	<i>Nasonia vitripennis</i>	5	1	2.2
Food remains	50	<i>Pachycrepoideus vindemmiae</i>	9	9	18.0
		<i>Muscidifurax raptor</i>	—	—	—
Human feces	111	<i>Spalangia cameroni</i>	1	1	0.3
		<i>Hemencyrtus herbertii</i>	5	1	0.9
Total	11.403	—	113	74	—

Note: The table was transcribed from the original article. Dash marks indicate cells where individual values were not clearly legible in the scanned table; the original total reported 113 parasitoids and 74 parasitized pupae.

The results show that *M. domestica* used several organic substrates in Itumbiara, but chicken manure was clearly the most important breeding substrate. This pattern is expected because poultry manure offers high moisture, organic matter, and microbial resources suitable for larval development. The high abundance of pupae in chicken manure indicates that this substrate may function as an important source of *M. domestica* populations in rural and peri-urban environments.

The parasitoid fauna associated with *M. domestica* was diverse, with species belonging mainly to

Encyrtidae, Eulophidae, and Pteromalidae. The predominance of *P. vindemmiae* is important because this species is a solitary pupal parasitoid with a broad host range and frequent association with synanthropic Diptera. Its occurrence in several substrates suggests ecological plasticity and potential importance in the natural regulation of fly populations. The occurrence of *N. vitripennis* also deserves attention because this species is a gregarious ectoparasitoid of fly pupae and may produce several individuals from a single host (Figure 15).



Figure 15: *Musca domestica* and its parasitoids associated with different organic substrates in Itumbiara, Goiás, Brazil. The illustration shows fly adults, larvae, pupae, and hymenopterous parasitoids emerging from or attacking host pupae in substrates such as chicken manure, bovine feces, human feces, bovine liver, chicken viscera, and food remains. This figure summarizes the host–parasitoid interactions recorded for *M. domestica* and its natural enemies in decomposing substrates

Therefore, for gregarious parasitoids, the number of emerged parasitoids must be interpreted carefully, because it may be higher than the number of parasitized pupae. Although the overall parasitism rate was low, the presence of several parasitoid species indicates that *M. domestica* populations are exposed to natural enemies in different substrates. The significant chi-square results reinforce that host availability and parasitism were not evenly distributed. Overall, the study confirms the importance of organic substrates, especially chicken manure, in maintaining *M. domestica* populations and their associated parasitoids (Marchiori, 2006b).

14. Pattern and time of Emergence of *Fannia Pusio* (Wiedemann) (Diptera: Fanniidae) in the Laboratory

A total of 491 adults of *F. pusio* emerged under laboratory conditions, including 288 males and 203 females. Adult emergence occurred over six days, but it was strongly concentrated between the second and fourth days. The distribution of total adult emergence differed

significantly among days ($\chi^2 = 423.73$; $df = 5$; $p < 0.0001$), indicating that emergence was not homogeneous during the observation period. The highest daily emergence occurred on the third day, when 45.6% of the adults emerged. This was followed by the fourth day with 23.0% and the second day with 21.6%. Lower emergence was recorded on the first, fifth, and sixth days, with 3.5%, 4.0%, and 1.4%, respectively. This pattern shows a clear temporal concentration of adult emergence, especially during the central period of the experiment.

Male emergence also varied significantly among days ($\chi^2 = 251.92$; $df = 5$; $p < 0.0001$). The highest percentage of males emerged on the third day (44.7%), followed by the second day (26.9%) and the fourth day (19.1%). Female emergence showed a similar pattern and also differed significantly among days ($\chi^2 = 185.23$; $df = 5$; $p < 0.0001$), with the highest emergence on the third day (46.5%), followed by the fourth day (26.9%) and the second day (16.3%) (Table 13).

Table 13: Emergence (%) of adult *F. pusio* under laboratory conditions at 27°C, 65 ± 5% relative humidity, and 12:12 light: dark photoperiod. Values represent the percentage of males, females, and total individuals emerging over six days. Data were extracted from Marchiori and Prado (1996)

Sex/category	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Male	4.7	26.9	44.7	19.1	3.8	0.8
Female	2.3	16.3	46.5	26.9	6.0	2.0
Individuals emerged/day (%)	3.5	21.6	45.6	23.0	4.0	1.4

Note: *F. pusio* emergence was concentrated mainly on the third and fourth days under the laboratory conditions reported in the original article.

The sex ratio differed significantly from the expected 1:1 proportion ($\chi^2 = 14.71$; $df = 1$; $p = 0.0001$), with males representing the majority of emerged adults. Overall, the results indicate that *F. pusio* emergence followed a synchronized pattern, with most adults emerging during the photo phase, especially between 10:00 and 12:00 h, as reported in the original study. The

emergence pattern of *F. pusio* showed a clear circadian rhythm under laboratory conditions. Most adults emerged during the light period, with a marked concentration between the second and fourth days. This indicates that adult emergence is not random, but temporally organized (Figure 16).



Figure 16: Adult and larval stages of *F. pusio*. The upper images show adult specimens in lateral and dorsal views, emphasizing the external morphology of the fly, while the lower image shows the larval stage with its characteristic segmented body and lateral projections. This figure illustrates the developmental stages used to support studies on the emergence pattern and biological characteristics of *F. pusio* under laboratory conditions

The predominance of emergence on the third day suggests that pupal development was relatively synchronized under controlled temperature, humidity, and photoperiod. Such synchronization may represent an adaptive advantage, reducing the time during which newly emerged adults are exposed to unfavorable conditions. The higher proportion of males indicates that males emerged more frequently than females under the experimental conditions. This pattern may be related to reproductive strategy, because earlier or more frequent male emergence can increase mating opportunities when females emerge shortly afterward.

Overall, the study demonstrates that *F. pusio* emergence is influenced by circadian rhythm and photoperiod. The strong concentration of adult emergence during daytime hours supports the importance of environmental cues in regulating the developmental biology of this species (Marchiori and Prado, 1996a).

15. Life table of *F. Pusio* in the Laboratory

The life-table parameters showed that temperature strongly influenced the population dynamics of *F. pusio* under laboratory conditions. The highest net reproductive rate was recorded at 27°C, with $R_0 = 48.18$, indicating that this temperature supported the greatest reproductive output. At 20°C, the net reproductive rate was intermediate, with $R_0 = 29.21$, whereas at 33°C the value decreased sharply to $R_0 = 3.79$.

The intrinsic rate of natural increase followed the same pattern. The highest R_m was observed at 27°C ($R_m = 0.1849$), followed by 20°C ($R_m = 0.1009$). The lowest value occurred at 33°C ($R_m = 0.0670$), showing that this higher temperature reduced the capacity for population increase. Generation time was longest at 20°C ($T = 33.41$ days) and shorter at 27°C ($T = 20.95$ days) and 33°C ($T = 19.88$ days) (Table 14).

Table 14: Summary of life-table parameters of *F. pusio* maintained at constant temperatures under laboratory conditions

Temperature (°C)	Development time egg-adult (days)	Eggs reaching maturity	Net reproductive rate (R_0)	Intrinsic rate of increase (R_m)	Generation time (T, days)	Biological interpretation
20	19.00	0.209	29.21	0.1009	33.41	Intermediate reproductive performance and longer generation time.
27	11.87	0.380	48.18	0.1849	20.95	Most favorable condition, with the highest reproductive and growth parameters.
33	10.16	0.203	3.79	0.0670	19.88	High temperature reduced reproductive performance and population growth.

