Bulletin of Insectology ***72*** *(2): 169-176, 2019*
ISSN 1721-8861 eISSN 2283-0332

New findings on biology and life cycle of *Pauropsylla buxtoni* for developing an integrated control program of the insect on fig trees

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Abstract

Pauropsylla buxtoni (Laing) (Hemiptera Psylloidea Triozidae) is a psyllid species of edible fig trees, it sucks the sap and induces galls on infested fig leaves. The main objectives of this research were: to study the biology of *P. buxtoni* and its life cycle on fig trees in terms of gall induction and initiation, growth and development of galls and nymphs during the infestation especially in the early stages of immatures' development. Results indicate that eggs of *P. buxtoni* are laid on the lower leaf surface. Each egg has a short pedicel inserted into the leaf tissue by the female ovipositor. New hatching nymphs of *P. buxtoni* suck the leaf sap and secrete large amounts of saliva so that they induce galls on the infested leaves in which they enter to develop inside. The *P. buxtoni* overwintering full-grown nymphs exit from the hosting galls in spring and moulting as adult. The insect undergoes one generation per year. A full description of the different life stages of the insect are provided. Illustrations needed for this description are included. These results constitute the base for developing an integrated control program that should be helpful to the fig producers to better manage this pest.

Key words: psyllids, gall development, gall induction, nymphs, life stages, leaf surface, saliva secretion, integrated control.

Introduction

Edible fig trees (*Ficus carica* L., Moraceae) are important fruit trees in the Palestinian Territories where the fig production is estimated at 70 tons annually (PMA, 2017). The major portion of this production is freshly consumed and the rest is consumed either in a dried form or used for jam production (Shtayeh *et al.*, 1991). Different fig cultivars, especially of local type, are grown in these territories, some of them need protection against insects, in general, and particularly from those that suck the sap and transmit diseases to other trees. Psyllids or jumping plant lice are among the sucking insects that heavily attack fig trees in many fig producing countries (Tuncer, 2002; Gençer *et al.*, 2007). Generally, there are two species of psyllid insects that infest fig trees worldwide. The first species is *Homotoma ficus* (L.) which is host-specific species on fig trees, it sucks the leaf sap but it does not induce galls on infested trees. The infested trees with *H. ficus* become weak and produce poor quality figs compared to healthy trees (Hollis and Broomfield, 1989; Tuncer, 2002; Gençer *et al.*, 2007). The second species is *Pauropsylla buxtoni* (Laing) that sucks the leaf sap and induces small, scattered galls on the infested leaves (Batta and Burckhardt, 2018). This species was described as *Trioza buxtoni* by Laing (1924) and then reported by Buxton (1924) as an insect pest on fig trees in the historical Palestine. This species induces large nipple-shaped galls aggregated in clusters on the infested leaves of fig trees, it causes heavy damage to infested trees in the Palestinian Territories (Batta and Burckhardt, 2018).

Batta and Burckhardt (2018) reported information on the taxonomy and biology of *P. buxtoni* such as description of the egg, nymph instars and adult, development of galls, duration of life cycle and number of generation per year, susceptibility of local fig cultivars to psyllid infestation, in addition to the phylogenetic relationships of *P. buxtoni*. However, no information was provided in the above-mentioned publication or elsewhere on certain biological aspects of *P. buxtoni* such as induction and initiation of galls by the first nymph instars of *P. buxtoni*, causes of gall development and enlargement, development of the nymphs inside the gall and their numbers, description of life cycle of *P. buxtoni* nymphs, description of eggs laid including the shape, place of laying, hatching process etc. Therefore, the main objective of the present research was to study the above-mentioned biological aspects that were not studied before or that are lacking enough information to explain them. Briefly, the main biological aspects that should be studied in the present research are the following: i) induction and initiation of *P. buxtoni* galls on fig leaves, ii) growth and development of galls and nymphs of *P. buxtoni* on the infested fig leaves, and iii) description of *P. buxtoni* life stages especially the new hatched nymphs.

