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Short Communication

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

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Abstract

Objective: Brazil is one of the leaders in the production of fruit in temperate climates but is still plagued by pests, particularly frugivorous flies. The damage caused by these insects results in reduced yields and difficulties in exporting due to quarantine barriers imposed by importing countries. The objective of this study was to investigate the main species of frugivorous flies of the Drosophilidae, Lonchaeidae and Tephritidae and their parasitoids in Brazil.

Methods: The collected fruits (orange, star fruit, guava, mango, and pitanga) were deposited on a layer of fine sand, in plastic containers, cylindrical, transparent, and open at the top. Each week, the pupae were separated from the substrate by flotation. They were removed and placed in glass flasks with fine sand at room temperature until the emergence of dipterans and/or their parasitoids.

Results: In Goiás, *Doryctobracon areolatus* (Szepligeti, 1911) (Hymenoptera: Braconidae) was the most frequent with 65.0%, and in Minas Gerais, *Trichopria anastrepha* Costa Lima, 1940 (Hymenoptera: Diapriidae) accounted for 44.4%. In Goiás, *Doryctobracon areolatus* presented the highest percentage of parasitism with 4.3%, and in Minas Gerais the percentage of parasitism in *Trichopria anastrepha* was 5.7%. In both states, *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) the largest species with the highest percentage of parasitism was *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae) with 96.7%. In *Psidium guajava*, L. (Myrtaceae) the percentage of natural parasitism was 16.7%, being 0.9% by *Aganapis pelleranoi* (Brèthes, 1924) 8.0%.

Conclusion: In Brazil, frugivorous flies are important pests of fruits and vegetables. Knowledge of the population fluctuation of these species in each biome is an important requirement for the adoption of pest control.

Keywords: insects, parasitoids, damage, flies, dipterans, hymenopterans

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1 INTRODUCTION

The expansion of frontiers for fruit growing causes changes in population dynamics and modifies the spatial distribution of fruit fly species. The Brazilian fruit industry covers an area of 2.3 million hectares planted and an annual production of more than 38 million tons. Brazil is the third largest fruit producer in the world, surpassed only by China (133 million tons) and India (58 million tons), generating 6 million direct jobs, 27% of the total agricultural labor employed in the country, and a gross domestic product of $11 billion[1].

This sector demands intensive and qualified labor, keeping people in the countryside and allowing a life dignity for farmers and their families on both small farms and large projects. However, Brazil ranks 20th among exporting countries. Tropical climate zone has great potential for year-round fruit production. The variations of temperature fluctuate by only 2 to 3% between the monthly averages, without the extremes seen in the Southeast and South regions of the country[1].

Fruit flies are one of the major problems faced by these producers. Fruit flies (Diptera: Tephritidae, Lonchaeidae and Drosophilidae) are among the main pests of world agriculture and are of particular concern to tropical developing countries that have an important role in fruit production of its trade balance. Essentially, the negative economic impacts of these insect pests are associated with direct damage and quarantine restrictions imposed by importing countries[2-4].

The Tephritidae family is one of the largest families of the Order Diptera. This family is one of the pests with a high economic impact in the world fruit industry because they attack the reproductive organs of plants, fruits with their pulp and flowers. These insects constitute an important group of pests in fruit production worldwide, as they have a life cycle in which their larval period develops inside the fruits, and generally feed on their pulp. Fruit flies are insects that cause high damage to fruit growers (Figure 1A)[5-7].

Some species of Lonchaeidae have been reported as pests of agricultural crops, causing economic damage by infesting fruits and/or flower buds. Therefore, interest in studying these insects has increased in recent years. Lonchaeidae from the Brazilian Amazon was developed to group and provide information on the diversity, distribution and hosts of Lonchaeidae species in the Legal Amazon (states of Acre, Amapá, Amazonas, Mato Grosso, Pará, Rondônia, Roraima, Tocantins and Maranhão) in a simple and accessible way (Figure 1B)[8-10].

