

Scanning Electron Microscopy of *Icerya aegyptiaca* (Douglas, 1890) (Hemiptera: Monophlebidae)

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ABSTRACT

The present study deals with the morphological study with both Light and Electron microscope of *Icerya aegyptiaca* (Douglas, 1890). It is revealed that the body regions are indistinct with convex dorsum and flattened venter. Body covered with tufts of white waxy secretions and surrounded with submarginal long processes which covers the ovisac. The external features of the adult female such as, antennae, eyes, legs and thoracic and abdominal spiracles with their measurements were described in details. The Ultrastructure of derm pores with their variations in size, type and distribution were also explained with measurements. Only one circatrix is recorded in this mealy bug.

Keywords: Ultrastructure- morphology, *Icerya aegyptiaca*, mealy bugs.

INTRODUCTION

Scale insects are important economically because they have long been known as one of the most destructive pests of agriculture, horticulture and forestry in several countries. (Williams & Watson, 1990; Miller & Davidson, 1990; Miller *et al.*, 2005).

Scale insects are divided into either 22 (Ben- Dov *et al.*, 2006) or about 30 extant families (Koteja, 1996, 2001; Miller *et al.*, 2007).

Scale insects and Mealy bugs comprise the super family Coccoidea in the Order Homoptera are divided traditionally into two informal groups, the primitive archaeococcoids in which adult female possess abdominal spiracles (Orthoidea) and the advanced neococcoids in which the adult female do not have abdominal spiracles (Borchsenias, 1956; Koteja, 1974).

The primitive scale insects were classified by Morrison, (1928) into two families i.e. Ortheziidae and Margarodidae. In Egypt, the family Margarodidae is represented by six species (Ezzat and Nada, 1986). Three of them are belonging to the genus *Icerya* Signoret, 1875; *Icerya aegyptiaca* (Douglas, 1890), *Icerya purchasi* Maskell, 1878, and *Icerya seychellarum* (Westwood, 1855), that were originally described by Morrison, (1928).

Gullan and Cook (2007) reported that the neococcoids form a monophyletic group supported by both morphological and genetic data. In contrast the

monophyly of the archaeococcoids is uncertain and the higher level ranks within it have been controversial.

Recent phylogenetic studies using molecular and morphological data conducted by Gullan and Cock (2007) supported the recognition of up to 15 extant families of archaeococcoids, including 11 families for the former Margarodidae. They also stated that relationships among most scale insect families are unresolved in phylogenetic trees based on nuclear DNA sequences, and most nodes in trees based on morphological characters. Later on, Unruh and Gullan (2008 a) published a molecular phylogeny of scale insects tribe Iceryini, they proved that four of the five genera belonging to this tribe are not monophyletic. In another investigation(2008 b) , they considered genus *Icerya* as a member of family Monophlebidae instead of Margarodidae and revealed that their hypothesis of relationships was supported by morphological characters of the adult females, especially features of the wax-extruding cuticular pores.

Aim of the present work is to study the ultrastructure of the external characteristic features of *Icerya aegyptiaca* (Douglas, 1890).

MATERIALS AND METHODS

Identification of Mealy bugs in practically all cases is based upon the adult female, (Carnes, 1907). Morrison's traditional classification (1928) was also based largely on the morphology of adult females, so the present study depends on adult females.

1-Preparing of the specimens for the Scanning Electron Microscopy (SEM):

Adult females of *Icerya aegyptiaca* (Douglas, 1890) were collected from leaves of croton, *Codiaeum variegatum*, in Alexandria.

The covering wax of the specimen was dissolved by using tetrahydrochlorofurane and chloroform 3:1. They were fixed in 2.5% glutaraldehyde. Then rinsed in phosphate buffer (PH=7.4) at room temperature, and post fixed with 2% O₂ SO₄ (1.5 h). After dehydration in a graded series of alcohols (50, 70, 90, 95 and 100% each for 15 min.) and than specimens were transferred to acetone 15 min. Specimens were then prepared for the Scanning Electron Microscope (dried at the critical point PELCOCPD2, mounted on a stub by means of a double sided sticky transparent tape, and coated with

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gold in the PELCO SC-6-duster. They were examined with Jeol GSM 5300, Scanning Electron Microscope in the Faculty of Science, Alexandria University.

