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Taxonomic and Faunistic Synthesis of Hymenopteran Parasitoids Associated with Diptera in Decomposing Organic Substrates in Brazil

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Abstract: This study synthesizes taxonomic and faunistic records of hymenopteran parasitoids associated with Diptera in decomposing organic substrates in Brazil. The focus is on host records, parasitoid families, substrate associations, parasitism rates, and the interpretation of solitary and gregarious parasitoids. The analyzed studies included Diptera and parasitoids collected from bovine feces, buffalo feces, human feces, chicken manure, chicken viscera, bovine kidney, bovine liver, fish, and fruits. Host pupae were obtained mainly by substrate exposure, baited traps, yellow pan traps, Malaise traps, and flotation, followed by individual rearing in gelatin capsules until the emergence of adult flies or parasitoids. The records involved Braconidae, Chalcididae, Diapriidae, Encyrtidae, Figitidae, and Pteromalidae, showing broad taxonomic diversity among parasitoids associated with dipteran hosts. Bovine and buffalo feces were especially important for dung-breeding Diptera, whereas animal-derived substrates and fruits supported additional host–parasitoid associations. Several records expanded known host ranges, substrate associations, and geographic distributions of parasitoids in Brazil. When quantitative data were available, parasitism rates and chi-square tests were used to support interpretation, but first-record and strictly taxonomic notes were treated descriptively. Gregarious parasitoids required special care because the number of emerged individuals may differ from the number of parasitized pupae. Overall, this synthesis highlights the importance of accurate taxonomy, host records, and substrate documentation for understanding parasitoid diversity and natural regulation of Diptera in Brazilian environments.

Keywords: Decomposing Substrates, Diptera, Faunistic Records, Host Records, Hymenoptera, Parasitoids, Taxonomy.

RESEARCH PAPER

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1.0. INTRODUCTION

Diptera associated with decomposing organic matter are important components of terrestrial ecosystems because they participate in nutrient recycling, organic matter decomposition, and energy transfer between trophic levels. However, several species that develop in feces, carrion-like substrates, manure, animal viscera, and fruits are also relevant from sanitary, veterinary, and agricultural perspectives. These flies may occur in livestock systems, rural environments, forest fragments, orchards, and areas modified by human activity, where they use different substrates for larval development (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Among the substrates used by Diptera, bovine feces, buffalo feces, human feces, chicken manure, animal tissues, and decomposing fruits are especially important because they support the development of immature stages and maintain local fly populations. In pasture and corral environments, fecal pats provide favorable conditions for dung-breeding Diptera, including species of Calliphoridae, Fanniidae, Muscidae, Sarcophagidae, Sepsidae, Sphaeroceridae, and Tephritidae. These substrates also attract natural enemies, especially hymenopteran parasitoids, which develop in or on immature stages of Diptera (Marchiori, 2021a; Marchiori, 2021b; 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 associated with organic substrates. Families such as Braconidae, Chalcididae, Diapriidae, Encyrtidae, Figitidae, and Pteromalidae include species that parasitize larvae or pupae of flies. These parasitoids may reduce host emergence and contribute to the natural regulation of synanthropic and economically important Diptera. Because many fly species are associated with disease transmission, livestock disturbance, fruit damage, or organic waste, the study of their parasitoids is relevant for biological control and ecological management (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The host–parasitoid associations recorded in Brazil involve different environments and substrates. Species such as *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae), *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae), *Paraganaspis egeria* Díaz, Gallardo & Walsh, 1996 (Hymenoptera: Figitidae), and *Spalangia nigra* Latreille, 1805 (Hymenoptera: Pteromalidae) and *Zaeucoila incompleta* (Kieffer, 1907) (Hymenoptera: Figitidae) have been recorded in association with dipteran hosts in decomposing substrates. Their occurrence demonstrates the diversity of parasitoid fauna associated with Diptera in Brazilian ecosystems (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Dipteran hosts also show strong ecological variation according to substrate type. Species such as *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae), *Archisepsis scabra* (Loew, 1861) (Diptera: Sepsidae), *Cyrtoneurina pararescita* Couri, 1995 (Diptera: Muscidae), *Peckia chrysostoma (*Wiedemann, 1830) (Diptera: Sarcophagidae), and *Sarcophagula occidua* (Fabricius, 1794) (Diptera: Sarcophagidae), have been reported from feces, animal tissues, or fruits. These hosts are important because they connect decomposing substrates with parasitoid populations and influence the structure of local natural enemy communities (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

In ecological studies involving parasitoids, correct taxonomic identification is essential. The first citation of each species should include the complete scientific name, authority, year, order, and family. Subsequent citations may use the abbreviated genus followed by the specific epithet. To avoid excessive repetition of taxonomic information in the text, the complete list of species cited in this article, including authority, order, family, first citation form, and abbreviated form, is presented in Table 1 (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The available experimental studies show that parasitoid occurrence may vary according to substrate, host species, environment, and exposure period. Some parasitoids show broad host associations, while others appear more restricted to particular hosts or substrates. In addition, gregarious parasitoids require careful interpretation because several individuals may emerge from a single host pupa. Therefore, the number of parasitoid individuals and the number of parasitized pupae must be treated separately when estimating parasitism rates (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

This article aims to synthesize taxonomic and faunistic records of hymenopteran parasitoids associated with Diptera in different organic substrates in Brazil. The study emphasizes parasitoid families, host–parasitoid associations, substrate records, parasitism rates, first records, and the correct interpretation of gregarious parasitoids. This focus distinguishes the article from broader ecological comparisons, because the central objective is to organize host records and parasitoid diversity rather than to compare all environments as a single ecological dataset.

2.0 MATERIALS AND METHODS

2.0.1. General Approach

This study was based on a synthesis of experimental surveys carried out under field and laboratory conditions to investigate Diptera and their hymenopteran parasitoids associated with different organic substrates in Brazil. The analyzed studies included collections performed in pasture, corral, forest, native vegetation, and anthropized environments, using standardized traps, exposed substrates, and laboratory rearing of immature Diptera.

The sampling procedures involved animal-derived substrates and decomposing organic resources, including bovine and buffalo feces, human feces, chicken manure, chicken viscera, bovine kidney, bovine liver, fish, and fruits. These substrates were used either as baits in traps or as breeding media for Diptera. After exposure in the field, the material was taken to the laboratory for the extraction of pupae and emergence of adult flies and parasitoids.

Dipteran pupae were generally obtained by flotation or by separation from sand or substrate residues. The pupae were counted and individually placed in gelatin capsules until the emergence of adult Diptera or parasitoids. Emerged insects were preserved in 70% alcohol and later identified using taxonomic keys and specialized literature.

2.0.2 Experimental Procedures

1. Black Cylindrical Traps with Baits

Adult flies and parasitoids were collected using black cylindrical traps adapted from Ferreira-type traps. These traps were made from dark cans measuring approximately 19 cm in height and 9 cm in diameter. Each trap had two lateral openings in the lower third, allowing insects to enter. A nylon funnel was attached to the upper part of the trap, and a plastic collection bag was fitted externally to retain the captured insects. Different substrates were used as baits, including human feces, bovine kidney, bovine liver, fish, chicken viscera, and fruits. The bait was placed inside the traps over a layer of soil or sand. In most experiments, five traps were used and suspended from trees approximately one meter above the ground, with spacing between traps to reduce interference among sampling units.

The collected insects were transported to the laboratory, killed with ethyl ether when necessary, and preserved in 70% alcohol for later identification. To obtain parasitoids, the remaining trap contents were transferred to plastic containers containing a sand layer suitable for larval pupation. After approximately 15 days, the sand was sieved, and the pupae were removed, counted, and individually placed in gelatin capsules until the emergence of flies or parasitoids.

2. Yellow Pan Traps

Yellow pan traps were used to collect Cynipoidea and other microhymenopterans in pasture and native vegetation. The traps consisted of yellow plastic basins, approximately 30 cm in diameter, placed at ground level and distributed randomly in the sampling area. Each basin contained a solution composed of approximately two liters of water, 2 mL of detergent, and 2 mL of formalin. The yellow color attracted flying insects, which fell into the solution. The material was collected weekly with a fine sieve and preserved in 70% alcohol for later sorting and identification.

3. Malaise Traps

Malaise traps were used to capture flying insects, especially Hymenoptera. These traps consisted of a tent-like mesh structure with a central barrier and a collecting chamber. Insects flying into the mesh were directed toward the upper collecting container, where they were retained in a preservative solution. The traps were installed in field environments such as pasture, forest, or native vegetation and remained active for extended periods. Samples were removed periodically, usually weekly or every two weeks, depending on the study design. The collected insects were preserved and later identified in the laboratory.

4. Bovine and Buffalo Feces

Bovine and buffalo feces were used as substrates for the development of Diptera and their parasitoids. Fresh feces were collected immediately after defecation, homogenized, and placed in circular supports or plastic containers to form standardized fecal pats. The fecal pats were exposed in pasture, corral, or other field environments for defined periods. After exposure, the feces were transported to the laboratory, where pupae were extracted mainly by the flotation method. The recovered pupae were counted and placed individually in gelatin capsules until adult flies or parasitoids emerged.

5. Chicken Manure

Chicken manure was collected from laying hens maintained in cage systems. Fresh feces were placed in containers and kept under controlled or semi-controlled conditions for a defined period, usually around 15 days, to allow larval development and pupation. After exposure, the substrate was processed by flotation to recover pupae. The pupae were counted and individualized in gelatin capsules until the emergence of Diptera or parasitoids.

6. Fruit-Fly Substrates

Fruits used for the collection of Tephritidae and their parasitoids included orange, star fruit, guava, mango, and pitanga. The fruits were placed over a layer of fine sand in transparent plastic containers. The containers were covered with organza secured with an elastic band to prevent contamination by other insects. The pupae were periodically separated from the sand or fruit residues by flotation in water. After removal, the pupae were dried, counted, and kept in glass containers with fine sand at room temperature until the emergence of fruit flies or parasitoids.

7. Flotation Method

The flotation method was used to separate Diptera pupae from feces, manure, fruit residues, or other organic substrates. The substrate was placed in water, allowing heavier debris to sink while pupae floated to the surface. Floating pupae were collected with a fine sieve, counted, and transferred individually to gelatin capsules. This method allowed the recovery of pupae from different breeding substrates and made it possible to estimate parasitism rates based on the emergence of adult flies or parasitoids.

2.0.3 Statistical Analyses

Parasitism rate was calculated as the number of parasitized pupae divided by the total number of pupae collected, multiplied by 100. When the parasitoid was gregarious, special care was taken to distinguish between the number of parasitoid individuals that emerged and the number of parasitized host pupae.

Frequency was calculated as the number of individuals of each taxon divided by the total number of individuals collected, multiplied by 100. The chi-square test was used when applicable to compare distributions among hosts, parasitoid species, substrates, environments, or exposure periods. Statistical significance was considered at the 5% probability level. No chi-square test was applied to strictly taxonomic articles or first-record notes when the available data did not provide suitable quantitative categories for statistical comparison. In such cases, the analysis focused on host records, substrate association, geographic distribution, and taxonomic relevance.

3.0. RESULTS

The analyzed experimental studies showed that decomposing organic substrates supported a diverse assemblage of Diptera and hymenopteran parasitoids in Brazil. To avoid excessive repetition of taxonomic information in the text, the complete scientific names, authorities, orders, families, and abbreviated forms used after the first citation are presented in Table 1. The main substrates recorded in the studies were bovine feces, bovine kidney, bovine liver, buffalo feces, chicken manure, chicken viscera, fish, and fruits.

Table 1: Taxonomic list of hosts Diptera and Hymenoptera parasitoids cited in the article, including authority, order, family, complete first citation, and abbreviated form used after the first mention

Species	Authority	Order	Family	First citation in the text	Subsequent citation
Aganaspis pelleranoi	(Brèthes, 1924)	Hymenoptera	Figitidae	*Aganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae)	*A. pelleranoi*
Anastrepha fraterculus	(Wiedemann, 1830)	Diptera	Tephritidae	*Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae)	*A. fraterculus*
Archisepsis scabra	(Loew, 1861)	Diptera	Sepsidae	*Archisepsis scabra* (Loew, 1861) (Diptera: Sepsidae)	*A. scabra*
Brachymeria podagrica	(Fabricius, 1787)	Hymenoptera	Chalcididae	*Brachymeria podagrica* (Fabricius, 1787) (Hymenoptera: Chalcididae)	*B. podagrica*
Brachymeria sp.		Hymenoptera	Chalcididae	*Brachymeria* sp. (Hymenoptera: Chalcididae)	*Brachymeria* sp.
Brontaea debilis	(Williston, 1896)	Diptera	Muscidae	*Brontaea debilis* (Williston, 1896) (Diptera: Muscidae)	*B. debilis*
Brontaea quadristigma	(Thomson, 1869)	Diptera	Muscidae	*Brontaea quadristigma* (Thomson, 1869) (Diptera: Muscidae)	*B. quadristigma*
Ceratitis capitata	(Wiedemann, 1824)	Diptera	Tephritidae	*Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae)	*C. capitata*
Chrysomya albiceps	(Wiedemann, 1819)	Diptera	Calliphoridae	*Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae)	*C. albiceps*
Chrysomya megacephala	(Fabricius, 1794)	Diptera	Calliphoridae	*Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae)	*C. megacephala*
Cyrtoneurina pararescita	Couri, 1995	Diptera	Muscidae	*Cyrtoneurina pararescita* Couri, 1995 (Diptera: Muscidae)	*C. pararescita*
Doryctobracon areolatus	(Szépligeti, 1911)	Hymenoptera	Braconidae	*Doryctobracon areolatus* (Szepligeti, 1911) (Hymenoptera: Braconidae)	*D. areolatus*
Fannia pusio	(Wiedemann, 1830)	Diptera	Fanniidae	*Fannia pusio* (Wiedemann, 1830) (Diptera: Fanniidae)	*F. pusio*
Haematobia irritans	(Linnaeus, 1758)	Diptera	Muscidae	*Haematobia irritans* (Linnaeus, 1758) (Diptera: Muscidae)	*H. irritans*
Musca domestica	Linnaeus, 1758	Diptera	Muscidae	*Musca domestica* Linnaeus, 1758 (Diptera: Muscidae)	*M. domestica*
Nasonia vitripennis	(Walker, 1836)	Hymenoptera	Pteromalidae	*Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae)	*N. vitripennis*

Species	Authority	Order	Family	First citation in the text	Subsequent citation
Neralsia splendens	(Borgmeier, 1935)	Hymenoptera	Figitidae	*Neralsia splendens* (Borgmeier, 1935) (Hymenoptera: Figitidae)	*N. splendens*
Ophyra aenescens	(Wiedemann, 1830)	Diptera	Muscidae	*Ophyra aenescens* (Wiedemann, 1830) (Diptera: Muscidae)	*O. aenescens*
Oxysarcodexia thornax	(Walker, 1849)	Diptera	Sarcophagidae	*Oxysarcodexia thornax* (Walker, 1849) (Diptera: Sarcophagidae)	*O. thornax*
Pachycrepoideus vindemmiae	(Rondani, 1875)	Hymenoptera	Pteromalidae	*Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae)	*P. vindemmiae*
Paraganaspis egeria	Díaz, Gallardo & Walsh, 1996	Hymenoptera	Figitidae	*Paraganaspis egeria* Díaz, Gallardo & Walsh, 1996 (Hymenoptera: Figitidae)	*P. egeria*
Peckia chrysostoma	(Wiedemann, 1830)	Diptera	Sarcophagidae	*Peckia chrysostoma* (Wiedemann, 1830) (Diptera: Sarcophagidae)	*P. chrysostoma*
Ravinia belforti	(Prado & Fonseca, 1932)	Diptera	Sarcophagidae	*Ravinia belforti* (Prado & Fonseca, 1932) (Diptera: Sarcophagidae)	*R. belforti*
Sarcophagula occidua	(Fabricius, 1794)	Diptera	Sarcophagidae	*Sarcophagula occidua* (Fabricius, 1794) (Diptera: Sarcophagidae)	*S. occidua*
Spalangia cameroni	Perkins, 1910	Hymenoptera	Pteromalidae	*Spalangia cameroni* Perkins, 1910 (Hymenoptera: Pteromalidae)	*S. cameroni*
Spalangia drosophilae	Ashmead, 1887	Hymenoptera	Pteromalidae	*Spalangia drosophilae* Ashmead, 1887 (Hymenoptera: Pteromalidae)	*S. drosophilae*
Spalangia nigra	Latreille, 1805	Hymenoptera	Pteromalidae	*Spalangia nigra* Latreille, 1805 (Hymenoptera: Pteromalidae)	*S. nigra*
Spalangia nigroaenea	Curtis, 1839	Hymenoptera	Pteromalidae	*Spalangia nigroaenea* Curtis, 1839 (Hymenoptera: Pteromalidae)	*S. nigroaenea*
Triplasta atrocoxalis	(Ashmead, 1896)	Hymenoptera	Figitidae	*Triplasta atrocoxalis* (Ashmead, 1896) (Hymenoptera: Figitidae)	*T. atrocoxalis*
Triplasta coxalis	(Ashmead, 1865)	Hymenoptera	Figitidae	*Triplasta coxalis* (Ashmead, 1865) (Hymenoptera: Figitidae)	*T. coxalis*
Zaeucoila incompleta	(Kieffer, 1907)	Hymenoptera	Figitidae	*Zaeucoila incompleta* (Kieffer, 1907) (Hymenoptera: Figitidae)	*Z. incompleta*

Note: The table is organized alphabetically by species. Genus-level records and records originally reported without species identification should be treated separately in the manuscript text to avoid incorrect binomial attribution.