The larvae of most species of the family Drosophilidae feed on microorganisms in spoiled fruits, slime fluxes, fungi, rotting cacti, or other decaying organic matter. The Drosophilidae family is represented by generally small flies and is distributed throughout the planet (it is cosmopolitan) (Figure 1C)[11-13].

In several countries, studies with a survey of fruit fly species are mainly based on trap collections and sporadic fruit sampling, and little is known about the hosts and infestation rates[14,15].

The survey with character traps allows for the analysis of the character quantitatively and qualitatively, while fruit collection allows assessment of infestation and population levels and determination of the association with hosts, as well as the abundance and diversity of natural enemies, which is not possible through the use of traps to capture the adults[16,17].

The objective of this study was to know the main species of frugivorous flies of the Drosophilidae, Lonchaeidae Tephritidae and their parasitoids in Brazil.

2 MATERIALS AND METHODS

The experiment was carried out in the municipalities of Itumbiara located in the State of Goiás and in Lavras in the State of Minas Gerais, Brazil. In Itumbiara, the collections were carried out on the farm of the Lutheran Institute of Higher Education in Itumbiara and in Lavras on the campus of the Federal University of Lavras. The collections were carried out from January to December 2001.

The collected fruits (orange, star fruit, guava, mango and pitanga) were deposited on a layer of fine sand, in plastic containers, cylindrical, transparent and open at the top. Each week, the pupae were separated from the substrate by flotation. They were removed and placed in glass flasks with fine sand at room temperature until the emergence of dipterans and/or their parasitoids. The experiments were carried out in 2001. Possible differences between the preference of parasitoids and flies for fruits were tested by Chi-square (Figure 2).

2.1 *Experiment with Psidium guajava, L.* (Myrtaceae)

The study was carried out in *P. guajava* crops located at Sítio Rio Grande in Divinopolis State of Minas Gerais. The experiment was performed from January to December 2002. The collected fruits were placed in plastic containers

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Figure 1. Specimens. A Specimen of Tephritidae family; B: Specimen of Lonchaeidae family; C: Specimen of Drosophilidae family. Sources: https://alchetron.com/Tephritidae, https://bugguide.net/node/view/1343473 and https://www.mapress.com/zt/article/view/zootaxa.5068.2.8

