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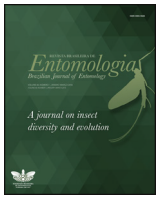

Prospection of potential bioagents against *Bactrocera carambolae* if it is detected in the São Francisco River Valley, Brazil

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ABSTRACT

The region of the São Francisco River Valley (VSF) in Brazil produces 90–95% of mangoes and grapes for export; therefore, they are subjected to international phytosanitary measures. *Bactrocera carambolae* Drew & Hancock, 1994 (Diptera: Tephritidae) is a present quarantine pest in Brazil, restricted to the states of Amapá, Roraima, and Pará. Surveys were conducted to identify parasitoids to be used against *B. carambolae*, in the event of its future detection in the region. A total of 1,292.80 kg of 14 different types of fruits were collected, resulting in the recovery of 27,367 puparia. From these puparia, 18,433 fruit flies (Tephritidae) emerged: 15,679 adults of *Ceratitis capitata* (Wiedemann, 1824) and 2,754 of *Anastrepha* spp. Additionally, 557 parasitoid specimens of seven species were recovered from Tephritidae family: *Tetrastichus giffardianus* Silvestri, 1915 (Hymenoptera: Eulophidae), *Doryctobracon areolatus* (Szépligeti, 1911) (Hymenoptera: Braconidae), *Utetes anastrephae* (Viereck, 1913) (Hymenoptera: Braconidae), *Asobara anastrephae* (Muesebeck, 1958) (Hymenoptera: Braconidae), *Ganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae), *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae), and *Spalangia simplex* Perkins, 1910 (Hymenoptera: Spalangiidae). Besides the abovementioned species, *Tropideucoila weldi* Lima, 1940 (Hymenoptera: Figitidae) emerged from unidentified host family (Tephritidae or Lonchaeidae). The parasitoids *A. anastrephae, G. pelleranoi, S. simplex, U. anastrephae*, and *T. weldi* were recorded for the first time in the state of Pernambuco (PE, Brazil). The predominant species were *D. areolatus* (45.27%) and *T. giffardianus* (23.38%), and their potential use as bioagents to control *B. carambolae*, in case of detection in the VSF, were discussed.

Introduction

Brazil is the third-largest fruit producer in the world (IBGE, 2020). The region of São Francisco River Valley (VSF) produces 90 to 95% of the country's mango and grape exports, with one of the largest agricultural Gross Domestic Products (GDP), at approximately US$161 million (Beling, 2017). This region, located in the drylands of the states of Pernambuco (mainly in the municipalities of Petrolina, Lagoa Grande, and Belém do São Francisco) and Bahia (mainly in the municipalities of Juazeiro, Curaçá, Sobradinho, Casa Nova, and Salitre), along the São Francisco River, comprises more than 70 thousand hectares of irrigated land (IBGE, 2020) cultivating banana (*Musa* spp.) (42,000 ha), mango (*Mangifera indica* L.) (11,000 ha), grape (*Vitis vinifera* L.) (≈7,000 ha), guava (*Psidium guajava* L.) (4,000 ha), and acerola (*Malpighia emarginata* DC.) fruit crops, among others (ABMRA, 2019). Most fruits, including mango, guava, acerola, and grape are hosts for fruit flies (Paranhos et al., 2023). The staggering of plantings, enabling year-round harvests, exacerbates the possibility of reproduction and proliferation of these pests, leading to significant direct and indirect losses associated with export restrictions (Paranhos et al., 2013).

Brazil is home to 129 native species of fruit flies belonging to the genus *Anastrepha* Schiner (Diptera: Tephritidae) (Zucchi and Moraes, 2025a), some of which are economically and quarantine significant, such as *A. fraterculus* (Wiedemann, 1830), *A. obliqua* (Macquart, 1835), and *A. grandis* (Macquart, 1846) (Marsaro Júnior et al., 2010; Zucchi and

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Moraes, 2025a). In addition, two exotic species are also present in the country: the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), and the carambola fruit fly, *Bactrocera carambolae* Drew & Hancock, 1994 (Diptera: Tephritidae). *Ceratitis capitata* was first detected in Brazil in 1901 (von Ihering, 1901) and is widely distributed throughout the country (Zucchi and Moraes, 2025b). The most recent records were in the states of Amapá (Costa et al., 2022) and Amazonas (Acioli et al., 2024).

Bactrocera carambolae is native to Indonesia, Malaysia, and Thailand (Vijaysegaran and Oman, 1991) and is considered a present quarantine pest (PQP) in Brazil, where its first introduction occurred in 1996 in the Northern region, specifically in the municipality of Oiapoque, state of Amapá (Castilho et al., 2019), probably originating from French Guiana. Its presence is currently restricted to the states of Amapá, Roraima, and Pará, under governmental control program conducted by the Ministry of Agriculture and Livestock (MAPA) (Brasil, 2018). However, Mingoti et al. (2023) presented a Brazilian territorial zoning map indicating the national areas most favorable to this PQP in at least one month per year, concluding that all regions of Brazil presented the favorability, with emphasis on the Northeastern region, among others.

The most commonly applied method for controlling *B. carambolae* is through the use of attract-and-kill toxic baits, consisting of wooden blocks soaked in a solution of methyl eugenol (an attractant) and malathion (an insecticide), which are suspended from the branches of the trees (Midgarden et al., 2016). The negative environmental impacts caused by the use of this insecticide and its damage to human health are reported in the literature (Bruxel, 2021; Silva and De Carli, 2021), which should lead to future restrictions on its usage (Silva et al., 2016). Hence, other control methods, including biological control strategies, must be considered.

