




Pupal parasitoids associated with *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in a semiarid environment in Brazil

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ABSTRACT

Fruit flies (Diptera: Tephritidae) are important pests that affect the fruit-growing worldwide. In the northeastern Brazil, where a semiarid climate predominates and the production of tropical fruits for export is concentrated, some fruit flies, including *Anastrepha* spp. and *Ceratitis capitata* (Wiedemann), are considered pests due to economic damage and quarantine restrictions. In several parts of the world, fruit fly population regulation is carried out with the support of hymenopteran parasitoids. In Brazil, some information exists about larval parasitoids of fruit flies, but little is known about pupal parasitoids of these tephritids, especially in Brazilian semiarid environments. Therefore, the main objective of this study was to know the pupal parasitoids associated with *C. capitata* in a semiarid environment in Brazil. The parasitoid survey was carried out in a semiarid region in the states of Rio do Norte and Ceará. To obtain the parasitoids, pupae (sentinels) of *C. capitata* from the laboratory, Applied Entomology Laboratory of Universidade Federal Rural do Semi-Árido, were exposed to natural parasitism in the field. Six parasitoid (Hymenoptera) species were obtained: *Pachycrepoideus vindemmia* (Rondani), *Spalangia simplex* Perkins, *Spalangia gemina* Boucek, *Spalangia leiopleura* Gibson, and *Spalangia impunctata* Howard (Pteromalidae); *Trichopria anastrephae* Lima (Diapriidae). These are the first records of pupal parasitoids associated with the fruit fly *C. capitata* in Brazil.

Introduction

Fruit flies (Diptera: Tephritidae) are considered pests that affect the fruit-growing worldwide (White and Elson-Harris, 1992; Sznyszewska and Tatem, 2014). The fruit flies oviposit inside the fruits and their larvae feed on the pulp, making them unsuitable for fresh consumption and industrialization. After completing their development, the larvae leave the fruits and bury themselves in the soil to pupate, after a few days the adults emerge (White and Elson-Harris, 1992). In Brazil, *Ceratitis capitata* (Wiedemann) and several species from genus *Anastrepha* Schiner are important, due to economic damages and quarantine restrictions (Malavasi and Zucchi, 2000; Vilela and Zucchi, 2015). Some *Anastrepha* species are endemic to Brazil, whereas *C. capitata* was first detected in the country in 1901 (Zucchi, 2000).

In semiarid regions of Brazil, where the Caatinga biome predominates and tropical fruit production is concentrated, *C. capitata* was detected

in the early 1990s and is currently considered one of the region's key fruit pests (Araujo et al., 2013). The Caatinga biome is characterized by its vegetation with xerophyte and deciduous plants. The climate is predominantly hot semi-arid (BSh type), with sparse and irregular rainfall (average annual rainfall of 600 mm), high average temperatures ($\pm 28^\circ\text{C}$) and low air humidity ($\pm 65\%$) (Sousa et al., 2019).

For the control of *C. capitata* populations, it is important to know its natural enemies that can be used in integrated management programs, and the parasitoids are among the main natural enemies of this fruit fly (Aluja et al., 2014). However, as most of the life cycle of *C. capitata* occurs protected inside the fruit (egg and larval stage) and buried in the soil (pupal stage), the control of this fruit fly and the survey of its parasitoids, especially pupal parasitoids, is difficult. The methodology currently used for the survey of fruit fly parasitoids, which is based on the collection of fruits, allows only the knowledge of the egg and larval parasitoids, but it is not possible to know the pupal parasitoids.

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In Brazil, fruit fly parasitoid surveys have been practically restricted to larvae parasitoids, and these surveys have indicated that hymenopteran parasitoids (Braconidae and Figitidae) are the most common in several regions of the country and are associated mainly with species of *Anastrepha* (Garcia and Corseuil, 2004; Souza-Filho et al., 2009; Silva et al., 2010; Souza et al., 2012; Taira et al., 2013).