The development time from egg to adult decreased as the temperature increased. Development lasted 19.00 days at 20°C, 11.87 days at 27°C, and 10.16 days at 33°C. However, faster development at 33°C was not associated with greater population performance, because reproductive output and intrinsic growth were markedly lower at this temperature. The fraction of eggs reaching maturity was highest at 27°C (0.380), supporting this temperature as the most favorable condition among those tested.

The results indicate that 27°C was the most favorable temperature for the development and population growth of *F. pusio*. This condition produced

the highest net reproductive rate, the highest intrinsic rate of increase, and the greatest proportion of eggs reaching maturity. These findings suggest that moderate warm temperatures favor both survival and reproduction in this species.

Although development was faster at 33°C, the reproductive parameters were strongly reduced. This indicates that high temperature accelerated immature development but imposed physiological stress that reduced fecundity, survival, or both. Therefore, development time alone is not sufficient to evaluate population performance; reproductive output and survival must also be considered (Figure 17).



Figure 17: Adults *F. pusio* in detailed morphological views. The upper images show the head in frontal view, the adult in lateral view, and a close-up of the wing, whereas the lower image shows the adult specimen in dorsal view. This figure highlights important external characteristics of *F. pusio* used in biological and taxonomic studies

At 20°C, population growth was slower because the generation time was longer. However, the species still maintained an intermediate reproductive rate, indicating tolerance to cooler laboratory conditions. The longer generation time at this temperature suggests delayed development and slower generational replacement. Overall, the life-table data show that *F. pusio* has greater demographic potential at 27°C. This information is important for understanding laboratory

rearing, seasonal population dynamics, and the ecological success of this species in environments where temperature influences development, survival, and reproduction (Marchiori and Prado, 1999a).

16. Development of Immature Stages of *F. Pusio*: Egg Hatching Under Different Temperatures

Egg hatching of *F. pusio* varied markedly among the tested temperatures. A total of 800 eggs was

evaluated at each temperature, and hatching ranged from very low values at the thermal extremes to higher values at intermediate and warm temperatures. The lowest hatching rates were recorded at 10°C, with 6 hatched eggs (0.75%), and at 37°C, with 8 hatched eggs (1.00%). In contrast, the highest hatching rates occurred at 33°C, with 580 hatched eggs (72.50%), followed by 20°C, with 554 hatched eggs (69.25%), and 35°C, with 522 hatched eggs (65.25%).

The distribution of hatched and non-hatched eggs differed significantly among temperatures ($\chi^2 = 2050.49$; $df = 15$; $p < 0.0001$), indicating that egg viability was strongly affected by temperature. Intermediate temperatures between 25°C and 31°C produced relatively stable hatching rates, ranging from 49.75% to 53.38%, whereas the extreme temperatures of 10°C and 37°C were clearly unfavorable for egg eclosion (Table 15).

Table 15: Egg hatching of *F. pusio* under different constant temperatures in laboratory conditions

Temperature (°C)	Eggs evaluated (n)	Eggs hatched (n)	Hatching rate (%)	Mean eclosion time (h)
10	800	6	0.75	132 ± 2.4
12	800	220	27.50	120 ± 1.8
14	800	374	46.75	84 ± 1.6
16	800	407	50.88	72 ± 2.5
18	800	274	34.25	60 ± 3.2
20	800	554	69.25	44 ± 4.5
22	800	354	44.25	36 ± 3.0
24	800	367	45.88	29 ± 2.2
25	800	427	53.38	27 ± 2.5
26	800	412	51.50	26 ± 3.6
27	800	426	53.25	24 ± 1.2
29	800	401	50.12	23 ± 3.1
31	800	398	49.75	20 ± 1.2
33	800	580	72.50	19 ± 1.0
35	800	522	65.25	22 ± 1.9
37	800	8	1.00	20 ± 0.9

Note: Each temperature was tested with 800 eggs. The chi-square test compared hatched and non-hatched eggs among temperatures

Mean eclosion time decreased as temperature increased. At 10°C, the mean eclosion time was 132 h, whereas at 33°C it was 19 h. Between 20°C and 33°C, embryonic development became progressively faster, with mean values decreasing from 44 h at 20°C to 24 h at 27°C and 19 h at 33°C. These results show that higher temperatures accelerated embryonic development, although very high temperatures at 37°C sharply reduced egg viability.

The results indicate that temperature was a decisive factor affecting both egg viability and eclosion time in *F. pusio*. The strong reduction in hatching at 10°C and 37°C suggests that thermal extremes impaired embryonic development. Therefore, these temperatures can be considered unsuitable for the successful development of the egg stage under the tested laboratory conditions (Figure 18).

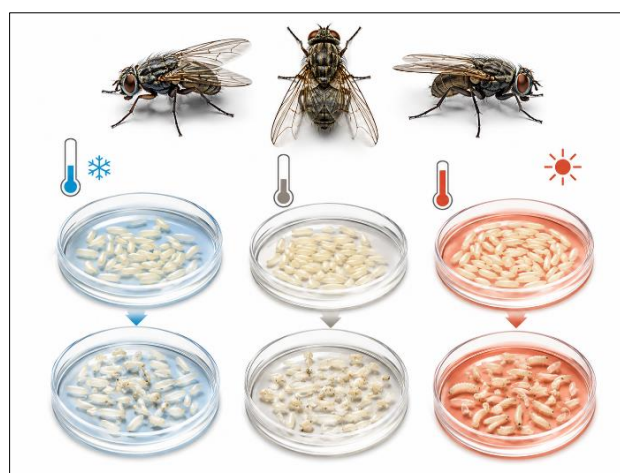


Figure 18: Eggs of *F. pusio* maintained under different temperature conditions in the laboratory. The illustration shows adult flies and egg batches exposed to low, intermediate, and high temperatures, highlighting the effects of temperature on egg development and hatching. This figure summarizes the initial stage of the life cycle of *F. pusio* under controlled experimental conditions

The highest hatching rates at 33°C and 20°C show that *F. pusio* can tolerate a relatively broad thermal range, but the response was not linear. Although the shortest eclosion time occurred at 33°C, high temperature did not always result in the highest viability, as demonstrated by the almost complete failure of hatching at 37°C. Thus, temperature accelerated development up to a physiological limit, beyond which egg survival was severely reduced.

The relatively stable hatching percentages between 25°C and 31°C suggest that this range is favorable for embryonic development and may reflect conditions commonly experienced by this species in warm environments. These findings help explain the abundance of *F. pusio* in warmer periods and reinforce the importance of temperature in regulating population dynamics. Overall, the egg stage showed both thermal sensitivity and developmental plasticity, with faster development at higher temperatures but reduced survival under extreme conditions (Marchiori and Prado, 1996b).

17. Effect of Temperature on the Development of Immature Stages of *F. Pusio* in the Laboratory

The immature development of *F. pusio* was influenced differently by temperature depending on the developmental stage evaluated. Larval growth, measured by body weight, did not show a statistically significant difference among the three temperatures tested ($F = 0.10$;

$p = 0.9067$). The larvae reached their maximum weight between 138 and 162 h at 20°C, whereas at 27°C and 33°C, the maximum larval weight was reached earlier, between 90 and 114 h.

