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Reanalysis of Diptera–Parasitoid Interactions in Decomposing Organic Substrates in Brazil Using Complementary Chi-Square Analyses

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Abstract: Synanthropic Diptera develop in decomposing organic substrates and include species of sanitary, veterinary, economic, and forensic importance. Their parasitoids are important natural enemies and may contribute to the regulation of fly populations in rural, urban, pasture, forest, and carrion-associated environments. This study synthesized experimental data from previous field and laboratory studies conducted in Brazil, mainly in Goiás and Minas Gerais, involving Diptera and their parasitoids associated with cattle dung, buffalo dung, human feces, food remains, animal tissues, fruit, and carcasses. The compiled datasets included information on host species, parasitoid species, substrate type, sampling environment, trap model, number of pupae, emerged parasitoids, and parasitism rates. In addition to organizing the original biological records, complementary chi-square analyses were applied to evaluate differences in parasitoid abundance, parasitism rate, substrate preference, trap efficiency, habitat, locality, dung exposure time, and host association. The results showed that Diptera and parasitoid occurrence varied according to substrate type, region, habitat, trap model, exposure time, and host availability. Higher parasitoid abundance was associated with cattle dung in pasture areas, older dung pats, and specific host–parasitoid associations. Organic substrates strongly influenced Diptera abundance, and different traps sampled different components of parasitoid communities. The complementary statistical analyses provided support for ecological patterns that had previously been interpreted mainly in descriptive form. Overall, the study reinforces the importance of parasitoids as natural enemies of synanthropic Diptera and highlights their potential relevance in biological control, sanitary management, veterinary entomology, and forensic studies involving decomposing organic substrates.

Keywords: Biological Control, Cattle Dung, Chi-Square Test, Decomposing Substrates, Host–Parasitoid Interaction; Synanthropic Flies.

RESEARCH PAPER

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1.0. INTRODUCTION

Synanthropic Diptera are insects of great sanitary, veterinary, economic, and forensic importance because many species develop in decomposing organic substrates and may occur in close association with humans, domestic animals, livestock systems, and urban environments. Cattle dung, buffalo dung, human feces, carrion, food remains, animal tissues, and fruit residues can act as breeding substrates for different fly families, including Calliphoridae, Fanniidae, Muscidae, Phoridae, Sarcophagidae, and Sepsidae. These substrates provide nutrients and suitable microhabitats for larval development, but they also create conditions for the occurrence of natural enemies, especially parasitoids associated with immature stages of Diptera (Marchiori, 2001a; Marchiori *et al.*, 2004a; Marchiori, 2014a; Jaume-Schinkel and Mengual, 2022).

Parasitoids are important natural regulators of fly populations because they attack larvae or pupae and reduce the emergence of adult Diptera. Several groups of Hymenoptera, such as Braconidae, Chalcididae, Diapriidae, Encyrtidae, Figitidae, and others, have been recorded in association with dipterous hosts developing in dung, carcasses, food remains, and other decomposing substrates. Some species, including *Brachymeria podagrica* (Fabricius, 1787) (Hymenoptera: Chalcididae), *Gnathopleura quadridentata* (Wharton, 1986) (Hymenoptera: Braconidae), *Hemencyrtus herbertii*, Ashmead 1900 (Hymenoptera: Encyrtidae), *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae), *Neralsia splendens* (Borgmeier, 1935) (Hymenoptera: Figitidae), *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae), *Spalangia cameroni* Perkins, 1910 (Hymenoptera:

Pteromalidae), *Spalangia drosophilae* Ashmead, 1885 (Hymenoptera: Pteromalidae), and *Spalangia nigroaenea* Curtis, 1839 (Hymenoptera: Pteromalidae) are especially relevant because of their association with flies of medical, veterinary, and forensic importance. Recent studies have reinforced the role of dipteran parasitoids as biological control agents and have shown that host specificity, competition, longevity, and fitness traits may influence their efficiency under natural and applied conditions (Marchiori *et al*., 2004b; Schuster and Sivakumar, 2024; Cingolani *et al*., 2025; Hopper and Leung, 2026; Teng *et al*., 2026).

The occurrence and abundance of Diptera and their parasitoids may vary according to substrate type, host availability, habitat structure, seasonality, exposure time, and sampling method. In pasture environments, the age of cattle dung can influence host development and parasitoid colonization, since older dung pats may contain more pupae available for parasitism. In urban and rural areas, the type of decomposing substrate can determine which Diptera species are attracted and which parasitoids can exploit them. In forensic environments, carrion-associated Diptera and their parasitoids may also influence immature fly survival and decomposition dynamics, which are relevant to ecological and forensic interpretations (Marchiori, 2014b; Jaume-Schinkel and Mengual, 2022; Guimarães *et al*., 2026; Huang *et al*., 2026).

Sampling methods are also important in studies of parasitoid diversity, because different traps may collect different components of the insect community. Malaise traps, yellow bowl traps, pitfall traps, baited traps, and dung-associated collection methods differ in their efficiency according to insect behavior, flight activity, body size, attraction to color, attraction to bait, and proximity to the host substrate. For this reason, comparisons among trap types and sampling environments are essential for understanding the structure of parasitoid communities. The use of complementary methods can increase the accuracy of surveys and improve the detection of host–parasitoid associations in forest fragments, pastures, corrals, urban environments, and decomposing organic substrates (Marchiori *et al*., 2004b; Marchiori, 2014c; Österman *et al*., 2026).

Although many previous studies have recorded parasitoids associated with Diptera in Brazil, several datasets were originally presented mainly in descriptive form, with emphasis on species occurrence, abundance, host association, and parasitism rate. Reorganizing these data and applying complementary statistical analyses can provide stronger support for ecological patterns involving region, habitat, substrate preference, trap efficiency, dung exposure time, parasitoid abundance, and parasitism rate. Therefore, the present study aims to compile and reinterpret data from previous studies on Diptera and their parasitoids associated with decomposing organic substrates in Brazil, emphasizing host–parasitoid associations, sampling methods, substrate use, parasitism rates, and complementary chi-square analyses applied to ecological patterns previously described mainly in descriptive terms.

2.0 MATERIALS AND METHODS

2.1 General Approach

This study is a synthesis of experimental research conducted under field and laboratory conditions, focusing on parasitoid–Diptera interactions in different environments in Brazil, especially in Goiás, Minas Gerais, and other sampled regions. Data were compiled from multiple studies that used standardized procedures, including baited traps, decomposing organic substrates, cattle dung exposure, pitfall systems, Malaise traps, yellow bowl traps, and laboratory rearing of Diptera pupae. All studies followed similar methodological steps: exposure or collection of organic substrates in the field, recovery of dipterous pupae, transfer of pupae to the laboratory, emergence monitoring, and identification of adult Diptera and parasitoids. Only studies conducted under field or semi-field conditions, involving decomposing substrates and reporting parasitoid emergence with quantitative data, were included in the synthesis.

The compiled database included information on host species, parasitoid species, substrate type, sampling environment, locality, number of pupae collected, number of parasitoids emerged, parasitized pupae, and parasitism rate. The analyses evaluated parasitoid diversity, host–parasitoid relationships, substrate use, trap efficiency, environmental variation, and differences in parasitism rates among studies. Although methodological variation among studies may influence the results, the use of standardized inclusion criteria allowed consistent comparisons among datasets.

2.2 Experimental Methods

1. Baited can traps/Ferreira (1978)/ Dark Can Trap

The experimental studies included in this synthesis used different field sampling methods to collect Diptera and their parasitoids in decomposing organic substrates and natural environments. The main substrates used were cattle dung, chicken manure, food remains, human feces, cattle kidney, fish, chicken, and cattle liver. These substrates were exposed under field or semi-field conditions to attract adult Diptera, allow larval development, and obtain pupae for laboratory rearing.

2. Tray/Substrate Traps

In studies using cattle dung, fresh bovine feces were collected immediately after defecation, homogenized, and placed in round plastic supports or exposed directly in pasture and corral areas. The fecal cakes remained exposed for defined periods, commonly up to 15 days, before being transported to the laboratory for pupal extraction. In experiments with chicken manure and food remains, the substrates were placed in plastic basins and maintained under field conditions until pupae could be recovered.

3. Carcass Trap

Pig carcasses were used as bait in forensic-related studies. The carcasses were exposed under environmental conditions inside metal mesh cages that allowed insect access but prevented interference by larger animals. Trays containing sand were placed below the carcasses to provide a pupation substrate. Pupae were collected from the sand using sieves and tweezers and were then taken to the laboratory.

4. Pitfall Systems, Yellow Bowl Traps (Moericke), and Malaise Traps

Different trap types were used according to the objective of each study. Ferreira-type traps consisted of dark cans with lateral openings, a nylon funnel at the top, and plastic bags for insect collection. These traps were baited with decomposing substrates and suspended approximately one meter above the ground. Pitfall systems, yellow bowl traps, and Malaise traps were also used. Yellow bowl traps consisted of yellow plastic basins containing water, detergent, and formalin, and were used mainly for sampling parasitoid Hymenoptera. Malaise traps were used to intercept flying insects in forest fragments and open environments. The insects were collected in a Malaise trap. The collecting flasks were put out at 6:00 P.M. and replaced by new ones at 7:00 A.M. every day for ten consecutive days, thus totaling 40 collections between May and August, 2005.

5. Flotation Method

After collection, substrates were processed in the laboratory to recover dipterous pupae, mainly by flotation, sieving, or manual collection. The pupae were counted and individually placed in gelatin capsules or acrylic vials until the emergence of adult flies or parasitoids. Emerged insects were preserved in 70% alcohol or sacrificed with ethyl ether, depending on the original method, and later identified using stereoscopic microscopy and taxonomic literature.

6. Food Scraps of Plant and Animal Origin (Washings)

The basins were exposed to the sun and shade for part of the day. To reduce the escape of the larvae, each basin was placed on a larger tray containing sand for the pupation of the larvae. The food scraps were placed in the field 12 times. The pupae were collected fortnightly from the food scraps, after fifteen days of permanence in the basins. The pupae were obtained by the flotation method. The collected pupae were individually placed in gelatin capsules and kept there until the emergence of the flies and/or parasitoids.

2.3. Analysis of Variance (ANOVA)

Results reported in the original articles were maintained when available and were used to support comparisons involving host preference, sampling period, parasitoid families, and abundance patterns. Statistical analyses originally reported in the reviewed studies were maintained. In studies where parasitoid preference for diurnal or nocturnal periods, host groups, trap types, or substrates had been analyzed by analysis of variance, the original ANOVA results were reported, including data transformation to $\sqrt{x} + 0.5$ and significance at the 5% probability level when stated by the authors. Complementary chi-square analyses were performed only when the reviewed articles presented quantitative data suitable for comparison and when no statistical test had been reported or when additional categorical comparisons were possible.

2.4. Note

Schematic illustrations. Wing venation was omitted or simplified in schematic figures for clarity and does not represent diagnostic morphological variation among parasitoid taxa.

3.0. RESULTS

The data were organized into comparative datasets according to locality, substrate, host species, parasitoid species, trap type, habitat, sampling period, and dung exposure time. Absolute abundance, relative frequency, number of pupae, number of parasitized pupae, and parasitism rate were calculated when the original data allowed these estimates. Parasitism rate was calculated using the formula: Parasitism rate (%) = number of parasitized pupae / total number of pupae × 100. Relative frequency was calculated as the number of individuals of each taxon divided by the total number of individuals in the sample, multiplied by 100.

Complementary chi-square analyses were performed to test differences in parasitoid abundance, parasitism rate, trap efficiency, substrate preference, host association, habitat, locality, and dung exposure time. These analyses were applied only when the available data were quantitative and suitable for comparison. When the dataset included only one parasitoid species, qualitative host associations, or incomplete numerical information, chi-square analysis was not applied. Statistical significance was considered when $p < 0.05$. The purpose of these complementary analyses was not to modify the original records, but to provide additional statistical support for ecological patterns previously described mainly in descriptive terms. Thus, the methodological approach combined original biological records with standardized data organization and statistical interpretation.

The datasets analyzed in the present study showed wide variation in the abundance of Diptera, pupae, parasitoids, host species, substrates, sampling environments, and trap types. The highest abundance of Diptera was recorded in the study using different organic substrates, with 4,080 specimens, especially in buffalo dung, human feces, fish, and cattle dung. In studies based on pupae collected from cattle dung, parasitism rates varied among localities, substrates, and sampling conditions, indicating that ecological and methodological factors influenced parasitoid occurrence.

In addition to compiling the original biological data from previous studies, the present manuscript performed complementary chi-square analyses to test patterns of variation in parasitoid abundance, parasitism rate, trap efficiency, substrate preference, exposure time, and host association. These analyses represent an additional contribution of the present study, because several of the original datasets were previously interpreted mainly in descriptive terms. Thus, the results presented here combine the original records with new statistical support for ecological patterns involving Diptera hosts and their parasitoids (Table 1).

Table 1: Summary of the datasets analyzed in the manuscript, including locality, substrate or sampling method, total sample size, main taxa or association, and principal result

Dataset/topic	Locality	Substrate or sampling method	Total sample	Main taxa or association	Main result
First occurrence of *Neralsia splendens*	Itumbiara, Goiás; Uberlândia, Minas Gerais	Bovine feces / dipterous pupae	26 pupae in one record; 98 parasitoids in the expanded dataset	*Neralsia splendens* is associated mainly with Sarcophagidae hosts	Low parasitism rate in Itumbiara and higher occurrence in Uberlândia.
Synanthropic Diptera associated with food remains	Urban/peri-urban environment	Decomposing food remains	425 pupae; 10 parasitized pupae	*Musca domestica* parasitized by *Pachycrepoideus vindemmiae* and *Muscidifurax raptor*	Overall parasitism rate of 2.4%; M. domestica was the only parasitized host.
Parasitoids in cattle dung, southern and central Goiás	Southern and central regions of Goiás	Cattle dung	987 pupae; 101 parasitoids	*Gnathopleura quadridentata, Spalangia* sp., *Aleochara notula, Nasonia vitripennis*	Southern Goiás had a higher abundance and parasitism rate than central Goiás.
Eucoilinae collected with Malaise traps	Itumbiara-GO, Araporã-MG, and Lavras-MG	Malaise traps in forest remnants	90 specimens	*Rhabddeucoela, Steleucoela, Zaeucoila,* and other Eucoilinae	Species composition varied among localities.
Cynipoidea collected with a yellow bowl and Malaise traps	Itumbiara, Goiás	Forest remnant; two trap types	56 Cynipoidea specimens	Eucoilinae predominated; *Zaeucoila* was abundant in yellow bowl traps	Yellow bowl traps collected more specimens than Malaise traps.
Calliphorid pupae from carcasses	Lavras, Minas Gerais	Pig carcasses in a native forest	595 Calliphoridae pupae	*Chrysomya,* and *Hemilucilia segmentaria*; *Trichopria, Spalangia,* and *Pachycrepoideus vindemmiae*	Parasitoid occurrence differed among recorded taxa.
Ophyra aenescens pupae	Goiás, Brazil	Traps baited with human feces	20 pupae; 4 parasitized pupae	*Brachymeria podagrica* parasitizing *Ophyra aenescens*	Parasitism rate of 20.0%; only one parasitoid species was recorded.
Diurnal and nocturnal parasitoid collections	Southern Goiás	Cattle dung; Malaise trap	135 parasitoids	Several hymenopteran superfamilies and families	No clear preference for the diurnal or nocturnal period was reported.
Chalcididae in the forest and pasture	Itumbiara, Goiás	Yellow pan traps in forest and pasture areas	121 specimens	*Haltichella, Conura*, and *Brachymeria* taxa	Pasture areas presented more

Dataset/topic	Locality	Substrate or sampling method	Total sample	Main taxa or association	Main result
					Chalcididae than forest areas.
Cattle dung exposure time	South Goiás	Dung pats exposed for 24 to 240 hours	100 dung pats; parasitoids recorded by exposure period	*Paraganaspis egeria* and *Spalangia drosophilae* were frequent	Older dung pats, especially 192-240 h, supported higher parasitoid occurrence.
Urban Diptera parasitoids	Goiânia, Goiás	Urban dipterous pupae	358 pupae; 82 parasitoids	*Brachymeria podagrica, Hemencyrtus herbertii, Tachinobia,* and *Pachycrepoideus vindemmiae*	*Brachymeria podagrica* was the dominant parasitoid.
Pasture and corral cattle dung	Itumbiara, Goiás	Cattle dung in pasture and corral areas	277 parasitoids	*Triplasta atrocoxalis, Spalangia nigroaenea, Spalangia cameroni,* and others	Pasture areas supported more parasitoids than corral areas.
Diptera associated with organic substrates	Itumbiara, Goiás	Cattle dung, buffalo dung, human feces, kidney, liver, fruit, and fish	4,080 Diptera specimens	*Palaeosepsis, Sarcophagula, Oxysarcodexia,* and other Diptera	Buffalo dung was the most attractive substrate.
Parasitoid families sampled in Araporã	Araporã, Minas Gerais	Yellow pan and Malaise traps	9,747 parasitoid specimens	Diapriidae, Ichneumonidae, Braconidae, and other families	Malaise traps collected more individuals than yellow pan traps.
Two trap models for Diptera parasitoids	Southern Goiás	Ferreira (1978) trap and pitfall traps	400 parasitoids	*Pachycrepoideus vindemmiae* in Ferreira trap; *Gnathopleura quadridentata* in pitfall traps	Trap type influenced parasitoid species composition.
Cattle dung in Goiânia, central Goiás	Goiânia, Goiás	Cattle feces / dipterous pupae	359 pupae; 23 parasitized pupae	*Aleochara notula, Nasonia vitripennis, Neralsia splendens,* and *Spalangia*	Overall parasitism rate of 6.4%.