Little is known about pupal fruit fly parasitoids and this information is concentrated to Brazil's central and southern regions, where some hymenopteran parasitoids, including *Pachycrepoideus vindemmiae* (Rondani), *Spalangia simplex* Perkins, *Spalangia endius* Walker, and *Spalangia gemina* Bouček (Pteromalidae); *Trichopria anastrephae* Lima and *Coptera haywardi* Loiacono (Diapriidae), were found parasitizing pupae of *Anastrepha* (Marchiori et al., 2000; Aguiar-Menezes et al., 2003; Uchôa-Fernandes et al., 2003; Garcia and Corseuil, 2004; Cruz et al., 2011). There are no records of pupal parasitoids associated with *C. capitata* (Paranhos et al., 2019). In semiarid regions of Brazil, the obtained information is related only to fruit fly larvae parasitoids (Araujo and Zucchi, 2002; Alvarenga et al., 2009; Araujo et al., 2015). However, knowing pupal parasitoids is important because these can be used to regulate the population of *C. capitata*. Therefore, this study's main objective was to know the pupal parasitoids associated with *C. capitata*, in a semiarid environment in Brazil.

Material and methods

To collect the parasitoids, live *C. capitata* (strain Vienna 8) pupae were exposed to parasitism throughout the studied zone using the sentinel pupae technique (Petersen and Watson, 1992). *Ceratitis capitata* (strain Vienna 8) pupae were obtained from the Applied Entomology Laboratory of Universidade Federal Rural do Semi-Árido (UFERSA) (Mossoró, Rio Grande do Norte, Brazil), as unirradiated 48 hours-old pupae.

The *C. capitata* pupae were exposed to natural field parasitism in transparent plastic containers (parasitism units) with a capacity of 500 ml (12 cm in diameter x 7 cm in height) with the upper part open. In each parasitism unit, 200 pupae (48 hours-old) were placed under a thin layer of vermiculite. The parasitism units were distributed in nine different locations of a semiarid region in the states of Rio Grande do Norte (RN) and Ceará (CE), Brazil: Municipality of Mossoró (RN) - Local 1 (5°12'34.3"S - 37°20'21.8"W), Local 2 (5°11'42.2"S - 37°18'48.0"W), Local 3 (5°11'52.8"S - 37°18'31.3"W), and Local 4 (5°03'44.5"S - 37°24'08.5"W); Municipality of Ipangaçu (RN) - Local 5 (5°32'6.60"S - 36°52'16.06"W); Municipality of Limoeiro do Norte (CE) - Local 6 (5°09'44.7"S; 38°06'21.6"W), Local 7 (5°08'44.2"S - 38°5'37.2"W), Local 8 (5°08'44.0"S; 38°05'53.0"W), and Local 9 (5°04'27.0"S; 38°06'21.6"W) (Table 1).

For each studied location, each 15 days one parasitism units was exposed in the field for 48 hours, during the period from May to September 2016. Then, a total of 90 parasitism units were used, with one parasitism unit per location and sampling date. To avoid predation by soil insects, the parasitism units were placed inside plastic trays (51 x 30 x 9.5 cm [l x w x h]) containing a solution of water and detergent (10%).

After field exposure, the parasitism units were retrieved to the laboratory, and the pupae were placed inside Petri dishes (10 cm in diameter x 1.5 cm in height) under a thin layer (4 cm) of moist vermiculite. The Petri dishes were then closed with voile fabric and kept in a climate-controlled room at a temperature of 25 ± 2°C, relative humidity of 70 ± 10% and a photoperiod of 12:12 h (L:D), where they remained until the emergence of adults (flies or parasitoids). Parasitoids that emerged were counted and fixed in 70% alcohol for later specific identification.

The parasitoids were identified based on Bouček and Heydon (1997), Gibson (2009) and Rueda and Axtell (1985) taxonomical keys. Voucher

specimens were deposited in the parasitoid hymenoptera collection at the Biological Institute, Campinas, São Paulo, Brazil.