Dung-based substrates were among the most productive systems for muscoid Diptera. Bovine and buffalo feces supported hosts belonging mainly to Calliphoridae, Muscidae, Sarcophagidae, Sepsidae, and Sphaeroceridae. These substrates also yielded parasitoids belonging mainly to Figitidae and Pteromalidae, indicating that livestock environments are important habitats for host–parasitoid interactions associated with decomposing organic matter. In cattle dung pats exposed in pasture, the abundance of flies varied according to the exposure period of the substrate. Sarcophagidae and Sepsidae represented the dominant groups, and the distribution of flies among exposure periods was not uniform. These results indicate that the decomposition stage of fecal pats influenced host availability and contributed to the temporal succession of dipteran communities. The studies with *A. scabra* showed that this host was parasitized in both bovine and buffalo feces.

After the first citation, *A. scabra* was used in the text. Parasitism was higher in bovine feces than in buffalo feces, and the parasitoid assemblage differed between substrates. This pattern indicates that substrate type may influence both host density and parasitoid occurrence. Several parasitoids were recorded from dung-breeding Diptera, including *P. egeria*, *Spalangia drosophilae* Ashmead, 1887 (Hymenoptera: Pteromalidae), *S. nigra*, *Spalangia nigroaenea* Curtis, 1839 (Hymenoptera: Pteromalidae), *Triplasta atrocoxalis* (Ashmead, 1896) (Hymenoptera: Figitidae), and *Triplasta coxalis* (Ashmead, 1865) (Hymenoptera: Figitidae). First records and new host associations contributed strongly to the results. *Zaeucoila incompleta* was recorded in pasture and native vegetation, and *Z. incompleta* showed no significant preference between these environments. *Neralsia splendens* (Borgmeier, 1935) (Hymenoptera: Figitidae) was recorded from *Oxysarcodexia thornax* (Walker, 1849) (Diptera: Sarcophagidae), expanding the known host and habitat association of *N. splendens*. *S. nigra* and *T. coxalis* were also recorded from *C. pararescita,* adding new host associations for these parasitoids in Brazil.

Animal-derived substrates such as bovine kidney, bovine liver, chicken viscera, and fish also supported important parasitoid records. These substrates favored Calliphoridae, Muscidae, and Sarcophagidae hosts and yielded parasitoids such as *Aphaereta* sp. (Hymenoptera: Braconidae), *Brachymeria podagrica* (Fabricius, 1787) (Hymenoptera: Chalcididae), *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae), and *P. vindemmiae*. The occurrence of gregarious parasitoids required careful interpretation of the numerical results. In records involving *N. vitripennis*, the number of emerged parasitoid individuals was not necessarily equivalent to the number of parasitized host pupae. Therefore, when parasitism rates were calculated, the number of parasitized pupae was considered separately from the total number of parasitoid individuals that emerged. Fruit-based substrates supported Tephritidae and their parasitoids. *A. fraterculus* was the main host associated with parasitoid emergence, while *C. capitata* showed lower parasitoid emergence. The principal parasitoids in this system were *Doryctobracon areolatus* (Szepligeti, 1911) (Hymenoptera: Braconidae), *Aganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae), and *P. vindemmiae*.

The chi-square analyses showed significant differences in several ecological comparisons, including host distribution across substrates, parasitoid distribution across host species, fly abundance across fecal exposure periods, and parasitized versus non-parasitized pupae. However, not all comparisons were significant. In some first-record studies, the available data supported only simple comparisons. In contrast, strictly taxonomic studies were not submitted to chi-square analysis because they did not provide suitable abundance categories. Overall, the results indicate that parasitoid diversity and parasitism patterns were influenced by substrate type, host identity, decomposition stage, and sampling method. Dung, animal-derived baits, and fruits each supported distinct host–parasitoid assemblages. The repeated occurrence of Braconidae, Chalcididae, Figitidae, and Pteromalidae shows that these families play an important role in the natural regulation of Diptera associated with decomposing organic matter in Brazil.

3.0.1. Statistical Analysis

The chi-square tests showed significant differences in most ecological comparisons involving parasitoid distribution, host association, substrate preference, and exposure period. In the study with muscoid Diptera collected from cattle dung pats, fly abundance differed significantly among exposure periods ($\chi^2 = 1085.8$; df = 56; $P < 0.0001$). The distribution among species/groups was also significant ($\chi^2 = 7048.27$; df = 7; $P < 0.0001$), as was the distribution among exposure periods ($\chi^2 = 173.88$; df = 9; $P < 0.0001$).

For microhymenopteran parasitoids collected in different substrates in a forest area, parasitoid preference differed significantly among substrates ($\chi^2 = 361.8$; df = 9; $P < 0.0001$) and among hosts ($\chi^2 = 1739.2$; df = 21; $P < 0.0001$). The distribution of parasitoid abundance among species was also unequal ($\chi^2 = 416.64$; df = 3; $P < 0.0001$).

In fruit-fly hosts, the comparison between parasitized and non-parasitized pupae was significant ($\chi^2 = 593.91$; df = 1; $P < 0.0001$). Parasitoid emergence was also significantly higher in *A. fraterculus* than in *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) ($\chi^2 = 33.33$; df = 1; $P < 0.0001$). The article also reported significant host- and parasitoid-preference patterns ($\chi^2 = 27.39$; df = 1; $P < 0.0001$; $\chi^2 = 15.7$; df = 1; $P < 0.0001$).

For *A. scabra*, parasitism differed significantly between bovine and buffalo feces ($\chi^2 = 19.15$; df = 1; $P < 0.0001$). The overall comparison between parasitized and non-parasitized pupae was also significant ($\chi^2 = 384.22$; df = 1; $P < 0.0001$), and parasitoid distribution among species was unequal ($\chi^2 = 18.92$; df = 5; $P = 0.0020$).

Several first-record studies also showed significant differences between parasitized and non-parasitized pupae, including *N. splendens* in *O. thornax* ($\chi^2 = 20.48$; df = 1; $P < 0.0001$), *S. nigra* in *C. pararescita* ($\chi^2 = 25.60$; df = 1; $P < 0.0001$), and *T. coxalis* in *C. pararescita* ($\chi^2 = 13.33$; df = 1; $P < 0.001$). In contrast, *N. vitripennis* in *P. chrysostoma* showed no significant difference because parasitized and non-parasitized pupae occurred in equal numbers ($\chi^2 = 0.00$; df = 1; P = 1.0000).

For *Z. incompleta*, no significant difference was observed between pasture and native vegetation ($\chi^2 = 0.71$; df = 1; P = 0.3980), indicating no clear environmental preference. Strictly taxonomic articles were not submitted to chi-square analysis because they did not provide suitable quantitative abundance data for statistical comparison. In those cases, the analysis focused on diagnostic characters, host records, and geographic distribution.

4.0. EXPERIMENTAL ARTICLES

1. Parasitoids Collected from Animal Feces in Brazil

A total of 6.900 dipteran pupae were recovered from the three substrates analyzed: 2.799 from chicken feces, 628 from bovine feces, and 3.473 from buffalo feces. From these pupae, 754 parasitoids emerged, corresponding to an overall parasitism rate of 10.9%. Chicken feces showed the highest parasitism rate, with 504 parasitoids emerging from 2.799 pupae (18.0%), followed by bovine feces, with 78 parasitoids from 628 pupae (12.4%), and buffalo feces, with 172 parasitoids from 3.473 pupae (5.0%). Host frequency was calculated as the number of pupae of each host divided by the total number of pupae in the same substrate. In contrast, parasitoid frequency was calculated as the number of individuals of each host-parasitoid association divided by the total number of parasitoids in the same substrate.

In chicken feces, host availability was dominated by *Musca domestica* L., 1758 (Diptera: Muscidae), which represented 74.4% of all pupae recovered from this substrate. Parasitoid emergence was also concentrated in this host, mainly through *P. vindemmiae*, which accounted for 68.8% of all parasitoids recorded in chicken feces in association with *M. domestica*. The corrected chi-square test indicated a highly significant association between dipteran hosts and parasitoid species in chicken feces (χ^2= 536.04; df = 36; P < 0.0001) (Table 2).

Table 2: Parasitoids associated with dipteran hosts collected from chicken feces in Brazil

Host species	No. of pupae	Frequency (%)	Parasitoid species	Parasitized pupae	Frequency (%)	Parasitism rate (%)
Chrysomya megacephala	500	17.9	*Nasonia vitripennis*	3	0.6	0.6
			Pachycrepoideus vindemmiae	3	0.6	0.6
			Spalangia endius	3	0.6	0.6
Fannia pusio	42	1.5	*Muscidifurax raptorellus*	2	0.4	4.8
			Pachycrepoideus vindemmiae	2	0.4	4.8
Musca domestica	2.083	74.4	*Muscidifurax raptorellus*	3	0.6	0.1
			Nasonia vitripennis	5	1.0	0.2
			Pachycrepoideus vindemmiae	347	68.8	16.7
			Spalangia endius	67	13.3	3.2
			Spalangia nigra	16	3.2	0.8
			Spalangia nigroaenea	2	0.4	0.1
			Spalangia sp.	15	3.0	0.7
			Tachinaephagus zealandicus	10	2.0	0.5
Palaeosepsis spp.	81	2.9	*Nasonia vitripennis*	2	0.4	2.5
			Pachycrepoideus vindemmiae	8	1.6	9.9
			Spalangia drosophilae	2	0.4	2.5
			Spalangia sp.	10	2.0	12.3
Ornidia obesa	93	3.3	*Pachycrepoideus vindemmiae*	2	0.4	2.2
			Spalangia cameroni	2	0.4	2.2
Total	**2.799**	**100.0**	**-**	**504**	**100.0**	**18.0**

In bovine feces, 78 parasitoids emerged from 628 pupae, producing a corrected total parasitism rate of 12.4%. The most frequent host was *C. pararescita*, representing 24.0% of the pupae, followed by *Palaeosepsis* spp. (Diptera: Sepsidae). (17.0%) and *O. thornax* (11.1%). *Gnathopleura quadridentata* Warton, 1986 (Hymenoptera: Braconidae) represented the highest parasitoid frequency in a single association, accounting for 25.6% of all parasitoids and occurring only in *O. thornax*. The host-parasitoid distribution in bovine feces was statistically significant (χ^2= 250.91; df = 77; P < 0.001) (Table 3).

Table 3: Parasitoids associated with dipteran hosts collected from bovine feces in Brazil

Host species	No. of pupae	Frequency (%)	Parasitoid species	Parasitized pupae	Frequency (%)	Parasitism rate (%)
Archisepsis scabra	40	6.4	*Spalangia drosophilae*	4	5.1	10.0
Brontaea debilis	56	8.9	*Spalangia cameroni*	1	1.3	1.8
			Spalangia nigroaenea	2	2.6	3.6
Brontaea quadristigma	49	7.8	*Kleidotoma nigra*	2	2.6	4.1
			Spalangia cameroni	1	1.3	2.0
			Spalangia drosophilae	1	1.3	2.0
			Spalangia endius	1	1.3	2.0
			Spalangia nigroaenea	5	6.4	10.2
			Spalangia nigra	3	3.8	2.0
			Spalangia nigroaenea	5	6.4	3.3
Chrysomya megacephala	51	8.1		-	-	-
Musca domestica	10	1.6	*Spalangia cameroni*	1	1.3	10.0
Oxysarcodexia thornax	70	11.1	*Gnathopleura quadridentata*	20	25.6	28.6
Palaeosepsis spp.	107	17.0	*Paraganaspis egeria*	2	2.6	1.9
			Spalangia drosophilae	2	2.6	1.9
			Spalangia endius	1	1.3	0.9
			Triplasta atrocoxalis	2	2.6	1.9
			Triplasta coxalis	8	10.3	7.5
			Trichopria sp.	1	1.3	0.9
Ravinia belforti	63	10.0		-	-	-
Sarcophagula occidua	31	4.9	*Pachycrepoideus vindemmiae*	5	6.4	16.1
			Spalangia cameroni	1	1.3	3.2
			Spalangia nigra	4	5.1	12.9
			Spalangia nigroaenea	6	7.7	19.4
Total	**628**	**100.0**	**-**	**78**	**100.0**	**12.4**

In buffalo feces, 172 parasitoids emerged from 3.473 pupae, resulting in the lowest corrected total parasitism rate among the substrates (5.0%). *Palaeosepsis* spp. was the dominant host, representing 56.1% of all pupae, followed by *S. occidua* with 26.8%. The highest parasitoid frequency in a single association was observed for *S. drosophilae* in *S. occidua*, corresponding to 19.2% of all parasitoids recovered from buffalo feces. The corrected chi-square test also showed a significant association between hosts and parasitoids in buffalo feces (χ^2= 146.21; df = 45; P < 0.001) (Table 4).

