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Trechnites flavipes Mercet (Hymenoptera: Encyrtidae): a parasitoid on *Pauropsylla buxtoni* comb. nov. (Hemiptera: Psylloidea) on *Ficus carica* L. (Moraceae)

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Abstract *Pauropsylla buxtoni* comb. nov. is a psyllid insect that infests fig trees (*Ficus carica* L.) in the Palestinian Territories. It sucks the plant sap and induces leaf galls in which it develops. In the present research, we identified an encyrtid parasitoid (*Trechnites flavipes* Mercet) on the immature stages of *P. buxtoni*. Morphological redescriptions of female and male adult of *T. flavipes* together with color illustrations are provided in this research. This is important for recognizable species, which might be used as a biocontrol agent of psyllid pests. Also, the mode of parasitism by this parasitoid is studied where host-parasitoid (*P. buxtoni*-*T. flavipes*) relationship is described. We observed that the female of *T. flavipes* parasitized the fourth or early fifth instars of *P. buxtoni* immatures with a mean percentage of natural parasitism ranging from 7.8 to 18.3% on the various fig cultivars grown in the Palestinian Territories. Significant differences in the means of percentage of parasitism on five cultivars were obtained. These differences may be related to the differences in susceptibility of these cultivars to *P. buxtoni* infestation. Also, the characteristics of these cultivars play an important role in this susceptibility. In conclusion, the results of this research may be exploited in the practical management of *P. buxtoni* on fig trees. The potential of *T. flavipes* as a biocontrol agent of *P. buxtoni* should be studied in depth in order to achieve an effective control of this insect pest.

Keywords Endoparasitoid · Host-parasitoid relationship · Encyrtidae · Chalcidoidea · *Ficus carica* · Parasitism · Triozidae · East Mediterranian

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Introduction

The common fig, *Ficus carica* L., is an important fruit tree in the Palestinian Territories where many local cultivars of this crop are grown. These cultivars are characterized by having large fruits with sweet taste, in addition to their adaptation to Mediterranean climate (Shtayeh et al. 1991). The national production of fig fruits in the Palestinian Territories is estimated at 700 tons per year (PMA 2017) where the fruits produced are usually consumed either freshly or in a dried form or being used for jam production. Fruits in a dried form may be exported or consumed locally (Shtayeh et al. 1991).

Pauropsylla buxtoni comb. nov. (combination nova) is a new combination proposed by Batta and Burckhardt (2018) who conducted a detailed study on the taxonomy of *Trioza buxtoni* (psyllid species described by Laing (1924) on fig trees). Authors of this combination suggested that the species of *Trioza* called *T. buxtoni* is congeneric with *Pauropsylla udei*, the type species of *Pauropsylla*.

Therefore, the above authors combined the genus *Pauropsylla* with the species *buxtoni* in one name referred to as "*Pauropsylla buxtoni* comb. nov." and used it in their study. However, *P. buxtoni* differs from other *Pauropsylla* species in the presence of short, though distinct genal processes (Batta and Burckhardt 2018).