3.0.1 Chi-Square Analysis

In cattle dung from the southern and central regions of Goiás, a total of 987 dipterous pupae and 101 parasitoids were recorded. The southern region presented higher abundance, with 628 pupae and 78 parasitoids, whereas the central region presented 359 pupae and 23 parasitoids. The parasitism rate was also higher in the southern region (12.4%) than in the central region (6.4%). The complementary chi-square analysis indicated a significant difference in parasitoid abundance between regions ($\chi^2 = 29.95$; df = 1; $p < 0.0001$) and also in parasitism rate ($\chi^2 = 9.03$; df = 1; $p = 0.0027$). These results indicate that the southern region supported greater parasitoid abundance and higher parasitism than the central region.

The analyses also showed that the sampling environment influenced parasitoid abundance. In cattle dung collected from pasture and corral areas in Itumbiara, Goiás, 277 parasitoids were obtained, with a higher number in pasture areas (188 individuals; 67.9%) than in corral areas (89 individuals; 32.1%). This difference was statistically significant ($\chi^2 = 35.38$; df = 1; $p < 0.0001$), indicating that pasture environments supported greater parasitoid abundance than corral environments. This pattern may be related to greater availability of exposed cattle dung, higher host density, and more favorable environmental conditions for parasitoid activity in pasture areas.

Trap type also influenced the abundance and composition of parasitoids. In the study with Cynipoidea collected in a forest remnant in Itumbiara, yellow bowl traps collected more specimens than Malaise traps, with 35 and 21 individuals, respectively. The difference between trap types was significant ($\chi^2 = 6.29$; df = 1; $p < 0.0001$). In addition, the distribution of Cynipoidea taxa differed significantly between trap types ($\chi^2 = 46.47$; df

= 11; $p < 0.0001$), indicating that each trap sampled different components of the parasitoid community. These results reinforce that the sampling method can affect both the number of parasitoids collected and the taxonomic composition observed in faunistic surveys.

In the comparison between diurnal and nocturnal sampling periods, 135 parasitoid specimens were collected in cattle dung in southern Goiás. Of these, 53 individuals were collected during the diurnal period and 82 during the nocturnal period. Although nocturnal collections were numerically higher, the original analysis indicated no significant preference of parasitoid specimens or families for either period. This suggests that parasitoid activity may occur during both periods and may depend on environmental conditions, host availability, microclimate, and the behavior of each parasitoid group.

The duration of cattle dung exposure in pasture strongly affected parasitoid occurrence. Parasitoids were more abundant in older dung pats, especially after 192 h, 216 h, and 240 h of exposure. The highest abundance was recorded at 240 h, followed by 192 h and 216 h. The chi-square analysis showed a highly significant difference among exposure periods ($\chi^2 = 88.07$; df = 9; $p < 0.0001$), indicating that dung age influenced parasitoid colonization and emergence. This result suggests that older dung pats provide more suitable conditions for the development of dipterous hosts and, consequently, increase the availability of pupae for parasitoid development.

Organic substrate also had a strong effect on Diptera abundance. Among the 4,080 Diptera specimens collected using different organic substrates, buffalo dung supported the highest abundance, followed by human feces, fish, and cattle dung. The chi-square analysis showed a highly significant difference among substrates ($\chi^2 = 9,325.6$; df = 6; $p < 0.0001$), demonstrating that substrate type strongly influenced fly attraction and occurrence. These results indicate that fecal substrates, especially buffalo dung, were more attractive or suitable for the sampled Diptera than animal tissues or fruit.

In urban environments, 358 dipterous pupae were collected in Goiânia, Goiás, from which 82 parasitoid individuals emerged. *B. podagrica* was the most abundant parasitoid, with 53 individuals, followed by *H. herbertii*, *Tachinobia* sp. (Hymenoptera: Eulophidae), and *P. vindemmiae*. The chi-square analysis showed a highly significant difference between parasitized and non-parasitized pupae ($\chi^2 = 158.61$; df = 1; $p < 0.0001$) and among parasitoid species ($\chi^2 = 66.00$; df = 3; $p < 0.0001$), with *B. podagrica* being the dominant species. These results show that urban environments can support parasitoid species associated with synanthropic Diptera, although parasitoid abundance may be concentrated in a few dominant taxa.

In the study of *Ophyra aenescens* (Wiedemann, 1830) (Diptera: Muscidae), 20 pupae were collected using traps baited with human feces, and four specimens of *B. podagrica* emerged, corresponding to a parasitism rate of 20.0%. The chi-square analysis showed a significant difference between parasitized and non-parasitized pupae ($\chi^2 = 7.20$; df = 1; $p = 0.0073$), with non-parasitized pupae being more frequent. Because only one parasitoid species was recorded, comparisons among parasitoid species were not applicable. Even so, the record indicates that *O. aenescens* may serve as a suitable host for *B. podagrica* under synanthropic conditions.

For calliphorid pupae collected from pig carcasses in Lavras, Minas Gerais, 595 pupae were recorded, mainly belonging to *Chrysomya* Robineau-Desvoidy, 1830 and *Hemilucilia segmentaria* (Fabricius, 1805) (Diptera: Calliphoridae). Three parasitoid taxa were obtained: *Trichopria* sp. (Hymenoptera: Diapriidae), *Spalangia* sp. (Hymenoptera: Pteromalidae), and *P. vindemmiae*. The chi-square test showed a significant difference in parasitoid occurrence among the recorded taxa ($\chi^2 = 15.38$; df = 2; $p < 0.001$), with *Trichopria* sp. being the most frequent parasitoid. These results are relevant because calliphorid flies are associated with decomposing carcasses and have forensic importance. At the same time, parasitoids may influence the survival of immature flies and population dynamics in carrion environments.

The analyses of parasitoid families and taxa collected with Malaise traps and yellow pan traps also showed significant variation in abundance and taxonomic composition. In Araporã, Minas Gerais, a total of 9,747 parasitoid specimens were collected, with Malaise traps capturing more individuals than yellow pan traps. In Itumbiara, Goiás, Cynipoidea abundance and composition also differed between trap types. These results indicate that the sampling method is an important factor affecting the observed structure of parasitoid communities, because different traps may capture insects with different flight behavior, body size, habitat use, and attraction responses.

In the article "First Occurrence of *Neralsia splendens* as parasitoids in Brazil", the analysis of variance showed that Neralsia splendens presented a statistically significant preference for hosts belonging to Sarcophagidae in both studied locations ($F = 5.59$; $P < 0.0001$). This result indicates that the host family influenced the occurrence of this parasitoid, suggesting a stronger association with sarcophagid hosts under the evaluated conditions. In the article "Parasitoid Hymenoptera collected during the diurnal and nocturnal periods in cattle dung in southern Goiás, Brazil", the analysis of variance indicated that the total number of specimens did not differ significantly between diurnal and nocturnal periods ($F = 0.73$; $P > 0.39$). Similarly, parasitoid families showed no significant preference for either sampling period (F = 1.26; P > 0.24). However, during the diurnal period, Ichneumonidae was the most abundant family, representing 56.5% of the individuals collected, with a statistically significant predominance in relation to the other families (F = 9.23; P < 0.0001).

In the study on parasitoid Hymenoptera collected during diurnal and nocturnal periods in cattle dung in southern Goiás, Brazil, the original analysis of variance was maintained. The preference of parasitoids for the diurnal or nocturnal period was evaluated using ANOVA, with data transformed to $\sqrt{x} + 0.5$ at the 5% probability level. The number of specimens did not differ significantly between periods (F = 0.73; P > 0.39), and parasitoid families also showed no significant preference for either period (F = 1.26; P > 0.24). However, during the diurnal period, Ichneumonidae was the most abundant family, representing 56.5% of the individuals collected, with significant predominance in relation to the other families (F = 9.23; P < 0.0001).

Overall, the results demonstrate that parasitoid abundance, parasitism rate, host association, and Diptera occurrence were strongly influenced by region, habitat, substrate type, trap type, exposure time, and host availability. The complementary chi-square analyses added in the present manuscript provided statistical support for patterns that were previously interpreted mainly in descriptive form. Therefore, the main innovation of the present study was the integration of dispersed biological records with additional statistical analyses, allowing a clearer interpretation of ecological patterns involving synanthropic Diptera and their parasitoids. These findings reinforce the ecological importance of parasitoids as natural enemies of Diptera in rural, urban, forensic, pasture, and decomposing organic substrate environments.

4.0. EXPERIMENTAL ARTICLES

1. First Occurrence of *Neralsia Splendens* as Parasitoids in Brazil

The hosts of Figitidae (Hymenoptera) are typically larvae of Cyclorrhaphous Diptera that develop in living plants or fungi, or in decomposing organic substrates such as rotting fruits, carrion, and dung. The hosts attacked also include aphid-feeding larvae of Chamaemyiidae (Diptera), parasitoids of aphids and psyllids belonging to Braconidae and Encyrtidae (Hymenoptera: Apocrita), as well as aphidophagous larvae of Neuroptera.

A total of 26 pupae of *Cyrtoneurina pararescita* (Couri, 1995) (Diptera: Muscidae) were collected from bovine feces, from which two specimens of *N. splendens* were recovered in Itumbiara, Goiás, Brazil. The parasitism rate was 0.35%, probably influenced by factors such as resource availability, host density, parasitoid searching capacity, and the proximity of agricultural areas. Forest and agricultural habitats may serve as important refuge and emergence sites for these parasitoids. *Neralsia splendens* may also present potential for host-range expansion and could therefore exert non-target effects on other beneficial parasitoids of Diptera (Figure 1) (van Noort, 2026).

Figure 1: Adult specimen of *Neralsia splendens*, a parasitoid associated with dipteran hosts developing in decomposing organic substrates. This species is considered an important natural enemy of synanthropic flies and may contribute to the biological regulation of Diptera populations. Source: Photographs and map illustration © Simon van Noort (Iziko Museums of South Africa), Matt Buffington, and Mattias Forshages

A total of 98 parasitoid specimens were obtained, of which 84 were collected in Uberlândia, Minas Gerais, and 14 in Itumbiara, Goiás. All identified parasitoids belonged to the species *N. splendens*. The parasitism rate in Uberlândia was 1.24%, whereas in Itumbiara it was 0.21%. In the article, the analysis of variance showed that *N. splendens* presented a statistically significant preference for hosts belonging to Sarcophagidae in both studied locations (F = 5.59; P < 0.0001). This result indicates that the host family influenced the occurrence of this parasitoid, suggesting a stronger association with sarcophagid hosts under the evaluated conditions.

The emergence of insecticide resistance has increased the need for alternative management strategies to control pest populations. In this context, natural regulators, such as parasitoids, are valuable biological control agents, as they reduce populations of pest insects (Marchiori and Linhares, 1999; Marchiori, 2014a).

2. Synanthropic Diptera Associated With Food Remains and Their Parasitoids

A total of 425 pupae of synanthropic Diptera associated with food remains were obtained, from which 101 adults emerged, corresponding to 23.8% of the collected pupae. Among the sampled pupae, 314 did not produce flies or parasitoids, while 10 pupae were parasitized, resulting in an overall parasitism rate of 2.4%. These results indicate that food remains constitute an important breeding substrate for synanthropic flies and may also support populations of natural enemies under suitable environmental conditions. The most frequent host species was *Megaselia* sp. (Diptera: Phoridae), representing 47.1% of the total collected pupae, followed by *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) (24.0%). The high abundance of *Megaselia* sp. may be associated with its strong capacity to exploit decomposing organic matter and rapidly colonize ephemeral substrates. Species of Phoridae are widely recognized as opportunistic colonizers of decaying substrates, especially in urban environments where organic waste is abundant.

Musca domestica L. 1758 (Diptera: Muscidae) represented 11.7% of the collected pupae and was the only host species parasitized, with 10 parasitized pupae and a parasitism rate of 20.0%. This elevated parasitism rate suggests that *M. domestica* may be a highly suitable host for parasitoid development, possibly due to its abundance, accessibility, and pupation behavior. Because *M. domestica* is one of the most important synanthropic flies worldwide, acting as a vector of pathogens of medical and veterinary importance, the occurrence of parasitoids attacking this species is of considerable ecological relevance (Table 2).

Table 2: Relationship of synanthropic flies associated with food remains and parasitism observed in collected pupae. Number of pupae, emerged adults, frequency, parasitism rate, and parasitized pupae

Host species	Total pupae	Parasitoid species	Parasitized pupae	Parasitism rate (%)
Chrysomya albiceps	1		0	0
Chrysomya megacephala	102		0	0
Fannia pusio	22		0	0
Hermetia illucens	50		0	0
Megaselia sp.	200		0	0
Musca domestica	50	*Pachycrepoideus vindemmiae*	9	18.0
	50	*Muscidifurax raptor*	1	2.0
Total	425		10	10.0

The parasitoids obtained were mainly *P. vindemmiae* and *Muscidifurax raptor* Girault & Sanders, 1910 (Hymenoptera: Pteromalidae), both recognized as important pupal parasitoids of synanthropic Diptera. *Pachycrepoideus vindemmiae* is considered a cosmopolitan and generalist parasitoid, capable of exploiting several dipteran families. This ecological plasticity increases its potential for use in biological control programs. Likewise, *M. raptor* has been widely reported as an efficient parasitoid of muscoid flies, particularly in livestock production systems (Figure 2).

Figure 2: Suspended metal trap containing decomposing food remains used to attract synanthropic flies and associated parasitoids. Arrows indicate representative adult fly and parasitoid wasp specimens collected on the substrate surface. This method is useful for ecological surveys and studies of biological control agents associated with Diptera

The absence of parasitism in the other recorded fly species may be related to low host density, reduced host suitability, interspecific competition, or environmental conditions unfavorable to parasitoid activity. In addition, some hosts may possess behavioral or physiological defenses that reduce successful parasitism. These factors demonstrate that parasitoid–host interactions are influenced by multiple ecological variables rather than host abundance alone (Figure 3).

Figure 3: Adult specimen of *Muscidifurax raptor*, a pupal parasitoid commonly associated with synanthropic Diptera. This species is an important natural enemy of muscoid flies and contributes to the biological regulation of pest populations. Source: https://pt.wikipedia.org/wiki/Ficheiro:Muscidifurax_raptor_laying_egg.jpg

Overall, the present results reinforce the ecological importance of parasitoids in decomposing organic substrates, where they may contribute to the natural suppression of fly populations. Food remains, frequently found in urban and peri-urban environments, may function not only as breeding sites for pest flies but also as habitats sustaining beneficial parasitoid communities. Further studies evaluating seasonal variation, host preference, and parasitoid efficiency under field conditions are necessary to better understand their role in integrated pest management programs (Marchiori and Silva, 2010).

3. Parasitoids of Dipterous Collected in Cattle Dung in the Southern and Central Regions of Goiás, Brazil

A total of 987 dipterous pupae and 101 parasitoids were collected from cattle dung in the southern and central regions of Goiás, Brazil. In the southern region, 628 pupae and 78 parasitoids were obtained, whereas in the central region, 359 pupae and 23 parasitoids were recorded. These results indicate a higher abundance of both hosts and parasitoids in the southern region. Table 4 shows that the most abundant parasitoid in the southern region was *G. quadridentata*, with 20 individuals, followed by *S. nigroaenea* (18 individuals) and *S. drosophilae*. In the central region, *Aleochara notula* Erichson, 1839 (Coleoptera: Staphylinidae) was the most abundant species, with 6 individuals, followed by *N. vitripennis* and *N. splendens*, with 4 individuals (Table 3).

Table 3: Parasitoid species collected from cattle dung in the southern and central regions of Goiás, Brazil

Taxonomic group	Parasitoid species	Southern region	Central region	Total
Coleoptera: Staphylinidae	*Aleochara notula*	0	6	6
Braconidae	*Gnathopleura quadridentata*	20	0	20
Diapriidae	*Trichopria* sp.	1	0	1
Figitidae	*Kleidotoma nigra*	2	0	2
	Neralsia splendens	0	4	4
	Paraganaspis egeria	2	1	3
	Triplasta atrocoxalis	2	0	2
	Triplasta coxalis	8	0	8
Pteromalidae	*Nasonia vitripennis*	0	4	4
	Pachycrepoideus vindemmiae	5	0	5
	Spalangia cameroni	4	2	6
	Spalangia drosophilae	7	4	11
	Spalangia endius	2	0	2
	Spalangia nigra	7	1	8
	Spalangia nigroaenea	18	0	18
	Spalangia sp.	0	1	1
Total		78	23	101

In the southern region, the highest parasitism rate occurred in *Oxysarcodexia thornax* (Walker, 1849) (Diptera: Sarcophagidae), with 20 parasitized pupae from 70 collected pupae, corresponding to 28.6%. Other relevant hosts were *Brontaea quadristigma* (Thomson, 1869) (Diptera: Muscidae), with a combined parasitism rate 14.3%, *Ravinia belforti* (Prado & Fonseca, 1832) (Diptera: Sarcophagidae), with 25 parasitized pupae from 63 collected pupae, and *Archisepsis scabra* (Loew, 1861) (Diptera: Sepsidae), with 10.0% parasitism rate (Figure 4) (Table 4).