The mean larval development time was 96.5 h at 20°C and 63 h at both 27°C and 33°C. Although larval development was numerically longer at 20°C, the difference among temperatures was not statistically significant ($F = 1.08$; $p = 0.3554$). Thus, the larval stage showed a similar developmental response under the three temperature conditions, despite the faster attainment of maximum weight at the higher temperatures.

In contrast, the pupal stage showed a significant response to temperature. Pupal development differed significantly among the three temperatures ($F = 12.54$; $p < 0.0005$). The beginning of pupation occurred between 210 and 234 h at 20°C, whereas at 27°C and 33°C it occurred earlier, between 138 and 162 h. The mean pupal period was 330 h at 20°C and 198 h at both 27°C and 33°C, indicating that higher temperatures shortened pupal development. Overall, the results show that temperature had a stronger effect on the pupal stage than on the larval stage. The shorter pupal period at 27°C and 33°C indicates accelerated development under warmer conditions, while the longer period at 20°C suggests slower immature development at lower temperatures (Table 16).

Table 16: Larval and pupal development of *F. pusio* maintained under controlled laboratory temperatures. Values summarize the development pattern reported for immature stages and the statistical interpretation of temperature effects

Temperature (°C)	Larval maximum weight reached after egg hatching (h)	Mean larval development time (h)	Beginning of pupal period (h)	Mean pupal development time (h)	Statistical interpretation
20	138–162	96.5	210–234	330	Larval development did not differ significantly among temperatures; pupal development was longer at 20°C.
27	90–114	63	138–162	198	Larval development was shorter than at 20°C; the pupal period was also shorter.
33	90–114	63	138–162	198	Larval development was similar at 27°C; pupal period was shorter than at 20°C.

The development of immature stages of *F. pusio* showed a temperature-dependent pattern, especially during the pupal stage. Although larval growth and larval development time did not differ significantly among temperatures, the larvae reached maximum weight earlier at 27°C and 33°C than at 20°C. This suggests that warmer temperatures accelerated early larval growth, even though the overall larval period did not differ statistically.

The pupal stage was more sensitive to temperature variation. The significant ANOVA result for pupal development indicates that temperature strongly affected the duration of this stage. At 20°C, pupal development was prolonged, whereas at 27°C and 33°C the pupal period was shorter. This pattern is consistent with the general biological response of insects, in which metabolic activity increases within favorable thermal limits and accelerates development (Figure 19).



Figure 19: Immature stages of *F. pusio* under laboratory conditions. The figure shows the first, second, and third larval instars, represented by progressively larger clear larvae, and the brown pupal stage. This sequence illustrates the post-embryonic development of *F. pusio*, used to evaluate larval and pupal development at different temperatures

The similarity between the pupal duration at 27°C and 33°C suggests that these temperatures supported faster development than 20°C. However, faster development at higher temperatures does not necessarily indicate better overall biological performance, because other parameters such as egg hatch, survival, longevity, and fecundity must also be considered. In the complete biological evaluation of *F. pusio*, temperature affects different stages in different ways.

These findings reinforce the importance of separating larval and pupal responses when evaluating immature development. The larval stage showed no significant statistical difference among temperatures, whereas the pupal stage was significantly affected. Therefore, the pupal stage appears to be a critical phase for understanding how temperature regulates the development and population dynamics of *F. pusio* under laboratory conditions (Marchiori, 1993).

18. Adult Longevity of *F. pusio* under Different Temperatures

Adult longevity of *F. pusio* varied markedly according to temperature. The highest mean longevity was recorded at 20°C, where adults survived for an average of 26.0 days. Longevity decreased at 27°C, with a mean of 18.5 days, and reached the lowest value at 33°C, with a mean of 12.0 days. The original statistical analysis indicated significant differences in adult longevity among the three temperatures, showing that survival decreased as temperature increased.

Sex also influenced longevity. At 20°C, females lived longer than males, with a mean longevity of 28.1 ± 14.9 days for females and 22.9 ± 7.9 days for males. This difference was statistically significant ($Z = 1.95$; $p < 0.0256$). At 27°C, females again showed greater longevity, surviving 22.4 ± 6.7 days, whereas males survived 14.4 ± 7.0 days ($Z = 6.04$; $p < 0.0001$). At 33°C, the same pattern was observed, with females surviving 14.0 ± 5.4 days and males 9.9 ± 3.7 days ($Z = 2.37$; $p < 0.0006$) (Table 17).

Table 17: Mean longevity in days of adult *F. pusio* maintained under constant laboratory temperatures. Values are presented as mean $\pm$ standard deviation for males, females, and total adults

Temperature (°C)	Male longevity (days)	Female longevity (days)	Total longevity (days)	Statistical interpretation
20	22.9 ± 7.9	28.1 ± 14.9	26.0 ± 7.0	Longest adult survival; females lived significantly longer than males ($Z = 1.95$; $p < 0.0256$).
27	14.4 ± 7.0	22.4 ± 6.7	18.5 ± 7.1	Intermediate longevity; females lived significantly longer than males ($Z = 6.04$; $p < 0.0001$).
33	9.9 ± 3.7	14.0 ± 5.4	12.0 ± 5.0	Shortest longevity; females lived significantly longer than males ($Z = 2.37$; $p < 0.0006$).

Survival curves also showed a progressive reduction in adult lifespan with increasing temperature. At 20°C, the last females died between 44 and 46 days, and the last males between 36 and 38 days. At 27°C, the

last females died between 30 and 32 days, and the last males between 24 and 26 days. At 33°C, the last females survived until approximately 18–20 days, whereas males survived until approximately 15–16 days. These results

confirm that adult survival followed a step-like decline and that higher temperatures accelerated mortality.

The longevity pattern observed for *F. pusio* indicates that temperature strongly affects adult survival. The longest survival occurred at 20°C, suggesting that

cooler laboratory conditions reduced metabolic expenditure and allowed adults to live longer. In contrast, the shortest longevity occurred at 33°C, indicating that high temperature increased physiological stress and accelerated adult mortality (Figure 20).

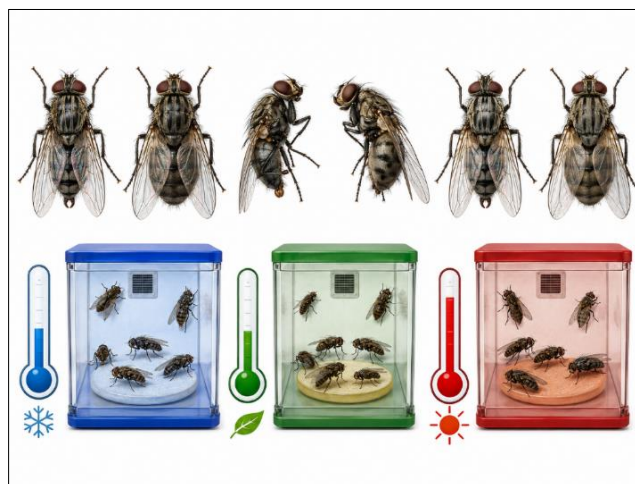


Figure 20: Adult *F. pusio* maintained under different laboratory temperature conditions. The figure illustrates adult males and females exposed to low, intermediate, and high temperatures, representing the experimental basis used to evaluate adult longevity. This image summarizes the influence of thermal conditions on survival and longevity patterns of *F. pusio* under controlled laboratory conditions

The consistently greater longevity of females in all temperatures is biologically relevant. Longer female survival may increase the reproductive period, allowing more opportunities for oviposition and contributing to population maintenance under favorable conditions. This pattern was especially evident at 27°C, where the difference between females and males was strongest.

