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Diversity and Ecological Interactions of Parasitoids Associated with Diptera and Hemiptera in Agricultural, Fruit-Based, and Synanthropic Environments

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Abstract: Diptera and Hemiptera include species of ecological and economic importance, particularly in agricultural, fruit-based, and synanthropic systems, where they may act as decomposers, pests, or hosts for parasitoids. Parasitoids are important natural enemies and contribute to the regulation of these insect populations. This study presents an integrative synthesis of experimental data from investigations involving dipteran and hemipteran hosts and their associated parasitoids under field and laboratory conditions. Data were compiled from studies conducted in agricultural environments, fruit substrates, decomposing organic matter, synanthropic habitats, and controlled laboratory systems. The analysis included host species, parasitoid species, substrate type, trap type, habitat, altitude, parasitism rates, and host–parasitoid associations. When quantitative data were available, complementary chi-square analyses were performed to evaluate differences in parasitoid abundance, host association, substrate use, habitat distribution, altitude, and trap efficiency. The results demonstrated high parasitoid diversity, mainly involving Braconidae, Chalcididae, Diapriidae, Encyrtidae, Figitidae, and Pteromalidae. Parasitism rates varied according to substrate type, host availability, and environmental conditions, with higher values observed in substrates rich in organic matter. Frugivorous systems and decomposing substrates provided favorable conditions for host development and supported diverse parasitoid communities. Trap-based sampling also showed differences in parasitoid abundance according to trap color. Overall, parasitoids exhibited both generalist and more specialized strategies, attacking different host developmental stages, including eggs, larvae, and pupae. The complementary statistical analyses added support to ecological patterns previously described mainly in descriptive terms. These findings reinforce the importance of parasitoids as biological control agents and support their use in sustainable pest management strategies.

Keywords: Biological Control, Diptera, Hemiptera, Host–Parasitoid Interaction, Parasitism, Synanthropic Insects.

RESEARCH PAPER

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INTERDUCTION

Diptera and Hemiptera are important components of agroecosystems, acting both as decomposers and as agricultural pests. Dipteran species are commonly associated with decomposing organic substrates and fruit-based systems, whereas hemipterans are frequently linked to crop damage, particularly in cultivated plants such as maize. Their occurrence in agricultural environments highlights the need to better understand their ecological interactions and population dynamics. Parasitoids play a fundamental role as natural enemies of these insects, contributing significantly to the regulation of their populations (Jaume-Schinkel and Mengual, 2022; Huang *et al*., 2026; Teng *et al*., 2026; Zhang *et al*., 2026).

These organisms attack different developmental stages, including eggs, larvae, and pupae, and exhibit a wide range of biological strategies, such as solitary and gregarious development. Egg parasitoids are commonly associated with hemipteran hosts, whereas larval and pupal parasitoids are frequently linked to dipteran species. The diversity and occurrence of parasitoids are influenced by environmental conditions, substrate type, habitat structure, and host availability. In fruit-based and agricultural systems, decomposing organic matter provides favorable conditions for dipteran development, which in turn supports parasitoid populations. In crop systems, hemipteran pests also serve as important hosts for parasitoids, reinforcing their ecological and economic relevance (Schuster and Sivakumar, 2024;

Peralta-Aragón *et al*., 2025; Rocha *et al*., 2025; Hopper and Leung, 2026; Österman *et al*., 2026).

Laboratory studies have further advanced the understanding of insect biology by enabling controlled observation of life cycles, reproductive behavior, host development, parasitoid emergence, and host–parasitoid interactions. These approaches complement field studies and are essential for developing effective biological control strategies. Overall, interactions among Diptera, Hemiptera, and their parasitoids form a complex ecological network shaped by environmental and biological factors. Understanding these relationships is essential for improving integrated pest management and promoting sustainable agricultural practices (Cingolani *et al*., 2025; Gudin *et al*., 2026; Guimarães *et al*., 2026; Huang *et al*., 2026; Hopper and Leung, 2026; Krčmar *et al*., 2026).

Although several studies have described parasitoids associated with Diptera and Hemiptera, many of them presented their results mainly in descriptive form. However, the quantitative data available in tables and results, such as abundance, host species, substrate type, trap type, number of pupae, number of parasitized pupae, and parasitism rates, allow complementary statistical analyses to be performed. In this context, chi-square tests can provide additional support for evaluating differences in parasitoid abundance, host association, substrate use, habitat distribution, altitude, and trap efficiency. Thus, the present synthesis not only compiles information from previous studies but also adds statistical support to ecological patterns that were not always tested in the original articles.

The present study aimed to synthesize information on Diptera, Hemiptera, and their associated parasitoids in agricultural, fruit-based, field, and laboratory systems. Additionally, this study aimed to evaluate host–parasitoid interactions, substrate associations, ecological patterns, and their importance for biological control in different environments, using complementary chi-square analyses whenever the available quantitative data allowed statistical comparison.

2.0. MATERIALS AND METHODS

2.0.1. General Approach

This study was conducted as an experimental review based on the compilation and synthesis of data obtained from multiple independent studies involving Diptera, Hymenoptera, and Hemiptera and their associated parasitoids in agricultural, fruit-based, and laboratory systems. The analyzed data were derived from previously conducted investigations carried out under both field and laboratory conditions in different environments, including crop areas, fruit substrates, and controlled experimental settings. These studies followed similar methodological approaches, including the exposure of substrates to allow insect colonization, the collection of immature stages such as eggs and pupae, and the rearing of specimens under laboratory conditions until adult emergence.

In the original studies, insect sampling was performed using standardized methods, including traps, direct collection from substrates, and exposure of organic materials. After collection, immature stages were isolated and maintained under controlled environmental conditions to allow the emergence of dipterans, hemipterans, and their associated parasitoids. For the present study, data from these investigations were compiled and organized into a unified dataset. The variables considered included host species, parasitoid species, substrate type, environment, and parasitism rates. Parasitism was calculated as the proportion of parasitized hosts relative to the total number of collected individuals.

The compiled data were analyzed descriptively to identify patterns of host–parasitoid associations, distribution across environments, and variation in parasitism rates. When available, statistical results reported in the original studies were considered to support the interpretation of parasitoid preferences and ecological interactions. This integrative approach provided a broader understanding of parasitoid–host relationships across different ecological contexts, offering a comprehensive perspective on their role in biological control.

2.0.2. Experimental Methods

1. Method 1

This study was conducted as a synthesis of experimental investigations carried out under field and laboratory conditions, focusing on insects and their associated parasitoids in agricultural and fruit-based systems. Data were obtained from multiple studies performed in different environments, including crop areas, fruit substrates, and controlled laboratory conditions. In field studies, insect sampling was performed using standardized trapping systems and direct collection from organic substrates, including decomposing fruits, agricultural environments such as maize crops, and animal feces (bovine, chicken, and buffalo). In addition, host substrates were exposed in the field to allow natural colonization by dipteran and hemipteran species.

Adult flies were collected using baited traps constructed from matte black-painted metal cans (approximately 19 cm in height and 9 cm in diameter), equipped with lateral openings for insect entry and nylon funnels connected to plastic collecting bags. Organic materials used as attractants included human feces, bovine liver, bovine kidneys, chicken viscera, fish, and fruits. Additionally, twelve colored traps (yellow, black, red, white, green, and blue) were used, with two replicates per color, suspended approximately 1 m above ground level, spaced 2 m apart. Collected material was

transported to the laboratory, where immature stages such as eggs and pupae were isolated. Pupae were recovered using sieving and flotation methods and individually placed in containers or gelatin capsules until the emergence of adult insects or parasitoids. Hemipteran eggs collected from host plants were also maintained under controlled conditions to allow parasitoid emergence.

2. Method 2

Frugivorous flies were maintained under laboratory conditions to evaluate their development, behavior, and interaction with parasitoids. Fruits such as mango, guava, and other substrates were used as oviposition media. The collected material was placed in containers with substrate suitable for pupation, and development was monitored until adult emergence. Laboratory conditions were maintained at controlled temperature, humidity, and photoperiod.

3. Analyses and Formulas

1. Data were analyzed to determine parasitism rates, calculated as the proportion of parasitized pupae relative to the total number of pupae (P = parasitized pupae / total pupae × 100).
2. Synanthropy was calculated using the index SI = (2a + b − 2c) / 2, where a represents the percentage of individuals collected in urban areas, b in rural areas, and c in forested areas.

2.4. Statistical Analysis

Quantitative data were extracted from the tables and results presented in the reviewed articles. When available, information on substrate type, trap type, habitat, altitude, host species, parasitoid species, number of pupae, number of parasitized pupae, number of emerged parasitoids, and parasitism rate was organized for comparison. Parasitism rate was calculated as the number of parasitized pupae divided by the total number of pupae, multiplied by 100.

Complementary chi-square tests were performed when the original data allowed statistical comparison among categories. These analyses were used to evaluate differences in parasitoid abundance, parasitism rates, substrate use, host association, habitat distribution, altitude, and trap efficiency. The tests were not applied when the available data were qualitative, incomplete, or insufficient for comparison. In several original studies, the numerical data were presented descriptively, but chi-square tests had not been performed or reported. Therefore, the complementary analyses applied in the present study were used to provide statistical support for ecological patterns previously described mainly in descriptive terms. Statistical significance was considered at $p < 0.05$.

2.5. Note

Figures include both photographic records and schematic illustrations. Wing venation was omitted or simplified in schematic figures for clarity and does not represent diagnostic morphological variation among parasitoid taxa (Table 1).

