Lozano-Sardaneta *et al.*
The Journal of Basic and Applied Zoology (2024) 85:13
https://doi.org/10.1186/s41936-024-00367-9

The Journal of Basic and Applied Zoology

RESEARCH

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Exochorionic pattern of ten sand fly species (Diptera: Psychodidae: Phlebotominae) from Mexico

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Abstract

Background Phlebotomine sand flies are of biological importance because of their role as vectors of several pathogens. Morphological identification faces challenges to separate related species; therefore, the study of immature stages, as the egg and its exochorion sculpturing pattern could provide useful characters for taxonomic and phylogenetic studies. In the Americas, morphological information of the egg exochorion obtained through scanning electron microscopy has become a complementary tool for taxonomic classification of sand fly species. The aim of this study was to examine and describe the exochorion pattern of eggs obtained from the abdomen of gravid wild females of 10 sand fly species collected in different areas of Mexico.

Results We describe the chorionic pattern of 10 sand fly species collected during the period 1997–2023, which was classified as (1) polygonal, (2) connected and unconnected parallel ridges, (3) verrucose, (4) volcano-like and (5) disperse, being the polygonal pattern the most common among several species of the genera *Dampfomyia*, *Micropygomia* and *Lutzomyia*.

Conclusions This study describes the exochorion pattern of eggs directly obtained from the abdomen of preserved female specimens, supporting that extraction of eggs directly from an abdomen of a collection specimen might be a viable alternative to gather information of taxonomic value. We describe the eggshell of *Bichromomyia olmeca olmeca*, *Dampfomyia deleoni*, *Micropygomyia cayennensis*, *Micropygomyia chiapanensis*, *Micropygomyia vindicator*, *Micropygomyia durani*, *Lutzomyia cruciata*, *Psathyromyia maya*, *Psathyromyia texana* and *Trichopygomyia triramula*. We detected differences with respect to previous descriptions of *Micropygomyia chiapanensis*, *Lutzomyia cruciata*, and *Psathyromyia texana*, providing new reference information.

Keywords Ootaxonomy, Morphology, Scanning electron microscopy, Leishmaniasis

Background

Phlebotomine sand flies (Diptera: Psychodidae) are dipterans of great biological relevance as vectors of *Leishmania*, *Bartonella*, and some arboviruses (Akhoundi et al., 2016), throughout the tropics and subtropics of the world. Therefore, in order to enrich surveillance programs of vector-borne diseases, it is necessary to have complementary information to enhance a reliable taxonomic identification. Morphological identification is based on adult males and females, which still holds limitations to correctly differentiate closely related species. In addition, immature stages have been little studied for

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most sand fly species, despite their relevance in taxonomic and phylogenetic studies (Alencar & Scarpassa, 2018; Jariyapan et al., 2022).

In the Americas, since 1975 the morphological description of the exochorion sculpturing of eggs through scanning electron microscopy (SEM) has resulted in a complementary tool for taxonomic classification of sand fly species both at generic and specific levels (Alencar & Scarpassa, 2018; Jariyapan et al., 2022; Ward & Ready, 1975). The study of the exochorion pattern is important because it is a layer that confers protection from the environment to the embryo, preventing desiccation and regulating gaseous exchange, which in several insect groups has taxonomic significance, besides that it conforms a character that reflects functional adaptations to different types of environment where sand flies oviposit, and could reflect phylogenetic relationships among sand fly species (Jariyapan et al., 2022; Pérez & Ogusuku, 1997; Ward & Ready, 1975). Although some species show a similar pattern, it is generally possible to observe specific details in the microanatomy of the eggs which allow to differentiate sibling species (Bahia et al., 2007).

To date, 556 sand fly species have been described in the New World, but only 58 species of the genera *Bichromomyia*, *Dampfomyia*, *Evandromyia*, *Lutzomyia*, *Micropygomyia*, *Migonemyia*, *Nyssomyia*, *Pintomyia*, *Psathyromyia*, and *Psychodopygus* have their chorionic pattern described, which is classified into nine types: (a) polygonal, (b) connected parallel ridges, (c) unconnected parallel ridges, (d) volcano-like, (e) elliptical, (f) reticular, (g) verrucose, (h) disperse, and (i) placoid (Alencar & Scarpassa, 2018; Galati & Rodrigues, 2023; Jariyapan et al., 2022). In Mexico, 52 sand fly species have been described but only the species *Dampfomyia beltrani* (unconnected parallel ridges), *Micropygomyia chiapanensis* (polygonal), and *Lutzomyia cruciata* (connected parallel ridges), have their chorionic pattern described (Montes de Oca-Aguilar et al., 2014, 2016, 2017). As morphological traits of eggs of Mexican sand flies are largely unknown, this work aims to describe the exochorion pattern of eggs obtained from the abdomen of 10 gravid wild female sand flies collected in different states of Mexico, through SEM.

Methods

Egg collection

Phlebotomine sand flies revised and analyzed were previously collected by Instituto de Diagnóstico y Referencia Epidemiológicos "Dr. Manuel Martínez Báez" (InDRE), during the period 1997–2023 using CDC (U.S. Centers for Disease Control) light trap, and Shannon traps, and were preserved in 70% alcohol at −20 °C. Sand flies were collected from several localities endemic of Leishmaniasis in the states of Guerrero, Nayarit, Nuevo León, Oaxaca, Quintana Roo, and Veracruz, Mexico. We separated and analyzed female sand flies that showed eggs in their abdomen (gravid females). Head and last segments of the abdomen of all selected female sand flies were dissected for morphological identification, and remaining parts of the abdomen were dissected for obtaining the eggs, which were individualized and preserved in ethanol 70%. Eggs were separated carefully as they are compacted within the abdomen. Inside the abdomen, eggs were coated with a sticky layer that holds the eggs together. This sticky coating makes it difficult to separate each egg, so not all available egg were recovered in several specimens.

