Qatar Univ. Sci. J. (1994), 14 (2): 303-309

SCANNING ELECTRON MICROSCOPY OF THE EGG-SHELL OF THE FLY *SYNTHESIOMYIA NUDISETA* (WULP)

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تركيب قشرة بيضة الذبابة سينثسيوميا نيود يسيتا بأستخدام الميكروسكوب الالكتروني الماسح

نجـــلاء زكي الألـــفي

تناول البحث دراسة التركيب الدقيق لقشرة بيضة ذبابة سينثسيوميا نيود يستيا . وأوضحت الدراسة أن القشرة الخارجية المغلفة للبيضة يوجد بها منحنيات وتجاويف تظهر على شكل شبكة فيما عدا المنطقة المحيطة بالقمة الأمامية . وأوضحت الدراسة أيضاً وجود قليل من الفتحات التنفسية في الجزء الخلفي من البيضة . وتتركب قشرة البيضة خارج خطوط الفقس من ثلاثة طبقات ، الطبقة الخارجية تتكون من أعمدة رأسية تنشأ من الطبقة الوسطى وهذه الأعمدة تتفرع عند القمة في مستوى سطح القشرة وتكون شبكة ذات ثقوب خماسية وسداسية الشكل . أما الطبقة المتوسطة فتتركب من شبكة خشنة ، وتتكون الطبقة الداخلية من أعمدة تتفرع وتكون أقراص غير منتظمة تختلف في مساحة سطحها .

وقد أوضحت الدراسة أن الخط الظهري المتوسط لقشرة البيضة محاط بخطي الفقس اللذان يظهران متوازيان ويمتدان من الجزء الأمامي إلى الجزء الخلفي من البيضة . التركيب الدقيق للخط الظهري الوسطى ويختلف عن بقية سطح قشرة البيضة في الطبقة الخارجية ويشبه تركيب الطبقة الداخلية في المنطقة خارج خط الفقس .

Key words: Egg-shell - *S. nudiseta* - Ultrastructure

ABSTRACT

The ultrastructure of the egg-shell of the fly *Synthesiomyia nudiseta* has been studied by scanning electron microscopy. The surface of the outer chorion is highly ridged, grooved and has a reticulate appearance except at the collar surrounding the micropyle. There are few aeropyles at the antimicropylar pole.

The structure of the egg-shell outside the hatching lines is composed of three layers. The outer layer consists of vertical columns that arise from the middle layer, these columns branch and anastomose at their apices in the plane of the shell and form a pentagonal and hexagonal network and that is the outer most wall of the shell. The middle layer is a coarse meshwork. The inner layer consists of vertical columns which branch to form irregular-shaped discs which vary in width.

The median dorsal strip enclosed by the hatching lines is very distinct, tapering and extends from the anterior end of the micropyle region to the posterior end of the antimicropyle region of the egg. The structure of the median dorsal strip is different from that of the chorion outside the hatching lines in the outermost layer. The structure of the median dorsal strip is similar to the inner layer of the chorion outside hatching lines.

INTRODUCTION

The fly *Synthesiomyia nudiseta* (Wulp) belongs to the Order Diptera, Suborder Cychorhapa, Family Muscidae and Subfamily Reinwardtiinae. *Synthesiomyia nudiseta* is very closely related to Muscine, differing mainly in various details of larval and adult morphology and in its tropical distribution[1]. Some biological studies were carried out on this fly [2,3,4,5,6,7,8 and 9]. The distribution of this fly is tropicopoliton between the 20 °C annual isotherms.

This fly has medical and veterinary importance owing to its significant role in causing wound myiasis. This myiasis is caused by the body invasion of humans or other animals by eggs and larvae. [10] summaries the medical significance of this species as an agent of human myiasis[5]. This fly sometimes lays eggs on open wounds where the eggs hatch out. Also, they lay eggs in the mouth or nose of a sleeping dirty person or in the orbits of eyes, anus and genital apertures of uncleaned small children, thus causing serious disorders.

