Tropical Biomedicine 36(1): 224–233 (2019)

Reliability of egg rafts electron micrographs for confirming the taxonomic status of *Culex pipiens* mosquitoes collected from Al-Ahsa, eastern Saudi Arabia

Shaalan, E.A.[1*], Bekhet, G.[2,3], Abdelmoaty, Z.[2,3] and Ahmad, N.W.[4]
[1]Zoology Department, Faculty of Science, Aswan University, Aswan 81528, Egypt
[2]Biological Science Department, Faculty of Science, King Faisal University, Al-Ahsa 31982, Saudi Arabia
[3]Zoology department, Faculty of Science, Alexandria University, Alexandria 21526, Egypt
[4]Medical Entomology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
[*]Corresponding author e-mail: essamshaalan@sci.aswu.edu.eg
Received 8 June 2018; received in revised form 12 November 2018; accepted 14 November 2018

Abstract. Mosquitoes are undesirable arthropods transmitting many diseases not only in Saudi Arabia but also worldwide. Identifying mosquito species relied for long time on both larval and adult characters whilst little or no attention was given to eggs. Electron microscopic studies of mosquito eggs are important as it is not only characterizing the external morphology of the eggs never seen by stereoscopic microscopes but also facilitates mosquito species identification. Accordingly, morphology and morphometric of *Culex pipiens* eggs collected from Al-Ahsa oasis, eastern Saudi Arabia were examined by scanning electron microscopy (SEM) for the first time in Saudi Arabia in the present work. Mosquito egg rafts were collected from breeding sites in Al-Ahsa by using of special long aquatic net. A portion of the rafts was reared for identification whilst the other portion was preserved in glutaraldehyde and prepared for SEM examination. Eggs appeared to be conical in shape with two ends, the anterior one that is represented with the micropyle is more tapered than the posterior end. The morphometrics gave many characteristics for the eggs such as length, width, proportion of length /width and so on. Eggs morphology and morphometrics were then compared to that of other *Culex* eggs. Our findings using SEM of the eggshell confirmed that the present mosquito species is *Cx. pipiens*. Scanning electron micrographs of any mosquito species eggs are valuable in correlating its fine structure that cannot be easily seen by light microscope and can assist in species separation. Thus, identifying medically important mosquito species is crucial in both mosquito and disease control.

INTRODUCTION

Mosquitoes are the most famous vectors of many diseases such as dengue (Khan *et al.*, 2008), malaria (Abdoon and Alsharani, 2003) and Rift valley fever (Al-Hazmi *et al.*, 2003). The previous diseases are the most prevalent diseases harbored by mosquitoes in Saudi Arabia whilst filaria is uncommon in Saudi Arabia, three cases have been reported during a period of 20 years (1981-2001) from the areas adjoining the Red Sea, in particularly the South-Western region (Haleem *et al.*, 2002). Identifying species of such medical importance is crucial in both disease and mosquito control. Mosquito surveys in Saudi Arabia pointed out the presence of 26 mosquito species (Shaalan *et al.*, 2017). Results of such surveys are sometimes contradictory as well as the recording of the species complex in particular *Cx. pipiens* and *Cx. univittatus* mosquitoes among Saudi Arabia mosquitoes (Harbach, 1985; Al-Khreji, 2005). *Culex pipiens* (including two forms *pipiens* and *molestus*) and *Cx. quinquefasciatus* are the most prevalent mosquitoes in temperate and tropical regions respectively (Dehghan *et al.*, 2011; Shaikevich *et al.*, 2016).

The contradictory findings of some mosquito surveys in Saudi Arabia alerting for utilizing other reliable techniques such as electron microscopy for accurate mosquito life stages identification in particular egg stage. It could be said that one advantage of utilizing scanning electron microscopes over more recent and accurate DNA based methods in insect identification and classification is that it provides researchers with information on some obscure structures that definitely neither seen by light microscopes nor isolated by DNA extraction method. Literatures revealed that such structures are helpful in mosquitos' eggs identification. Linley (1989a&b), Linley and Clark (1989) and Linley and Craig (1994) mentioned the morphology, development and physiology of some aedine mosquito eggs. Significant morphological differences among four *Ochlerotatus* mosquitoes (*Oc. albifasciatus*, *Oc. fluviatilis*, *Oc. scapularis* and *Oc. taeniorhynchus*) were found when their morphological characters were analyzed by Scanning Electron Microscope (Santos, 2013). Both morphology and morphometric variations of *Anopheles fluviatilis* (Sehrawat, 2014), *An. quadrimaculatus* (Linley *et al*., 1993), *An. nuneztovari* (Linley *et al*., 1996), *An. gambiae* complex (Lounibos *et al*., 1999) have been mentioned. Recently, Mello *et al*. (2014&2017a&b) have studied morphology of eggs of some *Coquillettidia* and *Psorophora* mosquitoes respectively. Similarly, Linley and Chadee (1991) and Alencar *et al*. (2003) have mentioned the structure of the *Haemagogus* mosquito. Eggs structure of *Cx. pipiens* have been studied by Chadee and Haeger (1986) and Sahlen (1990) whilst egg morphometrics are used to differentiate *Cx. quinquefasciatus* from *Cx. tritaeniorhynchus* (Suman *et al*., 2008).