Although 20°C favored adult longevity, longer survival does not necessarily indicate the best condition for population growth. The previous life-table analysis showed that 27°C produced the highest reproductive rate and intrinsic rate of increase. Therefore, adult longevity must be interpreted together with fecundity and development time. A lower temperature may extend adult survival, whereas an intermediate temperature may optimize reproduction and population increase.

Overall, the results demonstrate that adult longevity in *F. pusio* is temperature-dependent and sex-dependent. Females lived longer than males at all tested temperatures, and adult survival declined progressively from 20°C to 33°C. These findings contribute to understanding the population biology of *F. pusio* under laboratory conditions and help explain how temperature may influence the seasonal dynamics of this species (Marchiori and Prado, 1995).

19. Fecundity of *F. Pusio* under Different Laboratory Temperatures

Oviposition activity of *F. pusio* varied among the three temperatures evaluated under laboratory conditions. At 20°C, females showed oviposition peaks on the sixth day (6.2%), eleventh day (9.3%), and twenty-fifth day (6.2%) of adult life. At 27°C, two maximum reproductive peaks were observed, on the eighth day (9.5%) and the thirty-second day (9.6%). At 33°C, the maximum peak occurred on the seventh day (31.6%).

The pre-oviposition period was shorter at 27°C, with egg laying beginning after 48 hours, whereas females maintained at 20°C and 33°C began oviposition after 72 hours. Total egg production was highest at 27°C, with 342 eggs, followed by 20°C, with 324 eggs. The lowest egg production occurred at 33°C, with only 55 eggs. The mean number of eggs produced per female per day also differed among temperatures, with the highest value at 27°C (10.1 ± 7.6 eggs/day), followed by 20°C (7.0 ± 5.0 eggs/day) and 33°C (2.6 ± 3.9 eggs/day).

The original analysis indicated a significant difference in mean daily egg production among temperatures ($F = 13.87$; $p < 0.0001$). Therefore, fecundity was not homogeneous across the thermal conditions. The greatest reproductive performance occurred at 27°C, whereas 33°C strongly reduced oviposition. These results show that temperature directly affected the reproductive capacity of *F. pusio* females (Table 18).

Table 18: Egg productivity of *F. pusio* females maintained under different laboratory temperatures. The table summarizes the pre-oviposition period, total number of eggs produced, and mean daily oviposition recorded at 20°C, 27°C, and 33°C

Temperature (°C)	Pre-oviposition period	Total eggs	Eggs/female/day (mean $\pm$ SD)
20	72 h	324	7.0 $\pm$ 5.0
27	48 h	342	10.1 $\pm$ 7.6
33	72 h	55	2.6 $\pm$ 3.9

The results indicate that 27°C was the most favorable temperature for female fecundity in *F. pusio*. At this temperature, females showed the shortest pre-oviposition period, the highest total egg production, and the highest mean daily oviposition. This suggests that intermediate thermal conditions favored reproductive activity and allowed females to allocate more energy to egg production.

At 20°C, egg production was also relatively high, but the longer pre-oviposition period indicates slower physiological activation of reproduction. This pattern is consistent with the general effect of lower temperatures on insect metabolism, in which

development and reproductive processes tend to occur more slowly. Thus, although 20°C supported fecundity, reproduction was delayed compared with 27°C.

The sharp reduction in fecundity at 33°C indicates that high temperature had a negative effect on reproductive performance. Although development may be faster at warmer conditions, excessive temperature can reduce female longevity, impair ovarian activity, and decrease the number of eggs produced. In this study, the low total number of eggs and the low daily oviposition at 33°C show that this temperature was unfavorable for *F. pusio* reproduction (Figure 21).



Figure 21: Adult *F. pusio* and oviposition under different laboratory temperature conditions. The upper images show adult specimens, including a female during oviposition, while the lower images show egg masses deposited on the substrate under low, intermediate, and high temperatures. This figure summarizes the reproductive activity of *F. pusio* and illustrates the influence of temperature on oviposition and fecundity under controlled conditions

Overall, the fecundity results support the conclusion that temperature is a key factor regulating population growth in *F. pusio*. The combination of high egg production and faster reproductive onset at 27°C indicates that this temperature is close to the optimal

range for reproduction under laboratory conditions. These findings help explain the population dynamics of the species and provide useful information for laboratory rearing and biological studies of Fanniidae (Figure 22) (Marchiori and Prado, 1995).

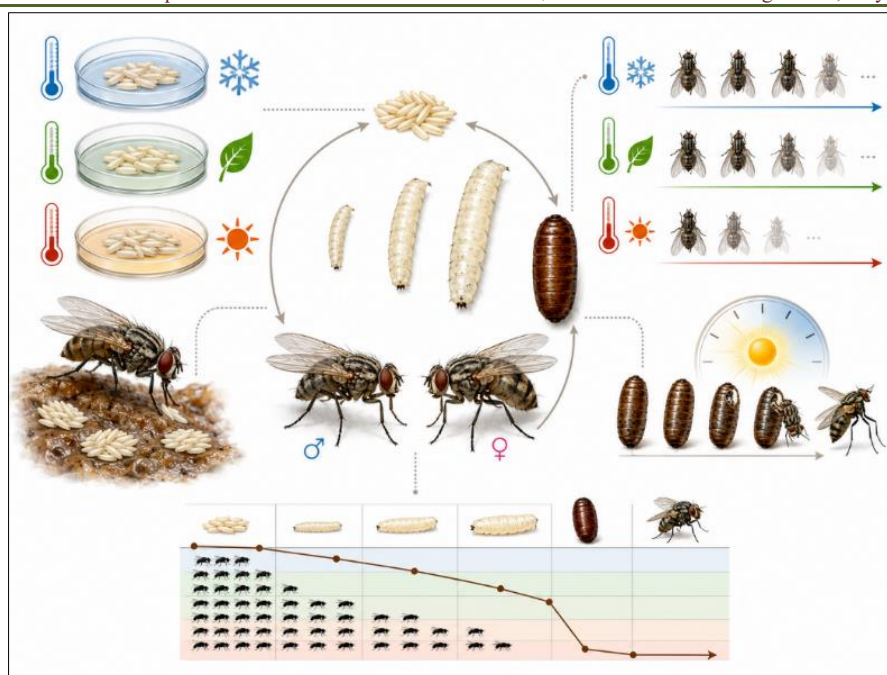


Figure 22: Integrative representation of the experimental studies on *F. pusio* under laboratory conditions. The figure summarizes the main stages and biological parameters evaluated in the species, including eggs under different temperatures, larval instars, pupal development, adult longevity, fecundity, life table parameters, and adult emergence pattern. This illustration provides an overall view of the life cycle and experimental factors used to study the biology of *F. pusio*

20. Occurrence of *Chelonus* sp. (Hymenoptera: Braconidae) collected in Itumbiara, Goiás, Brazil.

In Itumbiara, Goiás, Brazil, *Chelonus* sp. was recorded using yellow pan traps and a Malaise trap in areas of pasture and native vegetation. In 1998, yellow pan traps collected 622 Braconidae, of which 34 were *Chelonus* sp., representing 5.5% of the braconid fauna. Of these specimens, 25 were collected in pasture and nine in native vegetation. In 2000, the Malaise trap collected 213 Braconidae, including 15 *Chelonus* sp. specimens, representing 7.0%. Overall, 49 specimens of *Chelonus* sp. were recorded from 835 Braconidae, representing 5.9% of the total braconid sample.