Pauropsylla buxtoni is a specific psyllid that infests the cultivated fig in the Palestinian Territories. Infestation of fig trees with *P. buxtoni* causes large nipple-shaped galls that appear in large clusters on the upper leaf surface of infested trees (Fig. 1). The immatures of *P. buxtoni* develop inside the galls passing in five nymphal instars before the adults emerge (Fig. 2) (Batta and Burckhardt 2018). Heavy infestations of fig trees by the insect were reported in the northern part of the Palestinian Territories, causing yield reduction and leaf deformation due to presence of large clusters of galls on the upper surface of infested leaves (Batta and Burckhardt 2018). Almost similar type of infestations have been reported from Israel (Rivnay 1962; Avidov and Harpaz 1969) and Saudi Arabia (Burckhardt 1986; Burckhardt and Mifsud 1998). However, there are no available reports on the parasitoids of *P. buxtoni* on fig trees neither in the Palestinian Territories nor elsewhere. Our field observations carried out during the last two years in the fig orchards of the Palestinian Territories revealed the presence of a parasitoid on *P. buxtoni* immatures: that is a species of the genus *Trechnites*. Diagnosis for the genus *Trechnites* and key for the female was done for 5 species by Guerrieri and Noyes (2009). *Tricnites flavipes* was one of these species but its diagnosis by Guerrieri and Noyes (2009) did not contain neither morphological details nor colored illustrations. Therefore, one of the objectives of the present study is to identify then redescribe the species of this genus of parasitoid (*T. flavipes*) by giving full morphological descriptions with color illustrations and measurments. However, because there are no reports available on parasitoid species of *P. buxtoni* in the Palestinian Territories, the specific objectives of the present research are the following: i) to identify then redescribe the species of *Trechnites* parasitoid on *P. buxtoni* immatures by describing the morphological characteristics of male and female adults collected from fig orchards in the Palestinian Territories and providing color illustrations; ii) to describe the parasitic relationship between the parasitoid and its host (*P. buxtoni*); and iii) to determine the percentage of natural parasitism that may be caused by the parasitoid on *P. buxtoni* immatures on different local cultivars of fig orchards in the Palestinian Territories.

Materials and methods

Location of the study

Samples of infested leaves with galls of *P. buxtoni* were collected from different localities in different fig orchards in the Tulkarm district (32°31′25"N; 35°02′ 11.11″E) during October to January 2016–2017. These orchards are located in the Palestinian Territories and distributed in a large area situated at 320 m above sea level and 15 km distant from the eastern coast of the Mediterranean Sea.