Table 4: Parasitoids associated with dipteran hosts collected from buffalo feces in Brazil

Host species	No. of pupae	Frequency (%)	Parasitoid species	Parasitized Pupae	Frequency (%)	Parasitism rate (%)
Archisepsis scabra	310	8.9	*Paraganaspis egeria*	5	2.9	1.6
			Spalangia drosophilae	3	1.7	1.0
			Trichopria sp.	1	0.6	0.3
Brontaea debilis			*Spalangia cameroni*	2	1.2	1.6
			Spalangia nigroaenea	1	0.6	0.8
Cyrtoneurina pararescita	19	0.5	*Kleidotoma nigra*	6	3.5	0.3
			Paraganaspis egeria	12	7.0	0.6
			Spalangia cameroni	4	2.3	0.2
			Spalangia drosophilae	1	0.6	0.1
			Spalangia nigra	17	9.4	0.8
			Spalangia nigroaenea	1	0.6	5.3
			Triplasta atrocoxalis	19	11.0	1.0
			Triplasta coxalis	8	4.7	0.4

Host species	No. of pupae	Frequency (%)	Parasitoid species	Parasitized Pupae	Frequency (%)	Parasitism rate (%)
			Trichopria sp.	9	5.2	0.5
Sarcophagula occidua	931	26.8	*Paraganaspis egeria*	11	6.4	1.2
			Spalangia cameroni	8	4.7	0.9
			Spalangia drosophilae	33	19.2	3.5
			Spalangia endius	4	2.3	0.4
			Spalangia nigroaenea	11	6.4	1.2
			Trichopria sp.	16	9.3	1.7
Total	**3.473**	**100.0**	**-**	**172**	**100.0**	**5.0**

The comparison among substrates shows that chicken feces favored the highest parasitoid emergence and the highest overall parasitism rate. In contrast, buffalo feces supported the largest number of pupae but the lowest proportional parasitism. The inclusion of host and parasitoid frequencies clarifies that the dominant host was not always the substrate with the highest parasitism. Thus, host density, parasitoid preference, and substrate conditions acted together to determine parasitoid emergence. These findings reinforce the ecological importance of parasitoids in the natural regulation of synanthropic Diptera and support their relevance in biological control programs (Table 5).

Table 5: Comparative summary of parasitoid emergence, host frequency, and dominant parasitoids across the three substrates

Substrates	Total pupae	No. Parasitoids	Parasitism rate (%)	Most frequent host and frequency	Dominant parasitoid association and frequency	Chi-square (χ^2)
Bovine feces	628	78	12.4	*Cyrtoneurina pararescita*: 151 pupae (24.0%)	*Gnathopleura quadridentata* in *Oxysarcodexia thornax*: 20 (25.6%)	250.91; df = 77; P < 0.001
Buffalo feces	3.473	172	5.0	*Palaeosepsis* spp.: 1.948 pupae (56.1%)	*Spalangia drosophilae* in *Sarcophagula occidua*: 33 (19.2%)	146.21; df = 45; P < 0.001
Chicken feces	2.799	504	18.0	*Musca domestica*: 2,083 pupae (74.4%)	*Pachycrepoideus vindemmiae* in *Musca domestic*a: 347 (68.8%)	536.04; df = 36; P < 0.001
Total/overall	**6.900**	**754**	**10.9**	**-**	**-**	**-**

Numerical review confirmed the consistency of the main totals: 2.799 + 628 + 3,473 = 6.900 pupae and 504 + 78 + 172 = 754 parasitoids. The total parasitism rates were recalculated from the corrected totals as 18.0% for chicken feces, 12.4% for bovine feces, and 5.0% for buffalo feces. The general parasitism rate across all substrates was 10.9% (Figure 1) (Marchiori, 2021a).

Figure 1: Schematic illustration of parasitoid–dipteran associations in chicken, bovine, and buffalo feces in Brazil. The figure shows animal feces as larval substrates for dipteran development, with pupae exposed to parasitism by hymenopteran parasitoids. This integrative representation highlights the ecological importance of parasitoids in the natural regulation of synanthropic flies

2. *Eurytoma* sp. (Hymenoptera: Eurytomidae) as a Parasitoid of *Fannia Pusio* (Wiedemann, 1830) (Diptera: Fanniidae)

A total of 153 pupae of *F. pusio* were collected from chicken dung in Itumbiara, Goiás, Brazil. From these pupae, two specimens of *Eurytoma* sp. emerged, corresponding to a parasitism rate of 1.3%. The remaining 151 pupae were not parasitized and produced adult flies or did not yield parasitoids. This record represents the first report of *Eurytoma* sp. parasitizing pupae of *F. pusio* in Brazil. The chi-square test comparing parasitized and non-parasitized pupae showed a highly significant difference ($\chi^2 = 145.10$; df = 1; $P < 0.0001$). This result indicates that non-parasitized pupae predominated strongly in the sample. Nevertheless, the emergence of *Eurytoma* sp. confirms the association between this parasitoid and *F. pusio* under the environmental and substrate conditions evaluated in chicken dung.

The occurrence of *Eurytoma* sp. in pupae of *F. pusio* expands the knowledge of host–parasitoid associations involving synanthropic Diptera in Brazil. Although the parasitism rate was low, at 1.3%, the record is relevant because it documents a new association between a pteromalid parasitoid and a Fanniidae host developing in poultry manure. Chicken dung is an important breeding substrate for muscoid flies, especially in poultry production systems, and may favor the development of fly populations when manure accumulation is not properly managed (Figure 2).

Figure 2: Morphological features of a Eurytomidae parasitoid, showing the body in lateral view, the head in front lateral and dorsal views, the fore wing, the antenna, the metasoma in dorsal view, and the mesosoma in dorsal view. The figure highlights the main external structures useful for recognition of the parasitoid. This schematic presentation provides a clear visual summary of diagnostic body regions in Hymenoptera: Sources: Christopher Taylor and Josef Dvořák/http

The low parasitism rate observed in this study may be related to several ecological factors, including host density, availability of exposed pupae, moisture, the physical structure of the substrate, seasonal conditions, and the parasitoid's searching efficiency. Even with only two parasitoids recovered, the record is biologically important because the first host records help clarify the host range, distribution, and ecological plasticity of parasitoids associated with flies of sanitary and veterinary importance.

The significant chi-square value mainly reflects the numerical predominance of non-parasitized pupae in the sample. However, from an ecological perspective, the detection of *Eurytoma* sp. emerging from *F. pusio* pupae demonstrates that this parasitoid can exploit this host under field conditions. This finding contributes to the knowledge of natural enemies associated with fly pupae in animal manure. It reinforces the importance of surveys in livestock and poultry environments for identifying parasitoids that may be relevant to biological control programs (Marchiori, 2007).

3. Braconidae (Hymenoptera: Ichneumonoidea) Collected in a Native Forest Area in Itumbiara, Goiás, Brazil

A total of 49 specimens of Braconidae were collected with Malaise traps in a native forest fragment in Itumbiara, Goiás, Brazil. The specimens were distributed among 19 genera and 10 subfamilies. Cheloninae was the most abundant subfamily, represented by *Chelonus* sp., with 17 specimens and a frequency of 34.7%. Other frequent genera were *Bentonia* sp. with six specimens (12.2%), Braconidae sp. with five specimens (10.2%), and *Coccygidium* sp. with four specimens (8.2%). Most genera were represented by only one specimen, accounting for 2.0% of the total collected. This pattern indicates a community with few dominant taxa and several rare genera. The chi-square test confirmed that the distribution among genera was significantly uneven ($\chi^2 = 101.06$; df = 18; $P < 0.0001$) (Table 6).

Table 6: Braconidae collected with Malaise traps in a native forest area in Itumbiara, Goiás, Brazil

Taxonomic group	**Parasitoids species**	**Number of individuals**	**Frequency (%)**
Agathidinae	*Coccygidium* sp.	4	8.2
Cardiochilinae	*Toxoneuron* sp.	1	2.0
Cheloninae	*Chelonus* sp.	17	34.7
Doryctinae	*Heterospilus* sp.	1	2.0
	Megaloproctus sp.	1	2.0
	Moneuron sp.	1	2.0
	Notiospathius sp.	1	2.0
	Spathius sp.	1	2.0
Euphorinae	*Leiophron* sp.	1	2.0
Macrocentrinae	*Bentonia* sp.	6	12.2
	Distatrix sp.	1	2.0
	Diolcogaster sp.	1	2.0
	Dolichozele sp.	1	2.0
	Hypomicrogaster sp.	1	2.0
	Macrocentrus sp.	5	10.2
Opiinae	*Opius* sp.	1	2.0
	Stantonia sp.	2	4.1
Rogadinae	*Aleiodes* sp.	2	4.1
	Rogas sp.	1	2.0
Total	**—**	**49**	**100.0**

Regarding monthly occurrence, Braconidae were collected in almost all months except December. The highest frequency occurred in June, with 12 specimens, followed by March, with 10 specimens, April, with six specimens, and August, with five specimens. The monthly distribution was also statistically significant ($\chi^2 = 35.98$; df = 11; $P < 0.001$), showing that the occurrence of Braconidae varied throughout the year.

The predominance of *Chelonus* sp. indicates that Cheloninae was the most representative group of Braconidae in the sampled native forest fragment. This genus is widely recognized as a parasitoid of Lepidoptera and is frequently associated with hosts that occur in natural and agricultural environments. Its high frequency may reflect the availability of suitable hosts in the studied area, as well as favorable environmental conditions for parasitoid activity.

The presence of 19 genera in only 49 specimens suggests considerable taxonomic diversity, even with relatively low abundance. The occurrence of many genera represented by a single specimen may indicate a complex parasitoid assemblage, with several taxa occurring at low density. This pattern is common in parasitoid communities, especially in heterogeneous environments such as forest fragments, where microhabitats and host resources may vary spatially and seasonally.

The significant monthly variation suggests that Braconidae activity was not constant throughout the year. The highest abundance in March and June may be related to climatic conditions, host availability, vegetation structure, or seasonal changes in insect populations. The absence of specimens in December and the low number in September indicate periods of reduced parasitoid activity or lower capture efficiency (Table 7).

Table 7: Monthly frequency of Braconidae collected with Malaise traps in a native forest area in Itumbiara, Goiás, Brazil

Taxonomic group	**Taxonomic group**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**	**Total**	**Frequency (%)**
Agathidinae	*Coccygidium* sp.	0	0	2	1	0	1	0	0	0	0	0	0	4	8.2
Cardiochilinae	*Toxoneuron* sp.	0	0	0	1	0	0	0	0	0	0	0	0	1	2.0
Cheloninae	*Chelonus* sp.	2	0	7	1	0	4	0	1	0	2	0	0	17	34.7
Doryctinae	*Heterospilus* sp.	0	0	0	0	0	1	0	0	0	0	0	0	1	2.0
	Megaloproctus sp.	0	0	0	0	0	0	0	0	0	0	1	0	1	2.0
	Moneuron sp.	0	0	0	0	0	0	0	1	0	0	0	0	1	2.0
	Notiospathius sp.	0	0	0	0	0	1	0	0	0	0	0	0	1	2.0

Taxonomic group	Taxonomic group	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total	Frequency (%)
	Spathius sp.	0	0	0	0	0	1	0	0	0	0	0	0	1	2.0
Euphorinae	*Leiophron* sp.	0	0	0	0	1	0	0	0	0	0	0	0	1	2.0
Macrocentrinae	*Dolichozele* sp.	0	0	0	0	0	1	0	0	0	0	0	0	1	2.0
	Macrocentrus sp.	0	0	0	1	0	1	2	1	0	0	0	0	5	10.2
	Diolcogaster sp.	0	0	0	0	0	0	0	0	0	0	1	0	1	2.0
	Distatrix sp.	0	0	0	0	0	0	0	0	0	0	1	0	1	2.0
	Hypomicrogaster sp.	0	0	0	1	0	0	0	0	0	0	0	0	1	2.0
Opiinae	*Opius* sp.	0	0	0	0	0	0	0	1	0	0	0	0	1	2.0
	Bentonia sp.	2	2	0	0	1	1	0	0	0	0	0	0	6	12.2
	Stantonia sp.	0	0	0	0	0	1	0	0	1	0	0	0	2	4.1
Rogadinae	*Aleiodes* sp.	0	0	1	1	0	0	0	0	0	0	0	0	2	4.1
	Rogas sp.	0	0	0	0	0	0	0	1	0	0	0	0	1	2.0
Total	—	**4**	**2**	**10**	**6**	**2**	**12**	**2**	**5**	**1**	**2**	**3**	**0**	**49**	**100.0**

These results reinforce the ecological importance of native forest fragments as reservoirs of parasitoid diversity. Braconidae include many species with potential relevance in natural biological control, and their occurrence in preserved or semi-preserved habitats contributes to the maintenance of ecological interactions involving herbivorous insects and their natural enemies. Therefore, surveys using Malaise traps are important tools for documenting parasitoid diversity and understanding seasonal patterns of occurrence in tropical environments (Figure 3) (Marchiori and Penteado-Dias, 2002).

Figure 3: Adult specimen of *Chelonus* sp. associated with Malaise trap sampling of Braconidae in a native forest fragment. The image illustrates the dominant braconid genus recorded in the survey and the passive interception method used for parasitoid collection. This visual synthesis emphasizes the relevance of Malaise traps for documenting parasitoid diversity in forest environments

4. *Brontaea Debilis* (Thomson, 1896) (Diptera: Cyclorrhapha: Muscidae) as Host for *S. Drosophilae* in Brazil

A total of 92 pupae of *B. debilis* were obtained from bovine feces exposed in pastures at the Federal University of Goiás farm, Goiânia, Goiás, Brazil. From these pupae, 31 specimens of *S. drosophilae* emerged, corresponding to a parasitism rate of 33.7%. The remaining 61 pupae were not parasitized. The chi-square test showed a significant difference between parasitized and non-parasitized pupae ($\chi^2 = 9.78$; df = 1; $P < 0.01$). Although non-parasitized pupae predominated, the emergence of 31 parasitoids indicates a strong association between *S. drosophilae* and *B. debilis* under the sampled field conditions.

The record of *S. drosophilae* parasitizing pupae of *B. debilis* in bovine feces expands the knowledge of host–parasitoid associations involving muscid flies in Brazil. This association is ecologically relevant because *B. debilis* develops in animal feces, a substrate commonly associated with synanthropic flies of sanitary and veterinary importance.

The parasitism rate of 33.7% can be considered expressive, especially under field conditions, where parasitoid activity may be influenced by substrate exposure, host density, microclimatic conditions, and the capacity of the parasitoid to locate buried or protected pupae. The emergence of *S. drosophilae* from nearly one-third of the recovered pupae suggests that this parasitoid may play an important role in the natural regulation of *B. debilis* populations in cattle-associated environments (Figure 4).

Figure 4: Schematic illustration of *B. debilis* and its parasitoid *S. drosophilae* associated with bovine feces. The figure shows the fly, the parasitoid with four wings, the dipteran pupa, and bovine feces as the substrate where host development and parasitism occur. This integrative representation highlights the host–parasitoid association recorded in Brazil and its ecological relevance in cattle manure environments

The significant chi-square result reflects the numerical difference between parasitized and non-parasitized pupae. However, from a biological perspective, the presence of 31 parasitoids is highly relevant because it confirms the use of *B debilis* as a host by *S. drosophilae*. The article also notes that *B. debilis* had previously been recorded as a host for other parasitoids, but not for *S. drosophilae*, making this a new host–parasitoid record in Brazil.

These findings reinforce the importance of surveys in livestock systems, especially in bovine feces, for identifying natural enemies of flies. Parasitoids of the genus *Spalangia* Latreille, 1805, are important pupal parasitoids and may contribute to biological control programs aimed at reducing fly populations in animal production environments (Marchiori, 2021b).

5. Occurrence of *Brachymeria* sp as a Parasitoid of Diptera Collected in Different Substrata in Brazil

A total of 1.355 dipteran pupae were collected from five different substrates in Itumbiara, Goiás, Brazil: cattle kidney, cattle liver, chicken viscera, fish, and human feces. From these pupae, 188 specimens of *Brachymeria* sp**.** emerged, corresponding to an overall parasitism rate of 13.9%. Cattle kidneys attracted the highest number of *Brachymeria* sp.**,** with 75 parasitoids, representing 39.9% of the total parasitoids collected. In this substrate, the parasitoid emerged from pupae of *Hemilucilia segmentaria* (Fabricius, 1805) (Diptera: Calliphoridae), *O. thornax*, and *P. chrysostoma*. Chicken viscera were the second most productive substrate, with 62 parasitoids, mainly associated with *P. chrysostoma* and *Chrysomya* Robineau-Desvoidy 1830 (Diptera: Calliphoridae).

