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Morphological description of the pupa of *Aedes aegypti* (Diptera: Culicidae) using a scanning electron microscope

[*Descrição morfológica da pupa de Aedes aegypti (Diptera: Culicidae) usando um microscópio eletrônico de varredura*]

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ABSTRACT

Yellow fever mosquito, *Aedes aegypti*, has been associated with several severe human diseases. Larvae and adults of *Ae. aegypti* has been widely studied, but pupae have not received much attention. In this study, external morphology and chaetotaxy of *Ae. aegypti* pupae were investigated using scanning electron microscopy (SEM) to determine additional morphological characteristics for proper species identification. *Ae. aegypti* pupae exhibit high mobility with rapid submergence in response to external disturbances. Pupae contact air through paired respiratory horns. A pupal body is covered with a translucent cuticle composed of a cephalothorax and a narrow, articulated abdomen. Anterior region of cephalothorax is occupied by mouthparts and a flattened head. Adult's compound eyes are visible on both sides of their head. Before compound eyes, antennae emerge in upper part and are bent in reverse along sides of thorax. First abdominal segment has two straightforward palmate bundles, essential for maintaining pupal body above water's surface. Compared to other eight abdominal segments, ninth is relatively tiny; it holds a couple of movable appendages called paddles which are fundamental organs in pupal movement, and a midrib supports them. This morphological analysis of pupae attributes of *Ae. aegypti* is critical for controlling mosquito-borne diseases and taxonomy.

Keywords: *Aedes aegypti*, Morphology, SEM, Pupal stage

RESUMO

O mosquito da febre amarela, Aedes aegypti, tem sido associado a várias doenças humanas graves. As larvas e os adultos do Ae. aegypti foram amplamente estudados, mas as pupas não receberam muita atenção. Neste estudo, a morfologia externa e a caetotaxia das pupas de Ae. aegypti foram investigadas por meio de microscopia eletrônica de varredura (MEV) para determinar características morfológicas adicionais para a identificação adequada da espécie. As pupas de Ae. aegypti apresentam alta mobilidade com rápida submersão em resposta a distúrbios externos. As pupas entram em contato com o ar por meio de chifres respiratórios emparelhados. O corpo da pupa é coberto por uma cutícula translúcida composta por um cefalotórax e um abdômen estreito e articulado. A região anterior do cefalotórax é ocupada por peças bucais e uma cabeça achatada. Os olhos compostos do adulto são visíveis em ambos os lados da cabeça. Antes dos olhos compostos, as antenas emergem na parte superior e são dobradas em sentido inverso ao longo das laterais do tórax. O primeiro segmento abdominal tem dois feixes palmados retos, essenciais para manter o corpo da pupa acima da superfície da água. Comparado com os outros oito segmentos abdominais, o nono é relativamente pequeno; ele possui dois apêndices móveis chamados pás, que são órgãos fundamentais no movimento da pupa, e uma nervura mediana os sustenta. Essa análise morfológica dos atributos das pupas do Ae. aegypti é fundamental para o controle de doenças transmitidas por mosquitos e para a taxonomia.

Palavras-chave: Aedes aegypti, morfologia, MEV, estágio pupal

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INTRODUCTION

Mosquitoes play a crucial role in medical entomology because of their potential role as vectors of many pathogens. Yellow fever is a global trouble of public health, transmitted by the adult female mosquito, *Ae. aegypti* which is an essential vector for dengue fever, chikungunya, and zika (Bhatt *et al*., 2013). Pupae of different genera and even species differ in their external morphology and chaetotaxy. Although the larval and adult phases of other mosquito species have been thoroughly investigated and included in the key, an attempt has yet to be made to add the pupal stage owing to its short lifetime.

Ae. aegypti has been discussed in terms of its taxonomic status. Craig *et al*. (1961) were the first to examine its genetic variability. Then, because of its similarity in morphology to *Ae. vittatus*, it was included in the genus *Stegomyia* (Service, 1970). Hill (1921) observed morphological variations in *Ae. aegypti*. The author observed that *Ae. aegypti* populations that reproduced in the wild were more intensely pigmented than those linked to human settlements. In response to this implicit correlation between color and behavior, Mattingly (1957, 1958) and Diouf *et al*. (2020) reevaluate the biology and taxonomy of *Ae. aegypti.* Most early workers used gross morphological characteristics like the respiratory trumpets and swim paddles to classify pupae. Nuttall and Shipley (1901) and Wesché and Graham (1910) conducted two of the first morphological investigations of the mosquito pupal stage. In 1920, Macfie studied in detail the pupal setae of *Ae. aegypti*, known at that time as *Stegomyia fasciata*. Later researchers Macfie and Ingram (1920), Puri (1931), and Christophers (1933) used the same nomenclature to describe *Culex decans* Theo pupal phases.

Researchers have recently reported little on the morphology of mosquito pupae, including Tanaka (2003), Bickley and Harrison (1991) for *Ae. grossbecki*, and Reid (1963), Leung and Romoser (1979), Harrison and Peyton (1984), Rossi *et al*. (2006) for *Culex cuyanus*, Stein *et al*. (2009) for *Psorophora pallescens*, Kirti and Kaur (2011) for *Culex quinquefasciatus*, Kirti (2014) for *Anopheles stephensi*, Rivera-García and Ibáñez-Bernal (2018) for *Culex bidens*, and Bangher *et al*. (2022) for *Culex aliciae*. Following Macfie's research, the relevance of the setae as a foundation for pupal taxonomy attracted greater attention. Recent investigations reported the pupal chaetotaxy are Knight and Chamberlain (1948), Penn (1949), Melero-Alcábar (2006) for *Ae. geminus* and *Ae. vittatus*, and Rossi (2017) for *Anopheles annulipalpis*.