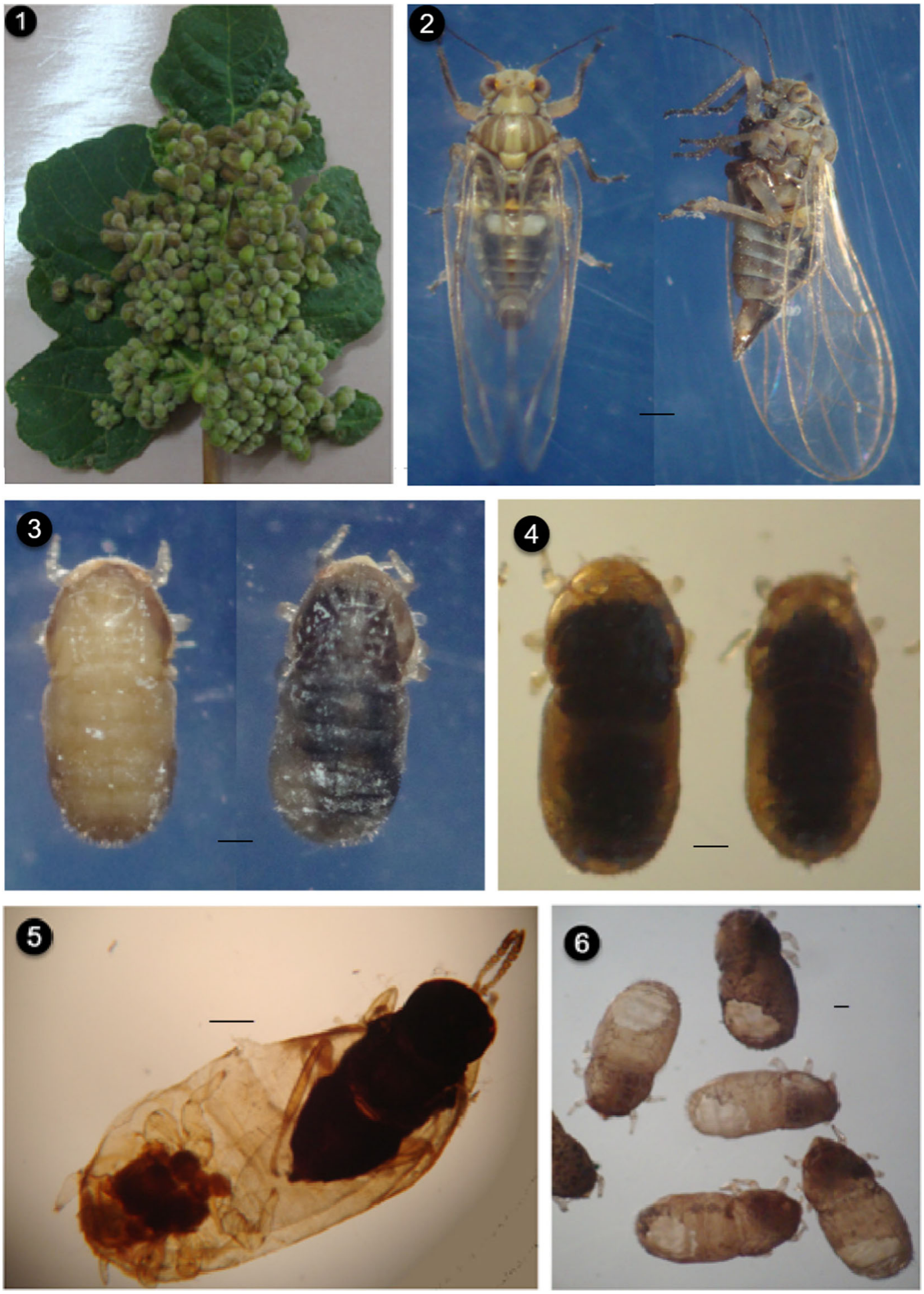
Identification of psyllid species

Psyllid species was identified as *Pauropsylla buxtoni*. This identification was carried out in a previous study (Batta and Burckhardt 2018). Voucher specimens of *P. buxtoni* are deposited in the collection of An-Najah National University, Palestinian Territories; and in Naturhistorisches Museum Basel (NMB), Switzerland.

Rearing psyllid parasitoid and its identification

For the purpose of identifying the parasitoid and photographing it, some of the collected leaves of fig trees with galls of *P. buxtoni* were incubated in closed transparent plastic bags at 25 °C under the fluorescent light for 96 h to promote emergence of adult parasitoids. Emerged parasitoid was identified as *T. flavipes*. This identification was based on morphological

Figs. 1–6 Parasitism of *Trechnites flavipes* on *Pauropsylla buxtoni*, **1.** Nipple-shaped galls of *P. buxtoni* on *Ficus carica* leaf, dorsal view (normal size), **2.** Adults of *P. buxtoni* (male and female adults; dorsal and lateral views) emerging from *P. buxtoni* galls, **3.** Parasitized immatures (fourth instar, right picture) of *P. buxtoni* with dark-brown or black color vesus unparasitized immatures (fourth instar, left picture) with yellowish color, **4.** *P. buxtoni* immatures (fourth instar) with a developing parasitoid inside (black appearance and could be seen through the integument of the parasitized host), **5.** *P. buxtoni* immature (fourth instar) with a parasitoid emerging from the interior part, ventral view, **6.** Exuviae, empty skins of *P. buxtoni* immatures that were parasitized by *T. flavipes*, with exit holes for the parasitoid at end of abdomen, dorsal view. **Scale bars = 100** μm (in the above photographs)



characteristics of female and male adult of the parasitoid and was confirmed by Dr. John Noyes (the Natural History Museum, London, UK). Voucher specimens of *T. flavipes* are deposited in An-Najah National

University, Palestinian Territories; Steinhardt Museum of Natural History, School of Zoology, Tel Aviv University, Israel; and in the Natural History Museum, London, UK.

Extraction and examination of *P. buxtoni* immatures

The collected samples of infested fig leaves showing galls of *P. buxtoni* were dissected under the microscope to extract the immatures of *P. buxtoni* then, the extracted immatures were preserved in 70% ethanol for being classified into parasitized or unparasitized immatures according to their color (dark-brown or black if parasitized or yellowish if unparasitized as in Figs. 3 and 4).