2-Preparing the Specimens for Light microscoping examination:

Wax secretions of the specimens dissolved in xylene, the body of the insect carefully punctured with a fine pin or a needle. Then heated in NaOH 10% using water bath to about 80°C for approximately one hour. The specimens were then washed several times in water, then transferred to a series of alcohol (35, 50, 70%) for 15 min. each. Specimens were mounted in Hoyer's solution and dried in oven at 50°C for two weeks.

RESULTS

General appearance:

Unmounted material:

The adult female of *Icerya aegyptiaca* (Douglas, 1890) is covered with lumps of white waxy secretions, about 20 waxy processes are surrounded the body margin, six short and stout processes on the anterior part of the body directed back wards followed by 12 -14 long slender tapered posterior extensions on the posterior body margin. These extensions which develops from the ventral surface of the abdomen giving it a fluted appearance.

Side mounted material:

Adult female oval to pyriform in shape, 5 mm long, yellowish orange in colour; legs, antennae and eyes dark brown.

Microscopical features:

The main body region often are indistinct and fused (Fig – 1), the dorsal side is usually convex with the ventral side flat. Antennae are eleven segmented, the last of which being by far the longest, while the segments from the sixth to the tenth are equal. The scape broad at base, becoming narrow distally (Fig-2) Lengths of the antennal segments 1 to 11, are measured in microns as follows (100, 70, 90, 40, 40, from sixth to tenth 50, 120) respectively. Eyes are simple and conical in shape, about 60 µm in diameter (Fig. 2 & 3). The mouth parts like all the superfamily Coccoidea consists of looped retracted stylets received into a long internal pouch (crumina) extending backwards from above the base of the labium into the thorax till the base of the abdomen (Fig. 4). The beak (labium) conical in shape, it is three segmented with setae measuring 220 µm at base, the two basal segments are very narrow (Fig.1, 4 & 5). Adult and nymphs are able to protrude their thread like mouth stylets from the head to insert them into plant tissue (Snodgrass, 1935). There are three pairs of well developed thoracic legs (Fig. 6 & 7),

coxa bears row of 4 special seta on its lower edge, the femur is comparatively swollen and stout (500 µm) in length. The tibia is slightly longer than femur (550 µm), while the tarsus is less than the half length of the tibia (200 µm), claw strong and curved (50 µm), tarsal and claw digitules not knobbed (Fig. 7)

There are two pairs of large heavily chitinized thoracic spiracles (Fig. 6 & 8) the small anterior one measured 90 µm in length and 50 µm in width while the large posterior one measured 140 µm long axis and 100 µm short axis. The abdominal spiracles much smaller than the thoracic ones (Fig. 9), they are three pairs lies ventrally at the end of the abdomen. They are relatively simple, consisting a bell – shaped atrium (Fig- 10) and a short narrow tube, the circular opening of which measured about 17.5 µm. The anal opening absent, except for its internal structure, appear dorsally at the posterior end of the abdomen. The opening itself is not differentiated from the adjacent derm, many strong erected setae directed towards the opening. Vulvar opening surrounding with extreme short setae and multilocular pores with bilocular or trilocular center and (8- 16) slightly reniform outer loculi with a thin inner rim; these pores form medial to submedial band anterior to vulvar opening (Fig. 11 A & B). The presence of the vulva is an indication of full maturity of the adult female. Circatrix appear ventrally posterior to the genital opening (vulva), it is round to oval clear membranous area without pores or setae (Fig. 12).

The derm pores serve as producers of the secreted substance which so frequently protects the insect. They considerably variable in type, size and distribution. The derm near thoracic spiracle orifice with multilocular pores measuring 5 – 7 µm each with bilocular center and 6 - 9 outer loculi (Fig. 8). Same type of multilocular pores are arranged between hind coxae forming a transverse ovisac band 3 or 4 pores wide, each with bilocular or trilocular center and 6 -8 outerloculi (Fig. 13 & 14). the pores of this band is responsible for the secretion of the ovisac, they secrete a thin waxy filaments which amalgamate together, thus forming the ovisac wall. Simple multilocular pores similar to pores of ovisac band, forming marginal clusters and covering derm on dorsal surface around other dorsal pores

Dorsal and ventral body setae are vary greatly in length (Fig.15). The dorsal setae are comparatively long and flagellat, with a bulb – like base, while the ventral setae are short and thin (Figs. 12 & 13).