In VSF, 12 species of *Anastrepha* have already been detected, yet *C. capitata* accounts for about 99% of all tephritid species recovered from fruits and traps (Paranhos et al., 2023). This prevalence imposes numerous phytosanitary requirements, thereby escalating the costs of exporting mangoes and grapes to the USA and Europe (Brasil, 2018). In the USA, restrictions for both fruits target *C. capitata* (MAPA Normative Instruction Nº 20 of July 13, 2010), while the European Union (EU) restricts all species of the genus *Anastrepha* only for mangoes (Implementing Directive (EU) 2019/523 of March 21, 2019). Miranda et al. (2015) estimated losses of 5% in mango exports in Brazil, a decade after the introduction of *B. carambolae* in production regions, considering only scenarios of phytosanitary treatment for fruit flies, amounting to over R$ 450 million. Regarding only the VSF, which accounts for 90 to 95% of Brazilian mango and grape exports, an annual loss of R$ 60 million was estimated in 2017 (Information provided by VSF Fruit Institute). Besides the quarantine barriers imposed by the international market, rigorous requirements for physical and chemical fruit qualities, notably the use of active ingredients of pesticides, further compound the challenge. Therefore, the use of biological control agents, such as parasitoids, is paramount for the efficient integrated management of fruit flies (Aluja et al., 2014).

In Brazil, thirty-one species of native and introduced parasitoids of fruit flies belong to Hymenoptera families, namely Braconidae, Diapriidae, Eulophidae, Figitidae, Pteromalidae, and Spalangiidae (Paranhos et al., 2019; Shimbori et al., 2020). Braconidae and Figitidae have the largest number of species (24) (Paranhos et al., 2023), all being koinobiont endoparasitoids which parasitize larvae, with their adults emerging from the host puparium (Wharton 1997a, 1997b; Ronquist et al., 2006). Among all native species, 23 were recovered only from *Anastrepha* species, which are also native to Brazil (Paranhos et al., 2023). In the Amazon region, three parasitoids from the Figitidae family and eight from the Braconidae family have already been found associated with *Anastrepha* species (Adaime et al., 2018). After 20 years of surveys, Costa et al. (2025) reported, for the first time in Brazil, native species parasitizing *B. carambolae* larvae, recording *Doryctobracon areolatus* (Szépligeti, 1911) (Hymenoptera: Braconidae), *Utetes anastrephae* (Viereck, 1913) (Hymenoptera: Braconidae), and *Ganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae), with a total parasitism rate of 1.8%. Therefore, these reports could infer that native parasitoid species tend to prefer native fruit flies.

Brazil has implemented three major biological control programs for fruit flies (Garcia and Ricalde, 2013). The first program began in 1937, with the importation of the exotic parasitoid *Tetrastichus giffardianus* Silvestri, 1915 (Hymenoptera: Eulophidae) for the classical biological control of *C. capitata* in the São Paulo State (Autuori, 1938), with limited releases in the field. The second program began in 1994, with the introduction of the exotic larval parasitoid *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae) for the control of *C. capitata* and *Anastrepha* spp. (Diptera: Tephritidae) (Walder et al., 1995; Garcia and Ricalde, 2013). The third program began in 2012, with the importation of the exotic parasitoid *Fopius arisanus* (Sonan, 1932) (Hymenoptera: Braconidae) as an alternative to control carambola fruit fly in the state of Amapá (Paranhos et al., 2013; Groth et al., 2016). The latter two programs aimed at applied biological control, involving augmentative releases of these parasitoids.

In Hawaii, renowned for its extensive biological control program targeting fruit flies, several parasitoid species have been introduced (Wharton, 1989). Nonetheless, only seven of these species have endured, six of which belonging to the Braconidae family [*F. arisanus, Fopius vandenboschi* (Fullaway, 1952), *Diachasmimorpha tryoni* (Cameron, 1911), *D. longicaudata*, *Psyttalia fletcheri* (Silvestri, 1916), and *P. incisi* (Silvestri, 1916)] and one to the Eulophidae family (*T. giffardianus*) (Vargas et al., 1993; Purcell, 1998).

Three exotic species of parasitoids introduced in Brazil (*T. giffardianus*, *D. longicaudata*, and *F. arisanus*) have successfully controlled *Bactrocera dorsalis* (Hendel, 1912) (Diptera: Tephritidae) in Hawaii, as well as other species of this same genus internationally (Vargas et al., 2012; Montoya et al., 2017), indicating their potential for also controlling *B. carambolae*. In addition, *B. dorsalis* is considered an Absent Quarantine Pest (AQP) in Brazil, being one of the 20 quarantine pests prioritized for research by Embrapa and the Department of Plant Health (DSV) of the Secretariat of Agricultural Defense (SDA) of MAPA (Fidelis et al., 2018). It is also noteworthy that *B. carambolae* is typically parasitized by *F. arisanus* in its native regions (Ramadan et al., 1992; Yuliadhi et al., 2022).

In the VSF, three species of parasitoids of fruit flies have been detected: a) *Doryctobracon areolatus* (Szépligeti, 1911) (Hymenoptera: Braconidae), native to the Americas and associated with 20 species of *Anastrepha* and *C. capitata* (Paranhos et al., 2019); b) *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae) associated with *A. fraterculus* (Paranhos et al., 2019) and *C. capitata* (Silva et al., 2020); and c) *T. giffardianus* often associated with *C. capitata* (Costa et al., 2005; Montes et al., 2011; Araújo et al., 2015; Carvalho et al., 2018). *Tetrastichus giffardianus* was not detected in the various surveys carried out throughout Brazil after its release in São Paulo State (SP) in 1937. Nevertheless, over the last two decades, this parasitoid has been frequently recovered from puparia of *C. capitata* (Costa et al., 2005; Montes et al., 2011; Araújo et al., 2015; Carvalho et al., 2018) and occasionally from *Anastrepha* (Araújo et al., 2016*),* mostly in the Northeastern region (Costa et al., 2005; Araújo et al., 2015, 2016; Carvalho et al., 2018), but it has also been detected in the Southeastern region (Montes et al., 2011).

