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Fecundidad y descripción morfológica de los huevos de *Psorophora cyanescens* (Coquillett, 1902) (Diptera: Culicidae) por microscopía electrónica de barrido

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ABSTRACT. Taxonomic studies on mosquitoes are based on the morphological description of larvae and adults. However, few studies have focused on the morphological description of eggs despite their taxonomic value. The description of mosquito eggs by scanning electron microscopy (SEM) allows more detailed descriptions of the ornamentation of the egg, which can provide diagnostic characters of the species. The objective of the work was to describe the eggs of *Psorophora cyanescens* by SEM and provide fecundity data for the species. In general, *Ps. cyanescens* eggs are elliptical and very wide. The exochorion is characterized by polygon pattern, where each polygon consists of longitudinal ridges joined by cross-ridges defining regular "hexagonal" areas. Each longitudinal ridge consisting by a small tubercle. The small tubercles are irregular, rectangular, rounded, or tubular. The ornamentation of the exochorion also has long and conical tubercles throughout the egg region. The micropylar apparatus

located in the anterior region of the egg has a prominent, continuous and thickness collar. *Psorophora cyanescens* laid mature eggs at 2.5 days post-feeding. The mean number of eggs per female was 82.20 (± 13.31). This is the first study that describes the ultrastructure of the morphology of the eggs of *Ps*. *cyanescens* by SEM. With the contribution of the present work, there are five species of the genus *Psorophora* whose eggs morphology is described by SEM: *Ps. albigenu, Ps. albipes, Ps. columbiae, Ps. cyanescens, and Ps. ferox*.

Key words: Culicidae; Scanning Electron Microscopy; SEM; mosquitoes; Mexico

RESUMEN. Los estudios taxonómicos sobre mosquitos se basan en la descripción morfológica de larvas y adultos. Sin embargo, son pocos los estudios enfocados en la descripción morfológica de los huevos a pesar de su valor taxonómico. La descripción de los huevos de mosquitos mediante microscopía electrónica de barrido (MEB) permite obtener imágenes más detalladas de la ornamentación del huevo, los cuales pueden proporcionar caracteres diagnósticos de la especie. El objetivo del trabajo fue describir los huevos de *Psorophora cyanescens* por MEB y proporcionar datos de fecundidad de la especie. En general, los huevos de *Ps*. *cyanescens* son de forma elíptica y muy anchos. El exocorión se caracteriza por un patrón de polígono, donde cada polígono consta de crestas longitudinales unidas por crestas cruzadas que definen áreas "hexagonales" regulares. Cada cresta longitudinal consiste en un pequeño tubérculo. Los pequeños tubérculos son irregulares, rectangulares, redondeados o tubulares. La ornamentación del exocorión también presenta largos tubérculos cónicos en toda la región del huevo. El aparato micropilar ubicado en la región anterior del huevo tiene un collar prominente, continuo y grueso. *Psorophora cyanescens* puso huevos maduros a los 2.5 días después de la alimentación sanguínea. El número promedio de huevos por hembra fue de 82.20 (± 13.31). Este es el primer estudio que describe la ultraestructura de la morfología de los huevos de *Ps*. *cyanescens* mediante MEB. Con el aporte del presente trabajo, son cinco las especies del género *Psorophora* que cuentan con la descripción de la morfología de los huevos mediante MEB: *Ps. albigenu, Ps. albipes, Ps. columbiae, Ps. cyanescens,* and *Ps. ferox*.

Palabras clave: Culicidae; Microscopía Electrónica de Barrido; MEB; mosquitos; México

INTRODUCTION

Psorophora (*Janthinosoma*) *cyanescens* (Coquillett, 1902) is a mosquito native to America. It has been reported in Argentina, Bolivia, Colombia, Guatemala, Guyana, Mexico, Nicaragua, Paraguay, Peru, Trinidad and Tobago, United States, Uruguay, and Venezuela (WRBU, 2021).