Results and discussion

A total of 194 parasitoids (Hymenoptera) belonging to six species and two families were obtained: *P. vindemmiae* (n= 142 | 73.2%), *S. simplex* (n= 32 | 16.5%), *S. gemina* (n= 13 | 6.7%), *Spalangia leiopleura* Gibson (n= 5 | 2.6%) and *Spalangia impunctata* Howard (n= 1 | 0.5%) (Pteromalidae); *T. anastrephae* (n= 1 | 0.5%) (Diapriidae) (Table 1).

The considerable diversity of Pteromalidae (Chalcidoidea) collected is possibly related to the fact that the main genera of pupal parasitoids of fruit flies in the world belong to this family (Noyes, 2014). Although most parasitoid species collected in this study had already been registered in Brazil, this is the first report of *S. impunctata* in the country. Among the pupal fruit fly parasitoids reported in Brazil, only *S. endius* (Aguiar-Menezes et al., 2003; Silva et al., 2003; Uchôa-Fernandes et al., 2003; Nicácio et al., 2011) and *C. haywardi* (Aguiar-Menezes et al., 2003) were not collected during the present study (Table 2).

Pachycrepoideus vindemmiae was the most common species in this survey, possibly due to its generalist behaviour (Marchiori et al., 2013; Zhao et al., 2013), which favors its presence in various environments and regions. In several parts of the world, records indicate that *P. vindemmiae* parasitizes species of fruit flies of economic importance in the genera *Anastrepha*, *Ceratitis*, *Bactrocera*, and *Rhagoletis* (Noyes, 2014). In some arid regions such as Tunisia and Benin, *P. vindemmiae* has been recorded parasitizing pupae of *C. capitata* (Harbi et al., 2015) and *Bactrocera invadens* Drew, Tsuruta & White (Vayssières et al., 2011).

Pachycrepoideus vindemmiae was introduced in the Americas from West Africa and India (Purcell, 1998) to control *Anastrepha* species and *C. capitata* (Ovruski et al., 2000). Due to its wide range of hosts, which includes at least 57 species in 14 dipteran families (Noyes, 2014), this parasitoid has spread to various countries in the Americas, where it is quite common in surveys. In Mexico, for example, *P. vindemmiae* was obtained from *Anastrepha striata* Schiner and *Anastrepha ludens* (Loew) (Sánchez-García et al., 2014). For *Ceratitis*, there are reports of this parasitoid associated with *C. cosyra* Walker, *C. silvestrii* Bezzi, *C. quinaria* Bezzi, *C. fasciventris* Bezzi, *C. anonae* Graham, *C. ditissima* Munro, and *C. capitata* in Africa (Vayssières et al., 2002). In Argentina, *P. vindemmiae* was obtained from *C. capitata* (Ovruski et al., 2006). In Brazil, *P. vindemmiae* has been reported parasitizing *Anastrepha* species in the central-western and southern regions of the country, in

Table 1.

Pupal parasitoids recorded in association with the fruit fly *Ceratitis capitata* in the States of Rio Grande do Norte (RN) and Ceará (CE), Brazilian semiarid.

Municipalities/States Local - Geographical coordinates	Parasitoid species (Number of parasitoids collected)
Mossoró/RN	
Local 1: 5°12'34.3"S; 37°20'21.8"W	<i>Pachycrepoideus vindemmiae</i> (106); <i>Spalangia leiopleura</i> (3)
Local 2: 5°11'42.2"S; 37°18'48.0"W	<i>P. vindemmiae</i> (4); <i>Spalangia simplex</i> (4); <i>S. leiopleura</i> (2)
Local 3: 5°11'52.8"S; 37°18'31.3"W	<i>S. simplex</i> (8); <i>Spalangia impunctata</i> (1)
Local 4: 5°03'44.5"S; 37°24'08.5"W	<i>P. vindemmiae</i> (3)
Ipangaçu/RN	
Local 5: 5°32'6.60"S; 36°52'16.1"W	<i>P. vindemmiae</i> (6)
Limoeiro do Norte/CE	
Local 6: 5°09'44.7"S; 38°06'21.6"W	<i>Spalangia gemina</i> (13)
Local 7: 5°08'44.2"S; 38°05'37.2"W	<i>P. vindemmiae</i> (23)
Local 8: 5°08'44.0"S; 38°05'53.0"W	<i>S. simplex</i> (20)
Local 9: 5°04'27.0"S; 38°06'21.6"W	<i>Trichopria anastrephae</i> (1)

Table 2.