This study aimed to expand the areas with surveys of parasitoids present in the region of the São Francisco River Valley (VSF) to prospect and identify species for potential application as biological control

agents against *B. carambolae,* in order to support public policies for the mitigation and control of this PQP if detected in the region.

Material and Methods

a) Study sites and fruit collection

Fruits exhibiting symptoms of fruit fly infestations were collected to retrieve parasitoids from the field. The symptoms considered included fruits with small holes in the skin, showing signs of softening and leaking liquid, which indicates the possible presence of fruit fly larvae in the stage preferred by parasitoids (Purcell et al., 1996).

Monthly collections of fruit infested by fruit flies were conducted from October 2017 to May 2019 in the Irrigation Districts of N4, N6, and Salitre, and from December 2021 to February 2023 in the Irrigation District of Bebedouro; all located in Petrolina-PE, except Salitre, which is situated in Juazeiro-BA. The host fruits collected were *Anacardium occidentale* L. (cashew), *Averrhoa carambola* L. (star fruit), *Citrus sinensis* (L.) Osbeck (sweet orange), *Diospyros kaki* L. F. (persimmon), *Malpighia emarginata* DC. (acerola), *Mangifera indica* L. (mango), *Vitis vinifera* (grape), *Psidium cattleyanum* Sabine (araçá), *Psidium guajava* L. (guava), *Pyrus communis* L. (pear), *Spondias dulcis* G. Forst. (cajá-manga), *Spondias purpurea* L. (seriguela), *Spondias tuberosa* Arruda (umbu), and *Terminalia catappa* L. (castanhola), according to the availability in each area. Mature fruits were randomly sampled from both the plant and the soil in groups weighing between 0.5 to 10 kg.

The fruits were collected at various locations within each district: two properties in N4 (site 1: 9°21'1211"S 40°41'3366"W and site 2: 9°21'06"S 40°42'6888"W); two in N6 (site 1: 9°17'4266"S 40°32'9888"W and site 2: 9°17'7922"S 40°32'3288"W); one in the Salitre District (9°32'4633"S 40°36'90"W); four in the District of Bebedouro (site 1: 9°06'8166"S 40°18'8955"W, site 2: 9°07'3444"S 40°18'8622"W, site 3: 9°06'08.7"S 40°17'11.3"W, and site 4 at Experimental Station of Embrapa: 9°08'12"S 40°17'96.8"W); as well as one at the Caatinga Experimental Station (9°03'96.2"S 40°19'68.7"W).

b) Laboratorial procedures

Following the collection, the fruits were taken to the Laboratory of Entomology at Embrapa Semiárido, in Petrolina-PE, where they were weighed and packed in plastic trays (40 × 60 cm), placed on a 5-cm layer of fine vermiculite and covered with voile-type fabric. Afterward, they were labeled with information including the type of fruit, weight, location, and date of collection.

The vermiculite was sieved after 8 and 15 days to recover puparia from the larvae that had exited the fruit pulp. The total amount of recovered puparia from each sample was divided in portions of twenty-five to thirty puparia per test tubes, properly identified as described above, along with a small amount of fine moistened vermiculite. Subsequently, the tubes were sealed with plastic film and placed in an air-conditioned room under controlled environmental conditions (Temperature = 27.0 ± 2.0 °C, humidity = 60 ± 10%, and 12 h lighting).

The emergence of adults (flies and parasitoids) was monitored daily. After natural death, the adults were quantified and transferred to bottles containing 70% ethanol for preservation and subsequent identification of the species.

Species identifications were conducted based on the following literature: Marinho et al. (2018) for Braconidae; Guimarães (2023) and Ovruski et al. (2007) for Figitidae (Eucoilinae); and Shimbori et al. (2020) for Eulophidae and Pteromalidae. The specimens were deposited at Oscar Monte Entomophagous Insect Collection of the Instituto Biológico, headquartered in Campinas-SP, Brazil, at the Biological Control Laboratory, under reference number IB-CBE-S-857.

As *T. giffardianus* is a gregarious species, while the other fruit fly parasitoids obtained are solitary, a simple comparison of the recovered numbers of specimens of each parasitoid species would lead to a biased result in favor of *T. giffardianus*. To avoid such error, utilizing the number of parasitized puparia rather than the number of parasitoid specimens obtained, is more appropriate. Given the large quantity of over 14 thousand puparia obtained, which requires extensive processing, individualizing fruit fly puparia was not feasible. Therefore, the number of puparia parasitized by *T. giffardianus* was estimated by using the average of individuals reared per puparium found in the literature. Pemberton and Willard (1918), Autuori (1942) and Ramadan and Wong (1990) reported 8.6, 10.5, and 6.7 individuals per puparium, respectively, which corresponds to an average of 8.6 ± 1.9 individuals per puparium.

Results

a) Pupa and fruit flies recovered from different fruit hosts

Two hundred and fifty-six samples of seven fruit trees were collected from October 2017 to May 2019 (20 months), totaling 799.40 kg of fruit. Among these samples, 14,603 puparia were obtained, from which 11,685 of Tephritidae family flies emerged. Specifically, 10,204 adults of *C. capitata* (87.33%) and 1,481 of *Anastrepha* spp. (12.67%) were associated with acerola, cajá-manga, guava, mango, seriguela, umbu, and grape (Table 1).