As stated in the preceding paragraph, none of the cited authors have conducted studies to describe the pupal morphology of *Ae. aegypti* using scanning microscopic (SEM) study. So, the purpose of this study is to provide a comprehensive description of the external morphology and chaetotaxy of *Ae. aegypti* pupae using SEM, as well as to identify new taxonomic features that would distinguish this species from similar ones, which could be essential for the control of *Ae. aegypti* mosquitoes and, consequently, the control of mosquito-borne diseases.

MATERIALS AND METHODS

For more than 15 generations, *Ae. aegypti* pupae were gathered from the lab colony of Heinrich University's Zoomorphology, Cell Biology, and Parasitology Institute in Düsseldorf, Germany. All *Ae. aegypti* mosquitoes were kept during the tests in a climate-controlled insectary using the guidelines described before (Yamany *et al*., 2021).

Pupae had their exuviae removed after their last molt, then preserved in 70% ethanol, dehydrated in 96% ethanol, and then frozen in 100% ethanol. Pupal exuviae were mounted on microscope slides using Canada balsam as a permanent mounting medium. All drawings were made with the aid of a camera lucida.

The pupae were killed with ethyl acetate, and the samples were fixed in 2.5% glutaraldehyde, washed for 4 hr at 4°C in buffered sodium cacodylate (pH 7.4), Samples were dehydrated by ascending series of ethyl alcohol (30, 50, 70, 95, and 100% for 5 min/each). The samples were dried using Critical point dryer “LEICA, EM CPD300” with liquefied CO_2 and then sputter-coated with gold-palladium in Auto fine coater (JEOL, JEC-3000FC). Examination and photography of the prepared samples were done by using Etec Autoscan at 10-kV JEOL scanning electron microscope (LEO1430VP).

The cephalothorax, trumpet, abdomen, and paddle of the pupae have been investigated. This study's morphological terminology and chaetotaxy are based on Harbach and Knight (1980, 1982). Measurements were taken using ImageJ 1.53e software (Wayne Rasband and contributors, National Institute of Health, USA). All measurements for different body parts were recorded in millimeters (mm) and expressed as a range followed by mean in parentheses.

RESULTS

The *Ae. aegypti* pupa with a translucent cuticle is composed of an anterior globular cephalothorax and a flexible abdomen, which is held under the cephalothorax and used during swimming. The range and mode of the setal branches of pupal setae and chaetotaxy are shown in Table (1) and Figures (1-5).

Table 1. Number of the branches for setae of *Ae. aegypti* pupa with average in parentheses

No. of seta	Cephalothorax	Segments of Abdomen								Paddle
		I	II	III	IV	V	VI	VII	VIII	
0	-	1	1	1	1	1	1	1	1	-
1	1-2 (2)	8-19 (10)	3-6 (4)	3-6 (4)	3-6 (4)	3-6 (4)	2-4 (3)	1-3 (2)	1-3 (2)	1
2	1-2 (2)	1	1-2 (1)	1	1	1-2 (2)	1	1	-	-
3	2-3 (2)	1-2 (1)	1-2 (1)	1-2 (1)	2-3 (2)	2-3 (2)	1-2 (1)	1-2 (1)	-	-
4	2-3 (3)	4-5 (4)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	-
5	2-3 (2)	2-3 (2)	3-4 (3)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	-	-
6	1-2 (1)	1-2 (1)	2-3 (2)	2-3 (2)	2-3 (2)	3-5 (4)	3-4 (3)	3-4 (3)	3-4 (3)	-
7	2-3 (2)	2-3 (2)	2-3 (2)	1-3 (3)	2-3 (3)	2-4 (3)	1	1	1	-
8	2-3 (3)	-	-	1-3 (2)	1-3 (2)	2-4 (3)	2-4 (3)	3-5 (4)	4-7 (6)	-
9	1-2 (1)	2-3 (2)	1-2 (2)	1	1	1	1	2-3 (2)	2-3 (2)	-
10	1-2 (2)	1	1-2 (2)	1	1	1	1	1	1-2 (2)	-
11	1	1	1	1	1	1	1	1	1	-
12	1-2 (2)	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	-	-	-	1	1	1	1	1	1	-

