



Systematics, Morphology and Biogeography

## Development and morphological characterization of the immature stages of *Tetrastichus giffardianus* Silvestri (Hymenoptera: Eulophidae)

 Elania Fernandes, Elton Araujo \*, Itala Souza, Mariana Souza, Glauber Nunes

Universidade Federal Rural do Semi-Árido, Departamento de Ciências Agronômicas e Florestais, Mossoró, RN, Brazil



### ARTICLE INFO

#### Article history:

Received 18 March 2019

Accepted 27 May 2019

Available online 12 June 2019

Associate Editor: Marcel Hermes

#### Keywords:

Biology

Biological control

Morphology

Parasitoid

Tephritidae

### ABSTRACT

The gregarious endoparasitoid *Tetrastichus giffardianus* Silvestri (Hymenoptera: Eulophidae) is a natural enemy of fruit flies. This parasitoid was previously used to successfully control *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Hawaii, USA. Despite its importance in the control of fruit fly pests, little is known about the development or characteristics of its preimaginal stages. The aim of this study was to observe the development and morphologically characterize the immature stages of *Tetrastichus giffardianus*. *Tetrastichus giffardianus* individuals were reared on *C. capitata* larvae/pupae under laboratory conditions at a temperature of  $25 \pm 2^\circ\text{C}$ , relative humidity of  $60 \pm 10\%$ , and 12-h photophase. Third-instar *C. capitata* larvae were exposed to parasitism for 24 h. After parasitism, the pupae were dissected every 24 h to evaluate the stage of development attained by *T. giffardianus*, and to record their morphological characteristics. A stereomicroscope was used to observe all the immature stages of *T. giffardianus*. The complete development of *T. giffardianus* under these conditions was completed within 14 days as follows: egg (duration  $\cong 1$  day); first ( $\cong 1$  day), second ( $\cong 1$  day), and third ( $\cong 2$  days) larval instars; pre-pupa ( $\cong 2$  days); and pupa ( $\cong 7$  days). The immature stages of *T. giffardianus* differed sufficiently in their shape, color, and size to allow morphological characterization.

© 2019 Sociedade Brasileira de Entomologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### Introduction

Biological control of fruit flies (Diptera: Tephritidae) with parasitoids (Hymenoptera) has been practiced in many regions of the world (Aluja et al., 2014; Ovruski et al., 2000). In the Neotropics, the parasitoids most commonly used against these flies belong to the families Braconidae, Chalcididae, Diapriidae, Eulophidae, Figitidae, and Pteromalidae (Aluja et al., 2009; Araujo et al., 2015; Núñez-Campero et al., 2014; Wharton and Yoder, 2019).

Eulophidae is a large family of hymenopteran insects, with over 4500 described species in some 300 genera (Noyes, 2003). Tetrastichinae is the largest subfamily of Eulophidae, which include parasitoids of more than 100 families of insects of different orders (LaSalle, 1994). *Tetrastichus* genus is the third largest in the subfamily Tetrastichinae, with cosmopolitan distribution, but the species (only three – *Tetrastichus giffardianus* Silvestri, *Tetrastichus giffardii* Silvestri and *Tetrastichus oxyurus* Silvestri) that have been most commonly associated with fruit-infesting Tephritidae are of African origin (LaSalle and Wharton, 2002).

*Tetrastichus giffardianus* is a gregarious fruit fly parasitoid. This species was introduced from West Africa to the Hawaiian Islands in 1914 to control populations of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), and quickly became established there (Purcell et al., 1996). During the 1920s and 1930s, this parasitoid was also introduced to Latin American countries in attempts to control *C. capitata* populations (Ovruski et al., 2000). However, owing to the lack of studies on *T. giffardianus* at the beginning of the last century, practically all of these later attempts to control *C. capitata* with this parasitoid failed.

*Tetrastichus giffardianus* penetrates larval exit holes and other openings in fruits to oviposit in larvae (third-instar) of tephritid fruit flies (Pemberton and Willard, 1918) and develops as a gregarious koinobiont endoparasitoid. The mean development time from egg to adult is 17.4 days at  $26^\circ\text{C}$  (Purcell et al., 1996). Sex ratios are strongly female-biased in populations, and mating occurs between siblings shortly after they emerge from the host (Purcell et al., 1996).