Taxonomic identification

For taxonomic identification we performed a semi-permanent mounting (Lozano-Sardaneta et al., 2023), and used the taxonomic keys of Galati (2019) and the abbreviation system proposed by Marcondes (2007). We followed the summarized information of exochorionic sculpture patterns proposed by Alencar and Scarpassa (2018).

SEM procedures

Eggs were treated for SEM examination at Laboratorio de Microscopía y Fotografía de la Biodiversidad of Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).The eggs were cleaned in ethanol 70%, then were dehydrated in an ascending series of ethanol (80% OH, 90% OH and 100% OH), and subjected to critical point drying in carbon dioxide (CO_2) in a machine Emitech k850. Eggs were then mounted on an aluminum support and coated with gold plated (QUORUM Q150R ES).

Data analysis

The eggs were observed and photographed in a Hitachi SU1510 scanning electron microscope (Hitachi, Japan). Pictures obtained were visualized and measured through the program Image Pro Plus-3D suite version 7.0 (Media Cybernetics, Maryland, USA), and then edited in Adobe Photoshop CS5 software.

Results

Sand flies analyzed

A total of 24 sand fly specimens, belonging to seven genera and 10 species were analyzed to determine chorionic pattern of the eggs. Generally, eggs obtained of the sand fly abdomens are elongate-ellipsoidal with rounded edges, as described for other species. Only *Bichromomyia olmeca olmeca* showed a different shape, since it was a rounder and smaller egg. In none of the eggs analyzed was it possible to observe the micropyle.

Chorionic patterns observed in the sand flies examined were: (1) polygonal, (2) connected and unconnected parallel ridges, (3) verrucose, (4) volcano-like, and (5) disperse, with the polygonal pattern as the most common in several species of the genera *Dampfomyia*, *Micropygomia* and *Lutzomyia* (Table 1).

In the case of *Bi. olmeca olmeca*, *Mi. chiapanensis* and *Pa. maya* we provided information of the eggs size and shape, however achieving only a partial description of the exochorion pattern, as eggs were apparently damaged during processing and it is likely the exochorion is incomplete. Therefore, complementary studies are necessary in these species.

Chorionic pattern

Detailed descriptions of the eggs of the ten sand fly species examined are presented below.

(a) *Bichromomyia olmeca olmeca* (Vargas & Díaz-Nájera). Color: dark brown. Shape: orbed. $N=3$. Size: length = 154–159 µm (154 ± 2.3); width = 78.4–89.5 µm (78.4 ± 4.2). Exochorion: Pattern observed is not clear because the egg is covered by a tissue layer that conceals exochorion. However, in some portions of the observed eggs it seems to have a polygonal pattern, with rectangular and square forms, although in the middle layer a rough pattern is observed (Fig. 1). Examined material: Eggs analyzed were collected on July 2022 in the locality Huay Pix, Othón P. Blanco, Quintana Roo (18° 31′ 04″ N; 88° 25′ 25″ W; 10 m).

(b) *Dampfomyia* (*Coromyia*) *deleoni* (Fairchild & Hertig). Color: brown. Shape: orbed. $N=5$. Size: length = 281–339 µm (281 ± 24); width = 59.9–91.7 µm (59.9 ± 11.5). Exochorion: Eggs showed a polygonal pattern, with rows of polygonal cells (size: mean length = 13 µm; mean width = 7.54 µm) perpendicularly to longitudinal axis of the egg, with

Table 1 Chorionic patterns of ten Mexican sand fly eggs examined by SEM

Species	Date	State	Exochorion pattern
Bichromomyia olmeca olmeca	July 2022	Quintana Roo	Unspecific* (polygonal)
Dampfomyia deleoni	June and September 2021	Quintana Roo	Polygonal
Micropygomyia cayennensis maciasi	March 2020 July 2009	Quintana Roo Guerrero	Polygonal
Micropygomyia chiapanensis	February 2001	Veracruz	Unspecific* (verrucose)
Micropygomyia vindicator	July 2009	Oaxaca	Polygonal
Micropygomyia durani	December 2019	Oaxaca	Polygonal
Lutzomyia cruciata	April 1998	Nayarit	Connected parallel ridges
Psathyromyia maya	May 2018	Quintana Roo	Unspecific* (disperse)
Psathyromyia texana	August 2023	Nuevo León	Polygonal
Trichopygomyia triramula	September 2001	Oaxaca	Volcano-like

* For these species the exochorion pattern was not conclusive, information about preliminary observation of the pattern is included in parentheses.

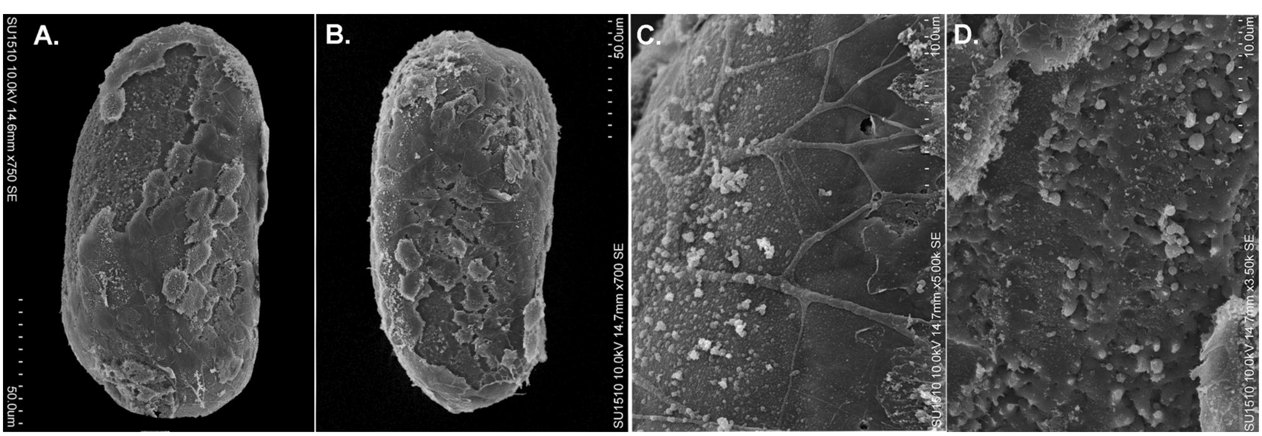

Fig. 1 Exochorion pattern of *Bichromomyia olmeca olmeca*: **A**, **B** Complete egg. **C** Polygonal pattern. **D** Zoom to middle layer