Table 1: Taxonomic list of the main species cited in the manuscript, with authority, order, family, first citation, and subsequent citation

Species	Authority	Order	Family	First citation in the text	Subsequent citation
Aganaspis pelleranoi	Brèthes, 1924	Hymenoptera	Figitidae	*Aganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae)	*A. pelleranoi*
Aleochara notula	Erichson, 1839	Coleoptera	Staphylinidae	*Aleochara notula* Erichson, 1839 (Coleoptera: Staphylinidae)	*A. notula*
Anastrepha fraterculus	Wiedemann, 1830	Diptera	Tephritidae	*Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae)	*A. fraterculus*
Brachymeria podagrica	Fabricius, 1787	Hymenoptera	Chalcididae	*Brachymeria podagrica* (Fabricius, 1787) (Hymenoptera: Chalcididae)	*B. podagrica*
Ceratitis capitata	Wiedemann, 1824	Diptera	Tephritidae	*Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae)	*C. capitata*
Cyrtoneurina pararescita	Couri, 1995	Diptera	Muscidae	*Cyrtoneurina pararescita* (Couri, 1995) (Diptera: Muscidae)	*C. pararescita*
Diceratataspis grenadensis	Ashmead, 1895	Hymenoptera	Figitidae	*Diceratataspis grenadensis* Ashmead, 1895 (Hymenoptera: Figitidae)	*D. grenadensis*

Species	Authority	Order	Family	First citation in the text	Subsequent citation
Doryctobracon areolatus	Szépligeti, 1911	Hymenoptera	Braconidae	*Doryctobracon areolatus* (Szépligeti, 1911) (Hymenoptera: Braconidae)	*D. areolatus*
Fannia pusio	Wiedemann, 1830	Diptera	Fanniidae	*Fannia pusio* (Wiedemann, 1830) (Diptera: Fanniidae)	*F. pusio*
Gryon gallardoi	Brèthes, 1914	Hymenoptera	Scelionidae	*Gryon gallardoi* (Brèthes, 1914) (Hymenoptera: Scelionidae)	*G. gallardoi*
Helicoverpa zea	Boddie, 1850	Lepidoptera	Noctuidae	*Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae)	*H. zea*
Heliothis subflexa	Quenée, 1852	Lepidoptera	Noctuidae	*Heliothis subflexa* (Quenée, 1852) (Lepidoptera: Noctuidae)	*H. subflexa*
Heliothis virescens	Fabricius, 1777	Lepidoptera	Noctuidae	*Heliothis virescens* (Fabricius, 1777) (Lepidoptera: Noctuidae)	*H. virescens*
Hyposoter exigua	Viereck, 1912	Hymenoptera	Ichneumonidae	*Hyposoter exigua* (Viereck, 1912) (Hymenoptera: Ichneumonidae)	*H. exigua*
Leptopilina boulardi	Barbotin *et al.,* 1982	Hymenoptera	Figitidae	*Leptopilina boulardi* Barbotin *et al.,* 1982 (Hymenoptera: Figitidae)	*L. boulardi*
Manduca sexta paphus	Cramer, 1779	Lepidoptera	Sphingidae	*Manduca sexta paphus* (Cramer, 1779) (Lepidoptera: Sphingidae)	*M. sexta paphus*
Microplitis croceipes	Cresson, 1872	Hymenoptera	Braconidae	*Microplitis croceipes* (Cresson, 1872) (Hymenoptera: Braconidae)	*M. croceipes*
Musca domestica	Linnaeus, 1758	Diptera	Muscidae	*Musca domestica* Linnaeus, 1758 (Diptera: Muscidae)	*M. domestica*
Nasonia vitripennis	Walker, 1836	Hymenoptera	Pteromalidae	*Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae)	*N. vitripennis*
Oxysarcodexia thornax	Walker, 1849	Diptera	Sarcophagidae	*Oxysarcodexia thornax* (Walker, 1849) (Diptera: Sarcophagidae)	*O. thornax*
Pachycrepoideus vindemmiae	Rondani, 1875	Hymenoptera	Pteromalidae	*Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae)	*P. vindemmiae*
Palaeosepsis pusio	Schiner, 1868	Diptera	Sepsidae	*Palaeosepsis pusio* (Schiner, 1868) (Diptera: Sepsidae)	*P. pusio*
Paraganaspis egeria	Díaz, Gallardo & Walsh, 1996	Hymenoptera	Figitidae	*Paraganaspis egeria* Díaz, Gallardo & Walsh, 1996 (Hymenoptera: Figitidae)	*P. egeria*
Sarcophagula occidua	Fabricius, 1794	Diptera	Sarcophagidae	*Sarcophagula occidua* (Fabricius, 1794) (Diptera: Sarcophagidae)	*S. occidua*
Spalangia cameroni	Perkins, 1910	Hymenoptera	Pteromalidae	*Spalangia cameroni* Perkins, 1910 (Hymenoptera: Pteromalidae)	*S. cameroni*
Spalangia drosophilae	Ashmead, 1885	Hymenoptera	Pteromalidae	*Spalangia drosophilae* Ashmead, 1885 (Hymenoptera: Pteromalidae)	*S. drosophilae*
Spalangia endius	Walker, 1839	Hymenoptera	Pteromalidae	*Spalangia endius* Walker, 1839 (Hymenoptera: Pteromalidae)	*S. endius*

Species	Authority	Order	Family	First citation in the text	Subsequent citation
Spalangia nigra	Latreille, 1805	Hymenoptera	Pteromalidae	*Spalangia nigra* Latreille, 1805 (Hymenoptera: Pteromalidae)	*S. nigra*
Spalangia nigroaenea	Curtis, 1839	Hymenoptera	Pteromalidae	*Spalangia nigroaenea* Curtis, 1839 (Hymenoptera: Pteromalidae)	*S. nigroaenea*
Trichogramma pretiosum	Riley, 1879	Hymenoptera	Trichogrammatidae	*Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae)	*T. pretiosum*
Trichopria anastrephaee	Lima, 1940	Hymenoptera	Diapriidae	*Trichopria anastrephaee* Lima, 1940 (Hymenoptera: Diapriidae)	*T. anastrephae*
Zaprionus indianus	Gupta, 1970	Diptera	Drosophilidae	*Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae)	*Z. indianus*

3.0. RESULTS

Studies conducted in agricultural environments, fruit-based substrates, and controlled laboratory conditions revealed a diverse assemblage of dipteran and hemipteran species and their associated parasitoids. Insects were collected from decomposing fruits, crop systems, and trapping methods, showing variation in abundance according to substrate type and environmental conditions (Table 2).

Table 2: Distribution of Diptera, Hemiptera, and parasitoids across environments and substrates. Distribution of insect hosts and parasitoids across different environments. The table summarizes substrates, host groups, and parasitoid families. It provides an integrated overview of field and laboratory observations

Environment	Substrate	Hosts	Parasitoids	Notes
Agricultural	Maize	Diptera and Hemiptera	Pteromalidae and Scelionidae	High interaction
Colored traps	Visual attraction	Diptera	Multiple	Color effect
Field	Organic matter	Diptera	Diapriidae	Variable density
Fruit system	Fruits	Frugivorous Diptera	Figitidae, Braconidae	High diversity
General	Mixed	All groups	All groups	Integrated data
Laboratory	Controlled	Diptera and Hemiptera	Multiple	Stable development
Trap system	Baited traps	Diptera	Multiple	Bait influence

Frugivorous flies and other dipterans associated with plant substrates were frequently recorded, particularly in decomposing fruits and agricultural systems such as maize. These environments provided favorable conditions for oviposition and larval development, increasing host availability for parasitoids. A wide diversity of parasitoid species was observed, mainly belonging to the families Braconidae, Diapriidae, Figitidae, Pteromalidae, and Scelionidae. The most representative species included are *Gryon gallardoi* (Brèthes, 1914) (Hymenoptera: Scelionidae), *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae), *Paraganaspis egeria* Díaz, Gallardo & Walsh, 1996 (Hymenoptera: Figitidae), *Spalangia cameroni* Perkins, 1910 (Hymenoptera: Pteromalidae), *Spalangia drosophilae* Ashmead, 1885 (Hymenoptera: Pteromalidae).

These parasitoids exhibited different biological strategies, including egg and pupal parasitism, as well as solitary and gregarious development. Parasitism rates varied according to host availability and environmental conditions. Statistical analyses indicated significant differences in parasitoid preferences for hosts and substrates (χ^2, $p < 0.05$). In addition, insect attraction to trap colors showed significant variation (ANOVA, $p < 0.0001$), although environmental and substrate factors had a stronger influence (Table 3).

Table 3: Parasitism patterns, statistical analysis, and ecological interpretation. Summary of parasitism, statistical analyses, and ecological patterns. The table includes variables, methods, and analytical approaches used. It integrates quantitative and qualitative results of the study

Variable	Description	Method	Analysis	Result
Development	Life cycle	Laboratory	-	Successful
Diversity	Species richness	Comparative	-	High
Environment	Substrate effect	Descriptive	-	Strong
Host preference	Parasitoid-host	Comparative	Chi-square	Significant

Variable	Description	Method	Analysis	Result
Interaction	Host-parasitoid	Descriptive	-	Complex
Parasitism	Rate calculation	Quantitative	-	Variable
Trap color	Attraction	Experimental	ANOVA	Significant

3.0.1. Chi-Square Analysis

Complementary chi-square analyses were performed when the reviewed articles presented quantitative data suitable for statistical comparison. These analyses were not originally reported in several studies, but the available tables allowed additional statistical testing of parasitoid abundance, host association, substrate use, habitat effect, altitude, and trap efficiency.

For *S. drosophilae* collected in different hosts and substrates in Goiás, parasitism differed significantly among substrates ($\chi^2 = 184.50$; df = 5; $p < 0.0001$). Parasitoid abundance also differed among substrates ($\chi^2 = 550.07$; df = 5; $p < 0.0001$), and the association between host species and substrate was significant ($\chi^2 = 856.18$; df = 35; $p < 0.05$). These results indicate that substrate type and host availability strongly influenced the occurrence of *S. drosophilae*.

In the study of gregarious parasitoids of muscoid Diptera collected in different substrates in Itumbiara, Goiás, the distribution of parasitoid individuals differed significantly among species ($\chi^2 = 379.91$; df = 2; $p < 0.0001$). Parasitoid abundance also differed among substrates ($\chi^2 = 733.43$; df = 6; $p < 0.0001$), showing that both parasitoid identity and substrate type influenced the observed patterns.

For frugivorous flies and their parasitoids, significant differences were observed in host–parasitoid relationships and parasitism patterns. These results indicate that parasitoid occurrence varied according to host species, fruit substrate, and locality, reinforcing the influence of ecological conditions on parasitoid activity.