Despite the importance of *T. giffardianus* as a natural enemy of fruit flies, information on its preimaginal development and the morphological characteristics of its immature stages is scarce. However, this information is important because it can be used as the basis for planning its rearing, to determine the ideal age at which to release these parasitoids during biological control

\* Corresponding author.

E-mail: [elton@ufersa.edu.br](mailto:elton@ufersa.edu.br) (E. Araujo).

programs, as well as to allow the immature phases of this species to be recognized and distinguished from those of other parasitoids.

The aim of this study was to describe the development and morphologically characterize the immature stages of *T. giffardianus* reared on *C. capitata* larvae/pupae under laboratory conditions to help future studies on this natural enemy of fruit flies.

## Material and methods

### Specimen sources and rearing procedures

The specimens used in this study were from *C. capitata* and *T. giffardianus* populations reared at the Laboratory of Applied Entomology, Universidade Federal Rural do Semi-Árido, Mossoró, Rio Grande do Norte, Brazil. The insects were reared in an air-conditioned room (temperature  $25 \pm 2^\circ\text{C}$ , relative humidity  $60 \pm 10\%$ , and 12-h photophase). The initial population of *C. capitata* was obtained from Biofábrica Moscamed Brasil, Juazeiro, Bahia. The specimens for the initial population of *T. giffardianus* were collected in chestnut fruits (*Terminalia catappa* L. – Combretaceae) infested with *C. capitata*, in the city of Mossoró.

*Ceratitis capitata* were reared in semitransparent plastic cages ( $27.6 \times 33.1 \times 48.7$  cm), with one side covered with voile fabric on which the females could oviposit. Food and water were available inside the cages through a 1:4 mix of brewer's yeast and sugar and two 250-ml plastic water bottles with absorbent tape (Spontex®), respectively. A plastic tray containing water was placed in the outer front part of the cage to collect eggs, just below the voile fabric. Eggs were collected daily and transferred onto an artificial diet to larval development and the subsequent formation of puparia, which were then used to establish new rearing cages. The artificial diet used was described by Albajes and Santiago-Alvarez (1980).

*Tetrastichus giffardianus* were reared following the methodology described by Fernandes (2018). Adult parasitoids were maintained in plastic cages ( $30 \times 30 \times 30$  cm) with a voile fabric opening ( $10 \times 10$  cm) at the top for ventilation and fed with granulated sugar (3 g) provided in a Petri dish (5 cm diameter  $\times$  0.5 cm high) and pure honey (1 ml) spread with brush in a paper filter.

Water was supplied through a 50-ml plastic water bottle with absorbent tape (Spontex®) attached for capillarity. To multiply the parasitoids, chestnut fruits were manually infested daily with laboratory-reared third instar larvae of *C. capitata*. The infested fruits were placed inside the cage and exposed to parasitism for 24 h. Posteriorly, the larvae were transferred into a container with artificial diet (Albajes and Santiago-Alvarez, 1980) until the formation of puparium. The pupae were then transferred into Petri dishes ( $8.5 \times 8.5 \times 2.5$  cm) containing moist vermiculite, where they were kept until the adult parasitoids emerged.

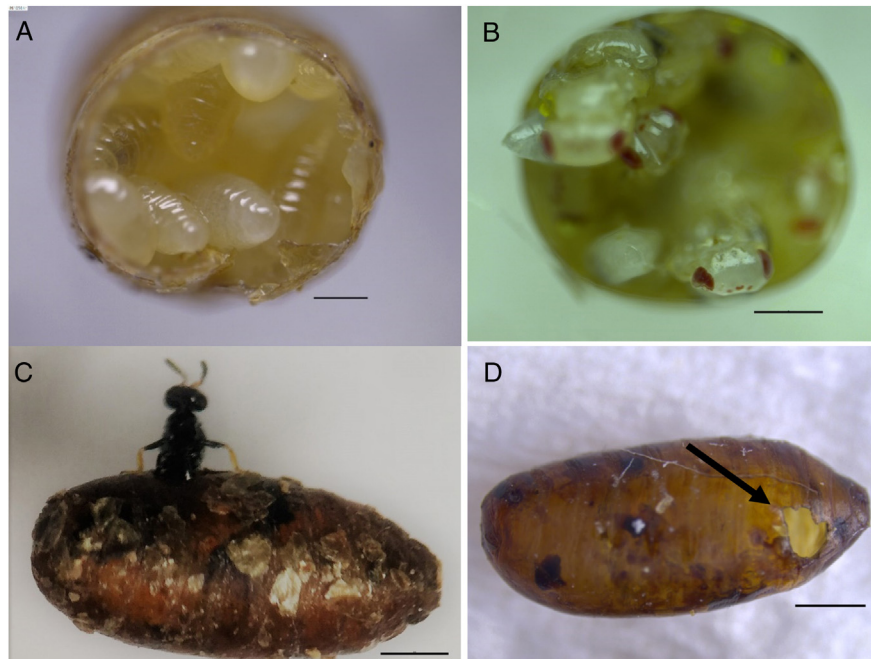
### Development and characterization of *Tetrastichus giffardianus*