Mello *et al*. (2017a&b) have mentioned and summarized the benefits of scanning electron microscopic (SEM) investigations of mosquito's eggs. Where it has facilitated more detailed descriptions of mosquito egg morphology compared to light microscopes (LM) and correlating these fine and obscure structures with mosquito species discrimination. Consequently, it will facilitate the direct identification of mosquito species rather than depending on laboratory rearing to more identifiable stages which is usually time consuming, costly, and risky particularly if the mosquito is a disease vector. Furthermore, egg characters produced by SEM can also be used for phylogenetic analyses and as a tool for the characterization of possible species complexes and for comparison between species (Pacheo *et al*., 2012; Sarmento *et al*., 2014). For instance, Soliman *et al*. (2014) distinguished the two forms of the Egyptian *Aedes* (*Ochlerotatus*) *caspius* species complex by their eggs ultrastructure. Eggs of *Ae. aegypti* and *Ae. albopictus* were differentiated by using morphological measurements produced by the scanning electron microscopy (SEM) (Suman *et al*., 2011). Sallum and Flores (2004) mentioned that ultrastructure of eggs of two morphologically similar species, *An. costai* and *An. mediopunctatus*, are distinct and reliable compared to similarity in adult, larval and pupal stages. Similarly, *Cx. tritaeniorhynchus summorosus* has been confirmed as separate species by Airi and Kaur (2015). Linley and Chadee (1990) used SEM of *Psorophora ferox* eggs to differentiate some populations. The ultrastructure of *Ps. ferox* populations from Florida (USA) and Trinidad were found clearly different in both the number and shape of the external chorionic tubercles in each chorionic cell. Likely, Mello *et al*. (2017b) compared eggs from different *Ps. ferox* populations. The Brazilian population was different from both Florida (USA) and Arena (Trinidad) populations. Populations differed considerably in tubercles morphology, external chorionic reticulum, micropylar collar, and micropyle. Likely, Suman *et al*. (2009) suggested that ecological variation may have influenced morphometrics of the egg of four strains of *Cx. quinquefasciatus* from different geographical areas of India. This phenomenon is also observed in eggs of *Anopheles* mosquitos' whereas Almeida *et al*. (2014) have concluded that eggs of *An. darlingi* were polymorphic and that some morphological patterns were regional.

A recent study has revealed that egg morphometrics are as reliable as PCR assays in differentiating sibling mosquito species. Tyagi *et al*. (2016) provided the first evidence on the efficacy of morphometrics produced by EM in identifying sibling species of the malaria vector *An. culicifacies* in addition to PCR assays. Results also implied the dissimilarity in eggs morphology of sibling mosquito species and possibility of separation by using electron microscopy.

Hence and based on the aforementioned information, the present study was designated to employ the scanning electron microscopic (SEM) studies of mosquito eggs in identifying and confirming status of *Culex pipiens* mosquitoes prevail in Al-Ahsaa, eastern Saudi Arabia.

MATERIALS AND METHODS

Mosquito eggs samples

Al-Ahsa is the largest oasis in the world with its 2.5 million date palms and located about 60 km inland from the coast of the arabian gulf, in the eastern Saudi Arabia. It has a dry, tropical climate, with long very hot summer (five-month) and a relatively cold winter. It has many springs and copious reserves of underground water which allowed the development of such large number of date palms.

Mosquito eggs were collected from breeding sites around Al-Ahsa with special aquatic net. Eggs were kept in the water from the breeding sites inside plastic jars and transformed to the laboratory then divided into two portions. First one left in the water from the breeding sites in the plastic jars and kept inside the insectary for hatching and rearing to 4^{th} instar larval stage. Hatched larvae were identified by using keys of Harbach (1985) and AL-Ahmad *et al*. (2011). The second portion was preserved in gluteraldehyde for scanning electron microscopic examinations (SEM).

Scanning Electron Microscopic Studies

For SEM studies, the egg raftes were prpared as the method descibed by Suman *et al*. (2009) and Mello *et al*. (2017a&b) but with slight modification. Eggs were fixed in 2.5% glutaraldehyde then in 1% osmium tetroxide, both with 0.1 M sodium cacodylate buffer at 7.2 pH. Eggs were washed in buffer then dehydrated in an increasing ethanol series, and dried using super-dry CO_2 in a Balzers device. Finally, eggs were mounted on metal supports, coated with gold, and examined using a JEOL JSM/6390LV scanning electron microscope (JEOL, Ltd., Akishima, Tokyo, Japan) at 100-6,500× magnification. Measurements were made directly on the images obtained. The measured attributes included egg length and width, micropyle diameter, corolla diameter, micropyle disc diameter, micropyle mound diameter and tubercle diameter. Both description and terminology of egg morphology in the present work were adopted from Harbach and Knight (1978).

