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MORPHOLOGICAL STUDY ON EGGS OF *SPILOSTETHUS PANDURUS* (SCOPOLI) (HETEROPTERA: LYGAEIDAE)

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Abstract

The milkweed bug, *Spilostethus pandurus* egg was re-described and photographed using light and scanning electron microscopes. Egg whole-mount showedthe different development stages from zero to 116 hrs. Egg dechorionation was unfeasible at the early stages, from 0-100 hrs. SEM showed the hatching lines and weakness lines, and chorion with 11-13 micropylar openings.

Key words: *Spilostethus,* Milkweed bugs, Lygaeidae, Egg, SEM, Hatching lines, Micropyles.

Introduction

Spilostethus pandurus subsp. *Pandurus* (Scopoli, 1763) of Family Lygaeidae, native to Albania, Austria, Bulgaria, Corsica, Crete, Crimea, Cyprus, Czech Republic, France, Germany, Greece, Hungary, Italy, Portugal, Russia, Sardinia, Spain, Switzerland, Yugoslavia, Algeria, Egypt, Libya, Morocco, Nigeria, Senegal, Sierra Leone, Slovakia, Sudan, Kenya, Arabia, Iran, Iraq, Israel & India causimg serious damage to seeds of plants (Döring, 2022). *S. pandurus* occurs in Egypt all year round (Awad *et al.* 2013).

The rear and maintain desirable experimental model in biological laboratories (Liu and Kaufman, 2009). Chorion forms a barrier to protect eggs and embryos from possible disadvantageous environmental influences. Chorion enables gas exchange and maintains proper humidity usually performed by distinct and structurally specified regions of chorion (Chapman, 2013). Its morphology reflected important evolutionary adaptations and characters used for phylogenetic considerations (Dominguez and Cuezzo, 2002).

The present study aimed to re-describe laboratory reared *S. pandurus eggs* by using light and scan electron microscopy.

Materials and Methods

Rearing: *S. pandurus* colony was initiated from nymphs and adults collected from sunflower plants *Helianthus annuus* (Asterales, Asteraceae) during April 2019, from Fayoum Governorate. Detailed descriptions of rearing technique were given (Elelimy *et al*, 2017). Stock culture of *S. pandurus* was maintained in cylindrical plastic containers (20 cm indiameter and 22 cm in height). The upper lids of the containers were fitted with fine mesh screen for ventilation. All the containers were tightly covered from above with muslin kept in place with rubber bands to prevent the insects from escaping. Insects were fed on peeled fresh sunflower seeds placed in the bottom of the containers. Water supplied from external container by means of cellucotton in Petri-dish for oviposition. The cellucotton containing masses of eggs were removed and new ones were introduced. Ten groups of 100 newly laid eggs were put into ten 50 cc beaker onto a piece of cotton and the beaker was covered. The time of hatching was recorded.

Experiments were done under control-ed laboratory conditions from June to September, at 30±2°C, 60±5% RH & 14:10 LD; as well as, in winter season, from November to February, at 22±2°C, 60±5% RH & 14:10 LD, and each was repeated 3 times.

Light microscopy: Laid eggs were examined under laboratory conditions, and adults were daily observed for laying eggs. Number of eggs laid by each female was recorded. They were separated with a fine brush, put on a wet cotton piece in Petri-dishes at room temperature (25-30°C). From 0-100 hours it was difficult to recognize embryonic development, eggs were dechorionated from 102-

106h (hour) in 7% hypochlorite solution for few seconds, washed with distilled water, fixed in warm Bouin's solution and then were examined and photographed. Photos were taken by Canon Power shot Camera G12 attached to Boeco Stereomicroscope.

Scanning electron microscopy: Eggs were fixed for an hour in 3% buffered pH 7.2 glutaraldehyde, washed for 2min in 7.2 pH phosphate buffer, dehydrated in ascendingethanol series, coated with gold by the Sputter coater, and then examined and photographed using SEM JEOL JSM 5200.