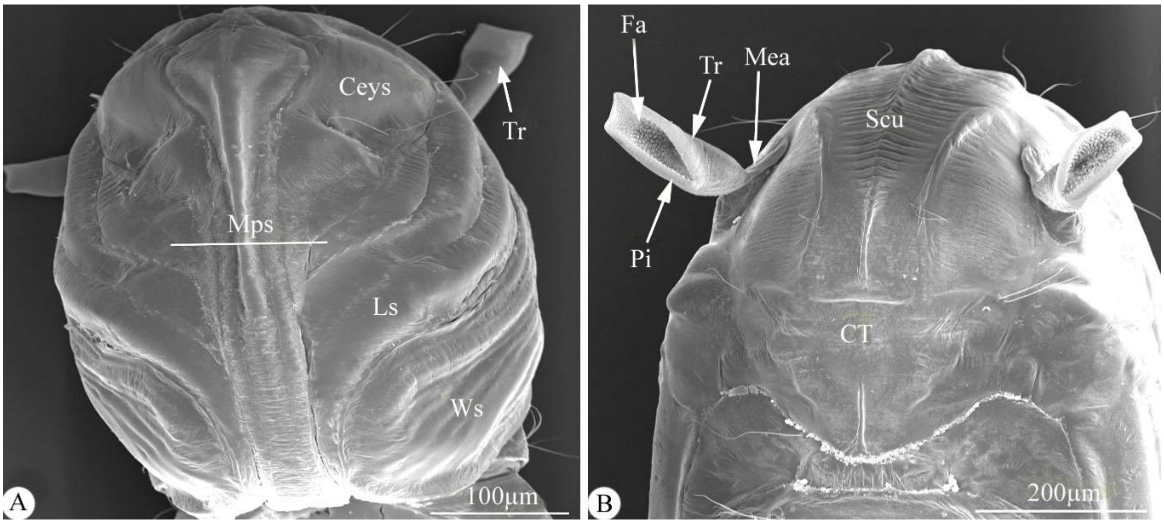

Figure 1. Scanning electron micrographs for the ventral (A) and dorsal (B) view of the cephalothorax in *Ae. aegypti* pupa. Note: Ceys, compound eye sheath; Tr, trumpet; Mps, mouthparts sheath; Ls, leg sheath; Ws, wing sheath; Fa, filter apparatus; Mea, meatus; Pi, pinna; CT, cephalothorax; Scu, scutum.

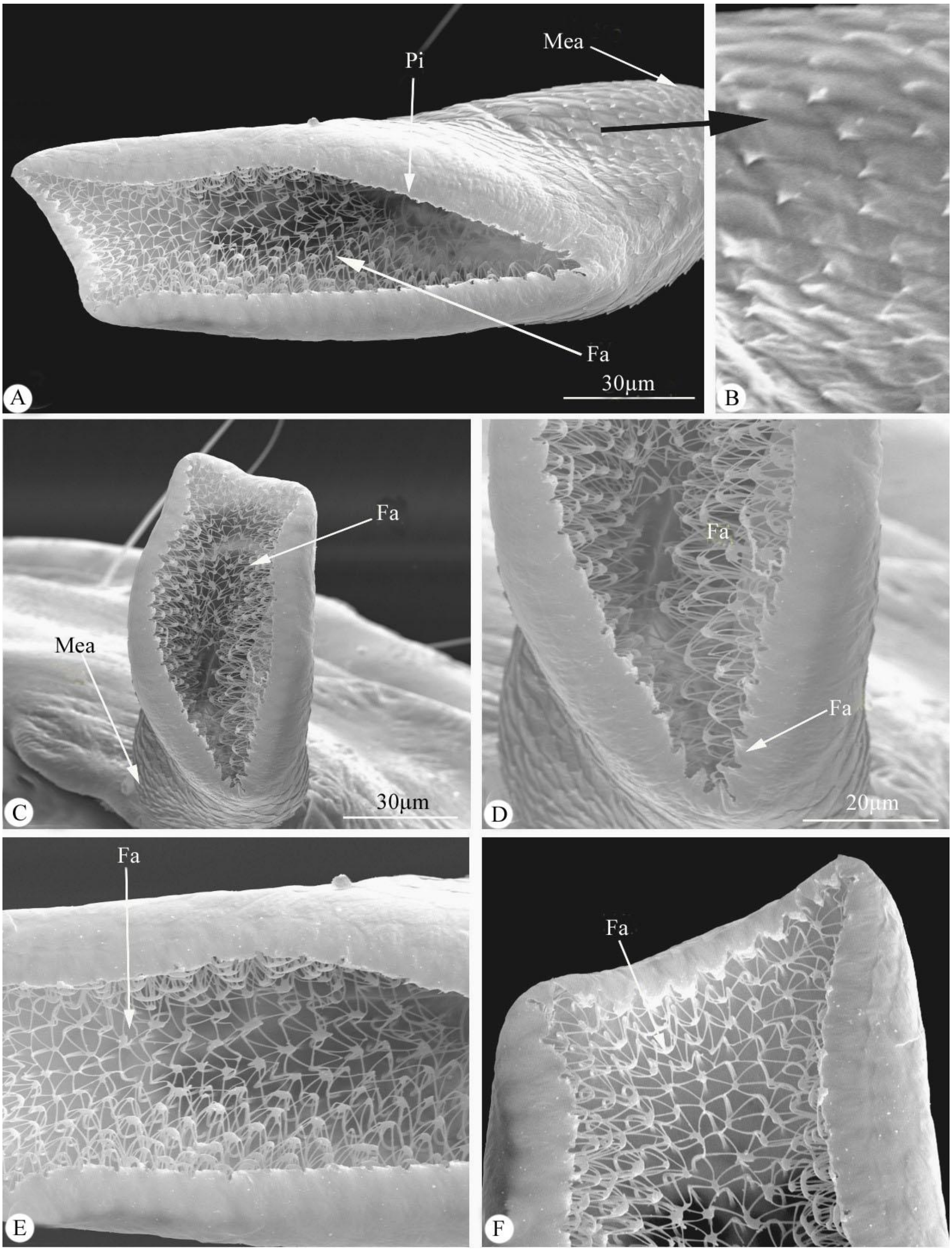

Figure 2. Scanning electron micrographs trumpet and related structures. (A, B) Trumpet's Pinna and Meatus. (C, D) Meso-posterior view of the left trumpet. (E, F) Filter apparatus of trumpet in *Ae. aegypti* pupa. Note, Fa, filter apparatus; Mea, meatus; Pi, pinna.