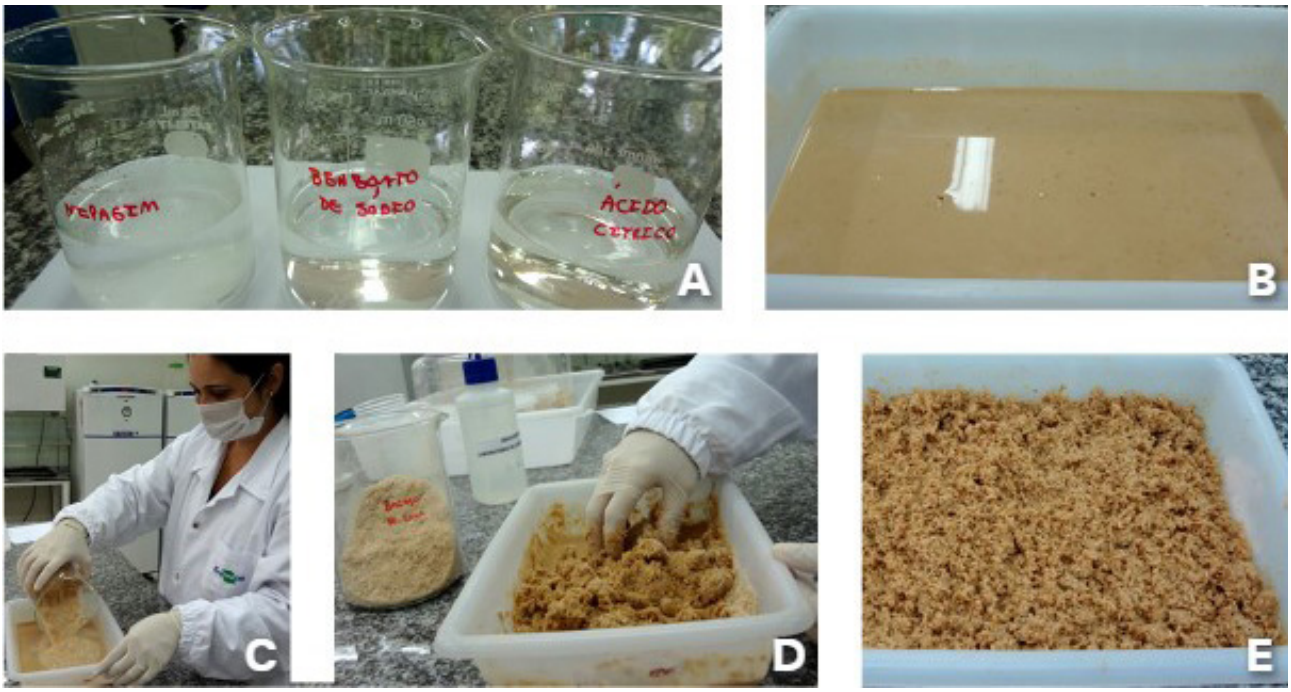

Figure 2. Fruit fly laboratory. A: Nipagim, sodium benzoate and citric acid dissolved in distilled water; B: Appearance of the artificial diet without the addition of sugar cane pomace; C: Increment from sugarcane bagasse to artificial diet; D: Mixture of cane cake to ingredients in the diet; E: Final aspect of artificial diet. Source: https://docplayer.com.br/56731187-Documentos-tecnicas-para-criacao-da-mosca-da-carambola-bactrocera-carambolae-drew-hancock-rio-para-pesquisa-cientifica.html

(five fruits for each container) 20cm high by 10cm in diameter, being deposited on a 5cm layer of fine autoclaved sand used in house construction, serving as a substrate for pupation. The opening of the containers was sealed with organza fabric tied with elastic to prevent the entry of other insects.

Weekly, the sand containing the fruit fly pupae was deposited in buckets of water, where the pupae were separated by the floating method. Then, they were removed from the water with the aid of a sieve and dried. After drying, they were counted and individualized in gelatin capsules (number 00) and kept in an acclimatized room at 25±2°C until the emergence of the fly adults and / or their parasitoids.

The percentage of parasitism was calculated: P = (parasitized pupae / total pupae) × 100.

3 RESULTS AND DISCUSSION

3.1 Fruit Flies (Tephritoidea) and Their Parasitoids

In the state of Goiás, the percentage of frequency of hosts was 64.0% for *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) (917/1516), 30.5% for *Ceratitis capitata* (Wiedemann, 1830) (Diptera: Tephritidae) (463/1516), and 9.0% for *Neosilba* sp. (Diptera: Lonchaeidae) (136/1516). *Anastrepha fraterculus* presented the highest frequency, probably due to its polyphagia (Table 1 and Figure 3).

Anastrepha fraterculus: This species of fruit fly is considered the main pest of many fruit species in Brazil. It is mostly found in avocado, plum, coffee, persimmon, citrus, fig, guava, apple, mango, passion fruit, quince, loquat, pear, peach, tomato, and grape. The adults lay their eggs on the fruits, and the larvae penetrate the fruit and feed from within, capable of destroying it completely.

Table 1. Frugivorous Flies and Their Collected Parasitoids and Their Parasitoids in Goiás, Brazil

Taxonomic Group	Number of Pupae	Parasitoids	Pupae Parasitized	% Parasitism
Anastrepha fraterculus	917	*Doryctobracon areolatus*	39	4.3
		Pachycrepoideus vindemmiae	02	0.2
		Aganaspis pelleranoi	26	2.8
Ceratitis capitata	463	*Doryctobracon areolatus*	02	0.4
Neosilba sp.	136	*Doryctobracon areolatus*	02	1.5
	1516		71	

Figure 3. *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae). Source: http://www.bio.ufpr.br/portal/pragasplantas/wp-content/uploads/sites/12/2013/11/frutiferas_resumida.pdf

Ceratitis capitata: Fruits attacked by flies show characteristic symptoms: around the place where the egg is laid, a halo appears approximately 2cm in diameter and dark in color. When the larvae hatch, this halo takes on a brownish color due to the rotting of the shell. Certain fungi develop on these destroyed tissues. The pest preferentially attacks fruits exposed to the sun. The pest predominates in coffee, where the eggs are deposited inside the ripe fruit and where the larva reaches its maturity, causing loss of coffee quality and great damage to the crop[18-20].