Results

The electronic microscopy of eggs showed that the milkweed bugs, *S. pandurus*, eggs were oval 0.67±0.02 x1.2±0.034 mm. Freshly laid ones pearl colored and became orange just before hatching. Anterior and posterior poles slightly rounded, anterior one surrounded by a crown of micropylar process numbered 11-13, Micropyles funnel shaped lay near egg anterior pole, funnel orifice measures 27-43µm as to age, Each micropylar opening with a singlesmall orifice with wide apical portion and narrow base.

Chorion thin, opaque without apparent sculptures lacking chorionic spines strong protecting outer layer, with longitudinal lines of weakness per hours before hatching, twolines one transverse and one longitudinal for embryo's emergence, incision line or hatching line straight longitudinal ones middle of anterior pole down to fore side. Egg burster helps nymph emerging attached at chorion anterior part, chorion removal from eggs after hatching.

Embryonic eggs development didn't appear in early stages, but just chorion and micropyles, with germ band located along ventral side facing yolk. At this stage, embryo consists of a thin sheet of cells surrounding egg mass, blastoderm thickened area at side plates fuse medially, and reverses its position and a germ bandrudiment after 28h (anatrepesis stage with shortening germ band). Egg development after 30h being cellular density to form blastoderm surface, the plates form germ band proper, and remaining cells contribute to extra-embryonic tissues.

Early site of invagination and glands on A5 & A6 tergites faint pigmentation, blastoderm stripes visible, six initialstripes correspond to mandibular followed by three thoracic segments, as chorion line of weakness.

Katatrepsis starts from 92-104h old eggs with amnion and serosa rapture fill peripheral space, two membranes firmly attached together at early Katatrepsis, but at mid Katatrepsis, head, antenna, legs, and abdomen bend backward. At late Katatrepsis, head near to anterior pole and serosa degenerates, and eggs dechlorinated after 100h to show appendages stackedtogether enclosed by body wall and mandibular and thoracic appendages development.

The details were given in figures (1, 2, 3, 4, 5, 6, 7, & 8)

Discussion

Hemipteran eggs were described by Butt (1949), Bhattacherjee (1959) and Slater and Sperry (1973) and other hemipteran species were described by Bianchi *et al.* (2011), Candan and Suludere (2000) and Candan *et al.* (2021). The morphological characteristic of the *Spilostethus pandurus* egg showed distinct differentiation of significant taxonomic importance in various insect orders (Canden *et al,* 2005). Generally speaking, eggshells are important to protect the developing embryo from external damage, parasites, predators, & desiccation (Margaritis, 1985). *Spilostethus pandurus* eggs aggregated together and firmly attached. Freshly laid ones about 30-50 eggs at atime were soft and gradually harden; these changes were due to egg capsule protein stabilization (Dosary *et al.,* 2010).

In the present study, the light and SEM microscopes clearly showed the external surface of *S. panders'* chorion, without any sculpturing or chorionic spines. This agreed with Canden *et al.* (2005) who reported that the surface of the egg of *Ceraleptus obtusus* was covered by perforated polygons and had chorionic spines. The chorion layer was th-

ick, usually of an electron densematerial and generally characterized by remarkable elasticity and resistance because it controls the interaction between environment and embryo through structures as micropyles or aeropyles. This agreed with Hinton (1961) for *Nepa cinerea* Linne, who found that the plastron holes communicate to the inner part of the horn where a meshwork is developed.

Also, in *S. pandurus* dechorionation treated by hypochlorite solution clarified embryonic structures of late developing. This agreed with Ibrahim and Elshewy (2020)

Undoubtedly, the egg cell structure is intimately related to embryonic pattern information, and as the chorion was laid during oogenesis, some provision was indicated to allow the entry of sperm when the egg is fertilized. This agreed with Turner and Mohowald (1976), Hinton (1981), Al-Dosary *et al.* (2010). Besides, the micropylar region plays an important role in sperm reception and activation of the egg cell and enables gas exchange of the developing embryo (Wolf *et al,* 2003).

In the present study, the micropylar projections in *S. pandurus* varied from 11-13 in numbers. In other heteropteran species, micropylar processes showed variation in numbers and sizes. This agreed with Esselbaugh (1946); Southwood (1956) and Javahery (1994) who dealt with *S. pandurus.* Besides, several micropylar apparatus have different set of openings for the spermatozoa penetration (Yamauchi and Yoshitake, 1984).

