Ultrastructure of the Chorion of *Echthistus cognatus* (Loew, 1849) (Diptera, Asilidae)[1]

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ABSTRACT. The fine structure of the *Echthistus cognatus* (Loew 1849) chorion was studied using both transmission (TEM) and scanning electron microscopes (SEM). Females were collected in the field and kept under laboratory conditions. Eggs were individually deposited in cotton batting. They were a light yellow color and elongated in shape after the deposition, with an average length of 1.5 mm and an average width of 0.4 mm. Except for the micropylar region, ridges extend all over the surface of the chorion. Aeropyles with different size and shape were present between ridges. There is a single micropylar opening at one pole of the egg. In the thin section, chorion forms a single layer. Chorionic ridges and sunken area are seen in the section.

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INTRODUCTION

Scanning electron microscopy is an ideal tool for detailed description of the micro-sculpture of insect eggs. Scanning microscopy of eggs allows the separation of Diptera species (Salkeld 1980; Kula 1988; Kuznetsov 1988; Linley and Chadee 1990; Sahlen 1990; Mouzaki and others 1991; Feliciangeli and others 1993; Greenberg and Singh 1995; Service and others 1997; Suludere and others 2000a,b; Choochote and others 2001). Although an extensive survey of morphological structures of insect eggs has been conducted (Hinton 1981; Margaritis 1985), little information concerning the surface structure of eggs of Asilidae is present. Some articles that cover robber fly ovipositon include a superficial description of the eggs (Lavigne 1963a,b, 1964, 1979, 1984; Dennis and Lavigne 1975, 1976a,b,c, 1979; Lavigne and Dennis 1975, 1980, 1985; Dennis and others 1986; Lavigne and Bullington 1984). Additionally, Castillo and others (1994) have described the eggs of six species representing the subfamilies Stenopogoninae, Asilinae, Laphriinae, and Dasypogoninae using scanning electron microscopy. Musso (1981) studied the morphology and development of the immature stages of some robber flies and classified the eggs into three groups; pigmented eggs, unlimited ones, and eggs covered with sand. According to Musso (1981) unpigmented eggs can be subdivided into two types: Ornamented eggs, such as *Machimus fimbriatus* (Meigen) and *Dystolmus kiesenwetteri* (Loew) and unornamented eggs are observed in *Machimus rusticus* and *M. pilipes* (Meigen). This paper is concerned with the investigation of the morphological structure of the eggs of *E. cognatus* with SEM and TEM.

MATERIALS AND METHODS

Deposited eggs of 10 female *E. cognatus* collected in Aksaray, Eskil Esmekaya village, Turkey, were examined. Females deposited eggs into cotton batting in plastic jars. The eggs were gently removed from the cotton fibers and prepared for SEM by the use of the previously described methodology (Suludere 1988). The cleaned and dried eggs were mounted with double-sided tape on SEM stubs and coated with gold in a Polaron SC 502 Sputter Coater. They were examined with a JEOL JSM 5410 Scanning Electron Microscope at 15 kV and photographed with Mitsubishi video copy thermal paper. Other eggs were fixed with 2.5% glutaraldehyde in a phosphate buffer (pH 7.2) for 2 hours and post-fixed with 1.0% osmium tetroxide in phosphate buffer (pH 7.2) for 1 hour. The samples were embedded in Glauert's araldite medium and the ultra thin sections were stained with Reynold's lead citrate following uranyl acetate. They were examined with a Zeiss EM 900 Transmission Electron Microscope operated at 80 kV.

RESULTS AND DISCUSSION

Robber flies show a highly diversified egg deposition behavior. The females of some species, such as *Mallophora fautrix* (Osten Sacken) (Alcock 1974), place their egg clusters at the tip of dried weed stems; *Megaphorus guildianus* (Williston) (Dennis and Lavigne 1975) deposit large number of eggs in clusters covered with a soft chalky-white material along dead stems of plants. Others such as *Efferia helenae* (Bromley) and *Diogmites angustipennis* Loew deposit their eggs in the soil (Lavigne and Holland 1969). *Antipalus varipes* Meigen cover the eggs with sand grains (Musso 1981). *M. rusticus* females lay multiple eggs inside the stems of graminaceous plants (Musso 1981). *M. rusticus* eggs are deposited singly into cotton batting under laboratory conditions and are pale yellow in color when deposited (Suludere and others 2000a). *E. cognatus* shows the same behavior and the eggs are light yellow color after deposition.