RESULTS

Eggs are black in color, elongate and conical in shape (Cr) with tapered posterior end (P) exposed to the atmosphere and round anterior end (A) in direct contact with the water surface (Fig. 1). The chorion consists of two very distinct layers, thicker inner one always referred to as the endochorion (EN) and outer layer always referred to as the exochorion (EC) (Fig. 2A).

The posterior end of the egg is devoid of the exochorion and hosts a slightly circular depression the micropyle (M) (Fig. 2A) that could be either empty or filled with either water droplets or lipid material according to Hinton (1968). The micropyle is surrounded by a collar (MC) followed by exochorionic tubercles (T) with diverse shapes (rectangular, pentagonal, hexagonal and octagonal) and assist in the adhesion of the eggs together within the egg raft. These tubercles are supported by chorionic bridges (Cb) (Fig. 2B). The majority of the exochorionic cells have ornamentation with an octagonal or hexagonal appearance, but sometimes this was pentagonal or rectangular (Fig. 2B).

The micropylar apparatus is located at the posterior end of the egg. It is consisting of a continuous micropylar collar or corolla

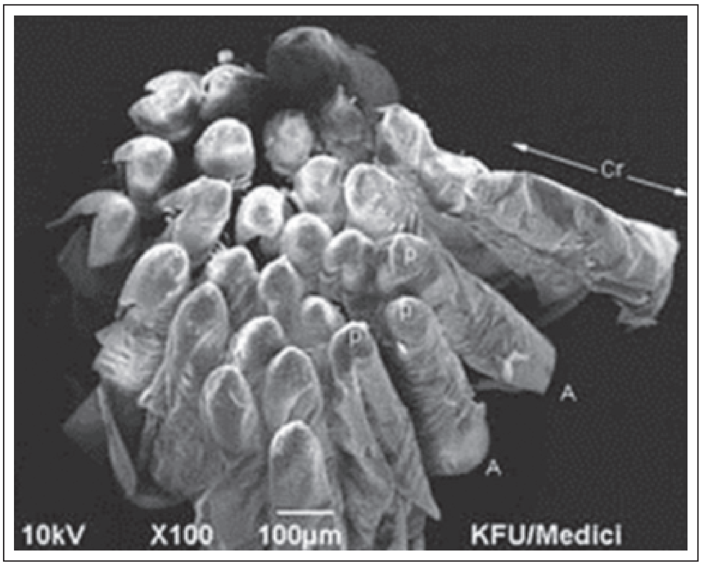

Figure 1. Micrograph showing external morphology of egg raft of *Culex pipiens*. Anterior end of entire egg (A), posterior end (P), conical structure (Cr).

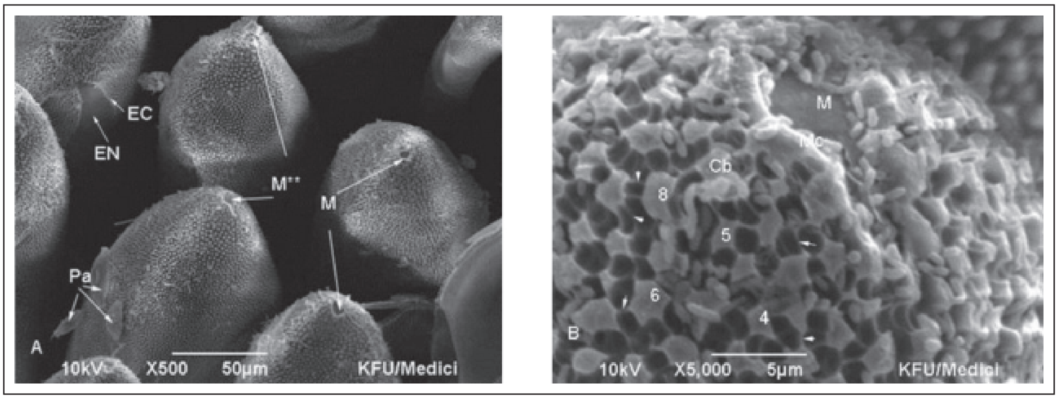

Figure 2. Micrograph showing micropyle (M), located in the posterior end of the egg. A) Showing empty micropyle (M) and filled micropyle (M**) with drop of water. The exo-, and endochorionic membranes (EC, EN). B) Showing micropyle (M), collar (Mc), exochorionic network bridges (head arrows, Cb) and flattened tubercles with variable angles (4-8).

(Mc) with an irregular surface (Fig. 3). In the center of the micropyle a micropylar disc (Md) is found (Fig. 3A) and possess a 3 lobed micropylar mound (Mmd) in its center (Fig. 3B). Tubercles of various sizes (small, medium, and large) are radially arranged in longitudinal rows around the micropylar apparatus (Fig. 3A&B) and directed from the micropylar disc downwards.

