MORPHOLOGICAL ANALYSIS OF *PANSTRONGYLUS GENICULATUS* EGGS (HETEROPTERA, REDUVIIDAE, TRIATOMINAE) FROM A CHAGAS' ENDEMIC AREA IN THE CENTER-WEST OF VENEZUELA

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Recibido: Junio 2011. Aprobado: Octubre 2011.
Publicado: Noviembre 2011.

ABSTRACT
As a classical point of view *Panstrongylus geniculatus* has been considered as a secondary vector of *Trypanosoma cruzi* protozoon, the etiological agent of Chagas Disease, because to its association with sylvatic areas, among other factors. However, in the last years it has been found repeatedly in domiciles of rural and urban environments, where it has been identified by observation of eggs, nymphs, exuviae and adult forms. The structural characteristics of the eggs could be useful to obtain information of interest that facilitate an identification and discrimination of species living in such environments. The aim of this work was to analyze by mean of optical and scanning electron microscopy, first filial generation eggs (F1) obtained from specimens of *P. geniculatus* maintained in laboratory conditions. The parental specimens of this colony were captured in Rio Claro, at the center-west of Venezuela. Analysis showed that length and maximum width eggs were 1.73 mm and 1.25 mm, respectively and a narrow neck were observed; 120 aeropyles were calculated in the spermatic gutter and 142 micropyles in the external region of the chorial rim on a prominent and convex operculum; polygonal cells covered with cement were observed on the operculum surface and on the shell. The structure of micropylar system could contribute to identify and distinguish the adaptations of this specie to different habitats.

Keywords: SEM; operculum; Chagas vector; micropilar system; eggshell.

RESUMEN
Panstrongylus geniculatus ha sido considerado tradicionalmente como un vector secundario del protozoo *Trypanosoma cruzi*, agente etiológico de la enfermedad de Chagas, debido entre otros factores, a su asociación a hábitats silvestres. Sin embargo reiteradamente en los últimos años es encontrado en los domicilios de ambientes rurales y urbanos, donde se le ha identificado por la observación de huevos, ninfas, exubias y adultos. Las características estructurales de los huevos podrían contribuir con información de interés para facilitar la identificación y discriminación de la especie que habita en tales ambientes. En el presente trabajo se analizaron mediante microscopía óptica y electrónica de barrido, los huevos de la F1 obtenida en condiciones de laboratorio a partir de adultos colectados en Rio Claro, en el centroccidente de Venezuela. Este análisis mostró que el largo y ancho máximo del huevo fueron 1,73 mm y 1,25 mm respectivamente, cuello evidente y estrecho, se calcularon numerosos aeropilos (120) en la gotera espermática y 142 micropilos en la cara externa del borde corial; opérculo convexo y prominente; celdas poligonales cubiertas de cemento tanto en la superficie del opérculo como del cascarón. La estructura del sistema micropilar podría contribuir a identificar y discriminar adaptaciones de la especie a diferentes hábitats.

Palabras clave: MEB; opérculo; vector de Chagas; sistema micropilar; cáscara del huevo.

INTRODUCTION

In Venezuela, vectorial transmission of Chagas disease has been associated with high rates of infection and colonization of the human dwelling by *Rhodnius prolixus*, who is traditionally considered the main agent of vectorial transmission of *Trypanosoma cruzi* in this country, while *Panstrongylus geniculatus* has been often considered as a secondary vector. *P. geniculatus* is a widely distributed specie in Central and South America and it has been associated with armadillos caves ([1, 2]). This species has been found distributed in rural areas of most states of Venezuela, however, it has not been frequently found in human dwelling. This triatomine is found frequently infected by *T. cruzi* ([3, 4, 5, 6]), moreover, immature and adults forms of *P. geniculatus* infected by this parasite has been found at urban areas of Caracas City and its surroundings ([7, 8, 9, 10]). These findings could suggest an adaptation of *P. geniculatus* to artificial environments and to reconsider its secondary role in the transmission of Chagas disease in Venezuela.