The distribution of *Chelonus* sp. between pasture and native vegetation in yellow pan traps was significant ($\chi^2 = 7.53$; $df = 1$; $p = 0.0061$), indicating higher occurrence in pasture. The number of *Chelonus* sp. specimens differed significantly between the two sampling periods/methods ($\chi^2 = 7.37$; $df = 1$; $p = 0.0066$), with more specimens recorded in yellow pan traps than in the Malaise trap. When *Chelonus* sp. was compared with the remaining Braconidae in the combined sample, the difference was highly significant ($\chi^2 = 925.34$; $df = 1$; $p < 0.0001$), showing that *Chelonus* sp. represented a small but clearly documented component of the braconid assemblage (Table 19).

Table 19: The table summarizes the number of Braconidae collected in yellow pan traps and Malaise traps, and the proportion represented by *Chelonus* sp. The records indicate the occurrence of the genus in pasture and native vegetation and support its first report for Goiás

Period	Sampling method	Environment	Total Braconidae	<i>Chelonus</i> sp. specimens	<i>Chelonus</i> proportion (%)	Main observation
Jan-Dec 1998	Yellow pan traps	Pasture and native vegetation	622	34	5.5	Twenty-five specimens were collected in the pasture, and nine in the native vegetation.
Feb-Nov 2000	Malaise trap	Native vegetation	213	15	7.0	The Malaise trap also recorded <i>Chelonus</i> sp., confirming its occurrence in the sampled area.
Total	Two sampling methods	Pasture/native vegetation	835	49	5.9	The combined records confirm <i>Chelonus</i> sp. in Itumbiara, Goiás.

The record of *Chelonus* sp. in Itumbiara is important because Braconidae are highly diverse parasitoids, and species of *Chelonus* sp. are mainly associated with eggs and larvae of Lepidoptera. The occurrence of this genus in Goiás expands knowledge of its distribution in Brazil and confirms the usefulness of field surveys for detecting parasitoid diversity in agricultural and semi-natural environments.

The higher number of specimens collected in the pasture may reflect differences in vegetation structure, host availability, or local microclimatic conditions. Pasture environments can contain herbaceous plants and lepidopteran hosts that favor the

activity of egg-larval parasitoids. However, the record in native vegetation also indicates that *Chelonus* sp. may occur in different habitats in the region.

The comparison between yellow pan traps and the Malaise trap suggests that both methods can detect *Chelonus* sp., although yellow pan traps collected more specimens in this study. Yellow pan traps are efficient for small hymenopterans attracted to color, whereas Malaise traps intercept flying insects moving through vegetation. Therefore, using different sampling methods increases the chance of detecting parasitoid taxa with different behaviors (Figure 23).

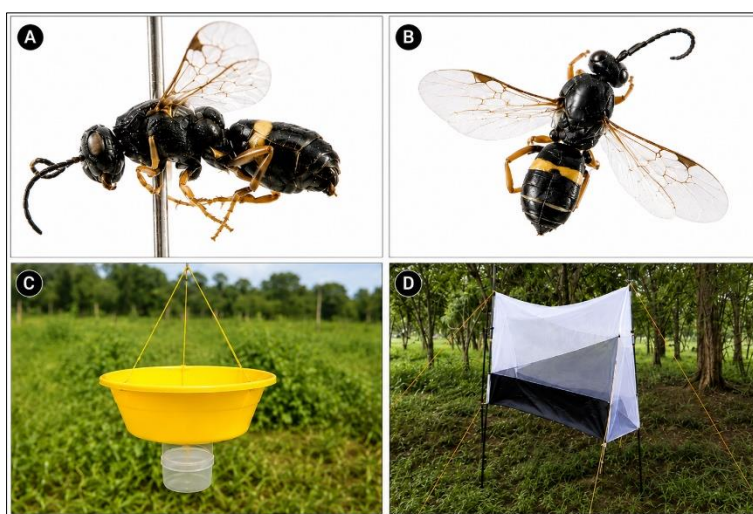


Figure 23: *Chelonus* sp. and collecting devices used in Itumbiara, Goiás, Brazil. (A) Adult specimen in lateral view. (B) Adult specimen in dorsal view. (C) Yellow pan trap used for field collection. (D) Malaise trap used for field collection in pasture and native vegetation areas. This figure summarizes the parasitoid morphology and the sampling methods employed in the study. Source: bobgaia/mage 13.954 views 1.677 times since download/ Connected users: 92 /LMDI — 2002-2026

Overall, the study represents a relevant faunistic record of *Chelonus* sp. in Goiás. Even though the genus represented a low proportion of all Braconidae, its detection contributes to the knowledge of parasitoid

distribution and reinforces the importance of surveying Hymenoptera in pasture and native vegetation (Figures 24-25) (Marchiori and Penteado-Dias, 2001).



Figure 24: Biological cycle of parasitism by *Chelonus* sp. in lepidopteran hosts. Females oviposit inside the eggs of the host insect, and the caterpillar emerges already parasitized. The parasitoid larva develops internally during host growth and completes its development near the prepupal stage, emerging from the caterpillar and causing host death

21: Parasitoids of Insects of Economic and Sanitary Importance Collected in Brazil

The comparative synthesis showed that organic substrates differed strongly in the number of host pupae or puparia obtained, parasitoid emergence, and parasitism rates. The five substrate groups with complete numerical data produced a total of 8.477 pupae or puparia, of which 1.022 yielded parasitoids. The overall parasitism rate was 12.1%.

Chicken feces produced 2.799 pupae and 504 parasitoids, corresponding to a parasitism rate of 18.0%. This substrate showed high parasitoid emergence and

supported strong host–parasitoid interactions, especially involving muscoid Diptera associated with poultry manure. Cattle dung produced 628 pupae and 78 parasitoids, with a parasitism rate of 12.4%, indicating that cattle dung also supported important host–parasitoid associations in pasture and corral systems. Buffalo feces produced the highest number of pupae, with 3.473, but only 172 parasitoids, resulting in a lower parasitism rate of 5.0%. These three animal-feces substrates together had been previously organized as a corrected dataset with 6,900 pupae, 754 parasitoids, and 10.9% overall parasitism (Table 20).