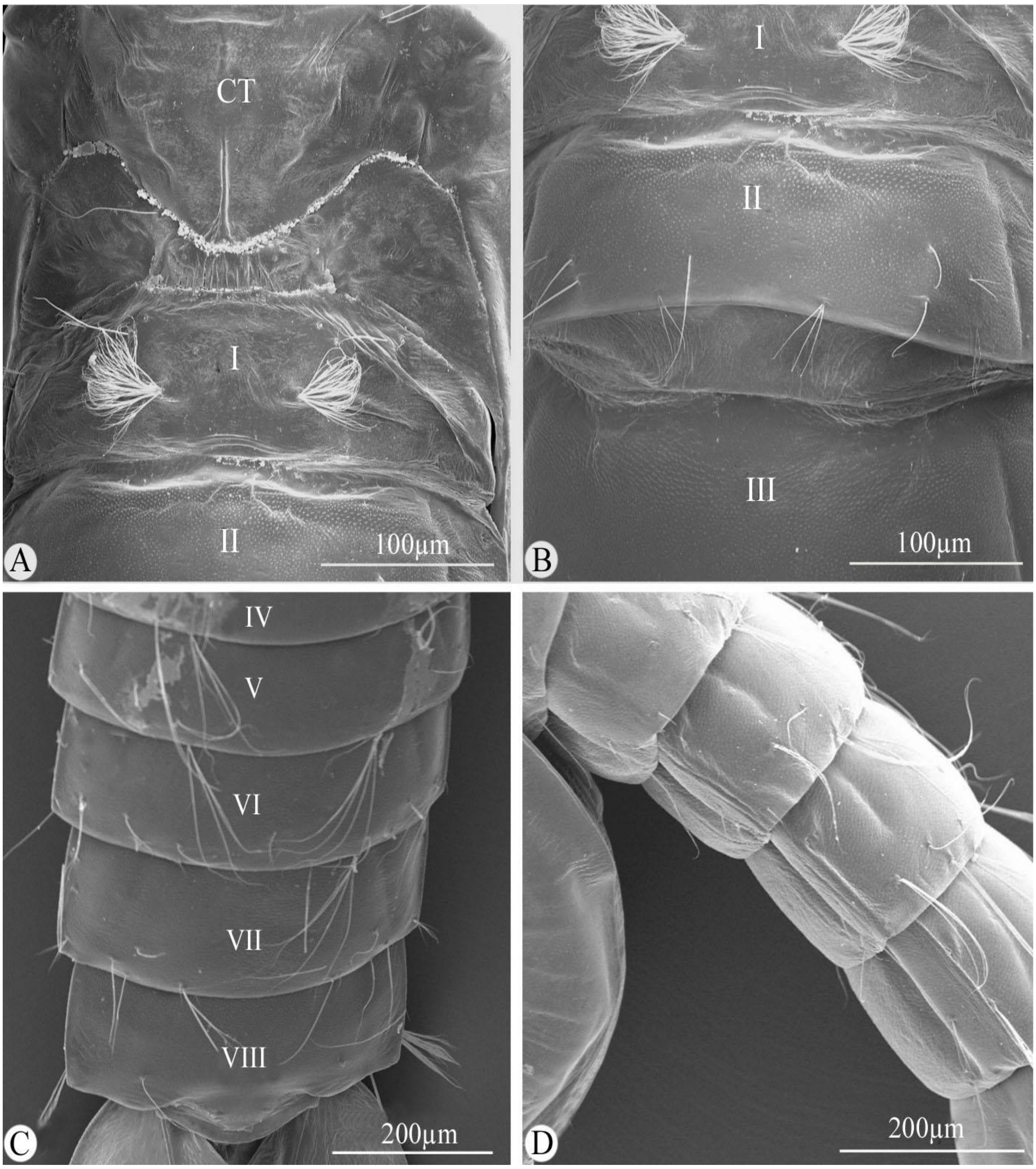

Figure 3. Scanning electron micrographs showing the abdomen with related structures. (A, B) Dorsal view of the abdominal segments I with floating hair (Fr), II, and III. (C) Ventral view of abdominal segments and terminal structure of female. (D) Lateral view of abdominal segments of *Ae. aegypti* pupa. Note, CT, cephalothorax.