Description of the adult parasitoid wasp

Morphological characteristics of female and male adult of the parasitoid (*Trechnites flavipes*) were described. The characteristics of antennae (especially the clava), wings, mesoscutum, scutellum, female ovipositor and male genitalia (aedeagus) were fully described and photographed. Comments relating to the differences and similarities of male and female adult of the parasitoid in relation to the other species of *Trechnites* were included in the description using the key of European species of *Trechnites* (Guerrieri and Noyes 2009), and the description of female adult of *T. brevivalvus* by Zu and Li (2016).

Determination of percentage of parasitism on *P. buxtoni* immatures

After the extraction of *P. buxtoni* immatures as indicated before, the immatures were examined in order to determine if they are parasitized or not. The criteria used for this determination is the color of the examined immatures: if dark-brown or black, they are parasitized as in Figs. 3, 4 and 5, but if yellowish, they are unparasitized as in Fig. 3. Moreover, when the adult parasitoid emerges from a parasitized immature, it leaves behind an empty skin with an exit hole called the exuvia (Fig. 6). The presence of these exuviae is another indication that the immature is parasitized. The percentage of parasitism by *T. flavipes* was thus determined by counting the number of parasitized and unparasitized immatures of *P. buxtoni*. Five local fig cultivars were used for determining the percentage of parasitism. For each cultivar, one hundred randomly chosen leaves were used for calculating the percentage of parasitism. Ten galls were randomly chosen per leaf for extracting and checking parasitized or unparasitized immatures. Therefore, the total number of galls dissected and checked per cultivar was 1000. The check was replicated 5 times by taking 100 infested leaves from 5 different fig orchards of the same cultivar. Mean percentage of parasitism for each cultivar was calculated and the means obtained were used for comparison of the parasitism on the tested cultivars.

Statistical analysis

Analysis of Variance (ANOVA) was used to test the differences between the values of mean percentage of parasitism on the different fig cultivars and Tukey's HSD test was used for means' separation.