This study is in agreement with that which is carried out by Unruh & Gullan (2008 b). Ezz *et al.*, (1974) and

Kosztarb (1996) described *Icerya purchasi* which is similar to great extent to *I. aegyptiaca* except the lacking of the quadrilocular disc pores with trilocular center. And the number of the circatrices which are three in *I. purchasi*, the central one is the largest while

in the present work, there is only one. Also, there were no distinguished body regions or tufts of hairs surrounding the body margin which are described by the previous authores.

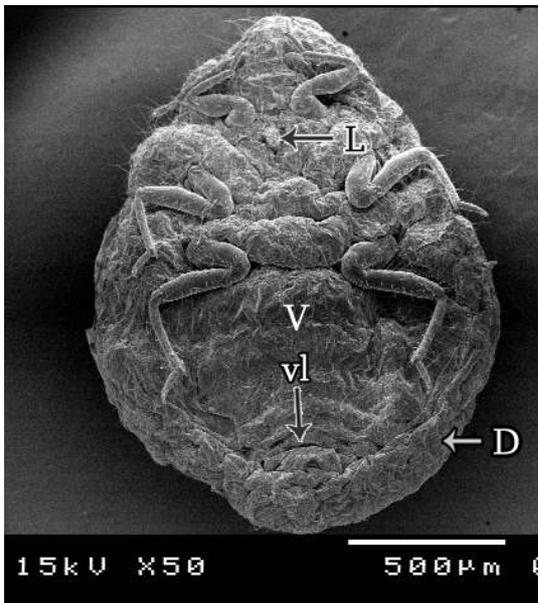


Figure 1. SEM micrograph of *Icerya aegyptiaca* female (D. dorsal side; L. labium; V. ventral side vl. Vulva).

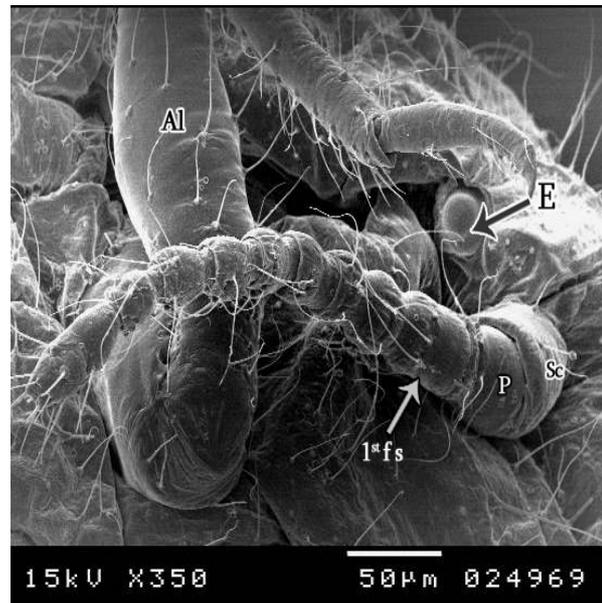


Figure 2. SEM micrograph showing antenna, eye and anterior leg (Al., anterior leg ; E. eye; P. pedicel; Sc. Scape and 1st fs. flagellar segments)



Figure 3. Light microscopic photograph showing the eleven segmented antenna and adjacent eye.

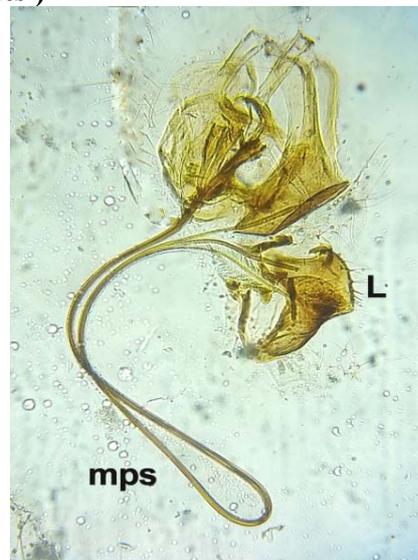


Figure 4. Light microscopic photograph showing the mouth parts(L. labium and mps. Mouth part stylets looped in crumena)

