

Morphological Study of the Soft Scale Insect *Protopulvinaria pyriformis* (Cockerell, 1894) (Hemiptera: Coccidae) Using Scanning Electron Microscopy

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ABSTRACT

Pyriiform scale, *Protopulvinaria pyriformis* (Cockerell, 1894) is an exotic insect pest. This pest represents a serious pest of fruit trees and ornamental plants in many parts of the world. This insect pest infests many host species belonging to many plant families. The entire life cycle is spent on the lower leaf surface and few individuals were also observed on the upper parts of the leaf surface and it is estimated that the fecundity of this scale insect reaches about 200 eggs per female. The major damage from excretion large quantity of honeydew, which support the growth of sooty mould fungus and leads to defoliation. The present study deals with morphological description for pyriiform scale by measuring the length and width of the different stages of nymph and adult female, since no males have been discovered for this insect and it reproduces by parthenogenesis. Also, some morphological characters of the adult female of this soft scale insect were studied by using a scanning electron microscope and photographing the ultrastructure of some parts such as, antennae, legs, marginal seta, bilocular pore, anal plate and wax producing pores. Results revealed that antennae consist of 8 segments, multilocular pores scattered around vulva and in transverse rows on abdomen.

Keywords: *Protopulvinaria pyriformis*, biology, SEM study.

INTRODUCTION

The pyriiform scale, *Protopulvinaria pyriformis* (Cockerell, 1894) in fact is considered to be a polyphagous soft scale insect with over one hundred host plants belonging to thirty-four families. It is belonging to the family Coccidae which contains many economically important insect pests of agriculture (Merrill, 1953; Gill, 1988; Williams & Watson, 1990; Hodgson & Henderson, 2000 and Miller & Miller, 2003). *P. pyriformis* as all scale insects generally feeding on the underside of leaves. It feeds on plant leaves by their sucking mouthparts to get phloem sap causing wilting and stunting of shoots. The secreted honeydew encourages growth of sooty moulds that cover the leaves and fruit, which reducing photosynthesis operation (Gullan, 1997). *P. pyriformis* is a pest of ornamentals and fruit trees like avocado, citrus and gardenia and has a wide range of host plants

(Ben-Dov, 1993). Heavy infestations may cause leaf margins to fold and premature leaf drop and yield reduction (De Meijer *et al.*, 1989 and Swirski *et al.*, 1997). In general, damage of feeding by scales insects cause yellowing, chlorosis and wilting of the affected parts of plant.

The soft scale insect *P. pyriformis* (Cockrell) was identified for the first time in Egypt by Karam (2013) who collected them from the heavily infested shrub *Schefflera* sp. (Family: Araliaceae). Many studies were carried out on different species of soft scale insects ultrastructure, but no sufficient morphological study was found on this insect. The scanning Electron Microscope (SEM) is advanced tool for studying the details of structures of insect cuticle (Zhang *et al.*, 2012). It is requires special preparation of the material but provides clear and excellent results (Ubero-Pascal *et al.*, 2010).

The aim of the present study is doing ultrastructure of *P. pyriformis* by using the scanning Electron Microscope (SEM).

MATERIAL AND METHODS

A. Collecting insect specimens:

The females of the pyriiform scale are used in this work were collected from guava and Schefflera shrubs in the Experimental Farm Station, Faculty of Agriculture, Alexandria University (31°12'24"N 29°59'39"E) at Abies region, Alexandria, Egypt. Immature stages and adult females were preserved in 70% ethyl alcohol and transferred to the laboratory of Department of Applied Entomology and Zoology, Faculty of Agriculture, Alexandria University, Egypt.

B. Preparation microscopic slides for mounting scale samples:

The method of mounting of slide for Coccoidea adapted from Kozarzhevskaya (1968) was used. Insects were killed and preserved in either 70-75% ethanol, or in lactic acid/ethanol: 2 parts 95% ethanol + 1 part 75% lactic acid. Ten individuals of adult females, eggs and nymphal instars were mounted in slides in Hoyer's solution. Measurements (length and width) of different

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stages were estimated by using micrometer lens and presented in the text as the mean.

C. Specimens identification:

The mounted specimens were identified by the author as *P. pyriformis* (Cockrell) according to the description and illustration of Hamon and Williams (1984). The genus was identified and certified by using the key of Hodgson (1994) and the species by the keys of Kuwana (1909); Takahashi (1955); Gill (1988); Tang (1991) and Hodgson (1994). Morphological characteristics of Coccidae adult which used in this work and helpful for identification, were according to Williams and Kosztarab (1972) (Fig. 1).