The scanning electron microscope (SEM) has been used previously to study the surface structure of the egg-shell in a number of dipteran insects. For example, ten genera of the subfamily *Muscinae* [11] *Leptohylemyia coarctata*[12]; *Culex pipiens*[13]; *Aedes aegypti*[14]; *A. triseriatus*[15]; five species of mosquitoes[16]; *Glossina spp.* [17]; *Delia radicums and Erioischa brassica* [18,19,20]; *Dermatobia hominis*[21] and *Anopheles albimanus*[22]. The eggs of *Muscina stabulans* and *Synthesiomyia nudiseta* were morphologically described[23].

Hinton [11] studied the respiratory structure of the egg-shell of ten genera of the Subfamily *Muscinae*. Also he described the structure of the respiratory system and plastron of the egg shells of *Calliphora erythrocephala* Meig. and *Lucilia sericata* Meig[24]. The micropylar and the antimicropylar ends of *Culex pipiens, Chrysopa carneastepha, Glossina spp* and *Delia radicum* were described [13, 17, 20, 25]..The main aim of the present work is to investigate the development of the fine surface structure, micropyle and antimicropyle regions of the mature egg of this fly by using SEM.

MATERIAL AND METHODS

Insects of *S. nudiseta* were reared in the laboratory as described by [1] and [26]. Mature deposited eggs were collected and fixed in 2.5% (w/vol) glutaraldehyde in 0.25 M Sorensen's phosphate buffer at pH 7.1 overnight at room temperature, given three 10 min. buffer washes and dehydrated in an ascending series of ethanol. The eggs were subsequently critically point dried using carbon dioxide as a transition fluid. The dry material was attached to a scanning stub using adhesive pressure sensitive tape and sputter coated with gold by 3 successive 1 min. bursts in an atmosphere of argon at 2 X 10 torr. All specimens were examined in a SEM operated at 20 kv and images recorded on plus-x pan 120 film.

OBSERVATIONS

Egg-shell surface structure

The mature deposited egg of *S. nudiseta* is elongate ovate, at most slightly concave dorsally and convex ventrally. It is creamy white, with a maximum length of 1.18 mm and has a diameter of approximately 0.3 - 0.4 mm (Fig. 1). The entire surface of the outer chorion is highly ridged and grooved (Fig. 2) except at the polar region surrounding the micropyle and antimicropyle poles. The surface of the chorion has a network appearance of pentagonal and hexagonal forms (Fig. 3).

The circular micropylar apparatus (approx. 3.0 um diam) at the anterior pole is quite prominent and the micropyle is situated in its center (Fig. 4). The chorion is depressed at the center to form the micropylar apparatus of the micropyle region (Figs. 4,5). The outer chorion at the micropyle region is modified to form a collar with an irregular groove around it (Figs. 4,5).

The outer layer of the chorion at the posterior pole of the egg-shell is modified to give rise a small number of aeropyles. The aeropyles at the antimicropyle region are generally rounded and sometimes appear oval-shaped (Figs. 6,7).

Chorion structure outside the hatching lines

The chorion along a mature egg consists of three distinct layers. The outermost layer appears as a pentagonal and hexagonal network (Fig. 3), derived from the vertical columns which arise from the middle layer. These vertical columns anastomose at their apices in the plane of the shell to form the outer network. The middle layer appears as a quite dense network of thick columns (Fig. 8). The columns extending out from the thick middle mesh-work were observed (Figs. 8,9). These columns branch as they travel inwards and form the inner layer which consists of irregularly shaped discs which vary in width (Fig. 9).

Structure of median strip between hatching lines

The median dorsal strip that is enclosed by the hatching lines is very distinct, tapering and extends from the anterior end of the micropyle pole to the posterior end of the antimicropyle pole of the egg (Figs. 10,11). The structure of this median dorsal strip is different from that of the chorion outside the hatching lines (Figs. 10,12). It consists of a thick middle mesh-work, the columns of which are up to 1.2 um thick at their widest point (Fig. 8). This outer layer resembles that described for the inner layer of the area outside the hatching lines (Fig. 11). At about the middle of the median dorsal strip, there is an open network of delicate fibres which form a pentagonal and hexagonal pattern. The interstices within the network vary in size from about 6.4 um wide (Fig. 13) and 136 um in length (Fig. 10). In some areas of the outer chorion, the discs fuse together to form a sponge-like structure (Figs. 14,15).