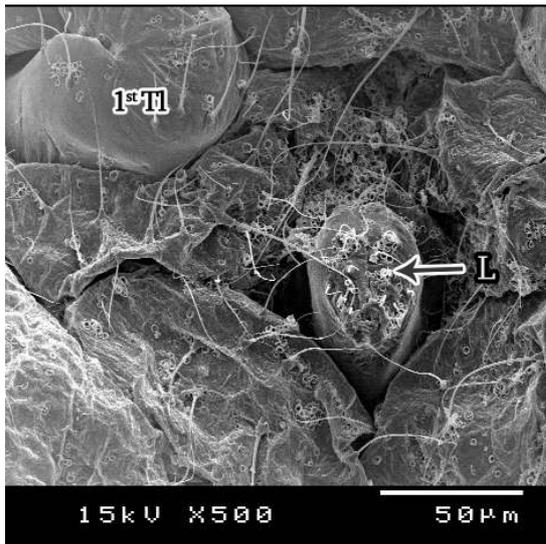


Figure 5. SEM micrograph showing labium (L.) and 1st thoracic leg (1st Tl.).

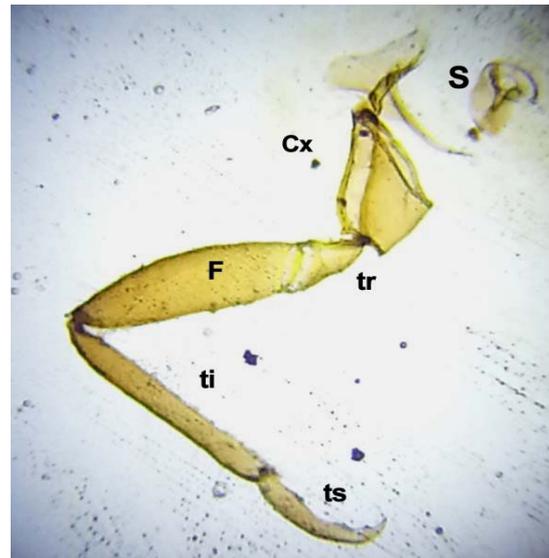


Figure 6. Light microscopic photograph showing leg and spiracle (cx. Coxa; F. femur; ti. Tibia; tr. Trochanter; ts. Ts. Tarsus. S. spiracle)

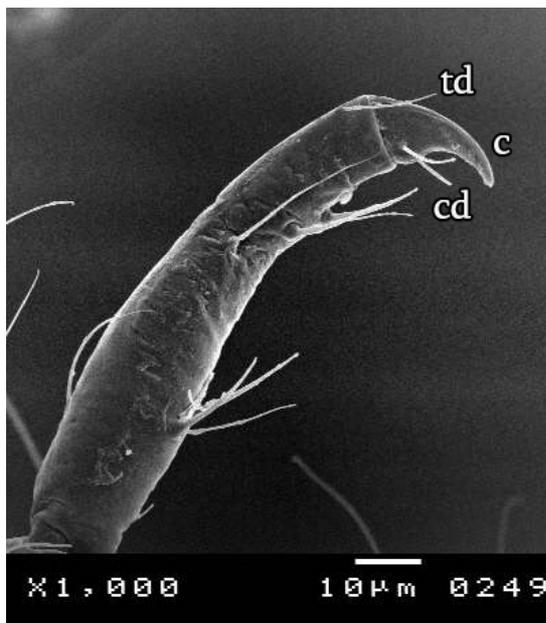


Figure 7. SEM micrograph showing one segmented tarsus and claw. (C. claw; cd. claw digitules and td. tarsal digitules)