pentagonal and rectangular forms. Ridges are narrow and slightly elevated, although well-defined, some weakly connected in the middle (Fig. 2). Examined material: Eggs analyzed were collected on June and September 2021 in Chetumal (18° 29′ 38″ N; 88° 17′ 52″ W; 100 m) and Nicolas Bravo (18° 27′ 33″ N; 88° 55′ 44″ W), Othón P. Blanco, Quintana Roo.

(c) *Micropygomyia* (*Micropygomyia*) *cayennensis maciasi* (Fairchild & Hertig). Color: light brown. Shape: oval. $N=2$. Size: length = 292–296 μm (292 ± 3); width = 52.6–64.8 μm (52.6 ± 8.6). Exochorion: Eggs showed a polygonal pattern, with rows of polygonal cells (size: mean length = 13 μm; mean width = 5.49 μm), rectangular forms along the egg. Ridges are formed by small and numerous rounded tubercles that define the cells, small protuberances lie within the polygonal cells (Fig. 3). Examined material: Eggs collected on March 2020 in Los Divorciados, Bacalar, Quintana Roo (18° 40′ 41″ N; 88° 23′ 31″ W, 40 m), and July 2009 in La Unión, Ayutla de los Libres, Guerrero (16° 49′ 10″ N; 99° 7′ 27″ W; 70 m).

(d) *Micropygomyia* (*Coquillettimyia*) *chiapanensis* (Dampf). Color: dark brown. Shape: oval. $N=4$. Size: length = 292–318 μm (292 ± 14); width = 67.3–95.9 μm (67.3 ± 13). Exochorion: According to the literature this species should present polygonal pattern, however in this study, no sculptures with ridges were observed on any of the eggs, but in some areas they were covered by small protuberances of different diameter giving a verrucose appearance (mean diameter = 0.5 μm) (Fig. 4). It is possible that the original pattern was lost during processing. Examined material: Eggs collected on February 2001 in Pozo de Arena, Ignacio de la Llave, Veracruz (18°37′32″N; 95° 59′ 13″ W; 7 m).

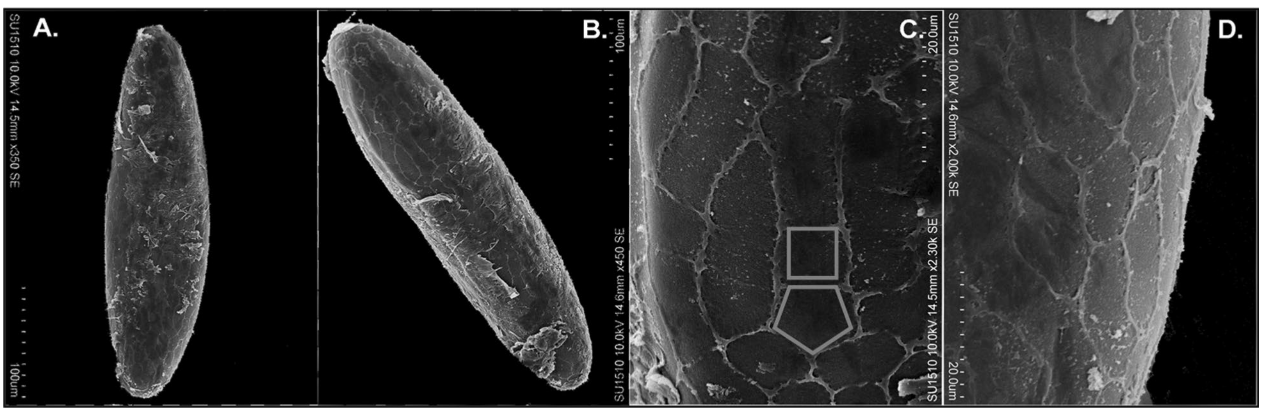

Fig. 2 Exochorion pattern of *Dampfomyia deleoni*: **A**, **B** Complete egg; **C**, **D** Polygonal pattern

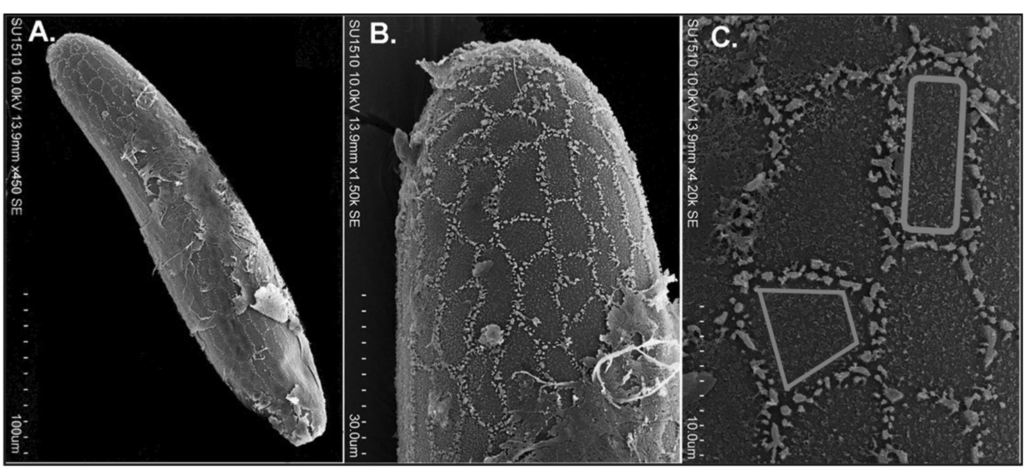

Fig. 3 Exochorion pattern of *Micropygomyia cayennensis maciasi*. **A** Complete egg; **B** Polygonal pattern in the apical area; **C** Zoom to the polygonal area