D. Scanning Electron Microscope (SEM):

For SEM preparation, the wax was dissolved in 10% Xylene and then dehydrated by soaking in a series of ethanol (80, 85 & 90%) for half an hour for each concentration. Finally, they were dried for 2 hours at room temperature. The following procedures were carried out at the SEM unit of Faculty of Science, Alexandria University, the dehydrated samples were glued on a SEM stub using double-sided sticky tape, some samples on the dorsal and others on ventral side of body. The Specimens were placed in sputter chamber scanning electron (JOELFC-1100E) to coat the samples with a very thin layer of gold. A computer controlled SEM (JEOL model JSM-IT200) was used to scan the samples from different angles to select the best micrographs.

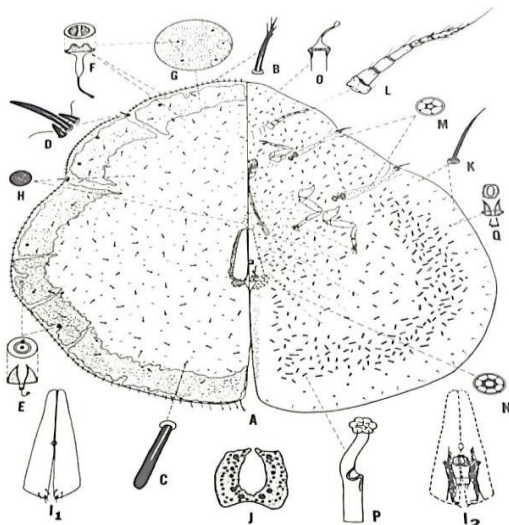


Fig.1. Morphological characteristics adult of *Protospulvinaria Pyriformis* according to Williams and Kosztarab (1972)

RESULTS AND DISCUSSION

Adult females of *Protospulvinaria Pyriformis* are similar to those of *Kilifia acuminata* (Signoret) (acuminate scale), *Kilifia americana* Ben-Dov and *Milviscutulus mangiferae* (Green), but differ from these three species by having extremely long sclerotised anal plates (Hamon & Williams 1984 and Gill 1988). But, *P. pyriformis* is most similar to *P. longivalvata* Green and *P. fukayai* (Kuwana). *P. pyriformis* differs from *P. fukayai* by having anal plates with the anterolateral margin at least four times as long as posterolateral margin (*P. fukayai* has the anterolateral margin less than three times as long as posterolateral margin). *P. pyriformis* differs from *P. longivalvata* by having the marginal setae fringed, dorsal setae numerous and 8–10 μm long (*P. longivalvata* has the marginal setae simple, dorsal setae sparse and 3–5 μm long). This scale pest (*P. longivalvata*) was recorded for the first time in Egypt in 2013 and more research is needed to explore more about this scale pest under Egyptian ecosystem.

Females of pyriform scale insect have 3 nymphal instars and no males are unknown in Egypt. The reproduction is parthenogenetic. This agree with Miller *et al.* (2015) who reported that many species of coccids are parthenogenetic. Also, Moznette (1922) recorded that males of *P. pyriformis* in Florida but this information needs confirmation.

Adults of *P. pyriformis* is pyriform in shape, this pyriform scale is typical of genus *Protospulvinaria*. The *P. pyriformis* is light greenish in younger females turned to light brown in older ones, which are dark brown with broad, reddish, mottled marginal bands. Sometimes with sclerotized areas around margin and surrounded by a thin white waxy fringe (Fig. 2A). Nymphs and young adults are clear yellowish. It is pointed anteriorly and broadly rounded posteriorly (pyriform), usually asymmetrical. This can be used in the field to distinguish at least the genus from other genera of Coccoidea (Hamon & Williams, 1984 and Gill, 1988). They are thin and flat, 299.5 μm in length and 249.5 μm in width (Fig. 2 B).

P. pyriformis reproduce by parthenogenesis (Ben-Dov, 1993; Swirski *et al.*, 1997 and Miller *et al.*, 2015). Eggs are deposited under females with very short posterior ovisac (Fig. 3A). Merrill (1953); Hamon & Williams (1984) and Gill (1988) they reported that during laying of egg-laying is produced in a narrow ovisac, visible as a white secretion around posterior margin. The egg is elongate, yellowish in colour and about 29.4 μm in length and 20.8 μm in width (Fig. 3B).