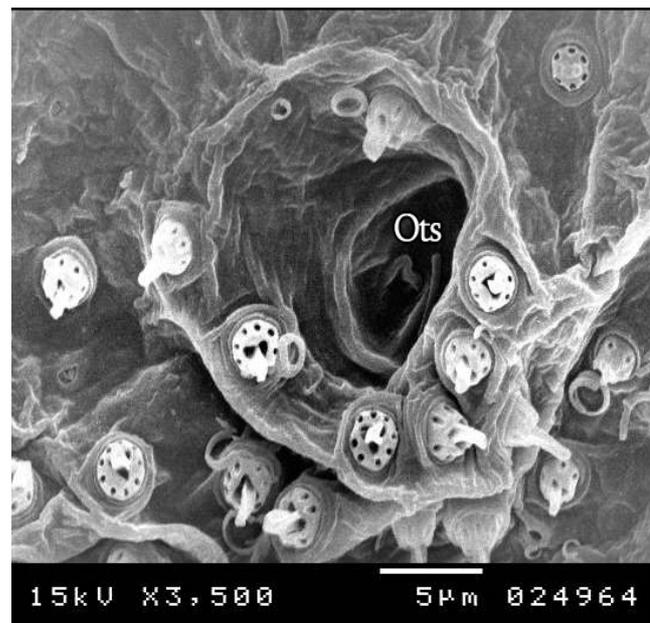


Figure 8. SEM micrograph showing orifice of thoracic Spiracles (Ots.)



Figure 9. Light microscopic photograph showing abdominal spiracles

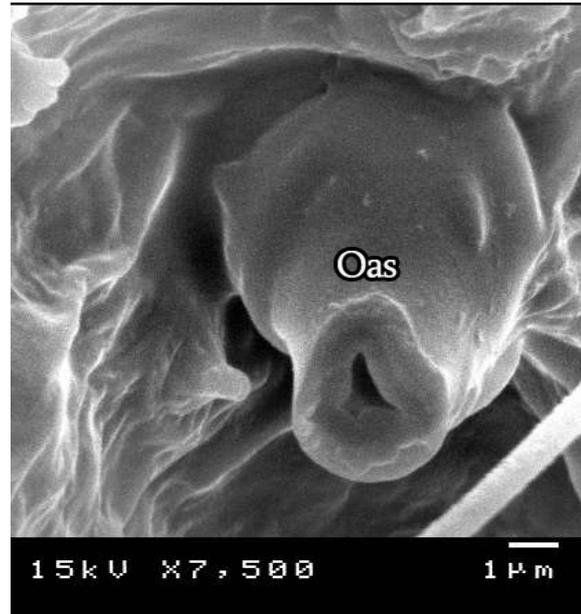


Figure 10. SEM micrograph showing bell shaped orifice of abdominal spiracles (Oas.)

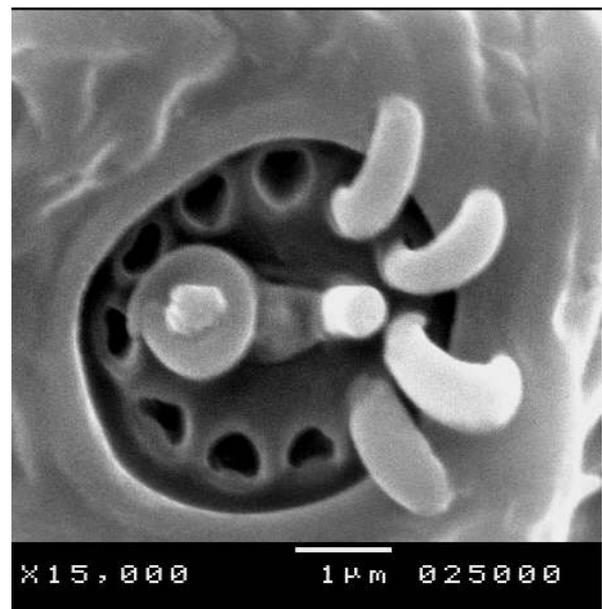
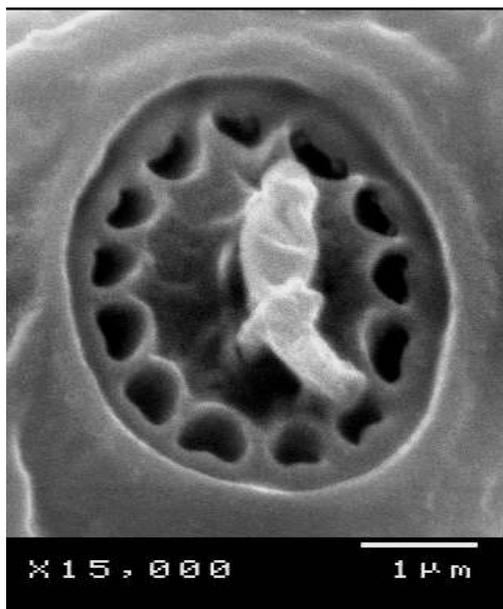