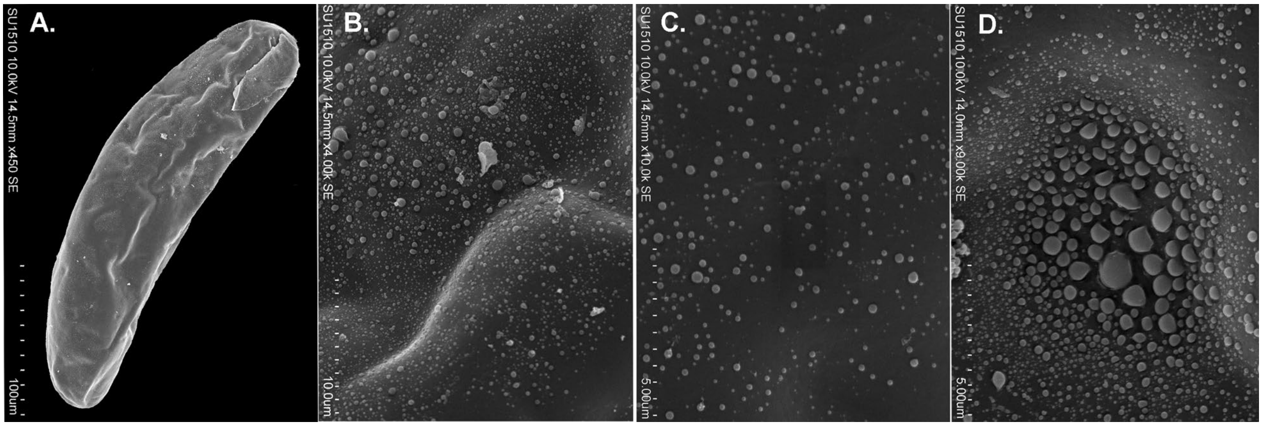

Fig. 4 Exochorion pattern of *Micropygomyia chiapanensis.* **A** Complete egg; **B**–**D** Zoom to the verrucose areas

(e) *Micropygomyia* (*Coquillettimyia*) *vindicator* (Dampf). Color: dark brown. Shape: orbed. *N* = 5. Size: length = 266–281 µm (266 ± 7.5); width = 59.3–60.7 µm (59 ± 0.7). Exochorion: Eggs show a polygonal pattern, with rows of polygonal cells (size: mean length = 14 µm, mean width = 5.9 µm) along the egg; ridges are narrow with small holes, inner area of polygonal cell is smooth (Fig. 5). Examined material: Eggs collected on July 2009 in El Zacatal, San Juan Guichicovi, Oaxaca (16° 56′ 29″ N; 95° 12′ 6″ W; 540 m).

(f) *Micropygomyia* (*Micropygomyia*) *durani* (Vargas & Diaz-Nájera). Color: pale brown. Shape: oval. *N* = 3. Size: length = 270 µm; width = 65.7 µm. Exochorion: Eggs show a polygonal pattern, with rows of oval cells and narrow (size: mean length = 14.81 µm, mean width = 6.92 µm) along the egg, some cells connected and other appear partially connected, inner space of cells appears smooth (Fig. 6). Examined material: Eggs collected on December 2019 in Cerro Gordo, Santa María Tonameca, Oaxaca (15°47′35″N; 96°35′38″W; 142 m).

(g) *Lutzomyia* (*Tricholateralis*) *cruciata* (Coquillett). Color: brown. Shape: oval. *N* = 2. Size: length = 273–287 µm (273 ± 10); width = 74.3–81.2 µm (74.3 ± 5). Exochorion: Eggs show an ornamentation of connected parallel ridges on ventral side (some connections are weak) and unconnected parallel ridges on lateral sides of the egg. The ridges are flattened, elongate and discontinuous, with semi rectangular cells (in some areas) and some hexagonal, that traverse the egg longitudinally. The inner area of polygonal cells has rugose aspect and ridges are made up of granular structures (mean length = 28 µm and mean wide = 8.85 µm). (Fig. 7). Examined material: Eggs collected on April 1998 in El Tepozal, Xalisco, Nayarit (21° 23′ 13″ N; 105° 3′ 57″ W; 300 m).

(h) *Psathyromyia maya* Ibáñez-Bernal, May-UC & Rebollar-Tellez. Color: dark brown. Shape: oval. *N* = 5. Size: length = 265–288 µm (265 ± 10);