Table 20: Comparative synthesis of pupae and parasitoids associated with organic substrates in Brazil

Substrate/sampling system	Total pupae	Parasitoids emerged	Parasitism rate (%)	Main interpretation
Bovine feces	628	78	12.4	Cattle dung supported muscoid Diptera and parasitoids associated with pasture and corral systems.
Bovine feces / exposure-time experiment	3.099	430	13.9	Field exposure of cattle dung showed temporal variation in Diptera emergence and parasitoid activity.
Buffalo feces	3.473	172	5.0	Buffalo dung supported high host abundance but lower proportional parasitism.
Chicken feces	2.799	504	18.0	Poultry manure supported high parasitoid emergence and strong host-parasitoid interactions.
Fruit substrates	774	48	6.2	Fruits supported Tephritidae and parasitoids of agricultural importance.
Human feces/pitfall traps	803	220	27.4	Human feces attracted synanthropic Diptera and showed high parasitoid diversity and parasitism.

Note: Values were organized from the previously revised datasets for the synthesis article. Rows marked “To organize” should be completed after the extraction of the detailed host-parasitoid data from the corresponding experimental articles. Parasitism rate was calculated as parasitoids or parasitized pupae divided by total pupae, multiplied by 100, according to the interpretation used in each dataset.

Human feces sampled with pitfall traps produced 803 pupae and 220 parasitoids, corresponding to the highest parasitism rate among the analyzed substrates (27.4%). This substrate supported a diverse parasitoid community and showed important associations with synanthropic Diptera, especially Sarcophagidae and other flies associated with fecal environments.

Fruit substrates produced 774 pupae and 48 parasitoids, with a parasitism rate of 6.2%. Although the parasitism rate was lower than that recorded in human feces, chicken feces, and bovine feces, fruits supported parasitoids associated with Tephritidae, which are important from an agricultural perspective.

The chi-square test comparing parasitized and non-parasitized pupae among substrates was highly significant ($\chi^2 = 462.11$; $df = 4$; $P < 0.0001$), confirming that substrate type strongly influenced parasitism. The overall comparison between parasitized and non-parasitized pupae was also significant ($\chi^2 = 4881.86$; $df = 1$; $P < 0.0001$), reflecting the predominance of non-parasitized pupae in the total dataset. Parasitoid emergence was not equally distributed among substrates

($\chi^2 = 643.30$; $df = 4$; $P < 0.0001$), with the highest number recorded in chicken feces and the lowest in fruits.

The comparative analysis indicates that decomposing organic substrates differ markedly in their capacity to support Diptera and their hymenopteran parasitoids. Human feces/pitfall traps showed the highest proportional parasitism, while chicken feces produced the greatest absolute number of parasitoids. Buffalo feces produced the largest number of host pupae but showed the lowest proportional parasitism among the animal feces substrates. This pattern indicates that high host abundance does not necessarily result in high parasitism.

Chicken feces were highly productive for parasitoid emergence, probably because poultry manure provides abundant organic matter, moisture, and suitable conditions for larval development of muscoid Diptera. The high parasitism rate observed in this substrate suggests that parasitoids can efficiently exploit hosts developing in poultry environments. This is especially relevant because flies associated with chicken manure may have sanitary and veterinary importance.

Bovine feces also supported relevant parasitoid emergence, although with lower total host abundance than chicken and buffalo feces. The parasitism rate in cattle dung indicates that pasture and corral systems may

act as important habitats for parasitoids of dung-breeding Diptera. These environments may maintain parasitoid populations that contribute to the natural regulation of flies associated with livestock (Figure 25).



Figure 25: Integrative representation of Diptera and their hymenopteran parasitoids associated with decomposing organic substrates and sampling traps used in experimental studies in Brazil. The figure shows adult flies with two wings, smaller parasitoids with four wings, and the main substrates evaluated, including bovine feces, buffalo feces, human feces, chicken viscera, fish, bovine organs, and fruits. The sampling systems represented include black cylindrical baited traps, yellow pan traps, Malaise traps, and pitfall traps, highlighting the relationship between substrates, host flies, parasitoids, and collection methods

Buffalo feces supported the largest number of pupae, but the proportional parasitism was low. This suggests that host density alone does not determine parasitoid success. Physical and chemical characteristics of buffalo dung, host species composition, moisture, decomposition rate, and parasitoid searching behavior may influence the lower parasitism rate observed in this substrate.

Human feces collected in pitfall traps showed the highest parasitism rate in the synthesis. This result is ecologically important because fecal substrates attract synanthropic flies of sanitary relevance. The high parasitoid emergence suggests that environments containing human fecal bait may reveal a rich assemblage of parasitoids associated with Sarcophagidae and other Diptera. However, in the synthesis article, this chapter should be treated as one component of a broader comparative analysis, avoiding duplication of a single-substrate study.

Fruit substrates showed lower overall parasitism, but are important because they involve Tephritidae and parasitoids of agricultural relevance. Even with fewer parasitoids, records from fruits contribute to the understanding of natural enemies

associated with fruit flies and may have implications for biological control in fruit production systems.

The significant chi-square results confirm that substrate type influenced parasitism and parasitoid emergence. The uneven distribution of parasitoids among substrates indicates that each organic resource creates a distinct ecological context for host–parasitoid interactions. Therefore, integrating different substrates in a synthesis article provides a stronger and more original contribution than treating one already analyzed chapter as a separate article.

22. Insects and Parasitoids Associated with Bovine Feces at Different Exposure Times in Brazil: A Synthesis of Diptera, Coleoptera, and Hymenopteran Natural Enemies

The distribution of Diptera among exposure periods differed significantly. The total number of Diptera collected at 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours was not homogeneous, showing a significant effect of exposure time on fly abundance ($\chi^2 = 173.88$; $df = 9$; $P < 0.0001$). This result indicates that the age of the fecal substrate influenced the colonization and emergence of Diptera. The distribution of Coleoptera among exposure periods was also highly significant ($\chi^2 = 332.51$; $df = 9$; $P < 0.0001$). This result shows that

coprophagous beetles were not evenly distributed during the exposure period and that their occurrence was strongly associated with particular stages of fecal decomposition (Table 20).

Parasitoid emergence also varied significantly among exposure periods ($\chi^2 = 172.05$; $df = 9$; $P < 0.0001$).

The highest parasitoid abundance occurred in feces exposed for 96 hours, followed by those exposed for 24, 120, 72, and 192 hours. This suggests that parasitoid activity depends on the availability of suitable host pupae, which changes according to the development time of Diptera in the fecal substrate (Table 21).

Table 21: Diptera extracted from bovine feces exposed in the field for different periods in Itumbiara, Goiás, Brazil

Taxonomic group	Species	24 h	48 h	72 h	96 h	120 h	Total	Frequency (%)
Muscidae	<i>Brontaea debilis</i>	70	11	7	8	8	151	4.9
	<i>Brontaea quadristigma</i>	0	6	0	0	0	56	1.8
	<i>Cyrtoneurina paraescita</i>	0	0	0	0	2	71	2.3
Sarcophagidae	<i>Ravinia belforti</i>	0	0	0	0	4	7	0.2
	<i>Sarcophagula occidua</i>	108	231	224	107	201	1213	39.1
Sepsidae	<i>Archiseopsis scabra</i>	1	1	0	0	2	29	0.9
	<i>Palaeosepsis</i> spp.	169	44	160	137	114	1558	50.3
Sphaeroceridae	Sphaeroceridae sp.	10	0	0	0	0	14	0.5
Total		358	293	391	252	331	3099	100.0
Taxonomic group		144 h	168 h	192 h	216 h	240 h	Total	Frequency (%)
Muscidae	<i>Brontaea debilis</i>	19	9	3	10	6	151	4.9
	<i>Brontaea quadristigma</i>	2	11	12	16	9	56	1.8
	<i>Cyrtoneurina paraescita</i>	0	8	26	17	18	71	2.3
Sarcophagidae	<i>Ravinia belforti</i>	0	2	0	0	1	7	0.2
	<i>Sarcophagula occidua</i>	56	54	142	13	77	1213	39.1
Sepsidae	<i>Archiseopsis scabra</i>	0	1	4	19	1	29	0.9
	<i>Palaeosepsis</i> spp.	347	98	181	164	144	1558	50.3
Sphaeroceridae	Sphaeroceridae sp.	0	0	4	0	0	14	0.5
Total		424	183	372	239	256	3099	100.0