Materials and methods

Collection of the insect and localities of the study

Samples of fig leaves infested with galls of *P. buxtoni* were collected in fig orchards in the Palestinian Territories. These localities are situated within the geographical area with the following coordinates: 32°31'25"N 35°02'11.11"E. The mean annual temperature in the sampled areas was 17.6 °C (5-38 °C) and the average annual relative humidity was 60.8% (45-98%) (PMA, 2017). Different local fig cultivars such as Khurtmani, Himari, Sewadi were included in this collection to represent the Palestinian population of the insect. The collected galls were used either for studying the develop-

ment of galls and nymphs or for extracting the different nymph instars living inside the galls. The collected immature stages were preserved in 70% ethanol and used for describing and photographing of these stages.

Induction and initiation of the insect galls

Collected samples of infested fig leaves with *P. buxtoni* were used for studying the induction and initiation of galls by nymphs using the following method: i) samples of young leaves were collected in early spring 2018 from fig trees that were infested with the insect in the previous growing season. This is because *P. buxtoni* has one generation per year and the infestation with the insect starts in early spring. ii) The collected leaves were inspected for the presence of eggs using a dissecting microscope, iii) if the eggs were present on the sampled leaves, they should be described and photographed then followed-up for the subsequent stages, iv) the subsequent collection of samples aimed at studying how the nymphs induces and initiates the gall. This was done by inspecting the collected leaves for the presence of the early instar nymphs with a dissecting microscope, v) describing and photographing the series of events that occur from egg hatching until the gall formation. Following-up of these events was done by successive collections of samples of infested leaves and inspecting them as mentioned above.

Development of galls and nymphs

Collected samples of infested fig leaves with *P. buxtoni* were also used for studying the growth and development of galls and nymphs during the infestation period (spring 2018) by the following method: i) after the early formation of the gall, the development of nymphs (from first to fifth) was followed-up under a dissecting microscope; ii) the development of the nymphs inside the gall was described and photographed along with the development and enlargement of the hosting galls. This was done by dissecting the galls to measure the length of each instar nymph and the corresponding length of the hosting galls. Mean and range of each instar length and its corresponding hosting galls were calculated. In short, a detailed study was conducted including the description and photographing of eggs and all nymphs (from first to fifth instars) during the development and swelling of hosting galls until reaching the maturity. The adults (males and females) that emerged from galls were also described and photographed.

Statistical analysis

The standard error of the mean (SE) was calculated and added to the mean length of *P. buxtoni* eggs, nymphs and adults. Similar calculations were carried out for the mean length of galls induced by *P. buxtoni*.

Results

Induction and initiation of the insect galls

During the inspection of eggs on young leaves, it was observed that the females of *P. buxtoni* laid their eggs on the lower leaf surface in a scattered form (figure 1). The eggs are very small (mean length: 0.16 ± 0.02 mm; range: 0.14-0.21 mm, n = 100). They are yellowish to whitish in colour, nearly oval in shape with long apical process and short pedicel at the bottom that could be inserted into the leaf tissues by the ovipositor (figures 2-3). After egg hatching, it was observed that the first instar nymphs of *P. buxtoni* crawls around on the lower leaf surface for 1 to 2 days then settles in a feeding site on the leaf and begins to suck the leaf sap (figure 4). The nymphs were observed under the dissecting microscope, feeding and injecting saliva on detached leaves recently infested (this behaviour is similar to that of other psyllid species reported by Raman, 1991; Burkhardt, 2005; Khattab and Khattab, 2005). Subsequently to the attack, the upper leaf surface rise up (in opposite direction of the feeding sites) in relation to the surrounding tissues in the form of an arch or a small nodule (figure 5). Therefore, the early gall forms by arching the leaf tissue over the nymphs in the feeding site (figure 6). Then, the new gall encloses around the nymph, due to the continuous secretion of saliva by the nymph inside. After that, the nymph inside the gall grows and develops into subsequent instars (second to fifth).

Development of galls and nymphs

After the formation of new gall with the first instar nymph inside, the nymph continues to feed on the sap of internal tissues of hosting gall and secretes additional amounts of saliva to induce swelling of the surrounding tissues, thus forming a small nipple-shaped gall (figure 7). The nymph continues to suck the sap and secretes saliva and then it develops inside the gall by moulting successively into the second, third, fourth and fifth instar. The subsequent growth of the insect causes an increase in the size and number of cells forming the gall hundreds of times and eventually a large and mature gall is produced (figure 8). The full grown gall becomes nipple-shaped with a closed distal end (upper part) and slit-like opening at the bottom of the lower part (figure 9). This opening is usually used for exiting from the gall of the full-grown fifth instar and then moulting as adult. The dissection of large number of galls (n = 100) has revealed the presence of one nymph per gall chamber.