It is the species of the genus *Neosilba*, which has the widest geographic distribution and host diversity in Brazil, being considered an important pest of fruits grown in the Northeast and Southeast regions of the country. Therefore, studies focused on this family should be encouraged, especially regarding ecology and biology. It should be noted that a significant part of the insect species in the region is still unknown to science (Table 1 and Figure 4)[21-23].

The frequency of parasitoids was 65.0% for *Doryctobracon areolatus* (Szepligeti, 1911) (Hymenoptera: Braconidae) (46/71), 2.8% for (2/71), and 37.0% for (26/71). The high frequency of *Doryctobracon areolatus* may be attributed to its ability to search for hosts in the larval stage (Figure 5).

The results shown in Table 1 demonstrate the small diversity of Diptera. The results suggest that *A. fraterculus* can be considered the most important pest of fruit trees. Regarding parasitoids (Table 1), *D. areolatus* can be considered the most important parasitoid of frugivorous. *Anastrepha fraterculus* is the only species found in all the hosts sampled.

The total percentage of parasitism was (71/1516) 4.7%. The highest percentage of parasitism was in *A. fraterculus* by the parasitoid *D. areolatus* with (39/917) 4.3%.

In Brazil, the native parasitoid *D. areolatus* stands out for its constant presence, the greater number of specimens obtained in most surveys carried out in the country, and the aggressiveness in the parasitism of fruit fly larvae of different stages[24-26].

The Chi-square calculation showed that *A. fraterculus* showed a preference for pitanga and *C. capitata* for guava (χ^2=27.39; GL=1; P<0.0001) with 5% significance *Neosilba* sp. (Diptera: Lonchaeidae) being more common in pitanga, accounting for 66.6% of individuals.

In the state of Minas Gerais, a total of 349 pupae of *Anastrepha* spp. 45 specimens were obtained from the following six parasitoids: *Trichopria anastrepha* Costa Lima, 1940 (Hymenoptera: Diapriidae) (20/45) 44.4%, *Leptopilina boulardi* Barbotin, Carton & Kelner-Pillault 1979 (Hymenoptera: Figitidae) (10/45) 22.2%, *Spalangia endius* Walker, 1839 (Hymenoptera: Pteromalidae) (6/45) 13.3%, *D. areolatus* (5/45) 11.1%, *Odontosema anastrepha* Borgameier, 1935 (Hymenoptera: Figitidae) (2/45) 4.4% and *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae) (2/45) 4.4%.

The genus *Trichopria* includes microhymenopterans that can be used for the biological control of flies, since they are parasitoids of immature stages of Diptera. *Trichopria anastrephae* is a species generalist, usually occurring in a single parasitoid by host puparium. (Table 2 and Figure 6)[27,28].