The exochorion is a thin porous layer with small and large, rounded or polygonal pores situated around the outside of the tubercles and connecting them forming a mesh or web like layer, but more often sheet and occupied with two distinct layers outer and inner and in between them there were an empty space (Fig. 3B). Tubercles size is seen decreasing in a descending order (Fig. 4). Tubercles of

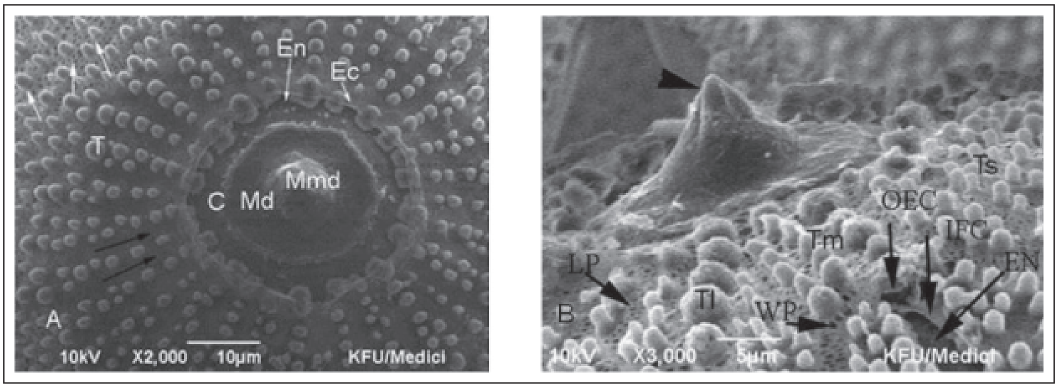

Figure 3. Micrograph showing the posterior end of the egg. A) Showing the micropylar apparatus of the posterior end; micropyle mound (Mmd), micropylar disk (Md), exochorionic sheet (EC) collar (C) and tubercles (T). Non porous between tubercles rows (black arrows) and porous between wheel like tubercles (white arrows). B) Lateral view showing trilobite micropyle mound (black head arrow) and large, medium and small sizes of chorionic tubercles (Tl, Tm, Ts respectively); ornamentation of the outer chorionic reticulum (OCR) showing the outer and inner layers of the exochorion (OEC, IEC) and endochorionic sheet (EN); arrangement of pores of the reticulum either in a line (LP) or in a wheel (WP).

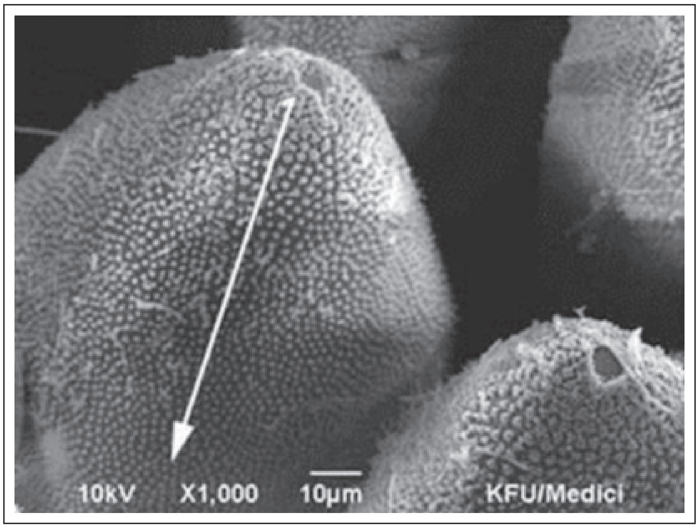

Figure 4. Showing the descending order of tubercles sizes (arrow).

the conical-shaped region are almost structurally differing than the tubercles of micropylar region, which is polygonal in anterior region (Fig. 5) whereas completely different in the middle and the posterior region. In the dorsomedian region, tubercles supported with chorionic network with different wheel pore sizes as large-, medium- or small-sized tubercles wheels are present (Fig. 5).

Additionally, comparing measurements of the present eggs shown in Table 1 to those of other *Culex* eggs such *Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus summorosus*, it reveals that present eggs belongs to the former mosquito species. This is coincidence with the identification of larvae that hatched from the second portion of the eggs that collected from the same breeding place and reared inside the insectary to the larval stage.

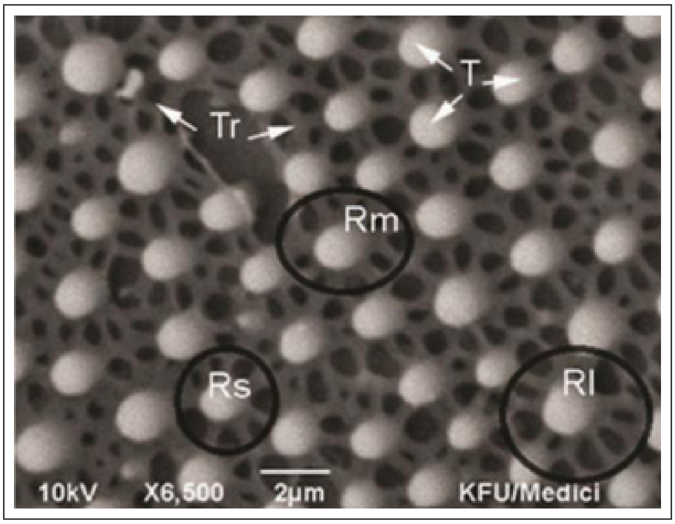

Figure 5. Showing sizes of wheel of tubercles. Large (Rl) with 11-15 pores, medium (Rm) with 8-10pores and small rings (RS) with 6-7 pores.