The highest host-specific parasitism rate was recorded in cattle liver, where 21 parasitoids emerged from 45 pupae of *P. chrysostoma*, resulting in a parasitism rate of 46.6%. In chicken viscera, *Chrysomya* spp. showed a parasitism rate of 45.0%, with nine parasitoids emerging from 20 pupae. Human feces also supported parasitoid emergence, especially from *P. chrysostoma* and *S. lamben*s.

The chi-square test indicated a significant preference of *Brachymeria* sp. among substrates (χ^2 = 2196.42; df = 24; $P < 0.0001$). In cattle kidneys, the parasitoid showed preference for *O. thornax* and *P. chrysostoma* (χ^2 = 352.20; df = 16; $P < 0.0001$). In chicken viscera, preference was recorded for *Chrysomya* spp. and *P. chrysostoma* (χ^2 = 149.70; df = 15; $P < 0.0001$). The comparison between parasitized and non-parasitized pupae was also significant (χ^2 = 707.16; df = 1; $P < 0.0001$), reflecting the predominance of non-parasitized pupae in the total sample.

The occurrence of *Brachymeria* sp. in different substrates demonstrates its ecological flexibility and its capacity to exploit dipteran hosts developing in decomposing organic matter. The parasitoid was recorded in cattle kidneys, chicken viscera, human feces, fish, and cattle liver, indicating that it can locate hosts across substrates with different physical and nutritional characteristics (Table 8).

Table 8: Hosts of *Brachymeria* sp. collected in an urban area using different substrates, Brazil

Substrates	Number of individuals	Host species	Host pupae (N)	Parasitism rate (%)
Cattle liver	21	*Peckia chrysostoma*	45	46.6
Cattle kidneys	3	*Hemilucilia segmentaria*	43	7.0
	11	*Oxysarcodexia thornax*	73	15.0
	61	*Peckia chrysostoma*	553	11.0
Chicken viscera	9	*Chrysomya* spp.	20	45.0
	2	*Oxysarcodexia thornax*	19	10.5
	51	*Peckia chrysostoma*	280	18.2
Fish	1	*Oxysarcodexia thornax*	39	2.6
Human feces	1	*Ophyra aenescens*	26	3.8
	1	*Oxysarcodexia thornax*	109	0.9
	19	*Peckia chrysostoma*	106	17.9
	8	*Sarcodexia lambens*	42	19.0
Total	**188**	**-**	**1.355**	**13.9**

Note: Total parasitism rate was recalculated as 188/1,355 x 100 = 13.9%.

The highest number of parasitoids was obtained from cattle kidneys, suggesting that this substrate may provide favorable conditions for host development and parasitoid activity. The associations with *O. thornax* and *P. chrysostoma* were especially important in this substrate. These flies belong to Sarcophagidae, a family frequently associated with decomposing organic material and of sanitary relevance. Although cattle liver produced fewer pupae, it showed the highest parasitism rate, with 46.6% in *P. chrysostoma*.

This result suggests that parasitism rate is not determined only by the total number of available hosts, but also by host suitability, substrate attractiveness, host exposure, and searching efficiency of the parasitoid. Chicken viscera also showed high parasitism, especially in *Chrysomya* spp. reinforcing the importance of animal-derived substrates for parasitoid–host interactions.

The significant chi-square results confirm that the distribution of *B. podagrica* was not random among substrates or hosts. These results support the interpretation that the parasitoid shows a preference for specific host–substrate combinations. From an ecological and applied perspective, this is relevant because parasitoids associated with synanthropic flies may contribute to the natural regulation of fly populations in urban, periurban, and livestock-associated environments (Figure 5).

Figure 5: Schematic representation of *Brachymeria* sp. associated with metallic synanthropic flies attracted to animal-derived substrates. The figure shows the parasitoid with enlarged flattened hind coxae, four wings without visible venation, and flies near the baited black cylindrical trap. This visual synthesis highlights the ecological association between parasitoids, decomposing substrates, and dipteran hosts in urban environments

The record of *Brachymeria* sp. /in several dipteran hosts reinforces the importance of surveys using decomposing substrates to identify natural enemies with potential value for biological control. Because many of the recorded hosts are flies of medical and veterinary importance, the presence of this parasitoid may contribute to integrated strategies aimed at reducing populations of synanthropic Diptera (Marchiori *et al.*, 2003a).

6. New Habitat for *Trichopria* sp. (Hymenoptera: Diapriidae) in Brazil

A total of 929 pupae of *S. occidua* were obtained from buffalo dung exposed in pastures in Itumbiara, Goiás, Brazil. From these pupae, 13 specimens of *Trichopria* sp. emerged, corresponding to a parasitism rate of 1.4%. The remaining 916 pupae were not parasitized. The chi-square test showed a highly significant difference between parasitized and non-parasitized pupae (χ^2 = 877.73; df = 1; P < 0.0001), indicating a strong predominance of non-parasitized hosts in the sample. Nevertheless, the emergence of *Trichopria* sp. from *S. occidua* confirms the occurrence of this parasitoid in buffalo dung (Figure 6).

Figure 6: Schematic illustration of the association between Diptera and a diapriid parasitoid in bovine feces. The image shows the dipteran host *S. occidua* (a) and the parasitoid *Trichopria* sp. (b) near the fecal substrate, representing the ecological environment where host development and parasitism occur. This visual synthesis highlights the role of animal feces as a habitat for synanthropic flies and their natural enemies in Brazil

The occurrence of *Trichopria* sp. in pupae of *S occidua* collected from buffalo dung represents an important ecological record for Brazil. Although the parasitism rate was low, at 1.4%, the finding is relevant because it reports a new habitat for this diapriid parasitoid. Buffalo dung, therefore, appears as an additional substrate supporting host–parasitoid interactions involving synanthropic Diptera. The low parasitism rate may be associated with variation in food resources, host density, substrate quality, exposure time, and the ability of the parasitoid to locate suitable puparia in the dung environment. Even with low emergence, the record suggests that *Trichopria* sp. can exploit *S. occidua* under field conditions.

The significant chi-square result mainly reflects the predominance of non-parasitized pupae. However, from a biological perspective, the detection of *Trichopria* sp. in buffalo dung is important because it expands knowledge of the ecological distribution and habitat use of this parasitoid. These findings reinforce the importance of surveys in animal feces, especially buffalo dung, for documenting natural enemies of flies with sanitary and veterinary relevance (Marchiori *et al.,* 2004a).

7. Species of Diptera of Medical, Sanitary and Veterinary Importance Collected from Buffalo and Cattle Dung in South Goiás, Brazil.

A total of 3.473 dipteran specimens were collected from buffalo dung, and 628 from cattle dung, in southern Goiás, Brazil. In buffalo dung, the specimens belonged to three families and six species, whereas cattle dung yielded four families and ten species. In buffalo dung, *Palaeosepsis* spp. was the dominant taxon, with 1.948 specimens and a frequency of 56.1%, followed by *S. occidua* with 931 specimens (26.8%) and *A. scabra* with 310 specimens (8.9%).

In cattle dung, *C. pararescita* was the most frequent species, with 151 specimens and a frequency of 24.0%, followed by *Palaeosepsis* spp. with 107 specimens (17.0%), *O. thornax* with 70 specimens (11.1%), and *R. belforti* with 63 specimens (10.0%). Species of medical and veterinary importance, such as *C. megacephala* and *M. domestica*, were recorded only in cattle dung in this survey (Table 9).

Table 9: Dipteran species collected from buffalo dung in southern Goiás, Brazil

Taxonomic group	Diptera species	Number of specimens collected	Frequency (%)
Muscidae	*Brontaea debilis*	127	3.7
	Brontaea quadristigma	138	4.0
	Cyrtoneurina pararescita	19	0.5
Sarcophagidae	*Sarcophagula occidua*	931	26.8
Sepsidae	*Archisepsis scabra*	310	8.9
	Palaeosepsis spp.	1.948	56.1
Total	**—**	**3.473**	**100.0**

The comparison between buffalo and cattle dung showed a highly significant difference in species distribution (χ^2 = 2105.69; df = 9; P < 0.0001). The goodness-of-fit tests also showed non-uniform distributions within each substrate, with strong dominance patterns in buffalo dung (χ^2 = 4807.62; df = 5; P < 0.0001) and cattle dung (χ^2 = 230.57; df = 9; P < 0.0001) (Table 10).

Table 10: Dipteran species collected from cattle dung in southern Goiás, Brazil

Taxonomic group	Dipteran species	Number of specimens collected	Frequency (%)
Calliphoridae	*Chrysomya megacephala*	51	8.1
Muscidae	*Brontaea debilis*	56	8.9
	Brontaea quadristigma	49	7.8
	Cyrtoneurina pararescita	151	24.0
	Musca domestica	10	1.6
Sarcophagidae	*Oxysarcodexia thornax*	70	11.1
	Ravinia belforti	63	10.0
	Sarcophagula occidua	31	4.9
Sepsidae	*Archisepsis scabra*	40	6.4
	Palaeosepsis spp.	107	17.0
Total	**—**	**628**	**100.0**

The results demonstrate that buffalo and cattle dung supported distinct dipteran assemblages. Buffalo dung was characterized by the strong dominance of *Palaeosepsis* spp. and *S. occidua*, indicating that this substrate favored species commonly associated with decomposing fecal material. In contrast, cattle dung showed a more balanced composition and included species of high medical, sanitary, and veterinary relevance, such as *C. megacephala* and *M. domestica*.

The significant chi-square result confirms that the distribution of species differed markedly between substrates. This difference may be related to substrate structure, moisture, nutritional composition, exposure conditions, and host preferences. The high abundance of Sepsidae in buffalo dung suggests that this substrate may provide favorable conditions for their development. In contrast, cattle dung supported a broader set of muscoid and Sarcophagidae flies.

From an applied perspective, the occurrence of dipteran species in both buffalo and cattle dung is important because several taxa are associated with synanthropic environments and may act as mechanical vectors of pathogens. Knowledge of the species composition in manure substrates contributes to epidemiological studies and to the development of more effective monitoring and control strategies in livestock systems (Figure 7) (Marchiori, 2014).

Figure 7: Schematic illustration of dipteran flies and hymenopteran parasitoids associated with buffalo and cattle dung in southern Goiás, Brazil. The figure shows adult flies on the fecal substrate and parasitoids with four wings, highlighting the ecological association between dung-breeding Diptera and their natural enemies. This integrative representation emphasizes the importance of buffalo and cattle dung as habitats for insects of medical, sanitary, and veterinary relevance

8. First Occurrence of the Parasitoid *K. nigra* in Pupae of *B. Quadristigma* in Cattle Dung in Brazil

A total of 210 puparia of *B. quadristigma* were collected from bovine feces in Panamá, Goiás, Brazil. From these puparia, 21 specimens of *K. nigra* emerged, while 189 pupae were not parasitized. Based on the values reported in the article, the corrected parasitism rate was 10.0%. The chi-square test showed a highly significant difference between parasitized and non-parasitized puparia (χ^2 = 134.40; df = 1; P < 0.0001), indicating a strong predominance of non-parasitized hosts. Nevertheless, the emergence of *K. nigra* confirms its association with *B. quadristigma* in bovine feces under field conditions (Figure 8) (van Noort, 2026).

Figure 8: *Kleidotoma nigra* associated with dipteran pupae in decomposing organic substrates in Brazil. The illustration shows the adult figitid parasitoid in detail, highlighting its general morphology and its role as a natural enemy of Diptera. This figure summarizes the occurrence of *K. nigra* as a parasitoid associated with substrates rich in organic matter. Sources: 10.11646/zootaxa. 3878.6.4 and https://commons.wikimedia.org/wiki/Category:Kleidotoma

The occurrence of *K. nigra* in puparia of *B. quadristigma* represents an important host–parasitoid record in Brazil. This finding expands the known biological associations of Eucoilinae parasitoids with muscoid Diptera developing in bovine feces. Although the majority of pupae were not parasitized, the recovery of 21 parasitoids demonstrates that *K. nigra* can exploit *B. quadristigma* as a host in cattle dung environments.

The corrected parasitism rate of 10.0% indicates moderate parasitoid activity in the sampled substrate. This value may reflect host availability, exposure time of fecal pats, microclimatic conditions, substrate quality, and the capacity of the parasitoid to locate suitable host pupae. The significant chi-square result mainly reflects the numerical dominance of non-parasitized pupae, but biologically, the record remains relevant because it documents a new association between the parasitoid and its dipteran host.

Bovine feces are important substrates for the development of synanthropic Diptera, including species of medical, veterinary, and sanitary relevance. Therefore, records of parasitoids associated with these hosts contribute to the understanding of natural biological regulation in livestock systems. The detection of *K. nigra* reinforces the ecological importance of Eucoilinae as natural enemies of dung-breeding flies and supports the need for additional surveys in cattle-associated environments (Marchiori *et al.,* 2004b).

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

A total of 695 dipteran pupae were collected from chicken viscera in Itumbiara, Goiás, Brazil. From these pupae, 145 parasitoid specimens emerged, corresponding to an overall parasitism prevalence of 20.0% as reported in the article. The parasitoid assemblage was dominated by *Hemencyrtus* sp., with 75 specimens, representing 52.0% of the parasitoids collected, followed by *B. podagrica*, with 62 specimens, corresponding to 42.8%.

The remaining parasitoids were less frequent. *Nasonia vitripennis* accounted for six specimens, and *P. egeria* was represented by two specimens. The distribution of parasitoids among taxa was highly significant (χ^2 = 117.32; df = 3; P < 0.0001), indicating strong predominance of *Hemencyrtus* sp. and *B. podagrica* in chicken viscera. The comparison between parasitoid emergence and non-parasitized pupae was also significant (χ^2 = 236.01; df = 1; P < 0.0001), reflecting the predominance of pupae from which no parasitoids emerged. However, the recovery of 145 parasitoids confirms that chicken viscera supported an important host–parasitoid community under the sampled field conditions (Tables 11-12).

Table 11: Reconstructed summary of parasitoid species collected from dipteran pupae in chicken viscera in Goiás

Taxonomic group	Parasitoid species	Number of specimens	Frequency (%)	Parasitism rate (%)
Encyrtidae	*Hemencyrtus* sp.	75	52.0	10.8
Chalcididae	*Brachymeria podagrica*	62	42.8	8.9
Pteromalidae	*Nasonia vitripennis*	6	4.1	0.9
Figitidae	*Paraganaspis egeria*	2	1.4	0.2-0.3
	Total	**145**	**100.0**	**20.0**

Note: Frequencies were recalculated from the total number of parasitoid specimens (n = 145). The overall parasitism rate reported in the text was 20.0%. Because *Hemencyrtus* sp. was reported as gregarious, the number of emerged parasitoid specimens should not be interpreted as exactly equivalent to the number of parasitized pupae.

Table 12: Host species and ecological information available from the scanned text

Taxonomic group	Host species	Information available in the text
Calliphoridae	*Chrysomya albiceps*	Medically and sanitarily important species; cited among preferred hosts for *Brachymeria podagrica*.
	Hemilucilia flavifacies	The host from which 14 parasitoid individuals were obtained; the high prevalence was related to gregarious parasitoid emergence.
Muscidae	*Musca domestica*	Medically and sanitarily important species; cited as the preferred host for *Nasonia vitripennis*.
Sarcophagidae	*Peckia chrysostoma*	Most abundant dipteran host, representing 40.3% of sampled insects; associated with three parasitoid species.
Sphaeroceridae	*Poecilosomella* sp.	The second most abundant dipteran host, representing 24.7% of sampled insects.