The total percentage of natural parasitism observed was

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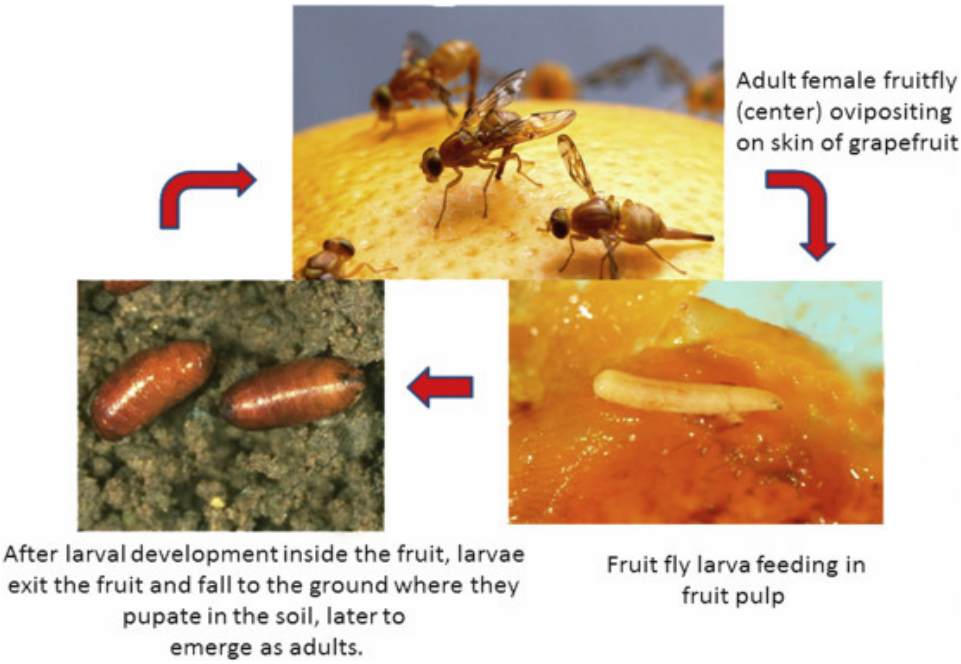

Figure 4. *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) life cycle. Source: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/anastrepha-fraterculus

Figure 5. *Doryctobracon areolatus* (Szepligeti, 1911) (Hymenoptera: Braconidae). Source: https://entnemdept.ufl.edu/creatures/beneficial/wasps/doryctobracon_areolatus.htm

13.0%, which may be attributable to the density of the hosts, the number of collections carried out, the size of the sample area, and the characteristics of the place chosen for collection, with vegetation that guarantees basic conditions to maintain a diversified fauna of host insects. In the work carried out in 27 municipalities, the percentage of parasitism was found to range from 0.007 to 42.86%, and a percentage of parasitism of 6.2% was identified in Goiás[29,30].

These results demonstrate that *T. anastrepha* can be considered one of the most important parasitoids of fruit flies in this region. Probably, this fact may be influenced by the parasitoid's search capacity and its density[31,32].

3.2 *Psidium guajava*, L. (Myrtaceae)

Zaprionus indianus Gupta, 1970 (Diptera: Drosophilidae) is considered to be secondary to more than 70 fruit species, due to its ability to attack and feed on decaying or mechanically damaged fruit. The fig fly, *Z. indianus* is an invasive species with a high colonization potential and high occurrence in anthropic places and is considered one of the most abundant species among the drosophilid community in Brazil (Figure 7)[33-35].

A total of 1068 individuals of *Z. indianus* were collected, including 04 specimens of *S. endius* 03 specimens of *L. boulardi* and 285 specimens of *P. vindemmiae* (Table 3 and Figure 7C)

The frequency of parasitoids was 1.9% of *S. endius* (4/215) 1.9%, 96.7% of *P. vindemmiae* (208/215) and 1.4% of *L. boulardi* (3/215). *Pachycrepoideus vindemmiae* showed a higher frequency due to its ability to search for hosts (Table 3). A total percentage of 20.1% of *P. vindemmiae* showed a higher percentage of parasitism in *Z. indianus*, possibly due to the higher food supply (Figure 8).

Pachycrepoideus vindemmiae is a solitary parasitoid of numerous Diptera. It has a wide geographic distribution and is also found in North America, Latin America, and Africa[36,37].

3.3 *Psidium guajava* (Myrtaceae)

The guava tree *P. guajava* is widely distributed throughout the tropical and subtropical regions of the world. In addition to the economic importance, its fruits have a high nutritional value with high levels of sugars, iron, calcium, phosphorus and vitamins A, B and C[38,39].