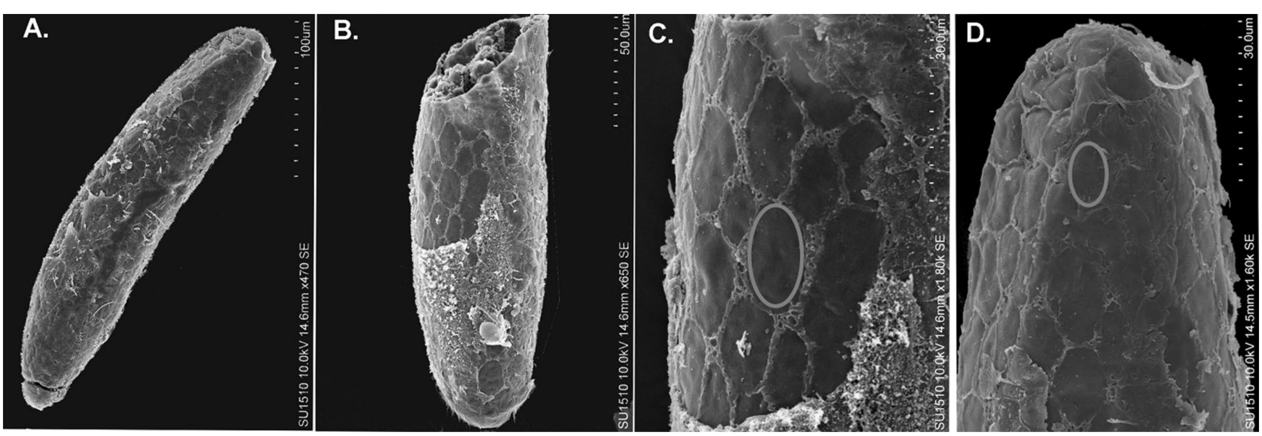

Fig. 5 Exochorion pattern of *Micropygomyia vindicator.* **A** Complete egg; **B** Polygonal pattern in the middle area; **C**, **D** Zoom to the polygonal area

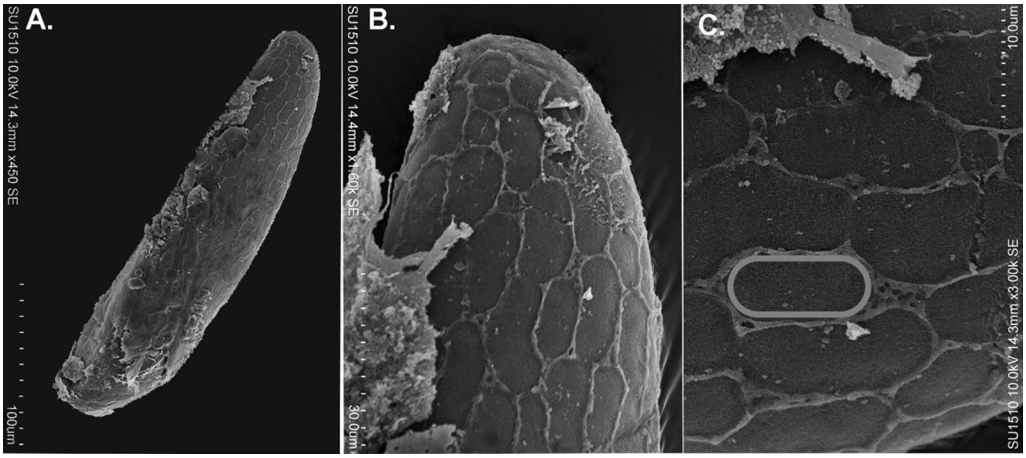

Fig. 6 Exochorion pattern of *Micropygomyia durani*. **A** Complete egg; **B**, **C** Zoom to the polygonal area

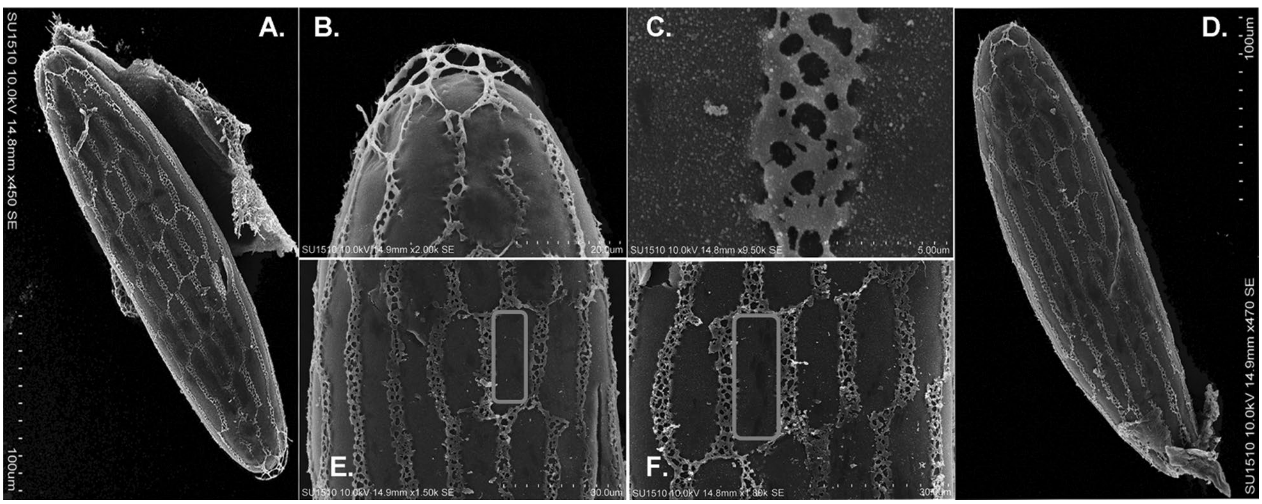

Fig. 7 Exochorion pattern of *Lutzomyia cruciata*. **A**, **D** Complete egg; **B** Apical area of the exochorion; **C** Zoom to the ridges and verrucose area; **E** Unconnected and connected parallel ridges; **D** Zoom polygonal pattern

width = 86–101 µm (86 ± 5.5). Exochorion: Eggs show a disperse pattern, without ridges with small and abundant disperse protuberances along the structure that forms parallel lines, which may be connected (Fig. 8), inner space between patterns is smooth. Examined material: Eggs collected on May 2018 in Dzula, Felipe Carrillo Puerto, Quintana Roo (19°35′5″N; 88°35′28″W; 40 m).