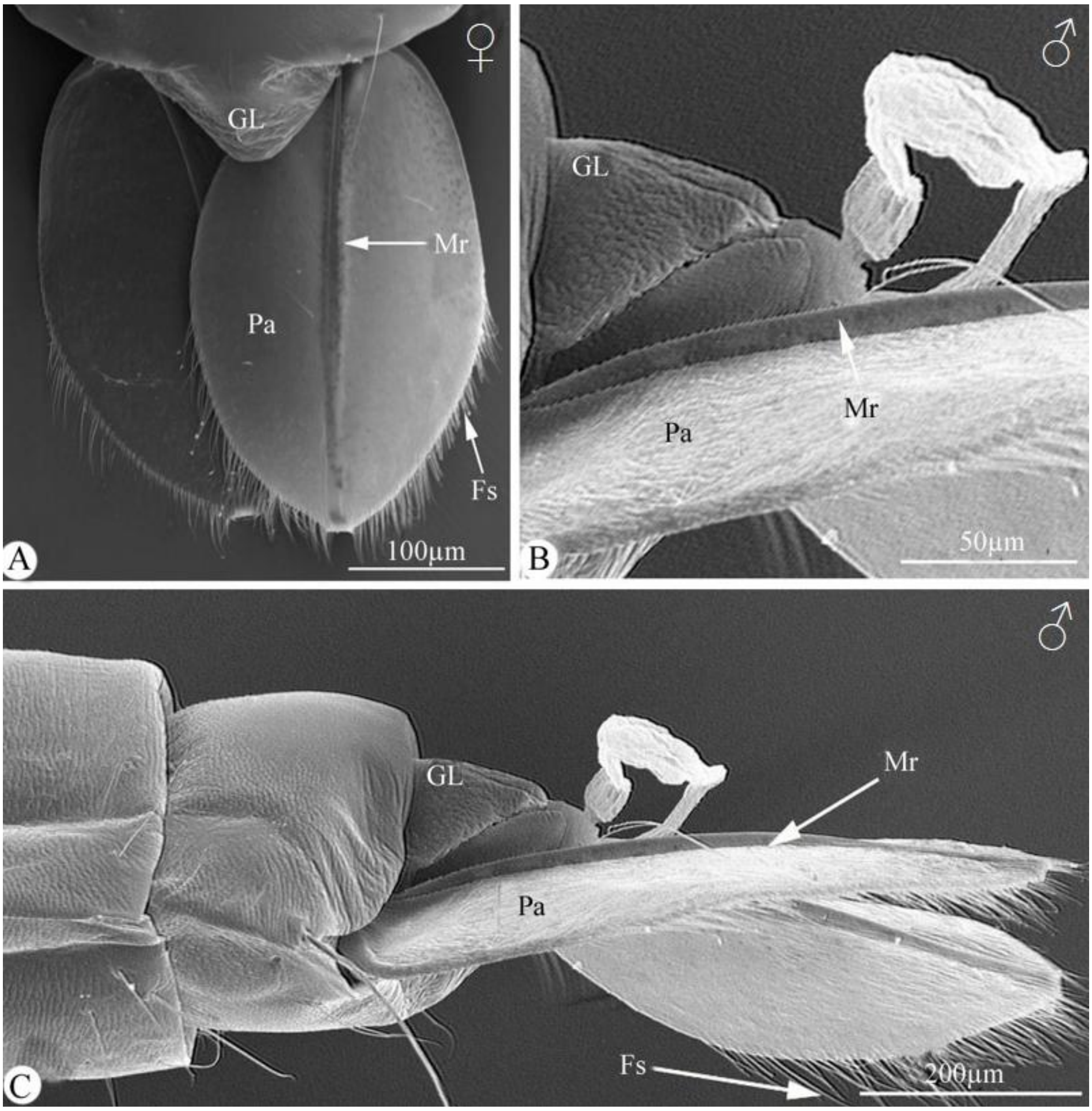

Figure 4. Scanning electron micrographs showing: (A) Ventral view of the terminal abdominal structure of females and paddles. (B, C) Lateral view of the terminal abdominal structure of males and paddles of *Ae. aegypti* pupa. Note: GL, genital lobe; Pa, paddle; Mr, midrib; Fs, filamentous spicule (fringes).