Triatomine eggs chorion structure has proved to be useful as a taxonomic tool at interspecific level ([11, 12, 13, 14, 15]) and also in investigations about variations between triatomine populations and spatial structure. Costa *et al.* [16] found differences in the perforation average of exocorion cells when several populations of *Triatoma brasiliensis* were compared. On the other hand, Rosa *et al.* [17] found differences in the diameter of the operculum, and in the length and width of the egg when wild and colony eggs of *P. megistus* were also compared; because of this finding they proposed a reduction in eggs size as a marker of domiciliation, assuming that colonization under laboratory conditions is analogous to insect adaptation to domiciles.

The aim of the present study is to expand knowledge about *P. geniculatus* from Venezuela, by mean of optical and scanning electron microscopy.

We studied shape, operculum, ultrastructure of the cephalic region and exchorion of the eggs obtained from a *P. geniculatus* population captured in human dwellings in the central-west- of Venezuela.

MATERIALS AND METHODS

Biological material: Eggs were obtained from laboratory colony F1 females, which it was formed from adults collected in La Frontera Rio Claro (located at N 9°55'41" and W 69°22'45") domiciles (Lara State-Venezuela) on May 2010. Colony was maintained at 28 ° C, 50% humidity and fed on hen. These specimens were identified according to Lent & Wygodzinsky key [1]. Embryonated, hatched eggs and opercula were washed three times with distilled water, dried at 26°C and then stored and maintained in Petri dishes at room temperature until use.

Shape index: Images from 20 embryonated eggs were obtained with a Leica lens S6D connected to a Canon digital camera. Distances A, B, C and D (see Figure 1A) and shape index B/D were measured.

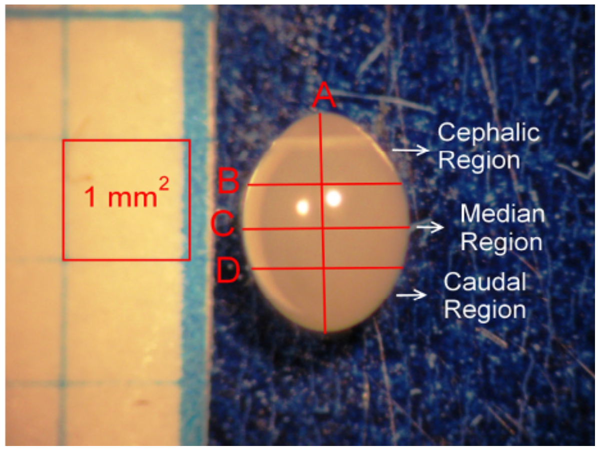

Fig. 1A. Egg of *Panstrongylus geniculatus* showing the distances A, B, C and D, as well as the different regions: cephalic, median and caudal.

Operculum: Operculum diameter and chorion rim were measured by mean of a stereomicroscope Wild Heerbrugg to 25x with a camera lucida. Chorion rim perimeter was calculated according to the formula π x diameter.

Scanning electron microscopy: by this technique, six embryonated and hatched eggs were analyzed with their respective operculum. They were fixed with tape on a metal stand, and metalized with gold for 100 seconds on an ionic coverter SPI-MODULE TM S-2500 operated at 10mA. The micrographs were taken with an electron microscope Hitachi S-2500 operated at 20 Kv. To analyze egg's morphology it was used a nomenclature proposed by Barata [18].

Statistical analysis: We calculated mean, standard deviation and coefficient of variation of operculum diameter, chorion rim, distances between micropyles and between aeropyles, distances A, B, C and D and shape index. Also maximun and minimun values of each parameter are given.

RESULTS

Entomological material: On the month of May 2010 there was passively captured 25 adult insects of *P. geniculatus*, 4 females and 21 males, 23 found inside the domiciles and 2 in the peridomiciles, 3 of them presented flagellate forms of *T. cruzi* in the intestinal contents , in which 2 were captured in the peridomicile and 1 in the domicile.