(i) *Psathyromyia* (*Forattiniella*) *texana* (Dampf). Color: brown. Shape: oval. *N* = 5. Size: length = 246–268 (246 ± 9.4) µm; width = 57.1–75 (51 ± 7.4) µm. Exochorion: Eggs show a polygonal pattern, rows of polygonal cells have a mean length = 13.4 µm and a mean width = 9.77 µm, because of their size the pattern seems rectangular along the egg; pattern was also observed at the light microscope. Ridges are formed by joined thin lines in a chain-like appearance (Fig. 9); space between this pattern is rough with some small protuberances (verrucose) of different sizes (mean sizes = 0.317–0.545 µm) (Figs. 5E, F and 6A, B). Examined material: Eggs collected on August 2023 in Las Adjuntas, Santiago, Nuevo León (25° 18.056′ N, 100° 08.286′ W; 731 m).

(j) *Trichopygomyia triramula* (Fairchild & Hertig). Color: brown. Shape: oval. *N* = 5. Size: length = 260–310 µm (260 ± 17); width = 58–71 µm (58 ± 5.6). Exochorion: Eggs show a rough appearance and several volcano-like structures (size: mean length = 9.24 µm, mean width = 4.48 µm, mean

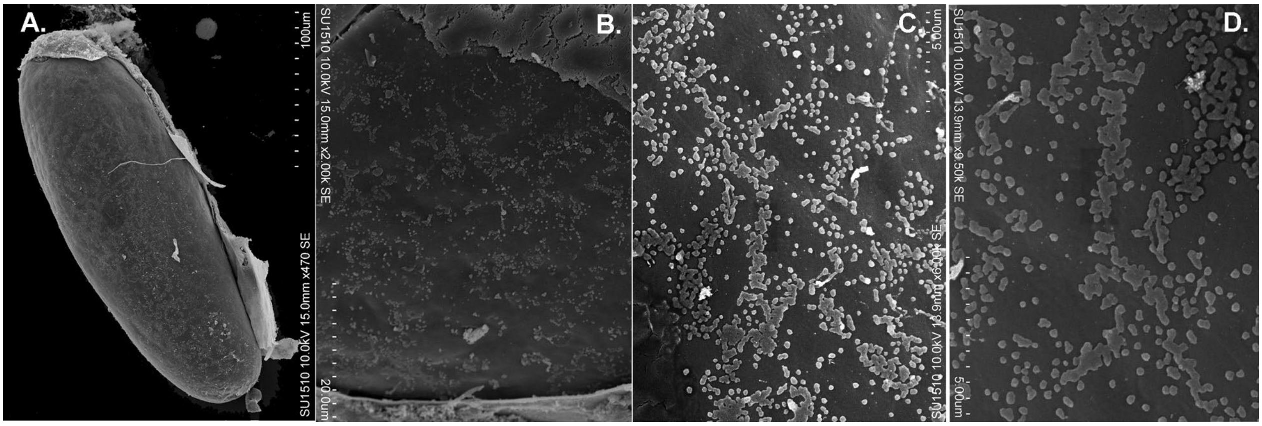

Fig. 8 Exochorion pattern of *Psathyromyia maya*. **A** Complete egg; **B** Disperse pattern; **C**, **D** Zoom to the disperse area

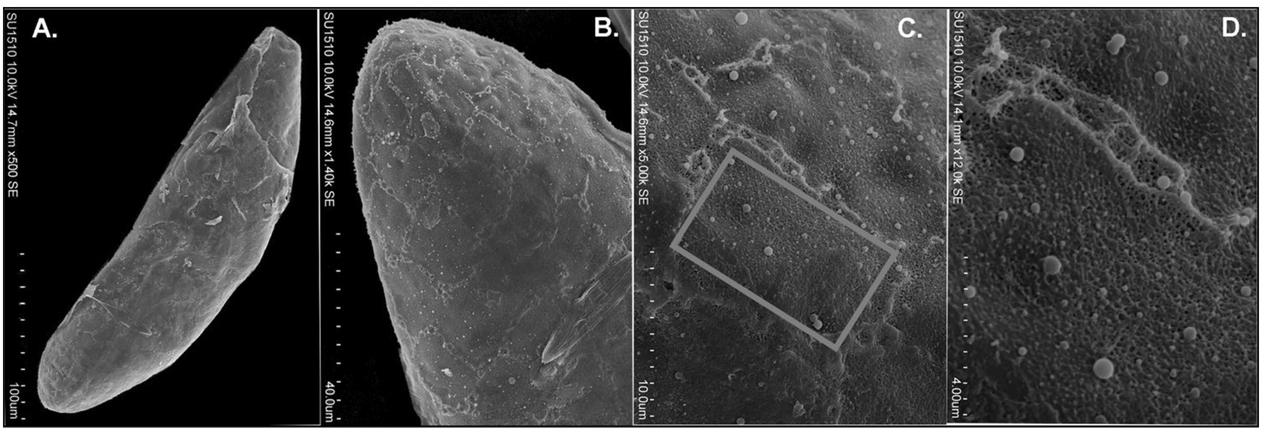

Fig. 9 Exochorion pattern of *Psathyromyia texana*. **A** Complete egg; **B** Apical area of the exochorion; **C** Zoom of polygonal area; **D** Zoom to the ridges of the cells

height = 3.17 μm), that are evenly distributed at a mean distance of 10.42 μm between each other. The volcano-like pattern forms diagonal lines across the egg. There are also flat and elongated silks or scales, in the form of a leaf, which seem to be inserted in the chorion of the egg, mainly in the volcano-like structure, this character also was observed in eggs of *Pa. texana* (Fig. 10). Examined material: Eggs collected on September 2001 in Santa Inés, Santa María Chimalapa, Oaxaca (16° 46′ 20.01″ N; −94° 48′ 16″ W; 263 m).