During the second gathering period, from December 2021 to February 2023 (15 months), 115 samples were gathered, totaling approximately 493.40 kg of various fruits collected from both plants and on the ground. Among these samples, 12,764 puparia were obtained from which 6,748 flies of the Tephritidae family emerged, consisting of 5,475 adult *C. capitata* (81.13%) and 1,273 *Anastrepha* spp. (18.87%) associated with acerola, araçá, persimmon, cashew, star fruit, castanhola, guava, orange, mango, pear, seriguela, and umbu (Table 1).

The exotic species *C. capitata* was predominant in both study periods, accounting for over 80% of all fruit fly specimens recovered from the fruits collected in the two collection periods. The level of infestation by fruit flies ranged from 0 (cajá-manga, orange, and cashew) to 98.52 (araçá) puparia/kg of fruit, with averages of 13.64 and 36.79 in the first (2017-2019) and second periods (2021-2023) of the survey, respectively. Generally, fruits with the highest infestations by these pests were guava, acerola, seriguela, umbu, and araçá (Table 1).

b) Pupa and parasitoids recovered from different fruit hosts

Regarding parasitoids, in the first survey period, from the 14,603 recovered puparia, 463 parasitoids were obtained (Table 1), belonging to four species from four families: a) *T. giffardianus*, with 394 specimens from guava, acerola, and seriguela; b) *D. areolatus*, with 66 specimens from guava and umbu; c) *Ganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae), with one specimen from acerola; and d) *P. vindemmiae*, with two specimens from guava (Table 2).

Two specimens of *Tropideucoila weldi* Lima, 1940 (Hymenoptera: Figitidae), one female and one male, were recovered from guava during the first collection period. However, this sample yielded 458 *Anastrepha* specimens, five *C. capitata*, and four specimens of the genus *Neosilba* (Diptera: Lonchaeidae), making it impossible to determine their fruit fly hosts, since representatives of both Diptera families emerged from the sample.

In the second collection period, from the 12,764 recovered puparia, 94 specimens of parasitoids belonging to seven species from five families were obtained (Table 1): a) *G. pelleranoi*, with 36 specimens recovered from guava, acerola, persimmon, and araçá; b) *D. areolatus* with 25 specimens from guavas and umbu-cajá; c) *T. giffardianus*, with nine

Table 1
Amount of fruits (kg), number of puparia, puparia per kilogram of fruit (avg±SE), number of adult fruit flies, number of parasitoids from tephritid fruit flies, and percentages of emergence (avg±SE) recorded in the two survey periods (October/2017 to May/2019 and December/2021 to February/2023), in São Francisco River Valley (VSF), Brazil.

Name of Fruit Plant		Amount (kg)	Puparia (n)	Puparia/ kg	Flies (n)	Parasitoids (n)	Emergence (%)
scientific	common	2017-2019 Survey					
Psidium guajava	Guava	295.41	10,675	36.14±0.48	8689	448	85.59±1.11
Malpighia emarginata	Acerola	127.87	1685	13.18±0.23	1214	8	72.52±1.30
Vitis vinifera	Grape	188.56	461	2.44±0.05	358	0	77.66±1.56
Mangifera indica	Mango	98.09	373	3.80±0.14	290	0	77.75±2.80
Spondias tuberosa	Umbu	68.37	1090	15.94±0.76	824	1	75.69±3.76
Spondias purpurea	Seriguela	13.29	319	24.00±4.0	310	6	99.06±16.23
Spondias dulcis	Cajá-manga	7.81	0	0	0	0	-
	Total	799.40	14,603	-	11,685	463	-
		2021-2023 Survey					
Psidium guajava	Guava	139.31	5481	39.34±1.27	3111	80	58.22±1.10
Malpighia emarginata	Acerola	65.60	4532	69.09±2.41	2045	6	45.26±1.60
Mangifera indica	Mango	189.66	952	5.02±0.22	549	0	57.67±2.43
Spondias tuberosa	Umbu	2.54	48	18.90±9.45	14	4	37.50±18.75
Spondias purpurea	Seriguela	19.19	605	31.53±33.09	293	0	48.43±4.85
Pyrus communis	Pear	46.28	134	2.90±0.48	106	0	79.10±13.18
Psidium cattleyanum	Araçá	8.15	803	98.52±16.42	532	3	66.63±11.04
Diospyros kaki	Persimmon	8.92	30	3.37±0.84	19	1	66.67±15.83
Averrhoa carambola	Star fruit	8.14	26	3.19±1.60	14	0	53.85±26.92
Citrus sinensis	Sweet orange	2.60	0	0	0	0	-
Anacardium occidentale	Cashew	2.11	0	0	0	0	-
Terminalia catappa	Castanhola	0.90	153	169.62	65	0	42.48
	Total	493.40	12,764	-	6,748	94	

Table 2
Parasitoid species recovered from tephritid fruit flies and number of parasitoids* from each fruit tree sampled during the collection periods from October 2017 to May 2019 (P1) and from December 2021 to February 2023 (P2), in São Francisco River Valley (VSF), Brazil.