For *Fannia pusio* (Wiedemann, 1830) (Diptera: Fanniidae) collected in Caldas Novas, Goiás, parasitism differed significantly among substrates ($\chi^2 = 7.64$; df = 2; $p = 0.0219$). Parasitoid abundance also differed among species ($\chi^2 = 15.00$; df = 3; $p = 0.0018$), and the association between substrate and parasitoid species was significant ($\chi^2 = 14.78$; df = 6; $p = 0.0220$). These results suggest that substrate type influenced the structure of the parasitoid assemblage associated with *F. pusio*.

In the study of Diptera collected at different altitudes in Serra de Caldas Novas, Goiás, total abundance differed significantly between altitudes ($\chi^2 = 64.53$; df = 1; $p < 0.0001$). The distribution of substrates also differed between altitudes ($\chi^2 = 104.07$; df = 4; $p < 0.0001$), and species distribution varied significantly between altitudes ($\chi^2 = 1040.58$; df = 12; $p < 0.0001$). These results indicate that altitude and substrate availability influenced Diptera composition.

For parasitoids collected in traps of different colors in southern Goiás, abundance differed significantly among trap colors ($\chi^2 = 58.45$; df = 5; $p < 0.0001$). Parasitoid abundance also differed among species ($\chi^2 = 218.27$; df = 3; $p < 0.0001$), and the association between trap color and parasitoid species was significant ($\chi^2 = 51.96$; df = 15; $p < 0.0001$). These results indicate that trap color and parasitoid species identity influenced capture patterns.

For Eucoilinae collected in a remnant of Cerrado Forest in Itumbiara, Goiás, the abundance of taxa differed significantly ($\chi^2 = 26.40$; df = 7; $p = 0.0004$), indicating uneven distribution among Eucoilinae species. Similarly, Eucoilinae collected in Lavras, Minas Gerais, also showed significant differences in taxon abundance ($\chi^2 = 26.53$; df = 6; $p = 0.0002$).

In the study involving agricultural pests and their parasitoids, parasitoid distribution differed significantly among the four pest hosts ($\chi^2 = 507.96$; df = 3; $p < 0.0001$). Abundance also differed among parasitoid species ($\chi^2 = 1977.30$; df = 10; $p < 0.0001$), showing that parasitoid occurrence was highly concentrated in specific host–parasitoid associations.

For Cynipoidea associated with bovine feces in native forest and pasture areas in Goiás, parasitism differed significantly among host species ($\chi^2 = 785.77$; df = 3; $p < 0.0001$). Parasitoid abundance also differed among taxa ($\chi^2 = 72.40$; df = 2; $p < 0.0001$), and the host–parasitoid association was significant ($\chi^2 = 88.60$; df = 6; $p < 0.0001$). However, the abundance of Figitidae did not differ significantly between pasture and native forest ($\chi^2 = 2.17$; df = 1; $p = 0.1407$), and the distribution of Figitidae taxa between these habitats was also not significant ($\chi^2 = 12.35$; df = 9; $p = 0.1945$).

For synanthropic Diptera collected in cattle feces in Brazil, the distribution of pupae differed significantly among forest, rural, and urban environments ($\chi^2 = 64.50$; df = 2; $p < 0.0001$). Abundance also differed among species ($\chi^2 = 125.48$; df = 3; $p < 0.0001$), and species distribution varied significantly among environments ($\chi^2 = 167.25$; df = 6; $p < 0.0001$). These results indicate that synanthropic patterns were strongly influenced by habitat type.

For microhymenopterans associated with *Musca domestica* L., 1758 (Diptera: Muscidae) collected on different substrates in Brazil, parasitism differed significantly among substrates ($\chi^2 = 346.32$; df = 5; $p < 0.0001$). Parasitoid abundance differed among taxa ($\chi^2 = 80.00$; df = 6; $p < 0.0001$), and the association between

substrate and parasitoid species was significant (χ^2 = 133.83; df = 30; $p < 0.0001$). These results show that substrate type strongly influenced parasitoid occurrence and host–parasitoid interactions.

Overall, the complementary chi-square analyses demonstrated that parasitoid abundance, parasitism rates, host associations, substrate use, habitat distribution, altitude, and trap efficiency were not randomly distributed in most datasets. These analyses provided additional statistical support for ecological patterns that had previously been described mainly in descriptive terms in the original studies.

Host–parasitoid relationships indicated both specificity and generalism. Egg parasitoids were mainly associated with hemipteran hosts, whereas pupal parasitoids were more frequently associated with dipteran species. Species of *Spalangia* showed broader host ranges, while others exhibited more specific associations. Laboratory studies confirmed that controlled conditions allowed the successful development of insects and parasitoids, enabling observation of life cycles and ecological interactions. Overall, the results demonstrate that parasitoid communities are structured by host availability, substrate type, environmental conditions, and sampling methodology, reinforcing their role in biological control.

4.0. EXPERIMENTAL ARTICLES

1. *Spalangia Drosophilae* Collected in Different Hosts and Substrates in Goiás

A total of 11.669 Diptera pupae were collected from six different substrates, from which 294 pupae were parasitized, resulting in the emergence of 354 specimens of *S. drosophilae*. The overall parasitism rate was 2.5%. This value may be influenced by host availability, substrate type, and the occurrence of more than one parasitoid individual emerging from some host pupae. The highest number of parasitoids was recorded in bovine feces, followed by buffalo feces, bovine liver, chicken manure, fish, and human feces (Table 4).

Table 4: Variations in parasitism rates, host associations, and distribution of *S. drosophilae* across different substrates in Goiás. The data emphasize the ecological adaptability of this parasitoid and its potential role in the biological control of Diptera populations

Substrate	Host species	Frequency	Number of parasitoids	Parasitized pupae	Parasitism rate (%)
Bovine feces	*Archisepsis scabra*	185	10	10	5.4
	Brontaea quadristigma	49	1	1	2.0
	Palaeosepsis spp.	1.611	181	181	11.2
	Sarcophagula occidua	2.833	27	27	1.0
Bovine liver	*Fannia pusio*	2.018	6	6	0.3
	Oxysarcodexia thornax	191	43	3	1.6
	Peckia chrysostoma	90	6	1	1.1
Buffalo feces	*Archisepsis* sp.	310	4	4	1.3
	Brontaea quadristigma	138	1	1	0.7
	Palaeosepsis spp.	1.948	1	1	0.1
	Sarcophagula occidua	931	33	33	3.5
Chicken manure	*Musca domestica*	591	1	1	0.2
Fish	*Oxysarcodexia thornax*	286	16	1	0.4
Human feces	*Musca domestica*	135	3	3	2.2
	Peckia chrysostoma	353	21	21	6.0
Total		**11669**	**354**	**294**	**2.5**

Parasitism rates varied among substrates, with values of 4.7% in bovine feces, 1.2% in buffalo feces, 0.2% in chicken manure, 4.9% in human feces, 0.4% in bovine liver, and 0.3% in fish. The highest parasitism rate among host groups was observed in *Palaeosepsis* spp. (Diptera: Sepsidae) in bovine feces, with 181 parasitized pupae from 1.611 pupae, corresponding to 11.2%. Human feces also showed an important parasitism rate, mainly because of the association of *S. drosophilae* with *Peckia chrysostoma* (Wiedemann, 1830) (Diptera: Sarcophagidae) (Figure 1).

Spalangia drosophilae is an important pupal parasitoid of Diptera and may contribute to biological control in agroecosystems and synanthropic environments. This parasitoid was recorded in association with several host species and substrates, demonstrating a broad host range and ecological adaptability. The number of emerged parasitoids was higher than the number of parasitized pupae in some records, indicating that multiple parasitoid individuals may emerge from a single host pupa under certain conditions.

Complementary chi-square analyses were performed using the quantitative data available in the table. Parasitism differed significantly among substrates (χ^2 = 184.50; df = 5; $p < 0.0001$), and parasitoid

abundance also differed significantly among substrates (χ^2 = 550.07; df = 5; p < 0.0001). Host preference analysis indicated that *S. drosophilae* was not randomly distributed among host-substrate associations (χ^2 = 856.18; df = 35; p < 0.05). These results support the interpretation that host species and substrate type influenced parasitoid occurrence.

These findings demonstrate that *S. drosophilae* have a wide host range and occur in multiple substrates, which favors its persistence in the environment and highlights its potential use in biological control programs (Figure 1) (Marchiori *et al.*, 2013a; Marchiori *et al.*, 2013b; Marchiori and Borges, 2014a; Marchiori, 2015; van Noort, 2026).

Figure 1: Adult female of *Spalangia drosophilae* Ashmead, 1887 (Hymenoptera: Pteromalidae) in lateral view, with enlarged details of the head and thorax and of the forewing. The figure highlights the principal external morphological characteristics of this pupal parasitoid associated with Diptera developing in decomposing organic substrates. Source: Simon van Noort (Iziko South African Museum)

2: *Aleochara Notula* Erichson, 1839 (Coleoptera: Staphylinidae) as a Natural Enemy of Dipterans in Chicken and Cattle Feces in Brazil

In cattle feces, 905 pupae of *Palaeosepsis* spp. from which three (3) specimens of *A. notula* emerged, presenting a rate of parasitism of 0.33%. In chicken feces during the period of August 2007, 600 pupae of *M. domestica* were collected, from which two specimens of *A. notula* emerged, presenting a percentage of parasitism of 0.3%. These values suggest that, although at present, the species exerts a limited parasitic impact under the conditions studied. Despite the low parasitism rates, *A. notula* plays a dual ecological role. During its larval stage, it acts as a parasitoid of dipteran pupae, while adults behave as predators of eggs and larvae.

This combination enhances its importance in regulating fly populations in decomposing organic substrates. The presence of *A. notula* in both chicken and cattle feces indicates its adaptability to different environments and substrates. However, the low frequency of parasitism may be influenced by factors such as host availability, environmental conditions, and competition with other natural enemies (Figure 2).