Table 1. Attributes of the present *Culex* species eggs

Attributes	Mean ± SE
Egg length	341.27 ± 9.7
Egg width	122.86 ± 0.63
Ratio (egg length/width)	2.78 ± 0.12
Micropylar diameter	7.77 ± 1.66
Corolla Diameter	29 ± 0.1
Micropylar Disc diameter	21.21 ± 0.03
Micropylar mound diameter	13.93 ± 1.22
Micropylar Tl diameter	2.8 ± 0.1
Micropylar Tl height	1.51 ± 0.02
Micropylar Tm diameter	1.65 ± 0.05
Micropylar Tm height	1.05 ± 0.04
Micropylar Ts diameter	1 ± 0.01
Micropylar Ts height	0.80 ± 0.09

DISCUSSION

Several SEM studies investigating morphology of mosquito eggs surface have shown significant influence in mosquito species identification (Linley, 1989b; Lounibos *et al.*, 1999; Choochote *et al.*, 2001; Alencar *et al.*, 2003 & 2005; Suman *et al.*, 2008; Santos, 2013). This is due to SEM complements traditional classification because invisible structures by light microscopes could be important for morphological characterization of mosquito eggs. Such structures are formed by desiccation on the egg surface and are characteristic for each mosquito species. Exochorion ornamentation of mosquito eggs is an excellent and reliable character for making comparisons and differentiating species among mosquitoes (Alencar *et al.*, 2003 & 2005; Sehrawat, 2014; Soumare & Ndiaye, 2005). It has been suggested that the function of these ornamentations are adaptive structures that covering and protecting the embryo, preventing it from desiccation and regulating embryonic gaseous exchange (Soumare and Ndiaye, 2005).

The discrimination of Culicidae species relies on the exochorion ornamentation that shows significant differences (Alencar *et al.*, 2003 & 2005; Suman *et al.*, 2008; Santos-Mallet *et al.*, 2009 & 2010) whilst the micropylar apparatus was the most characteristic feature for species confirmation in *Anopheles* (Lounibos *et al.*, 1999; Rodriguez *et al.*, 2002) and *Culex* (Suman *et al.*, 2008; Airi & Kaur, 2015) mosquitoes.

Morphologically, description and dimensions of the present eggs are found to be similar to the description of *Cx. pipiens* eggs by Sahlen (1990) who mentioned that eggs are conical in shape and slightly curved in their long axis. The whole egg surface was ornamented with polygonal outer chorionic cells (OCC), containing large central, medium and small peripheral tubercles (OCTs), except the micropylar apparatus region (Fig. 3B). These tubercles acting as protective and adhesive structures (Suman *et al.*, 2008) and providing support to the egg shell from sudden forces exerted by either the water waves and/or embryonic movement (Sahlen, 1990) in egg rafts of *Culex* mosquitoes. Such egg rafts play an important role in egg flotation on the water surfaces (Soumare and Ndiaye, 2005). The adhesion occurs by the interlocking of the larger tubercles on the surface of one egg between the smaller ones on the surface of the opposite egg and therefore provide necessary stability in the egg-rafts (Sahlen, 1990). In *Aedes* mosquitoes, tubercles contribute to the adhesion of the eggs to the substrate (Santos, 2013).

The dimensions and densities of tubercles found on the present eggs were different to some extent from that of *Cx. quinquefasciatus*. Data in table (1) showing that all attributes of the present *Culex* eggs are smaller than that of *Cx. quinquefasciatus* (Suman *et al.*, 2008) except for micropylar mound (Mmd) diameter (13.94 and 13.30 µm respectively) and medium and small micropylar tubercles diameter (1.65, 1.0 and 1.13, 0.74 µm respectively). Such larger diameters particularly micropylar mound of the present eggs compared to *Cx. quinquefasciatus* could be due to its trilobite structure and implying in the same time to another species of the *Cx. pipiens* complex rather than *Cx. quinquefasciatus*. Additionally, the height of the tubercles ranged from 0.80 to 1.51 µm which is nearly similar to height of *Cx. pipiens* tubercles that measured 0.90-1.60 µm (Sahlen, 1990).