Control of this pest in the guava crop has been carried out through the application of pesticides when the fruits are still small, with a moratorium 30 days before

Table 2. Fruit Fly and Its Parasitoids Collected in Minas Gerais State, Brazil

Host / Puparium Number	Parasitoids	Frequency	Parasitism (%)
Anastrepha spp. / 349	Braconidae:		
	Doryctobracon areolatus	05	1.4
	Diapriidae:		
	Trichopria anastrepha	20	5.7
	Figitidae:		
	Leptopilina boulardi	10	2.9
	Odontosema anastrepha	02	0.6
	Pteromalidae:		
	Pachycrepoideus vindemmiae	02	0.6
	Spalangia endius	06	1.7
Total		45	

Table 3. Parasitoids of *Zaprionus Indianus* Gupta, 1970 (Diptera: Drosophilidae) Collected in Goiás and Minas Gerais, Brazil

Host / Puparium Number	Parasitoids	Frequency	Parasitism (%)
Zaprionus indianus / 1068	*Spalangia endius*	04	0.4
	Pachycrepoideus vindemmiae	208	19.5
	Leptopilina boulardi	03	0.3
		215	

Figure 6. *Trichopria anastrepha* Costa Lima, 1940 (Hymenoptera: Diapriidae). Source: https://www.sciproveg.com/?p=3370&lang=en

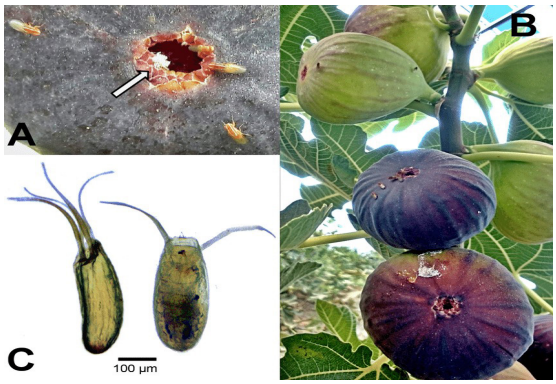

Figure 7. *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) on fig fruits. A: Oviposition of *Z. indianus*; B: Fig fruits susceptible to *Z. indianus*; C: *Z. indianus* (left) and (right) eggs. The arrow in Figure 2A points to eggs at the entrance of the ostiole. Source: https://www.researchgate.net/figure/Zaprionus-indianus-on-fig-fruits-A-Oviposition-of-Zaprionus-indianus-B-fig-fruits_fig2_321928658

harvest. However, due to the strong requirements of the importing countries regarding the absence of pests and chemical residues, allied to the environmental awareness of the rural producer, other control alternatives have been sought, highlighting the bagging of fruit, the release of the sterile males, and the use of parasitoids[38,39].

Little knowledge is available to the action of fruit fly parasitoids in Brazil. The main Hymenoptera Parasitica groups of these flies belong to the Braconidae, Figitidae and Pteromalidae families. Among the parasitoids used in the biological control of fruit flies, those belonging to the Figitidae family, Eucoilinae subfamily, have received great academic attention due to their efficiency as a control agent, in addition to their wide geographic distribution and high potential for use in pest management programs[39,40].

One hundred and ninety pupae of *Anastrepha* sp. were collected, from which 38 parasitoids of 38 pupae belonging to the Figitidae family emerged, including 20 *Aganapis pelleranoi* (Brèthes, 1924) and 18 *Diceratasspis grenadensis* Ashmead, 1896, with a percentage of natural parasitism around 16.7%. The frequency of parasitoids was *A. pelleranoi* (20/38) 52.6% and (18/38) 47.4%.