In the present study, the SEM, showed lateral hatching lines, split after complete development of the embryo. These lines' appeared within 46h before eggs hatching. Generally, the hatching lines were demonstrated in the eggs of some dipterous flies (Endris *et al,* 1987) to facilitate lateral hatching lines of eggs. In the present study, segmentation and appendages appeared 40 & 106h, respectively. This agreed with Liu and Kaufman (2004) who reported the segmentation in *Oncopeltus fasciatus* occurred in two phases during the blastoderm and germ band stages.

In the present study, the SEM clarified the hatching lines and the appendages of the developing embryo. Also, Liu and Kaufman (2004) in *O. fasciatus* reported that as early as 36h the blastoderm separated into six segments. They added that the milkweed bugs underwent intermediate germ band segmentation resembling the *Drosophila* blastoderm, and that the milkweed bugs showed invaginated type referring to cell movement and gave rise to the germ band.

Conclusion

The study showed the different stages during embryonic development in *Spilostethus pandurus.* These given data showed the ability of *Spilostethus pandurus* process of the egg hatching.

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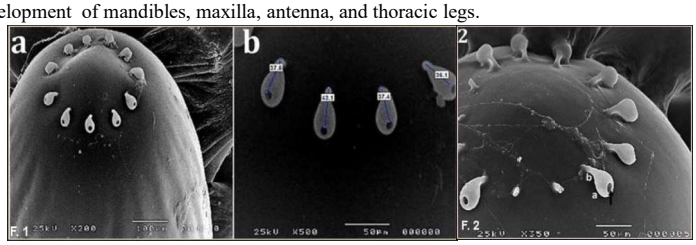
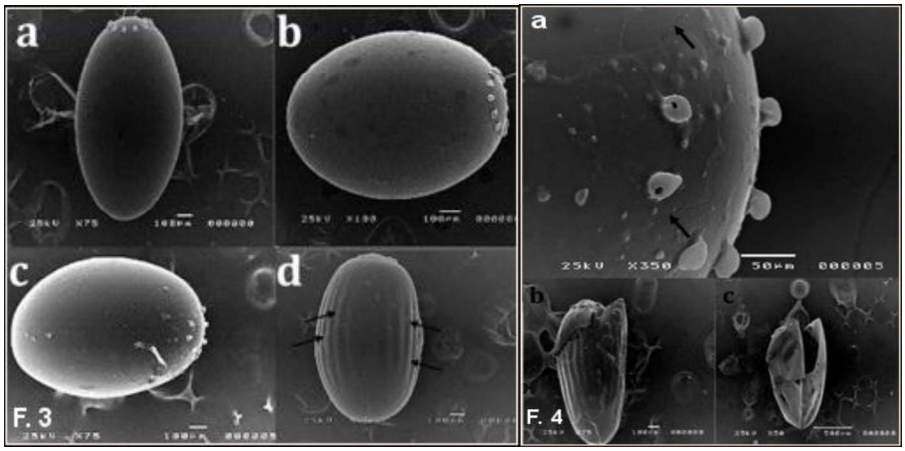
Explanation of figures

Fig.1: SEM of *S. pandurus* egg showed (a) micropylar region, and (b) micropylar process.
Fig. 2: SEM of egg showed micropylar process in theapical portion, (a) black arrow showed orifice and (b) basal stalk.
Fig. 3: SEM of egg showed (a) dorsal view, (b) lateralview, (c) smooth exochorion layer, and (d) black arrows lines weakness.
Fig. 4: SEM of egg showed (a) hatching lines, and (b-c) chorion rupture of nymph's emergence 100-116h.
Fig. 5: Eggs showed (a) 0-2 h old with singlenucleus in center, (b) 5 h old nuclear division, (c) 12-15 h old cleavage of cells in cytoplasm, (d) 18 h old egg.
Fig. 6**:** Eggs showed (a) 22 h old blastoderm formation, (b) 28 h old embryo anatrepesis phase, (c)30 h old cellular density.
Fig. 7: Eggs showed (a) 36-40 h old black arrows invagination and glands on abdomen A5 &A6. (b) 36-40 h old 6 stripes from mandibles to 3 thoracic segments. (c) 46 h old lines of weakness.
Fig. 8: Eggs showed (a) 116-112 h old development. (b) 116-118 h old embryo development of mandibles, labium, antenna, and thoracic legs. (c) 106-112 h old development of mandibles, maxilla, antenna, and thoracic legs.