Although all morphological characters and morphometrics of the present eggs are looking alike that of *Cx. pipiens* and different from *Cx. quinquifasciatus*, *Cx. tritaeniorhynchus* and *Cx. summorous* eggs, the very distinctive tri-lobed micropylar mound which have been shown for the first time in the present eggs highlighting the importance of this finding and the reliability of SEM in differentiating such *Cx. pipiens* mosquito complex. The micropylar mound appeared as either flat not protruding outwards as in *Cx. quinquifasciatus* and *Cx. tritaeniorhynchus* (Suman *et al.*, 2008) or evaginated outwards to form a conical structure as in *Cx. summorous* eggs (Airi and Kaur, 2015). Additionally, the half of the egg rafts that separated and left to hatch and develop to 4th instar larval stage was identified as *Cx. pipiens* according to keys of Harbach (1985) and AL-Ahmad *et al.* (2011). Finally, present eggs were collected from breeding sites characterized by stagnant water that are supplied with algae and subjected to sun rays which are characteristic to breeding sites of *Cx. pipiens* in Saudi Arabia as mentioned by AL Ashry *et al.* (2018).

According to all the previously mentioned information it could be said that the present eggs are belonging to *Cx. pipiens* mosquitoes and the tri-lobed micropylar mound has been shown for the first time in the present work.

CONCLUSION

Complete knowledge of the egg morphology of different mosquito species through SEM is not only useful in correlating its fine structure with the invisible ones examined under ordinary microscope, but also can assist in species differentiation. Such SEM studies may allow for the emergence of Pictorial keys for mosquito eggs that should be helpful in studies involving eggs recovered from ovitraps or soil samples. Our findings using SEM of the eggshell tubercles and the characteristic tri-lobed micropyle mound seen for the first time indicated that the present mosquito species is *Cx. pipiens*.

Acknowledgement. The authors want to express their gratitude to Deanship of scientific research, King Faisal University, Saudi Arabia for supporting the present work (Grant No.: 130167). Our gratitude goes to the project technician, Mr. Youssif Al-Gassim, Biological sciences Department, College of Science, King Faisal University, Saudi Arabia for his assistance in collecting mosquitoes. We also appreciated to Dr. Walid Al-Melhim and technician Hani Al-Rosassi, Electron Microscope Unit, College of Medicine, King Faisal University, Saudi Arabia for their assistance in preparing samples and conducting micrographs.

REFERENCES

Abdoon, A.M. & Alshahrani, A.M. (2003). Prevalence and distribution of anopheline mosquitoes in malaria endemic areas of Asir region, Saudi Arabia. *Eastern Mediterranean Health Journal* **9**(3): 240-247.

Airi, M. & Kaur, S. (2015): Confirmation of *Culex* (*Culex*) *tritaeniorhynchus summorosus* (Diptera: Culicidae) as a separate species. *Journal of Vector Borne Diseases* **52**(3): 219-223.

AL-Ahmad, A.M., Sallam, M.F., Khuriji, M.A., Kheir, S.M. & Azari-Hamidian, S. (2011). Checklist and pictorial key to fourth-instar larvae of mosquitoes (Diptera: Culicidae) of Saudi Arabia. *Journal of Medical Entomology* **48**(4): 717-737.

AL Ashry, H.A., Kenawy, M.A. & Shobrak, M. (2018). Ecological aspects of the bancroftian filariasis vectors, *Culex pipiens* and *Cx. quinquefasciatus* (Diptera: Culicidae) in Hail, Saudi Arabia. *International Journal of Mosquito Research* **5**(1): 25 - 32.

Alencar, J., Guimarães, A.E., Mello, R.P., Lopes, C.M., Dégallier, N. & Santos-Mallet, J.R. (2003): Scanning electron microscopy of eggs of *Hemagogus leucocelaenus* (Diptera: Culicidae). *Revista De Saude Publica* **37**(5): 658-661.

Alencar, J., Guimarães, A.E., Gil-Santana, H.R. & Santos-Mallet, J.R. (2005). Scanning electron microscopy of eggs of *Ochlerotatus (Protomacleaya) terrens* Walker. *Journal of the American Mosquito Control Association* **21**(4): 355-359.

Al-Hazmi, M., Ayoola, E.A., Abdurahman, M., Banzal, S., Ashraf, J., El-Bushra, A., Hazmi, A., Abdullah, M., Abbo, H., Elamin, A., Al-Sammani, E., Gadour, M., Menon, C., Hamza, M., Rahim, I., Hafez, M., Jambavalikar, M., Arishi, H. & Aqeel, A. (2003). Epidemic Rift Valley Fever in Saudi Arabia: A Clinical study of severe illness in humans. *Clinical Infectious Diseases* **36**(3): 245-252.

Al-Khreji, M.A. (2005). Survey and distribution of mosquito species (Diptera: Culicidae) and description of its habitat in Riyadh district, Kingdom of Saudi Arabia. M. Sc. Thesis, King Saud University; Kingdom of Saudi Arabia.

Almeida, F., Suesdek, L., Motoki, M.T., Bergo, E.S. & Sallum, M.A.M. (2014). Morphometric comparisons of the scanning electron micrographs of the eggs of *Anopheles* (Nyssorhynchus) *darlingi* Root (Diptera: Culicidae). *Acta Tropica* **139**: 115-122.

Chadee, D.D. & Haeger, J.S. (1986). A description of the egg of *Culex* (*Culex*) *nigripalpus* Theobald from Florida, with notes on five egg rafts (Diptera: Culicidae). *Mosquito Systematics* **18**(3): 288-292.