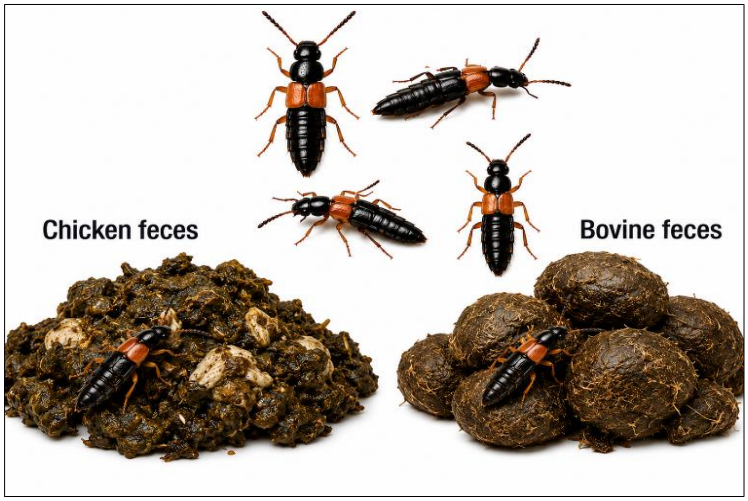

Figure 2: Dorsal view of an adult specimen of *Aleochara.* The image shows the characteristic elongated body, reduced elytra, and exposed abdominal segments. These morphological features are typical of rove beetles associated with decomposing organic substrates. The figure emphasizes adaptations related to its ecological role as a predator and parasitoid of dipteran pupae. Species of this genus are important natural enemies contributing to biological control in agroecosystems

These findings reinforce the potential of *A. notula* as a biological control agent, although its effectiveness may depend on ecological conditions. Therefore, further studies are necessary to understand its role better and optimize its use in integrated pest management programs targeting dipteran populations (Marchiori, 2021a).

3. Occurrence of Microplitis Croceipes (Cresson, 1872) (Hymenoptera: Braconidae: Microgastrinae) as Parasitoid of Manduca Sexta Paphus (Cramer, 1779) (Lepidoptera: Sphingidae) in Southern Goiás, Brazil

A total of 43 *M. croceipes* adults were obtained from two (2) larvae of *M. sexta paphus*; the parasitism rate was 4.7%. Microgastrinae are solitary or gregarious koinobiont endoparasitoid larvae belonging to almost all families of Lepidoptera. *Microplitis croceipes*, an important larval parasitoid, has been widely used as a model organism in studies on insect learning and foraging behavior. Like other parasitoid wasps, *M. croceipes* uses olfactory and visual cues to locate and oviposit in hosts such as *Heliothis subflexa* (Quenée, 1852) (Lepidoptera: Noctuidae), *Heliothis virescens* (Fabricius, 1777) (Lepidoptera: Noctuidae), and *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae). It also uses these cues to locate food resources, and their efficiency is enhanced through associative learning (Figure 3).

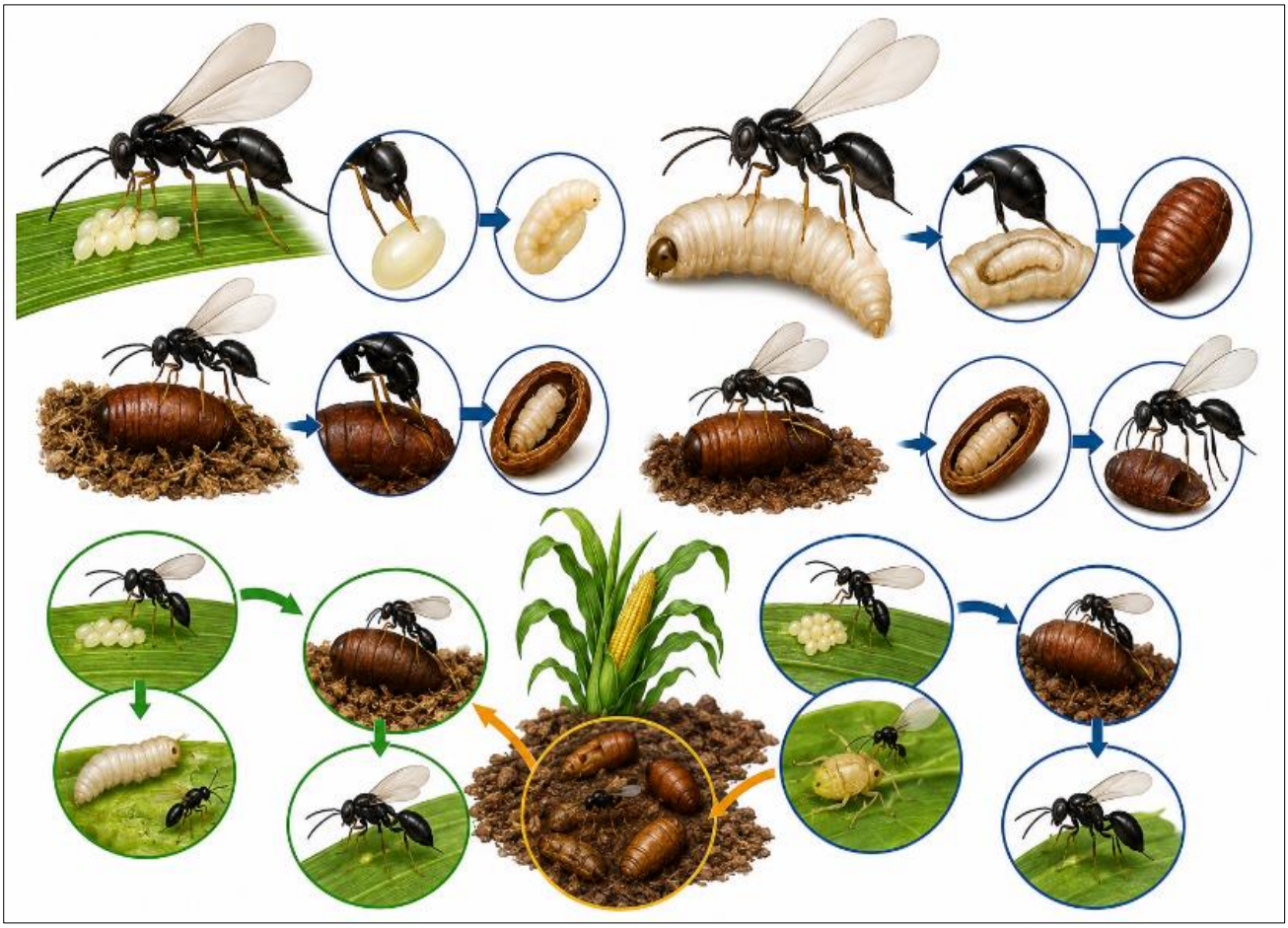

Figure 3: Developmental interactions between hymenopteran parasitoids and their insect hosts in a maize agroecosystem. The figure illustrates parasitoid oviposition, development within eggs, larvae, and pupae, adult emergence, and the recycling of parasitoid populations in the agricultural environment. These interactions contribute to the natural regulation of insect pests

4. Frugivorous Flies of the Drosophilidae, Lonchaeidae, and Tephritidae and Their Parasitoids in Brazil

A total of 1.516 pupae were collected in Goiás, from which 71 parasitoids emerged, yielding an overall parasitism rate of 4.7%. The study revealed that frugivorous flies and their parasitoids showed distinct patterns of occurrence and parasitism according to host species, substrate, and geographic region. In Goiás, *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) was the most frequent species, representing 60.5% of the total pupae collected, followed by *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) with 30.5%, and *Neosilba* sp. (Diptera: Lonchaeidae) with 9.0%. This pattern is probably related to the polyphagous behavior of *A. fraterculus* and its capacity to exploit different fruit hosts.

Parasitoid diversity in Goiás was relatively low. *Doryctobracon areolatus* (Szépligeti, 1911)

(Hymenoptera: Braconidae) was the most abundant parasitoid, with 43 individuals, representing 60.6% of all parasitoids recorded in this dataset. *Aganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae) represented 36.6%, and *P. vindemmiae* represented 2.8%. The highest parasitism rate was recorded for *D. areolatus* in *A. fraterculus* (4.3%). Complementary chi-square analyses showed significant differences in parasitism among host species ($\chi^2 = 36.02$; df = 2; $p < 0.0001$) and in parasitoid abundance among species ($\chi^2 = 35.86$; df = 2; $p < 0.0001$), indicating that parasitoid occurrence was not evenly distributed among hosts or parasitoid taxa (Table 5).

Table 5: Distribution of fruit fly species and their parasitoids in Goiás, Brazil. The table presents host–parasitoid relationships and parasitism levels. *A. fraterculus* and *D. areolatus* were the most representative species

Host species	Number of pupae	Frequency (%)	Parasitoid species	Parasitized pupae	Frequency (%)	Parasitism rate (%)
Anastrepha fraterculus	917	60.5	*Aganaspis pelleranoi*	26	36.6	2.8
			Doryctobracon areolatus	39	55.0	4.3
			Pachycrepoideus vindemmiae	2	2.8	0.2
Ceratitis capitata	463	30.5	*Doryctobracon areolatus*	2	2.8	0.4
Neosilba sp.	136	9.0	*Doryctobracon areolatus*	2	2.8	1.5
Total	**1.516**	**100**		**71**	**100**	**4.7**

A total of 349 pupae of *A. fraterculus* were obtained in Minas Gerais, from which 45 parasitoids emerged, resulting in an overall parasitism rate of 13.0%. Parasitoid diversity was higher in this region, with six parasitoid taxa recorded. *Trichopria anastrephae* Lima, 1940 (Hymenoptera: Diapriidae) was the most frequent parasitoid, with 20 individuals (44.5%), followed by *Leptopilina boulardi* Barbotin *et al.*, 1982 (Hymenoptera: Figitidae: Eucoilinae) with 10 individuals (22.2%), and *Spalangia endius* Walker, 1839 (Hymenoptera: Pteromalidae) with six individuals (13.4%). The complementary chi-square analysis showed significant differences in parasitoid abundance among taxa ($\chi^2 = 30.87$; df = 5; $p < 0.0001$). The parasitism rate was significantly higher in Minas Gerais than in Goiás ($\chi^2 = 32.79$; df = 1; $p < 0.0001$), suggesting regional differences in parasitoid activity and host availability.