1- Description the instars of female *P. pyriformis*:

First-instar of nymph:

Body of the first-instar crawler of *P. pyriformis* is about 62.2 µm in length and 40.4 µm in. It is oval in shape, yellowish in colour with a thin covering of dry white wax with wide short anal cleft. The first instar or "crawler" is definitely the dispersal stage and is generally the most active developmental stage in the soft scales (Fig. 4). Legs are well developed. Each antenna is well developed. Same result was also found in the body of the first-instar crawler of the Coccidae, *Pulvinaria psidii* (Beshr *et al.*, 2009).

Second-instar of female nymph:

Body elongate oval, widest across abdomen; derm is translucent to greenish in colour, with a fairly dense covering of white wax over the head and thorax and another over the abdomen, body elongate oval; 145.5 µm long; 116.5 µm wide; anal cleft short (Fig. 5).

Third-instar of female nymph:

Body oval, widest across abdomen, lightly convex dorsally; derm greenish in colour, with the waxy texture somewhat similar to that of the late second-instar. Body elongate oval; 185 µm long; 161.5 µm with wide short anal cleft (Fig. 6).



A



B

ovisac

Fig.3. *P. pyriformis* (A) Light microscope photo of eggs and (B) ovisac



A



B

Fig.2. Female of *P. pyriformis* (A) microscopic photograph (B) SEM micrograph

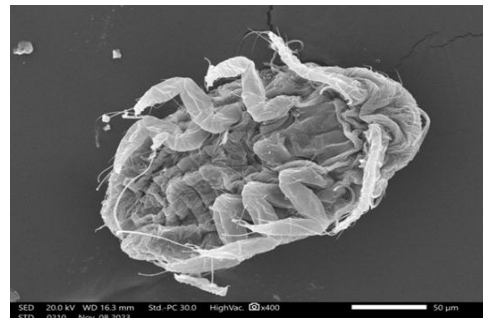


Fig.4. SEM micrograph of *P. pyriformis* first instar nymph (crawler)



Fig.5. Second nymphal instar of *P. pyriformis*



Fig.6. Third nymphal instar of *P. pyriformis*

2- Ultrastructure of *P. pyriformis* female using SEM:

Anal plates (Fig. 7A) are the extremely long and narrow they are at the center of body of *P. pyriformis* (at least 4 times longer than wide). These agree with Karam (2013), each plate is with a Y shaped which supporting bar. Anterior end of the anal plates is lying over mesothorax. Each anal plate has three or four apical setae without discal setae and 1 or 2 small subapical setae. Anal fold has two pairs of long setae located on anterior margin and none laterally are shorter and smaller than others, and two irregular rows of translucent wax pores. Miller *et al.* (2015) reported that preopercular pores are inconspicuous and restricted to anterior part of anal plates.

Williams and Kosztrab (1972) reported that anal fold had four or five fringe seta and anal rings had probably 6 setae present. Also, they reported that anal ring was rectangular in outline with 10 hairs, but 2 pairs of this body seta rod-shaped, with blunt apices, randomly scattered over dorsum (Fig. 7B).

The number of sub marginal tubercles are 6-12 around entire body (Fig. 7C). Williams and Kosztrab (1972) reported that location of tubercles was varied between individuals. Dorsal tubercles are rather small and in a sparse submarginal ring, with two or three pairs on abdomen, one pair between stigmatic clefts and 0 or one pair on prothorax and the head.

Pores: Minute bilocular are pores scattered over derm. In heavily sclerotized submarginal derm (Fig. 7C), Williams and Kosztrab (1972) reported that minute bilocular pores were scattered with a slender filament. We found another pore on dorsum occurs near the edge (Fig. 7D). Dorsal pores all are minute and sunken and simple, with a long inner filament, located in most areolations. Preopercular pores each are small and flat with granulated surface located in a group of 11-33 pores which located around anterior end of anal plates and extending slightly anteriorly.

Antennae (Fig. 8A) is consist of eight segments, Williams and Kosztrab (1972) reported that each antenna are consisted of seven or eight segments, 4th

segment with a pseudo-articulation and apical segment is rather long and total length is 264-318 μm . Mouthparts often is displaced slightly to one side and labium with four pairs of setae and width 70-90 μm .

Legs (Fig. 8B) are well developed with tibiotarsal scleroses and articulation. Claws are with non a denticle; claw digitules both are broad and shorter than tarsal digitules. The dimension's trochanter and femur 200-208 μm but tibia is 124-150 μm and tarsus is 72-82 μm .