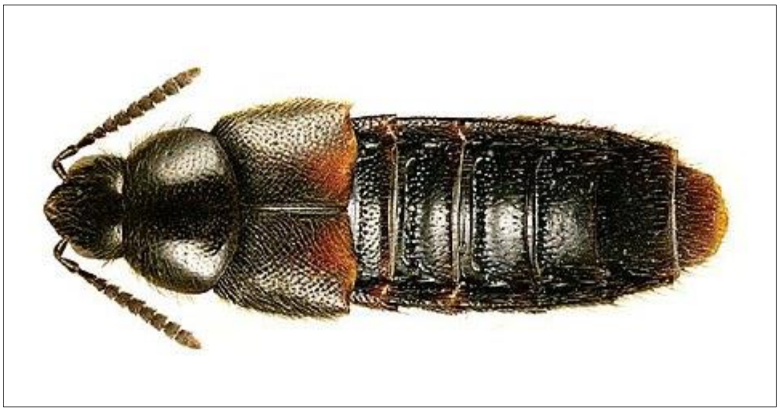

Figure 4: It is a beetle from the *Aleochara* (Staphylinidae), recognized as a natural enemy, acting as a parasitoid of fly pupae in buffalo feces, according to studies conducted in Goiás, Brazil. This species is a predator in the adult stage and a parasitoid in the larval stage. Source: https://en.wikipedia.org/wiki/Aleocharinae

Table 4: Host species, parasitoids, and parasitism rates recorded in cattle dung from the southern region of Goiás, Brazil

Host species	Pupae collected	Parasitoid species	Parasitized pupae	Parasitism rate (%)
Archisepsis scabra	40	*Spalangia drosophilae*	4	10.0
Brontaea debilis	56	*Spalangia cameroni*	1	1.8
		Spalangia nigroaenea	2	3.6
Brontaea quadristigma	49	*Kleidotoma nigra*	2	4.1
		Spalangia cameroni	1	2.0
		Spalangia drosophilae	1	2.0
		Spalangia endius	1	2.0
		Spalangia nigroaenea	5	10.2
Cyrtoneurina pararescita	151	*Spalangia nigra*	3	2.0
		Spalangia nigroaenea	5	3.3
Musca domestica	10	*Spalangia cameroni*	1	10.0
Oxysarcodexia thornax	70	*Gnathopleura quadridentata*	20	28.6
Palaeosepsis sp.	107	*Paraganaspis egeria*	2	1.9
		Spalangia drosophilae	2	1.9
		Spalangia endius	1	0.9
		Triplasta atrocoxalis	2	1.9
		Triplasta coxalis	8	7.5
		Trichopria sp.	1	0.9
Ravinia belforti	63	*Pachycrepoideus vindemmiae*	5	7.9
		Spalangia cameroni	1	1.6
		Spalangia nigra	4	6.3
		Spalangia nigroaenea	6	9.5
Total	**628**		**78**	**12.4**

In the central region, the highest parasitism rate was recorded for *C. pararescita*, mainly due to *N. vitripennis*, which parasitized 4 pupae, corresponding to 9.8%. In addition, *Palaeosepsis* spp. (Diptera: Sepsidae) showed a relevant parasitism rate, especially by *A. notula*, which parasitized 6 pupae, corresponding to 7.5%. The comparison between regions indicates that the southern region supported a greater abundance and diversity of parasitoids than the central region (Figure 5) (Table 5).

Table 5: Host species, parasitoids, and parasitism rates recorded in cattle dung from the central region of Goiás, Brazil

Host species	Pupae collected	Parasitoid species	Parasitized pupae	Parasitism rate (%)
Brontaea debilis	77	*Spalangia drosophilae*	2	2.6
Brontaea quadristigma	83	*Spalangia drosophilae*	1	1.2
		Spalangia cameroni	2	2.6
		Spalangia nigra	1	1.3
		Spalangia sp.	1	1.3
Cyrtoneurina pararescita	41	*Neralsia splendens*	2	4.9
		Nasonia vitripennis	4	9.8
Palaeosepsis spp.	80	*Aleochara notula*	6	7.5
		Spalangia drosophilae	1	1.3
Sarcophagula occidua	78	*Neralsia splendens*	2	2.6
		Paraganaspis egeria	1	1.3
Total	**359**		**23**	**6.4**

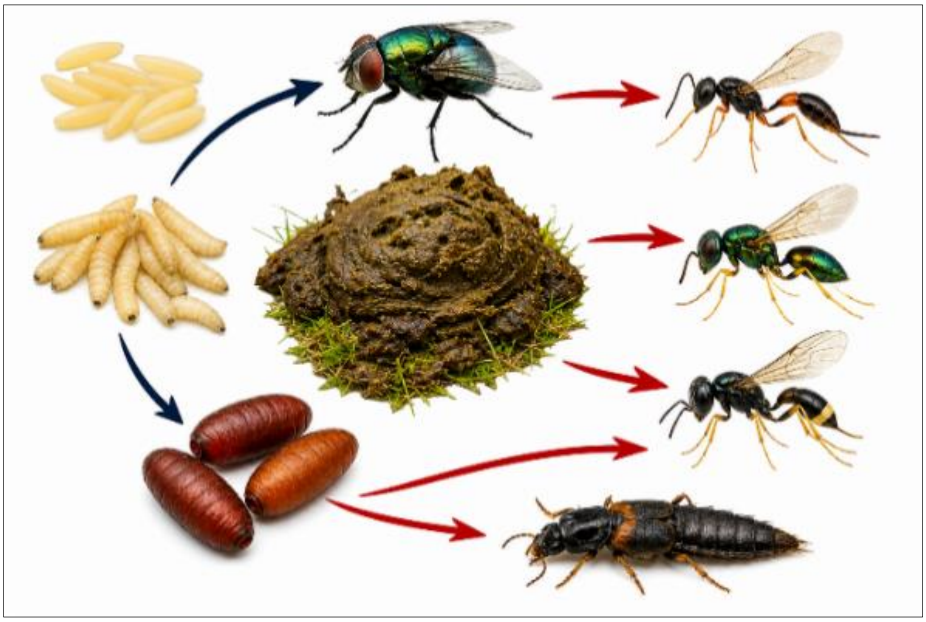

Figure 5: Life cycle of dipterous flies associated with cattle dung and their principal parasitoids recorded in the southern and central regions of Goiás, Brazil. Immature stages developing in dung serve as hosts for hymenopteran parasitoids and coleopteran natural enemies

This difference may be related to local ecological conditions, host availability, dung persistence, and environmental suitability for parasitoid development. There was a highly significant difference in parasitoid abundance between the southern and central regions of Goiás ($\chi^2 = 29.95$; df = 1; $p < 0.0001$), with more parasitoids collected in the southern region. The parasitism rate also differed significantly between regions ($\chi^2 = 9.03$; df = 1; $p = 0.0027$), being higher in the southern region (12.4%) than in the central region (6.4%).

Overall, the results demonstrate that cattle dung acts as an important substrate for dipterous hosts and their parasitoids in Goiás. The occurrence of several parasitoid taxa, especially *A. notula, G quadridentata*, *N. vitripennis*, *S. drosophilae,* and *S. nigroaenea*, reinforces the role of these natural enemies in regulating synanthropic fly populations associated with livestock environments (Figure 6) (Marchiori, 2014b).

Figure 6: Schematic representation of cattle dung in pasture environments as a breeding substrate for synanthropic Diptera and as a source of pupae parasitized by natural enemies. The figure illustrates the association between cattle dung, fly development, pupal stages, and parasitoid emergence in grazing systems

4. Eucoilinae (Hymenoptera: Figitidae) Parasitoids Collected in Itumbiara-GO, Araporã-MG, and Lavras-MG using a Malaise Trap

A total of 90 specimens of Eucoilinae parasitoids were collected in three forest remnants located in Itumbiara-GO, Araporã-MG, and Lavras-MG between February and October 2002. Lavras-MG presented the highest abundance, with 34 individuals (37.8%), followed by Araporã-MG with 30 specimens (33.3%) and Itumbiara-GO with 26 individuals (28.9%). The greater number of specimens obtained in Lavras-MG may be associated with more favorable vegetation structure, microclimatic conditions, and greater host availability for parasitoid development.

The relatively lower abundance recorded in Itumbiara-GO may reflect reduced habitat complexity or lower densities of dipterous hosts. Since Eucoilinae are parasitoids of Diptera larvae developing in decomposing substrates, their occurrence is strongly influenced by the availability of organic matter and host populations. These results reinforce the ecological importance of preserved forest fragments as reservoirs of parasitoid diversity and natural biological control agents (Table 6).

Table 6: Absolute numbers are presented by locality, and the relative frequency was calculated from the total of 90 collected specimens

Species or genus	Itumbiara-GO	Araporã-MG	Lavras-MG	Frequency (%)	Total
Aganaspis pelleranoi	1	1	8	11.1	10
Dettmeria	1	0	4	55.6	5
Dieucoila	2	0	0	5.6	2
Kleidotoma	0	0	2	2.2	2
Odonteucoila	5	1	1	7.8	7
Odontosema anastrepha	0	1	0	1.1	1
Paraganaspis egeria	0	0	3	3.3	3
Prosaspicera	0	1	0	1.1	1
Rhabddeucoela	7	9	0	17.8	16
Steleucoela	2	2	14	20.0	18
Triplasta	0	1	0	1.1	1
Tropideucoila	1	1	0	2.2	2
Trybliographa	1	0	0	1.1.	1
Trybliographa infuscata	1	0	0	1.1	1
Zaeucoila	5	4	2	12.2	11
Zaeucoila triangulifera	0	2	0	2.2	2
Zaeucoila unicarinata	0	7	0	7.8	7
Total	**26**	**30**	**34**	**100**	**90**

Among the species collected, *Rhabddeucoela* accounted for 7.7% in Itumbiara-GO and 10.0% in Araporã-MG, while *Steleucoela* was the dominant species in Lavras-MG, representing 15.5% of the specimens collected. These genera are recognized as important parasitoids of larvae associated with decomposing substrates and fruit-infesting Diptera. Species distribution among the three localities was statistically significant ($\chi^2 = 82.04$; df = 32; $P < 0.05$), indicating heterogeneity in species composition and abundance.

This variation suggests that local environmental characteristics, vegetation type, altitude, and host availability influenced the structure of Eucoilinae communities in each municipality. The use of Malaise traps proved efficient for capturing Eucoilinae parasitoids in native vegetation areas. This method is particularly useful for surveys of Hymenoptera fauna because it allows continuous interception of flying insects and standardized comparisons among habitats. The present results contribute to the knowledge of parasitoid diversity in Brazil and reinforce the potential role of Eucoilinae species in biological control programs against pestiferous Diptera (Figure 7) (Marchiori, 2004b).

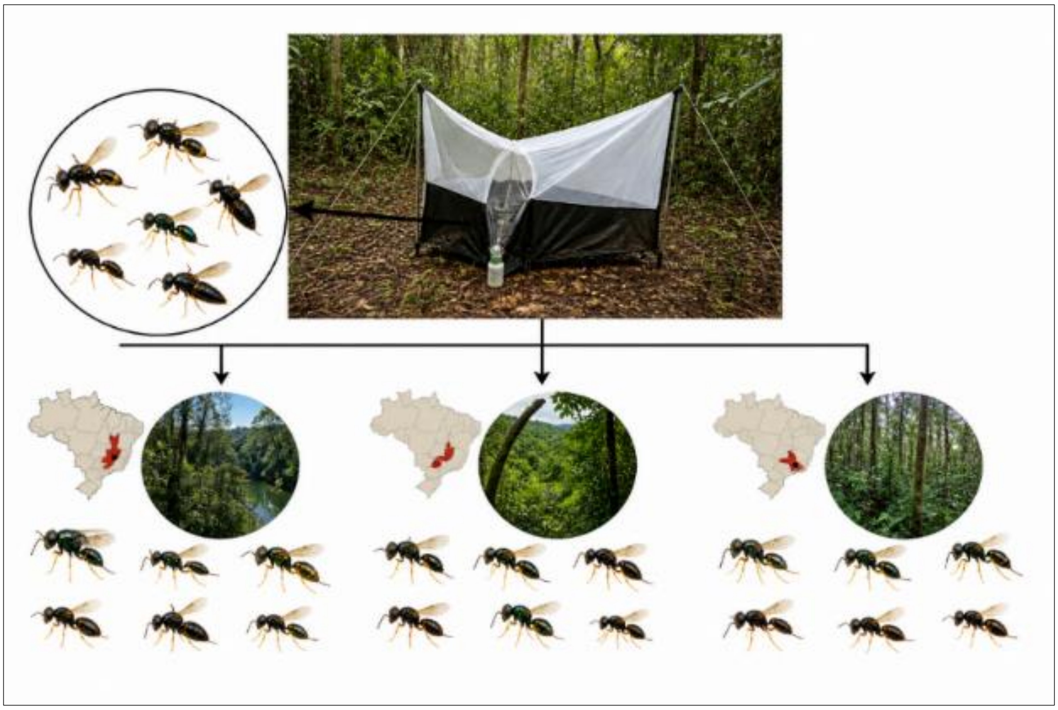

Figure 7: Malaise trap installed in native forest remnants used for the collection of Eucoilinae parasitoids in Itumbiara-GO, Araporã-MG, and Lavras-MG, Brazil. The figure illustrates the sampling method, representative habitats, and comparative abundance of parasitoid species among the three studied localities

5. Cynipoidea Collected in a Forest Remnant Using a Yellow Bowl and Malaise Traps in Itumbiara, Goiás

A total of 56 Cynipoidea specimens were collected in a forest remnant in Itumbiara, Goiás, using a yellow bowl and Malaise traps between January and September 2000. Of the total collected individuals, 55 specimens (98.2%) belonged to Eucoilinae, whereas only one specimen (1.8%) belonged to Figitinae. These results demonstrate the clear predominance of Eucoilinae in the studied environment, confirming the ecological importance of this group in forest remnants of the Cerrado region.

The yellow bowl trap was more efficient than the Malaise trap for collecting Cynipoidea, capturing 35 specimens, whereas the Malaise trap collected 21 individuals. This difference was statistically significant ($\chi^2 = 6.29$; df = 1; $P < 0.0001$), indicating that trap type strongly influenced sampling success. The greater efficiency of the yellow bowl trap may be associated with its visual attractiveness and passive interception of small hymenopterans flying near the vegetation layer. The distribution of Cynipoidea taxa differed significantly between trap types ($\chi^2 = 46.47$; df = 11; $p < 0.0001$), indicating that Malaise and yellow bowl traps sampled different taxonomic components of the community. (Table 7).

Table 7: Cynipoidea collected in a forest remnant in Itumbiara, Goiás, using Malaise and yellow bowl traps. Absolute abundance, frequency of each taxon, and distribution according to sampling method

Taxonomic group	Malaise	Yellow bowl trap	Frequency (%)	Total
Acanthaegilips	1	0	1.8	1
Aganaspis pelleranoi	1	0	1.8	1
Dettmeria	2	0	3.6	2
Dicerataspis	1	0	1.8	1
Dioucoula	0	1	1.8	1
Odonteucoila	1	9	17.9	10
Paraganaspis egeria	2	4	10.7	6
Steleucoela	10	0	17.9	10
Trybliographa	1	0	1.8	1
Triplasta	0	1	1.8	1
Zaeucoila	0	20	35.7	20
Zaeucoila unicarinata	2	0	3.6	2
Total	**21**	**35**	**100**	**56**

Among the Eucoilinae species, *Zaeucoila* sp. was the most abundant taxon, with 20 collected individuals, corresponding to 35.7% of the total sample. Other representative taxa were *Odonteucoila* sp., with 10 specimens (17.9%), *Steleucoela* sp., with 10 individuals (17.9%), *Paraganaspis egeria* Díaz, Gallardo & Walsh, 1996 (Hymenoptera: Figitidae) with six specimens (10.7%), and *Dettmeria* sp. with two individuals (3.6%). These genera are recognized as parasitoids of Diptera larvae and may contribute to the natural regulation of host populations in forest ecosystems (Figure 8).

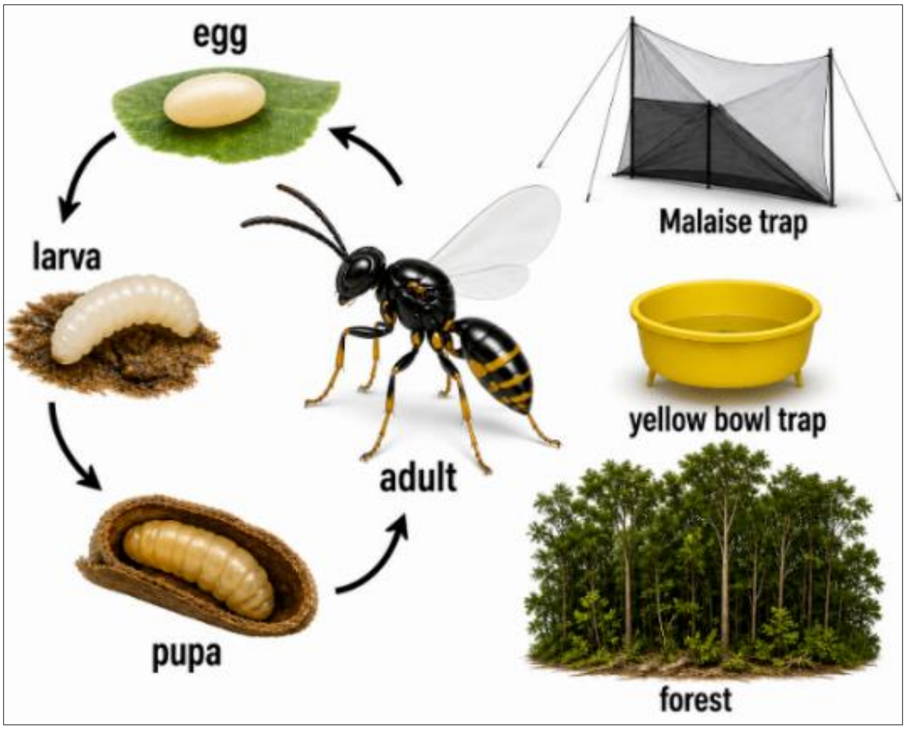

Figure 8: Comparative illustration of the life cycle of Eucoilinae parasitoids and the principal sampling methods used in forest remnants of Itumbiara, Goiás, Brazil. Developmental stages are shown from egg to adult, together with Malaise and yellow bowl traps used for field collections

Species preference in relation to trap type was also significant ($\chi^2 = 28.63$; GL = 11; $P < 0.0001$). *Dettmeria*, *Steleucoela*, and *Zaeucoila unicarinata* Ashmead, 1903 (Hymenoptera: Figitidae) were more frequently collected with the Malaise trap, whereas *Odonteucoila*, *P. egeria*, and *Zaeucoila* were more abundant in the yellow bowl trap. These results indicate behavioral differences among taxa in relation to flight pattern, attraction to color, and microhabitat use (Figure 9) (Matthew *et al.*, 2017).