Note: This table summarizes only host information explicitly visible in the scanned text. The complete original host table was not visible in the uploaded scan.

The parasitoid community associated with flies developing in chicken viscera was characterized by the strong dominance of *B. podagrica* and *Hemencyrtus* sp. Together, these taxa represented most of the parasitoids obtained, indicating that chicken viscera may provide favorable conditions for host development and parasitoid activity. The occurrence of *Hemencyrtus* sp. as the most abundant parasitoid is particularly relevant because the text indicates that this genus is gregarious, with several individuals emerging from the same puparium. This biological trait helps explain the high number of parasitoid specimens and the high frequency attributed to this taxon (Figure 9).

Figure 9: Schematic illustration of a black cylindrical trap baited with chicken viscera, showing dipteran flies and Hymenoptera parasitoids associated with this substrate. The figure highlights the trap model used for field collections, the animal bait employed for attraction, and the occurrence of parasitoids with four wings around synanthropic flies. This integrative representation emphasizes the ecological association between decomposing chicken viscera, dung-breeding flies, and their natural enemies in Goiás, Brazil

Brachymeria podagrica was also highly frequent, reinforcing its ecological importance as a parasitoid associated with dipteran hosts in decomposing animal-derived substrates. The lower frequencies of *N. vitripennis* and *P. egeria* suggest that these species were present but less dominant in this particular substrate. The significant chi-square results confirm that parasitoid occurrence was not evenly distributed among taxa and that non-parasitized pupae predominated numerically. Even so, the overall parasitoid emergence demonstrates the importance of chicken viscera as a substrate for studying parasitoid–Diptera interactions. These findings contribute to the understanding of natural enemies of synanthropic flies and may support future biological control studies in environments where decomposing animal residues attract Diptera of sanitary and veterinary relevance (Marchiori *et al.,* 2003b).

10. Study of the Attractiveness of Their Hosts by Parasitoids in Chicken Feces in Goiás, Brazil

In Itumbiara, Goiás, 3.085 flies were collected from chicken feces, and 310 parasitized pupae were recorded. The parasitoid fauna was associated mainly with *M. domestica*, which was the most important host in this locality. The highest number of parasitized pupae was recorded for *S. endius* in *M. domestica*, with 143 pupae parasitized, followed by *P. vindemmiae* with 52 and *S. cameroni* with 38.

In Morrinhos, Goiás, 2,815 flies were collected, and 785 parasitized pupae were recorded. As in Itumbiara, *M. domestica* was the dominant host and supported the highest parasitoid diversity and abundance. The most frequent association was *P. vindemmiae* with *M. domestica*, with 347 parasitized pupae, followed by *N. vitripennis* with 188 and *M. raptorellus* with 101.

The chi-square tests indicated significant differences in host attractiveness to parasitoids in both localities. In Itumbiara, the association between parasitoids and hosts was significant ($\chi^2 = 23.09$; df = 40; $P < 0.05$). In Morrinhos, the association was also significant and much stronger ($\chi^2 = 711.80$; df = 36; $P < 0.05$). These results indicate that parasitoids did not occur randomly among hosts but showed a preference for specific host species, especially *M. domestica* (Tables 13-14).

Table 13: Diptera and their parasitoids collected in chicken feces in Itumbiara, Goiás, Brazil

Taxonomic group	Host species	No. of specimens	Parasitoid species	Parasitized pupae	Frequency (%)	Parasitism rate (%)
Fanniidae	*Fannia pusio*	153	*Eurytoma* sp.	2	0.6	1.3
			Pachycrepoideus vindemmiae	9	2.9	5.9
Muscidae	*Musca domestica*	1.982	*Aphaereta* sp.	1	0.3	0.1
			Nasonia vitripennis	11	3.5	0.6
			Pachycrepoideus vindemmiae	52	16.8	2.6
			Spalangia cameroni	38	12.3	1.9
			Spalangia drosophilae	1	0.3	0.1
			Spalangia endius	143	46.1	7.2
			Spalangia nigra	3	1.0	0.2
			Spalangia nigroaenea	10	3.2	0.5
			Spalangia sp.	21	6.8	1.1
			Tachinaephagus zealandicus	1	0.3	0.1
	Ophyra aenescens	529	*Nasonia vitripennis*	1	0.3	0.2
			Spalangia endius	4	1.3	0.8
Sarcophagidae	*Sarcodexia lambens*	222	*Pachycrepoideus vindemmiae*	2	0.6	0.9
			Tachinaephagus zealandicus	9	2.9	4.1
Syrphidae	*Ornidia obesa*	226	*Pachycrepoideus vindemmiae*	2	0.6	0.9
Other		4	-	-	-	-
Total	**-**	**3.085**	**-**	**310**	**100.0**	**-**

Note: Frequency (%) was calculated using the total number of parasitoids in the table (n = 310). The scanned table shows a printed total of 3.085 host specimens; however, the visible host values sum 3.116, indicating a numerical inconsistency in the source scan.

Table 14: Diptera and their parasitoids collected in chicken feces in Morrinhos, Goiás, Brazil

Taxonomic group	Host species	No. of Specimens	Parasitoid species	Parasitized pupae	Frequency (%)	Parasitism rate (%)
Calliphoridae	*Chrysomya megacephala*	500	*Nasonia vitripennis*	3	0.4	0.6
			Pachycrepoideus vindemmiae	3	0.4	0.6
			Spalangia endius	3	0.4	0.6
Fanniidae	*Fannia pusio*	42	*Muscidifurax raptorellus*	2	0.3	4.8
			Pachycrepoideus vindemmiae	2	0.3	4.8
Muscidae	*Musca domestica*	2.083	*Muscidifurax raptorellus*	101	12.9	4.8
			Nasonia vitripennis	188	23.9	9.0
			Pachycrepoideus vindemmiae	347	44.2	16.7
			Spalangia endius	67	8.5	3.2

Taxonomic group	Host species	No. of Specimens	Parasitoid species	Parasitized pupae	Frequency (%)	Parasitism rate (%)
			Spalangia nigra	16	2.0	0.8
			Spalangia nigroaenea	2	0.3	0.1
			Spalangia sp.	15	1.9	0.7
			Tachinaephagus zealandicus	10	1.3	0.5
Sepsidae	*Palaeosepsis* spp.	81	*Nasonia vitripennis*	2	0.3	2.5
			Pachycrepoideus vindemmiae	8	1.0	9.9
			Spalangia drosophilae	2	0.3	2.5
			Spalangia	10	1.3	12.3
Syrphidae	*Ornidia obesa*	93	*Pachycrepoideus vindemmiae*	2	0.3	2.2
			Spalangia cameroni	2	0.3	2.2
Other		16	—	—	—	—
Total	**—**	**2.815**	**—**	**785**	**100.0**	**27.9**

Note: Frequency (%) was calculated from the total number of parasitized pupae (785). The overall parasitism rate was calculated as 785/2,815 × 100 = 27.9%.

The results show that chicken feces supported a diverse assemblage of dipteran hosts and their parasitoids in both Itumbiara and Morrinhos. *M. domestica* was the most important host in the two localities, both because of its abundance and because it supported the greatest number of parasitoid associations. This pattern is relevant from a sanitary and veterinary perspective, since *M. domestica* is a synanthropic species capable of developing in several organic substrates and is frequently associated with human and animal environments.

The dominance of parasitoids associated with *M. domestica* suggests that this host plays a central role in maintaining parasitoid populations in poultry environments. In Itumbiara, *S. endius* was the most frequent parasitoid, whereas in Morrinhos, *P. vindemmiae* was dominant. This difference indicates that parasitoid composition may vary between localities, probably due to environmental conditions, host availability, substrate characteristics, and management practices in each poultry system (Figure 10).

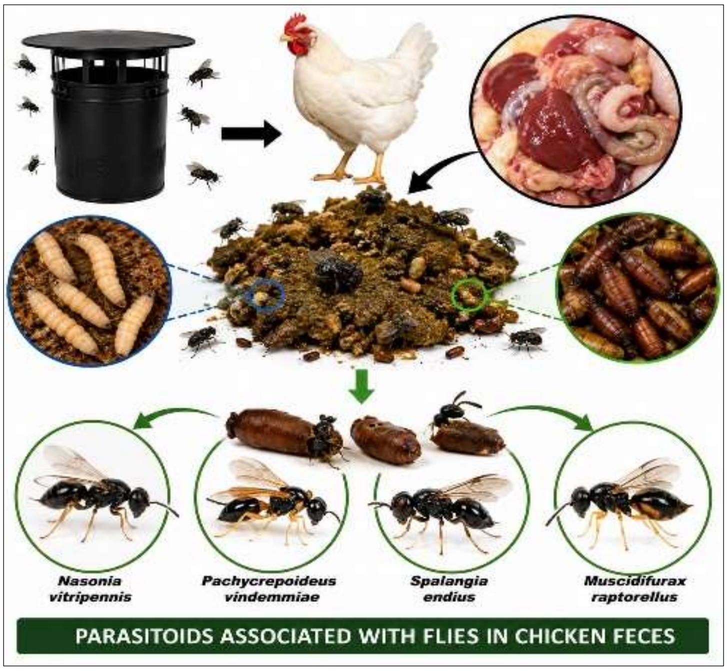

Figure 10: Schematic illustration of parasitoids associated with flies developing in chicken feces in Goiás, Brazil. The figure shows the black cylindrical trap, chicken feces as the breeding substrate, dipteran flies, pupae, and hymenopteran parasitoids with four wings. This integrative representation highlights the ecological interaction between synanthropic flies and their natural enemies in poultry environments

The significant chi-square values confirm that parasitoid distribution was not random. Instead, parasitoids showed different levels of attraction or preference for particular hosts. In Morrinhos, the high chi-square value reflects the strong concentration of parasitoids in *M. domestica*, especially through *M. raptorellus, N. vitripennis*, and *P. vindemmiae*. In

Itumbiara, although the association was also significant, the distribution was less concentrated than in Morrinhos.

These findings reinforce the importance of chicken feces as a substrate for the development of synanthropic flies and as a habitat for their natural enemies. The parasitoids recorded in this study may contribute to the natural regulation of fly populations, especially in poultry farms. Therefore, surveys of host–parasitoid associations in animal feces are important for understanding ecological interactions and for identifying species with potential use in biological control programs (Marchiori, 2013).

11. Diptera and Their Parasitoids Collected from Fish Bait in Goiás

From March 2001 to April 2002, a total of 140 pupae of Diptera were collected from fish bait in Goiás, Brazil. From these pupae, 43 parasitoid specimens emerged, resulting in an overall parasitism rate of 30.7%. The remaining 97 pupae were not parasitized. Among the dipteran hosts, *P. chrysostoma* was the most abundant species, representing 60.7% of the sampled insects. The parasitoid fauna was dominated by *P. vindemmiae*, which represented 60.4% of the parasitoids collected, followed by *B. podagrica*, with 29.3%. The text also reports parasitism rates of 2.3%, 7.2%, and 60.5% for *B. podagrica*, *N. vitripennis*, and *P. vindemmiae*, respectively.

The chi-square test comparing parasitized and non-parasitized pupae showed a significant difference ($\chi^2 = 20.83$; df = 1; $P < 0.0001$), indicating the predominance of non-parasitized pupae. However, the parasitism rate of 30.7% indicates expressive parasitoid activity in fish bait. Host preference by *P. vindemmiae* was also significant ($\chi^2 = 29.90$; df = 4; $P < 0.0001$), especially in association with *O. thornax* and *S. lambens*.

The results show that fish bait is an important substrate for attracting synanthropic Diptera and their parasitoids. The predominance of *P. chrysostoma* indicates that this sarcophagid fly is strongly associated with decomposing fish substrate, probably because this material provides favorable conditions for larval development. The high frequency of *P. vindemmiae* demonstrates its ecological importance as a parasitoid of Diptera in decomposing organic substrates. This species was the dominant parasitoid in the sample and showed a significant preference for specific hosts, especially *O. thornax* and *S. lambens*. This pattern suggests that host identity and substrate type may influence parasitoid attraction and successful emergence.

The presence of *B. podagrica* and *N. vitripennis* also reinforces the diversity of parasitoids associated with fish bait. Although they were less frequent than *P. vindemmiae*, their occurrence contributes to the natural regulation of fly populations in environments where decomposing animal material is available. The significant chi-square results confirm that parasitism was not randomly distributed. From an ecological perspective, the study reinforces the importance of decomposing fish bait as a microhabitat for host–parasitoid interactions. These findings are relevant for understanding the biology of synanthropic flies and for identifying parasitoids with potential use in biological control programs (Figure 11) (Marchiori and Silva Filho, 2005a).

Figure 11: Schematic illustration of a black cylindrical trap baited with fish, showing dipteran flies, pupae, and Hymenoptera parasitoids associated with this substrate in Goiás, Brazil. The figure highlights decomposing fish as an attractive resource for synanthropic Diptera and the occurrence of parasitoids with four wings around the bait and host pupae. This integrative representation emphasizes the ecological association between fish bait, dung-breeding flies, and their natural enemies

12. *C. Pararescita* New Host Record for the Parasitoid *T. coxalis* in Brazil

A total of 30 pupae of *C. pararescita* were obtained from cattle dung in Itumbiara, Goiás, Brazil. From these pupae, five specimens of *T. coxalis* emerged, corresponding to a parasitism rate of 16.7%. This record represents the first occurrence of *T. coxalis* parasitizing *C. pararescita* in cattle dung in Brazil. The chi-square test showed a significant difference between parasitized and non-parasitized pupae ($\chi^2 = 13.33$; df = 1; $P < 0.001$), indicating the predominance of non-parasitized pupae. However, the emergence of five parasitoids confirms the association between *T. coxalis* and *C. pararescita* under field conditions.

The occurrence of *T. coxalis* in pupae of *C. pararescita* expands the known host range of this Figitidae parasitoid in Brazil. Although the sample size was small, the parasitism rate of 16.7% indicates that this parasitoid was able to exploit *C. pararescita* in cattle dung, confirming a new host–parasitoid association. Cattle dung is an important microhabitat for the development of synanthropic Diptera and their natural enemies. This substrate provides food resources and protected conditions for immature flies, while also attracting parasitoids that use dipteran larvae or pupae as hosts. Therefore, records obtained from cattle dung are relevant for understanding ecological interactions in livestock environments (Figure 12).

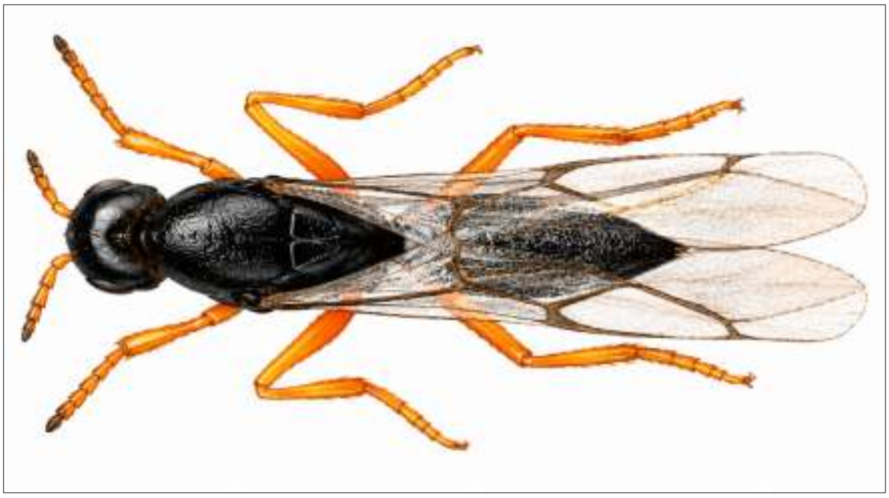

Figure 12: Schematic representation of Eucoilinae (Hymenoptera, Cynipoidea, Figitidae), a figitid parasitoid associated with pupae of *C. pararescita* in cattle dung in Brazil. The parasitoid is shown as a small hymenopteran with four wings, emphasizing its role as a natural enemy of dung-breeding Diptera. This illustration highlights the ecological importance of Eucoilinae parasitoids in the natural regulation of flies in livestock environments. Source: Forshage, M. & Nordlander, G. (2008). Identification key to European genera of Eucoilinae (Hymenoptera, Cynipoidea, Figitidae). *Insect Systematics & Evolution, 39*, 341-359

The significant chi-square result reflects the predominance of non-parasitized pupae, but this does not reduce the biological importance of the record. First host records are valuable because they clarify the host range, ecological plasticity, and distribution of parasitoids associated with flies of sanitary and veterinary relevance. This finding reinforces the importance of surveys in cattle dung for identifying parasitoids with potential relevance in natural biological control. The detection of *T. coxalis* associated with *C. pararescita* contributes to the knowledge of Eucoilinae parasitoids and their role in regulating dung-breeding Diptera populations (Marchiori *et al.,* 2004c).