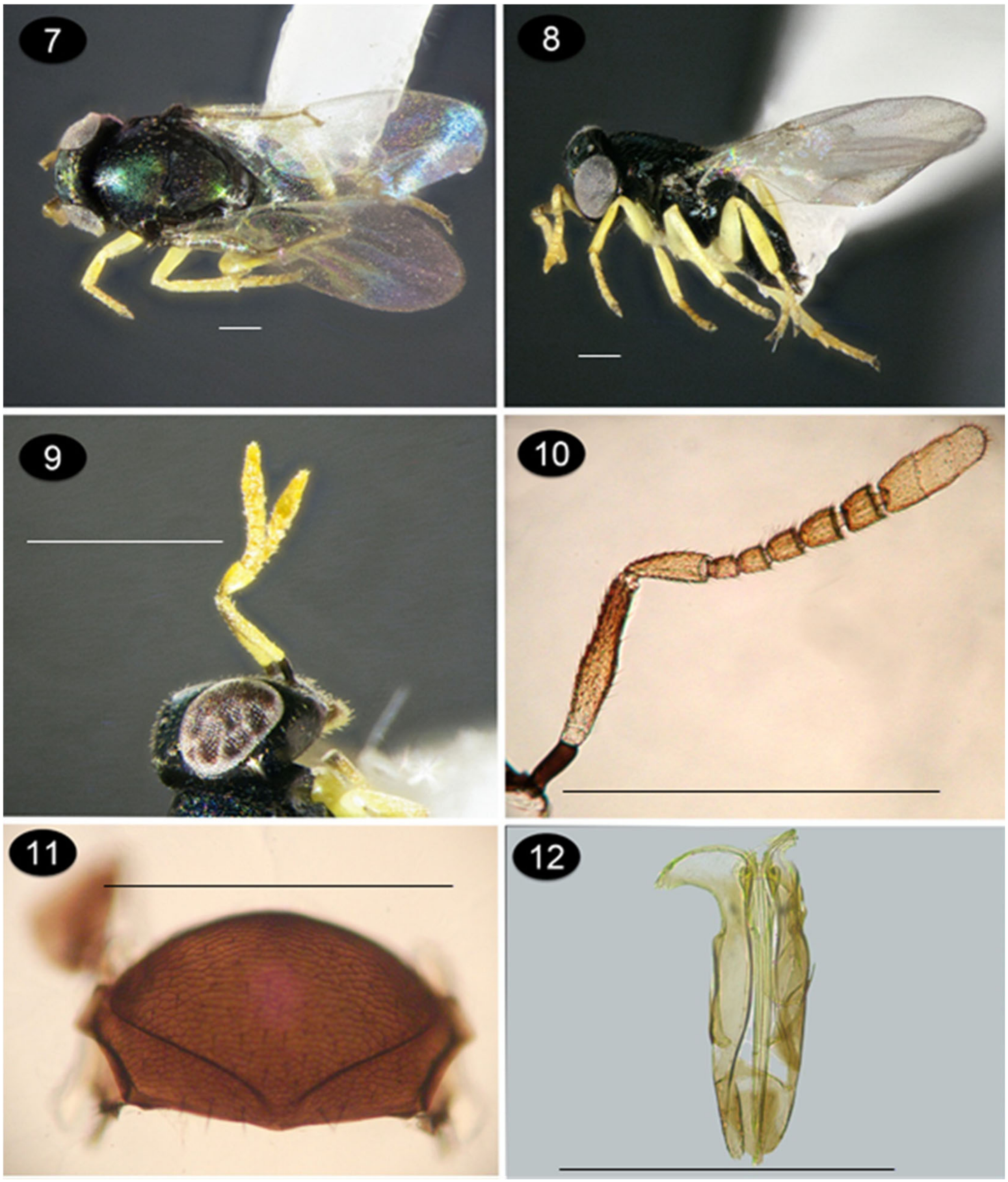
Results

Description of the parasitoid *Trechnites flavipes* (Mercet, 1921)

For identification of the parasitoid and description of its morphological characteristics, 110 adults (50 females and 60 males) of *T. flavipes* were used. The collected adults were originated from fig orchards of the Tulkarm district (32°31′25"N; 35°02′11.11″E) reared *ex Pauropsylla buxtoni,* October to January 2016–2017 (Y. Batta).

Female Length of body 1.07–1.2 mm. Body dark brown, pronotum, mesoscutum and scutellum with green metallic tint and brown gaster (Fig. 7), legs entirely yellow (Fig. 8). Head with purple vertex, frons and scrobes, lower face shining green. Eyes reddish. Antenna with brown radicle (Fig. 9), scape and pedicel brownish, clava yellow or yellow brownish. Antenna (Fig. 10). Scape 6.1–6.3× as long as broad, pedicel 2.3–2.4× as long as broad, F1 1.0× as long as broad, F2 1.08× as long as broad, F3 1.1× as long as broad, F4 1.0× as long as broad, F5 1.0× as broad as long, clava 2.6× as long as broad. Clava 3.6× as long as F5. Mesoscutum finely reticulate and 1.8× as broad as long (Fig. 11). Ovipositor with visible gonostyli 4.2× as long as broad (Fig. 12).

Comments Female of *T. flavipes* (our specimens) similar to female of *T. flavipes* in Guerrieri and Noyes (2009)



Figs. 7–12 Female adult of *Trechnites flavipes* (Mercet), **7.** Body, dorsal view, **8.** Body, lateral view, **9.** Head with antennae, lateral view, **10.** Antenna, **11.** Sculpture of mesoscutum, dorsal view, **12.** Ovipositor with visible gonostyli, dorsal view. **Scale bars = 100 µm** (in the above photographs)

which is characterized by having complete notaular lines on mesoscutum, antenna and legs entirely yellow, scape 6.1–6.3× as long as broad (5.3–6.0× in *T. flavipes*), F1 1.0× as long as broad and 0.35× as long as pedicel (0.36× in *T. flavipes*), clava 3.6× as long as F5 (3.4× in *T. flavipes*), right gonostylus (frontal view) of ovipositor 4.2× as long as broad, left gonostylus (lateral view) (Fig. 12) 2.6× as long as broad. On Fig. 4 (p.234) gonostylus (frontal view) in *T. flavipes* (Guerrieri and Noyes 2009) 4.8× as long as broad.