Third-instar *C. capitata* larvae were exposed to *T. giffardianus* adults in rearing cages for a period of 24 h for parasitism as described above. The fly larvae were then transferred to Petri dishes ( $9.0 \times 1.5$  cm) containing artificial diet and covered with plastic film until they pupated. Then the pupae were transferred to Petri dishes containing moist vermiculite that were closed with plastic film and kept in an air-conditioned room (temperature  $25 \pm 2^\circ\text{C}$ , relative humidity  $60 \pm 10\%$ , and 12-h photophase).

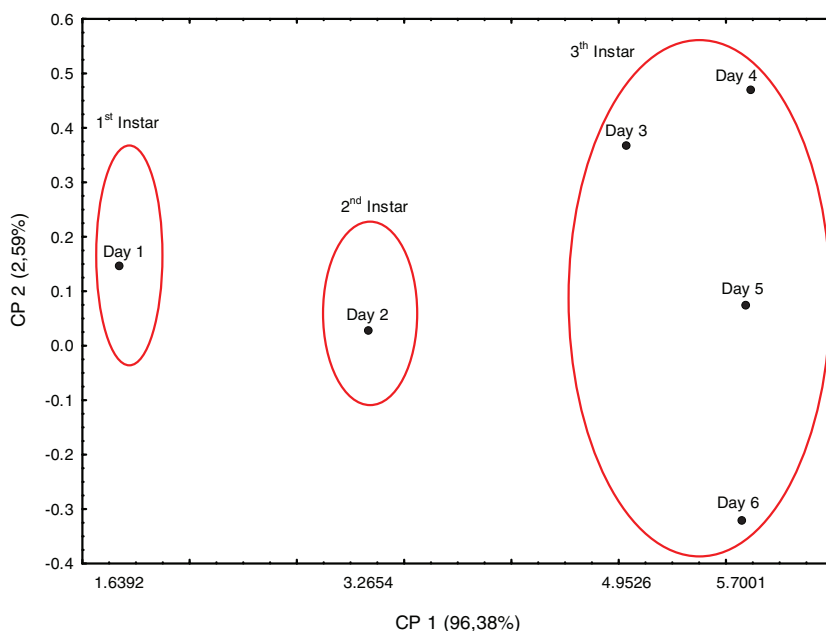
Six hours after the end of the parasitism period, ten fly pupae were removed every 24 h from the Petri dishes and dissected under a stereomicroscope using tweezers and probes to observe the time duration of each developmental stage of the parasitoid (egg, larval instars, pre-pupa and pupa). A total of 140 *C. capitata* pupae were dissected and the immature parasitoids were counted and analyzed. From each dissected puparium, ten immature parasitoids were analyzed, totaling 1400 immature specimens, which were then measured and used for the morphological characterization of each developmental stage, including shape, color, and size of individuals sampled at each of the distinct phases.

To determine the number of larval instars of *T. giffardianus*, a multivariate analysis was carried out using the K-Means method, based on the width of the cephalic capsule, length and width of the larva body, using the GENES software package (Cruz, 2013).

Measurements and photographs of the immature stages were obtained with a stereomicroscope (Leica S8 APO, Singapore, Repub-



**Fig. 1.** *Tetrastichus giffardianus* larvae (A) and pupae (B) inside a *Ceratitis capitata* puparium; (C) emergence of adults; (D) *C. capitata* puparium with *T. giffardianus* emergence hole.