Figure 9: *Zaeucoila* Ashmead, 1903 (Hymenoptera, Figitidae), parasitoids of Agromyzidae (Diptera). Digital painting of a female Zaeucoila. Source: Doi: 10.4289/0013-8797.119.3.317 and *Proceedings of the Entomological Society of Washington, 119*(3), 317-397

The use of both trap models increased the diversity of collected species, demonstrating that combined sampling methods are recommended for inventories of parasitoid Hymenoptera. The present study contributes to the knowledge of Cynipoidea fauna in Brazil and reinforces the importance of preserved forest remnants as reservoirs of parasitoid biodiversity and potential biological control agents (Marchiori *et al.,* 2000).

6. Hymenoptera Parasitoids of Calliphorids of Forensic Interest: New Occurrences

In July 2004, 595 pupae of Calliphoridae were collected from pig carcasses exposed in a native forest area in Lavras, Minas Gerais, Brazil. Of this total, 465 pupae belonged to *Chrysomya* spp., and 130 to *H. segmentaria*, representing 78.2% and 21.8% of the collected material, respectively. Three parasitoid taxa emerged from these pupae: *P. vindemmiae, Spalangia* sp., and *Trichopria* sp., confirming new host records for calliphorids of forensic interest (Table 8).

Table 8: Calliphorid pupae collected from pig carcasses in Lavras, Minas Gerais, Brazil. Host frequency, parasitoid occurrence, and parasitism rates are shown according to the original data

Host species	Pupae	Frequency (%)	Parasitoid species	Parasitism rate (%)
Chrysomya	465	78.2	*Trichopria* sp.	2.3
Hemilucilia segmentaria	130	21.8	*Spalangia* sp.	0.8
		21.8	*Pachycrepoideus vindemmiae*	0.8
Total	**595**	**100**		

The chi-square test showed a significant difference in parasitoid occurrence among the recorded species ($\chi^2 = 15.38$; df = 2; $p < 0.001$), with *Trichopria* sp. being the most frequent parasitoid emerging from calliphorid pupae. This result indicates that parasitoid occurrence was not evenly distributed among the species collected. The highest number of parasitoids was obtained from pupae of the genus *Chrysomya,* from which genus *Trichopria* sp. emerged, with a parasitism rate of 2.3%. In *H. segmentaria*, two parasitoids were recorded: *P. vindemmiae* and *Spalangia* sp., each with 0.8% parasitism rate. These results are important because calliphorids are strongly associated with decomposing carcasses and have forensic relevance, while their parasitoids may influence immature fly survival and population dynamics in natural environments (Figure 10) (Silva *et al.,* 2005)

Figure 10: Decomposition of a rodent carcass on forest soil showing colonization by calliphorid flies, parasitoid Hymenoptera, and predatory Coleoptera. Immature and adult insect stages associated with carrion succession are illustrated under natural field conditions

7. Parasitoids of *Ophyra Aenescens* Collected in Goiás, Brazil

A total of 20 pupae of *O. aenescens* were obtained in the State of Goiás, Brazil, using traps baited with human feces. From these pupae, four adult specimens of *B. podagrica* emerged, corresponding to a parasitism rate of 20.0%. This result demonstrates that *O. aenescens* may serve as a suitable host for parasitoid development under synanthropic conditions (Figure 11).

Figure 11: Adult specimen of *Ophyra aenescens,* a synanthropic fly associated with decomposing organic matter and animal waste. This species may act as a mechanical vector of pathogenic microorganisms affecting animals and humans. Source: https://www.biocont-profi.cz/ophyra-aenescens/

The relatively elevated parasitism rate observed in this study indicates that parasitoids may contribute to the natural regulation of muscoid fly populations. Species of genus *Ophyra* Albuquerquei, 1985 (Diptera: Muscidae) are of medical and sanitary importance because they develop in decomposing organic matter and may occur in urban and rural environments. Therefore, the occurrence of natural enemies associated with this species is ecologically relevant. *Ophyra aenescens* species are synanthropic flies commonly associated with decomposing organic matter, animal manure, and urban waste. These insects may act as mechanical vectors of pathogenic microorganisms, carrying bacteria, fungi, protozoa, and helminth eggs to food, water, animals, and human environments. Therefore, their presence represents a potential sanitary risk for livestock production systems and public health. In addition, high populations of *Ophyra* may indicate poor hygiene and inadequate waste management conditions (Figure 12).

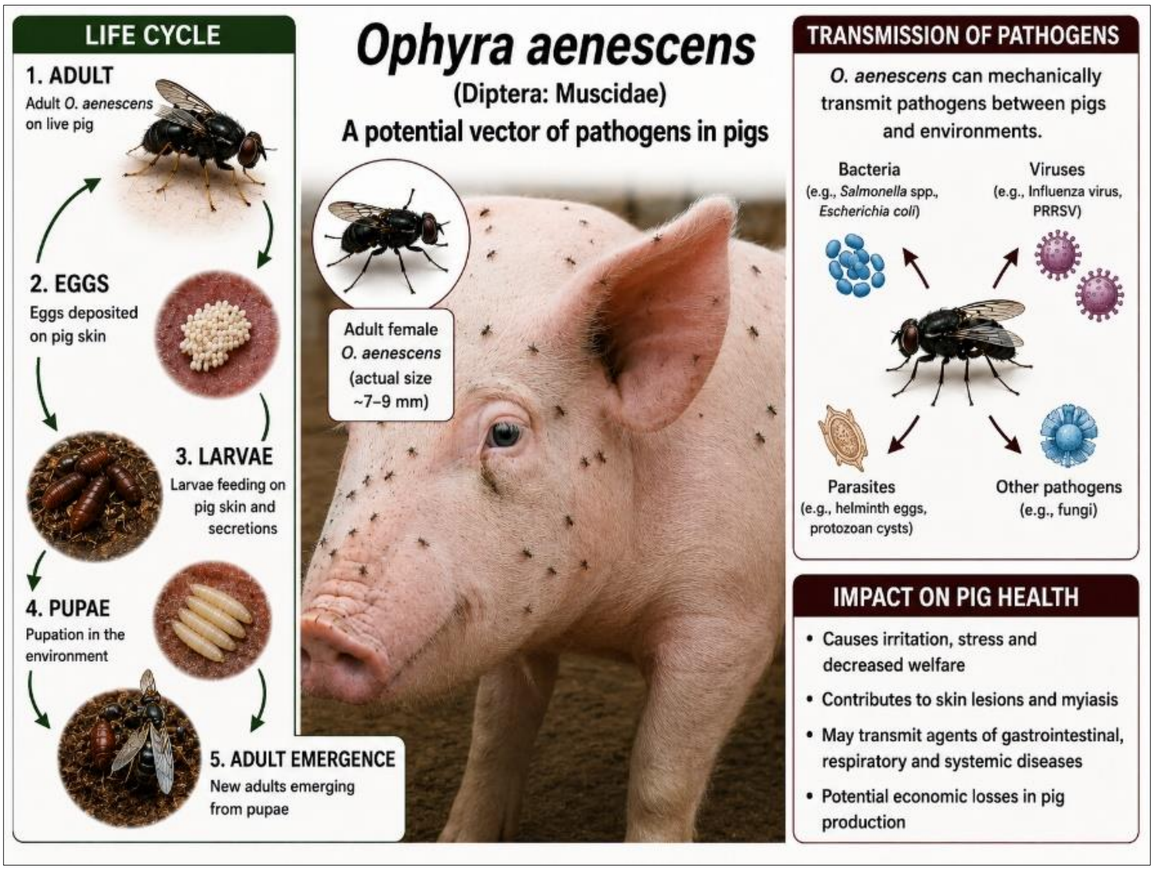

Figure 12: The figure illustrates the life cycle of *O. aenescens*, including adult, egg, larval, pupal, and adult emergence stages, as well as its occurrence on live pigs. This synanthropic fly may act as a mechanical vector of pathogens between pigs and contaminated environments, including bacteria, viruses, parasites, and fungal agents. Its presence on animals may cause irritation, stress, skin lesions, myiasis risk, reduced welfare, and potential economic losses in pig production

In Forensic Entomology, insect evidence is widely used in criminal investigations to estimate the Postmortem Interval (PMI), an important parameter for reconstructing the circumstances surrounding death. In this context, flies of several families within the order Diptera are especially relevant because they frequently colonize carcasses and use them as breeding substrates. The developmental stages of these insects, particularly larvae, provide valuable biological information for forensic analyses (Figure 13).

Figure 13: Larvae of *Ophyra aenescens* developing on decomposing organic substrates under natural conditions. These immature stages actively participate in organic matter breakdown and may occur in environments of sanitary importance studies. Source: https://desinsectador.com/2020/03/06/gran-cantidad-de-Hydrotaea-aenescens-Diptera-Muscidae-en-granjas-de-porcino/

PMI is commonly based on larval growth and accumulated thermal units expressed as degree-days. Since the mid-nineteenth century, Forensic Entomology has progressed considerably and has become an important auxiliary tool in criminal investigations. Studies conducted in different regions of Brazil have reported decomposer species such as *C. albiceps*, *O. aenescens*, and *O. thornax*, demonstrating regional variation in carrion-associated insect fauna. These findings reinforce the scientific and practical importance of forensic entomology in modern criminal science. There was a significant difference between parasitized and non-parasitized pupae of *O. aenescens* ($\chi^2 = 7.20$; df = 1; p = 0.0073), with non-parasitized pupae being more frequent. Important note: As only one parasitoid has been recorded, *B. podagrica*, it does not make sense to compare abundance between parasitoid species in this article.

The use of baited traps proved effective for obtaining pupae and parasitoids under field conditions. Sampling methods based on attractive baits are useful tools for surveys of Diptera and their natural enemies, especially in ecological studies involving medically important flies. This study represents the first record of *B. podagrica* parasitizing pupae of *O. aenescens* in Brazil. New host-parasitoid records are valuable because they increase knowledge of biodiversity, ecological interactions, and natural control agents associated with synanthropic insects (Figure 14) (Marchiori *et al.,* 2003a).

Figure 14: Adult specimen of *Brachymeria podagrica*, a pupal parasitoid associated with synanthropic Diptera. This species is recognized as an important natural enemy of muscoid flies developing in decomposing organic substrates. Sources: Prince George's Co., Maryland (7/11/2017). Determined by Brad Barnd/Bug Guide. Photo by Barbara Thurlow

8. Parasitoid Hymenoptera Collected During the Diurnal and Nocturnal Periods in Cattle Dung in Southern Goiás, Brazil

A total of 135 specimens of parasitoids were collected. These were distributed among seven superfamilies and sixteen families of Hymenoptera. Chalcidoidea exhibited the greatest family diversity, and this group, together with Ichneumonoidea, predominated in the native forest ecosystem. Among the individuals collected, 39.3% were in the diurnal period and 60.7% in the nocturnal period (Table 8). The analysis of variance indicated that the total number of specimens did not differ significantly between diurnal and nocturnal periods ($F = 0.73$; $P > 0.39$). Similarly, parasitoid families showed no significant preference for either sampling period ($F = 1.26$; $P > 0.24$). However, during the diurnal period, Ichneumonidae was the most abundant family, representing 56.5% of the individuals collected, with a statistically significant predominance in relation to the other families ($F = 9.23$; $P < 0.0001$). During the diurnal period, the family with the most individuals collected was Ichneumonidae, accounting for 56.5% of the individuals collected. During the nocturnal period, the family with the most individuals collected was Encyrtidae, accounting for 31.7% of the individuals collected (Table 9).

Table 9: Relation of superfamilies and families of parasitoid Hymenoptera and frequency of specimens collected during the diurnal and nocturnal periods using a Malaise trap in Itumbiara, State of Goiás

Superfamilies	Families	N. diurnal	Frequency (%)	N. nocturnal	Frequency (%)	Total
Chrysidoidea	Bethylidae	0	0	4	4.9	4
	Dryinidae	2	3.8	1	1.2	3
Chalcidoidea	Chalcididae	0	0	2	2.4	2
	Encyrtidae	3	5.7	26	31.7	29
	Eulophidae	0	0	1	1.2	1
	Eupelmidae	1	1.9	3	3.7	4
	Mymaridae	2	3.8	5	6.1	7
	Pteromalidae	0	0	1	1.2	1
	Torymidae	0	0	1	1.2	1

Superfamilies	Families	N. diurnal	Frequency (%)	N. nocturnal	Frequency (%)	Total
Cynipoidea	Figitidae	0	0	1	1.2	1
Evanoidea	Evaniidae	2	3.8	1	1.2	3
Ichneumonoidea	Braconidae	6	11.3	4	4.9	10
	Ichneumonidae	30	56.5	20	24.5	50
Platygrastroidea	Platygastridae	0	0	1	1.2	1
	Scelionidae	6	11.3	5	6.1	11
Proctotrupoidea	Diapriidae	1	1.9	6	7.3	7
Total		53	100	82	100	135

These results contribute to the knowledge of the entomofauna of parasitic Hymenoptera in the Itumbiara region in the southern part of the State of Goiás (Figure 15) (Marchiori *et al.,* 2007).

Figure 15: The Ichneumonidae family is widely studied and used in the control of agricultural and forestry pests, helping to reduce dependence on insecticides. These insects are essential for the balance of terrestrial ecosystems, and monitoring their biodiversity helps to understand climate and land-use impacts. Source: Miroslav Fiala and chatgpt.com

9. Parasitoids of the Family Chalcididae (Insecta: Hymenoptera) Collected in Goiás, Brazil

A total of 121 specimens of parasitoids belonging to the family Chalcididae were collected using yellow pan traps in forest and pasture areas of Itumbiara, Goiás, Brazil. Among the captured individuals, 32 specimens (26.4%) were obtained in forest areas, whereas 89 specimens (73.6%) were collected in pasture areas. Therefore, under the sampling conditions adopted in this study, a higher number of Chalcididae individuals was recorded in pasture environments (Figure 16).

Figure 16: Representative schematic illustration of *Conura* spp. showing the typical external morphology of adult parasitoids, including compact body shape, enlarged hind femora, membranous wings, and segmented antennae, characters commonly associated with species of this genus. Sources: Photo#823759, Efram Goldberg, and Iowa State University

The most abundant taxon was Haltichella sp. 4, with 19 specimens (15.7%), followed by *Conura* sp. 1, with 16 specimens (13.2%), and *Brachymeria pandora* (Crawford, 1914), with 12 specimens (9.9%). These three taxa together represented an important proportion of the total sample, indicating greater numerical occurrence in the surveyed habitats.

Other representative taxa included *Brachymeria annulata* (Fabricius, 1793) with 9 specimens (7.4%), *Haltichella perpulchra* (Walsh, 1861) with 10 specimens (8.3%), and *Haltichella rhyacioniae* Gahan, 1927, with 7 specimens (5.8%). The remaining taxa were represented at low frequencies, many with one to four individuals, indicating the presence of several less abundant species in the sampled environments. Some species were recorded in both habitats, while others occurred only in forest or only in pasture areas. This distribution indicates variation in occurrence among habitats; however, as the original study did not present inferential statistical analyses, these differences should be interpreted only as descriptive patterns of abundance and occurrence (Table 10).

Table 10: Rebuilt dataset of Chalcididae collected in forest and pasture areas of Itumbiara, Goiás, Brazil. Absolute abundance (n) and relative frequency (%) are shown for each habitat

Taxon	Forest number	Forest (%)	Pasture number	Pasture (%)	Total
Brachymeria annulata	1	3.1	8	9.0	9
Brachymeria pandora	1	3.1	11	12.4	12
Brachymeria sp.1	0	0	4	4.5	4
Conura sp.1	2	6.2	14	15.7	16
Conura sp.2	0	0	1	1.1	1
Conura sp.3	0	0	1	1.1	1
Conura sp.4	0	0	2	2.2	2
Conura sp.5	0	0	1	1.1	1
Conura sp.6	0	0	1	1.1	1
Conura sp.7	0	0	1	1.1	1
Conura sp.8	0	0	2	2.2	2
Conura sp.9	0	0	2	2.2	2
Conura sp.10	1	3.1	1	1.1	2
Haltichella ornaticipae	4	12.5	2	2.2	6
Haltichella perpulchra	3	9.4	7	7.9	10
Haltichella rhyacioniae	4	12.5	3	3.4	7
Haltichella sp.1	0	0	4	4.5	4
Haltichella sp.2	2	6.2	0	0.0	2
Haltichella sp.3	1	3.1	2	2.2	3
Haltichella sp.4	4	12.5	15	16.9	19
Haltichella sp.5	3	9.4	0	0.0	3
Haltichella sp.6	3	9.4	1	1.1	4
Dirhinus sp.	2	6.2	3	3.4	5
Melanosmicra sp.1	0	0	1	1.1	1
Melanosmicra sp.2	1	3.1	2	2.2	3
Total	**32**	**100**	**89**	**100**	**121**

There was a highly significant difference in Chalcididae abundance between forest and pasture habitats ($\chi^2 = 26.85$; df = 1; $p < 0.0001$), with a greater number of specimens collected in pasture areas. Interpretation: There was a significant difference between the habitats. The pasture showed a greater abundance of Chalcididae (89 individuals) than the forest (32 individuals). The presence of Chalcididae species in both forest and pasture areas demonstrates that these environments support representatives of this parasitoid group in the studied region. In addition, the results confirm that yellow pan traps were effective for collecting Chalcididae and useful for faunistic surveys in contrasting habitats. Overall, the study contributes to the knowledge of parasitoid diversity in central Brazil and provides baseline information on the occurrence of Chalcididae in managed and natural environments (Figure 17) (Marchiori *et al.,* 2003b).