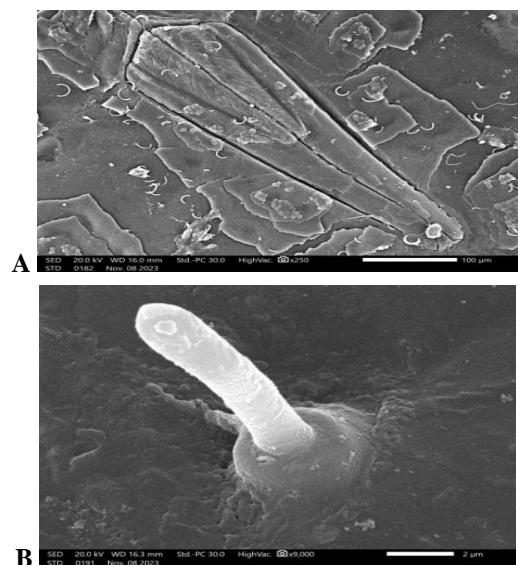
Multilocular pores (Fig. 8C) they are with 6 loculi scattered around vulva and in transverse rows located on abdomen. Williams and Kosztrab (1972) reported that multilocular pores most had 7 loculi, some 6-9 loculed.

Tubular duct, there are two types of tubular ducts present: short ones with slender filament predominantly near antennae bases but this duct did not described here and longer ones (Fig. 8D) with broader filament in sub marginal band around posterior portion of venter. This type is located near sub marginal band around body scattered over posterior part of venter.

Marginal seta is (Fig. 8 E) numerous, fimbriate except few slender, pointed setae near anal cleft. Williams and Kosztrab (1972) reported that marginal setae were from 18-28 between spiracular setae.

Marginal setae are spinose and each one with a broadly fimbriate apex and a well-developed, broad basal-socket; each setae is 12-28 μm long with 20-30 setae on each side between stigmatic clefts.

Thoracic seta/ventral sub marginal setae are (Fig. 8 F) long, slightly, straight (F1) or curved (F2) and pointed. Spiracular setae is (Fig. 8.G) in groups of 3, medial seta 2.5 to 3X longer than lateral setae.



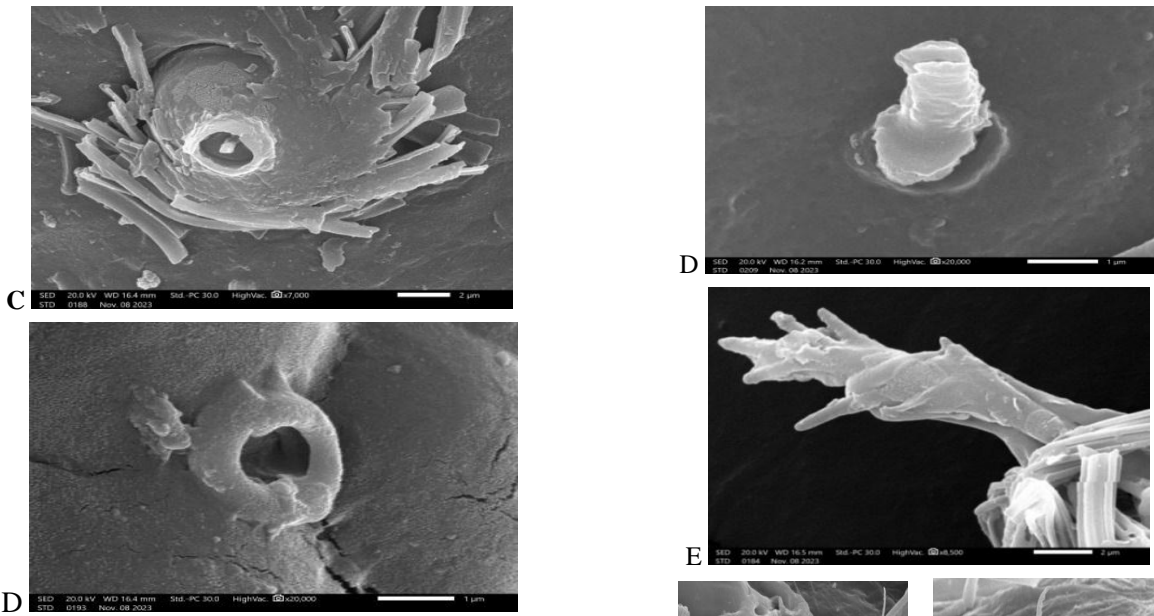


Fig.7. Scanning electron micrographs of *P. pyriformis* (A):Anal plates, (B):Dorsal body seta, (C):Sub marginal tubercles,(D): Minute bilocular pore and (E): dorsal pore near the edge