When all insects were considered together, including Diptera, Coleoptera, and parasitoids, total abundance also differed significantly among exposure periods ($\chi^2 = 329.66$; $df = 9$; $P < 0.0001$). The highest total abundance was recorded at 24 hours, followed by 48, 192, 72, and 144 hours. These results indicate that bovine feces are rapidly colonized after deposition and remain biologically active across several exposure periods.

The abundance of Diptera differed significantly among taxonomic groups ($\chi^2 = 7048.27$; $df = 7$; $P < 0.0001$). *Palaeosepsis* spp. and *S. occidua* were the dominant groups, representing most of the dipteran fauna collected from bovine feces. This pattern indicates that Sarcophagidae and Sepsidae are strongly adapted to dung-breeding environments (Table 22).

Table 22: Parasitoids and associated Coleoptera collected from Diptera pupae in bovine feces in Itumbiara, Goiás, Brazil

Taxonomic group	Family	Taxon	Number of individuals	Frequency (%)
Hymenoptera	Diapriidae	<i>Trichopria</i> sp.	20	4.8
	Figitidae	<i>Kleidotoma nigra</i>	1	0.2
		<i>Neralsia splendens</i>	2	0.4
		<i>Paraganaspis egeria</i>	191	44.4
		<i>Triplasta atrocaxalis</i>	51	11.9
		<i>Triplasta coxalis</i>	5	1.2
	Pteromalidae	<i>Spalangia cameroni</i>	1	0.2
		<i>Spalangia drosophilae</i>	98	22.8
		<i>Spalangia endius</i>	3	0.7
		<i>Spalangia nigra</i>	6	1.4
		<i>Spalangia nigroaenea</i>	14	3.3
		<i>Spalangia</i> sp.	26	6.0
Coleoptera	Staphylinidae	<i>Aleochara notula</i>	12	2.7
Total			430	100.0

The distribution of parasitoids among species was also highly significant ($\chi^2 = 1087.01$; $df = 12$; $P < 0.0001$).

Paraganaspis egeria was the most abundant species, followed by *S. drosophilae* and *T. atrocaxalis*.

This uneven distribution indicates that only a few parasitoid species were responsible for most of the parasitism observed in Diptera pupae. The distribution of Coleoptera among species was highly significant ($\chi^2 = 18854.14$; $df = 13$; $P < 0.0001$), reflecting the strong dominance of *A. aequalis* among the Scarabaeidae. This result shows that Coleoptera communities in bovine feces were also structured by a few highly abundant species.

In the broader comparison among substrates included in the synthesis, parasitism differed significantly among chicken feces, bovine feces, buffalo feces, human feces collected with pitfall traps, fruit substrates, and bovine feces exposed for different periods ($\chi^2 = 453.48$; $df = 5$; $P < 0.0001$). Parasitoid emergence was also not equally distributed among these substrates ($\chi^2 = 718.61$; $df = 5$; $P < 0.0001$). These results confirm that substrate type strongly influenced both host availability and parasitoid occurrence (Table 23).

Table 23: Temporal synthesis of Diptera, Coleoptera, and Hymenoptera parasitoids in bovine feces according to field exposure time

Exposure time (h)	Diptera	Coleoptera	Total reported	Hymenoptera parasitoids from Diptera pupae
24	358	552	973	63
48	293	494	824	37
72	391	300	736	45
96	252	282	638	104
120	331	261	651	59
144	424	247	710	39
168	183	245	437	9
192	372	320	737	45
216	239	284	528	5
240	256	244	524	24
Total	3,099	3,229	6,758	430

Note: Tables were formatted in black and white for insertion into the experimental synthesis article. In Table 4, the total reported corresponds to Diptera + Coleoptera + parasitoids from Diptera pupae. The exposure-time experiment emphasizes that substrate age and temperature may influence oviposition, larval development, pupation, emergence of flies, parasitoid activity, and associated Coleoptera abundance.

Temperature is an important ecological factor influencing the colonization of decomposing organic substrates by flies, parasitoids, Acarina, and Coleoptera. In substrates such as bovine feces, buffalo feces, poultry manure, animal tissues, and fruits, temperature may affect oviposition, egg hatching, larval development, pupation, adult emergence, and parasitoid activity. Therefore, the number of Diptera, parasitoids, coprophagous Coleoptera, and associated mites collected at different exposure periods may reflect not only substrate availability, but also the thermal conditions acting on immature development and adult behavior.

The experiments with bovine feces showed that Acarina, Coleoptera, Diptera, and parasitoids varied according to the exposure time of fecal pats in the field. The highest population peaks occurred mainly during the first and intermediate exposure periods, indicating that fresh and partially decomposed fecal pats were more attractive and biologically active for arthropod colonization. These patterns may be associated with higher moisture, fermentation, odor emission, microbial activity, and temperature conditions favorable to oviposition, larval development, beetle activity, mite occurrence, and parasitoid searching behavior.

Diptera responded strongly to the exposure time of fecal pats. The significant variation among exposure periods indicates that substrate age is an important factor regulating fly colonization. Fresh and intermediate-aged

feces may provide more favorable conditions for oviposition and larval development because they retain moisture, release volatile compounds, and maintain suitable nutritional conditions. The dominance of *Palaeosepsis* spp. and *S. occidua* confirms the importance of Sepsidae and Sarcophagidae in dung-breeding communities. These flies are well adapted to fecal substrates and may reach high abundance in pasture systems.

Coleoptera were also strongly influenced by exposure time. Scarabaeidae were among the most important beetles and likely contributed to the physical transformation of fecal pats. Their activity may reduce the availability of breeding sites for flies by breaking, burying, and aerating the fecal material. Staphylinidae and Histeridae may also be important in this system because many species are associated with predation or with arthropod communities in decomposing substrates. Therefore, Coleoptera may influence Diptera abundance and parasitoid access to host pupae both directly and indirectly.

Acarina also formed part of the arthropod fauna associated with bovine feces. The occurrence of Macrochelidae indicates that fecal pats may function as microhabitats for mites associated with decomposing organic matter and immature stages of Diptera. Although Acarina were less abundant than Diptera and Coleoptera, their presence reinforces the ecological complexity of

bovine feces as a temporary substrate that supports different trophic levels.

Parasitoid emergence was significantly associated with exposure time, indicating that parasitoids respond to temporal changes in host availability. Parasitoids depend on the presence of suitable host larvae or pupae, and these stages occur only during specific periods of Diptera development. Therefore, the temporal pattern of parasitoid emergence reflects the interaction between substrate age, fly development, host availability, and parasitoid searching behavior.