**Fig. 2.** Number of larval instars of *Tetrastichus giffardianus* determined by multivariate analysis based of the cephalic capsule width, length and width of the larval body, using the K-Means method.

lic of Singapore) equipped with a DMC 2900 digital camera using LAS version 4.6 (Leica Microsystems Imaging Solutions) software at 50× magnification.

## Results

### Development of immature stages

The development of *T. giffardianus* began with the oviposition of a group of eggs (average of 13.7) inside a third-instar *C. capitata* larva. After embryonic development, one *T. giffardianus* larva hatched from each viable egg and developed gregariously with other larvae (Fig. 1A) inside the host. Three instars were found to occur, which were characterized based on width of the cephalic capsule, length and width of the larval body (Fig. 2). After larval development, the pre-pupal and later the pupal stages were observed (Fig. 1B). The adult parasitoids emerged using their mandibles to make a single circular hole in the puparium wall (Fig. 1C), through which the parasitoids passed (Fig. 1D). Males and females emerged within a short time interval of each other.

The complete preimaginal developmental period (egg to adult) was 14 days long under the conditions used in this study (temperature  $25 \pm 2^\circ\text{C}$ , relative humidity  $60 \pm 10\%$ , and 12-h photophase). The duration of each developmental stage was as follows: egg  $\cong 1$  day, first larval instar  $\cong 1$  day, second larval instar  $\cong 1$  day, third larval instar  $\cong 2$  days, pre-pupa  $\cong 2$  days, and pupa  $\cong 7$  days.

### Morphological characterization of immature stages

The eggs are elongated, slightly cylindrical in shape with a thin, white chorion. A central opaque area is visible, showing the growing larva inside the egg (Fig. 3A). This species is gregarious, and an average of 13.7 eggs per host pupa was observed. The mean egg length was 0.20 mm (Table 1).

The first larval instar is hymenopteriform and has 13 segments plus a cephalic capsule without apparent mandibles. The body is translucent, and a digestive canal gradually becomes filled with fat globules from feeding on the host's body (Fig. 3B, C). This larval instar has little mobility, and the only visible movement is

the contraction and expansion of the body. Mean larval length is 0.27 mm, mean width is 0.15 and mean cephalic capsule width is 0.04 (Table 1).

The second larval instar has a glabrous and elongated body, and the segments are more distinct than in the previous instar (Fig. 3D). The cephalic capsule little differentiated from the other segments of the body. Mean larval length is 0.59 mm, mean width is 0.26 and mean head capsule width is 0.08.

The third and last larval instar is white/yellowish in color. Mature (third-instar) larvae increase in size and have an elongated shape (Fig. 3E), but they are not very different from previous instars. In this instar, larvae occupy the entire puparium, with none of the host's body tissues remaining. A dark meconium can be seen through the cuticle at the caudal end. Mean larval length is 1.19 mm, mean width is 0.50 and mean head capsule width is 0.12.

The pre-pupa is still white/yellowish in color; during this stage, the insect stops growing and begins the definition of the adult body shape (Fig. 3F). The first part of the body to take shape is the metasoma. A dark oval pile of meconium can be seen through the cuticle at the caudal end. Mean pre-pupal length is 1.36 mm.

The pupae are of the exarate type, shaped like adults, do not move when touched and present reddish-brown eyes. The pupae are of at the beginning of the pupal stage, the immature body is white/yellowish in color, but over time the pigmentation becomes darker until the standard adult color is attained. Pupae have a well-defined head, mesosoma, and metasoma (Fig. 3G, H, I). The antennae, ocelli, and mouthparts are fully visible. Pupae are formed with the head facing the cephalic end of the host puparium. Sexual dimorphism is not very apparent, although females do have a much larger metasoma than the males. Mean pupal length is 1.33 mm.

## Discussion

### Development of the immature stages

Owing to the low number of known gregarious species of fruit fly parasitoids and the general lack of information on the immature stages of these natural enemies (Ovruski et al., 2006; Purcell et al., 1996; van Achterberg et al., 2012; Viggiani et al., 2007), the imma-



**Fig. 3.** Immature stages of *Tetrastichus giffardianus*. Egg (A); first-instar larva (B, C); second-instar larva (D); third-instar larva (E); pre-pupa (F); pupa at three different stages of melanization (G, H, I). Scale bar = 0.1 mm.