Regarding host preference, the original analysis indicated that *A. fraterculus* showed a preference for pitanga, whereas *C. capitata* was more associated with guava. In addition, *Neosilba* sp. was more frequently found in pitanga (66.6%; $\chi^2 = 27.39$; df = 1; $p < 0.05$). These associations demonstrate that host selection is influenced by fruit characteristics and ecological conditions, which directly affect infestation levels and parasitoid interactions (Table 6).

Table 6: Diversity of parasitoids associated with *A. fraterculus* in Minas Gerais, Brazil. The table summarizes frequency and parasitism percentages. *Trichopria anastrephae* showed the highest frequency and parasitism rate

Host specie	Number of pupae	Parasitoid species	Frequency (%)	Number of parasitoids	Parasitism rate (%)
Anastrepha fraterculus	349				
		Doryctobracon areolatus	11.1	5	1.4
		Leptopilina boulardi	22.2	10	2.9
		Odontosema anastrepha	4.4	2	0.6
		Pachycrepoideus vindemmiae	4.4	2	0.6
		Spalangia endius	13.4	6	1.7
		Trichopria anastrephae	44.5	20	5.7
Total	**349**		**100**	**45**	**13.0**

In studies involving *Psidium guajava* L. 1753 (Myrtales: Myrtaceae), *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) was highly abundant, with 1.068 individuals collected. Among its parasitoids, *P. vindemmiae* was dominant, representing 96.7% of parasitoid specimens and showing a parasitism rate of 20.1%. This high efficiency is probably related to its broad host range and strong ability to locate pupae. Other parasitoids, such as *L. boulardi* and *S. endius,* were present at lower frequencies. The parasitoids of Figitidae, particularly *A. pelleranoi* and *Diceratasspis grenadensis* Ashmead, 1895 (Hymenoptera: Figitidae), were associated with *A. fraterculus*, with a total parasitism rate of 16.7%. The relatively high parasitism observed in this system may be linked to favorable environmental conditions and host density (Figure 4).

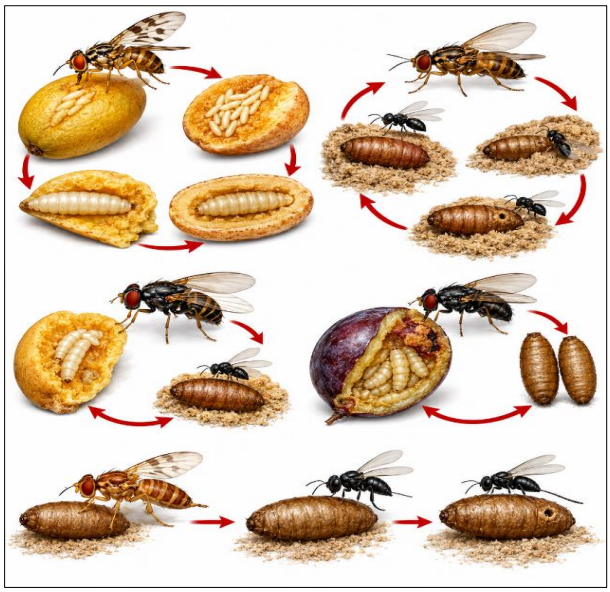

Figure 4: Life cycles of frugivorous flies developing in fruit substrates and their associated parasitoids. The figure illustrates oviposition, larval development within fruits, pupation, adult emergence, and the attack of parasitoids on immature stages, contributing to the natural biological control of fly populations

Overall, the results demonstrate that parasitoid communities vary significantly across regions and hosts, influencing the level of natural control of frugivorous flies. Species such as *D. areolatus*, *P. vindemmiae,* and *T. anastrephae,* and others, stand out as important regulators of dipteran populations. The complementary chi-square analyses added statistical support to patterns that were originally described mainly in descriptive terms. Understanding these interactions is essential for improving integrated pest management strategies and enhancing sustainable fruit production systems (Silva *et al.*, 2003; Marchiori, 2014c; Marchiori, 2023).

5. Parasitoids of *F. Pusio* Collected in Caldas Novas, Goiás, Brazil

The results showed that parasitoid species associated with *F. pusio* varied according to substrate type and ecological conditions. A total of 325 pupae were obtained, from which 24 pupae were parasitized, resulting in an overall parasitism rate of 7.4%. This level of parasitism indicates moderate natural regulation of *F. pusio* populations in the studied environment (Table 7).

Among the parasitoids, *P. vindemmiae* was the most frequent species, with 14 parasitized pupae, representing 58.3% of the total parasitoids recorded. *Spalangia nigra* Latreille, 1805 (Hymenoptera: Pteromalidae) showed the highest parasitism rate in a single substrate, with 5 parasitized pupae in bovine liver, corresponding to 7.7%. This result suggests that bovine liver may provide favorable conditions for this parasitoid-host interaction (Table 7).

Table 7: Parasitoids of *F. pusio* collected in different substrates in Caldas Novas, Goiás, Brazil. Distribution of parasitoids associated with *F. pusio* across substrates, with the number of pupae, parasitized pupae, and parasitism percentages

Substrate	Total pupae	Frequency (%)	Parasitoid species	Parasitized pupae	Parasitism rate (%)
Bovine liver	65	20.0	*Pachycrepoideus vindemmiae*	3	4.6
			Spalangia drosophilae	2	3.1
			Spalangia nigra	5	7.7
Fish	23	7.1	*Pachycrepoideus vindemmiae*	1	4.4
Human feces	237	72.9	*Pachycrepoideus vindemmiae*	10	4.2
			Paraganaspis egeria	3	1.3
Total	**325**	**100**	**-**	**24**	**7.4**

Parasitism differed among substrates. Bovine liver presented the highest substrate-level parasitism, with 10 parasitized pupae from 65 pupae (15.4%), followed by human feces, with 13 parasitized pupae from 237 pupae (5.5%), and fish, with 1 parasitized pupa from 23 pupae (4.4%). This indicates that substrate type influenced host availability and parasitoid occurrence.

Complementary chi-square analyses supported these differences. Parasitism differed significantly among substrates ($\chi^2 = 7.64$; df = 2; p = 0.0219),

parasitoid abundance differed among species ($\chi^2 = 15.00$; df = 3; p = 0.0018), and the association between substrate and parasitoid species was significant ($\chi^2 = 14.78$; df = 6; p = 0.0220). These results indicate that parasitoid distribution was not random and was influenced by substrate type and parasitoid identity (Figure 5).

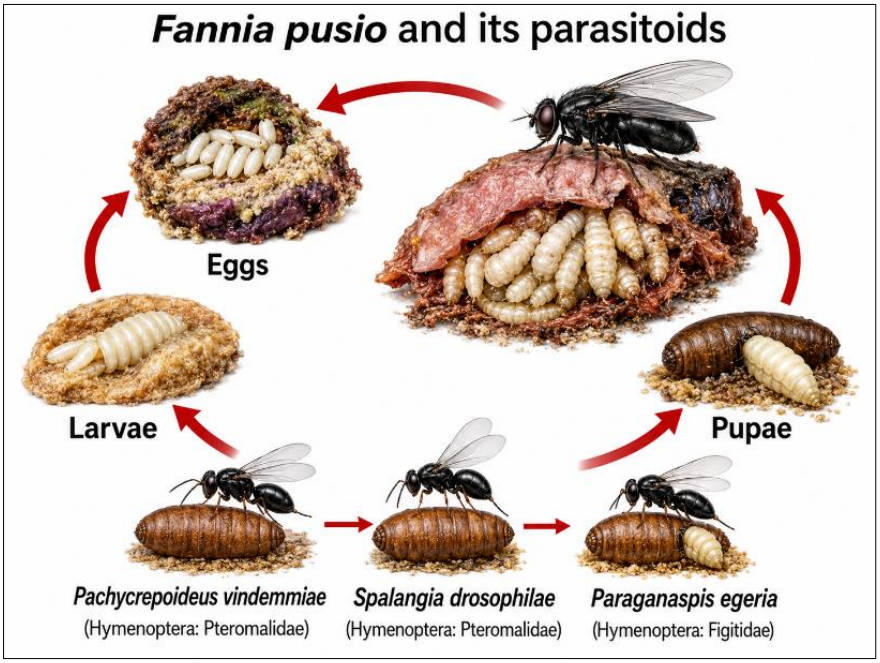

Figure 5: Life cycle of *F. pusio* and its associated parasitoids under natural conditions. The figure illustrates the developmental stages from egg to adult, highlighting the pupal phase where parasitism occurs. Parasitoid species such as *P. vindemmiae, P. egeria*, *S. drosophilae,* and *S. nigra,* contribute to the natural regulation of fly populations

Substrate preference significantly influenced parasitoid occurrence. *Paraganaspis egeria* was recorded only in hosts collected from human feces, whereas *S. drosophilae* and *S. nigra* were associated with bovine liver. *Pachycrepoideus vindemmiae* was the only species recorded in all three substrates, reinforcing its broad ecological adaptability and its importance as a generalist parasitoid of synanthropic flies (Table 8).

Table 8: Summary of parasitoid diversity and parasitism rates. The table highlights differences among species. *Spalangia nigra* showed the highest parasitism rate

Substrates	Parasitoid species	Number of individuals	Frequency (%)	Parasitism rate (%)
Bovine liver, fish, and human feces	*Pachycrepoideus vindemmiae*	14	58.3	4.3
Bovine liver	*Spalangia drosophilae*	2	8.3	0.6
	Spalangia nigra	5	20.8	7.7
Human feces	*Paraganaspis egeria*	3	12.5	0.9
Total		**24**	**100**	**7.4**

The results also highlight differences in parasitic strategies among species. *Pachycrepoideus vindemmiae* is a solitary parasitoid with a wide host range, while figitid species such as *P. egeria* may parasitize earlier developmental stages of dipteran hosts. Additionally, *S. drosophilae* are known to parasitize pupae of small dipterans, reinforcing its role in regulating populations of synanthropic flies.Overall, the study demonstrates that parasitoid assemblages associated with *F. pusio* are structured by substrate type and species-specific biological traits. The dominance of *P. vindemmiae* and the high parasitism efficiency of *S. nigra* emphasize their ecological importance and potential application in biological control programs targeting muscoid flies (Marchiori *et al.*, 2005a).