Description of *P. buxtoni* nymphs and adults

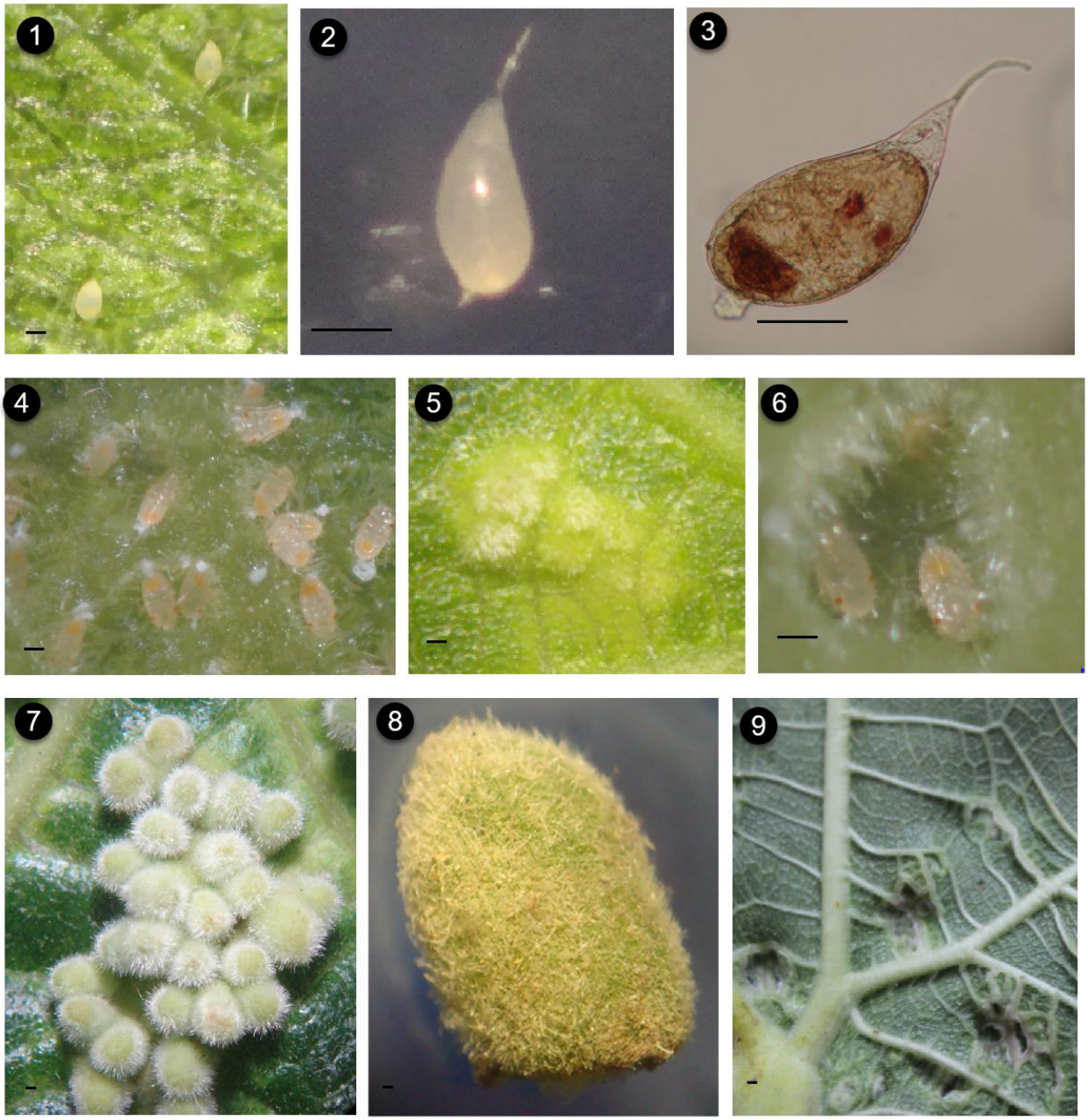
The dissection of *P. buxtoni* galls that were collected from infested leaves of fig orchards revealed the presence of five nymph instars.