DISCUSSION

In *S. nudiseta,* the shell outside the hatching lines consists of three layers, a situation reported previously in *D. radicum*[19,20]. The outer surface of the chorion is ridged; the ridges are developed along the boundaries between follicular cells. Thus the shell may have a superficial pentagonal and hexagonal pattern of ridges. Similar observations have been made in dipteran and other insects[11, 14, 15, 20, 21, 23, 27]. Those authors reported the existence of a canaliculus hexagonal network of air canals to act as a respiratory system. In sections, the canals of the hexagonal and pentagonal system are recognizable as distinctly higher and wider spaces in the inner layer. The film of air functions as a plastron when the egg is submerged in water. Also, the plastron resists wetting by both the hydrostatic pressure and the surface

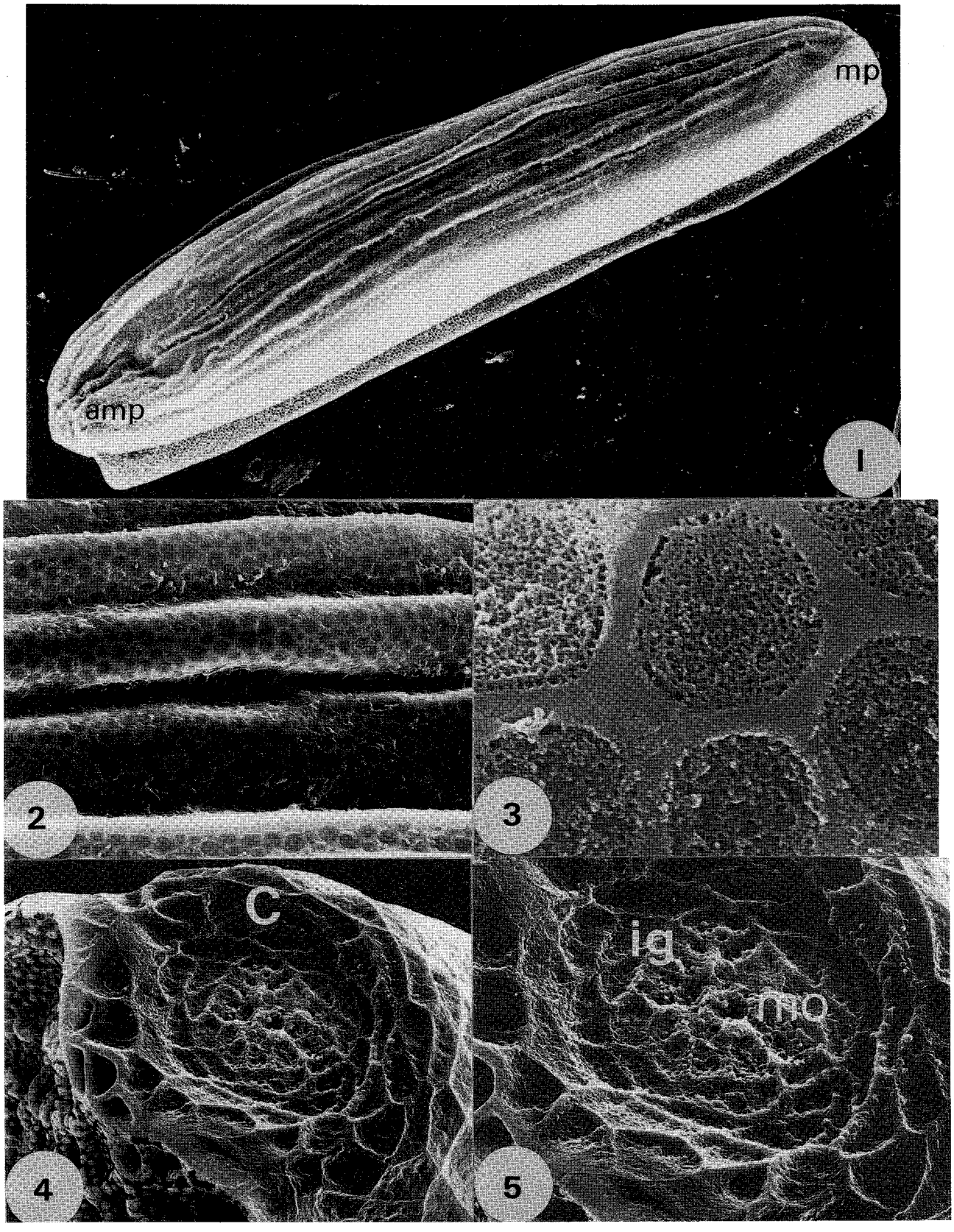

Fig. 1: Mature deposited egg of *Syntheiomyia nudiseta.* Micropyle pole (mp) and antimicropyle pole (amp) X 275.
Fig. 2: The outer chorion illustrating the highly riged and grooved surface X 1865.
Fig. 3: High magnification of the outer chorion surface. The chorion appeares as an open pentagonal and hexagonal network X 22889
Fig. 4: The micropylar region. The outer chorion is modified to form a collar (c) arround it X 2250.
Fig. 5: High magnification of the micropyle region showing that surface of the chorion appears as an irregular grooves (ig) around the micropyle opening (mo) X 4000.
Fig. 6: The posterior region of the egg showing the antimicropyle pole (amp) with round aeropyle (ap) X 758.
Fig. 7: The antimicropyle region showing the median dorsal strip extended to the antimicropyle pole. Note the aeropyles (ap) X 1500