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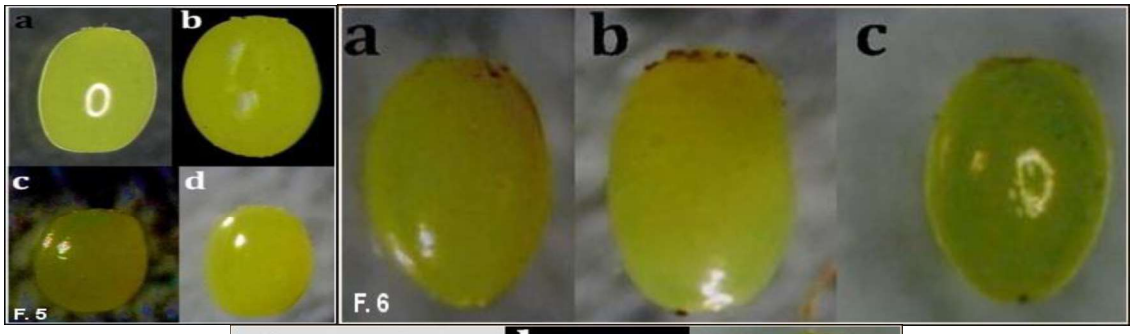
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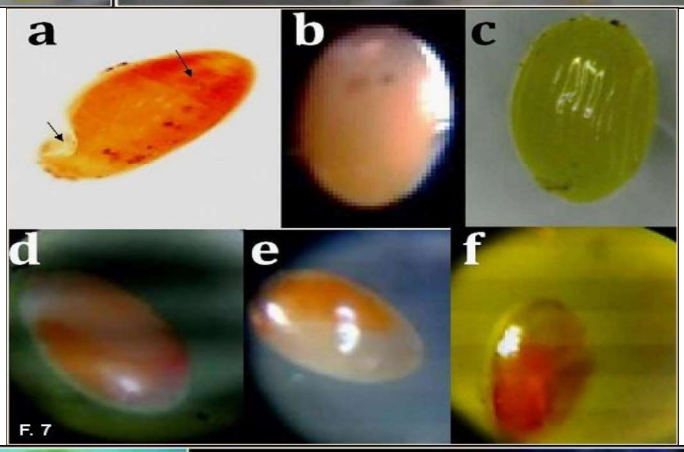
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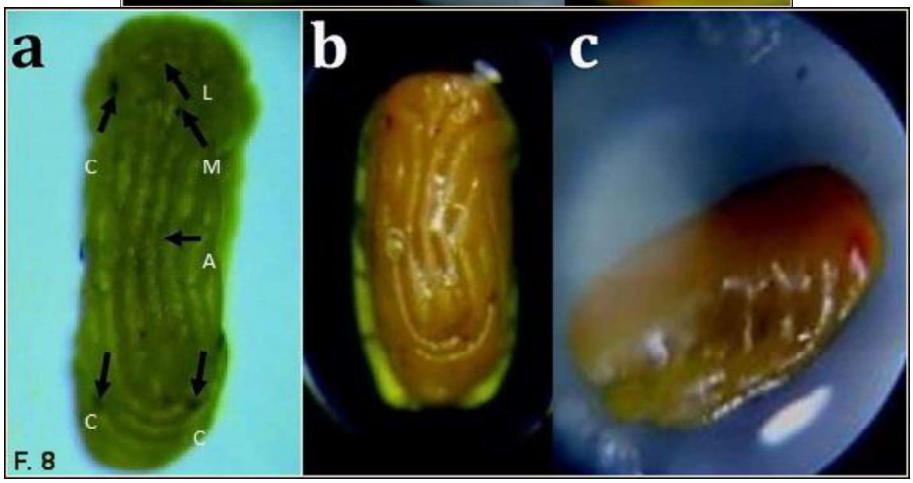
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