6. Parasitoids of Diptera Collected in Traps of Different Colors from the Southern Part of Goias State

A total of 88 parasitoid individuals were collected using traps of different colors. *Brachymeria podagrica* was the most frequent species, with 82 individuals, corresponding to 93.2% of all parasitoids recorded. Total abundance varied among trap colors, with the highest number of individuals recorded in black traps (37), followed by yellow (20), blue (15), white (12), red (3), and green traps (1). The greater number of

individuals in black traps may be related to greater heat absorption, faster bait decomposition, and stronger odor emission, which can increase the attraction of dipteran hosts and their parasitoids.

The original analysis indicated no significant preference for trap color when total attraction was evaluated by analysis of variance ($F = 0.772$; $p = 0.58$). However, the complementary chi-square analysis applied in the present review showed that parasitoid abundance differed significantly among trap colors ($\chi^2 = 58.45$; $df = 5$; $p < 0.0001$). This result indicates that the captures were not evenly distributed among colors, although color alone may not explain parasitoid attraction because bait decomposition, odor emission, temperature, and environmental conditions may also influence parasitoid activity (Table 9).

Table 9: Parasitoids of Diptera collected in traps of different colors in southern Goiás State. The table presents the frequency and relative abundance of each parasitoid according to trap color

Trap color	Parasitoid species	Number of individuals	Frequency (%)
Black	*Brachymeria podagrica*	37	42.0
Blue	*Brachymeria podagrica*	15	17.1
Green	*Brachymeria podagrica*	1	1.1
Red	*Brachymeria podagrica*	2	2.3
	Spalangia endius	1	1.1
White	*Brachymeria podagrica*	10	11.4
	Spalangia cameroni	2	2.3
Yellow	*Brachymeria podagrica*	17	19.3
	Brachymeria sp.	3	3.4
Total		**88**	**100**

Brachymeria podagrica was recorded in all trap colors and was the dominant parasitoid in the dataset. The abundance among parasitoid taxa also differed significantly ($\chi^2 = 218.27$; $df = 3$; $p < 0.0001$), reflecting the strong predominance of *B. podagrica*. Other parasitoids were collected in lower numbers: *Brachymeria* sp. was recorded in yellow traps, *Spalangia cameroni* Perkins, 1910 (Hymenoptera, Pteromalidae) in white traps, and *S. endius* in red traps. The association between trap color and parasitoid species was significant ($\chi^2 = 51.96$; $df = 15$; $p < 0.0001$), although this result should be interpreted with caution because some species were represented by few individuals (Figure 6).

Figure 6: Arrangement of six trap models used for capturing Diptera and associated parasitoids under field conditions. The figure illustrates standardized traps painted in different colors to evaluate their influence on parasitoid capture

The results summarized in Table 10 confirm that black traps had the highest capture rate, followed by yellow traps, whereas green traps had the lowest number of parasitoids. This pattern suggests that trap color may

influence capture efficiency, but the strong predominance of *B. podagrica* indicates that parasitoid biology and host availability are also important factors. The low frequencies of *S. cameroni* and *S. endius* indicate that these species occurred occasionally in the traps sampled (Table 10)

Table 10: Analysis of parasitoid abundance according to trap color. The table summarizes total captures, relative abundance, dominant species, and general observations for each trap color

Trap color	Total parasitoids	Relative abundance (%)	Dominant species	Observation
Black	37	42	*Brachymeria podagrica*	Highest capture
Blue	15	17	*Brachymeria podagrica*	Moderate capture
Green	1	1.1	*Brachymeria podagrica*	Lowest capture
Red	3	3.4	*Brachymeria podagrica*	Low capture
White	12	13.6	*Brachymeria podagrica*	Moderate capture
Yellow	20	22.7	*Brachymeria podagrica*	High attraction
Total	**88**	**100**		

Overall, the results indicate that parasitoid assemblages were influenced by trap color, but also by environmental and substrate-related factors. The predominance of *B. podagrica* highlights its ecological relevance and potential use in biological control programs targeting dipteran populations. The complementary chi-square analyses provided statistical support for differences in total abundance among trap colors and for the dominance of *B. podagrica*, while the original analysis indicated no significant color preference when evaluated by analysis of variance. Together, these results suggest that combined methodological and ecological factors should be considered when interpreting parasitoid capture patterns (Figure 7) (Marchiori *et al.*, 2009).

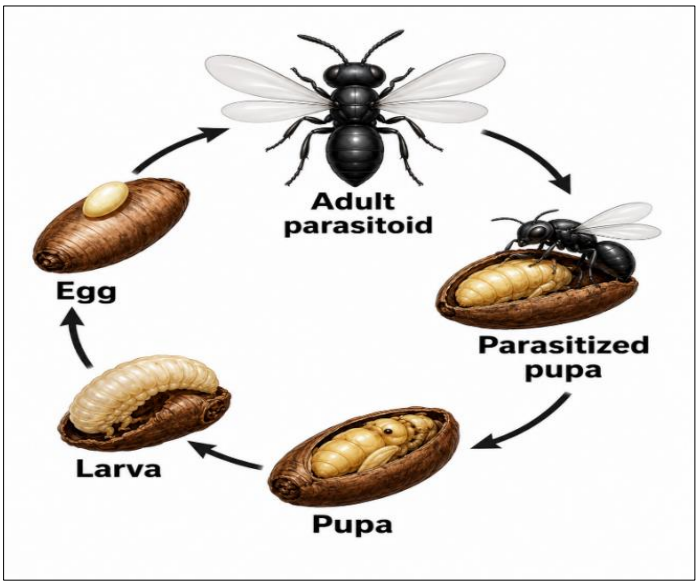

Figure 7: Life cycle of *B. podagrica*, illustrating development from immature stages to the adult parasitoid. The species develops in dipteran pupae and emerges as an adult, highlighting its role as a pupal parasitoid and natural enemy of synanthropic flies

7. Superparasitism of *Spalangia Nigroaenea* Curtis, 1839 (Hymenoptera: Pteromalidae) in Bovine Feces in Brazil

The species of *Spalangia* are primarily parasitoids of flies from the families Calliphoridae, Chloropidae, Drosophilidae, Muscidae, and Sarcophagidae, and in various parts of the world, making their use in biological control possible. Many species are known to develop in hosts that live in feces, decaying meat, and plant tissues. *S. nigroaenea* behaves as a parasitoid of flies from the Sepsidae. The results demonstrated the occurrence of superparasitism by *S. nigroaenea* in pupae of *Cyrtoneurina pararescita* (Couri, 1995) (Diptera: Muscidae) collected from bovine dung. A total of two pupae of *C. pararescita* were obtained, and from each pupa, two parasitoid individuals of *S. nigroaenea* emerged.

This finding confirms that more than one individual of the same parasitoid species can successfully develop within a single host, characterizing superparasitism under natural conditions. The occurrence of superparasitism suggests that *S. nigroaenea* may adopt flexible reproductive strategies depending on host availability. In situations where host density is low, females may oviposit multiple eggs in the

same host, increasing the chances of offspring survival. This behavior may represent an adaptive strategy to maximize reproductive success in environments with limited resources (Figure 8).

Figure 8: Superparasitism of *S. nigroaenea* developing within pupae of *C. pararescita* under natural conditions. The figure illustrates multiple parasitoid individuals emerging from a single host pupa, characterizing superparasitism. This reproductive strategy may occur under low host availability and contributes to the regulation of dipteran populations in bovine dung

The study also reinforces the ecological role of *S. nigroaenea* as a pupal parasitoid of muscoid Diptera associated with decomposing organic matter such as bovine feces. This species has been previously reported parasitizing hosts from several dipteran families, demonstrating its importance in natural biological control processes. Although based on a limited number of observations, this study represents the first record of superparasitism by *S. nigroaenea* in *C. pararescita* pupae in Brazil. This contributes to the understanding of parasitoid biology and highlights the need for further studies to evaluate the ecological and evolutionary implications of this behavior in natural populations (Marchiori *et al.,* 2005b).

8. Synanthropy of Dipterans Collected in Cattle Feces in Brazil

A total of 372 dipteran pupae were collected from bovine feces in three environments: forest, rural, and urban areas. Of these, 107 pupae were obtained from the forest area, 194 from the rural area, and 71 from the urban area, corresponding to 28.8%, 52.2%, and 19.0% of the total, respectively. The rural environment showed the highest abundance of dipterans associated with cattle feces. The complementary chi-square analysis indicated that the distribution of pupae among environments was not uniform ($\chi^2 = 64.50$; df = 2; $p < 0.0001$).

Among the identified species, *Sarcophagula occidua* (Fabricius, 1794) (Diptera: Sarcophagidae) was the most frequent, with 157 individuals (42.2%), followed by *Palaeosepsis pusio* (Schiner, 1868) (Diptera: Sepsidae) with 130 individuals (34.9%), *Archisepsis scabra* (Loew, 1861) (Diptera: Sepsidae) with 66 individuals (17.7%), and *Brontaea quadristigma* (Thomson, 1869) (Diptera: Muscidae) with 19 individuals (5.1%). Species abundance differed significantly among taxa ($\chi^2 = 125.48$; df = 3; $p < 0.0001$), indicating that the dipteran community associated with bovine feces was dominated by a few species (Table 11).

Table 11: Abundance of dipteran species collected from cattle feces in forest, rural, and urban areas in Brazil, with total number of individuals and synanthropic tendency

Host species	Forest (N)	Rural (N)	Urban (N)	Total	Synanthropy tendency
Archisepsis scabra	51	15	0	66	Strongly negative / forest-associated
Brontaea quadristigma	0	13	6	19	Strongly positive
Palaeosepsis pusio	26	101	3	130	Moderately positive
Sarcophagula occidua	30	65	62	157	Moderately positive
Total	**107 (28.8%)**	**194 (52.2%)**	**71 (19.0%)**	**372**	----------

The distribution of species varied significantly among environments ($\chi^2 = 167.25$; df = 6; $p < 0.0001$). *Archisepsis scabra* was predominantly recorded in forest areas, whereas *P. pusio* was more abundant in rural areas. In contrast, *B. quadristigma* and *S. occidua* were more frequently associated with rural and urban environments, suggesting greater tolerance to anthropogenic conditions (Table 12).