Macroscopic aspects: The eggs of *P. geniculatus* analyzed were laid as individuals forms and not attached to the substrate; they were ovoid, rounded at the caudal region and conic at the cephalic region, pearl color when embryonated and more transparent when hatched; they showed a remarkably lateral groove (Figures 1A, 1B).

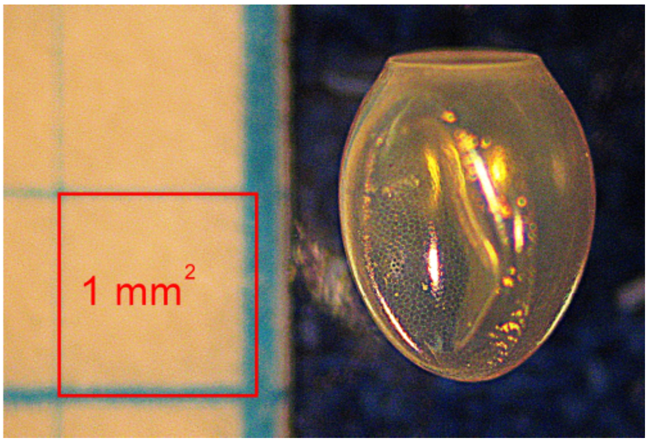

Fig. 1B. Egg of *Panstrongylus geniculatus* showing the lateral groove.

The average distances A, B, C and D were 1.73 mm, 1.16 mm, 1.25 mm and 1.18 mm, respectively and 0.98 average shape index. Below the operculum it was noticeable the endochorial membrane (Figure 2, Table 1).

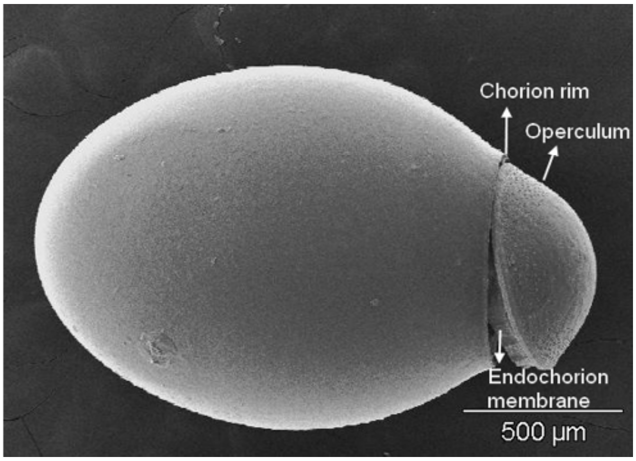

Fig. 2. Whole egg of *Panstrongylus geniculatus* showing the operculum, chorion rim and endochorion membrane.

Microscopic aspects: Operculum. It was convex (Figure 3), polygonal cells were visible on the surface covered with a perforated layer of cement on the border and smooth on the rest of the surface (Figures 4-6). The average diameter of the operculum was 0.72 mm while chorion rim was 0.76 mm. The average perimeter of the chorion rim was 2.28 mm (Figure 7).

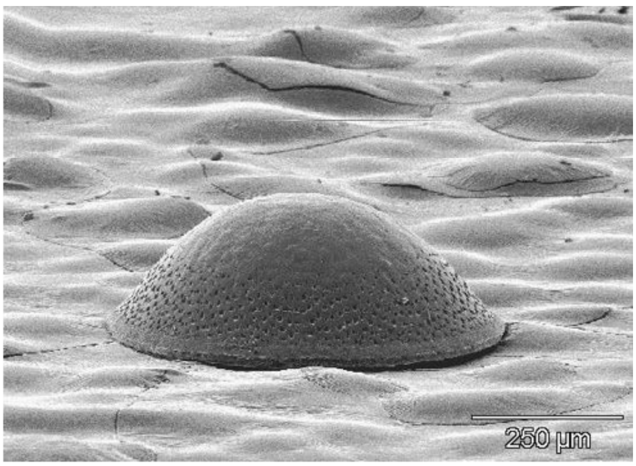

Fig. 3. Operculum of *Panstrongylus geniculatus* from a lateral view.