13. Species of Fruit Flies (Diptera: Tephritidae) and Their Parasitoids in Itumbiara, Goiás, Brazil

A total of 774 fruit-fly pupae were obtained from fruits collected in Itumbiara, Goiás, Brazil. Among these, 384 pupae belonged to *A. fraterculus*, 12 to *Anastrepha striata* Schiner, 1868 (Diptera: Tephritidae), and 378 to *C. capitata*. The most abundant fruit-fly species was *A. fraterculus*, representing 49.6% of the total, followed by *C. capitata* with 48.8% and *A. striata* with 1.5%. A total of 48 parasitoids emerged from the pupae, corresponding to an overall parasitism rate of 6.2%. *A. fraterculus* yielded 44 parasitoids, with a parasitism rate of 11.5%, whereas *C. capitata* yielded 4 parasitoids, with a parasitism rate of 1.1%. No parasitoids emerged from *A. striata*.

The parasitoid fauna was dominated by *D. areolatus*, which represented 43 individuals and was associated with both *A. fraterculus* and *C. capitata*. The other parasitoids were *A. pelleranoi*, with 3 individuals, and *P. vindemmiae*, with 2 individuals, both recorded only from *A. fraterculus*. The chi-square test confirmed a significant difference between parasitized and non-parasitized pupae ($\chi^2 = 593.91$; df = 1; $P < 0.0001$) and also showed that parasitoid emergence was significantly higher in *A. fraterculus* than in *C. capitata* ($\chi^2 = 33.33$; df = 1; $P < 0.0001$) (Figure 13) (Table 15).

Table 15: Fruit fly hosts and their parasitoids collected in Itumbiara, Goiás, Brazil

Taxonomic group	Host species	No. of pupae	Frequency (%)	Parasitoid species	No. of parasitoids	Frequency (%)	Parasitism rate (%)
Tephritidae	*Anastrepha fraterculus*	384	49.6	*Doryctobracon areolatus*	39	81.3	10.2
				Aganaspis pelleranoi	3	6.3	0.8
				Pachycrepoideus vindemmiae	2	4.2	0.5
Subtotal	*Anastrepha striata*	12	1.6	—	44	91.7	11.5
				—	0	0.0	0.0
	Ceratitis capitata	378	48.8	*Doryctobracon areolatus*	4	8.3	1.1
Subtotal		378	48.8	—	4	8.3	1.1
Total	**—**	**774**	**100.0**	**—**	**48**	**100.0**	**6.2**

Note: The scanned text reports 384 puparia of *A. fraterculus*, 12 of *A. striata*, and 378 of *C. capitata*, totaling pupae. The original printed table included only parasitized hosts; the non-parasitized Anastrepha striata row was added here to close the total. Frequencies and parasitism rates were recalculated from the available numbers.

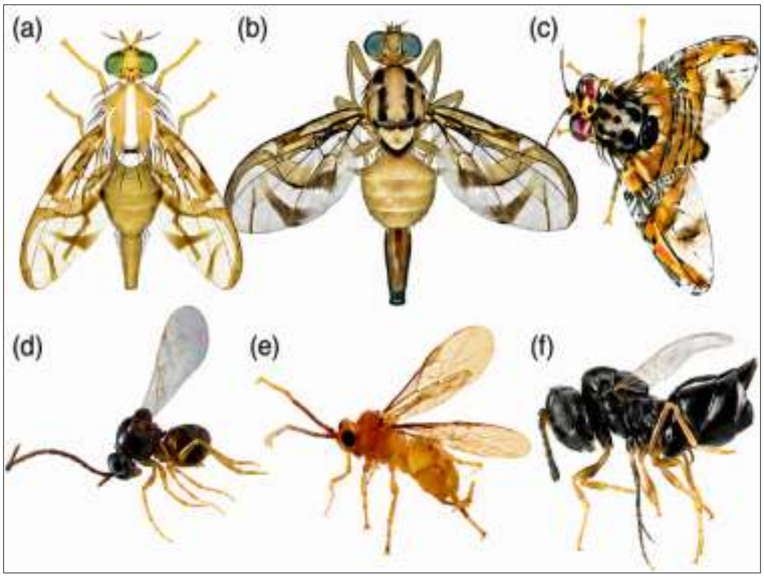

Figure 13: Morphological representation of fruit flies and their parasitoids collected in Itumbiara, Goiás, Brazil: (A) *A. fraterculus*; (B) *A. striata*; (C) *C. capitata*; (D) *A. pelleranoi*; (E) *D. areolatus*; and (F) *P. vindemmiae*. The figure shows the main tephritid hosts and parasitoid species associated with fruit-fly puparia in the studied region. This visual synthesis highlights the diversity of host–parasitoid associations recorded in fruit flies of economic importance

The results indicate that *A. fraterculus* was the most abundant fruit-fly species collected in Itumbiara and also the main host for parasitoids. Although *C. capitata* showed a similar number of pupae, its parasitism rate was much lower. This difference suggests that host identity, fruit species, host suitability, and parasitoid preference may influence parasitoid emergence in fruit-fly communities. The dominance of *D. areolatus* confirms its importance as a natural enemy of fruit flies in the region. This parasitoid was the only species recovered from both *A. fraterculus* and *C. capitata*, indicating broader host use when compared with *A. pelleranoi* and *P. vindemmiae*, which were associated only with *A. fraterculus*.

The higher parasitism observed in *A. fraterculus* may be related to its association with native or commonly available host fruits, especially guava, as reported in the article. The significant chi-square values reinforce that fruit-fly species and parasitoid emergence were not randomly distributed. Instead, the data suggest a stronger ecological relationship between *A. fraterculus* and its parasitoids, particularly *D. areolatus*. These findings are relevant for biological control studies because fruit-fly parasitoids can contribute to the natural regulation of pest populations. The occurrence of braconid and figitid parasitoids in Itumbiara reinforces the importance of local surveys for identifying natural enemies associated with economically important fruit flies in Brazil (Figure 14) (Marchiori *et al.,* 2000a).

Figure 14: Schematic illustration of decomposing fruits infested by fruit flies, showing carambola, guava, mango, pitanga, orange, and peach as substrates for larval development. The figure highlights adult fruit flies flying and resting on the fruits, enlarged larvae inside the decomposing tissues, and parasitoids associated with the infested fruits, including yellow braconids and black parasitoids with four wings. This integrative representation emphasizes host fruits, dipteran infestation, and parasitoid activity in fruit-fly systems of economic importance

14. *N. Vitripennis,* a Gregarious Parasitoid in Pupae of *P. chrysostoma* in Brazil

A total of 60 pupae of *P. chrysostoma* were collected using traps baited with bovine liver in a forest area of Parque da Serra de Caldas Novas, Goiás, Brazil. From these pupae, 30 yielded the gregarious parasitoid *N. vitripennis*, corresponding to a parasitism rate of 50.0%. The chi-square test comparing parasitized and non-parasitized pupae showed no significant difference ($\chi^2 = 0.00$; df = 1; P = 1.0000), because both categories had the same number of pupae. However, the parasitism rate was high, indicating strong parasitoid activity under the sampled conditions.

The occurrence of *N. vitripennis* in *P. chrysostoma* pupae represents an important record of parasitoid activity in a forest environment in Goiás, Brazil. The parasitism rate of 50.0% is expressive and suggests that *N. vitripennis* can successfully exploit *P. chrysostoma* pupae under field conditions. The gregarious habit of *N. vitripennis* is biologically relevant because several parasitoid individuals may emerge from a single host pupa. This characteristic can increase the number of parasitoids produced per host and may contribute to the natural regulation of fly populations in environments where suitable hosts are available (Figure 15).

Figure 15: Schematic illustration of the developmental cycle of *N. vitripennis* associated with pupae of *P. chrysostoma* in Brazil. The figure shows the successive immature stages, the host fly puparium, the oviposition process, the gregarious development of the parasitoid within the pupa, and the emergence of adult parasitoids. This integrative representation highlights the biological cycle of *N. vitripennis* and its host–parasitoid association with *P. chrysostoma*

Although the chi-square result was not significant, this does not reduce the ecological importance of the record. The equal number of parasitized and non-parasitized pupae reflects statistical balance between the two categories, while the 50.0% parasitism rate indicates a strong host–parasitoid association. This finding reinforces the importance of surveys using animal-derived baits, such as bovine liver, for detecting parasitoids associated with sarcophagid flies. The record also contributes to the knowledge of natural enemies of Diptera of medical and veterinary importance in Brazil (Marchiori, 2005b).

15. Cynipoidea parasitoids of dung-flies in Brazil

15.1. Identification key to Cynipoidea parasitoids of dung flies in Brazil

1. Scutellum without a characteristic raised cup on the disc; third metasomal tergite shorter than the fourth and not tongue-shaped. → Figitinae.

1'. Scutellum with a characteristic raised cup on the disc; third and fourth metasomal tergites fused. → Eucoilinae

15.2. Figitinae

2. Cheeks usually margined; antenna 13-segmented in females and 14-segmented in males; notauli usually present and recurrent; scutellum without a cup on the disc; metasoma sessile in most species; third tergite shorter than the fourth, not liguliform, and without a hairy ring at the base.
→ *Neralsia splendens*

15.3. Eucoilinae

3. Third metasomal tergite without a hairy ring and striate at the base; radial cell entirely open along the anterior margin; scutellar disc smooth or nearly smooth; scutellar cup narrow.
→ *Triplasta*
3a. Female antenna with a three-segmented club.
→ *Triplasta atrocoxalis*
3b. Female antenna with a five-segmented club.
→ *Triplasta coxalis*
4. Third metasomal tergite with a hairy ring and smooth at the base; radial cell open or closed along the anterior margin.
→ go to 5.
5. Scutellum bearing a sharp spine on the dorsal surface of the cup; mesoscutum smooth; radial cell closed; wings clouded.
→ *Acanthuecola.*
5a. Posterior surface of scutellum smooth, with two longitudinal keels behind the cup; wings with scarce discal and marginal setae.
→ go to 6
5b. Posterior surface of scutellum smooth or slightly rugose, with two longitudinal keels behind the cup; wings with abundant discal and marginal setae.
→ go to 7
6. Lateral keels behind the scutellar cup are straight; the spine of the cup is posteriorly directed.
→ *Acanthuecola armata.*
6'. Lateral keels behind the scutellar cup are concave outward; the spine of the cup is not posteriorly directed.
→ *Acanthuecola brevispina.*
7. Scutellar cup oval and narrow; first sector of the radius perpendicular to the anterior wing margin; facial lateral striation reduced to the antennal base.
→ *Acanthuecola coprophila*
7'. Scutellar cup circular and broad; first sector of the radius oblique to the anterior wing margin; facial lateral striation extending to the lower border of the eyes.
→ *Acanthuecola fumipennis*
8. Scutellum without a sharp spine on the dorsal surface of the cup.
→ go to 9
9. Scutellum conical in dorsal view, gradually tapering to a point; cup normal, not reaching two-thirds of the disc; radial cell partly or entirely open on the anterior margin.
→ *Coneucoela brasiliensis*
9'. Scutellum is truncated or rounded posteriorly.
→ go to 10
10. Scutellum truncate posteriorly; disc longitudinally striate; radial cell open; forewing emarginate distally; cup narrow.
→ *Kleidotoma nigra.*
10'. Scutellum rounded posteriorly; disc areolate-rugose; radial cell closed.
→ go to 11
11. Female antenna with six segments and a conspicuous club; scutellar disc isolated.
→ *Paraganaspis egeria*
11'. Female antenna with seven segments, without a conspicuous club; scutellar disc punctate.
→ *Aganaspis fulvicincta* (Figure 16).

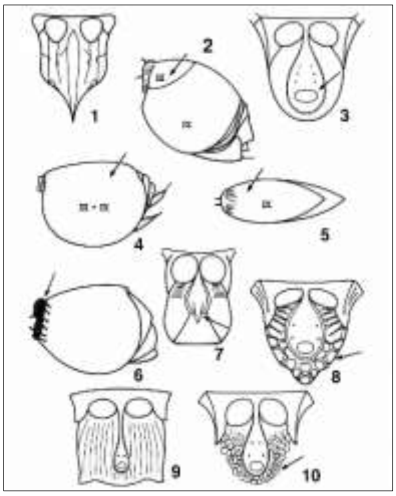

Figure 16: Diagnostic morphological characters used in the identification key for Cynipoidea parasitoids associated with dung-breeding flies in Brazil: 1, 3, 7–10, scutellum; 1, *Neralsia*; 3, Eucoilinae; 7, *Acanthuecola*; 8, *Coneucoela*; 9, *Kleidotoma*; 10, *Paraganaspis*; 2, 4–6, metasoma; 2, Figitinae; 4, Eucoilinae; 5, *Triplasta*; 6, *Acanthuecola*. The plate highlights external structures used to separate Figitinae and Eucoilinae, especially the scutellum, scutellar cup, metasomal tergites, and diagnostic surface features. These characters support the taxonomic identification of Cynipoidea parasitoids associated with dung flies in Brazil

The taxonomic study recorded Cynipoidea parasitoids associated with dung-breeding flies in Brazil, mainly species of Eucoilinae and Figitinae. The article emphasized diagnostic morphological characters used for identification, especially structures of the scutellum, scutellar cup, radial cell of the forewing, antennae, and metasomal tergites. The identification key separates Figitinae from Eucoilinae primarily by the presence or absence of the scutellar cup and by the structure of the third and fourth metasomal tergites. Within Eucoilinae, the key distinguished genera and species, such as *G. fulvicincta, K. nigra, P. egeria, T. atrocoxalis,* and *T. coxalis,* are based on antennal segmentation, scutellar shape, wing venation, and surface sculpture.

The study is also ecologically relevant because many of the listed parasitoids are associated with flies that develop in cattle dung. These flies may have sanitary and veterinary importance, and their parasitoids contribute to natural regulation in livestock environments. Therefore, even though the article is taxonomic, it supports biological control studies by improving the identification of natural enemies. The inclusion of this article in the review is justified because it provides the taxonomic foundation needed to interpret host–parasitoid records reported in other ecological studies. Without reliable identification keys, records of parasitoid occurrence, host association, and geographic distribution may become uncertain. Thus, this article strengthens the taxonomic basis of the broader synthesis on parasitoids of Diptera in Brazil (Table 16).