Females of *Ps*. *cyanescens* are persistent biters on humans and livestock and are rather difficult to detach from the host once they have started to take a blood meal (Snow *et al*., 1960). The first adults appear about a week after heavy rains and may persist for two or three weeks thereafter (Snow *et al*., 1960). Although, to our knowledge, no pathogen has been isolated from *Ps*. *cyanescens*, its importance lies in the fact that they are annoying and voracious blood biters. In Mexico, pools of *Ps*. *cyanescens* were tested for RNA of *Flavivirus*, *Alphavirus*, and *Orthobunyavirus*, and were negative (Farfan-Ale *et al*., 2009; 2010). However, its vectorial capacity cannot be ruled

out because other members of *Psorophora* are potential vectors of arboviruses. Rocio and West Nile viruses were isolated from *Ps*. *ferox* in Brazil and USA, respectively (de Souza Lopes *et al*., 1981; Kulasekera *et al*., 2001). In Argentina, Western Equine Encephalitis virus was isolated from *Psorophora pallescens* Edwards, 1922 (Mitchell *et al*., 1987), and in Colombia, Venezuelan equine encephalitis virus was found in *Psorophora confinnis* Lynch Arribálzaga, 1891 (Hoyos-López *et al*., 2016).

Most taxonomic studies on mosquitoes are based on the morphological description of larvae and adults. On the contrary, minor importance has been paid to egg morphology despite than exochorion pattern in mosquitoes has taxonomic value because allowed to identify of the species complex and has been useful in phylogeny studies (Bosworth *et al*., 1983; Reinert *et al*., 2009). Furthermore, the description of mosquito eggs by scanning electron microscopy (SEM) allows more detailed descriptions of the ornamentation of the egg exochorion, and which provide diagnostic characteristics for the species (Bosworth *et al*., 1983). Currently, in Mexico there is no study that provides data on the morphological patterns of the exochorion of mosquito eggs of even the most important medical and veterinary species.

Genus *Psorophora* includes 49 species divided into the subgenera *Grabhamia* (16 species), *Janthinosoma* (23 species), and *Psorophora* (10 species) (Harbach, 2013; WRBU, 2021). Currently, eggs of three species of *Janthinosoma*, and one of *Grabhamia* have been described by SEM (Bosworth *et al*., 1983; Pacheco *et al*., 2012, de Mello *et al*., 2017; 2018). Currently, no descriptions of the morphological patterns of the exocorion of mosquito eggs have been made in Mexico. Therefore, to contribute to the state of knowledge about the exochorionic pattern of *Psorophor*a, we used the SEM technique to describe *Ps*. *cyanescens* eggs and to provide fertility data from females collected in Yucatan, Mexico.

MATERIALS AND METHODS

***Study site*.** The mosquitoes were captured in the forest near the community of Ekmul (20° 57′ 54′′ N, 89° 20′ 56′′ W), located 27 km east of Merida city, the capital of Yucatan state. Yucatan has a distinct rainy (May-October) season and a dry season (November-April). In the rainy season, the mean rainfall is 1,000 mm, and the mean temperature is 27.5 °C. In the dry season, the mean rainfall is 300 mm, and the mean temperature is 25.1 °C (INEGI, 2021).

***Mosquito collections*.** Adults of *Psorophora* were captured in October 2020 (rainy season) using a backpack-mounted aspirator (Prokopack Aspirator®, model 1419, John W. Hock Company). The mosquitoes were captured in a low deciduous forest. The area is populated by trees typical of the region, such as *Alvaradoa amorphoides*, *Bursera simaruba*, *Ehretia tinifolia*, *Havardia albicans*, *Leucaena leucocephala*, *Mimosa bahamensis*, *Piscidia piscipula*, and the *Bromelia karatas plant*. Mosquitoes were captured during flight or when they landed on the entomologist. The capture was made between 16 and 18 hours. Mosquitoes were transported alive to the Laboratorio de Arbovirología at Universidad Autónoma de Yucatán and identified according to species using a published identification key (Darsie Jr & Ward, 1981).

Females of *Ps*. *cyanescens* (n = 10) were blood-feed on anesthetized mice (Animal Ethic authorization: CEI082015-CIR-UADY). Females were allowed to develop their eggs; they were individually kept in cardboard boxes at 27 °C and 70% RH and were fed sugar solution (10%) through cotton pads. A plastic container (7 x 7 x 5 cm) filled with 50 ml of de-chlorinated water (Hycel®) was used as the oviposition site.

***Scanning Electron Microscopy of eggs of Ps. cyanescens*.** The SEM methodology was similar to previous publications, with some modifications (Pacheco *et al*., 2012; de Mello *et al*., 2017). Briefly, the eggs were removed from the filter paper using a brush and were fixed in 2% glutaraldehyde and postfixed in 1% cacodylate buffer. Subsequently, the eggs were dehydrated in a series of increasing ethanol concentrations and critical point dried with CO_2. The eggs were then sputter-coated with gold. The SEM micrographs were obtained with a Digital Scanning Microscope (Hitachi VP-SEM SU1510, Hitachi High Technologies America, Inc.) at the Laboratorio Nacional de Biodiversidad, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

The morphology of the eggs was described using specialized terminology (Harbach & Knight, 1980). Egg measurements are given in micrometers and expressed as the mean and standard deviation.