**Table 1**  
Measurements (mm) of the immature stages of *Tetrastichus giffardianus* in larvae/pupae of *Ceratitidis capitata*. Temperature  $25 \pm 2$  °C, relative humidity  $60 \pm 10\%$  and photophase of 12 h. Sample number ( $n = 100$ ).

Stages	Body length		Body width		Cephalic capsule width	
	Mean	Range	Mean	Range	Mean	Range
Egg	0.20	0.17–0.21	–	–	–	–
L1	0.27	0.19–0.36	0.15	0.09–0.19	0.04	0.03–0.05
L2	0.59	0.40–0.78	0.26	0.19–0.38	0.08	0.06–0.12
L3	1.19	0.82–1.60	0.50	0.34–0.82	0.12	0.09–0.15
Pre-pupa	1.36	0.96–1.89	–	–	–	–
Pupa	1.33	0.81–1.80	–	–	–	–

L1, 1st larval instar; L2, 2nd larval instar; and L3, 3rd larval instar.

ture development of *T. giffardianus* (a gregarious endoparasitoid) has been mainly compared with that of solitary endoparasitoid species in the discussion below.

The development of *T. giffardianus* is similar to that of the solitary endoparasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), meaning that it begins with the egg phase, goes through three similar larval instars, a pre-pupal and a pupal stage after which it reaches the adult stage. However, we observed that in *T. giffardianus* the emergence of males and females occurs almost simultaneously, whereas in *D. longicaudata* males and females emerge on separate days (Paladino et al., 2010).

The duration of the preimaginal period of *T. giffardianus* observed in this study was shorter than the duration reported by Pemberton and Willard (1918). These authors reported that the length of the period from the egg to adult phase in *T. giffardianus* was 24–31 days in cold months, and about 18 days in warmer months. As Pemberton and Willard (1918) did not report details about the parasitoids and the climatic conditions in which they were reared, so the shorter developmental period observed in this study was likely due to the differences in the parasitoid lineage or climatic conditions (temperature, relative humidity and/or photoperiod) used in comparison with their study. Additionally, the development of parasitoids may be influenced by other factors, such as size, age, and host quality (Lawrence, 1990).

The duration of each immature stage of *T. giffardianus* (egg, larvae, pre-pupa, and pupa) was shorter than the duration reported for the equivalent immature stages of *Aganaspis daci* (Weld) (Hymenoptera: Figitidae) (Tormos et al., 2013), and *D. longicaudata* (Paladino et al., 2010), both of which were reared on *C. capitata* hosts at a constant temperature of 25 °C.

#### Morphological characterization

Despite the lack of information on the characteristics of the preimaginal stages of gregarious fruit fly parasitoids, we were able to verify that the characteristics of newly deposited *T. giffardianus* eggs are different from those of other larval-pupal parasitoids. For example, the eggs of the solitary endoparasitoid *A. daci* are larger than *T. giffardianus*; they measure approximately 0.51 mm in size, and each have a peduncle in the anterior extremity that disappears after 48 h (Tormos et al., 2013), which was not seen in eggs of *T. giffardianus*.

*Tetrastichus giffardianus* larvae presented morphological characteristics that are different from those of the solitary endoparasitoid *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae), in which the larvae are long and cylindrical, with the last abdominal segment in the shape of a tail (Murillo et al., 2016). The presence of a caudal appendix allows solitary endoparasitoid larvae to travel through the host's hemolymph to find and destroy competing parasitoid larvae (Harvey et al., 2013). The absence of a caudal appendix in the larvae of gregarious parasitoids like *T. giffardianus* is an important evolutionary feature, since it allows the larvae to

remain practically immobile during their development, facilitating the gregarious habit of these parasitoids (Pexton and Mayhew, 2004).

The three larval instars of *T. giffardianus* were characterized by increasing sizes and changes in the size of the cephalic capsule. Some studies on parasitoids have reported that the only differences among larval stages are apparent in size increases, mandible changes, and cephalic capsule dimension changes (Parra and Hadad, 1989; Tormos et al., 2013). The presence of developed mandibles in many solitary endoparasitoid larvae is a characteristic that can differentiate them from *T. giffardianus* larvae. In general, solitary endoparasitoids have well-developed mandibles in the first larval instar because they use the mandibles to pierce and kill competing parasitoids inside the same host (Harvey et al., 2013; Wang et al., 2008).