F i r s t i n s t a r

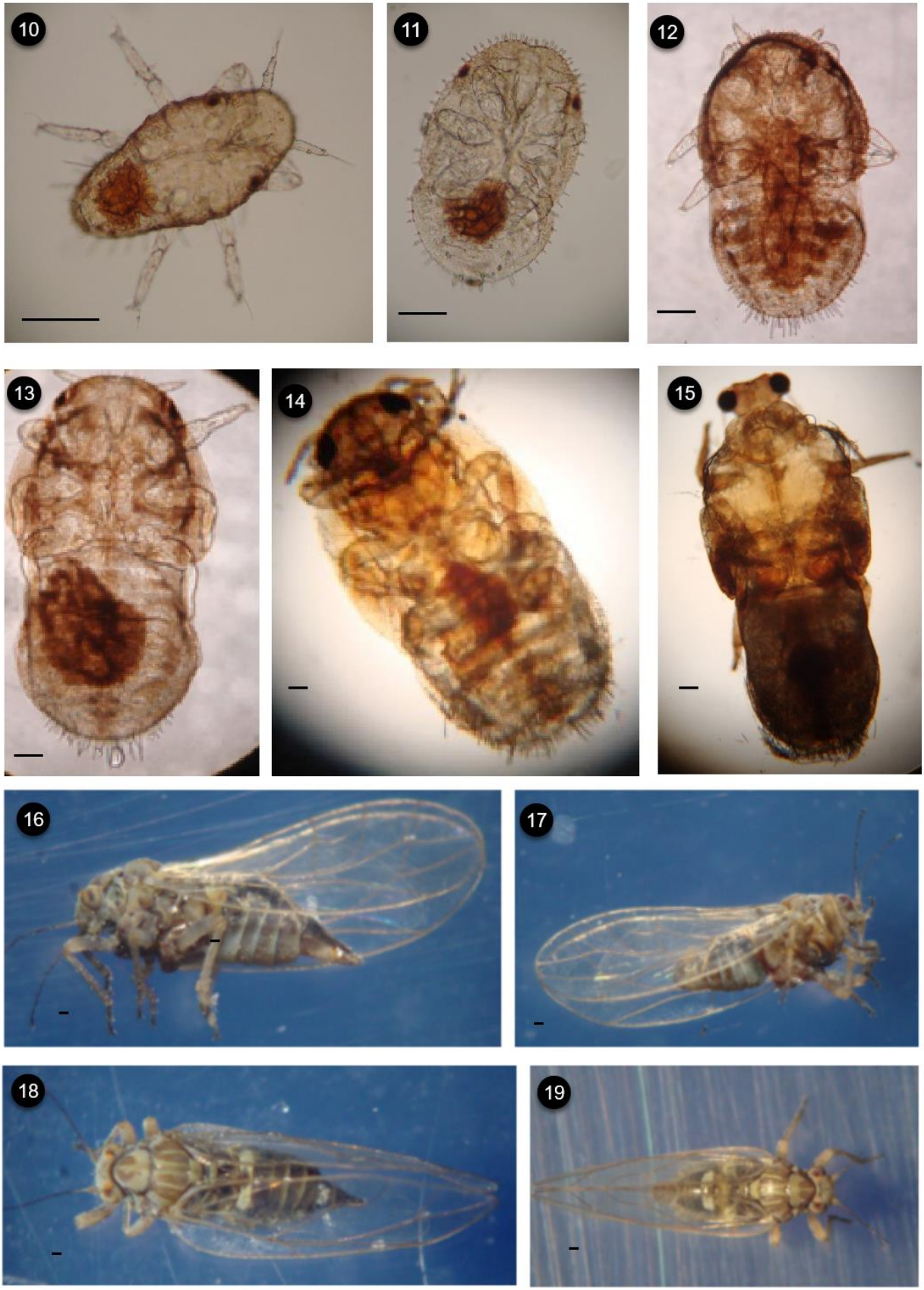
Oval to elongate, with no distinct body regions. It is yellowish in colour with red eyes and prominent rostrum used for sucking the plant sap. It has six legs but with no cerci or wing pads. It moves around on the leaf surface for 1 to 2 days then, it settles and begins to suck the leaf sap. It induces gall in which it enters then it develops into the subsequent nymph instars. The first instar is tiny in size with a mean body length of 0.26 ± 0.05 mm and a range of 0.21-0.33 mm (figure 10) (n = 100). The mean length of the corresponding hosting galls is 0.53 ± 0.11 mm (range was 0.31-0.65 mm) (n = 100 galls).