Female of *T. flavipes* (our specimens) similar to *T. brevivalvus* (Zu and Li 2016) characterized by having complete notaular lines on mesoscutum, antenna and legs entirely yellow, scape 6.1–6.3× as long as broad but differs in having clava 2.6× as long as broad (2.38× in *T. brevivalvus*). Gonostylus of ovipositor 2.0× as long as broad in *T. brevivalvus* (not mentioned about position and not figured by Zu and Li (2016) although it is the only distinct character, which differentiates this species from *T. flavipes*. The females of both species can be separated from all other known species of *Trechnites* by having the complete notaular lines, 3-segmented clava and yellow legs.

Male Length of body 0.98–1.1 mm. Color of body similar to female (Fig. 13). Antenna yellow in male (Fig. 14). Scape 5.3–5.4× as long as broad, pedicel 2.3–2.4× as long as broad, F1 1.1× as long as broad, F2 1.08× as long as broad, F3 1.1× as long as broad, F4 1.1×, F5 1.1× as broad as long (transverse), clava 3.3× as long as broad. Clava 2.9× as long as F5. Genitalia with aedeagus 4.6× as long as broad, digiti with 3 spines and 4 on right digitus, parameras not developed (Fig. 15). Scutellum finely reticulate and 1.1× as broad as long (Fig. 16). Venation of fore wing: brown, located at the base of the hyaline fore wing (Fig. 17), approximately 2.3× as long as broad.

In view of the fact that there are small discrepancies between some of the above ratios and those given for *T. flavipes* in the work of Guerrieri and Noyes (2009), we sent specimens of our parasitoid adults to Dr. John Noyes (the Natural History Museum, London, UK) for comparison of our specimens with their specimens of *T. flavipes* (pers. Comm. 26 Febr. 2018). Dr. Noyes wrote us the following: “I am fairly sure that your specimens are *T. flavipes*”. He also added “There are some very small differences in sculpture on the mesonotum and scutellum but I do not think that these are significant. That is not to say someone at a later date will compare DNA sequences from different parts of the species range and find that they are (subtly) different and suggest they are different species, but that (to me) is very subjective and a very different matter. I am happy for you to call them *T. flavipes*”.

Comments Male of *T. flavipes* (our specimens) is similar to the male of *T. flavipes* (Guerrieri and Noyes 2009), characterized by having scape 5.3× as long as broad (5.6× in *T. flavipes*), genitalia: digiti with 3 spines, size of body 0.98–1.1 mm (0.9–1.2 mm in *T. flavipes*). Male of *T. brevivalvus* is unknown according to Zu and Li (2016).

Distribution Armenia, Azerbaijan and Turkey (Japoshvili and Noyes 2005), Czeck Republic, Denmark, France, Hungary, Italy, Kazakhstan, Mongolia, Poland, Russia, Slovakia, Spain (Japoshvili and Noyes 2006), Norway (Hansen and Japoshvili 2013), new record from the Palestinian Territories (present research, 2018).

Hosts Caillardia sp., *Cyanophila* sp. (Psylloidea) and Aphididae (Hemiptera) (Trjapitzin 1989), *Eulecanium franconicum* (Coccidae) (Japoshvili and Noyes 2006). The record from *Eulecanium franconicum*as as a host is doubtful (John Noyes, pers. comm., Dec. 2017), new record *Pauropsylla buxtoni* (Hemiptera: Psylloidea) (present research, 2018).