Parasitoid species	Guava		Acerola		Persimmon		Seriguela		Umbu		Araçá		Total	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Doryctobracon areolatus	65	21	0	0	0	0	0	0	1	4	0	0	66	25
*Tetrastichus giffardianus**	44	1	1	0	0	0	1	0	0	0	0	0	46	1
Ganaspis pelleranoi	0	27	1	5	0	1	0	0	0	0	0	3	1	36
Pachycrepoideus vindemmiae	2	10	0	0	0	0	0	0	0	0	0	0	2	10
Spalangia simplex	0	10	0	1	0	0	0	0	0	0	0	0	0	11
Utetes anastrephae	0	2	0	0	0	0	0	0	0	0	0	0	0	2
Asobara anastrephae	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Estimated total	111	72	2	6	0	1	1	0	1	4	0	3	115	86

**Estimated number for T. giffardianus based on average of individuals reared per puparium obtained from literature (8.6 individuals/puparium).*

specimens from guava; d) *Spalangia simplex* Perkins, 1910 (Hymenoptera: Spalangiidae), with 11 specimens from guava and acerola; e) *Utetes anastrephae* (Viereck, 1875) (Hymenoptera: Braconidae), with two specimens from guava; f) *P. vindemmiae*, with 10 specimens from guava; g) *Asobara anastrepha*e (Muesebeck, 1958) (Hymenoptera: Braconidae), with only one specimen recovered from guava (Table 2).

In both collection periods, specimens of *C. capitata* and many species of genus *Anastrepha* were obtained from most samples, making it impossible to determine from which species of Tephritidae fruit fly the parasitoids emerged.

Photos of the eight species from five families of parasitoids recovered in the VSF are presented in the Fig. 1.

Comparing the two collection periods, notable differences were observed in parasitoid populations. In the first period, 463 parasitoids were obtained from guava, acerola, umbu, and seriguela, which belong to four species: *T. giffardianus, D. areolatus, P. vindemmiae*, and *G. pelleranoi* (Table 2). However, in the second period, despite collecting 40% fewer fruits, 94 parasitoids were recovered from guava, acerola, umbu, araçá, and persimmon, belonging to seven species, namely: *G. pelleranoi*, *D. areolatus*, *T. giffardianus*, *S. simplex*, *U. anastrephae*, *P. vindemmiae,* and *A. anastrepha*e (Table 2).

Among the seven parasitoid species recovered from Tephritidae family found in these surveys, the predominant parasitoid species in the first collection period was *D. areolatus*, followed by *T. giffardianus, whereas* in the second period, *G. pelleranoi* was predominant, followed by *D. areolatus* (Table 2 and Fig. 2). Overall, considering both collection periods, *D. areolatus* and *T. giffardianus* were recovered from 45.27 and 23.38% of the parasitized puparia, respectively (Fig. 2 and Table 3).

More than 90% of the parasitoids originated from guavas, considering both collection periods, encompassing three of the four species found in the first period and all seven species in the second collection period (Table 2 and Fig. 3). Considering the total number of parasitoid species, four recovered from Tephritidae family and one with unidentified host family (Tephritidae or Lonchaeidae) were reported for the first time in Pernambuco, namely *A. anastrephae*, *G. pelleranoi*, *S. simplex*, *U. anastrephae*, and *T. weldi*, respectively.

Discussion

The majority of fruit flies (Tephritidae) recovered in São Francisco River Valley (VSF) were *C. capitata* (80%) and just about 20% belonged to