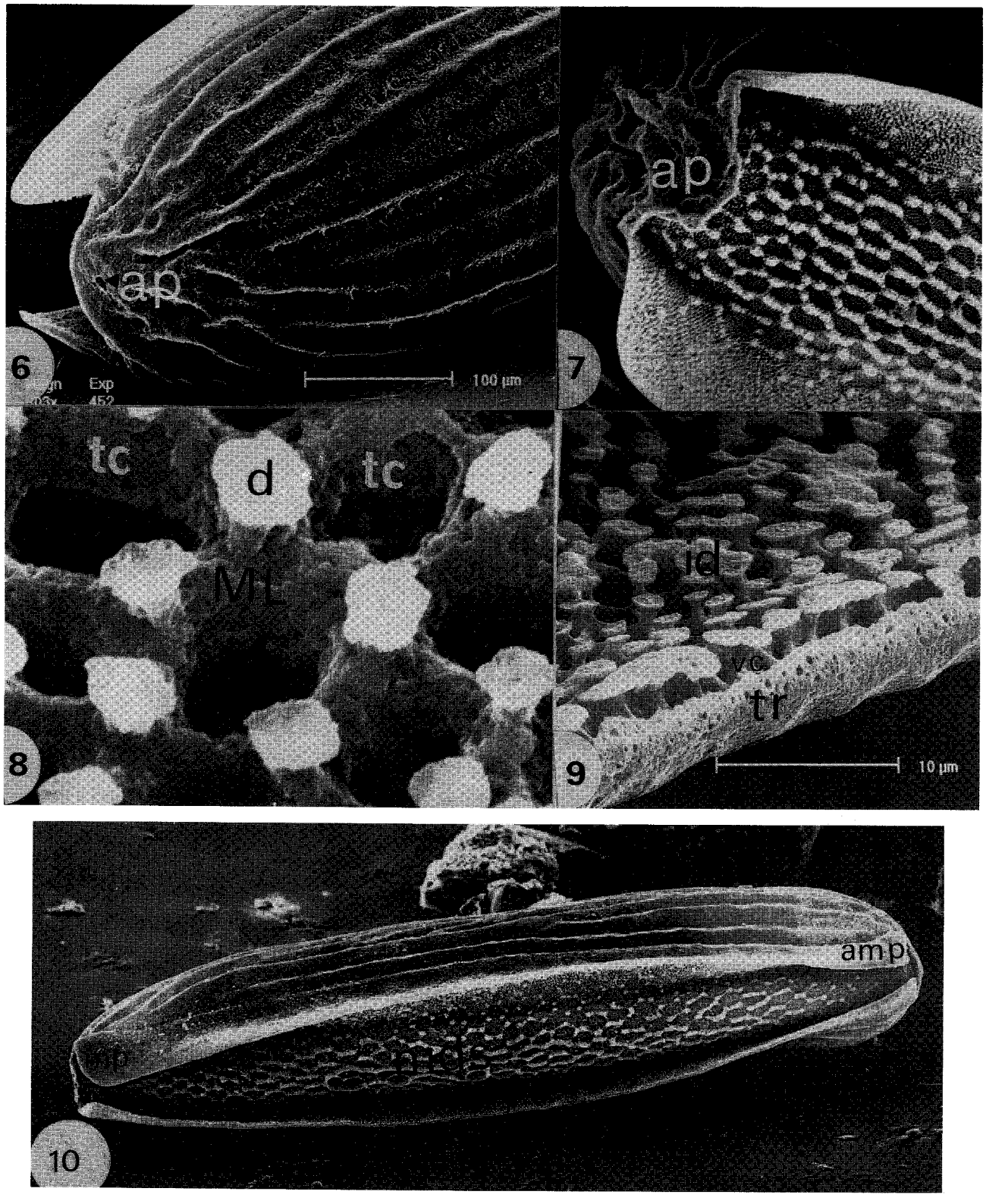

Fig. 8: Top view of the middle meshwork layer (ML) show that the disc (d) arises from the middle layer. Note the thick column (tc) X 20387.

Fig. 9: Side view of the inner layer outside the hatching lines show the vertical column (vc) branch to form irregular discs (id), thick ridge (tr) X 10000.

Fig. 10: Lateral view of mature deposited egg showing the median dorsal strip (mds) extend from the micropyle pole (mp) to the antimicropyle pole (amp) of the egg X 242.