Pupal parasitoids recorded in association with fruit flies (*Anastrepha* spp. and *Ceratitis capitata*) in Brazil.

Parasitoid species	Municipalities/States	Hosts (Tephritidae)	References
Pteromalidae			
<i>Pachycrepoideus vindemmiae</i>	Mossoró/RN**	<i>Ceratitis capitata</i> ***	
	Ipanguaçu/RN**	<i>C. capitata</i> ***	
	Limoeiro do Norte/CE**	<i>C. capitata</i> ***	
	Itumbiária/GO	<i>Anastrepha fraterculus</i>	Marchiori et al. (2000)
	Presidente Prudente/SP	<i>Anastrepha</i> spp. and/or <i>C. capitata</i>	Montes et al. (2011)
	Seropédica/RJ	<i>A. fraterculus</i> and/or <i>A. sororcula</i>	Aguiar-Menezes et al. (2003)
	Pelotas/RS	<i>A. fraterculus</i>	Salles (1996)
<i>Spalangia endius</i>	Divinópolis/MG	<i>Anastrepha</i> spp.	Silva et al. (2003)
	Not mentioned/MS	<i>Anastrepha</i> spp. and/or <i>A. obliqua</i>	Uchôa-Fernandes et al. (2003)
	Seropédica/RJ	<i>A. fraterculus</i> and/or <i>A. sororcula</i>	Aguiar-Menezes et al. (2003)
	Passo do Lontra/MS	<i>A. alveatoides</i>	Nicácio et al. (2011)
<i>Spalangia gemina</i>	Divinópolis/MG	<i>Anastrepha</i> spp.	Silva et al. (2003)
	Limoeiro do Norte/CE**	<i>C. capitata</i> ***	
	Not mentioned/MS	<i>Anastrepha</i> spp. and <i>A. obliqua</i>	Uchôa-Fernandes et al. (2003)
<i>Spalangia simplex</i>	Seropédica/RJ	<i>A. fraterculus</i> and/or <i>A. sororcula</i>	Aguiar-Menezes et al. (2003)
	Mossoró/RN**	<i>C. capitata</i> ***	
	Limoeiro do Norte/CE**	<i>C. capitata</i> ***	
<i>Spalangia leiopleura</i>	Jaboticabal/SP	<i>A. serpentina</i>	Fernandes et al. (2013)
	Mossoró/RN**	<i>C. capitata</i> ***	
<i>Spalangia impunctata</i> *	Mossoró/RN**	<i>C. capitata</i> ***	
Diapriidae			
<i>Coptera haywardi</i>	Seropédica/RJ	<i>A. fraterculus</i> and/or <i>A. sororcula</i>	Aguiar-Menezes et al. (2003)
<i>Trichopria anastrephae</i>	Limoeiro do Norte/CE**	<i>C. capitata</i> ***	
	Capão do Leão/RS	<i>A. fraterculus</i>	Cruz et al. (2011)
	Not mentioned/SC	<i>A. fraterculus</i>	Garcia and Corseuil, 2004
	Seropédica/RJ	<i>A. fraterculus</i>	Aguiar-Menezes et al., 2001
	Una/BA	<i>Anastrepha</i> spp.	Souza-Filho et al. (2007)
	Divinópolis/MG	<i>Anastrepha</i> spp.	Silva et al. (2003)
	Not mentioned/RJ	<i>Anastrepha</i> sp. and <i>A. serpentina</i>	Lima (1940)

* First reports in Brazil; ** New distribution reports; *** New reports of association with fruit flies in Brazil.