Figure 11 A&B. SEM micrograph showing the multilocular pores with 11- 13 reniform loculi with bilocular center surrounding the vulva(wax filaments extruded from some loculi)



Figure 12. Light microscopic photograph, arrow indicating the rounded oval circatrix

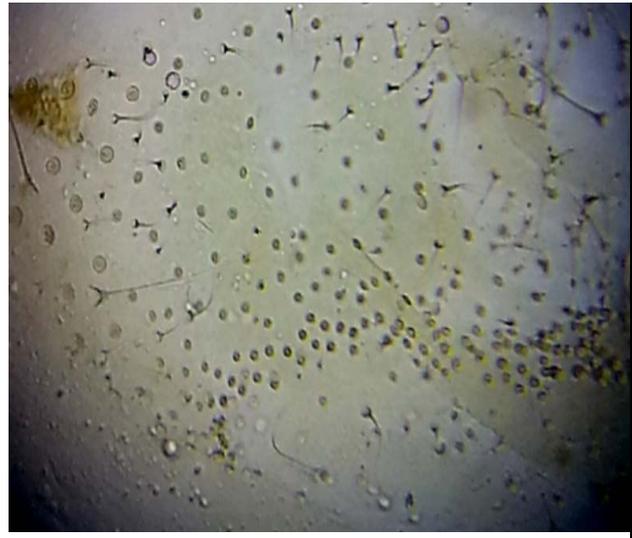


Figure 13. Light microscopic photograph, showing the transverse multilocular ovisac band

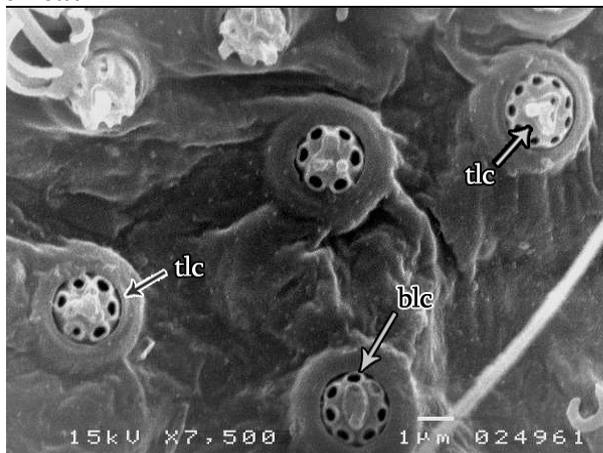


Figure 14. SEM micrograph showing the multilocular pores with 6 - 9 loculi with bilocular (blc) or trilocular (tlc) center of ovisac band

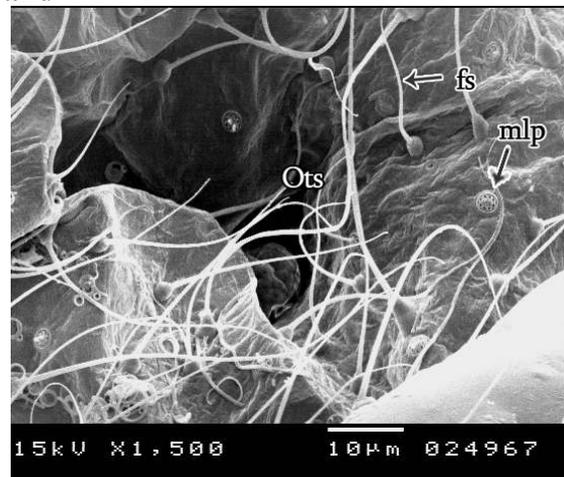


Figure 15. SEM micrograph showing variable length of dorsal Flagellate setae (fs). Flagellate seta; mlp. multilocular pores

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REFERENCES

- Ben-Dov, Y., D.R. Miller, and G.A.H. Gibson. 2006. Scale Net. <http://www.sel.barc.usda.gov/scalent/scalent.htm> (Access ed 5 August and 10 September 2007)
- Borchsenias, N. 1956. The question of the course of evolution of coccoidea (Insecta, Homoptera). *Zool. zhur.* 35; 546 – 553. (In Russian)
- Carnes, E.K. 1907. The Coccoidea of California Calif. State Comnt. Hort. Bien. Rept. 2(1905-106): 155 – 222.