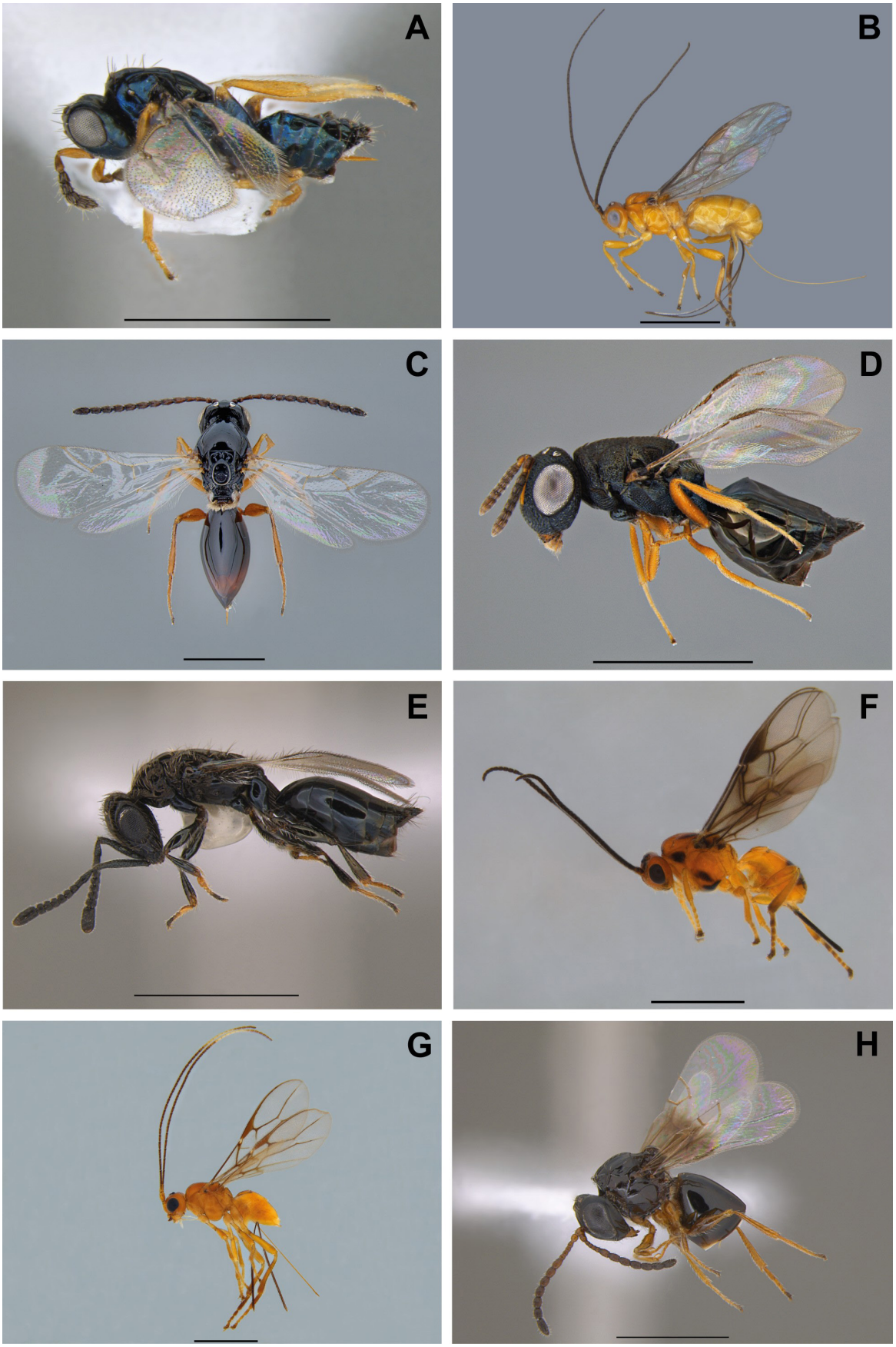

Figure 1 Females of all parasitoid species recovered in São Francisco River Valley (VSF), Brazil, considering all collection periods and all sampled fruits: A. *Tetrastichus giffardianus*; B. *Doryctobracon areolatus*; C. *Ganaspis pelleranoi*; D. *Pachycrepoideus vindemmiae*; E. *Spalangia simplex*; F. *Utetes anastrephae*; G. *Asobara anastrephae*; H. *Tropideucoila weldi*. Scale bar: 1 mm (A, C, D, E, H), 2 mm (B, F, G). Photos: V. A. Costa.

the genus *Anastrepha*, the same found by Paranhos et al. (2023). Among all kinds of fruits, guava was the most infested and better for recovering fruit fly pupa, from which were obtained more than 90% of parasitoids, including all species detected in this study. According to Araújo and Zucchi (2003), the levels of infestation in guava vary between 35 and 118 puparia/kg of fruit, confirming the high level of infestation by fruit flies in this fruit. Similarly, high levels of infestation in guavas (116 puparia/kg) were obtained in the semiarid region of Northern Minas Gerais (Canal et al., 1998).

Several authors have previously cited guava as the fruit with the greatest diversity of fruit fly species (Malavasi and Morgante, 1981; Raga et al., 2005). Furthermore, it can be inferred that the greater diversity of parasitoids is associated with the higher diversity of fruit fly species in guava. Studies conducted in Peru identified four species of parasitoids associated to guavas and correlated the highest parasitism levels with high fruit fly infestations (Salazar-Mendoza et al., 2021).

In total, we found seven species of parasitoids (*T. giffardianus*, *D. areolatus, U. anastrephae, A. anastrephae, G. pelleranoi*, *P. vindemmiae*, and *S. simplex*) belonging to five families of the order Hymenoptera (Eulophidae, Braconidae, Figitidae, Pteromalidae, and Spalangiidae). These parasitoids were recovered from Tephritidae fruit fly puparia and one more, *T. weldi*, from Lonchaeidae or Tephritidae family flies. From them, five species were first time detected in the state of Pernambuco, namely *A. anastrephae*, *G. pelleranoi*, *S. simplex, U. anastrephae,* and *T. weldi*. All of them are usually registered as tephritid parasitoids, except *T. weldi* whose hosts are usually from Lonchaeidae family. Lima (1940) cited *Neosilba* sp. (Diptera: Lonchaeidae) as its host in the Federal District, while Souza et al. (2012) identified three species of *Anastrepha* in the state of Minas Gerais as its host.

Comparing the results with information from literature conducted in similar climatic condition in Brazil revealed distinct findings. In the interior of Ceará State, nine species of fruit fly parasitoids were found: *A. anastrephae*, *D. areolatus*, *Opius bellus* Gahan, 1930 (Hym.: Braconidae), *U. anastrephae*, *Spalangia gemina* Bouček, 1963 (Hymenoptera, Pteromalidae), *S. simplex*, *P. vindemmiae*, *Trichopria anastrephae* Lima, 1940 (Hym.: Diapriidae), and *T. giffardianus* (Araújo et al., 2015). In a semiarid region of the Cerrado (similar to Savannah Bioma), located on Northern Minas Gerais State, nine species of parasitoids were recovered: *D. areolatus*, *D. brasiliensis* (Szépligeti, 1911), *U. anastrephae*, *O. bellus*, *A. anastrephae*, *Asobara obliqua* (Papp, 1969) (Braconidae, Alysiinae), *G. pelleranoi*, *T. weldi*, and *P. vindemmiae* (Souza et al., 2012). In both regions above mentioned, *D. areolatus* was the predominant species.

Among the seven species of parasitoids found in the VSF (Table 3), six have been previously reported in Ceará (*A. anastrephae*, *D. areolatus*, *P. vindemmiae*, *T. giffardianus*, *S. simplex,* and *U. anastrephae*) (Araújo et al.,