Figure 17: Schematic representation of yellow pan trap sampling used for collecting parasitoids of the family Chalcididae associated with cattle dung in pasture areas of Goiás, Brazil. The illustration highlights parasitoid activity around dung pats and trap attraction under field conditions

10. Relationship between Duration of Cattle Dung Exposure in Pastures and the Occurrence of Dipterous Insects and Their Parasitoids in South Goiás, Brazil

A total of 430 parasitoid individuals were recovered from 100 cattle dung pats exposed in pasture areas for different time intervals ranging from 24 to 240 hours. The results demonstrated that dung age influenced parasitoid occurrence, with marked variation in abundance throughout the exposure periods. The highest number of parasitoids was recorded in dung exposed for 240 hours (31 individuals), followed by 192 hours (27 individuals) and 216 hours (18 individuals). In contrast, lower abundance values were observed during the earliest exposure periods, with 12 individuals at 24 hours, 6 at 48 hours, and no parasitoids recorded at 72, 96, and 120 hours. These findings indicate that older dung pats provided more favorable conditions for parasitoid colonization.

Among the parasitoids collected, *P. egeria* was the most frequent species, occurring in several exposure periods and reaching 18 individuals at 240 hours. *S. drosophilae* were also highly representative, especially in dung exposed for 144, 192, 216, and 240 hours. Additional taxa such as *Muscidifurax* sp. (Hymenoptera: Pteromalidae), *S. cameroni*, *Spalangia endius* Walker, 1839 (Hymenoptera: Pteromalidae), and *Trichopria* sp. were mainly associated with later exposure periods, reinforcing the ecological importance of aged cattle dung as a reservoir of parasitoid diversity (Table 11).

Table 11: Relationship between duration of and occurrences of cattle dung exposure in pastures, and its average crustal thickness in South Goiás, Brazil

Exposure time of feces (hours)	The average thickness of the crust (mm)	Standard deviation (mm)
24	1.67	±0.70
48	3.00	±1.00
72	3.89	±1.45
96	5.56	±2.13
120	6.00	±1.12
144	8.56	±1.24
168	10.22	±1.72
192	11.22	±1.39
216	15.11	±1.45
240	20.22	±1.09

Parasitoid richness also increased in older dung pats. At 144 hours, four taxa were recorded, while at 240 hours, five taxa were found, including *Muscidifurax* sp., *S. cameroni*, and *S. endius*. This suggests that longer exposure periods favor the development of dipteran hosts and, consequently, increase the opportunities for parasitoid establishment (Table 12).

Table 12: Relationship between duration of cattle dung exposure in pastures, its average crustal thickness, and occurrences of dipterous insects and their parasitoids in south Goiás, Brazil

Exposure time of feces (hours)	Parasitoids espcies	Frequency (%)	Total
24	*Paraganaspis egeria*	12	12
48	*Paraganaspis egeria*	6	6
72		0	
96		0	
120		0	
144	*Paraganaspis egeria*	2	15
	Spalangia cameroni	1	
	Spalangia drosophilae	8	
	Trichopria sp.	1	
168	*Paraganaspis egeria*	3	
192	*Paraganaspis egeria*	15	27
	Spalangia drosophilae	6	
	Trichopria sp.	6	
216	*Paraganaspis egeria*	12	18
	Trichopria sp.	3	
	Spalangia drosophilae	3	
240	*Paraganaspis egeria*	18	31
	Muscidifurax sp.	1	
	Spalangia cameroni	6	
	Spalangia drosophilae	3	
	Spalangia endius	3	

There was a highly significant difference in parasitoid abundance among cattle dung exposure periods ($\chi^2 = 88.07$; df = 9; $p < 0.0001$), indicating that dung exposure duration influenced parasitoid occurrence. The highest parasitoid abundance was recorded at 240 h, followed by 192 h and 216 h. Interpretation: Yes, there was a significant difference between the exposure times. The highest values occurred in older stool samples, mainly 240 h, 192 h, and 216 h. The exposure for 192 to 240 hours supported the greatest parasitoid occurrence and diversity, highlighting the ecological relevance of decomposing cattle dung as a habitat for natural enemies of dipterous insects (Figure 18) (Marchiori *et al.,* 2003c).

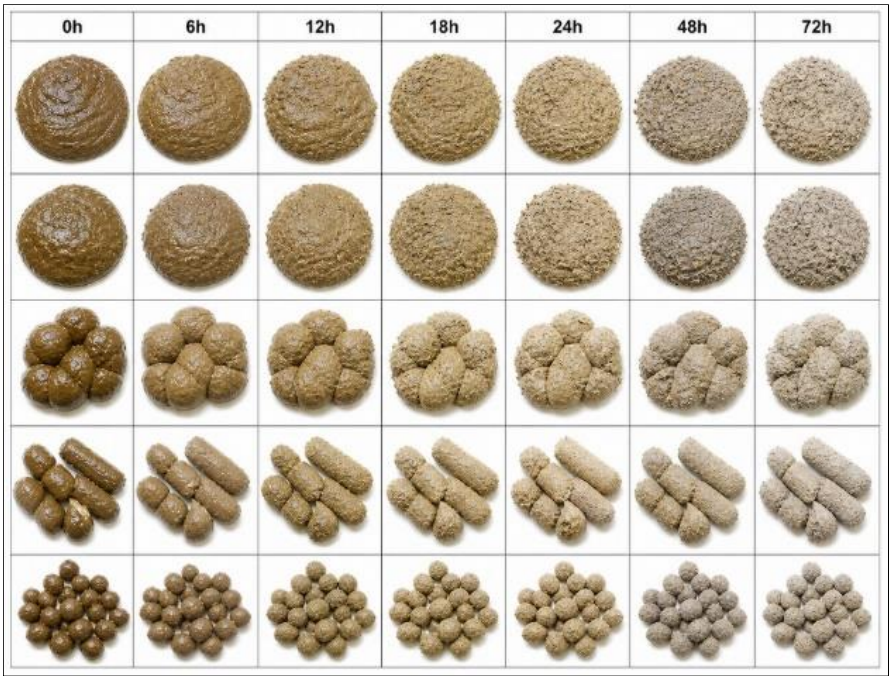

Figure 18: Schematic representation of progressive changes in cattle dung morphology and crust thickness during different exposure periods in pasture conditions, showing gradual dehydration, surface hardening, and color lightening with increasing time after deposition

11. Parasitoids of Diptera Collected in the Urban Area of Goiânia, Goiás, Brazil

A total of 358 dipterous pupae were collected in the urban area of Goiânia, Goiás, Brazil, from which 82 parasitoid individuals emerged. Among the collected pupae, 82 were parasitized, resulting in an overall parasitism rate of 23%. These results demonstrate the occurrence of natural enemies of synanthropic flies in urban environments and confirm the presence of parasitoid species associated with medically and veterinary important Diptera. The parasitoid species with the highest abundance was *B. podagrica,* with 53 individuals, representing the dominant species in the sample and accounting for the higher rate of parasitism (39.0%). This finding indicates that *B. podagrica* was the most successful parasitoid associated with the hosts collected in the studied area (Table 13).

Table 13: A total of 358 dipterous pupae were collected in the urban area of Goiânia, Goiás, Brazil, from which 82 parasitoid individuals emerged

Taxonomic group	Number of pupae	Parasitoids species	Number of individuals	Parasitism rate (%)
Chryomya albiceps	8	0	0	0
Fannia pusio	155	0	0	0
Peckia chrysostoma	25			
Sarcodexia lambens	137	*Brachymeria podagrica*	53	39.0
		Hemencyrtus herbertii	13	9.5
		Pachycrepoideus vindemmiae	1	0.7
		Tachinobia sp.	11	8.0
Synthesiomyia nudiseta	33	*Pachycrepoideus vindemmiae*	04	12.1
Total	**358**		**82**	**23.0**

Other parasitoids recorded in lower abundance were *H. herbertii* with 13 individuals, *Tachinobia* sp. with 11 individuals, and *P. vindemmiae* with 5 individuals. The presence of multiple parasitoid taxa demonstrates that different hymenopteran groups exploit dipterous pupae in urban habitats, contributing to the natural regulation of fly populations. Regarding host association, the highest number of parasitoids emerged from pupae of *Sarcodexia lambens* (Wiedemann, 1830) (Diptera: Sarcophagidae), which was the principal host species recorded. This result suggests that the abundance or suitability of this dipteran species favored parasitoid development, especially for *B. podagrica*.

There was a highly significant difference between parasitized and non-parasitized pupae ($\chi^2 = 158.61$; df = 1; $p < 0.0001$), with non-parasitized pupae being more frequent. Parasitoid abundance differed significantly among species Parasitoid abundance differed significantly among species ($\chi^2 = 66.00$; df = 3; $p < 0.0001$), with *B. podagrica* being the most abundant parasitoid collected in the urban area of Goiânia, Goiás, Brazil (Figure 19).

Figure 19: Schematic representation of an urban environment in Goiânia, Goiás, Brazil, illustrating synanthropic flies and their hymenopteran parasitoids associated with anthropized habitats. The figure highlights the occurrence of natural enemies of Diptera in densely populated urban areas

The relatively low diversity of parasitoid species observed in the urban area may be associated with environmental simplification and the reduced availability of breeding substrates compared with rural ecosystems. Even so, the parasitoid fauna detected in this study indicates that urban environments can maintain species capable of acting as biological control agents of pest flies. Overall, the results reinforce the ecological importance of parasitoids as natural enemies of Diptera in anthropized areas. The occurrence of these species in Goiânia highlights their potential contribution to reducing fly populations of sanitary relevance and provides useful information for integrated pest management programs (Marchiori *et al.,* 2019).

12. Parasitoids of Diptera Associated with Cattle Dung Collected in Pasture and Corral Areas in Itumbiara, Goiás, Brazil

A total of 277 (188+89) parasitoid individuals were recovered from dung-breeding flies collected in pasture and corral environments in Itumbiara, Goiás, Brazil. Of this total, 188 individuals (67.9%) were obtained from pasture areas, whereas 89 individuals (32.1%) were collected from corrals. These values indicate that pasture environments supported a higher abundance of parasitoids than corral areas during the sampling period. Among parasitoids collected in pasture areas, *Triplasta atrocoxalis* (Ashmead, 1895) (Hymenoptera: Figitidae) was the most abundant species, with 63 individuals (33.5%), followed by *S. drosophilae* with 30 individuals (15.9%) and *S. nigroaenea* with 38 individuals (20.2%). In the corrals, the dominant species were *S. cameroni* (17 individuals, 19.1%), *S. nigroaenea* (17 individuals, 19.1%), and *T. atrocoxalis* (26 individuals, 29.2%) (Table 14).

Table 14: Parasitoids and their respective hosts were collected from cattle dung in pasture and corral areas in Itumbiara, Goiás, Brazil

Parasitoid species/Pasture	Number	Frequency (%)	Parasitoid species/ Corral	Number	Frequency (%)
Aleochara notula	3	1.6	*Paraganaspis egeria*	8	9.0
Paraganaspis egeria	8.018	9.6	*Triplasta atrocoxalis*	26	29.1
Triplasta atrocoxalis	63	33.5	*Neralsia splendens*	4	4.5
Neralsia splendens	1	0.5	*Muscidifurax raptor*	2	2.2
Muscidifurax zaraptor	1	0.5	*Pachycrepoideus vindemmiae*	3	3.4
Spalangia cameroni	21	11.2	*Spalangia cameroni*	17	19.1
Spalangia drosophilae	30	15.9	*Spalangia drosophilae*	5	5.6
Spalangia endius	6	3.2	*Spalangia endius*	7	8.0
Spalangia nigra	1	0.5	*Spalangia nigroaenea*	17	19.1
Spalangia nigroaenea	38	20.2			
Total	**188**	**100**		**89**	**100**

The predominance of *T. atrocoxalis* in both environments suggests that this species is an important natural enemy of dung-breeding flies in the study region. Likewise, species of *Spalangia* were highly represented, reinforcing their recognized importance as pupal parasitoids of synanthropic Diptera. Table 17 demonstrates that several parasitoids were associated with more than one host species. *Spalangia cameroni* and *S. nigroaenea* showed broad host associations, whereas *T. atrocoxalis* and *P. egeria* were mainly related to *Palaeosepsis* spp. and *S. occidua* (Table 15).

Table 15: Parasitoids and their respective hosts were collected from cattle dung in pasture and corral areas in Itumbiara, Goiás, Brazil

Parasitoid species	Host species	Environment 1	Environment 2
Aleochara notula	*Sarcophagula occidua*	Pasture	------
Muscidifurax zaraptor	*Brontaea quadristigma*	Pasture	------
Muscidifurax raptor	*Palaeosepsis* spp.; *Sarcophagula occidua*	------	Corral
Neralsia splendens	*Sarcophagula occidua*	Pasture	Corral
Pachycrepoideus vindemmiae	*Musca domestica*; *Sarcophagula occidua*	------	Corral
Paraganaspis egeria	*Palaeosepsis* spp.; *Sarcophagula occidua*	Pasture	Corral
Spalangia cameroni	*Brontaea quadristigma*; *Brontaea debilis*; *Palaeosepsis* spp.; *Sarcophagula occidua*; *Musca domestica*	Pasture	Corral
Spalangia drosophilae	*Palaeosepsis* spp.; *Sarcophagula occidua*	Pasture	------
Spalangia endius	*Brontaea quadristigma*; *Sarcophagula occidua*	Pasture	------
Spalangia nigroaenea	*Brontaea quadristigma*; *Brontaea debilis*; *Cyrtoneurina pararescita*; *Palaeosepsis* spp.; *Sarcophagula occidua*	Pasture	Corral
Trichopria sp.	*Palaeosepsis* spp.; *Sarcophagula occidua*	Pasture	------
Triplasta atrocoxalis	*Palaeosepsis* spp.; *Sarcophagula occidua*; *Cyrtoneurina pararescita*	Pasture	Corral

There was a highly significant difference in parasitoid abundance between pasture and corral areas ($\chi^2 = 35.38$; df = 1; $p < 0.0001$), with a greater number of individuals collected in pasture environments. The higher abundance of parasitoids in pasture areas may be related to more favorable ecological conditions for host colonization and parasitoid development. Host occurrence also differed between pasture and corral environments, with some Diptera hosts recorded in both environments and others restricted to one substrate type. Because host associations were presented qualitatively, parasitoid–host and host–substrate relationships were interpreted descriptively rather than tested by chi-square.

Parasitoid–host associations varied between pasture and corral areas. Some parasitoids, such as *S. cameroni*, *S. nigroaenea*, *P. egeria*, and *T. atrocoxalis*, were associated with more than one host species, indicating broader host use. Overall, the results indicate that cattle dung supports a diverse parasitoid community in both habitats, with potential importance for the natural biological control of dung-breeding flies (Figure 20) (Marchiori *et al.,* 2009).

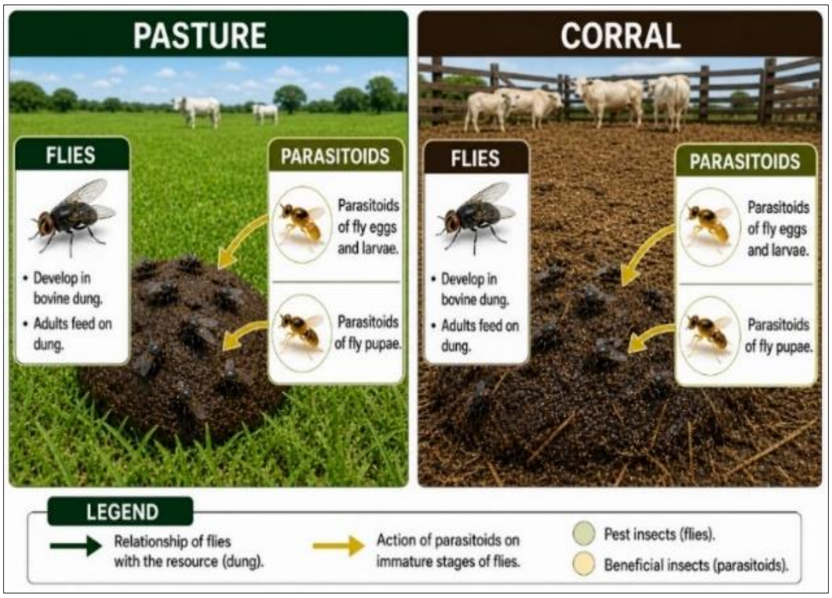

Figure 20: Comparative schematic representation of cattle dung in pasture and corral environments, showing the occurrence of synanthropic flies and their parasitoids. The figure illustrates dung as a breeding substrate for Diptera and highlights the role of parasitoids as natural enemies in both habitats

13. Diptera of Economic and Sanitary Importance Associated with Different Organic Substrates in Itumbiara, Goiás, Brazil

A total of 4,080 Diptera specimens were collected in traps baited with different organic substrates between May 1998 and April 1999. The highest number of individuals was obtained from buffalo dung 2,601 specimens; 63.8% of the total), followed by human feces (707) 17.3%, fish (300) 7.4%, and cattle dung (293) 7.2%. Lower abundances were recorded in traps containing cattle liver (82), 2.0%, cattle kidney (65), 1.6%, and fruit (32), 0.8% (Table 16).