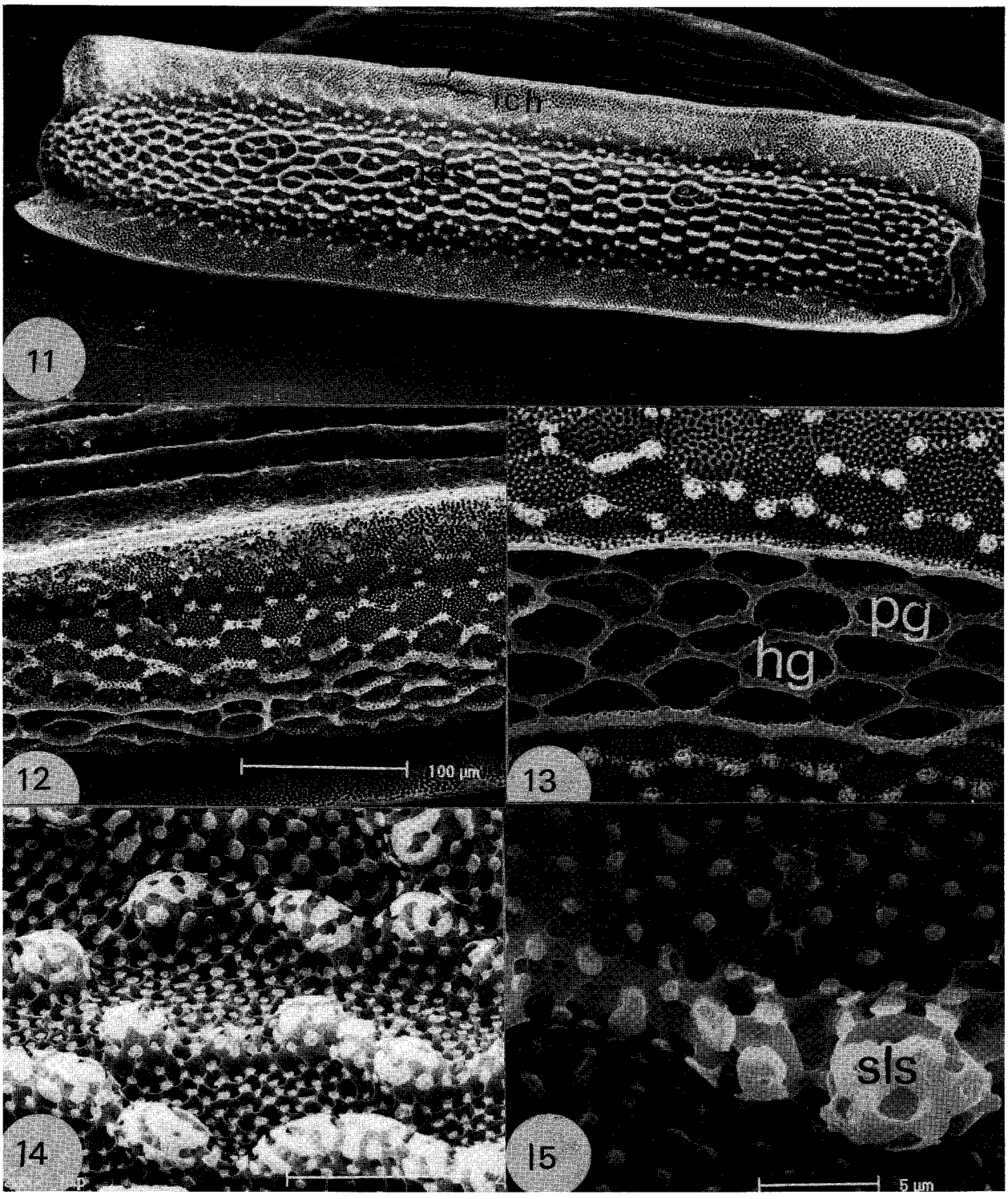

Fig. 11: Mature deposited egg showing the hatching lines open upwards and downwards. Inner chorion (ich), median dorsal strip (mds) X 321.
Fig. 12: Side view through the chorion at the median dorsal strip between the hatching lines X 792.
Fig. 13: High magnification of the median dorsal strip illustrates a pentagonal (pg) and hexagonal (hg) meshwork X 4704.
Fig. 14: The outer chorion at the median dorsal strip showing the irregular discs fuse together X 2866
Fig. 15: High magnification of the outer chorion at the median dorsal strip showing the irregular discs fuse together to from sponge like structure (s/s) X 7810.

active materials to which it is normally exposed[11,13,14, 28]. The chorion appears to provide mechanical protection and a route for the entry of oxygen[19,20 and 27].

The outer layer of the shell at the enterior region is modified to form an irregular groove at the micropyle. The chorion has invaginated inwards at the center to form the micropylar apparatus. Also, the outer layer of the chorion at the posterior pole of the egg is modified to give a small number of aeropyles. The structure of the respiratory system of the chorion is quite remarkable for its very long and narrow aeropyles[11,20]. Direct communication between the ambient air and the inner layer of air in the shell is restricted to the aeropyles and the micropylar opening.

The whole surface of the egg-shell is highly ridged and grooved except at the collar surrounding the micopyle and the area between the hatching lines. The hatching lines are on the median of the dorsal surface and extend from the micropyle to the antimicropyle; throughout most of their length they are more or