the states of Rio Grande do Sul (Salles, 1996), Goiás (Marchiori et al., 2000), Rio de Janeiro (Aguiar-Menezes et al., 2003) and Minas Gerais (Silva et al., 2003). *Pachycrepoideus vindemmiae* was also reported to parasitize fruit flies in São Paulo (Montes et al., 2011). However, it was unclear whether the parasitoids were obtained from *Anastrepha* species or *C. capitata*. In the present study, *P. vindemmiae* was recorded for the first time parasitizing pupae of *C. capitata* (Table 2) in a semiarid region of Brazil, states of Rio Grande do Norte and Ceará (Fig. 1).

The diversity of *Spalangia* species (*S. simplex*, *S. gemina*, *S. leiopleura*, and *S. impunctata*) in this survey shows that these parasitoids can be common in semiarid environment. *Spalangia* species are typically generalists and parasitize pupae of various dipteran families, such as Muscidae, Calliphoridae, Sarcophagidae, Drosophilidae, and Tephritidae (Gibson, 2009; Beitia et al., 2016). In Brazil, there are records of *Spalangia* parasitizing fruit flies only in the southeastern and center-west regions (Aguiar-Menezes et al., 2003; Silva et al., 2003; Uchôa-Fernandes et al., 2003; Nicácio et al., 2011; Fernandes et al., 2013). Therefore, these are the first reports of *Spalangia* species associated with pupae of fruit flies (*C. capitata*) in a semiarid region of Brazil (Fig. 1).

In Africa, there are reports of *S. simplex* parasitizing pupae of various *Ceratitis* species (Vayssières et al., 2002). In the Americas, *S. simplex* has been observed to parasitize *Anastrepha* species, such as *A. ludens* in Mexico, *Anastrepha suspensa* (Loew) in the United States and *Anastrepha acidusa* (Walker) in Puerto Rico (Gibson, 2009). In Brazil, *S. simplex* was only obtained from pupae of *Anastrepha serpentina* (Wiedemann) in the state of São Paulo (Fernandes et al., 2013). Thus, this is the first record of *S. simplex* in association with pupae of *C. capitata* in Brazil (Table 2).

Spalangia gemina was described based on specimens collected in India, Thailand, Malaysia, Fiji, Mauritius, and Venezuela, indicating the species' widespread distribution worldwide (Bouček, 1963). In Asia and Africa, there are records of *S. gemina* parasitizing *Bactrocera dorsalis* (Hendel) and *B. cucurbitae* (Coquillett) (Bouček, 1963; Vayssières et al., 2001). In Brazil, *S. gemina* was obtained from *Anastrepha* species in the states of Mato Grosso do Sul (Uchôa-Fernandes et al., 2003) and Rio de Janeiro (Aguiar-Menezes et al., 2003). Therefore, this is the first report of *S. gemina* parasitizing *C. capitata* pupae in Brazil (Table 2).

The species *S. leiopleura* was described based on specimens collected in North America and Mexico (Gibson, 2009). Recently this species was reported in Brazil in the state of Minas Gerais. However, this report was based on specimens collected in traps, so it was not possible to know its host (Juliato et al., 2017). In this study, *S. leiopleura* was collected from *C. capitata* pupae in Rio Grande do Norte, so this is the first record of the association of *S. leiopleura* with a host of the Tephritidae family in Brazil.

Spalangia impunctata is possibly a circumtropical species (Gibson, 2009). This species was described based on specimens obtained from *Drosophila* Fallén pupae in India (Sureshan and Farsana, 2014). As reported, this is the first record of *S. impunctata* in Brazil. The specimens of *S. impunctata* were obtained from pupae of *C. capitata* in Rio Grande do Norte. This shows that Brazilian semiarid regions may contain species not yet reported in Brazil.