The general characteristics of the pre-pupae of *T. giffardianus* are similar to those of other species of fruit fly endoparasitoids, which are characterized by movement deceleration, evacuation of the host character (meconium), and onset of eye pigmentation (Murillo et al., 2015; Tormos et al., 2009). However, pre-pupae of *T. giffardianus* (1.3 mm) are smaller than those of *D. longicaudata* (2.8 mm) (Paladino et al., 2010), *D. areolatus* (4.3 mm) (Murillo et al., 2015), and *A. daci* (2.1 mm) (Tormos et al., 2013).

The pupal stage of *T. giffardianus* presents many of the characteristics (antennae, coloration, legs, etc.) that will be present in the adults, similar to that in the pupae of *D. longicaudata* (Paladino et al., 2010). Sexual dimorphism is not very visible in the *T. giffardianus* pupal stage, in contrast to the pupal stage of *D. longicaudata*, in which females present a well-developed ovipositor, and males have longer antennae and valves that correspond to the external parts of their reproductive system in the ventral-caudal region (Paladino et al., 2010). In addition, *T. giffardianus* pupae are smaller than those of *D. longicaudata* (Paladino et al., 2010).

#### Conclusion

This study shows for the first time the development pattern and morphological characteristics of the immature stages of *T. giffardianus*. The information obtained can contribute to a better understanding of the biology and morphology of the preimaginal stages of this parasitoid, and may help to improve the rearing of colonies of this species in the laboratory. In addition, the characterization of this species' immature phases may aid future taxonomic studies on the immature stages of parasitoids and research on interspecific competition. However, more detailed studies on *T. giffardianus* should be carried out to evaluate the potential of this parasitoid to be used in the biological control of *C. capitata* in the Brazilian semiarid.

#### Conflicts of interest

The authors declare no conflicts of interest.