Discussion

Studies of the egg exochorion until now have described nine morphological chorionic patterns for specimens of the subfamily Phlebotominae in the Americas. We present the exochorion pattern of ten sand fly species from different regions in Mexico. For the species *Mi. chiapanensis* and *Lu. cruciata* there are previously described patterns in Mexico, while for *Pa. texana* a pattern is known from the United States, besides this species having a wide distribution in Mexico.

Chorionic patterns in sand flies have been little studied because it is difficult to obtain eggs in natural conditions or to establish laboratory colonies (Noguera et al., 2003). In this study, we corroborate that it is possible to obtain an egg chorionic pattern without laying the female to spawn, instead dissecting the abdomen of a gravid female to extract the eggs directly (Jariyapan et al., 2022). Although this method is efficient and economic, yielding morphological information of sand fly eggs, in some cases eggs are difficult to separate, probably because the chorion, as a secretion of the ovarian follicular cells, is viscous, in order to foster adherence of egg to a substrate when laid. Apparently, within the female, this viscous layer joins the egg, which makes it difficult to separate

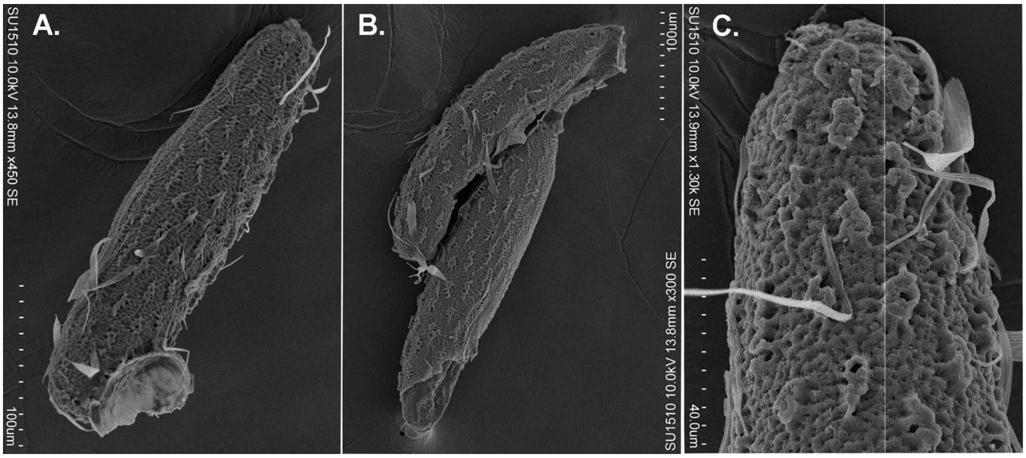

Fig. 10 Exochorion pattern of *Trichopygomyia triramula*. **A**, **B** Complete egg, **C** Zoom to the volcano-like pattern

one egg from another and in some cases made it difficult to visualize the chorionic pattern. The chorion consists of three layers: an inner thin layer, an intermediate thick porous layer and an outer layer bearing a sculptured pattern (Pérez & Ogusuku, 1997). However, we observed and additional layer that covers the pattern, in some cases preventing these areas to be observed in the eggs of *Lu. cruciata*, *Pa. maya* and *Mi. vindicator*. We were unable to observe micropyles on eggs directly obtained from a female abdomen. However, previous studies state that this structure is usually surrounded by an ornamented area that could be used as species-specific characters for sand fly ootaxonomy (Fausto et al., 2001; Pérez & Ogusuku, 1997).

We observed five chorionic patterns, with the polygonal pattern as the most common between the species analyzed. This pattern has been previously recorded in at least 29 species, presenting cells with different forms such as rectangular, pentagonal, and hexagonal, with morphology varying according to genus or species (Alencar & Scarpassa, 2018; Montes de Oca-Aguilar et al., 2014, 2016). Variation in chorionic pattern may also contribute to understanding adaptations to different types of environments of oviposition sites, since differences in the size of the ridges (high or smooth) has been recorded depending on the need for water storage (Jariyapan et al., 2022; Ward & Ready, 1975). Therefore, complementary environmental information is needed to allow establishing oviposition sites according to variation of chorionic pattern.

In the present study, species with a polygonal pattern were *Da. deleoni*, *Mi. cayennensis*, *Mi. durani*, *Mi. vindicator* and *Pa. texana*. Previously, Endris et al. (1987) stated that *Pa. texana* presents a polygonal pattern, yet they did not provide evidence nor a description, therefore we do not know whether the chorionic pattern in the Mexican specimens is similar to the previously recorded one from United States. However, we observed that the ridges show thin lines with square-like shapes, with the rough area between each ridge showing small protuberances.

In the sand fly genus *Dampfomyia* a pattern of parallel ridges (connected and unconnected) has been previously recorded in the species *Da. anthophora* (Endris et al., 1987) and *Da. beltrani* (Montes de Oca-Aguilar et al., 2014). However, in *Da. deleoni* collected from Quintana Roo, we observed a polygonal pattern characterized by pentagonal and rectangular cells. It is common for species of the same genus to show a similar pattern, however different patterns may be present within the same genus, for instance in the genus *Nyssomyia* some species present a polygonal pattern (e.g., *Ni. antunesi*, *Ni. yuilli*) and other parallel ridges (e.g., *Ni. whitmani*, *Ni. intemedia*) (Alencar & Scarpassa, 2018).