less parallel. The chorion splits along haching pleats when the larva hatches. It is thought that the hatching pleats and dorsal strip have a plastronic effect [9]. The chorion between the hatching lines is similar as elsewhere except in the outer layer. Columns arising from the middle layer branch at their apices to form irregular shaped discs. Similar observations have been made in the eggs of *D. radicum*[18,20], *Polietes lardaria*[11], *Calliphora erythrocephala*[12] and *Orthellia caesrion* [19]. In the present investigation, an open network of delicate fibers forming a pentagonal and hexagonal pattern was observed. This structure may be the first part to split and the larva emerges from it at the beginning of the hatching process. Rodriguez *et al.* [22] showed that egg hatching in *Anopheles albimamus* is aided by a chisel-shaped spine. This hatching tooth is surrounded by a thin flexible membrane fixed to a groove in the head of the larva. Increased intracranial pressure may force the spine against the egg shell until a fissure is produced. Further opening of the egg is achieved by movements of the head and the entire body of the larva.

In conclusion, the present work illustrates the first attempt to describe the ultrastructure of the egg-shell of *S. nudiseta*. Because of its medical importance further work is needed to study the details of developmental stages of eggs by transmission electron microscopy.

ACKNOWLEDGEMENT

I would like to express my deep gratitude to Dr. I.S. El-Noami, Dean of the Faculty of Science, University of Qatar and to Professor A.M. Kamal, for the kind facilities extended to me during the performance of this work.

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