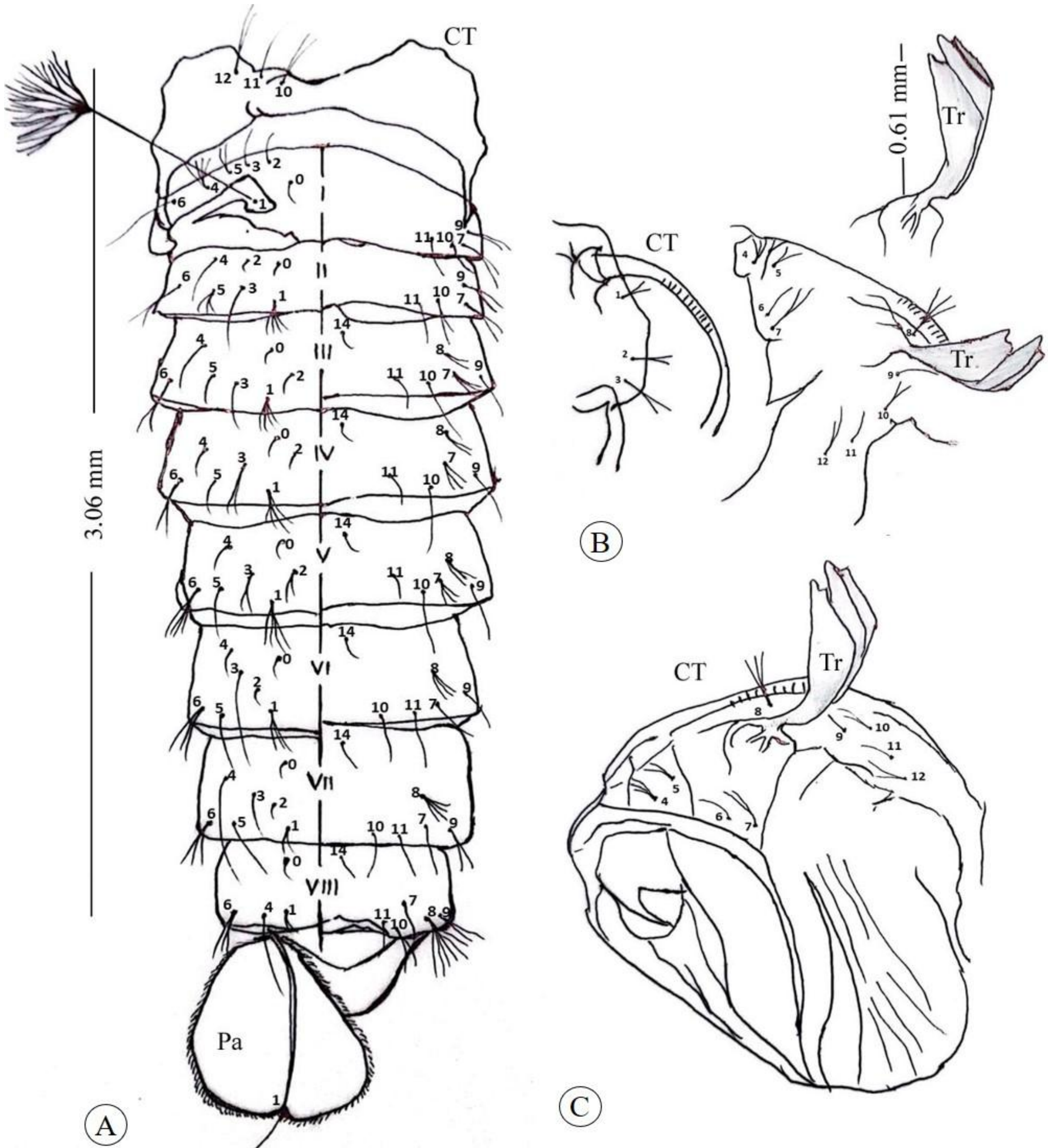

Figure 5. Pupa of *Ae. Aegypti*. (A) Metanotum, abdomen, and paddle (Pa); I-VIII abdominal segments. (B, C) Cephalothorax (CT) and trumpet (Tr).

Cephalothorax: The cuticle was light brown, and it is composed of one unit of a head with mouthparts sheaths and a thorax with both wings and legs sheaths. At the front, mouthparts are flexed posteriorly beneath the flattened head along the cephalothorax ventral side. In front of adult compound eyes, which are visible on both sides of the head, a couple of antennae are oriented to the back over the thorax's sides. The cephalothorax's anterior surface is covered by a wide convex scutum. The crest-shaped ridge, located dorsally along the median line, produces the weakness line, also known as the ecdysial line along which the cuticle may divide before adult emergence. Long and tubular respiratory trumpets arise from the scutum's sides, and their bases are flexible, so they can be moved quickly to the water. The wing's base is located just after the respiratory trumpets, where wing sheaths move down along the sides of the cephalothorax. Leg sheaths are visible between the sheaths of wings and mouthparts, with tarsi wrapping beneath the wing apex (Figs. 1a-c, 5b,c).

A part of the dorsal shield that corresponds to the adult's prothoracic lobe bears the setae 4–7, which are located on either side above the curved antenna. Setae 4 and 5 are positioned slightly anterior to setae 6 and 7. These structures are indicative of the prothoracic hairs found in the adult. Setae 8 and 9 of the mesothoracic region are located on the scutum's convex surface. Specifically, arrow 8 is located slightly posterior to the trumpet and dorsal, while arrow 9 is adjacent to the wing's origin line (Fig. 5c). The positioning of metathoracic setae 10, 11, and 12 is in a linear arrangement on either side of the metathoracic plate, which is located internally to the more expanded section that represents the haltere (Fig. 5c). It is a common occurrence for setae 10 and 11 to exhibit bifurcation, while arrow 11 typically remains singular. Setae 4, and 8 were usually triple with 2 to 3 branches, and 6, and 9 were single. Arrow 0 was not detected.

Trumpet: An elongated pair of respiratory horns located on the dorsolateral side of the cephalothorax. The trumpet is angusticorn type. The moveable trumpet contains a mesothorax spiral. It appeared to be a simple funnel with a wide mouth near and at the apex and thin walls that led directly to the spiracles. The trumpet surface is reticulate with a proximal tracheoid. The trumpet length is twice as wide as at the apex. The trumpet apex is cut off oblique to create pinna, while its remainder is called meatus. At the trumpet apex, a network of spicules covers the atrial wall and creates a perforated plate termed a filter apparatus. Trumpet walls appear two-layered; the outer and inner walls are well separated (Figs. 1c, 2, 5b,c). It has a length of 0.53-0.69 (0.61) mm and is a moderate shade of brown. Pinna length equals 0.34–0.38 mm of the trumpet length; the tracheoid region is significantly darker, extending approximately 0.47 mm from the base.

Abdomen: It is comprised of nine segments, flattened from dorsal to ventral; the final segments are relatively tiny holding paddles. It was 2.33–2.95 (2.626) mm in length. The sclerotized segments are joined together by intersegmental membranes and can move freely on each other, but they cannot move laterally. To keep the pupal body hovering above the water's surface, the first segment has a couple of palmate setae 1 with around 7 (4-9) robust primary branches, each segmented into distinct fine 8-19 secondary branches (Figs. 3a,b). Laterally and ventrally, the first abdominal segment is mostly membrane. Between the sterna and the terga, the abdominal segments II-VII consist of about equal-sized rings of sclerotized tissue; pleural membranes are absent. Segment VIII, with its dorsal median caudal lobe of tergum IX, is smaller than the preceding segments. The paddles are made from the lateral lobes of tergum IX, and the transverse stripe of tergum IX and sternum IX are invisible (Figs. 3c,d, 5a).