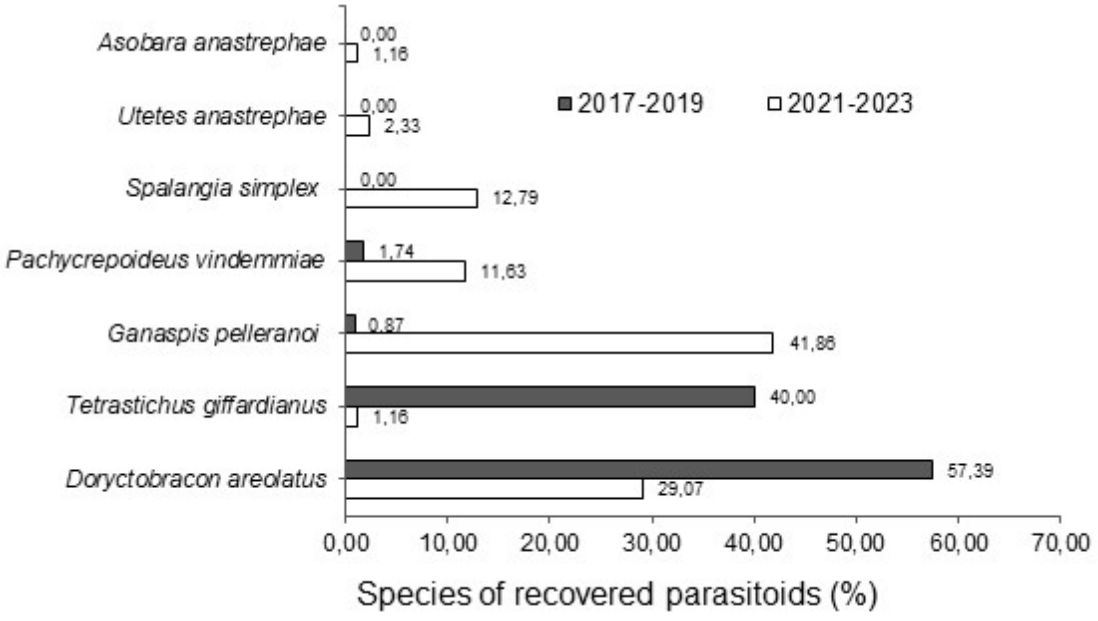

Figure 2 Relative abundance of parasitoid species (percentage) reared from tephritid fruit flies, considering all fruit trees collected from October/2017 to May/2019, and from December/2021 to February/2023, in São Francisco River Valley (VSF), Brazil.

Table 3
Parasitoid species recovered from tephritid fruit flies, total number of parasitized puparia*, and percentage of puparia attacked by each parasitoid species per total of parasitized puparia during the collection periods from October 2017 to May 2019 (P1) and from December 2021 to February 2023 (P2), in São Francisco River Valley (VSF), Brazil.

Parasitoid species	Parasitized puparia per parasitoid species	Puparia attacked by each parasitoid species per total of parasitized puparia (%)
Doryctobracon areolatus	91	45.27
Tetrastichus giffardianus	47	23.38
Ganaspis pelleranoi	37	18.41
Pachycrepoideus vindemmiae	12	5.97
Spalangia simplex	11	5.47
Utetes anastrephae	2	1.00
Asobara anastrephae	1	0.50
Total	201	100.00

**Estimated number for T. giffardianus based on average of individuals reared per puparium obtained from literature (8.6 individuals/puparium).*

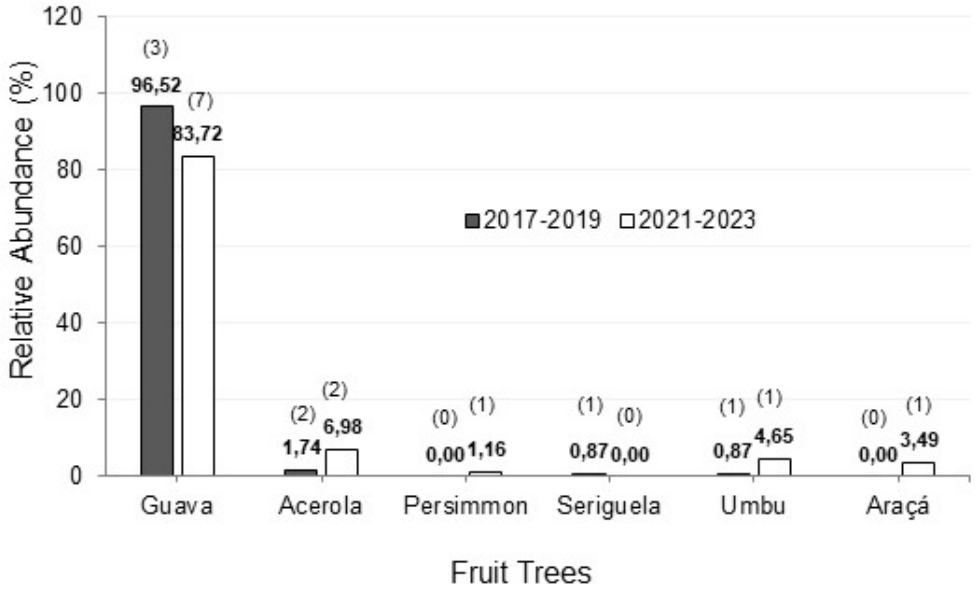

Figure 3 Relative abundance of parasitoids (percentage) recovered from tephritid fruit flies, considering all species, in the different fruit trees collected from October/2017 to May/2019 and from December/2021 to February/2023 in São Francisco River Valley (VSF), Brazil. Fruits without parasitism were not included. The respective numbers of parasitoid species found per fruit tree evaluated are presented above the bars, in parentheses.

2015) and five in the North of Minas Gerais (*A. anastrephae, G. pelleranoi, D. areolatus, P. vindemmiae,* and *U. anastrephae*) (Souza et al., 2012). The four common to the three regions studied are *A. anastrephae*, *D. areolatus*, *P. vindemmiae*, and *U. anastrephae*. Among the species found in the VSF, only *G. pelleranoi* was not reported in Ceará, while *S. simplex* and *T. giffardianus* were not found in the Northern Minas Gerais. The species detected in Ceará that were not observed in the VSF include *O. bellus, S. gemina,* and *T. anastrephae.* Similarly, the species identified in the North of Minas Gerais but not registered in the VSF were *D. brasiliensis, O. bellus,* and *As. obliqua*.