RESULTS

Measurement and morphology of eggs of *Ps. cyanescens*. In general, the eggs of *Ps*. *cyanescens* are black, elliptical in shape, and very wide. On average, the eggs measure 734.26 µm (± 33.52) in length and 245 µm (± 12.96) in width, with an egg index (length/width ratio) of 3 ± 0.26 µm (Table 1; Fig. 1). The exochorion is characterized by polygon pattern, where each polygon consists of longitudinal ridges joined by cross-ridges that define regular "hexagonal" areas. Each longitudinal ridge consisting by a small tubercle. The small tubercles are irregular, rectangular, rounded, or tubular, and their densities ranged from 28 to 36 tubercles per cell (Figs. 2A, B). The ornamentation of exochorion also presents long and conical tubercles throughout the egg region. Long tubercles are located at the base of the chorionic cell. The micropylar apparatus located in the anterior region of the egg and has a prominent, continuous and thickness collar (Fig. 2C).

Fecundity of *Ps. cyanescens*. *Psorophora cyanescens* laid mature eggs at 60 hrs. post-feeding (2.5 days). Of the 35 females, only ten laid a total of 822 eggs. The mean number of eggs per female was 82.20 (± 13.31).

Table 1. Average egg size of mosquitoes of the subgenus *Janthinosoma*. SD, Standard deviation value.

Species	Mean and SD length (µm)	Mean width (µm)	Egg index (µm)	Author
Ps. cyanescens	734.26 ± 33.52	245 ± 12.96	3.00	In this work
Ps. ferox	816.8	205.6	3.97	de Mello *et al*., 2018
Ps. albigenu	586.4	172.3	3.40	Pacheco *et al*., 2012
Ps. albipes	538.67	157.66	3.42	de Mello *et al*., 2017