Table 16: Relative frequency of Diptera collected in traps containing cattle dung, buffalo dung, human feces, cattle kidney, cattle liver, fruit, and fish

Taxonomic Group	Cattle dung	Buffalo dung	Human feces	Cattle kidney	Cattle liver	Fruit	Fish	Total
Calliphoridae								
Chrysomya albiceps	0	0	60	5	0	0	6	71
Chrysomya megacephala	0	0	13	0	0	0	20	33
Drosophilidae								
Zaprionus indianus	0	0	0	0	0	31	0	31
Fanniidae								
Fannia pusio	0	0	118	27	51	1	0	197
Muscidae								
Brontaea debilis	52	0	0	0	0	0	0	52
Brontaea quadristigma	31	117	21	0	0	0	0	169
Cyrtoneurina pararescita	50	0	0	0	0	0	0	50
Musca domestica	10	0	0	7	1	0	0	18

Taxonomic Group	Cattle dung	Buffalo dung	Human feces	Cattle kidney	Cattle liver	Fruit	Fish	Total
Synthesiomyia nudiseta	0	0	0	0	3	0	0	3
Sarcophagidae								
Euboettcheria collusor	0	0	29	0	0	0	0	29
Oxysarcodexia thornax	0	0	217	22	0	0	244	483
Peckia chrysostoma	0	0	135	04	27	0	30	196
Ravinia belforti	63	0	0	0	0	0	0	63
Sarcodexia lambens	0	0	114	0	0	0	0	114
Sarcophagula occidua	0	929	0	0	0	0	0	929
Sepsidae								
Archisepsis scabra	27	120	0	0	0	0	0	147
Palaeosepsis spp.	60	1435	0	0	0	0	0	1495
Total	**293**	**2601**	**707**	**65**	**82**	**32**	**300**	**4080**

Among the Diptera families recorded, Sepsidae was the most abundant, mainly due to the high occurrence of *Palaeosepsis* spp. (1,495 individuals) and *Sarcophagula occidua* (Fabricius, 1794) (Diptera: Sarcophagidae) (929 individuals), especially in buffalo dung. These results indicate a strong preference of these species for decomposing fecal substrates, particularly herbivore dung. Within Sarcophagidae, the most abundant species was *O. thornax* (483 individuals), followed by *P. chrysostoma* (196 individuals) and *S. lambens* (114 individuals) (Figure 21A).

Figura 21A: They are generally found near manure or decaying plant and animal matter. Many species resemble ants, having a "waist" and a shiny black body. Many Sepsidae have a curious habit of flapping their wings, most evident by the dark spots at the wingtips. Source: Determined by Salvador Vitanza and Confirmed by John F. Carr

Most sarcophagid flies were associated with human feces and fish, suggesting attraction to protein-rich or highly decomposed organic matter. Species of Muscidae were mainly associated with cattle and buffalo dung. *B. quadristigma* (169 individuals) and *B. debilis* (52 individuals) were especially frequent in these substrates, while *M. domestica* showed broader substrate use, occurring in cattle dung, cattle kidney, and cattle liver. Fruit was the least attractive substrate and was almost exclusively colonized by *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) (31 individuals), confirming the affinity of Drosophilidae flies for fermenting vegetal material.

There was a highly significant difference in Diptera abundance among organic substrates (χ^2 = 9,325.6; df = 6; p < 0.0001), indicating that substrate type strongly influenced fly attraction and occurrence. Interpretação: Houve diferença significativa entre os substratos, com maior abundância de Diptera em buffalo dung, followed by human feces, fish e cattle dung. Overall, the results demonstrate that different organic substrates strongly influence the composition and abundance of Diptera communities. Fecal substrates, particularly buffalo dung, supported the highest richness and abundance, whereas animal tissues and fruit attracted more specialized groups. These findings reinforce the ecological importance of substrate type in determining Diptera distribution patterns (Figure 21B) (Marchiori *et al.,* 2003c).

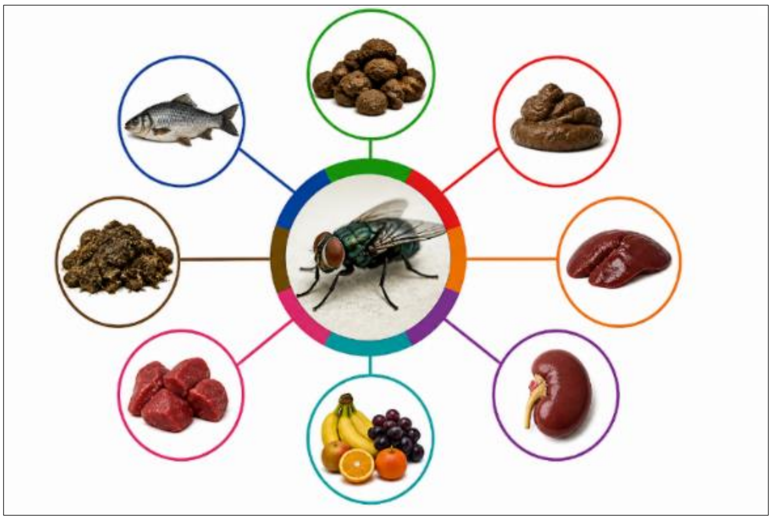

Figure 21B: Schematic representation of organic substrates used as baits for the collection of Diptera of economic and sanitary importance in southern Goiás, Brazil, including cattle dung, buffalo dung, human feces, cattle kidney, cattle liver, fruit, and fish

14. Survey of Families of Parasitoids Collected in Araporã, Minas Gerais, Brazil, Using Yellow Pan and Malaise Traps

A total of 9,747 parasitoid specimens belonging to several hymenopteran families were collected in Araporã, Minas Gerais, Brazil, between February and October 2002 using yellow pans and Malaise traps. Of this total, 6,341 individuals (65.1%) were captured in Malaise traps, whereas 3,406 individuals (34.9%) were obtained using yellow pan traps, indicating greater efficiency of Malaise traps for sampling parasitoid communities. Among the parasitoids collected with yellow pan traps (Table 19), the most abundant family was Diapriidae, with 909 individuals (26.7%), followed by Bethylidae (415; 12.2%), Encyrtidae (391; 11.5%), and Ichneumonidae (351; 10.3%). These results suggest that yellow pan traps were particularly effective for attracting small parasitoids associated with low vegetation and ground-level activity (Table 17).

Table 17: Families of parasitoids collected in yellow pan traps in Araporã, Minas Gerais, Brazil, from February to October 2002

Families	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Total
Agaonidae	0	1	0	2	0	1	0	0	0	4
Bethylidae	93	96	31	39	27	28	33	53	15	415
Braconidae	127	68	38	15	9	10	4	12	0	283
Ceraphronidae	21	13	5	6	4	1	2	9	0	61
Chalcididae	15	10	2	5	4	7	17	32	2	94
Diapriidae	334	97	77	156	72	45	50	53	25	909
Dryinidae	0	0	0	0	1	0	1	10	0	12
Encyrtidae	94	42	41	119	31	27	11	25	1	391
Eucharitidae	0	0	0	1	0	0	1	0	0	2
Eulophidae	22	25	91	23	2	7	9	13	11	203
Eupelmidae	2	0	1	8	2	5	3	27	1	49
Eurytomidae	0	0	0	0	0	1	0	3	3	7
Evaniidae	8	2	2	0	1	0	1	2	13	29
Figitidae	73	34	8	6	4	1	3	6	25	160
Ichneumonoidae	77	70	58	44	18	9	15	46	14	351
Megaspilidae	0	0	0	0	1	1	0	0	0	2
Mymaridae	0	0	0	0	1	0	0	0	0	1
Platygastridae	28	5	1	1	2	4	1	2	0	44
Pteromalidae	48	13	2	1	1	5	3	3	0	76
Scelionidae	64	76	12	3	2	6	3	3	0	169
Scelobythidae	1	0	0	0	0	0	0	0	0	1
Torymidae	43	71	15	3	1	4	3	2	0	142
Total	1050	623	384	432	183	163	160	301	110	3406

In Malaise traps (Table 2), Ichneumonidae was the dominant family, with 2,224 individuals (35.1%), followed by Braconidae (1,214; 19.1%), Diapriidae (526; 8.3%), Eulophidae (510; 8.0%), and Bethylidae (471; 7.4%). The higher abundance recorded in Malaise traps reflects their greater capacity to intercept flying insects moving through the environment (Figure 22) (Table 18).

Figure 22: Diapriidae are primarily gregarious endoparasitoids of Diptera pupae, but in this study, Trichopria sp. appears as a solitary parasitoid. Species of the genus *Trichopria* are usually parasitoids of immature stages of Diptera. Source: https://en.wikipedia.org/wiki/Diapriidae

Table 18: Families of parasitoids collected in a Malaise trap in Araporã, Minas Gerais, Brazil, from February to October 2002

Families	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Total
Agaonidae	0	0	0	0	1	1	0	0	0	2
Bethylidae	48	61	50	72	64	33	37	54	52	471
Braconidae	140	224	208	221	97	134	96	55	39	1214
Ceraphronidae	1	2	3	4	0	0	0	0	0	10
Chalcididae	47	55	75	66	44	32	24	19	18	380
Diapriidae	109	87	74	95	53	41	32	21	14	526
Dryinidae	3	2	1	0	0	0	1	0	0	7
Encyrtidae	27	24	29	32	14	9	8	5	3	151
Eucharitidae	0	0	1	0	0	0	0	0	0	1
Eulophidae	118	91	80	73	44	39	28	22	15	510
Eupelmidae	6	4	5	3	2	2	1	1	0	24
Eurytomidae	4	3	2	1	0	1	0	0	0	11
Evaniidae	12	10	8	7	5	4	3	2	1	52
Figitidae	96	84	72	61	39	28	22	17	11	430
Ichneumonidae	312	298	276	265	233	219	201	233	187	2224
Megaspilidae	2	1	1	1	0	0	0	0	0	5
Mymaridae	1	1	0	0	0	0	0	0	0	2
Platygastridae	61	48	39	32	21	19	13	10	7	250
Pteromalidae	73	64	52	41	29	18	12	9	7	305
Scelionidae	102	87	74	63	42	31	20	16	11	446
Scelobythidae	0	1	0	0	0	0	0	0	0	1
Torymidae	47	39	28	21	14	9	5	3	2	168
Total	1209	1186	1006	995	702	601	503	467	372	6341

There was a highly significant difference in parasitoid abundance between trap types ($\chi^2 = 883.7$; df = 1; $p < 0.0001$), with Malaise traps collecting more individuals than yellow pan traps. The two traps differed significantly. Seasonal variation was also observed throughout the sampling period. In both trapping methods, the highest numbers of parasitoids were generally recorded during the first months of sampling, especially in February, March, and April, followed by a progressive decline toward October. This pattern may be associated with climatic conditions favorable to parasitoid activity during warmer and wetter months (Figure 23).

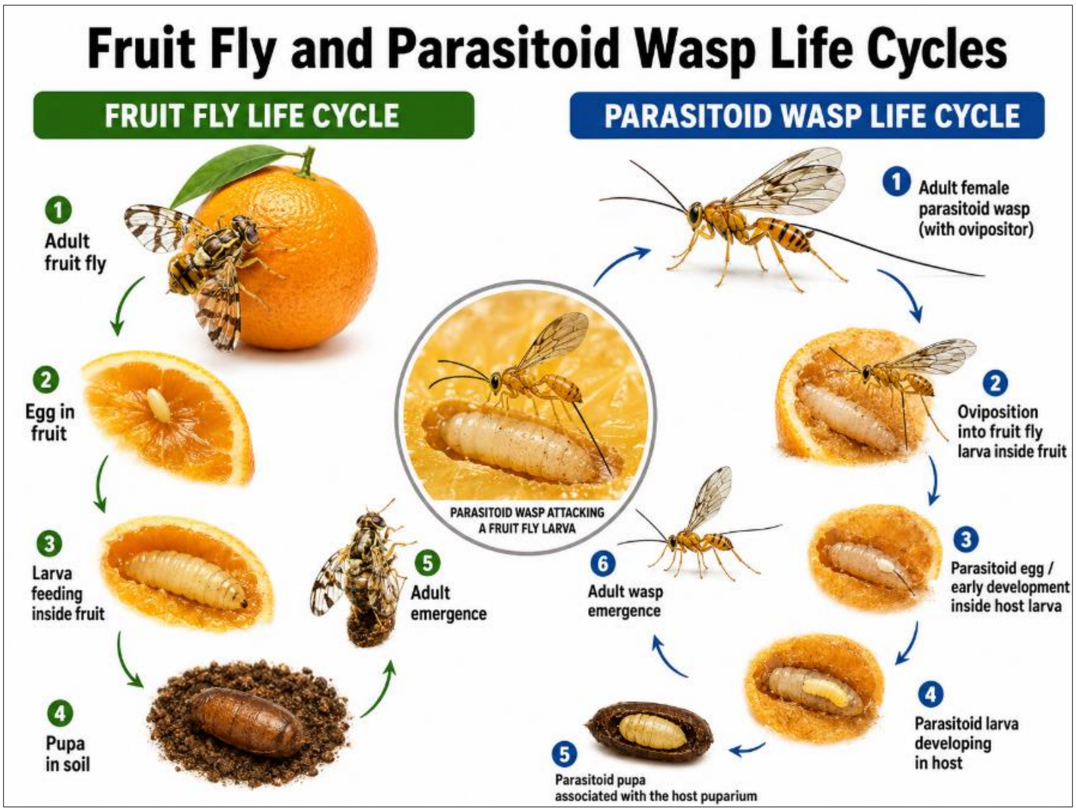

Figure 23: Life cycles of a fruit fly and its parasitoid wasp. The figure illustrates the developmental stages of the fruit fly, including adult, egg, larva, pupa, and adult emergence, and the parasitoid wasp cycle, showing oviposition into the fruit fly larva, parasitoid development inside the host, pupation associated with the host puparium, and adult wasp emergence. The central panel highlights the female parasitoid wasp attacking a fruit fly larva inside the fruit

Note: Family Braconidae collected in a Malaise trap in Araporã, Minas Gerais, Brazil, from February to October 2002. Total number of specimens: n = 1,214

The predominance of Ichneumonidae, Braconidae, and Diapriidae demonstrates the ecological importance of these groups in natural and agricultural ecosystems, where they act as parasitoids of several insect hosts. Their abundance indicates a potentially significant role in the natural biological regulation of phytophagous and decomposer insect populations. Overall, the results show that trap type strongly influences parasitoid capture. Malaise traps were more efficient in terms of abundance and diversity, whereas yellow pan traps complemented sampling by efficiently capturing smaller parasitoid taxa. Therefore, the combined use of both trap types is recommended for more comprehensive parasitoid surveys (Marchiori *et al.*, 2003d).

15. *Peckia Chrysostoma* and Its Parasitoids Collected in the South of the State of Goiás

A total of 1,819 pupae of *P. chrysostoma* were collected from five substrates in southern Goiás, Brazil. From these pupae, 1,742 parasitoid individuals emerged, and 377 pupae were parasitized, resulting in an overall parasitism rate of 20.7%. The highest parasitism rate was recorded in bovine liver, with 141 parasitized pupae out of 306, corresponding to 46.1%. This substrate also produced the highest number of parasitoids, with 1,054 individuals, mainly due to the high abundance of *N. vitripennis*, which accounted for 945 individuals in bovine liver.

The second-highest parasitism rate occurred in chicken viscera, with 96 parasitized pupae from 384 pupae, corresponding to 25.0%. In this substrate, *B. podagrica* was the most representative parasitoid, with 85 individuals, followed by *H. herbertti* with 61 individuals. In human feces, 273 pupae were collected, with 44 parasitized pupae and a parasitism rate of 16.1%. The parasitoids recorded in this substrate were *B. podagrica*, *N. vitripennis*, and *S. drosophilae*, with *S. drosophilae* showing the highest frequency in this substrate (Figure 24).

Figure 24: *Nasonia vitripennis* is one of four known species under the genus *Nasonia* - small parasitoid wasps that afflict the larvae of parasitic carrion flies such as blowflies and flesh flies. Source: *Nasonia vitripennis* Wikipedia

The lowest parasitism rate was observed in fish, with 27 parasitized pupae out of 303, corresponding to 8.9%, although this substrate yielded 400 parasitoid individuals. This result was strongly influenced by the occurrence of *Aphaereta* sp. (Hymenoptera: Braconidae), which produced 362 parasitoid individuals from only 12 parasitized pupae, indicating gregarious development. In the bovine kidney, 553 pupae were collected, with 69 parasitized pupae, resulting in a frequency of 12.5%. The most abundant parasitoid in this substrate was *B. podagrica*, with 61 individuals, followed by *S. endius* and *P. vindemmiae*.

Among the parasitoid species, *N. vitripennis* was the most abundant, mainly because of its high occurrence in bovine liver. *B. podagrica* was also widely distributed, occurring in all substrates and showing high abundance in bovine liver, chicken viscera, human feces, fish, and bovine kidney. This broad occurrence suggests that *B. podagrica* is an important parasitoid associated with *P. chrysostoma* in different decomposing substrates. There was a highly significant difference in the occurrence of *P. chrysostoma* pupae among substrates ($\chi^2 = 125.95$; df = 4; $p < 0.0001$), indicating that substrate type influenced the abundance of this fly species. The frequency of parasitized *P. chrysostoma* pupae differed significantly among substrates ($\chi^2 = 176.15$; df = 4; $p < 0.0001$), showing that substrate type influenced the parasitism pattern. The highest number of pupae was recorded in bovine kidney, followed by chicken viscera, bovine liver, fish, and human feces (Table 19).