Table 12: Synanthropy index of dipteran species collected from cattle feces in Brazil. Positive values indicate association with human-modified environments, whereas negative values indicate preference for more preserved habitats

Species	Urban (%) (a)	Rural (%) (b)	Forest (%) (c)	Synanthropy Index (SI)	Interpretation
Archisepsis scabra	0.0	22.7	77.3	-65.9	Strongly negative (forest-associated)
Palaeosepsis pusio	2.3	77.7	20.0	+21.1	Moderately positive
Brontaea quadristigma	31.6	68.4	0.0	+65.8	Strongly positive
Sarcophagula occidua	39.5	41.4	19.1	+41.1	Moderately positive

Note. SI = (2a + b − 2c) / 2.

The synanthropy index revealed distinct ecological preferences among species. *Archisepsis scabra* presented a strongly negative synanthropy index, indicating a clear association with forest environments and avoidance of anthropized areas. In contrast, *B. quadristigma* showed a strongly positive synanthropy index, demonstrating a strong association with human-modified environments. *P. pusio* and *S. occidua* exhibited moderately positive indices, indicating adaptation to environments influenced by human activity (Figure 9).

Figure 9: Distribution of synanthropic dipteran species across forest, rural, and urban environments associated with bovine feces. The figure illustrates differences in species occurrence along an environmental gradient influenced by human activity, ranging from forest-associated species to species adapted to urban and rural conditions

These findings show that environmental conditions and human influence structure dipteran communities associated with bovine feces. The significant chi-square results support the interpretation that species occurrence was not randomly distributed among environments. The variation in synanthropy indices demonstrates that some species are more adapted to anthropogenic environments, while others remain associated with more preserved habitats. This ecological differentiation is important for understanding the role of dipterans in disease transmission and environmental monitoring (Marchiori, 2021b).

9. Techniques for Collecting and Breeding Diptera Muscomorpha in the Field and Laboratory

The results highlight that the use of standardized techniques for collecting and rearing Diptera Muscomorpha in the laboratory is essential for obtaining reliable biological and ecological data. Field collection using nets and manual capture from organic substrates proved to be an effective method for obtaining adult specimens, which were subsequently anesthetized and transferred to controlled laboratory conditions. The rearing methodology demonstrated that the combination of appropriate environmental conditions and nutritional substrates directly influences the development and survival of flies. The mixture of powdered milk, sugar, and brewer's yeast was identified as an optimal diet for adults, significantly increasing longevity and reproductive performance. In addition, fermented organic substrates used for oviposition provided adequate humidity and temperature, promoting successful egg development and larval growth (Figure 10).

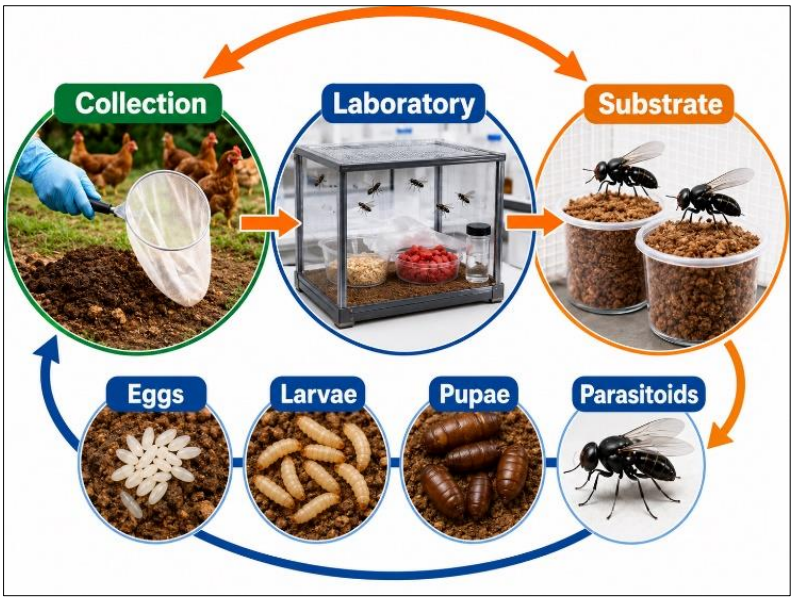

Figure 10: Schematic representation of the laboratory cycle of Diptera Muscomorpha from collection to adult emergence. The figure illustrates the sequential stages of collection, rearing, oviposition, and development under controlled conditions. This process enables studies on the life cycle, reproduction, and population dynamics of flies

The study also emphasizes that maintaining controlled environmental parameters, such as temperature (27°C), relative humidity (approximately 60%), and photoperiod (12:12), is fundamental for standardizing experimental conditions and ensuring consistent results. These conditions allowed the complete development of the life cycle from egg to adult under laboratory settings. However, challenges were observed during rearing, particularly contamination by other fly species, mainly from the family Phoridae. These species competed for resources and negatively affected larval development due to their rapid life cycle and high feeding activity. Collected fruits, orange, star fruit, guava, mango, and pitanga were placed individually over a 5 cm layer of sterilized fine sand in transparent cylindrical plastic containers 600 mL, open at the top to allow aeration. The fruits were previously inspected to ensure the presence of infestation symptoms, such as oviposition marks or larval galleries. The containers were maintained under laboratory conditions, approximately 25 ± 2°C, 70 % RH, and natural photo period to allow larval development and subsequent pupation (Figure 11).

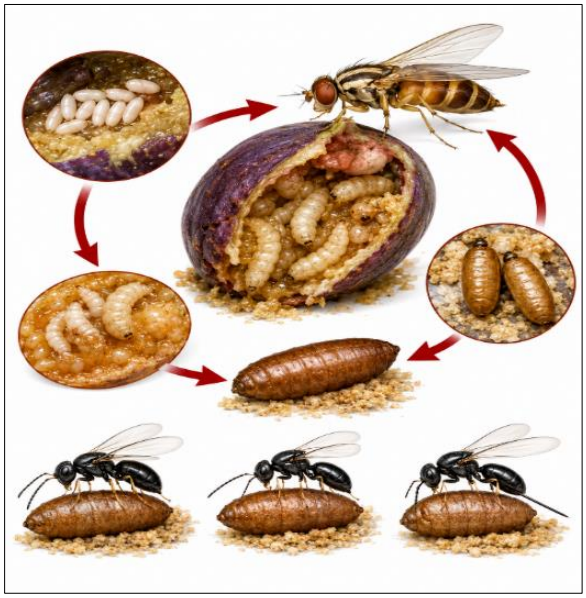

Figure 11: Life cycle of frugivorous flies associated with decomposing fruits and their interaction with parasitoid species. The figure illustrates developmental stages from egg to adult, including larval and pupal phases, within the substrate

As larvae exited the fruits, they burrowed into the sand layer to pupate. The substrate was examined weekly, and pupae were separated from the sand using the flotation method with water. Recovered pupae were carefully removed, counted, and transferred to glass vials containing a thin layer of sterilized fine sand to maintain humidity. Each pupa was kept under controlled conditions until the emergence of adult dipterans and/or parasitoids. Emerging insects were collected daily, identified to the lowest possible taxonomic level, and preserved in 70% ethanol for further analysis (Figure 12).

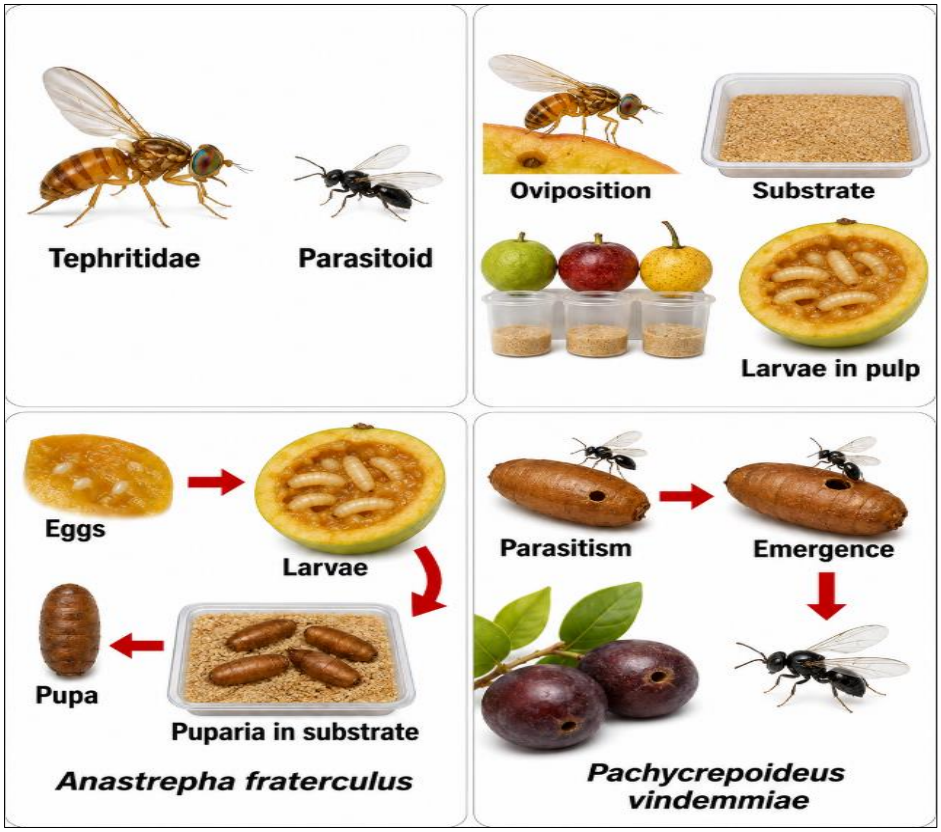

Figure 12: Developmental cycle of *A. fraterculus* in fruit substrates and its association with *P. vindemmiae*. The figure illustrates oviposition on fruits, egg and larval development within the pulp, migration of mature larvae to the substrate, puparium formation, pupal parasitism, and adult parasitoid emergence

This highlights the importance of careful colony management and monitoring to avoid interference in experimental outcomes. Overall, the findings demonstrate that well-defined collection and rearing techniques are crucial for studies involving Diptera, enabling investigations into life cycle, fecundity, longevity, and population dynamics. These methods are fundamental for advancing research in medical and veterinary entomology, as well as for supporting biological control strategies (Marchiori, 2021c).