Description of host-parasitoid (*P. buxtoni* - *T. flavipes*) relationship

During dissection of galls induced by *P. buxtoni* immatures on the leaves of fig cultivars, we observed that some of the fourth and early fifth instars of these immatures were characterized by having partially or entirely dark-brown to black appearance (Figs. 3, 4 and 5). This is attributed to the internal development of the parasitoid into a larva or pupa, compared to the yellowish appearance of the unparasitized immatures (i.e. fourth instar; Fig. 3). This indicates that the adult female *T. flavipes* lays her eggs inside *P. buxtoni* immatures and the hatched larva of the parasitoid develops internally as an endoparasitoid. This parasitoid is thus living and developing inside the host then, emerging as an adult after approximately 28–35 days of egg laying (Batta 2018). We also observed that, in the parasitized immatures, the pupa of the parasitoid lies in an opposite direction in relation to the head of the parasitized immatures (Fig. 5) so that, upon emergence, the adult hatches by rupturing the posterior part of the last abdominal segments of the parasitized immatures leaving behind an empty skin with an exit hole, the exuvia (Fig. 6). It is important to note that in comparison with the body length of *T. flavipes* adults (0.98 to 1.2 mm; $n = 100$ specimens of male and female adults), the susceptible host stage of *P. buxtoni* (fourth or early fifth instar) has a larger body length (1.41 to 1.58 mm; n = 100 specimens). This reflects the characteristics of endoparasitoids that live and develop inside their hosts.

Percentage of parasitism on *P. buxtoni* immatures

The mean percentage of natural parasitism by *T. flavipes* on *P. buxtoni* immatures varied from 7.8 to 18.3%

Figs. 13–17 Male adult of *Trechnites flavipes* (Mercet), **13.** Body, lateral view, **14.** Antenna, **15.** Genitalia, aedeagus and digiti with spines, **16.** Sculpture of scutellum, dorsal view. **17.** Fore wing, base venation. **Scale bars = 100 μm** (in the above photographs)

(Table 1). The highest mean percentage of parasitism was found on Himari fig cultivars (18.3%) and the lowest on Khurtmani fig cultivars (7.8%). The percentages of the other tested cultivars were intermediate between these two extremes. Differences between the means of percentage parasitism on the above-mentioned cultivars were significant (at $p < 0.05$; Table 1), so this may suggest that there is a certain type of susceptibility in parasitism by *T. flavipes* on *P. buxtoni* infesting various fig cultivars. This susceptibility is most probably relating to the intensity of infestation by *P. buxtoni* on the different local cultivars. The characteristics of these cultivars may affect this susceptibility.

Discussion

To date, no record on the distribution of *T. flavipes* has been noted for the Middle East, and this research thus presents the first record of this encyrtid parasitoid in this region. A new record of *T. flavipes* parasitism on *P. buxtoni* immatures is thus reported here for the Palestinian Territories (new host record on Psylloidea: Hemiptera). However, the parasitoid has been reported on different host insects rather than *P. buxtoni* such as *Caillardia* sp., *Cyanophila* sp. (Psylloidea) and Aphididae (Homoptera) by Trjapitzin (1989), *Eulecanium franconicum* (Coccidae) by Japoshvili and Noyes (2006). It has been also distributed in other countries outside the Middle East such as Armenia, Azerbaijan and Turkey by Japoshvili and Noyes (2005), Czeck Republic, Denmark, France, Hungary, Italy, Kazakhstan, Mongolia, Poland, Russia, Slovakia, Spain by Japoshvili and Noyes (2006), Norway by Hansen and Japoshvili (2013).

Table 1 Percentage of parasitism by *Trechnites flavipes* on *Pauropsylla buxtoni* immatures infesting different local fig cultivars in the Palestinian Territories

Mean % parasitism on *P. buxtoni* immatures on different fig cultivars ($n = 100$ leaves per cultivar, ten galls per leaf were inspected and 1000 gall per cultivar checked) [a]

Cultivars	Mean ± SEM [b]	Range (lower and upper limits of parasitism %)
Biadi	10.2 ± 3.1 **a** (Mean = 102/1000)	6–12
Himari	18.3 ± 3.9 **bc** (Mean = 183/1000)	11–35
Khidari	15.4 ± 4.1 **b** (Mean = 154/1000)	8–22
Khurtmani	7.8 ± 2.8 **a** (Mean = 78/1000)	5–18
Mowazi	8.6 ± 2.2 **a** (Mean = 86/1000)	5–10

[a] Gall dissection was performed to check if *P. buxtoni* immatures are parasitized or not; five replicates per cultivar were used (for more details, refer to M& M, page 6, lines 116–123)