Choochote, W., Jitpakdi, A., Sukontason, K., Suntaravitun, T., Wongkamchai, S., Sukontason, K. & Pitasawat, B. (2001): Scanning electron microscopy of *Aedes lineatopennis* (Diptera: Culicidae) Eggs. *Journal of Medical Entomology* **38**(5): 753 - 755.

Dehghan, H., Sadraei, J. & Moosa-Kazemi, S.H. (2011). The morphological variations of *Culex pipiens* (Diptera: Culicidae) in central Iran. *Asian Pacific Journal of Tropical Medicine* **4**(3): 215 - 219.

Haleem, A., Al Juboury, M. & Al Husseini, H. (2002). Filariasis: A report of three cases. *Annals of Saudi Medicine* **22**(1-2): 77-79.

Harbach, R.E. (1985). Pictorial keys to the genera of mosquitoes, subgenera of *Culex* and the species of *Culex (Culex)* occurring in southwestern Asia and Egypt, with a note on the subgeneric placement of *Culex deserticola* (Diptera:

Culicidae). *Mosquito Systematics* **17**(2): 83-107.

Harbach, R.E. & Knight, K.L. (1978). A mosquito taxonomic glossary. XV. The egg. *Mosquito Systematics* **10**: 249 - 298.

Hinton, H.E. (1968). Structure and protective devices of the egg of the mosquito *Culex pipiens*. *Journal of Insect Physiology* **14**(2): 145-161.

Khan, N.A., Azhar, E.I., El-Fiky, S., Madani, H.H., Abuljadial, M.A., Ashshi, A.M., Turkistani, A.M. & Hamouh, E.A. (2008): Clinical profile and outcome of hospitalized patients during first outbreak of dengue in Makkah, Saudi Arabia. *Acta Tropica* **105**(1): 39-44.

Linley, J.R. (1989a). Scanning electron microscopy of the egg of *Aedes* (*Protomacleaya*) *triseriatus* (Diptera: Culicidae). *Journal of Medical Entomology* **26**(5): 474-478.

Linley, J.R. (1989b). Comparative fine structure of the eggs of *Aedes albopictus*, *Ae. aegypti* and *Ae. bahamensis* (Diptera: Culicidae). *Journal of Medical Entomology* **26**(6): 510-521.

Linley, J.R. & Chadee, D.D. (1990). Fine structure of the eggs of *Psorophora columbiae*, *Ps. cingulata* and *Ps. ferox* (Diptera: Culicidae). *Proceedings of Entomological Society of Washington* **92**(3): 497-511.

Linley, J.R. & Chadee, D.D. (1991). Fine structure of the eggs of *Haemagogus equinus* and *Hg. janthinomys* (Diptera: Culicidae). *Journal of Medical Entomology* **28**(3): 434-445.

Linley, J.R. & Clark, G.G. (1989). Egg of *Aedes* (*Gymnometopa*) *mediovittatus* (Diptera: Culicidae). *Journal of Medical Entomology* **26**(4): 252-255.

Linley, J.R. & Jr Craig, G.B. (1994). Morphology of long- and short-day eggs of *Aedes atropalpus* and *Ae. epactius* (Diptera: Culicidae). *Journal of Medical Entomology* **31**(6): 855-867.

Linley, J.R., Kaiser, P.E. & Cockburn, A.F. (1993). A description and morphometric study of the eggs of species of the *Anopheles quadrimaculatus* complex (Diptera: Culicidae). *Mosquito Systematics* **25**: 124-147.

Linley, J.R., Lounibos, L.P., Conn, J., Duzak, D. & Nishimura, N. (1996). A description and morphometric comparison of eggs from eight geographic populations of the South American malaria vector *Anopheles* (Nyssorhynchus) *nuneztovari* (Diptera: Culicidae). *Journal of the American Mosquito Control Association* **12**(2 Pt 1): 275-292.

Lounibos, L.P., Coetzee, M., Duzak, D., Nishimura, N., Linley, J.R., Service, M.W., Cornel, A.J., Fontenille, D. & Mukwaya, L.G. (1999). A description and morphometric comparison of eggs of species of the *Anopheles gambiae* complex. *Journal of the American Mosquito Control Association* **15**(2): 157-185.

Mello, C.F., Santos-Mallet, J.R., Gleiser, R.M. & Alencar, J. (2017a). Ultrastructure and morphometry of the egg of *Psorophora albipes* (Theobald, 1907) (Diptera: Culicidae). *Zootaxa* **4317**(1): 196-200.

Mello, C.F., Santos-Mallet, J.R., Tátila-Ferreira, A. & Alencar, J. (2017b). Comparing the egg ultrastructure of three *Psorophora ferox* (Diptera: Culicidae) populations. *Brazilian Journal of Biology* http://dx.doi.org/10.1590/1519-6984.171829.

Mello, C.F., Santos-Mallet, J.R., Morone, F., Guimaraes, A.E., Marcondes, C.B. & Alencar, J. (2014). Ultrastructure of the egg of *Coquillettidia juxtamansonia* (Chagas, 1907) (Diptera: Culicidae). *Journal of Vector Ecology* **39**(1): 219-221.