10: Parasitoids of Dipterans and Hemipterans Collected to Cultivate on Maize in Brazil

The results demonstrated the occurrence of parasitoids associated with both dipteran and hemipteran hosts collected in maize crops in Itumbiara, Goiás. A total of 41 eggs of *Leptoglossus zonatus* Dallas, 1852 (Hemiptera: Coreidae) were collected, from which 11 individuals of *G. gallardoi* and 2 individuals of *Brasema* sp. (Hymenoptera: Eupelmidae) emerged. These findings indicate that egg parasitoids play an important role in regulating populations of hemipteran pests in maize fields. The overall parasitism rate observed in *L. zonatus* eggs was 31.7%, with *G. gallardoi* accounting for 26.8% and *Brasema* sp. for 4.8%. The higher parasitism by *G. gallardoi* suggests that this species is a key natural enemy of *L. zonatus*, likely due to its specialization in parasitizing hemipteran eggs. This reinforces its potential importance in biological control programs targeting agricultural pests.

In addition to hemipteran hosts, parasitoids were also obtained from dipteran pupae. Six pupae of *Allograpta* sp. (Diptera: Syrphidae) were collected, from which 9 gregarious individuals of Pteromalidae sp. and 2 solitary individuals of *Conura* sp. (Hymenoptera: Chalcididae) emerged. The total parasitism rate in dipteran pupae was 18.3%, with Pteromalidae sp. accounting for 15.0% and *Conura* sp. for 33.3%. These results demonstrate the diversity of parasitoid strategies, including both gregarious and solitary development (Figure 13).

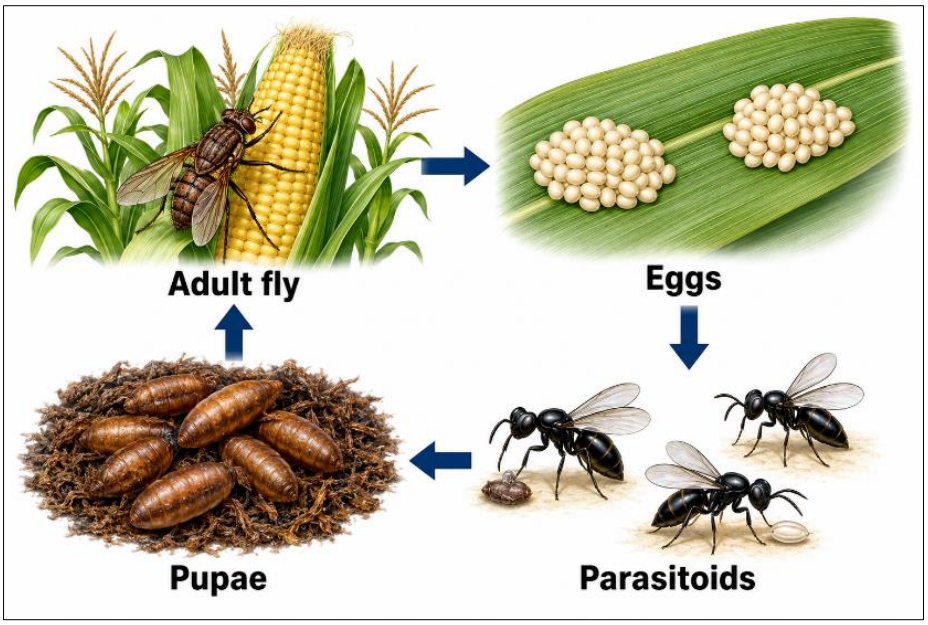

Figure 13: Interactions between parasitoids and their dipteran and hemipteran hosts in maize agroecosystems. The figure illustrates parasitism occurring in both egg and pupal stages, highlighting different developmental strategies. These interactions contribute to the natural regulation of insect populations in agricultural environments

The study highlights that parasitoid species exhibit different host preferences and developmental behaviors depending on the host stage. Egg parasitoids such as *G. gallardoi* are associated with hemipteran hosts, while pupal parasitoids such as Pteromalidae sp. and *Conura* sp. are associated with dipteran hosts. This diversity of interactions contributes to the natural regulation of insect populations in agroecosystems. Overall, the findings emphasize the ecological importance of parasitoids in maize cultivation, where they act as natural enemies of both dipteran and hemipteran pests. Understanding these host–parasitoid relationships is essential for developing sustainable pest management strategies and reducing reliance on chemical control methods (Marchiori, 2021d).

5.0. DISCUSSION

Studies on parasitoid–host interactions in Diptera have consistently demonstrated structured ecological associations and significant potential for biological control. Early investigations reported the occurrence of parasitoids such as *S. nigroaenea* and highlighted reproductive strategies such as superparasitism, which may increase survival under conditions of limited host availability (Marchiori *et al.,* 2005b). During the same period, host–parasitoid relationships involving species such as *F. pusio* emphasized the importance of pupal parasitoids in regulating dipteran populations (Marchiori *et al.,* 2005a). Subsequent studies demonstrated that environmental conditions and sampling methods, including trap characteristics, strongly influence parasitoid diversity and capture efficiency (Marchiori *et al.,* 2009). Additional research expanded these findings by documenting parasitoid occurrence across different ecological contexts, including agricultural systems, thereby improving the understanding of host associations and species distribution (Marchiori, 2014b; Marchiori, 2014c; Marchiori, 2015).

Later studies reinforced the ecological importance of species such as *S. drosophilae*, which exhibit adaptability across different hosts and substrates (Marchiori, 2015). More recent research has focused on applied aspects, particularly in agricultural systems, where parasitoids are associated with both dipteran and other pest hosts, highlighting their role in integrated pest management. Methodological advances, especially the use of standardized laboratory techniques, have enabled more precise investigations of life cycles, parasitoid behavior, host emergence, and species identification (Marchiori, 2021c). Furthermore, studies on synanthropy have shown that environmental gradients influence dipteran distribution patterns and, consequently, parasitoid occurrence (Marchiori, 2021b). The inclusion of other natural enemies, such as coleopteran predators, has also broadened the understanding of biological control agents within these systems (Marchiori, 2021a; Marchiori, 2021d).

An important contribution of the present study was the application of complementary chi-square analyses when the reviewed articles presented quantitative data suitable for statistical comparison. In several original studies, numerical information was available in tables, including abundance, host species, substrate type, trap type, number of pupae, number of parasitized pupae, and parasitism rates; however, formal chi-square tests were not always performed or reported. Therefore, the present study used these available datasets

to test patterns of parasitoid abundance, host association, substrate use, habitat distribution, altitude, and trap efficiency. These analyses did not modify the original biological records, but added statistical support to ecological patterns that had previously been interpreted mainly in descriptive terms (Marchiori, 2014b; Marchiori, 2014c; Marchiori, 2015).

More recent studies have incorporated frugivorous fly systems, demonstrating the diversity of Diptera and their associated parasitoids in fruit-based environments and reinforcing their ecological and applied importance (Marchiori, 2023). The results of the present study are consistent with these findings, showing that parasitoid communities are structured primarily by host availability, substrate type, and environmental conditions. Higher parasitism rates observed in substrates rich in organic matter indicate that resource availability influences host development and parasitoid activity. Similarly, the variation in parasitoid abundance among trap colors and trap types indicates that sampling methodology can affect the observed composition of parasitoid communities (Marchiori, 2023).

The complementary statistical analyses also reinforced that many parasitoid–host and parasitoid–substrate associations were not randomly distributed. Significant chi-square results were observed for several datasets involving parasitism among substrates, abundance among parasitoid species, host–parasitoid associations, distribution among environments, altitude effects, and trap efficiency. These findings indicate that parasitoid occurrence depends on multiple interacting factors, including host density, substrate quality, habitat structure, decomposition stage, and sampling method. However, some comparisons were not statistically significant, showing that not all observed numerical differences necessarily represent strong ecological separation among groups (Marchiori, 2014b; Marchiori, 2014c; Marchiori, 2015).

Overall, the accumulated evidence confirms that parasitoids associated with Diptera play a fundamental role in regulating insect populations across different ecological systems. Their occurrence in agricultural, fruit-based, livestock, forest, pasture, and synanthropic environments highlights their relevance for both natural and applied biological control programs. Species such as *B. podagrica, H. herbertii, N. vitripennis*, *P. vindemmiae*, *S. drosophilae*, and *T. anastrephae* demonstrate different levels of host specificity, ecological plasticity, and biological control potential. These findings support the development of more sustainable pest management strategies and reinforce the role of parasitoids as key agents in natural and applied biological control programs (Figure 14) (Marchiori, 2023).

Figure 14: Integrative representation of hymenopteran parasitoids associated with dipteran hosts in agricultural environments and decomposing organic substrates. The figure illustrates host location, oviposition, parasitoid development in immature host stages, pupal parasitism, adult emergence, and the contribution of parasitoids to the natural regulation of fly populations

6.0. CONCLUSION

The synthesis of studies involving Diptera, Hemiptera, and their associated parasitoids revealed high species diversity and complex ecological interactions across agricultural, fruit-based, synanthropic, field, and laboratory systems. Parasitoids played a fundamental role in regulating host populations, acting at different developmental stages and exhibiting both generalist and more specialized strategies. The results indicated that host availability, substrate type, trap characteristics, and

environmental conditions were key factors influencing parasitoid occurrence, abundance, and parasitism rates. Agricultural and fruit-based systems provided favorable conditions for the development of hosts and, consequently, supported diverse parasitoid communities. The integration of data from multiple studies allowed a broader understanding of host–parasitoid relationships, revealing patterns that may not be evident in isolated investigations. In addition, the quantitative data available in the reviewed articles made it possible to perform complementary chi-square analyses, which were not applied or reported in several original studies. These analyses added statistical support to ecological patterns previously described mainly in descriptive terms, reinforcing the non-random nature of host association, substrate use, habitat distribution, altitude effects, trap efficiency, and parasitoid abundance. Overall, this approach highlights the importance of combining field and laboratory data with complementary statistical analyses to better understand parasitoid ecology and to support sustainable biological control and integrated pest management strategies.

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