Table 1. *Panstrongylus geniculatus* eggs measure. Shape index: B/D; DCR: diameter of chorion rim; DOP: diameter of operculum; DMP: distance intermicropyles; NMP: number of micropyles; DAP: distance interaeropyles; NAP: number of aeropyles.

	Distances				Shape Index	DCR	DOP	Perimeter	DMP(NMP)	DAP(NAP)
	A	B	C	D						
Average	1,73	1,16	1,25	1,18	1,26	0,72	0,76	2,28	0,016(142)	0,019(120)
St. Des.	0,08	0,02	0,02	0,02	0,07	0,01	0,04	0,03	0,001	0,03
Max.	2,07	1,21	1,3	1,22	1,51	0,74	0,8	2,32	0,018	0,024
Min.	1,66	1,12	1,21	1,13	1,19	0,71	0,68	2,24	0,013	0,013
Var.Coef.	4,95	2,5	1,92	1,77	5,63	1,6	5,21	1,56	12	0,017
N	20	20	20	20	20	4	11	4	6	8

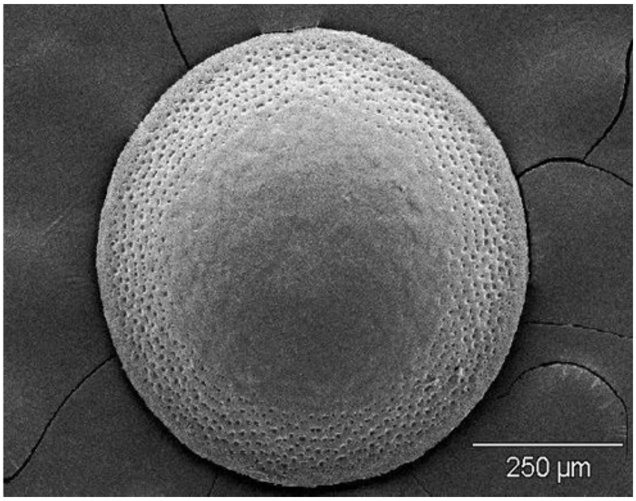

Fig. 4. Operculum of *Panstrongylus geniculatus* from a top view.

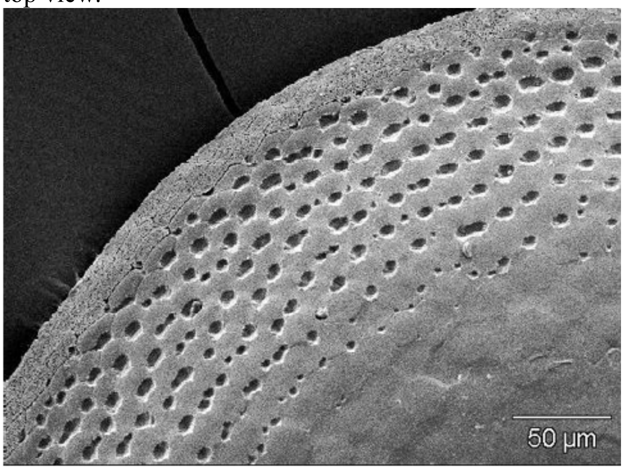

Fig. 5. Detail of the operculum of *Panstrongylus geniculatus.*

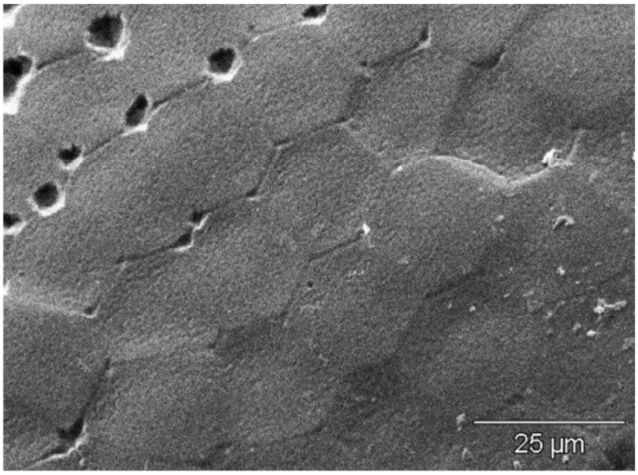

Fig. 6. Polygonal cells on the operculum of an egg of *Panstrongylus geniculatus.*