Of all the parasitoid species registered in our study as present in the VSF, only three had been previously registered as tephritid parasitoids in this region: *D. areolatus*, *T. giffardianus*, and *P. vindemmiae* (Paranhos et al., 2023). In the present study the most abundant parasitoid species obtained were *D. areolatus*, followed *by T. giffardianus* and *G. pelleranoi.*

Doryctobracon areolatus is the most common native parasitoid in Brazil, found throughout the country, with numerous host fruit fly species, particularly belonging to the *Anastrepha* genus (Paranhos et al., 2019; Zucchi and Moraes, 2025a). Its use as a bioagent in biological control programs against *A. fraterculus* in Southern Brazil has been recommended (Gonçalves et al., 2018; Paranhos et al., 2019). This native parasitoid has already been assigned Reference Specification number 54 (Portaria DOU, Nº 784, of April 19, 2023) as a bioagent to control *C. capitata* and *Anastrepha* spp. Recently, *D. areolatus* has been recovered from *B. carambolae* pupae (Costa et al., 2025). Although the current low parasitism rate, it was the most abundant species compared to the other two (*U. anastrephae*, and *G. pelleranoi*), all of which found in the Jari Valley, located between the South of the state of Amapá and the North of the state of Pará, Brazil (Costa et al., 2025). These findings arouse interest in further research to assess its potential use as a biological control agent.

The gregarious endoparasitoid *T. giffardianus* has been recovered in the Northeastern region parasitizing *C. capitata* (Costa et al., 2005; Montes et al., 2011; Araújo et al., 2015, 2016; Carvalho et al., 2018). Garcia et al. (2022) indicated favorability towards this parasitoid in all regions of the country, mainly in the Northeast, where the VSF is located. Additionally, the higher occurrence of *C. capitata* (> 80%) compared to the genus *Anastrepha* spp. (< 20%) in the VSF is a positive aspect to be considered for its use in this region, given the affinity of this parasitoid to this species of fruit flies (Carvalho et al., 2018). Moreover, the literature has demonstrated its parasitism efficiency on several species of fruit flies of the genus *Bactrocera* abroad (Vargas et al., 1993; LaSalle and Wharton, 2002; Vargas et al., 2012; Montoya et al., 2017).

Therefore, among all parasitoids found in VSF, *T. giffardianus* is the most promising to be used in a biological control program against *B. carambolae*, if detected in this region. Besides the potential effectiveness against *Bactrocera* species, this species is readily found in the VSF associated with guavas infested with *C. capitata* (Carvalho et al., 2018; Paranhos et al., 2023), making it highly suitable for establishing a laboratory colony. Thus, this parasitoid could serve as a viable strategy for augmentative biological control of *B. carambolae* in the event of its detection in the VSF.

Furthermore, for successful domestication of wild insects under artificial conditions, an initial colony should be started with at least 500 wild individuals (Parra et al., 2002). For that it is recommended to collect a large amount of guava in the first semester of the year, when a higher quantity of parasitoids was recovered, in order to obtain a large number of *T. giffardianus* to start a colony for mass-rearing in the future.

The biology of *T. giffardianus* on *C. capitata* and its rearing system for small colonies have already been described (Pemberton and Willard, 1918; Autuori, 1942; Carabajal-Paladino et al., 2010; Fernandes et al., 2019, 2021). Hence, the results indicate favorable conditions for using the parasitoid *T. giffardianus* in the VSF, since the location, the most suitable fruit host, and the most appropriate time of year to carry out collections directed towards this parasitoid were determined.

In addition to this parasitoid, two other parasitoid species that have also been introduced into Brazil (*D. longicaudata*, which parasitizes 3rd instar larvae of Tephritidae in general, and *F. arisanus*, which parasitizes eggs and newly hatched larvae, mainly of the genera *Bactrocera* and *Ceratitis*) could also be used very successfully to control *B. carambolae*

in the northern states of Brazil, where the species is present and under official control of MAPA.

For parasitoids to be released in the field, they must be registered in the MAPA. Among the exotics already introduced, *D. longicaudata* has been registered in Brazil, while *T. giffardianus* has not yet been registered for commercial use; however, it is considered a well-established exotic species in the country and is therefore eligible for registration. In contrast, the registration of *F. arisanus* has not yet been possible due to more restrictive regulations introduced since its importation in 2012 (Paranhos et al., 2019; Shimbori et al., 2023), including those governing the importation of new species. However, with the recent approval of the new bioinputs law (http://www.in.gov.br/web/dou/-/lei-n-15.070-de-23-de-dezembro-de-2024-603825827), it is hoped that this parasitoid species can be registered for use in Brazil. This is particularly significant because *F. arisanus* is the parasitoid that contributes most to the reduction of the population of *B. dorsalis* (Clausen, 1978; Leblanc et al., 2013).

It is noteworthy that these three exotic species are also effective in controlling *C. capitata*, currently the most prevalent fruit fly species in the VSF. Regarding *Anastrepha* species, which cause major problems in Southern Brazil, the exotic parasitoid *D. longicaudata* has been highly effective in controlling them, serving as an excellent complement to the native *D. areolatus* previously mentioned.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

Conceptualization, BdAGP; methodology, BdAGP, MYFO, and JVAC; data curation, BdAGP, VAC, EMS; writing - original draft preparation, BdAGP, JSMP, CRdJ, RA, and MCPYP; writing - review and editing, BdAGP, VAC, RA, and MCPYP. All authors have read and agreed to the published version of the manuscript.

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