The eggs of *E. cognatus* are elongated; they are 1.5 mm in average length and 0.4 mm in average width (Fig. 1a). Scanning electron microscopy studies revealed different features of both sides of the egg. General egg sculpture disappears in the side where micropyle takes place (Fig. 1b) and a micropylar opening is located at the middle of this region. In this region,

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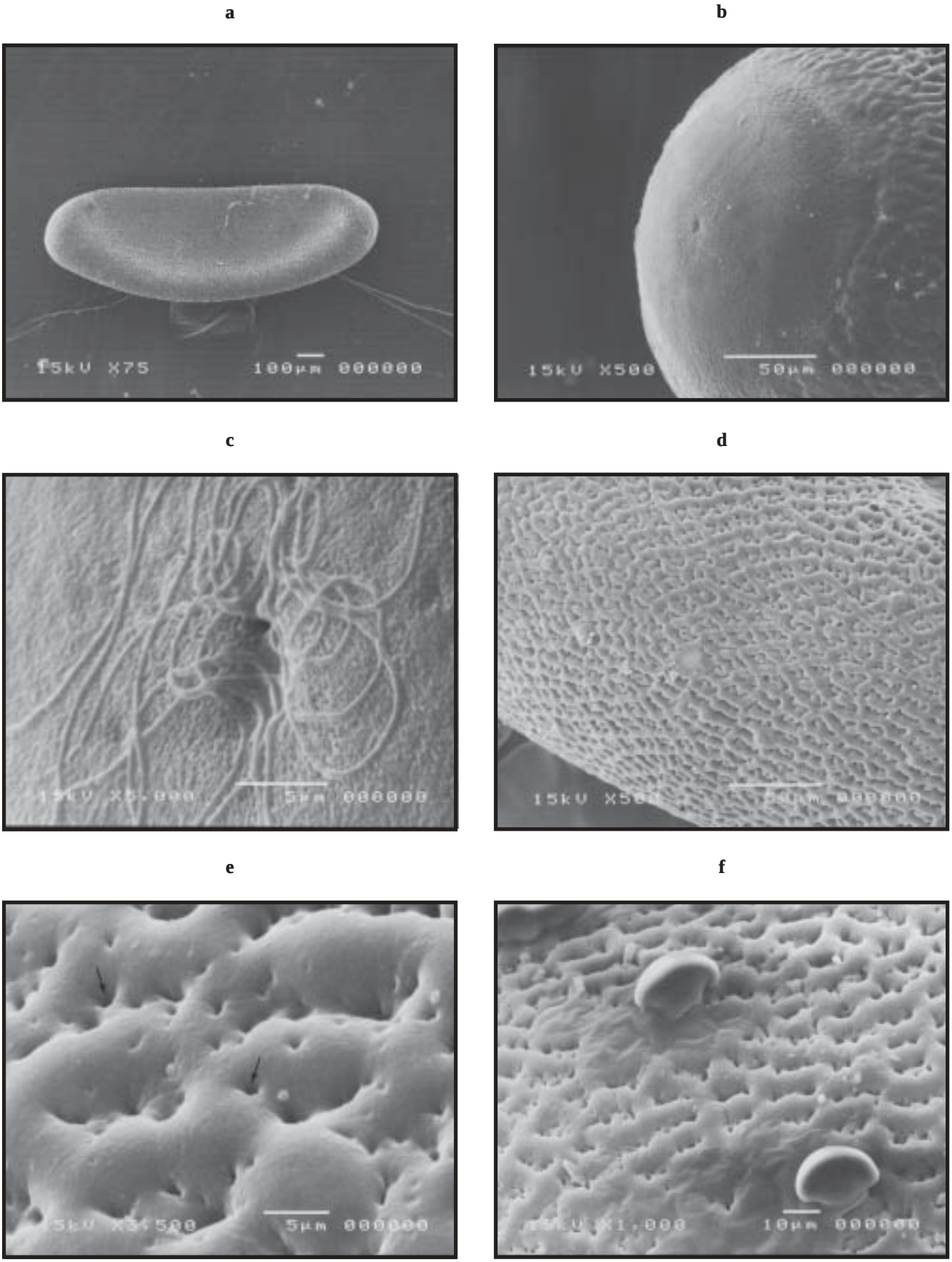