Trichopria anastrephae was described based on specimens obtained from pupae of *Anastrepha* sp. and *A. serpentina* in Rio de Janeiro, Brazil (Lima, 1940). Of the 12 *Trichopria* species cataloged in Brazil, *T. anastrephae* was the only one reported to parasitize *Anastrepha*

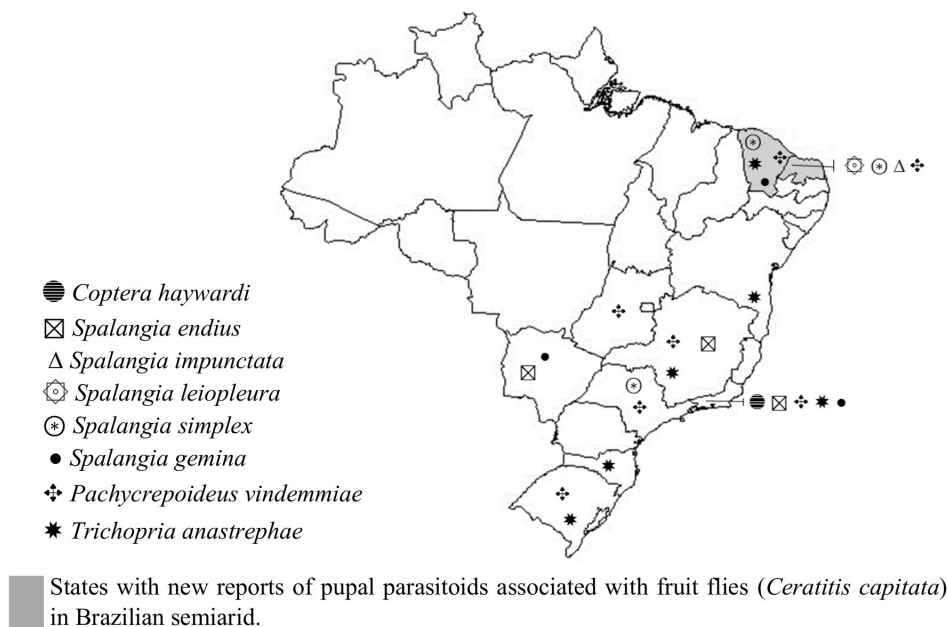


Figure 1 States with recordings of pupal parasitoids associated with fruit flies (*Anastrepha* spp. and / or *Ceratitis capitata*) in Brazil.

(Santis, 1980; Margaria, 2019). *Trichopria anastrephae* has been observed parasitizing fruit flies (*Anastrepha*) in the states of Rio de Janeiro (Lima, 1940; Aguiar-Menezes et al., 2001), Minas Gerais (Silva et al., 2003), Rio Grande do Sul (Garcia and Corseuil, 2004; Cruz et al., 2011) and Bahia (Souza-Filho et al., 2007). Therefore, this is the first record of *T. anastrephae* in a semiarid environment, and it is also the first time that *T. anastrephae* has been observed parasitizing *C. capitata* pupae in Brazil (Table 2).

In Brazil, the few reports of fruit fly pupae parasitoids known are concentrated in the midwestern and southern regions, which indicate that surveys of these parasitoids have been neglected in various regions of the country. In this study, six species of parasitoids are recorded for the first time, obtained from pupae (sentinels) of *C. capitata* distributed in a semiarid environment in northeastern Brazil. This information extends reports of pupae parasitoids associated with fruit flies in Brazil and records the presence of *S. impunctata* in the country for the first time. In addition, reports were made from pupae of *C. capitata*, which is an important exotic species that is spreading in Brazilian semiarid regions.

Therefore, the information obtained shows that if surveys are expanded, new reports of parasitoids associated with fruit fly pupae may be recorded in various regions of the country. Thus, further studies on these pupal parasitoids should be performed, aiming at their use in the management of *C. capitata* in fruit orchards located in semiarid regions of Brazil.

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Conflicts of interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This study was carried out following ethical standards.

Author contribution statement

BKA Silva, HM Silva and EC Fernandes carried out sampling of the parasitoids. VA Costa identified the parasitoids and reviewed the article. EL Araujo coordinated the study and wrote the final version of the article.

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