Table 19: Parasitoids of *Peckia chrysostoma* collected from different substrates in southern Goiás, Brazil

Bait	Number *P. chrysostoma*	Parasitoid species	Number of parasitoids	Number of parasitized pupae	Frequency (%)
Bovine kidney	553	*Brachymeria podagrica*	61	61	11.0
		Pachycrepoideus vindemmiae	2	2	0.4
		Spalangia endius	6	6	1.1
Total	**553**		**69**	**69**	**12.5**
Bovine liver	306	*Brachymeria podagrica*	106	106	34.6
		Gnathopleura quadridentata	3	3	1.0
		Nasonia vitripennis	945	32	10.5
Total	**306**		**1054**	**141**	**46.1**
Chicken viscera	384	*Brachymeria podagrica*	85	85	22.1
		Hemencyrtus herbertti	61	9	2.3
		Nasonia vitripennis	25	2	0.5
Total	**384**		**171**	**96**	**25.0**
Fish	303	*Aphaereta* sp.	362	12	4.0
		Brachymeria podagrica	7	7	2.3
		Hemencyrtus herbertti	13	3	1.0
		Nasonia vitripennis	16	3	1.0
		Pachycrepoideus vindemmiae	2	2	0.7
Total	**303**		**400**	**27**	**8.9**
Human feces	273	*Brachymeria podagrica*	19	19	7.0
		Nasonia vitripennis	5	1	0.4
		Spalangia drosophilae	24	24	8.8
Total	**273**		**48**	**44**	**16.1**
Total general	1819		1742	377	20.7

Overall, the results demonstrate that substrate type influenced both parasitism rate and parasitoid composition. Bovine liver showed the highest parasitism rate, whereas fish showed a lower parasitism rate but high parasitoid emergence due to gregarious parasitoids. These findings reinforce the ecological importance of parasitoids as natural enemies of *P. chrysostoma* and support their potential relevance in the biological control of synanthropic flies (Figure 25) (Marchiori *et al.,* 2004b).

Figure 25: Schematic representation of *Peckia chrysostoma*, its pupal stage, parasitoids, and the main substrates used for collection in southern Goiás, Brazil. The figure highlights bovine liver, chicken viscera, human feces, fish, and bovine kidney as substrates associated with the occurrence of *P. chrysostoma* and its parasitoids

16. Microgastrinae (Hymenoptera: Braconidae) Collected from Native Forests and Pasture in Southern Goiás, Brazil

In the 520 samplings performed from January to December 1998, 67 specimens of Microgastrinae were collected. Of these, 49 individuals (73.1%) were collected in pastures and 18 individuals (26.9%) in forest areas. There was a significant difference in Microgastrinae abundance between habitats ($\chi^2 = 14.34$; df = 1; $p < 0.001$), with a higher number of specimens recorded in pastures (Table 20).

Table 20: Number and relative frequency of Microgastrinae specimens (Hymenoptera: Braconidae) collected with yellow pan traps in forest and pasture areas in southern Goiás, Brazil

Taxonomic Group	Pasture	Pasture %	Forest	Forest %	Frequency (%)	Total
Apanteles sp.	18	36.7	1	5.6	14.9	19
Cotesia sp.	7	14.3	3	16.7	9.0	10
Diolcogaster sp.	2	4.1	4	22.2	38.8	6
Glyptapanteles sp.	19	38.8	7	39.9	1.5	26
Promicrogaster sp.	1	2.0	0	0	7.5	1
Pseudapanteles sp.	2	4.1	3	16.7	28.4	5
Total	**49**	**100**	**18**	**100**	**100**	**67**

The most frequent taxon was *Glyptapanteles* sp., with 26 specimens (38.8%), followed by *Apanteles* sp. with 19 specimens (28.4%) and *Cotesia* sp. with 10 specimens (14.9%). The least frequent taxon was *Promicrogaster* sp., with one specimen (1.5%). These results suggest that *Glyptapanteles* sp. may represent an important group of parasitoids associated with Lepidoptera larvae in southern Goiás (Figure 26).

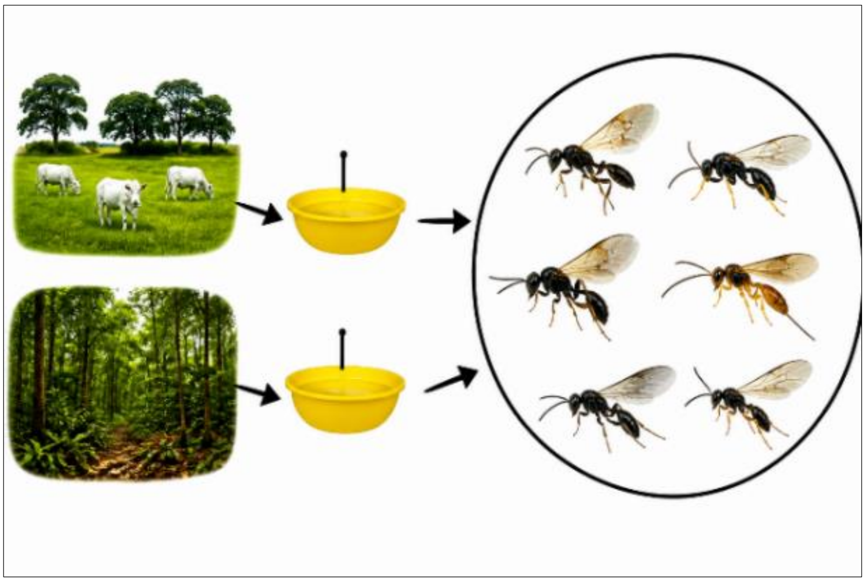

Figure 26: Schematic representation of Microgastrinae sampling in native forest and pasture areas of southern Goiás, Brazil, using yellow bowl traps. The figure illustrates the two habitats, the trap system, and the collection of parasitoid wasps associated with each environment

This study collected only 13.6% of this total. The higher number of specimens collected in pastures was probably due to an increase in attraction promoted by the traps in this area. In the forests, the trees' canopy may have reduced the incidence of sunlight, thus reducing the reflection of the yellow color of the containers (Figure 27) (Marchiori, 2014c; Abdoli *et al.,* 2021; van Noort, 2026).

Figure 27: The genus *Glyptapanteles* Ashmead (1904) specimens were collected using Malaise traps. Microgastrinae parasitoids are known to be important agents in the biological control of agricultural and forestry pests. Microgastrinae parasitizes almost exclusively Lepidoptera larvae. Source: Simon van Noort (Iziko South African Museum)

17. Diversity of Parasitoids Collected in an Area of Forest and Pastures in Southern Goiás, Brazil

A total of 793 parasitoids were collected in yellow pan traps in forest and pasture areas in southern Goiás, Brazil. Of these, 364 individuals (45.9%) were collected in the forest and 429 individuals (54.1%) in pastures. There was a significant difference in parasitoid abundance between habitats ($\chi^2 = 5.33$; df = 1; p = 0.021), with a higher number of individuals collected in pasture areas. The most abundant parasitoid species was *Trichopria anastrephae* Costa Lima,1940 (Hymenoptera: Trichopria), with 332 individuals, corresponding to 41.8% of the total sample. This species was frequent in both habitats, with 159 individuals in forest areas and 173 individuals in pastures, indicating broad occurrence in the studied environments (Table 21).

Table 21: Parasitoids collected in yellow pan traps in forest and pasture areas in southern Goiás, Brazil

Family	Parasitoid species	Forest	Pasture	Total
Braconidae	*Apanteles* sp.	18	1	19
	Aphaereta sp.	17	7	24
	Asobara sp.	25	3	28
	Bentonia longicornis	1	64	65
	Chelonus sp.	9	25	34
	Cotesia sp.	7	3	10
	Dinotrema sp.	0	2	2
	Diolcogaster sp.	2	4	6
	Glyptapanteles sp.	19	7	26
	Microcrasis sp.	0	1	1
	Phaenocarpa sp.	13	1	14
	Promicrogaster sp.	1	0	1
	Pseudapanteles sp.	2	3	5
Diapriidae	*Trichopria anastrephae*	159	173	332
	Trichopria sp.	0	3	3
Figitidae	*Aganaspis pelleranoi*	0	2	2
	Agrostocynips sp.	0	1	1
	Dettmeria sp.	0	3	3
	Dieucoila sp.	1	1	2
	Neralsia splendens	0	1	1
	Odonteucoila sp.	9	1	10
	Paraganaspis egeria	4	5	9
	Triplasta atrocoxalis	1	6	7
	Zaeucoila sp.	20	15	35
Pteromalidae	*Spalangia cameroni*	8	32	40
	Spalangia drosophilae	0	1	1
	Spalangia endius	1	0	1
	Spalangia nigra	1	1	2
Scelionidae	*Gryon gallardoi*	46	63	109
Total		**364**	**429**	**793**

Among Braconidae, the most frequent species was *Bentonia longicornis* Achterberg, 1992 (Hymenoptera: Braconidae: Orgilinae) with 65 individuals, most of them collected in pastures. Other representative braconids included *Chelonus* sp., *Asobara* sp., *Glyptapanteles* sp., and *Aphaereta* sp., showing that this family was well represented in both forest and pasture habitats. In Figitidae, *Zaeucoila* sp. was the most abundant taxon, with 35 individuals, followed by *Odonteucoila* sp. and *P. egeria*. In Pteromalidae, *S. cameroni* was the dominant species, with 40 individuals, mainly collected in pastures (Figure 28).

Figure 28: Schematic representation of parasitoid Hymenoptera collected with yellow pan traps in forest and pasture areas in southern Goiás, Brazil. The figure illustrates the two sampling environments and the occurrence of parasitoids associated with these contrasting habitats

Overall, the results indicate that pasture areas supported a slightly higher abundance of parasitoids than forest areas. However, both habitats contributed to parasitoid diversity, reinforcing the importance of maintaining forest remnants and pasture environments as reservoirs of parasitoid Hymenoptera in southern Goiás (Marchiori *et al.,* 2014d).

18. Parasitoids of *Chrysomya Megacephala* (Fabricius) Collected in Itumbiara, Goiás

A total of 70 pupae of *C. megacephala* were obtained from bovine kidney used as bait in Itumbiara, Goiás, Brazil. Of these, 13 pupae were parasitized, resulting in an overall parasitism rate of 18.6%. A total of 122 parasitoid individuals emerged from the parasitized pupae. Three parasitoid species were recorded: *Nasonia vitripennis*, *B. podagrica*, and *P. vindemmiae*. The most abundant species was *N. vitripennis*, with 115 individuals, parasitizing six pupae and representing 8.6% parasitism rate. This high number of individuals indicates gregarious development, since several parasitoids emerged from a small number of parasitized pupae (Table 22).

Table 22: Parasitoids of *Chrysomya megacephala* collected in Itumbiara, Goiás, Brazil. The table shows the number of pupae obtained from bovine kidney

Substrate	Pupae	Parasitoid species	Number of parasitoids	Parasitized pupae	Parasitism rate (%)
Bovine kidney	70	*Nasonia vitripennis*	115	6	8.6
		Brachymeria podagrica	6	6	8.6
		Pachycrepoideus vindemmiae	1	1	1.4
Total	**70**		**122**	**13**	**18.6**

Brachymeria podagrica also parasitized six pupae, with six individuals recorded, corresponding to 8.6% parasitism rate. *Pachycrepoideus vindemiae* was less frequent, with one individual emerging from one parasitized pupa, corresponding to 1.4% parasitism rate (Figure 29).

Figure 29: Schematic representation of *Chrysomya megacephala* associated with bovine kidney substrate, showing pupal development and the emergence of hymenopteran parasitoids. The figure highlights the interaction between synanthropic flies, decomposing animal tissue, and parasitoids recorded in Itumbiara, Goiás, Brazil

Overall, the results show that bovine kidney can support the development of *C. megacephala* and its parasitoids. The occurrence of these natural enemies highlights their ecological importance in regulating synanthropic fly populations of sanitary relevance in urban environments (Marchiori, 2004a).

19. *Trichopria Anastrephae, a* Parasitoid of Diptera Collected in Native Forest and Pasture Areas in Itumbiara, Goiás, Brazil

A total of 371 specimens of *T. anastrephae* were collected using yellow pan traps in areas of native forest and pasture in Itumbiara, Goiás, Brazil. The species was recorded in both environments, with 173 individuals (51.1%) collected in pasture areas and 159 individuals (47.9%) in forest areas. There was no significant difference in the abundance of *T. anastrephae* between pasture and forest areas ($\chi^2 = 0.59$; df = 1; p = 0.44), indicating a similar distribution of the species between habitats. The results indicate that *T. anastrephae* occurs in both habitats, showing a relatively similar distribution between forest and pasture environments. However, a slightly higher abundance was observed in pasture areas, which may be related to greater exposure of traps, increased light incidence, and environmental conditions that favor parasitoid activity (Figure 30).

Figure 30: Schematic representation of male and female *Trichopria anastrephae*, highlighting sexual dimorphism in antennal morphology. The male is characterized by longer and more setose antennae, whereas the female has shorter antennae; antennal contact between individuals is represented as part of courtship behavior

The species showed seasonal variation, with population peaks associated with warmer and more humid periods of the year, suggesting that climatic factors such as temperature and rainfall influence its population dynamics. The decrease in abundance during cooler and drier months reinforces the sensitivity of this parasitoid to environmental conditions. Overall, *T. anastrephae* can be considered a common and well-distributed parasitoid in both forest and pasture habitats, indicating its ecological importance in regulating dipteran populations in these environments (Marchiori and Penteado-Dias, 2001b).

20. The Parasitoid *Conura* Sp. in Pupae *of Allograpta Obliqua* Say (Diptera: Syrphidae) Collected from Corn Plants in Itumbiara

This study reports the first occurrence of *Conura* sp. parasitizing pupae of *A. obliqua* collected in maize crops in Itumbiara, Goiás, Brazil. During the sampling period, six pupae of *A. obliqua* were obtained, of which two produced parasitoids belonging to *Conura* sp., resulting in a parasitism rate of 33.3% (Figure 31).

Figure 31: *Conura* sp. parasitizing pupae of *A. obliqua* collected in maize crops in Itumbiara, Goias, Brazil. Source: Photo 2444351, (c) manimiranda

The occurrence of *Conura* sp. associated with *A. obliqua* is relevant because syrphid larvae are important predators of immature stages of agricultural pests, especially aphids. Therefore, parasitism rate on *A. obliqua* pupae may influence the natural regulation of pest populations indirectly, by affecting a beneficial predator species present in maize crops. Although only a small number of pupae were obtained, the record expands the knowledge of host associations involving Chalcididae in Goiás. The presence of *Conura* sp. in maize fields indicates that agricultural environments may support parasitoid diversity and complex trophic interactions involving herbivorous insects, predators, and parasitoids (Figure 32).

Figure 32: Aphids are the primary diet of the syrphid *Allograpta obliqua* fly larvae. The syrphid fly eggs are the white cylindrical eggs circled. They will hatch within 2-3 days and go to work. http/www.facebook.com/groups/TheBeautifulMonarch/posts/3541806315946773/

Overall, these findings contribute to the knowledge of parasitoid fauna in the state of Goiás and reinforce the importance of recording new host–parasitoid associations in agroecosystems. Such information is useful for understanding local biodiversity and the ecological relationships that may affect biological control processes (Figure 33) (Marchiori *et al.,* 2001c).

Figure 33: Schematic representation of the ecological interaction among aphids, larvae of *Allograpta obliqua*, pupae of *A. obliqua*, and the parasitoid *Conura* sp. in maize plants. The figure illustrates *A. obliqua* as a predator of aphids and *Conura* sp. as a parasitoid associated with the pupal stage of this syrphid fly

21. *Hemencyrtus Herbertii* Collected in Brazil

A total of 1,336 dipteran pupae were collected between March 2001 and June 2006 in Brazil, from which 31 pupae were parasitized by *H. herbertii*, resulting in an overall parasitism rate of 2.3%. The host species with the highest number of pupae was *Euboettcheria collusor* (Curran e Walley, 1934) (Diptera: Sarcophagidae), with 412 pupae, corresponding to 30.8% of the total host sample, followed by *P. chrysostoma* with 280 pupae (21.0%) and *Lucilia eximia* (Wiedemann,1819) (Diptera, Calliphoridae) (1.1%) with 268 pupae (20.1%). Among the hosts recorded, the highest parasitism rate was observed in *S. lambens*, with 5 parasitized pupae out of 17 collected, corresponding to 29.4%. This result indicates that, although this host was poorly represented in terms of abundance, it showed the greatest susceptibility or suitability for parasitism rate by *H. herbertii* (Table 23).

Table 23: Hosts of the parasitoid *Hemencyrtus herbertii* collected in Brazil. The table presents the number and relative frequency of dipteran pupae obtained for each host species

Taxonomic group	Hosts species	Number of pupae	Host Frequency	Number of pupae parasitized	Parasitism rate (%)
Calliphoridae	*Chrysomya albiceps*	74	5.5	3	4.1
	Chrysomya megacephala	76	5.7	1	1.3
	Hemilucilia flavifacies	16	1.2	3	18.8
	Lucilia eximia	268	20.1	3	1.1
Muscidae	*Euboettcheria collusor*	412	30.8	1	3.2
	Musca domestica	20	1.5	1	5.0
	Ophyra aenescens	64	4.8	3	4.7
Sarcophagidae	*Oxysarcodexia thornax*	109	8.2	2	1.8
	Peckia chrysostoma	280	21.0	9	3.2
	Sarcodexia lambens	17	1.3	5	29.4
Total		**1336**	**100**	**31**	**2.3**

Other hosts with relevant parasitism rates included *Hemilucilia flavifacies* (Engel, 1931) (Diptera: Calliphoridae), with 3 parasitized pupae from 16 collected (18.8%), *M. domestica*, with 1 parasitized pupa from 20 collected (5.0%), and *Ophyra* sp., with 3 parasitized pupae from 64 collected (4.7%). In contrast, lower parasitism rates were observed in *L. eximia* (1.1%), *C. megacephala* (1.3%), and *O. thornax* (1.8%). The greatest number of parasitized pupae was recorded in *P. chrysostoma*, with 9 parasitized pupae, although its parasitism rate was only 3.2% because of the relatively high number of pupae collected. This shows that host abundance and parasitism rate percentage do not always follow the same pattern: some abundant hosts may show low parasitism rates, whereas less abundant hosts may present proportionally higher parasitism rate (Figure 34).