Seta 0 was detected in all abdominal segments as a single tiny arrow. Setae 1-II, III, IV, and V are moderately long, with approximately 4 (3–6) main sturdy branches, 1-VI usually triple (2–4). Arrow 2 short was detected in all segments single while not seen in segment VIII and observed in segment V double. In the first abdominal segment, arrow 3 is usually single and is often just a few cell widths apart from arrow 1. They are sometimes separated laterally. Arrow 3-II, IV, and VI are somewhat long but short in the other abdominal segments and absent in the eighth. Arrow 4 is often located near arrow 5 in the seventh abdominal segment. However, they are sometimes separated laterally. In segments V, VI, and VII, arrow 6 is usually the longest of the respective segments; in segments II-IV, it is shorter than in segments I. Arrow 8-VIII always with 6 (4-6) branches. Arrow 9-I, VII, and VIII are usually two (2-3) branches. Arrow 6-II, III, and IV are frequently double (2–3), and arrow 6-VI-VIII are invariably triple (3–4). Setae 12 and 13 were not detected in all segments. Arrow 14 was not seen in segments I, and II while observed in segments III- VIII single. The median caudal lobe was simple and observed without setae. The segments from I to VIII feature the presence of a small arrow denoted as arrow 0. Segment VIII laterally exhibits a prominent, multi-branched arrow denoted as arrow 8. This arrow comprises six robust branches of approximately equivalent length, each bearing delicate lateral filamentous branches. Arrow 1 is typically characterized by multiple branches and an oblique placement within arrow 4 on segment III.

Anal lobe: Proctiger is a small rudiment of segments XI and X, is noticed ventral to a median caudal lobe. It is widened in female mosquitoes and carries a distinguishable couple of cercal lobes. The adult genitalia is enclosed by a sheath that emerges between the paddle bases, along with the genital lobe (Figs.1b, 5b,c). The genital lobe in males is notably larger and partitioned, with a substantial median fissure that extends near its base, resulting in almost complete separation (Figs. 4b,c). In females, the genital lobe is comparatively smaller and less pointed than that in males and is separated by the median fissure. The pupal sex can be determined easily by the genital lobe's size and shape compared to the paddles' size and median fissure level.

Paddles: The paddles are often round 0.78–1.13 (0.92) mm but can also be roughly oblong with a narrower base. The pupal paddles are the primary locomotory organs and are supported by a median longitudinal thickening, commonly referred to as the midrib, the middle longitudinal reinforcement. The apical arrow, which measures approximately one-quarter of the paddle's length, is situated at the end of the midrib. Fringes of tiny filamentous or dentiform spicules occur around the paddle's edge, these structures are relatively larger on the external portion of the paddle hair. A critical morphological feature for taxonomy (Figs. 4, 5a) was the translucent spiculate midrib which is evident except towards the tip. The paddles are characterized by a single, unbranched, elongated primary arrow. The entity commonly known as the "paddle seta" is formally designated as "seta 1". Arrow 2 was absent (Figs. 3, 5a).

A second method might be employed in the lab to distinguish between the sexes. Male pupae were much smaller than female pupae, and the abdomen of female pupae was roughly the same diameter as cephalothorax when larvae were created overfed and in aerated water. Since female larvae grow into pupae slower than males, sexing may be employed with high precision based on external characteristics, such as body size and shape.

DISCUSSIONS

Ae. aegypti mosquitoes have become a severe public health crisis worldwide due to the frequency of the diseases they spread. Yellow fever, Dengue, and chikungunya are vector-borne diseases whose geographic distributions quickly spread, placing them at the forefront of public health concerns (Gardner *et al*., 2012; Bhatt *et al*., 2013). Numerous studies have been conducted on the *Ae. aegypti* mosquito since it was discovered that it transmitted yellow fever to humans.

Based on this description, specific morphological characteristics are observed to differentiate *Ae. aegypti* from other closely related *Aedes* species, *Ae. geminus* and *Ae. vittatus*- both previously studied by Melero-Alcíbar (2005, 2006), *Cx. cuyanus* Rossi *et al*., 2006, *Ps. pallescens* Stein *et al*., 2009, *Anopheles annulipalpis* Rossi, 2017, and *Cx. bidens* Dyar Rivera-Garcia and Ibáñez-Bernal, 2018.

Comparatively, little is known about the taxonomic characteristics of the many pupal stages of the Culicidae, although the morphological traits of distinct mosquitoes are well recognized. The pupal stage of *An. stephensi* has been thoroughly addressed by Harrison and Peyton (1984). Christophers (1933), Baisas (1936), Crawford (1938), Satchell (1948), Christophers (1960), Reid (1963), Becker *et al*. (2010) for mosquito species, Kirti and Kaur (2011) for *Cx. quinquefasciatus*, and Kirti (2014) for *An. stephensi* showed that mosquito pupae of different genera have different exterior morphology and chaetotaxy.