Temperature is also important for parasitoids because host availability depends on the developmental rate of fly larvae and pupae. When temperature accelerates the development of immature Diptera, it may change the period during which hosts are suitable for parasitism. In addition, parasitoid emergence, searching behavior, and reproductive activity may also be affected by thermal variation. Thus, the interaction between substrate age and temperature may help explain differences in parasitism rates among exposure periods.

The importance of temperature is supported by studies on fly biology, such as those involving *F. pusio*, in which egg hatching and immature stages, including larvae and pupae, are strongly dependent on thermal conditions. Although higher temperatures may accelerate immature development, they do not necessarily improve population performance. Therefore, temperature should be considered a key environmental variable in studies of Diptera, parasitoids, Coleoptera,

and Acarina associated with decomposing organic matter.

Among parasitoids, *P. egeria* was the most abundant species, followed by *S. drosophilae* and *T. atrocotalis*. The high frequency of these parasitoids indicates their ecological importance in bovine feces environments. Although parasitism was not uniformly distributed among host species or exposure periods, the presence of several parasitoid taxa suggests that cattle dung can maintain a diverse natural enemy community.

The integration of Acarina, Coleoptera, Diptera, and Hymenoptera parasitoids in the same synthesis strengthens the ecological interpretation of the article. Bovine feces should not be viewed only as a breeding substrate for flies, but as a temporary ecosystem where decomposition, host development, parasitoid activity, beetle colonization, mite occurrence, and environmental factors interact. This broader view helps explain the variation in abundance and parasitism observed among exposure periods.

Overall, the significant chi-square results confirm that arthropod abundance and parasitoid emergence were not random. Exposure time, substrate condition, host availability, decomposition stage, and probably temperature influenced the structure of the community. These findings reinforce the importance of studying decomposing organic substrates as ecological systems that support both pest species and their natural enemies (Figures 26, 27) (Marchiori, 2002; Marchiori, 2003c).



Figure 26: Integrative representation of bovine feces exposed in the field over time, showing adult flies, smaller hymenopterous parasitoids with four wings, coprophagous beetles, larvae, and pupae associated with the substrate. The figure illustrates the ecological succession occurring in cattle dung, highlighting the interaction between host Diptera, parasitoids, and Coleoptera during different stages of decomposition and exposure. This visual synthesis emphasizes the importance of bovine feces as a microhabitat for oviposition, immature development, parasitoid emergence, and beetle activity in pasture environments



Figure 27: Integrative representation of experimental studies on Diptera, parasitoids, and associated substrates in Brazil. The figure shows decomposing organic substrates, field traps, laboratory rearing conditions, adult flies, eggs, larvae, pupae, and hymenopteran parasitoids associated with fly hosts. The parasitoids are represented with four wings, emphasizing their role as natural enemies of Diptera in organic substrates and laboratory systems

5.0. DISCUSSION

Acarina and Coleoptera also formed an important component of the arthropod fauna associated with bovine feces. The occurrence of Histeridae, Macrochelidae, Scarabaeidae, and Staphylinidae indicates that fecal pats function not only as breeding sites for Diptera but also as microhabitats for coprophagous, predatory, and associated arthropods. Coleoptera may contribute to dung fragmentation, burial, aeration, and nutrient recycling, while Acarina may be associated with decomposing organic matter and immature stages of Diptera. Therefore, the presence of these groups reinforces the ecological complexity of bovine feces as a temporary substrate (Marchiori, 1994; Marchiori, 2013; Marchiori, 2014; Marchiori, 2017; Marchiori, 2021; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Host-parasitoid associations were strongly influenced by the type of substrate and by the dipteran host species. Some parasitoids occurred in several substrates, while others showed more restricted associations with specific hosts. This pattern was evident in records involving *G. quadridentata*, *P. egeria*, *P. vindemmiae*, *S. drosophilae*, and other parasitoids associated with muscoid and sarcophagid flies. The significant chi-square results observed in several datasets indicate that parasitoid abundance and parasitism rates were not randomly distributed among hosts or substrates (Marchiori, 1994; Marchiori, 2013; Marchiori, 2014; Marchiori, 2017; Marchiori, 2021; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The high parasitism recorded in some host species, especially in *P. chrysostoma*, demonstrates that parasitoids may exert strong natural pressure on Diptera populations under favorable conditions. However, parasitism varied widely among studies, suggesting that host availability, substrate quality, exposure time, habitat structure, and local environmental conditions may affect parasitoid performance. Therefore, the biological control potential of these parasitoids should be interpreted according to each host-substrate system (Marchiori, 1994; Marchiori, 2013; Marchiori, 2014; Marchiori, 2017; Marchiori, 2021; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The laboratory studies with *F. pusio* showed that temperature is a central factor regulating development, survival, reproduction, and population growth. Egg hatching was reduced at thermal extremes, while intermediate temperatures favored higher viability. Although higher temperatures accelerated immature development, this did not necessarily result in better population performance. The life-table results indicated that 27°C was the most favorable temperature for population increase, whereas 33°C reduced reproductive performance despite shortening development time (Marchiori, 1994; Marchiori, 2013; Marchiori, 2014; Marchiori, 2017; Marchiori, 2021; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Adult emergence in *F. pusio* was concentrated over a short period and occurred mainly during the photophase. This pattern suggests that emergence is synchronized with light conditions, which may favor mating, dispersal, and reproductive activity. The male-biased sex ratio and the significant differences in emergence among days indicate that the species has a defined emergence rhythm under laboratory conditions (Marchiori, 1994; Marchiori, 2013; Marchiori, 2014; Marchiori, 2017; Marchiori, 2021; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Overall, the results show that the biology of Diptera and their parasitoids is shaped by substrate type, host identity, environmental conditions, and temperature. The field studies highlight the diversity and ecological importance of parasitoids in decomposing substrates, while the laboratory studies with *F. pusio* provide detailed information on development, fecundity, longevity, and emergence. Together, these findings contribute to the understanding of Diptera ecology and may support future studies on biological control, forensic entomology, and the management of synanthropic flies.

6.0. CONCLUSION

The experimental studies showed that decomposing organic substrates are important environments for the development of Diptera and for the occurrence of their parasitoids. Animal tissues, carcasses, chicken manure, feces, and fruits supported different host-parasitoid associations, with parasitism varying according to substrate type, host species, exposure time, and environmental conditions. In bovine feces, Acarina, Coleoptera, Diptera, and Hymenopteran parasitoids formed a dynamic arthropod community, showing that fecal pats function as temporary microhabitats for breeding, feeding, predation, decomposition, and natural regulation. The records of parasitoids such as *G. quadridentata*, *P. egeria*, *P. vindemmiae*, *S. drosophilae*, and other taxa reinforce the ecological importance of Hymenoptera as natural enemies of synanthropic flies. In the laboratory, *F. pusio* showed clear biological responses to temperature, with 27°C being the most favorable condition for population growth, fecundity, and life-table performance. Although higher temperatures accelerated development, they did not necessarily improve reproductive success. Overall, the results demonstrate that substrate availability, fecal age, host identity, parasitoid activity, associated arthropod fauna, and temperature are key factors shaping Diptera biology and their natural regulation, contributing to studies on biological control, medical-veterinary entomology, decomposition ecology, and Forensic Entomology.

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