S e c o n d i n s t a r

This nymph is similar in morphological characteristics to the first instar, it is bigger in size and has an oval shape with two distinct body regions, without wing pads. Nymphs live inside the gall and suck the sap of the mesophyll layer of the gall chamber (this behaviour is similar to that of other psyllid species reported by Luft and Paine, 1997). Its mean body length is 0.45 ± 0.08 mm and its range is 0.35-0.53 mm (figure 11) (n = 100). The mean length of the corresponding hosting galls is 0.95 ± 0.14 mm (the range is 0.69-1.10) (n = 100 galls).



Figures 1-9. Induction, initiation and development of galls of *P. buxtoni* on *F. carica* leaves: **1)** eggs of *P. buxtoni* laid on the lower leaf surface viewed under a dissecting microscope, **2)** enlarged view of *P. buxtoni* egg observed on the lower leaf surface under a dissecting microscope (higher magnification) showing long apical process and short pedicel at the bottom, **3)** *P. buxtoni* egg near hatching viewed in a prepared slide under a compound microscope showing the developing embryo inside, **4)** hatched nymphs (first instar) of *P. buxtoni* on the lower surface of leaves, **5)** enlarged upper view of early formed gall induced by *P. buxtoni* first instar nymph showing an elevated tissues in form of an arch on the upper leaf surface, **6)** first instar nymph on the lower leaf surface where they appear in a depressed area (feeding site) sucking the sap, this area is located in the opposite site of upper arched area. **7)** Upper view of nipple-shaped galls, **8)** mature, nipple-shaped gall, **9)** lower view of mature galls. Scale bars = 100 µm.



Figures 10-19. Development of *P. buxtoni* nymphs on *F. carica* leaves: **10)** first instar nymph viewed in a prepared slide, **11)** second instar nymph extracted from hosting gall, viewed in a prepared slide, **12)** third instar nymph viewed in a prepared slide, **13)** fourth instar nymph, **14-15)** fifth instar nymphs (ventral and dorsal views, respectively), **16-17)** adult lateral view (female and male, respectively), **18-19)** dorsal view of female and male, respectively. Scale bars = 100 µm.

T h i r d i n s t a r

It is yellowish in colour, it has oval shape with two distinct body regions, without wing pads (figure 12). Its mean body length is 0.72 ± 0.10 mm and its range is 0.62-0.88 mm (n = 100). It lives inside the gall. The mean length of the corresponding hosting galls is 1.62 ± 0.20 mm (the range is 1.41-1.93 mm) (n = 100 galls).

F o u r t h i n s t a r

It lives inside the gall and has an oval shape and yellow colour. It differs from the previous instars by having three distinct body regions (head, thorax and abdomen) and little wing pads. It has a mean body length of 0.98 ± 0.03 mm and a range of 0.95-1.08 mm (figure 13) (n = 100). The mean length of the corresponding hosting galls is 2.45 ± 0.25 mm and the range is 2.18-2.78 mm (n = 100 galls).

F i f t h i n s t a r

This instar represents the full-grown nymph (figures 14-15). It has been fully described by Batta and Burckhardt (2018). Shortly, it has three distinct body regions, it is yellowish to greyish in colour with short wings and large red compound eyes. It has a mean body length of 2.35 ± 0.19 mm and a range of 1.98-2.55 mm (n = 100). The mean length of the corresponding hosting galls is 4.95 ± 0.18 mm and the range is 4.75-5.42 mm (n = 100 galls). At the end of this instar, the full-grown nymph comes out from the hosting gall and outside moult as adult.