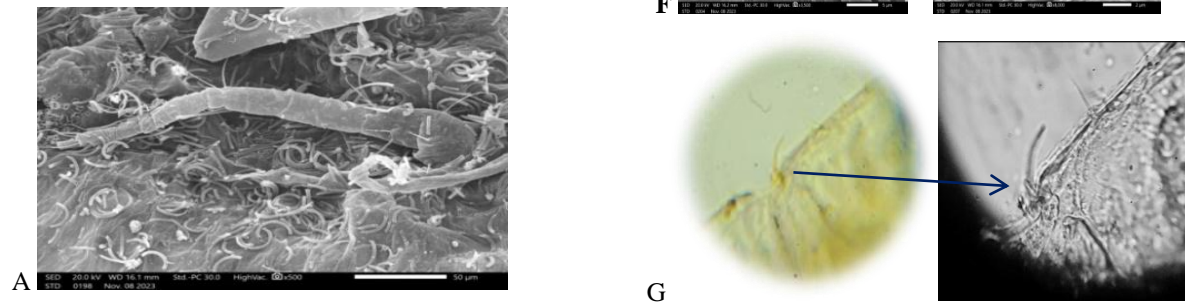


Fig.8. Scanning Electron Micrographs (SEM) of *P. pyriformis* showing:-(A): The eight segmented antenna (Ant.) with sensory setae on each segment, (B): the legs, (C): Multilocular pores, (D):Wax filament produced from tubular duct, (E): Marginal seta, (F): ventral sub marginal setae and (G): Spiracular setae

CONCLUSION

This work showed the description of different stages of Pyriform scale, *Protapulvinaria pyriformis* (Cockerell, 1894) by measuring the length and width of the nymph and adult female, Also, some morphological characters of the adult female of this soft scale insect were studied by using a scanning electron microscope and photographing the ultrastructure of some parts such as, antennae, legs, marginal seta, bilocular pore, anal plate and wax producing pores. This morphological study may help entomologists for identifying Pyriform

scale soft scale insect because no sufficient morphological study was found on this insect in Egypt.

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الملخص العربي

دراسة مورفولوجية للحشرة القشرية القلبية الرخوة *Protopulvinaria pyriformis*

(Cockerell, 1894) (Hemiptera: coccidae) باستخدام الميكروسكوب الإلكتروني الماسح

شاهنده على سمير إبراهيم، حنان محمد رمضان، محمد عوض العريان، أمانى مصطفى ابوشال، محمد السيد توفيق

المورفولوجية وصف الحشرة من خلال قياس طول وعرض المراحل المختلفة للحوريات والأنثى البالغة حيث لا يوجد لهذه الحشرة ذكور وتتكاثر بالتوالد البكرى. وأيضا تم دراسة بعض الصفات المورفولوجية للأنثى البالغة لهذه الحشرة القشرية وذلك باستخدام الميكروسكوب الإلكتروني الماسح وتم تصوير بعض الأجزاء مثل قرون الأستشعار وشعيرات الجسم والصفحة الشرجية والفتحات المنتجة للشمع. وأوضحت النتائج أن قرن الأستشعار يتكون من ٨ عقل وفتحات إفراز الشمع المنتشرة حول فتحة الشرج وفي صفوف عرضية على البطن.

تعتبر الحشرة قلبية الشكل *Protopulvinaria pyriformis* (Cockerell, 1894) آفة خطيرة حيث تمثل هذه الآفة خطورة كبيرة على أشجار الفاكهة ونباتات الزينة في أجزاء كبيرة من العالم. تصيب هذه الآفة الحشرية العديد من العوائل النباتية التي تنتمي إلى العديد من العائلات النباتية. تقضى هذه الحشرة دورة حياتها بأكملها على السطح السفلى للأوراق كما يوجد عدد قليل من الأفراد على السطح العلوى وتشير تقديرات خصوبة الأنثى إلى ما يقرب من ٢٠٠ بيضة لكل أنثى. ويكمن الضرر الرئيسى للحشرة فى إفراز كمية كبيرة من الندوة العسلية التي تساعد فى نمو فطر العفن الأسود والذي يؤدي إلى تساقط الأوراق. وتتناول هذه الدراسة