[b] Means of % parasitism followed by different letters are significantly different at $p < 0.05$ according to ANOVA and "Tukey's HSD test" for means' separation

The growth and development of *P. buxtoni* immatures and of the galls caused by them on fig trees were studied in detail by Batta and Burckhardt (2018). The authors reported five developmental instars of *P. buxtoni* immatures, in addition to four phases of gall development. They also reported that the immatures of *P. buxtoni* developed inside those galls that were initially induced by the first instar. One of the phases of gall development of *P. buxtoni* is the dehiscence phase, in which a slit-like opening is formed in the lower part of the gall. This opening is usually used for emergence of the fully developed fifth instar of *P. buxtoni* from the gall before molting into the adult stage, it is also used by *T. flavipes* females to enter the gall and lay their eggs on the *P. buxtoni* immatures (fourth or early fifth instars). To the best of our knowledge, no similar reports are available to compare with, although a very few reports are present on parasitism of other species of *Trechnites* on certain psyllid species such as *Trechnites insidiosus* (Crawford) that parasitizes on the immatures of pear psylla (*Cacopsylla pyricola* Förster, Hemiptera: Psyllidae) (McMullen 1966). In the latter case, this small-sized parasitoid lays its eggs in the body of its host (third or fourth instar nymphs) by inserting its short ovipositor into the host, after which the hatched larvae develop internally, transforming the attacked nymphs into mummies from which the adult parasitoids emerge (Unruh et al. 1995; McMullen 1966). A similar mode of parasitism was reported for other chalcidoid endoparasitoids that parasitize on other psyllid species, for examples *Tamarixia radiata* that parasitizes on the Asian citrus psyllid (*Diaphorina citri* Kuwayama) (Kohno et al. 2002). *Tamarixia radiata* can cause a high percentage of parasitism in *D. citri* immatures and hence may play an important role in pest management of this psyllid; *Tamarixia aguacatensis* on avocado psyllid (*Trioza aguacate* Hollis and Martin, Hemiptera: Triozidae) (Yefremova et al. 2014); *Psyllaephagus bliteus* parasitizing on red gum lerp psyllid (*Glycaspis* *brimblecombei* Moore, Hemiptera: Psyllidae) infesting *Eucalyptus camaldulensis* (Berti Filho et al. 2003; Bella and Rapisarda 2013); *Tamarixia schina* on *Calophya schini* (Hemiptera: Calophyidae) infesting *Schinus molle* (Anacardiaceae) and *T. dahlsteni* on *Trioza eugeniae* (Hemiptera: Triozidae) infesting *Syzygium paniculatum* (Myrtaceae) (Zuparko et al. 2011).

Our present research demonstrates that the female of *T. flavipes* lays her eggs inside the host (fourth or early fifth instar), after which the hatched larvae develop internally and transform the hosts into blackish mummies after killing them. Following a short period of pupation in the host, they then emerge as adults leaving behind the empty host skins. Also, we demonstrated that the percentage of parasitism on *P. buxtoni* by *T. flavipes* was significantly different from one local fig cultivar to another. These differences could be attributed to the differences in susceptibility of these cultivars to *P. buxtoni* infestation since in our previous study (Batta and Burckhardt 2018), we have demonstrated that there were significant differences in susceptibility of the same local fig cultivars to *P. buxtoni* infestation. It is important to mention that although the mean percentage of natural parasitism found in the present study was relatively low (from 7.8 to18.3%), *T. flavipes* could play an important role in pest management of *P. buxtoni*. Further research is necessary to determine the exact biocontrol efficiency of the parasitoid on *P. buxtoni* immatures infesting fig trees. This can be achieved by mass rearing of the parasitoid and its subsequent application against the host insect.

In conclusion, the descriptions provided here of *T. flavipes* female and male adult and its mode of parasitism on *P. buxtoni* immatures, together with the determination of percentage of natural parasitism on different fig cultivars, contribute important data for the pest management of this insect. The potential of *T. flavipes* as a biocontrol agent for practical application against *P. buxtoni* should thus be studied in depth in order to achieve an effective management of this insect pest.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interests.

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