A d u l t

As described by Batta and Burckhardt (2018), adults (figures 16-19) have the fore wings with clear light brown veins in which the vein R+M+Cu would trifurcate into veins R, M and Cu. Both sexes have long antennae with 10 segments and 2 apical antennal setae (figures 29-31). Body colour is brownish-grey. The female has prominent ovipositor at the end of abdomen, whereas the male has aedeagus curving upward. The males have a mean body length of 3.65 ± 0.13 mm and a range of 3.50-3.80 mm (n = 100 males), whereas the females have a mean body length of 3.92 ± 0.21 mm and a range of 3.70-4.20 mm (n = 100 females).

Supplementary descriptions

The body size, shape, colour, regions, presence or absence of wing pads, etc. were the main characters that have been used in the present study to separate the nymph instars of *P. buxtoni*. Other characters could be supplemented such as the number of antennal setae, size of antennal segments, characteristics of caudal plate and wing pads, etc. Therefore, illustrations are provided in figures 20-41. Differences between the nymph instars and adult of *P. buxtoni* can be observed in the size of antennal segments and their setae (figures 20-25). The details of antennal segments and number of antennal setae of the fifth instar nymphs (figures 26-28) can be compared with adults (figures 29-31) (10 short antennal segments with 1 antennal seta versus 10 long antennal segments with 2 antennal setae, respectively). Similar differences can be observed in the other characters such as the characters of the caudal plate (figures 32-36) and wing pads (figures 37-41).

Development of galls and *P. buxtoni* nymphs

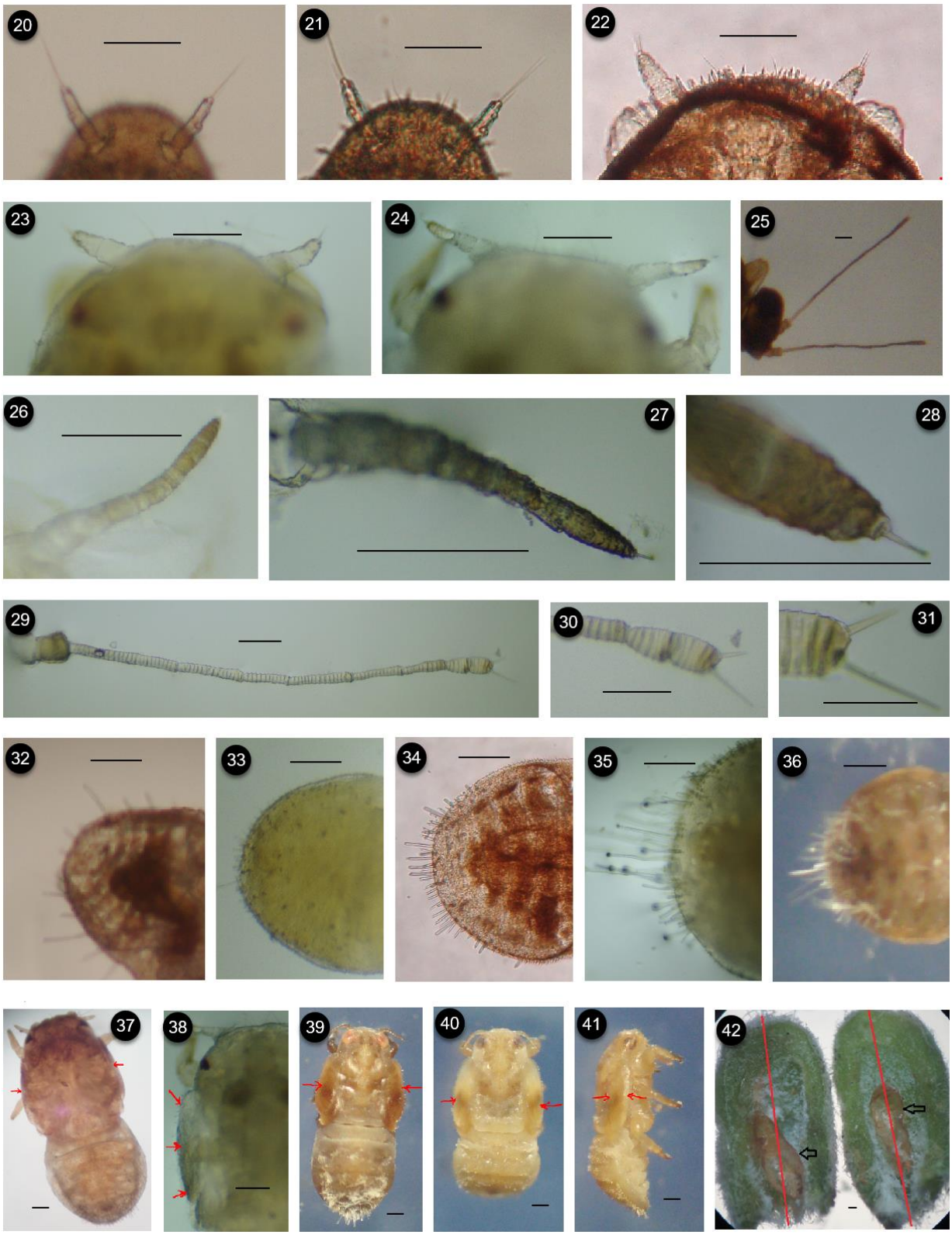
When the nymphs increased in size and developed from one instar to another, the hosting gall grew and increased in size accordingly. Therefore, the mean length of the gall (figure 42) increased from 0.53 to 4.95 mm (n = 100 galls) during the insect development from 0.26 mm to 2.35 mm of body length (n = 100). The most important increase in the gall size can be observed during the swelling phase of the gall (when the insect reached the fifth instar). The mean length of full grown gall was 4.95 mm and for the mean length of the insect in last instar living inside the gall was 2.35 mm.