The parasitic percentage of *A. pelleranoi* was 0.9%

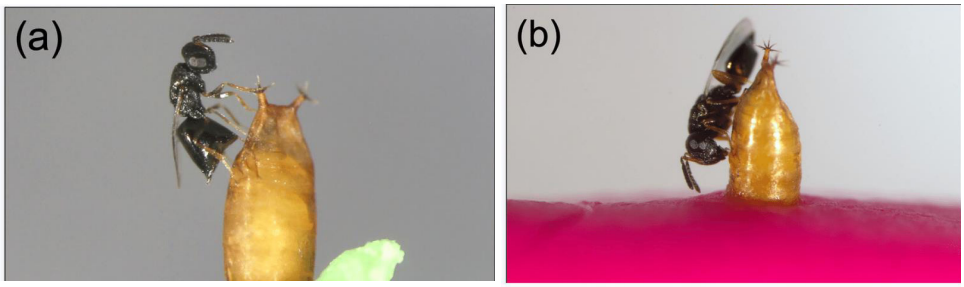

Figure 8. *Pachycrepoideus vindemmiae* (Rondani, 1875). a: Female wasp inserting her ovipositor through the *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) pupal case and performing evaluation of the host prior oviposition; b: Female wasp host-feeding on hemolymph of a *D. suzuki* pupa following ovipositor withdrawal from the pupal case. Source: https://www.researchgate.net/figure/Pachycrepoideus-vindemmiae-Hymenoptera-Pteromalidae-attacking-pupae-of-spotted-wing_fig1_334637885

Figure 9. *Aganapis pelleranoi* (Brèthes, 1924). Source: http://www.waspweb.org/cynipoidea/figitidae/Eucoilinae/Aganaspis/Aganaspis_pelleranoi.htm

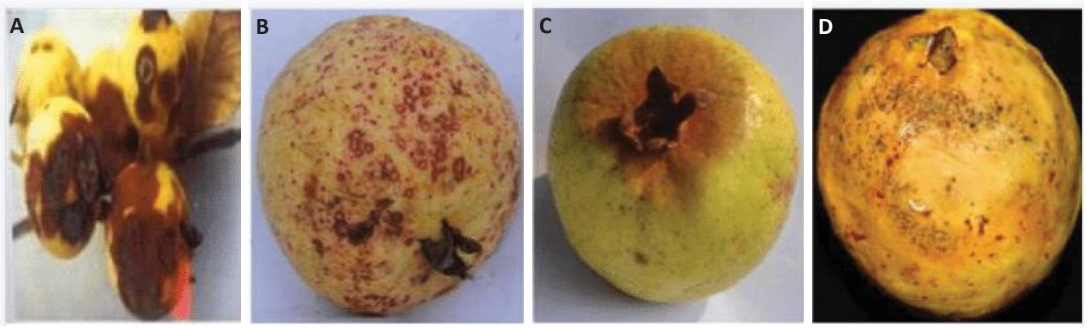

Figure 10. Fruits affected by guava diseases. A: Anthracnose; B: Algal spot; C: Styler end rot; D: Fruit fly. Source: https://www.researchgate.net/figure/Fruits-affected-by-guava-diseases-a-Anthracnose-b-Algal-spot-c-Styler-end-rot-d_fig1_353632026

and *D. grenadensis* 8.0%. This percentage may be due to the density of the hosts, the number of collections carried out, the size of the sample area or the characteristics of the place chosen for collection, where vegetation exists that ensures the basic conditions for maintaining the diversity of host insects.

Aganapis pelleranoi is widely distributed in Brazil. In a survey carried out by this author on Eucoilinae species in Brazil, this species was the most abundant, representing 29.9% of all Eucoilinae associated with frugivorous fly larvae. *Aganapis pelleranoi* was released as a biological control agent for fruit flies in Tucumán province in Argentina (Figures 9-11)[38-42].

The genus *Diceratapis* is currently represented by a single species, *D. grenadensis*. This species was recorded for the first time in Brazil in 1999 in the state of São Paulo, then in Pará, Goiás and Minas Gerais. Species of the *Diceratapis* are potential parasitoids of Drosophilidae larvae, since they are much smaller than those associated with larvae of Tephritoidea. Species of the genera *Diceratapis* and *Leptopilina* are the most commonly found Eucoilinae associated with drosophilid larvae-pupae, constituting important natural enemies of this family (Figure 12)[42-46].