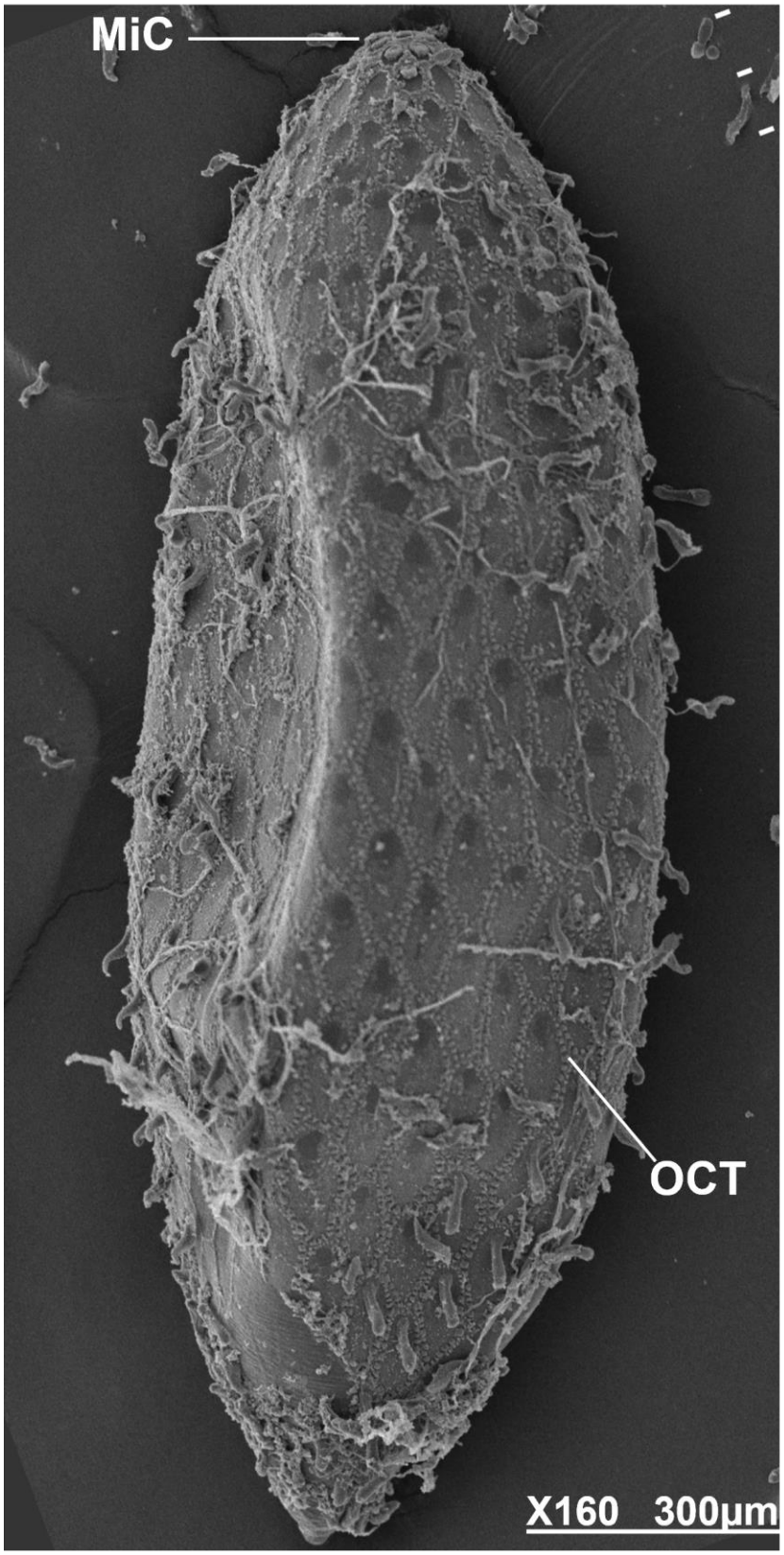

Figure 1. Ventral (upper) view, anterior end at the top of the entire eggs of *Psorophora cyanescens*. MiC, micropylar collar; OCT, outer chorionic tubercle.