Pacheo, J.B., Santos-Mallet, J.R., Guimaraes, A.E., Costa, J.M. & Alencar, J. (2012). Ultrastructure and morphometry of the egg of *Psorophora albigenu* Lutz, 1908 (Diptera: Culicidae). *Micron* **43**(2-3): 418-421.

Rodriguez, M.H., Chavez, B., Ulloa, A. & Arredondo-Jimenez, J.I. (2002). Fine structure of the eggs of *Anopheles* (*Anopheles*) *punctimacula*. *Journal of the American Mosquito Control Association* **18**(1): 1-9.

Sahlen, G. (1990). Egg raft adhesion and chorion structure in *Culex pipiens* L. (Diptera: Culicidae). *International Journal of Insect Morphology and Embryology* **19**(5/6): 307-314.

Shaalan, E.A., Abdelsalam, S., Elmenshawy, O. & Al-Kahtani, M.A. (2017). Mosquito vectors survey reveals new record of *Culiseta subochrea* in AL-Ahsaa oasis, Saudi Arabia. *Asian Pacific Journal of Tropical Diseases* **7**(2): 106-111.

Sallum, M.A.M. & Flores, D.C. (2004). Ultrastructure of the eggs of two species of *Anopheles* (*Anopheles*) Meigen (Diptera, Culicidae). *Revista Brasileira de Entomologia* **48**(2): 185-192.

Santos, J.S.S. (2013). Ultrastructure of eggs the mosquitoes of the genus *Ochlerotatus* (Diptera: Culicidae). 67p. Dissertation (Master Science in Animal Biology, Animal Biology). Instituto de Biologia, Departamento de Biologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil.

Santos-Mallet, J.R., Gleiser, R.M., Alencar, J., Marques, W.A., Sarmento, J.S., Müller, G.A. & Marcondesa, C.B. (2009): Scanning electron microscopy of the eggs of *Ochlerotatus albifasciatus* (Diptera: Culicidae). *Journal of Medical Entomology* **46**(5): 980-985.

Santos-Mallet, J.R., Müller, G.A., Gleiser, R.M., Alencar, J., Marques, W.A. & Sarmento, J.S. (2010). Scanning electron microscopy of the eggs of *Aedes scapularis* from southern South America. *Journal of the American Mosquito Control Association* **26**(2): 205-209.

Sarmento, J.S., Marcondes, C.B., Alencar, J., Oliveira, E.M., Mello, C.F., Freitas, V.F. & Santos-Mallet, J. (2014): Scanning electron microscopy of eggs of *Georgecraigius fluviatilis* (Lutz) (Diptera: Culicidae, Aedini). *Zootaxa* **3784**(5): 591-595.

Sehrawat, N. (2014). Scanning electron microscopic observations of eggs of *Anopheles fluviatilis* (T) mosquito (Diptera: Culicidae). *Research and Reviews: Journal of Zoological Sciences* **2**(4): 6 - 12.

Shaikevich, E.V., Vinogradova, E.B., Bouattour, A. & de Almeida, A.P.G. (2016). Genetic diversity of *Culex pipiens* mosquitoes in distinct populations from Europe: contribution of *Cx. quinquefasciatus* in Mediterranean populations. *Parasites and Vectors* **9**: 47.

Soliman, B.A., Wassim, N.M. & Linley, J.R. (2014). Distinguishing the two forms of Egyptian *Aedes* (*Ochlerotatus*) *caspius* Pallas species (Diptera: Culicidae) by ultra-structure micrographs of eggs. *Journal of the Egyptian Society of Parasitology* **44**(1): 71-77.

Soumare, M.L. & Ndiaye, M. (2005). Ultrastructural studies of mosquito ovogenesis. *Tissue and Cell* **37**(2): 117-124.

Suman, D.S., Shrivastava, A.R., Parashar, B.D., Pant, S.C., Agrawal, O.P. & Prakash, S. (2008). Scanning electron microscopic studies on egg surface morphology and morphometrics of *Culex tritaeniorhynchus* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research* **104**(1): 173-176.

Suman, D.S., Shrivastava, A.R., Parashar, B.D., Pant, S.C., Agrawal, O.P. & Prakash, S. (2009). Variation in morphology and morphometrics of eggs of *Culex quinquefasciatus* mosquitoes from different ecological regions of India. *Journal of Vector Ecology* **34**(2): 191-199.

Suman, D.S., Shrivastava, A.R., Pant, S.C. & Parashar, B.D. (2011). Differentiation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) with egg surface morphology and morphometrics using scanning electron microscopy. *Arthropod Structure and Development* **40**(5): 279-483.

Tyagi, V., Sharma, A.K., Dhiman, S., Srivastava, A.R., Yadav, R., Sukumaranet, D., Agrawal, O.P. & Veer, V. (2016). Malaria vector *Anopheles culicifacies* sibling species differentiation using egg morphometry and morphology. *Parasites and Vectors* **9**: 202.