Figure 11. Female fruit fly laying eggs on guava fruit. a: Ludhiana; b: Fruit fly-infested guava fruit on tree; c: Fruit fly-infested guava fruits in glass jar; d: Maggots of fruit fly developed from eggs in fruit; e: Collection of maggots from infested guava fruits; f: Rearing of maggots in artificial diet in laboratory; g: Maggots feeding on artificial diet; h: pupae of fruit fly developed from maggot. Source: https://ejbpc.springeropen.com/articles/10.1186/s41938-020-00345-7

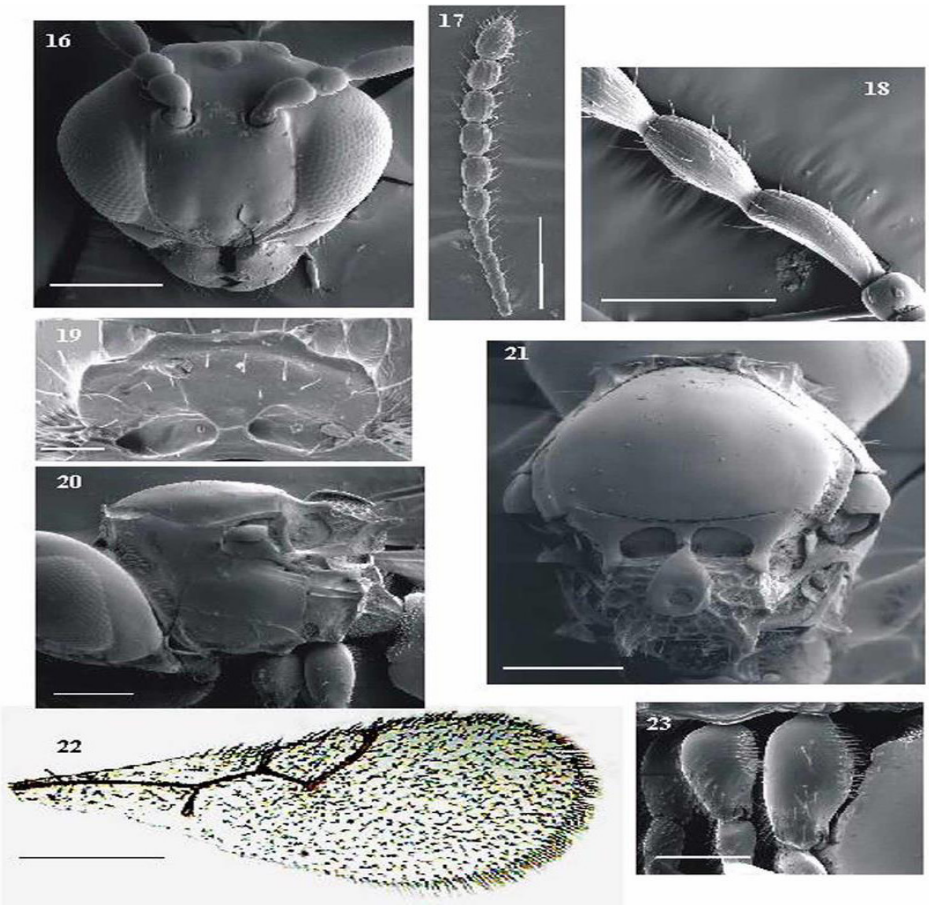

Figure 12. *Diceratapis grenadensis* Ashmead, 1896. 16: Head, anterior view; 17: Female antenna; 18: Flagellomeres 1 and 2 of male; 19: Pronotal plate; 20: Head, mesosoma and anterior part of metasoma, lateral view; 21: Mesosome, dorsal view; 22: Forewing; 23: Metacoxa. Source: https://www.researchgate.net/figure/FIGURES-16-23-Diceratapis-grenadensis-16-Head-anterior-view-140x-100m-17_fig2_262413490

Knowledge about the diversity and distribution of these groups of natural enemies is essential, as these parasitoids contribute to regulating the frugivorous dipteran populations.

4 CONCLUSION

In Brazil, frugivorous flies are important pests of fruits and vegetables. Knowledge of the population fluctuation of these species in each biome is an important

requirement for the adoption of pest control.

Acknowledgements
Not applicable.

Conflicts of Interest
The author declared no conflict of interest.

Author Contribution
Marchiori CH contributed to the manuscript and approved the final version.

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