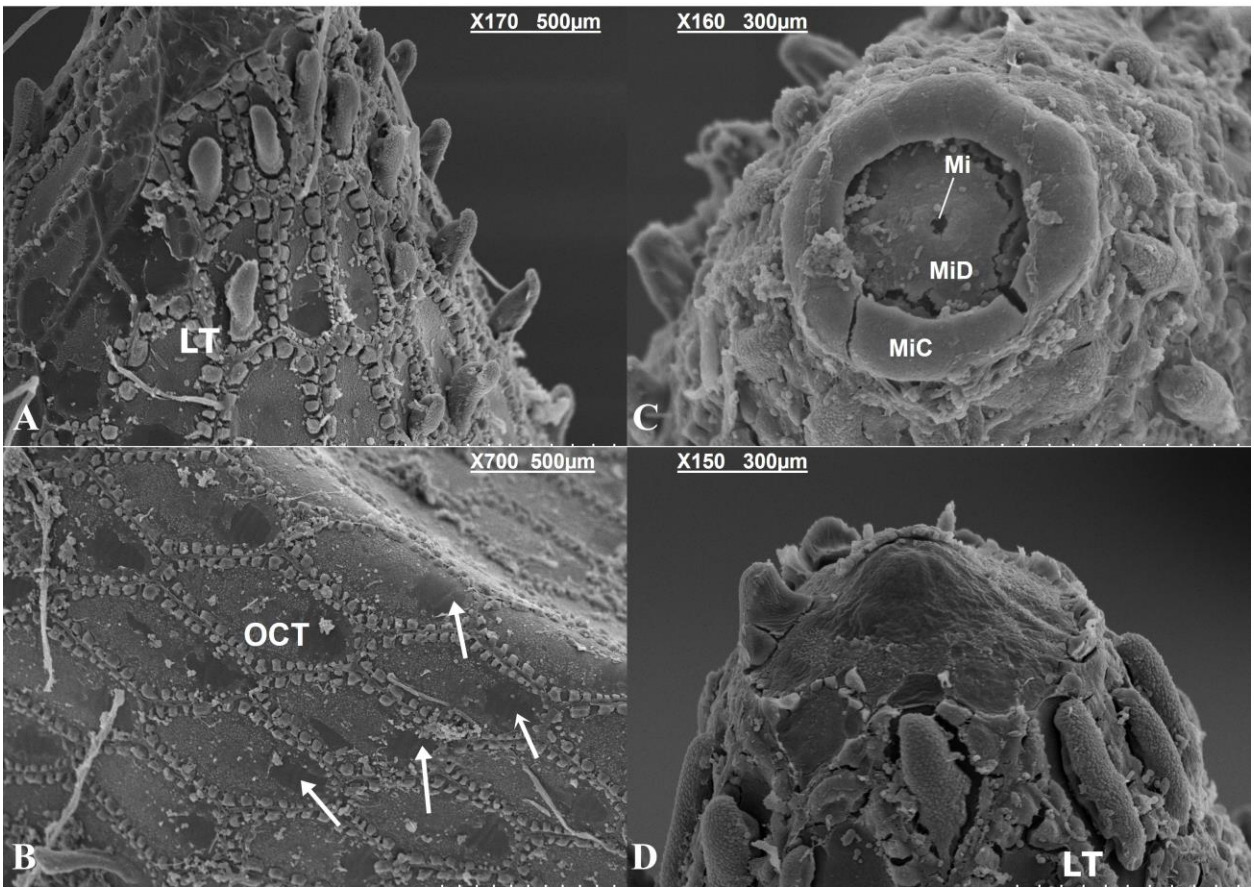

Figure 2. Egg of *Psorophora cyanescens*. A) typical ornamentation of the outer chorionic reticulum showing long tubercles (LT) located at the base of the chorionic cell; B) Outer chorionic reticulum (OCT), the shadows indicated by the white arrows indicate the absence of long tubercles; C) anterior region of the egg showing the micropylar apparatus, formed by a micropyle (Mi), micropylar disc (MiD), and micropylar collar (MiC); D) posterior region of the egg showing long tubercles (LT).

DISCUSSION

With the contribution of the present work, there are currently five species of the genus *Psorophora*, to which SEM describes the morphology of the eggs. Previously in the subgenera *Janthinosoma*, eggs of *Psorophora ferox* (von Humboldt, 1819), *Psorophora albigenu* (Lutz, 1908), and *Psorophora albipes* (Theobald, 1907) were described by SEM (Pacheco *et al*., 2012; de Mello *et al*., 2017; 2018). While in the subgenus *Grabhamia*, eggs of *Psorophora columbiae* (Dyar & Knab, 1906) were also described by this technique (Bosworth *et al*., 1983).

The eggs length of *Ps*. *cyanescens* (734.26µm) is similar to *Ps*. *ferox* (816.8 µm), but it is longer than *Ps*. *albigenu* (586.4 µm) and *Ps*. *albipes* (538.67 µm). However, the egg of *Ps*. *cyanescens* is wider compared to the mentioned species (Table 1). Hexagonal-shaped chorionic cells are similar to the eggs of *Ps*. *cyanescens* and *Ps*. *albigenu* (Pacheco *et al*., 2012). *Psorophora ferox* combines hexagonal and pentagonal shapes (de Mello *et al*., 2018), and *Ps*. *albipes* has pentagonal shapes (de Mello *et al*., 2017). Conical tubercles are common structures in exochorion ornamentation in *Psorophora* (Pacheco *et al*., 2012; de Mello *et al*., 2017; 2018). However, tubercles

vary intra and inter-species. de Mello and collaborators (2018) observed differences in tubercles number, length, and width in *Ps*. *ferox* populations from the United States, Trinidad and Tobago, and Brazil. In the present study, the long and conical tubercles of *Ps*. *cyanescens* were not measured. Further studies should consider the morphometry of mosquito egg tubercles.

The time between blood feeding and egg laying was 2.5 days for *Ps*. *cyanescens*, it is a very short period compared to seven days for *Psorophora howardii* (Coquillett, 1901), *Psorophora ciliata* (Fabricius, 1794), and *Ps*. *ferox* (Zhu *et al*., 2014). The temperature difference could explain part of the explanation. In the present study, egg maturation was conducted in a slightly warmer environment (27 °C) compared to the previous study (26 °C). The fecundity of *Ps*. *cyanescens* and *Ps*. *ciliata* is similar, both lay an average 82 and 81 eggs, respectively (Zhu *et al*., 2014). In contrast, *Ps*. *howardii* lays an average of 56 eggs. It was observed that a single blood feeding is sufficient for *Ps*. *cyanescens* to develop and mature eggs. In contrast, at least two blood meals are necessary for *Ps*. *howardii* oviposition (Gerberg *et al*., 1994).

The results indicate that with SEM it is possible to identify distinctive eggs characteristics between species of the subgenus *Janthinosoma*. There are currently five species of the genus *Psorophora*, to which SEM describes eggs morphology: *Ps*. *albigenu*, *Ps*. *albipes*, *Ps*. *columbiae*, *Ps*. *cyanescens*, and *Ps*. *ferox*.

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