FIGURE 1. a) The general view of *Echthistus cognatus* egg, SEM photo; b) Micropylar opening and micropylar region of *E. cognatus* egg; c) Micropylar opening in the micropylar region of egg; sperm tails remained in outside micropylar region of egg; d) Ridges extend all over the surface of chorion; e) Ridges extend in the surface of chorion and aeropyles (→); f) Mushroom-like structures in the surface of chorion.

micropyle (a special opening near the anterior end of the chorion which serves as a gateway for the entry of sperm during fertilization) is slightly collapsed and this side contains only one micropyle. In some eggs, tails of sperm cells trying to pass through the egg were noticeable (Fig. 1c). Although Castillo and others (1994) have shown the presence of one micropyle in the eggs of *Cnodolomyia* sp. (Fabricus) and *Archilestroides guimareaensis* (Artigas and Papavero), the eggs of *Paratractia dasypus* (Wiedemann) and *Taperigna diogmitiformis* (Artigas and Papavero) displayed two micropyles. On the other hand *Dissmeryngodes anticus* (Wiedemann) did not have any micropyle in their eggs. Suludere and others (2000a) found aeromicropylar openings in the eggs of *M. rusticus.* These findings clearly reveal the high level of diversity at species level from the same family. The surface of chorion *E. cognatus* eggs is covered with ridges, except in the micropylar region (Fig. 1d). There are aeropyles (microscopic pores that allow respiratory exchange of oxygen and carbon dioxide with relatively small loss of water) with different sizes and numbers between these ridges. In the collapse between the ridges, aeropyles with several numbers and sizes generally were shown to take place (Fig. 1e). There are also mushroom-like structures observed on many parts of the egg surface, however, functions of the structures are not clear at the present time (Fig. 1f).

Musso (1981) subdivided the eggs of *M. fimbriatus* and *Dystolmus kiesenwetteri* into unpigmented and ornamented eggs, and he described similar structures on their surfaces. However, Musso (1981) did not mention any such structure appearing on the eggs of *M. rusticus* he studied. Lawson and Lavigne (1984) described very small, rounded projections and scattered larger, elevated bodies on the surface of eggs of *Colepia abludo* Daniels. Clements and Skidmore (1998) also observed similar formations on the surface of eggs of *Asilus crabroniformis* L., a species closely related to *M. rusticus*; they appear to be natural, possibly a yeast contamination. Sites and Nichols (1999) reported that numerous globules adhered to the surface of eggs of *Ambrysus montandoni* La Rivers (Naucoridae, Hemiptera) and suggested that although these globules might be artifacts, they persist even after sonication in 100% acetic acid. Suludere and others (2000a) observed that, on the surface of eggs of *M. rusticus*, small rounded bodies cover much of the surface except one of the poles. They vary in size and shape and are connected to each other; some are single. The rounded bodies are intermixed with larger, dome-like projections with sloping sides, smooth apical surfaces, and a rounded or ovoid opening that may lead to a hollow interior. Suludere and others (2000a) found that the thin sections chorion of *M. rusticus* is composed of exochorion and endochorion. Exochorion shows a fibrous matrix with homogeneous electron dense rounded bodies and smaller electron dense oval structures embedded into it. Endochorion differs from the exochorion in the degree of homogenity of the rounded bodies and the density of its structure. Upon examination of a thin section of chorion of *E. cognatus*, the chorion is observed to be a discernible single layer. Chorionic ridges and sunken areas are apparent in the section. The inner layer shows trabecular vertical columns (Fig. 2).

FIGURE 2. Chorionic ridges and sunken areas in the section of chorion of *E. cognatus* (→). The inner layer is trabecular (➔), TEM photo × 12500.

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