Regarding the species of *Micropygomyia* analyzed (*Mi. cayennensis*, *Mi. durani*, *Mi. vindicator*), they showed a polygonal pattern with clear differences between them; these three descriptions conform new exochorion records for this genus. In the species *Mi. trinidadensis* and *Mi. vexator* it has also been recorded a polygonal pattern, but the elliptical (*Mi. venezuelensis*) and reticular (*Mi. absonodonta*) patterns also have been described (Alencar & Scarpassa, 2018). We observed a different pattern for *Mi. chiapanensis*, besides that a previous study described the eggs of this species with a polygonal pattern with square, pentagon and hexagon forms, in specimens collected from the locality Actopan, Veracruz (Montes de Oca-Aguilar et al., 2016). The specimens analyzed in this study, from Ignacio de la Llave, Veracruz, showed a verrucose pattern in some areas of the eggs.

Since it has been recorded that species of *Micropygomyia* generally show a polygonal pattern, we believe likely that the eggs lost this third layer during sample processing, turning out that only the verrucose pattern of the intermediate layer was observed. Complementary studies are necessary to explore the variability in the morphology of the eggs of these species.

The chorionic pattern of unconnected and connected parallel ridges of *Lu. cruciata* eggs has been previously recorded (Endris et al., 1987; Montes de Oca-Aguilar et al., 2017). According to its distribution, this species may present variation in characteristics of its chorionic pattern. In Mexico, three exochorionic patterns related to *Lu. cruciata* exist until now, which are classified into three morphotypes: (1) TACH (from Chiapas) with polygonal ridges, (2) AVER (from Veracruz) with connected parallel and longitudinal ridges, and (3) HOYU (from Yucatán) showing ridges with weak connections. Based on the patterns observed in our samples, we propose a new morphotype named Nayarit (XANAY), characterized by connected parallel granular ridges on the ventral side and unconnected parallel granular ridges on laterals of the egg. Since *Lu. cruciata* is widely distributed in Mexico and plays an important role in the transmission of *Leishmania*, this new morphological information may prove useful for the classification of this species.

The volcano-like pattern has been previously described in species of *Bichromomyia* and *Psychodopygus*, and it has been suggested that this pattern could be associated to species that deposit their eggs in damp microhabitats (Alencar & Scarpassa, 2018; Fausto et al., 2001; Ward & Ready, 1975). Now, such pattern is also described for the first time in *Trichopygomyia*, although unlike the other genera, eggs of *Tr. triramula* are smaller than the other species. In relation to scales observed in the exochorion we are unsure whether they are part of the ornamentation or not, these being the first time these structures are recorded. In Mexico, *Tr. triramula* has been only recorded from Chiapas and Veracruz (Ibáñez-Bernal & Durán-Luz, 2022), and now we record it from Oaxaca for the first time. This species is not considered of epidemiological relevance in the transmission of *Leishmania* in Mexico.

We were unable to determine a pattern in *Bi. olmeca olmeca*, yet we observed that the first layer showed a polygonal pattern, although in the middle layer some areas of the egg have a rugose pattern that appears similar to the volcano-like pattern, so complementary information would help confirm this finding. A previous study has determined that the egg of the species *Bichromomyia olmeca nociva* and *Bichromomyia flaviscutellata* has the volcano-like pattern. However, from our study, size and shape of the egg provide the most significant differences, since unlike the other species, in *Bi. olmeca olmeca* the egg showed an oval shape and is smaller (156 × 83 µm) compared to the other species which report it twice as long (392–410 µm) but with a similar width (82–86 µm) (Alencar & Scarpassa, 2018). The species *Bi. olmeca* is morphologically classified into the subspecies *Bi. olmeca olmeca*, *Bi. olmeca bicolor*, and *Bi. olmeca nociva*. Recently, it has been suggested that *Bi. olmeca olmeca* should be raised to species status because its genetic differences with other subspecies (Lozano-Sardaneta et al., 2023). We encourage efforts to explore sculpturing pattern and distinguishing traits of the egg in species of the genus *Bichromomyia*, which include species of high significance in the transmission of *Leishmania* spp. in the Americas (Lozano-Sardaneta et al., 2023).

We provide evidence for the first time on the exochorionic pattern of *Pa. maya*, which we classified as disperse. However, this classification should be considered preliminary, as in our SEM image we observed an apparent extra outer layer, given the possibility that the egg may contain another layer with another pattern that was lost during processing of the material, so complementary information is necessary to elucidate the actual pattern.

Conclusions

This study includes the description of the exochorion pattern of eggs directly obtained from the abdomen of female specimens from Mexico, supporting that extraction of eggs directly from an abdomen might be an option to complement taxonomic descriptions. We performed a morphological description of the eggshell of 10 species using this method, including *Bi. olmeca olmeca*, *Da. deleoni*, *Mi. cayennensis*, *Mi. chiapanensis*, *Mi. vindicator*, *Mi. durani*, *Lu. cruciata*, *Pa. maya*, *Pa. texana* and *Tr. triramula*. Notably, we detected differences with respect to previous descriptions in species such as *Mi. chiapanensis*, *Lu. cruciata*, and *Pa. texana*, which provide additional information to the classification of immature stages. Further studies on exochorion patterns would enrich morphological and phylogenetic knowledge of sand fly species.

Abbreviations

CO_2 Carbon dioxide
m Meter
µm Micrometer
N Number

Acknowledgements

Yokomi N. Lozano Sardaneta is currently a postdoctoral researcher under a fellowship from Programa de Becas Posdoctorales DAGAPA-UNAM 2023-2024.

Author contributions

YNLS conceptualization, methodology, writing—original draft, writing—review & editing, visualization, formal analyses; HH methodology, formal analyses, writing—review & editing; BMG methodology, formal analyses, writing—review & editing; ACR formal analyses, data interpretation, writing—review & editing, resources, supervision.

Funding
Dirección General de Asuntos del Personal Académico (DGAPA-UNAM), Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT), project IN216824 "Biodiversidad de Diptera y Neuropteroidea de la Reserva de la Biósfera Tehuacán-Cuicatlán con herramientas novedosas".

Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 22 December 2023 Accepted: 7 May 2024
Published online: 13 May 2024

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