Table 16: Taxonomic summary of Cynipoidea parasitoids associated with dung flies in Brazil

Taxonomic group	Species	Main diagnostic character	Host/substrate information
Eucoilinae	*Acanthuecola armata*	Scutellar cup with posteriorly directed spine	Associated with dung-fly environments
	Acanthuecola brevispina	Scutellar cup spine not posteriorly directed	Associated with dung-fly environments
	Acanthuecola coprophila	Narrow oval scutellar cup; radial sector perpendicular	Associated with coprophilous habitats
	Acanthuecola fumipennis	Broad circular scutellar cup; radial sector oblique	Associated with dung-fly environments
	Coneucoela brasiliensis	Conical scutellum in dorsal view; radial cell open	Associated with dung-breeding Diptera
	Ganaspis fulvicincta	Female antenna without a conspicuous club; scutellar disc punctate	Associated with Diptera hosts
	Kleidotoma nigra	Scutellum truncate posteriorly; forewing distally emarginate	Associated with muscid hosts in cattle dung
	Paraganaspis egeria	Female antenna with conspicuous club; scutellar disc isolated	Associated with muscoid Diptera
	Triplasta atrocoxalis	Female antenna with three-segmented club	Associated with the pupae of dung flies
	Triplasta coxalis	Female antenna with five-segmented club	Associated with dung-breeding Diptera
Figitinae	*Neralsia splendens*	Scutellum without a cup; metasoma generally sessile	Associated with dung-breeding Diptera

Note: The table summarizes the principal taxa and diagnostic characters presented in the taxonomic key for Cynipoidea parasitoids of dung flies in Brazil.

The knowledge of Cynipoidea parasitoids in Brazilian livestock environments. This article differs from the ecological studies because its main contribution is taxonomic rather than numerical. The identification key is central because it allows the recognition of Cynipoidea parasitoids associated with dung flies in Brazil using external morphological characters. For this reason, no chi-square test was applied, since the article does not present abundance data suitable for statistical comparison.

The use of characters such as the scutellar cup, radial cell, antennal club, and metasomal structure is especially important in Cynipoidea taxonomy. These structures allow reliable separation of closely related taxa and help standardize identification in studies involving parasitoids of synanthropic Diptera. The study is also ecologically relevant because many of the listed parasitoids are associated with flies that develop in cattle dung. These flies may have sanitary and veterinary importance, and their parasitoids contribute to natural regulation in livestock environments. Therefore, even though the article is taxonomic, it supports biological control studies by improving the identification of natural enemies.

The inclusion of this article in the review is justified because it provides the taxonomic foundation needed to interpret host–parasitoid records reported in other ecological studies. Without reliable identification keys, records of parasitoid occurrence, host association, and geographic distribution may become uncertain. Thus, this article strengthens the taxonomic basis of the broader synthesis on parasitoids of Diptera in Brazil (Figures 17-18) (Marchiori *et al.,* 2000b).

Figure 17: Schematic illustration of the hosts of *P. egeria* collected in bovine and buffalo dung in southern Goiás, Brazil. The figure shows multiple dipteran pupae associated with dung-breeding flies, a flattened black parasitoid with four wings, and one adult emerging from a host pupa. This integrative representation highlights the host range of *P. egeria* and its association with pupae developing in cattle and buffalo dung

Figure 18: *Coneucoela brasiliensis* Kieffer, 1909 (Hymenoptera: Figitidae) is a species of parasitoid wasp belonging to the Eucoilinae subfamily of the Figitidae family. They act as natural enemies of dipterans and play a fundamental role in biological control, mainly by parasitizing fly larvae that develop in bovine feces

From a biological control perspective, the study is relevant because parasitoids associated with dung-breeding flies may contribute to the natural regulation of synanthropic Diptera populations. Even at low parasitism rates, records of host range and substrate use are important for understanding parasitoid ecology and for selecting potential natural enemies for future studies. Overall, this article expands the knowledge of *P. egeria* in Brazil by documenting its hosts in bovine and buffalo feces and reinforcing its role as a natural enemy of flies associated with livestock environments (Marchiori, 2009).

16. Muscoid Dipterous Collected from Cattle Dung Pats in Pastures in Itumbiara, Goiás, Brazil

A total of 3.099 muscoid Diptera were collected from cattle dung pats exposed in pasture for different periods in Itumbiara, Goiás, Brazil. The specimens were distributed among Muscidae, Sarcophagidae, Sepsidae, and Sphaeroceridae. The most abundant group was *Palaeosepsis* spp., with 1.558 specimens, representing 50.3% of the total collected, followed by *S. occidua*, with 1,213 specimens, corresponding to 39.1%. Together, *Palaeosepsis* and *S. occidua* represented 89.4% of all specimens collected, indicating strong dominance of these dung-breeding Diptera in the sampled pasture environment. Other species were less frequent, including *B. debilis* with 151 specimens (4.9%), *C. pararescita* with 71 specimens (2.3%), and *B. quadristigma* with 56 specimens (1.8%).

Regarding exposure time, the highest abundance was recorded in fecal pats exposed for 144 h, with 424 specimens (13.7%), followed by 72 h, with 391 specimens (12.6%), and 192 h, with 372 specimens (12.0%). The lowest abundance occurred at 168 h, with 183 specimens (5.9%). The chi-square test reported in the article showed that the distribution of flies among exposure periods was highly significant ($\chi^2 = 1085.8$; df = 56; $P < 0.0001$). The complementary chi-square tests also confirmed significant differences among species/groups ($\chi^2 = 7048.27$; df = 7; $P < 0.0001$) and among exposure periods ($\chi^2 = 173.88$; df = 9; $P < 0.0001$) (Table 17).

Table 17: Abundance of muscoid Diptera collected from cattle dung pats after different exposure periods in Itumbiara, Goiás, Brazil

Taxonomic group	Species	24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h	216 h	240 h	Total	Frequency (%)
Muscidae	*Brontaea debilis*	70	11	7	8	8	19	9	3	10	6	151	4.9
	Brontaea quadristigma	0	6	0	0	0	2	11	12	16	9	56	1.8
	Cyrtoneurina pararescita	0	0	0	0	2	0	8	26	17	18	71	2.3
Sarcophagidae	*Ravinia belforti*	0	0	0	0	4	0	2	0	0	1	7	0.2
	Sarcophagula occidua	108	231	224	107	201	56	54	142	13	77	1,213	39.1
Sepsidae	*Archisepsis scabra*	1	1	0	0	2	0	1	4	19	1	29	0.9
	Palaeosepsis spp.	169	44	160	137	114	347	98	181	164	144	1,558	50.3
Sphaeroceridae	*Coproica* sp.	10	0	0	0	0	0	0	4	0	0	14	0.5
Total	—	**358**	**293**	**391**	**252**	**331**	**424**	**183**	**372**	**239**	**256**	**3.099**	**100.0**

Note: Frequency (%) was calculated from the total number of specimens (n = 3,099). The total row gives the number of specimens collected at each exposure period.

The results demonstrate that cattle dung pats in pasture are highly suitable substrates for the development of muscoid Diptera. The strong predominance of *Palaeosepsis* spp. and *S. occidua* suggests that these taxa are especially well adapted to this substrate in the pasture conditions of Itumbiara, Goiás. The high abundance of *Palaeosepsis* spp. agrees with the ecological behavior of Sepsidae, which are commonly associated with animal feces and may colonize dung during different stages of decomposition. The occurrence of *Palaeosepsis* spp. Across several exposure periods indicates that this group can exploit cattle dung over a relatively broad temporal window (Figure 19).

Figure 19: Muscoid Diptera collected from cattle dung pats exposed for different periods in pastures in Itumbiara, Goiás, Brazil. The figure shows cattle dung as the breeding substrate and adult flies associated with this microhabitat, representing the main dipteran fauna recorded in the study. This visual synthesis highlights the ecological importance of bovine feces for the development and temporal succession of muscoid flies in pasture environments

Sarcophagula occidua was also highly abundant and occurred in large numbers, especially in feces exposed for 48, 72, 120, and 192 h. This pattern indicates that sarcophagid flies may respond strongly to changes in dung age, moisture, and decomposition stage. The abundance of these flies is relevant because several muscoid Diptera associated with livestock environments may have sanitary or veterinary importance. The significant chi-square results indicate that fly occurrence was not randomly distributed among species or exposure periods. Instead, each taxon showed different levels of association with specific dung exposure times.

This suggests that the decomposition stage is an important factor structuring dipteran communities in cattle dung. From an ecological perspective, the study reinforces the importance of cattle dung as a dynamic microhabitat, supporting different fly species according to substrate age and environmental conditions. These results are useful for understanding fly succession in pasture systems and may support future studies on parasitoids and natural biological control in livestock environments (Marchiori *et al.,* 2003c).

17. New Host and Habitat for *N. splendens* in Brazil

A total of 50 pupae of *O. thornax* were collected from human feces using pitfall traps in Itumbiara, Goiás, Brazil. From these pupae, nine specimens of *N. splendens* emerged, corresponding to a parasitism rate of 18.0%. The chi-square test showed a significant difference between parasitized and non-parasitized pupae ($\chi^2 = 20.48$; df = 1; $P < 0.0001$), with predominance of non-parasitized pupae. Nevertheless, the emergence of *N. splendens* confirms a new host association with *O. thornax* and a new habitat record in human feces (Figure 20).

Figure 20: The figure highlights the parasitoid associated with the sarcophagid host and the fecal substrate used as the habitat for larval development and parasitism. This record represents a new host and habitat for *N. splendens* in Brazil and reinforces the ecological importance of parasitoids in the natural regulation of synanthropic Diptera. Source: SEMUBC HYM-7926 -Spencer Entomological Collection and https://www.zoology.ubc.ca/entomology/main/Hymenoptera/Figitidae/index-gen.php

The occurrence of *N. splendens* parasitizing pupae of *O. thornax* represents an important record for Brazil. This association expands the known host range of this Figitidae parasitoid and confirms its ability to exploit sarcophagid hosts developing in human feces. The parasitism rate of 18.0% is relevant because it was obtained from a limited sample of 50 pupae and represents a new ecological record. Although most pupae were not parasitized, the emergence of nine parasitoids indicates that *N. splendens* may participate in the natural regulation of dung- and feces-associated Diptera.

Human feces function as an attractive substrate for synanthropic flies and may support interactions between dipteran hosts and parasitoids. This record is therefore important from both ecological and sanitary perspectives, since *O. thornax* belongs to Sarcophagidae, a family frequently associated with decomposing organic matter and environments of medical and veterinary relevance.

The significant chi-square result reflects the numerical predominance of non-parasitized pupae. However, the biological significance of the study lies primarily in documenting a new host and habitat for *N. splendens*. These findings reinforce the value of field surveys using different baited substrates to detect parasitoids with potential relevance to natural biological control (Marchiori *et al.,* 2006a).

18. New Host-Parasitoid Relation in Brazil

A total of 40 pupae of *C. pararescita* were collected from cattle dung in Itumbiara, Goiás, Brazil. From these pupae, four adults of *S. nigra* emerged, corresponding to a parasitism rate of 10.0%. The chi-square test showed a significant difference between parasitized and non-parasitized pupae ($\chi^2 = 25.60$; df = 1; $P < 0.0001$), with predominance of non-parasitized pupae. However, the emergence of *S. nigra* confirms a new host-parasitoid association in Brazil.

The occurrence of *S. nigra* parasitizing pupae of *C. pararescita* represents an important new host record in Brazil. Although the parasitism rate was 10.0%, the finding is biologically relevant because it expands the known host range of this pteromalid parasitoid (Figure 21).

Figure 21: Schematic representation of *S. nigra*, a pteromalid parasitoid recorded from pupae of *C. pararescita* collected in cattle dung in Itumbiara, Goiás, Brazil. The figure highlights the parasitoid associated with the muscid host and the bovine feces substrate, where host development and parasitism occur. This record represents a new host–parasitoid association in Brazil and reinforces the ecological importance of *Spalangia* species as natural enemies of dung-breeding Diptera

Cattle dung is an important substrate for the development of muscoid flies and their natural enemies. The association between *C. pararescita* and *S. nigra* reinforces the ecological importance of bovine feces as a microhabitat where host development and parasitism occur. The significant chi-square result reflects the predominance of non-parasitized pupae in the sample. Nevertheless, the record remains important because first host associations help clarify the biology, host range, and potential ecological role of parasitoids in livestock environments.

This study contributes to the knowledge of *Spalangia* species in Brazil and supports the importance of surveys of parasitoids associated with dung-breeding Diptera. Such parasitoids may contribute to the natural regulation of fly populations of medical and veterinary relevance (Marchiori, 2006b).

19. Parasitoids of Muscoid Diptera Collected In Different Substrates in a Forest Area in Goiás

A total of 684 hymenopteran parasitoids were obtained from 830 pupae of muscoid Diptera collected in different substrates in a forest area in Itumbiara, Goiás, Brazil. The overall parasitism rate reported in the article was 82.4%, probably influenced by the occurrence of gregarious parasitoids, mainly *Aphaereta* sp. and *N. vitripennis*. The most abundant parasitoids were *Aphaereta* sp., with 360 individuals, representing 52.6% of the parasitoids collected, and *N. vitripennis*, with 231 individuals, corresponding to 33.8%. *Brachymeria podagrica* accounted for 68 individuals and *P. vindemmiae* for 25 individuals.

The substrates showed different parasitoid associations. Human feces and fish showed the highest parasitoid diversity, whereas fish presented the highest parasitism prevalence, mainly due to the occurrence of gregarious *Aphaereta* sp. The corrected values in Table 18 indicate that the number of parasitized pupae was lower than the number of emerged parasitoid individuals in gregarious species, which explains the need to distinguish “parasitoid individuals” from “parasitized pupae”. The chi-square test confirmed significant parasitoid preference among substrates ($\chi^2 = 361.8$; df = 9; $P < 0.0001$) and among hosts ($\chi^2 = 1739.2$; df = 21; $P < 0.0001$). The complementary chi-square test also showed that parasitoid abundance was not evenly distributed among species (Table 18) ($\chi^2 = 416.64$; df = 3; $P < 0.0001$).

Table 18: Corrected host pupae and parasitism rates of parasitoids collected in different substrates in a forest area in Itumbiara, Goiás, Brazil

Substrate	Host species	No. of host pupae	Parasitoid species	Parasitized pupae	Parasitism rate (%)
Bovine kidneys	*Fannia pusio*	40	*Pachycrepoideus vindemmiae*	2	5.0
	Chrysomya albiceps	120	*Nasonia vitripennis*	8	6.7
Chicken viscera	*Chrysomya megacephala*	6	*Brachymeria podagrica*	2	33.3
	Oxysarcodexia thornax	33	*Brachymeria podagrica*	10	30.3
			Nasonia vitripennis	2	6.1
	Peckia chrysostoma	104	*Brachymeria podagrica*	34	32.7
			Nasonia vitripennis	23	22.1
Fish		43	*Nasonia vitripennis*	5	11.6
		42	*Aphaereta* sp.	10	23.8
			Brachymeria podagrica	7	16.7
	Musca domestica	236	*Pachycrepoideus vindemmiae*	18	7.6

Substrate	Host species	No. of host pupae	Parasitoid species	Parasitized pupae	Parasitism rate (%)
Human feces	*Ophyra* sp.	20	*Brachymeria podagrica*	11	55.0
	Sarcophagula occidua	47	*Pachycrepoideus vindemmiae*	5	10.6
Total	—	**691**	—	**137**	**19.8**

Note: Substrates are arranged alphabetically. The host-pupae total was calculated once for each host within each substrate; repeated host values were not double-counted. Parasitized pupae were corrected from the notes below the original table, especially for gregarious parasitoids.