Life cycle

In the fig orchards, the adults appeared at the beginning of spring season when the overwintering nymphs comes out from its hosting galls to develop into the adult. Mated females lays their eggs on young leaves that unfold as a result of bud break at the end of winter season or beginning of spring season during March. The nymphs developed throughout the year to reach the adult stage in the beginning of the next spring. Therefore, the duration of insect life cycle lasted for one year and the insect produced one generation per year.

Discussion and conclusions

The eggs of *P. buxtoni* are laid in an erect position on the lower leaf surface of fig leaves with insertion of egg pedicel into the leaf tissues by the female ovipositor. Boujou and Nguyen (1974) found that the females of Triozidae (Psylloidea) do not bury their eggs into the host tissues partially or completely in contrast to the gall-inducing females of *Phacopteron lentiginosum* Buckton (Phacopteronidae Psylloidea) which bury their eggs in the leaves of *Garuga pinnata* Roxb. (Burseraceae) in southern India (Raman, 1987). The first instar nymphs of *P. buxtoni* crawl on the leaf surface for one or two days and then settle on the leaf to suck the sap and secrete large amount of saliva which induces the formation of the galls in which they enter for further development. This behaviour is similar to that of other psyllid species reported by Raman (1991), Burkhardt (2005) and Khattab and Khattab (2005). Also, the results on egg laying of *P. buxtoni* on the lower leaf surface and settlement of hatched immatures on this surface in certain feeding sites are in agreement with the results obtained on other psyllid species such as *Trioza jambolanae* Crawford (Triozidae) (Raman, 1991) and *Diaphorina truncata* Crawford (Psyllidae) (Balakrishna and Raman, 1992) where the first instar nymphs of these species settle on the abaxial side of the leaves of host plants to initiate gall formation. Moreover, the present observations on *P. buxtoni* are in agreement with Luft and Paine (1997) who stated that, after settling, the immatures of *Trioza eugeniae* Froggatt feed on the primordial-mesophyll parenchyma cells lying immediately below the abaxial epidermis by inserting their stylets through stomata.



Figures 20-42. Supplementary characters for separation of the instars of *P. buxtoni* nymphs: **20-24)** antennae of the first, second, third, fourth and fifth instar nymphs, **25)** antennae of adults, **26-28)** details of the antenna of fifth instar nymphs (10 short antennal segments with 1 antennal seta), **29-31)** details of the antenna of adults (10 long antennal segments with 2 antennal setae), **32-36)** details of the caudal plate of the first, second, third, fourth and fifth instar nymphs, **37)** little wing pad of the fourth instar nymph, **38)** enlarged view of wing pad of the fourth instar nymph, **39)** wing pad of the early fifth instar nymph, **40-41)** short wings of the full-grown fifth instar nymph, **42)** length of mature gall. Scale bars = 100 µm.