Figure 34: Chemotic representation of gregarious emergence of *Hemencyrtus herbertii* from a dipteran pupa. The figure illustrates multiple parasitoid individuals emerging from the same host puparium, highlighting the gregarious parasitic behavior of this encyrtid parasitoid

Overall, the results indicate that *H. herbertii* is associated with several dipteran hosts of medical and veterinary importance. The occurrence of this parasitoid in different host families suggests ecological flexibility and reinforces its potential relevance as a natural enemy of synanthropic flies in Brazil (Marchiori, 2019a).

22. Parasitoids of Diptera of Importance to Public Health, Medical, Veterinary, and Economic Collected in Two Different Types of Traps in Southern Goiás, Brazil

A total of 400 parasitoids were collected, of which 180 individuals were obtained using the Ferreira (1978) trap and 220 individuals using pitfall traps. In the Ferreira (1978) trap, the most abundant species was *P. vindemmiae*, with 100 individuals. In contrast, in the pitfall traps, the dominant species was *G. quadridentata*, with 157 individuals. The chi-square test showed a significant difference in the distribution of parasitoid species between the two trap types ($\chi^2 = 322.04$; df = 9; $p < 0.001$). This result indicates that the composition of parasitoids varied according to the collection method used. The distribution of parasitoid species differed significantly between trap types, as indicated by the chi-square test, suggesting that parasitoid composition was influenced by the collection method (Table 24) (Ferreira, 1978).

Table 24: Taxonomic groups and species of parasitoids associated with Diptera collected using Ferreira (1978) traps and pitfall traps in southern Goiás, Brazil

Taxonomic groups	Parasitoides species	Traps Ferreira (1978)	Frequency (%)	Pitfall trap	Frequency (%)
Braconidae	*Gnathopleura quadridentata*	22	12.2	157	71.4
Chalcididae	*Brachymeria podagrica*	21	11.7	0	0
Encyrtidae	*Hemencyrtus herbertii*	23	12.8	0	0.
Figitidae	*Neralsia* sp.	0	0.0	43	19.5
	Paraganaspis egeria	1	0.6	0	0.0
	Tribliographa sp.	0	0	17	7.7
Pteromalidae	*Nasonia vitripennis*	13	7.2	0	0
	Pachycrepoideus vindemmiae	100	55.6	0	0
	Spalangia endius	0	0	2	0.9
	Spalangia nigroaenea	0	0	1	0.5
Total		**180**	**100**	**220**	**100**

Pitfall traps were mainly associated with *G. quadridentata*, *N.* sp., and *Tribliographa* sp., whereas the Ferreira (1978) trap collected higher numbers of *P vindemmiae*, *H. herbertii*, *B. podagrica*, and *N. vitripennis*. These differences may be related to the structure of each trap, the type of bait used, and the searching behavior of each parasitoid species (Figure 35).

Figure 35: Schematic representation of the two trap models used for collecting Diptera and their parasitoids in southern Goiás, Brazil. On the left, the Ferreira (1978) and the Pitfall trap

Although the chi-square result was significant, it should be interpreted with caution because some species had low abundance or were absent in one of the trap types. Even so, the data suggest that the two collection methods sampled different parasitoid assemblages associated with Diptera pupae (Marchiori, 2013a).

23. Dipterous Parasitoids Collected from Cattle Dung in Goiânia, Central Goiás, Brazil

A total of 359 Diptera pupae were collected from cattle feces in Goiânia, Goiás, Brazil, from March 2012 to February 2013. Of these, 23 pupae were parasitized, resulting in an overall parasitism rate of 6.4%. The highest monthly parasitism rate was observed in June, with 60.0%, followed by May with 22.2%, July with 19.5%, and April with 18.2%. No parasitism rate was recorded from August to February, except in September, when one parasitized pupa was recorded (Table 25).

Table 25: Taxonomic groups and species of Diptera parasitoids collected monthly from cattle dung samples between March 2012 and February 2013 in Goiânia, Goiás, central Brazil

Taxonomic group	Parasitoid species	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Frequency (%)	Total
Staphylinidae	*Aleochara notula*	0	0	0	0	5	0	1	0	0	0	0	0	26.1	6
Figitidae	*Neralsia splendens*	0	1	0	0	3	0	0	0	0	0	0	0	17.4	4
	Paraganaspis egeria	0	1	0	0	0	0	0	0	0	0	0	0	4.3	1
Pteromalidae	*Nasonia vitripennis*	0	0	4	0	0	0	0	0	0	0	0	0	17.4	4
	Spalangia cameroni	0	2	0	0	0	0	0	0	0	0	0	0	8.7	2
	Spalangia drosophilae	1	0	2	1	0	0	0	0	0	0	0	0	17.4	4
	Spalangia nigra	0	1	0	0	0	0	0	0	0	0	0	0	4.3	1
	Spalangia sp.	0	1	0	0	0	0	0	0	0	0	0	0	4.3	1
Total		**1**	**6**	**6**	**1**	**8**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**100.0**	**23**

The most frequent parasitoid species was *A. notula*, with six individuals, representing 26.1% of all parasitoids collected. Other species recorded were *N. splendens* and *N. vitripennis*, each with four individuals, followed by *S. drosophilae*, also with four individuals. Lower abundances were observed for *S. cameroni*, *P. egeria*, *S. nigra*, and *Spalangia* sp. The highest parasitism rate by parasitoid species was observed for *N. vitripennis* parasitizing pupae of *C. pararescita*, with 9.8%. *Aleochara notula* also showed an important association with *S. occidua*, with 7.7% parasitism rate based on the corrected calculation. These results indicate that the parasitism rate was not evenly distributed among host species (Table 26).

Table 26: Monthly parasitism rate of Diptera pupae collected from cattle dung samples between March 2012 and February 2013 in Goiânia, Goiás, central Brazil

Taxonomic group	Parasitoid species	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.
Number of pupae examined	50	33	9	10	41	25	69	38	8	24	38	10	359
Parasitized pupae	1	6	2	6	8	0	1	0	0	0	0	0	23
Parasitism rate (%)	2.0	18.2	22.2	60.0	19.5	0.0	1.4	0.0	0.0	0.0	0.0	0.0	6.4

Note: September parasitism rate was recalculated as 1/69 x 100 = 1.4%.

The chi-square test indicated a significant association between Diptera host species and parasitoid species ($\chi^2 = 47.28$; df = 8; $p < 0.05$). This result suggests that parasitoid occurrence was influenced by the host species available in cattle feces. Therefore, the parasitoid community showed host-related variation, with some parasitoids occurring only in specific Diptera species (Table 27).

Table 27: Number of Diptera pupae, parasitoid species, number of parasitized pupae, and parasitism rate recorded in cattle dung samples collected in Goiânia, Goiás, central Brazil

Diptera species	Number of pupae	Parasitoid species	Parasitized pupae	Parasitism rate (%)
Brontaea quadristigma	83	*Spalangia drosophilae*	1	1.2
Brontaea debilis	77	*Spalangia drosophilae*	2	2.6
		Spalangia cameroni	2	2.6
		Spalangia nigra	1	1.3
		Spalangia sp.	1	1.3
Cyrtoneurina pararescita	41	*Neralsia splendens*	2	4.9
		Nasonia vitripennis	4	9.8
Palaeosepsis spp.	80	*Spalangia drosophilae*	1	1.3
Sarcophagula occidua	78	*Aleochara notula*	6	7.7
		Neralsia splendens	2	2.6

		Paraganaspis egeria	1	1.3
Total	**359**		**23**	**6.4**

Note: The parasitism rate for *A. notula* in *S. occidua* was recalculated as 6/78 x 100 = 7.7%.

The results also show that parasitoid activity was concentrated in a few months, mainly from March to July. This pattern may be related to seasonal changes in environmental conditions, such as temperature, humidity, rainfall, and the availability of cattle dung as a breeding substrate for Diptera. The presence of parasitoids associated with synanthropic flies reinforces their ecological importance as natural enemies and their potential role in biological control programs (Figure 36) Marchiori, 2013b).

Figure 36: Schematic representation of Diptera hosts and their parasitoids associated with cattle dung. Adult Diptera are shown with two wings, while parasitoid adults are represented with four wings. The figure illustrates the association between cattle dung, Diptera pupae, and parasitoid emergence.

5.0. DISCUSSION

Parasitoids associated with Diptera represent an important component of natural regulation in decomposing organic substrates, especially in cattle dung, carrion, food remains, human feces, and other materials frequently colonized by synanthropic flies. The results of the present study reinforce that these substrates are not only breeding sites for Diptera of sanitary, veterinary, economic, and forensic importance, but also ecological reservoirs for natural enemies. This interaction is relevant because many dipterous hosts develop rapidly in ephemeral substrates, while parasitoids depend on host availability, substrate persistence, and favorable environmental conditions to complete their development. Therefore, the occurrence of parasitoids in rural, urban, pasture, forest, and forensic environments indicates their ecological importance in regulating fly populations (Marchiori, 2001a; Marchiori *et al*., 2004a; Marchiori, 2014b; Jaume-Schinkel and Mengual, 2022; Cingolani *et al*., 2025).

An important contribution of the present manuscript was the organization of data from previous studies and the complementary application of chi-square analyses to datasets that were originally interpreted mainly in descriptive form. This approach allowed differences in parasitoid abundance, parasitism rate, host association, sampling environment, trap type, substrate preference, and dung exposure time to be statistically tested. Thus, the present study does not change the original biological records but adds statistical support to ecological patterns observed among Diptera and their parasitoids. This is especially relevant for faunistic and ecological surveys, where numerical differences are often described without formal testing. The results therefore, strengthen the interpretation that parasitoid distribution is not random, but influenced by ecological, methodological, and biological factors (Marchiori, 2014a; Schuster and Sivakumar, 2024; Cingolani *et al*., 2025).

Regional and environmental differences were important factors affecting parasitoid abundance and parasitism. The higher abundance recorded in the southern region of Goiás compared with the central region suggests that local conditions may influence host availability and parasitoid establishment. Similarly, pasture areas supported more parasitoids than corral areas, indicating that open grazing environments may provide greater availability of dung pats, more heterogeneous microhabitats, and increased opportunities for host colonization. These differences may also be related to dung moisture, vegetation cover, exposure to sunlight, livestock density, and seasonal variation. The significant chi-square results support the interpretation that parasitoid occurrence differs among regions and habitats, reinforcing that environmental context must be considered when evaluating parasitoid communities associated with dung-breeding Diptera (Marchiori, 2014c; Marchiori, 2014d; Österman *et al*., 2026).

The type of organic substrate strongly influenced Diptera occurrence and, consequently, the availability of hosts for parasitoids. Fecal substrates, especially buffalo dung, human feces, and cattle dung, supported high numbers of Diptera, while animal tissues and fruit attracted different fly groups. This pattern reflects the feeding and oviposition preferences of Diptera because each substrate differs in moisture, odor, nutrient composition, microbial activity, and decomposition stage. Substrate preference is especially important for synanthropic and necrophagous flies, many of which use decomposing organic matter as a larval resource. The significant difference among substrates shows that host abundance is strongly linked to resource type, and this directly affects parasitoid opportunities for host location and parasitism (Marchiori, 2001b; Marchiori *et al*., 2004b; Jaume-Schinkel and Mengual, 2022; Guimarães *et al*., 2026; Huang *et al*., 2026).

Dung exposure time also played an important role in parasitoid occurrence. Older cattle dung pats, mainly those exposed for longer periods, supported higher parasitoid abundance and richness. This may be explained by the progressive development of dipterous larvae and pupae inside the dung, which increases host availability for pupal parasitoids. In the earliest exposure periods, immature Diptera may still be in larval stages or absent, reducing opportunities for parasitoid development. As the dung ages, changes in crust thickness, humidity, texture, and microbial decomposition may create more favorable conditions for host pupation and parasitoid activity. Therefore, dung age can be considered a key ecological variable structuring the interaction between Diptera hosts and parasitoids in pasture systems (Marchiori, 2014a; Cingolani *et al*., 2025).

The sampling method also affected the abundance and composition of parasitoids. Different traps, such as Malaise traps, yellow bowl traps, pitfall traps, and baited traps, capture insects according to their flight behavior, attraction response, body size, habitat use, and daily activity. The significant differences observed between trap types indicate that no single method is sufficient to represent the entire parasitoid community. Yellow bowl traps may be more efficient for visually attracted and low-flying hymenopterans, whereas Malaise traps intercept flying insects moving through vegetation. Baited traps and dung-associated methods are more directly linked to host substrates and may therefore be more effective for detecting host–parasitoid relationships. These results show that combined sampling methods are important for increasing the accuracy of parasitoid surveys (Marchiori *et al*., 2004a; Marchiori, 2014b; Österman *et al*., 2026).

The occurrence of several parasitoid taxa associated with Diptera pupae demonstrates the diversity of natural enemies in decomposing substrates. Species such as *A. notula, B. podagrica*, *G. quadridentata, N. vitripennis*, *N. splendens, P. vindemmiae*, *S. cameroni*, *S. drosophilae*, and *S. nigroaenea* were important in different datasets. Some parasitoids were associated with specific hosts, while others occurred in more than one host or substrate, suggesting different degrees of host specificity. This variation is important for biological control because generalist parasitoids may attack several pest fly species, whereas more specialized parasitoids may be useful in specific ecological contexts. Recent studies also indicate that competition among parasitoids, host specificity, longevity, and fitness traits can influence their potential as biological control agents (Schuster and Sivakumar, 2024; Cingolani *et al*., 2025; Hopper and Leung, 2026; Teng *et al*., 2026).

Overall, the present study demonstrates that parasitoid abundance, parasitism rate, host association, and Diptera occurrence are influenced by multiple interacting factors, including region, habitat, substrate type, trap type, dung exposure time, and host availability. The complementary chi-square analyses provided statistical support for patterns that were previously mainly descriptive, representing a methodological advance in the interpretation of these datasets. These findings reinforce the importance of parasitoids as natural enemies of synanthropic Diptera in rural, urban, pasture, and forensic environments. They also indicate that future studies should integrate standardized sampling, host identification, parasitoid identification, seasonal monitoring, and statistical analysis to improve the understanding of host–parasitoid interactions and their potential application in biological control programs (Figure 29) (Marchiori, 2014c; Marchiori, 2019b; Jaume-Schinkel and Mengual, 2022; Schuster and Sivakumar, 2024; Cingolani *et al*., 2025; Teng *et al*., 2026).

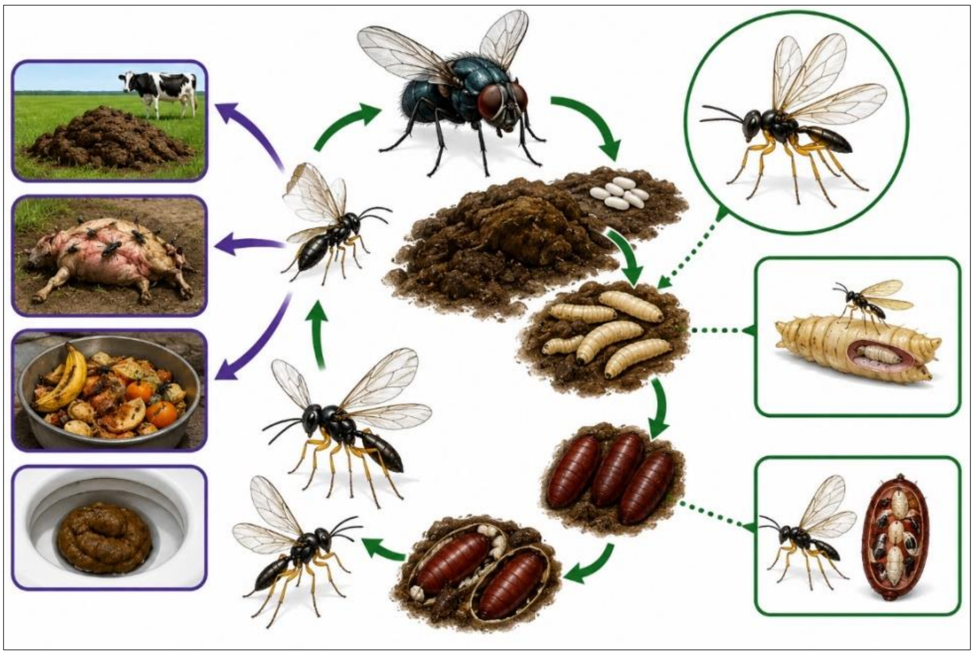

Figure 29: Integrated representation of parasitoid–Diptera interactions in decomposing organic substrates. The illustration shows different substrates, including cattle dung, food remains, carrion, and fruit, acting as breeding sites for Diptera and as sources of larvae and pupae attacked by parasitoids. Adult Diptera, immature stages, pupae, and parasitoid emergence are represented to illustrate the ecological relationships involved in the natural regulation of synanthropic fly populations

6.0. CONCLUSION

The present study demonstrated that decomposing organic substrates support diverse communities of Diptera and their parasitoids in rural, urban, pasture, forest, and forensic environments. Parasitoid abundance, parasitism rates, host associations, and Diptera occurrence varied according to region, habitat, substrate type, trap model, exposure time, and host availability. The complementary chi-square analyses applied in this manuscript provided statistical support for patterns that had previously been interpreted mainly in descriptive form, strengthening the ecological interpretation of host–parasitoid relationships. Overall, the results reinforce the importance of parasitoids as natural enemies of synanthropic Diptera and highlight their potential relevance in biological control, sanitary management, veterinary entomology, and forensic studies involving decomposing organic substrates.

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