The results demonstrate that different animal-derived substrates support distinct parasitoid assemblages associated with muscoid Diptera. Fish was especially important because it yielded a high number of *Aphaereta* sp., a gregarious braconid parasitoid. This explains the high number of parasitoid individuals and the elevated parasitism values reported in the article. The correction of Table 2 is important because gregarious parasitoids may produce several individuals from a single host pupa. Therefore, the number of emerged parasitoid individuals should not always be interpreted as the number of parasitized pupae. This distinction is essential for calculating the parasitism rate correctly (Figure 22).

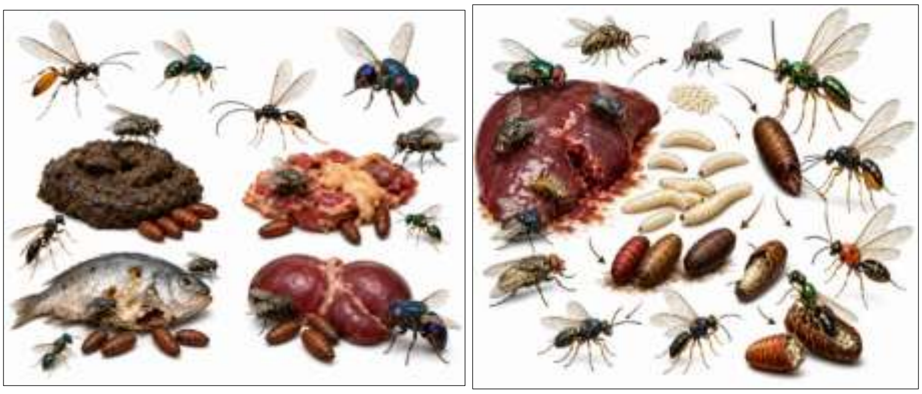

Figure 22: Schematic illustration of microhymenopteran parasitoids associated with muscoid Diptera collected from different substrates in a forest area in Itumbiara, Goiás, Brazil. The figure shows fecal and animal-derived substrates, including dung, chicken viscera, fish, and bovine kidneys, together with flies, host pupae, and parasitoids with four wings. This integrative representation highlights the diversity of substrates used by muscoid flies and the ecological association of their parasitoids in the studied environment

Brachymeria podagrica occurred in several substrates, indicating ecological flexibility and broad association with muscoid hosts. *Nasonia vitripennis* was also important, especially in bovine kidney and chicken viscera, reinforcing its role as a common parasitoid of Diptera pupae in decomposing organic substrates. The significant chi-square values indicate that parasitoids were not randomly distributed among substrates or hosts. Instead, each parasitoid showed different levels of association with particular hosts and substrates. These patterns may be influenced by host availability, substrate quality, decomposition stage, moisture, and parasitoid searching behavior.

Overall, this study reinforces the importance of forest environments and decomposing substrates such as human feces, chicken viscera, fish, and bovine kidney as habitats for muscoid flies and their natural enemies. The records are relevant for understanding parasitoid ecology and for identifying species with potential use in biological control of synanthropic Diptera (Marchiori *et al.,* 2003d).

20. First Report of *Z. Incompleta* as a Parasitoid of Diptera in Brazil

During 1998, a total of 35 specimens of *Z. incompleta* were collected using yellow water traps placed in pasture and nearby native vegetation in Itumbiara, Goiás, Brazil. Of these, 15 specimens were collected in pasture and 20 specimens in native vegetation. The specimens were collected mainly from April to July, with the highest numbers recorded in April and June. The chi-square test showed no significant difference between pasture and native vegetation (χ^2 = 0.71; df = 1; P = 0.3980), indicating that *Z. incompleta* did not show a statistically significant preference for either environment.

This record represents the first occurrence of *Z. incompleta* as a parasitoid of Diptera in Brazil and contributes to the knowledge of Eucoilinae parasitoid fauna in the country. The first record of *Z. incompleta* in Brazil is important from both taxonomic and ecological perspectives. The genus *Zaeucoila* includes Neotropical Eucoilinae associated with immature stages of Cyclorrhapha Diptera, and the detection of this species in Goiás expands its known geographic distribution.

The absence of a significant preference for pasture over native vegetation suggests that *Z. incompleta* may occur in both environments. This pattern may reflect the movement of parasitoids between open pasture areas and nearby native vegetation, especially where dipteran hosts are available in organic substrates. Native vegetation near pastures may serve as a refuge or reservoir for parasitoids, while pasture areas provide substrates, such as cattle dung, that support dipteran development. Therefore, both environments may contribute to the maintenance of parasitoid populations in livestock landscapes (Figure 23) (van Noort, 2026).

Figure 23: Schematic representation of *Z. incompleta*, a parasitoid recorded as a parasitoid of Diptera in Brazil. The figure highlights the adult parasitoid in lateral view, showing the elongated antennae, transparent wings with visible venation, and the dark body morphology characteristic of Cynipoidea. This illustration supports the record of *Z. incompleta* in pasture and native vegetation environments sampled with yellow water traps in Itumbiara, Goiás, Brazil

Although the study did not provide host-level parasitism data, the occurrence of *Z. incompleta* in yellow water traps indicates the presence of this parasitoid in environments associated with dung-breeding Diptera. This record strengthens the importance of faunistic surveys for documenting parasitoid diversity and distribution in Brazil (Marchiori and Oliveira, 2001).

21. Occurrence of Microhymenopteran Parasitoids of *A. Scabra* on Bovine and Buffalo Dung

A total of 479 pupae of *A. scabra* were obtained from bovine and buffalo feces in southern Goiás, Brazil. Of these, 169 pupae were collected from bovine feces and 310 pupae from buffalo feces. In bovine feces, 19 parasitoids emerged from *A. scabra* pupae, corresponding to a parasitism rate of 11.2%. The parasitoids recorded in this substrate were *S. drosophilae*, with 10 individuals, *S. nigroaenea*, with one individual, and *T. atrocoxalis*, with eight individuals.

In buffalo feces, six parasitoids emerged from *A. scabra* pupae, corresponding to a parasitism rate of 1.9%. The species recorded were *P. egeria* (4 individuals), *S. drosophilae* (1 individual), and *Trichopria* sp. (1 individual). The chi-square test showed a significant difference in parasitism between bovine and buffalo feces ($\chi^2 = 19.15$; df = 1; $P < 0.0001$). The overall comparison between parasitized and non-parasitized pupae was also significant ($\chi^2 = 384.22$; df = 1; $P < 0.0001$), reflecting the predominance of non-parasitized pupae. Parasitoid distribution among species was also unequal ($\chi^2 = 18.92$; df = 5; $P = 0.0020$) (Figure 24) (Silva and Pollet, 2020).

Figure 24: Schematic representation of the family Sepsidae, showing adult flies, larvae, and pupae associated with decomposing organic substrates. The figure highlights the ecological importance of sepsid flies in fecal environments, especially cattle and buffalo dung, where immature stages develop and become available to parasitoids. This illustration emphasizes the role of Sepsidae as important hosts in studies of hymenopteran parasitoids associated with dung-breeding Diptera in Brazil. Source: Publications scientifiques du Muséum national d'Histoire naturelle

The results show that *A. scabra* pupae collected from bovine and buffalo feces supported a small but diverse assemblage of microhymenopteran parasitoids. The highest parasitism rate was recorded in bovine feces, where *S. drosophilae* and *T. atrocoxalis* were the most frequent parasitoids. The difference between bovine and buffalo feces was statistically significant, suggesting that substrate type may influence parasitoid occurrence. This pattern may be related to differences in dung composition, moisture, decomposition stage, host density, or attractiveness of the substrate to parasitoids.

Spalangia drosophilae occurred in both bovine and buffalo feces, indicating ecological flexibility. In contrast, *S. nigroaenea* and *T. atrocoxalis* were recorded only from bovine feces, while *P. egeria* and *Trichopria* sp. were recorded only from buffalo feces. This suggests that parasitoid assemblages may vary according to substrate and local host availability.

Although the overall parasitism rates were relatively low, the records are important because they expand knowledge of parasitoids associated with *A. scabra* in livestock environments. Such parasitoids may contribute to the natural regulation of dung-breeding Diptera, especially in pasture systems where bovine and buffalo feces serve as breeding substrates (Marchiori, 2006c).

5.0. DISCUSSION

The results of this synthesis demonstrate that decomposing organic substrates represent important microhabitats for Diptera and their hymenopteran parasitoids in Brazil. The main contribution of this article is taxonomic and faunistic: it organizes parasitoid families, host records, substrate associations, and parasitism information reported in experimental studies. Bovine feces, buffalo feces, human feces, chicken manure, chicken viscera, bovine kidney, bovine liver, fish, and fruits supported different host–parasitoid systems. These substrates varied in physical structure, moisture, nutrient availability, decomposition stage, and attractiveness to flies, which helps explain differences in host occurrence and parasitoid records communities (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Bovine and buffalo feces were among the most important substrates for muscoid Diptera, especially species of Muscidae, Sarcophagidae, Sepsidae, and Sphaeroceridae. The dominance of *Palaeosepsis* spp. and *S. occidua* in cattle dung confirms that these taxa are strongly adapted to dung environments. The abundance of these flies across different exposure periods suggests that fecal pats provide suitable conditions for colonization over several stages of decomposition. However, the temporal distribution of flies was not uniform, indicating that the age of the fecal substrate plays an important role in structuring dipteran communities (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The significant chi-square values recorded for fly abundance among exposure periods reinforce the importance of substrate age in determining host availability. Fresh and intermediate-aged feces generally supported higher numbers of flies, probably because they provide higher moisture and more accessible nutritional resources for larval development. As decomposition progresses, the physical and chemical characteristics of the substrate change, influencing both fly colonization and parasitoid activity. Therefore, dung exposure time must be considered an important ecological variable in studies involving dung-breeding Diptera and their parasitoids (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The parasitoid fauna recorded in the analyzed studies was taxonomically diverse, including representatives of Braconidae, Chalcididae, Diapriidae, Encyrtidae, Figitidae, and Pteromalidae. This diversity indicates that several parasitoid lineages exploit dipteran hosts in decomposing substrates. Species such as *Aphaereta* sp., *B. podagrica, N. vitripennis, N. splendens, P. vindemmiae, P. egeria, S. drosophilae, S. nigra*, and *T. coxalis* showed faunistic relevance because they were repeatedly associated with hosts of sanitary, veterinary, or agricultural importance (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The occurrence of gregarious parasitoids, especially *Aphaereta* sp. and *N. vitripennis*, requires careful interpretation of parasitism data. In gregarious species, several parasitoid individuals may emerge from a single host pupa. Therefore, the number of emerged parasitoids is not always equivalent to the number of parasitized pupae. This distinction is essential to avoid overestimating parasitism rates. In the present synthesis, corrected interpretations were applied whenever possible, separating parasitoid abundance from parasitized host pupae (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Several studies showed significant host or substrate preferences. For example, parasitoid distributions differed among substrates such as bovine kidney, chicken viscera, fish, and human feces. These differences may reflect host availability, host suitability, parasitoid searching behavior, and the chemical attractiveness of each substrate. Chicken viscera and fish were especially important for Calliphoridae and Sarcophagidae hosts and supported high parasitoid emergence in some studies. These substrates are rich in decomposing animal protein and may attract both flies and parasitoids in large numbers (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The records involving *P. egeria* are particularly important because this species was associated with several hosts in bovine and buffalo dung. Although its parasitism rates were generally low, its broad host range suggests ecological flexibility. The association with *A. scabra, B. quadristigma, C. pararescita, H. irritans, Palaeosepsis* spp., and *S. occidua* indicates that *P. egeria* may exploit different dipteran families in livestock environments. Such flexibility is relevant for understanding parasitoid persistence in heterogeneous habitats (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

First records and new host associations also contributed significantly to the synthesis. Records of *T. coxalis* in *C. pararescita*, *S. nigra* in *C. pararescita, N. splendens* in *O. thornax*, and *Z. incompleta* in Brazil expanded the known host range, habitat use, and geographic distribution of these parasitoids. Even when parasitism rates were low, these records are biologically important because they document new ecological interactions and provide baseline information for future (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Fruit-fly systems showed a different ecological pattern when compared with animal-derived substrates. In fruits, *A. fraterculus* and *C. capitata* were the main hosts, while *A. pelleranoi*, *D. areolatus*, and *P. vindemmiae* were recorded as parasitoids. The higher parasitism observed in *A. fraterculus* suggests that host identity and fruit species influence parasitoid success. These findings are relevant because fruit flies are economically important pests, and their parasitoids may contribute to natural biological control in fruit production systems (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

The taxonomic article included in this synthesis plays a different but complementary role. Unlike the ecological studies, it does not provide abundance data suitable for chi-square analysis. However, it provides diagnostic characters and an identification key for Cynipoidea parasitoids associated with dung flies in Brazil. This taxonomic foundation is essential because accurate identification supports all ecological interpretations. Without reliable taxonomy, host records, distributional data, and biological control evaluations may become uncertain (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

Overall, the evidence indicates that substrate type, host species, environmental conditions, and sampling method strongly influence parasitoid diversity and parasitism rates. Bovine feces and buffalo feces supported rich communities of dung-breeding Diptera and parasitoids, while animal-derived baits such as bovine kidney, bovine liver, chicken viscera, and fish revealed additional host–parasitoid associations. Fruits supported economically important Tephritidae and their parasitoids. Together, these systems show that decomposing organic substrates function as important ecological arenas for interactions between flies and their natural enemies (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

From an applied perspective, the parasitoids recorded in these studies may contribute to the natural regulation of synanthropic flies. Many of the dipteran hosts are associated with livestock, human environments, decomposing organic matter, or fruit production. Therefore, understanding their parasitoids is relevant for integrated pest management and biological control. Although not all parasitoids showed high parasitism rates, their repeated occurrence across substrates and environments suggests that they are important components of natural enemy communities in Brazil (Marchiori, 2021a; Marchiori, 2021b; Malheiros and Marchiori, 2026; Marchiori and Malheiros, 2026a; Marchiori and Malheiros, 2026b; Marchiori and Malheiros, 2026c).

In conclusion, the analyzed studies show that Brazil harbors a diverse fauna of parasitoids associated with Diptera in decomposing substrates. The combination of ecological surveys, first host records, taxonomic identification, and statistical analyses provide a broad view of host–parasitoid interactions in dung, carrion-like substrates, poultry environments, and fruits. These findings reinforce the need for continued field surveys, accurate taxonomic identification, and careful numerical analysis to improve the understanding of parasitoid ecology and their potential role in biological control.

6.0. CONCLUSION

The experimental records analyzed in this study demonstrate that decomposing organic substrates in Brazil support a diverse fauna of Diptera and hymenopteran parasitoids. Bovine feces, buffalo feces, human feces, chicken manure, animal-derived substrates, and fruits acted as important breeding sites for flies and as ecological reservoirs for their natural enemies. The parasitoids recorded belonged mainly to Braconidae, Chalcididae, Diapriidae, Encyrtidae, Figitidae, and Pteromalidae, showing broad faunistic and taxonomic importance. Several records expanded the known distribution, host range, and substrate associations of parasitoids in Brazil. The synthesis also emphasizes that gregarious parasitoids require careful numerical interpretation because parasitoid abundance and the number of parasitized pupae are not always equivalent. Overall, these records reinforce the importance of accurate taxonomy, standardized methods, and host documentation for understanding parasitoid diversity and the natural regulation of Diptera in Brazilian environments.

Figure 25: Integrative schematic illustration of decomposing organic substrates used by Diptera and associated hymenopteran parasitoids in Brazil. The figure shows bovine feces, buffalo feces, human feces, chicken manure, chicken viscera, bovine liver, bovine kidney, fish, and fruits as breeding substrates for dipteran larvae and pupae, as well as sites for parasitoid activity. Adult flies, immature stages, pupae, and black four-winged parasitoids are represented to emphasize host development and host–parasitoid associations in decomposing organic matter

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