## References

- Albajes, R., Santiago-Alvarez, C., 1980. Influencia de la temperatura en el desarrollo de *Ceratitidis capitata* (Wied.). *Anales INIA Ser. Agric.* 13, 183–190.
- Aluja, M., Sivinski, J., Ovruski, S., Guillén, L., López, M., Cancino, J., Torres-Anaya, A., Gallegos-Chan, G., Ruíz, L., 2009. Colonization and domestication of seven species of native New World hymenopterous larval-prepupal and pupal fruit fly (Diptera: Tephritidae) parasitoids. *Biocontrol Sci. Technol.* 19, 49–79.
- Aluja, M., Sivinski, J., Van Driesche, R., Anzures-Dadda, A., Guillén, L., 2014. Pest management through tropical tree conservation. *Biodivers. Conserv.* 23, 831–853.
- Araujo, E.L., Fernandes, E.C., Silva, R.L.R., Ferreira, A.D.C.d.L., da Costa, V.A., 2015. Parasitoides (Hymenoptera) de moscas-das-frutas (Diptera:Tephritidae) no Semiárido do Estado do Ceará, Brasil. *Rev. Bras. Frutic.*, <http://dx.doi.org/10.1590/0100-2945-188/14>.
- Cruz, C.D., 2013. GENES – a software package for analysis in experimental statistics and quantitative genetics. *Acta Sci. Agron.* 35, 271–276.
- Fernandes, E.C., (Doctoral thesis) 2018. Aspectos Bioecológicos de Parasitoides (Hymenoptera) da mosca-do-mediterrâneo (Diptera: Tephritidae). Universidade Federal Rural do Semi-Árido, pp. 92.
- Harvey, J.A., Poelman, E.H., Tanaka, T., 2013. Intrinsic inter- and intraspecific competition in parasitoid wasps. *Annu. Rev. Entomol.* 58, 333–351.
- LaSalle, J., 1994. North american genera of tetrastichinae (Hymenoptera: Eulophidae). *J. Nat. Hist.* 28, 109–236.
- LaSalle, J., Wharton, R.A., 2002. The identity and recognition of African *Tetrastichus* species (Hymenoptera: Eulophidae) associated with fruit flies (Diptera: Tephritidae). *J. Nat. Hist.* 10, 297–304.
- Lawrence, P.O., 1990. The biochemical and physiological effects of insect hosts on the development and ecology of their insect parasites: an overview. *Arch. Insect Biochem. Physiol.* 13, 217–228.
- Murillo, F.D., Cabrera-Mireles, H., Barrera, J.F., Liedo, P., Montoya, P., 2015. *Doryctobracon areolatus* (Hymenoptera, Braconidae) a parasitoid of early developmental stages of *Anastrepha obliqua* (Diptera, Tephritidae). *J. Hymenopt. Res.* 46, 91–105.
- Murillo, F.D., Liedo, P., Nieto-López, M.G., Cabrera-Mireles, H., Barrera, J.F., Montoya, P., 2016. First instar larvae morphology of Opiinae (Hymenoptera: Braconidae) parasitoids of *Anastrepha* (Diptera: Tephritidae) fruit flies. Implications for interspecific competition. *Arthropod. Struct. Dev.* 45, 294–300.
- Noyes, J.S., 2003. Universal Chalcidoidea Database [WWW Document]. <https://www.nhm.ac.uk/our-science/data/chalcidooids/database/> (accessed 5.22.19).
- Núñez-Campero, S.R., Aluja, M., Rull, J., Ovruski, S.M., 2014. Comparative demography of three neotropical larval-prepupal parasitoid species associated with *Anastrepha fraterculus* (Diptera: Tephritidae). *Biol. Control* 69, 8–17.
- Ovruski, S., Aluja, M., Sivinski, J., Wharton, R., 2000. Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the Southern United States: diversity, distribution. Taxonomic status and their use in fruit fly biological control. *Integr. Pest Manag. Rev.* 5, 81–107.
- Ovruski, S.M., Schliserman, P., De Coll, O.R., Peñaloza, C., Oroño, L.E., Colin, C., 2006. The establishment of *Aceratoneuromyia Indica* (Hymenoptera: Eulophidae) in three biogeographical Regions of Argentina. *Florida Entomol.* 89, 270–273.
- Paladino, L.Z.C., Papeschi, A.G., Cladera, J.L., 2010. Immature stages of development in the parasitoid wasp, *Diachasmimorpha longicaudata*. *J. Insect Sci.* 10, 56.
- Parra, J.R.P., Hadad, M.L., 1989. Determinação do número de instares de insetos.
- Pemberton, C.E., Willard, H.F., 1918. A contribution to the biology of fruit-fly parasites in Hawaii. *J. Agric. Res.* 15, 419–465.
- Pexton, J.J., Mayhew, P.J., 2004. Competitive interactions between parasitoid larvae and the evolution of gregarious development. *Oecologia* 141, 179–190.
- Purcell, M.F., Van Nieuwenhoven, A., Batchelor, M.A., 1996. Bionomics of *Tetrastichus giffardianus* (Hymenoptera: Eulophidae): an endoparasitoid of tephritid fruit flies. *Environ. Entomol.* 25, 198–206.
- Tormos, J., Beitia, F., Böckmann, E.A., Asís, J.D., Fernández, S., 2009. The preimaginal phases and development of *Pachycrepoideus vindemmiae* (Hymenoptera, Pteromalidae) on Mediterranean fruit fly, *Ceratitidis capitata* (Diptera, Tephritidae). *Microsc. Microanal.* 15, 422–434.
- Tormos, J., Pedro, L., De Beitia, F., Sabater, B., Asís, J.D., Polidori, C., 2013. Development preimaginal phases and adult sensillar equipment in aganaspis parasitoids (Hymenoptera: Figitidae) of fruit flies. *Microsc. Microanal.* 19, 1475–1489.
- van Achterberg, K., Teixeira, T., Oliveira, L., 2012. *Aphaereta ceratitivora* sp. n. (Hymenoptera, Braconidae), a new parasitoid of *Ceratitidis capitata* (Wiedemann) (Diptera, Tephritidae) from the Azores. *Zookeys* 222, 1–9.
- Viggiani, G., Bernardo, U., Sasso, R., 2007. Description of *Baryscapus silvestrii*, n. sp. (Hymenoptera: Eulophidae), a new gregarious parasitoid of the olive fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) in southern Italy. *Boll. Lab. Ent. Agr. Filippo Silvestri* 61, 63–70.
- Wang, X.G., Bokonon-Ganta, A.H., Messing, R.H., 2008. Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: effect of co-evolutionary history on competitive superiority. *Biol. Control* 44, 312–320.
- Wharton, R., Yoder, M., 2019. Parasitoids of Fruit-Infesting Tephritidae [WWW Document]. <http://paroffit.org/public/site/paroffit/home> (accessed 5.9.19).