A new finding was also obtained in the present research regarding to how the nymphs of *P. buxtoni* induce and initiate the galls on the fig leaves. This induction and initiation take place during the feeding of the nymphs on leaves which causes the upper leaf epidermis to rise in relation to the surrounding tissues (opposite side of feeding sites of the immatures on lower leaf surface). Therefore, the folding appearance was observed on the upper leaf surface in the form of small nodules or young galls. Raman (1991) stated that the gall induction and initiation by sucking insects could be attributed to the shifts in the feeding sites within the gall, synchronized with the development of the nymphs. Several species of psyllid insects induce galls during sap sucking of their host plants, the hackberry psyllids (*Pachypsylla celtidis* Riley) and eucalyptus psyllids (*Schedotrioza marginata* Taylor) are two well-known gall-forming psyllids that induce galls on the leaves of hackberry and eucalyptus trees (Taylor, 1987; Yang and Mitter, 1994; Leatherman, 2010; Straka *et al.*, 2010). Gall induction by these psyllid species is attributed to the severe salivary injection by the first instar nymphs (Burckhardt, 2005; Khattab and Khattab, 2005). Raman (1991) found that the first instar nymphs of *T. jambolanae* insert their relatively short stylets into mesophyll layer through stomata and then inject large amounts of saliva, thus stimulating the division activity in the metaplasied cell(s). The injected saliva of *T. jambolanae* contains high-molecular weight proteins (Carango *et al*., 1988), mitogenic lipids (Farmer, 2000) and high concentrations of phytohormones (Straka *et al.*, 2010). The injected saliva activates and regulates growth by triggering novel patterns of differentiation, which follow at the site of metaplasied cells, thus initiating gall formation by increasing the thickness of leaf tissues on this site (Hodkinson, 1984; Harper *et al*., 2004).

The current paper demonstrates the development and growth of *P. buxtoni* galls in relation to the development and growth of the insects living inside. Although of using different cultivars and having a different set of data, the present study confirms what has been already obtained by Batta and Burckhardt (2018). This result could be attributed to the enlargement and swelling of galls containing the nymphs (first to fifth instars) which they feed on the sap of mesophyll layer of the hosting galls then they continue to suck the sap and secrete saliva that cause the swelling of the hosting galls. Raman (1993) attributed the increase in the size and number of cells forming the gall hundreds of times to the subsequent growth of the insect living inside. Therefore, a huge increase in the gall size occurred and eventually large and mature galls are produced. Furthermore, Raman (2011) summarized the sequence of steps in the morphogenesis of insect-induced plant galls as follows: osmotic-shock related stress → establishment of either one or a group of metaplasied cells → growth promotor-mediated cell expansion → commitment of the metaplasied cell(s) starting the "novel" cell-cycle patterns, cell multiplication, and programmed differentiation. He also indicated that galls control manifestations that are usually symmetrical (radial or bilateral) when mature, and each gall is unique because the insect species that induces the gall determines its shape.

The results obtained in the present paper on biology and life cycle of *P. buxtoni* could be used effectively for developing an integrated control program of *P. buxtoni* on edible fig trees. The program should include the following components: 1) to identify the natural enemies of the insect such as predators and parasitoids that can be used in a biological control program, 2) to test the efficacy of a series of selective insecticides against adults and nymphs, 3) to test the efficacy of other control measures such as using yellow sticky traps for attracting adults that emerge in early spring, 4) to destroy the infested fig leaves that remain attached to the trees from the previous growing season. These leaves remain attached to the trees even during the winter season and constitute a source of infestation for the next growing season, 5) to try to isolate some pathogens that may infect *P. buxtoni* such as the entomopathogenic fungi and then test their efficacy in a biocontrol program, 6) to try to cultivate resistant fig cultivars during the establishment of new fig orchards. The findings of the present research are thus new and should be helpful in developing an integrated control program for this insect on edible fig trees, in addition to help the fig producers to better managing of this pest.

Acknowledgements

I would like to thank the Deanship of Scientific Research at An-Najah National University for the financial support of this research (Ref. number of the grant: AN-NU-1819-Sc019). Many thanks for the native English speaker who has helped in editing the English of this paper.

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Received January 10, 2019. Accepted May 31, 2019.