- Ezz, A.I., A.H. EL-KIIL , A.G. Saad, and A. Wahab.1974. Morphological and Ecological studies on *Icerya purchsi* Mask. In Egypt.2nd Egt. Pest . cont. cong. Alexandria. Vol. 11, Alex – Univ. 10–12 sept. 1974 : 617 - 657
- Ezzat, Y. M., and S.M. A. Nada. 1986. List superfamily Coccoidea as know to exist in Egypt. Bull. Lab. Entomol Agr. "Fillippo silvestri" : 43 : 85 – 90.
- Gullan, P.J., and L.G. Cook. 2007. Phylogeny and higher classification of the scale insects (Hemiptera : Sternorrhyncha: coccoidea) www.mapress.com/Zootaxa 1668 :313-425
- Kosztarab, M.1996. Scale Insects of Northeastern North America: Identification, Biology, and distribution. Virginia Museum of Natural History, Martineville. Special publication No.3, 650 pp.
- Koteja, J. 1974. On the phylogeny and classification of the scale insects (Homoptera, Coccinae) (discussion based on the morphology of the mouth parts). Acta zoologica Cracoviensia. 19: 267 – 326.
- Koteja, J.1996. Scale insects (Homoptera: Coccinae) a day after. In: Schaefer, C.W. (Ed.), Studies on Hemipteran phylogeny. Proceedings of Thomas Say Publications in Entomology, Entomological society of America, Lanham, Maryland, pp. 65 – 88.
- Koteja, J.2001. Essays on coccids (Hemiptera: Coccinae): paleontology without fossils. Prace Museum ziemi, 46, 41 – 53.
- Miller, D.R., and J.A. Davidson. 1990. A list of the armored scale insects pests. In: Rosen, D. (Ed), World Crop pests, Volume 4 B, Armored Scale Insects: their Bilology, Natural Enemies and control. Elsevier, Amsterdam, pp. 299- 306.
- Miller, D.R., M.E. Gimpel, and A. Rung. 2005. A systematic Catalogue of the Cerococcidae, Halimococcidae, Kermesidae, Micrococcidae, Ortheziidae, Phenacoleachiidae, Phoenicococcidae and Stictococcidae (Hemiptera: coccoidea) of the world. Intercept Limited, wimborne.
- Miller, D.R., A. Rung, G.L. Venable, and R.J. Gill. 2007. Scale Insects: identification tools for species of quarantine importance scale families. ARS & APHIS, USAD. Avail able from :http://www.sel.barc.usda.gov/Scale keys/scale insectsHome/ scaleInsects Families. Htmi (accessed5Septmeber2007)
- Morrison, H. 1928. A classification of the higher groups and genera of the coccid. Family Margarodidae. U.S. Dept. Agric. Tech. Bull. 52: 1 – 240 , 7.
- Snodgrass, R.E. 1935. Principles of insect morphology, McGRAW-Hill Book Comany, Inc. New York and London pp.667.
- Williams, D.J. , and G.W. Watson. 1990 . The scale Insects of the Tropical South Pacific Region. Pt. 3; The soft scales (Coccidae) and other families. CAB International Institute of Entomology, London, 272 pp.
- Unruh, C.M., and P.J. Gullan. 2008 a. Molecular data reveal convergent reproductive strategies in iceryine scale insects (Hemiptera: coccoidea: Monophlebidae), allowing re-interpretation of morphology and revised generic classification. Systematic Entomology. 33: 8 – 50.
- Unruh, C.M., and P.J. Gullan. 2008 b. Identification guide 2 species in the scale insect tribe Iceryini (coccoidea: Monophlebidae) Zootaxa 1803, Mangolia press; 1 – 106

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