Since the pupal stage of *Ae. aegypti* is crucial to comprehend this vector species' life cycle; this study's primary objective was to identify and distinguish the pupae of *Ae. aegypti* using SEM to control mosquito-borne diseases and mosquito taxonomy. A new taxonomy may help to identify this species by its distinctive trumpet shape, abdominal segments with their setae, genital lobe changes between the sexes, and paddle shape.

The results, together with earlier ones, support that mosquito pupae are very agile, with a large globular cephalothorax and a smaller articulated abdomen that is retained flexible when swimming, unlike the pupae of most other insects. Mouthparts observed at the front of the cephalothorax within the flattened head. The anterior dorsal surface of the cephalothorax is found to be elongated by the large mesothorax and its convex scutum. The pupal body is held upright on the surface of the water by a pair of palmate setae on the first segment of the abdomen. The trumpet's base is pliable enough to be lifted to the water's surface relatively easily. These findings support the assumption of Melero-Alcbar (2005, 2006) for *Ae. geminus* and *Ae. vittatus*, and Becker *et al*. (2010) for *Anopheles* sp.

The trumpet is an angusticorn appendage of the cephalothorax. Simple funnel with narrow walls leading to spiracles. The trumpet's length doubles its width. Pinna is the trumpet tip, while Meatus is the remainder. A network of spicules covers the atrial wall at the trumpet apex, creating a filter apparatus. These results confirm a previous hypothesis of Harbach and Knight (1980) reported that the trumpet of *Ae. aegypti* is somewhat angled away from the stem along its longest vertical axis. When it's closed, it gives the impression of a wide-open skinny funnel. Christophers (1933) claimed that the Anophelini tribe used a narrow funnel with a cutout on one side. Upon being raised to the surface, the Anophelini trumpet is said to be so poorly articulated that it may be easily put by surface forces (Christophers, 1960).

According to Reid (1963), the complex trumpets might have been folds instead of protrusions. As Tanaka (2003) stated, the trumpets of *Aedes* species are cylindrical, ranging from two to five times as broad as they are long. It has been reported by Crawford (1938) for *Anopheles barbirostris*, Kirti and Kaur (2011) for *Cx. quinquefasciatus*, and Kirti (2014) for *An. stephensi* that the trumpet's tip is cut off indirectly to form the pinna and the meatus. However, the exact nature of this mechanism was not clear. The culicine mosquito pupae have a long, cylindrical respiratory trumpet, as reported by Becker *et al*. (2010). Anopheline mosquito pupae have shorter, wider, and flap-like respiratory trumpets. According to Ameen and Talukdar (1974), the length of a medium-sized membranous trumpet is around three times its apex width. For *Anopheles vagus*, arrow 1 was medium in length and lacks branching.

Present research reveals that the last two abdominal segments—out of the nine—are quite short and house the terminal paddles. Paddles are made from the lateral lobes of tergum IX. Arrow 1 emerged with about four (3–6) major, strong branches from segments II to VIII. The first segment has a couple of palmate setae 1 with about seven (4–9) robust primary branches, each segmented into distinct fine eight–nineteen secondary branches to keep the pupal body floating above the water's surface. Arrow 0 was found to be a single, very small arrow in every abdominal segment. Arrow 9–VIII usually has 6 (5-7) branches and arrow 6-V, VI-VIII always with 4 (3–5) branches. Single detection of short arrow 2 was recorded in all segments except segments VIII and IX, whereas double detection was observed only in segment V. Normal separation between arrow 3 and arrow 1 is just a few cells wide. Arrow 4 is often positioned close to arrow 5 in the seventh abdominal segment, although they may be laterally separated in certain cases. The median caudal lobe was simple and observed without setae.

The data provided here seems to support the theory of Melero-Alcábar (2006) on *Ae. geminus* and *Ae. vittatus*, as well as Becker *et al*. (2010) on *Anopheles* species. Like the *Anopheles* larvae, the pupal body is supported by a pair of palm trees on the first abdominal segment (1-I), allowing it to stand upright on the water’s surface. The genital lobe is a minor rudiment of segments XI and X; it is broader in female mosquitoes and has a somewhat different pair of cercal lobes, but it is much more significant in male mosquitoes, clearly separated, and doesn't entirely split along the median fissure near its base. Pupae of different sexes are readily identified by comparing the proportions of their genital lobe to those of their paddles and median fissures. Near the midrib end, there is an apical arrow. The paddle margin is fringed with short spines or spicules with a taxonomic value. These findings support the assumption of Tanaka (2003) for *Aedes* sp. that the paddle is often rounded out to the apical half of the inner edge. As revealed by Becker *et al*. (2010), the paddles of *Chaoborid* pupae resembled those of mosquitoes but included three ribs instead of two. The paddle is wide, the distal rime is unclear, and the midrib is somewhat darker, as described by Kirti and Kaur (2011) for *Cx.*

quinquefasciatus and Kirti (2014) for *An. stephensi*.

CONCLUSION

The mosquito pupae are less identified than the larvae and adults. In our knowledge of the pupal stages of *Ae. aegypti*, there is a large gap. This humble study attempted to fill this gap by investigating the morphology and chaetotaxy of the pupal stage with the help of the scanning electron microscope (SEM) to determine additional taxonomic features. This study will help in describing the morphology of the pupal body, trumpet, paddles, and the genital lobe's size and shape to identify this species from similar ones and distinguish the pupal sex, which may help assess the mosquito vectorial ability as well as both the controlling mosquito-borne diseases and for mosquito taxonomy.

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