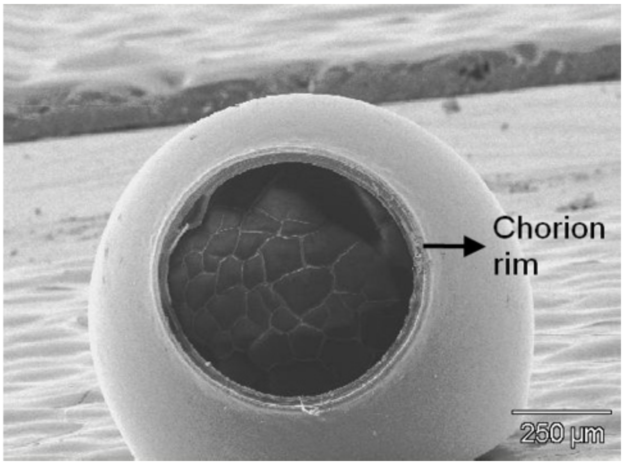

Fig. 7. Hatch egg of *Panstrongylus geniculatus* showing the chorion rim.

Cephalic region: A neck was seen immediately below the chorion, measuring about 3-4 um (Figures 8-9) and below it was observed polygonal cells covered with a layer of cement (Figure 10).

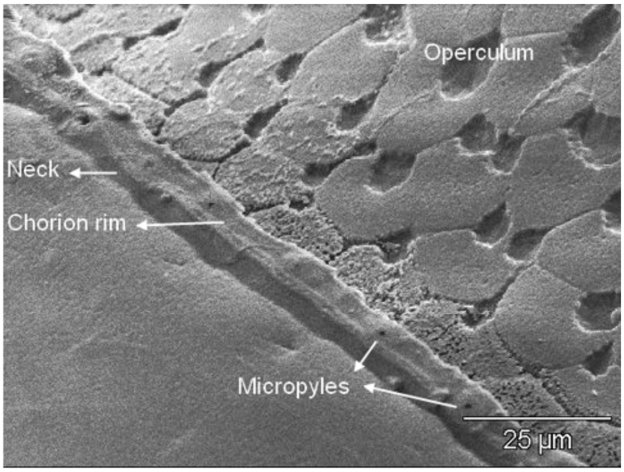

Fig. 8. Detail of an embryonated egg of *Panstrongylus geniculatus* displaying the chorion rim, operculum, micropyles and neck.

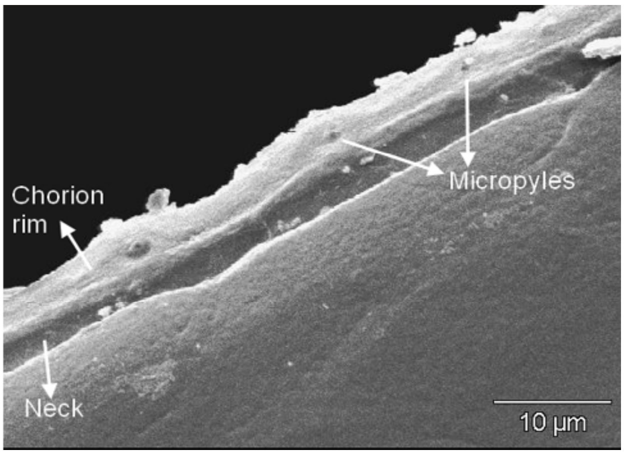

Fig. 9. Detail of a hatched egg of *Panstrongylus geniculatus* showing the chorion rim, neck and micropyles.

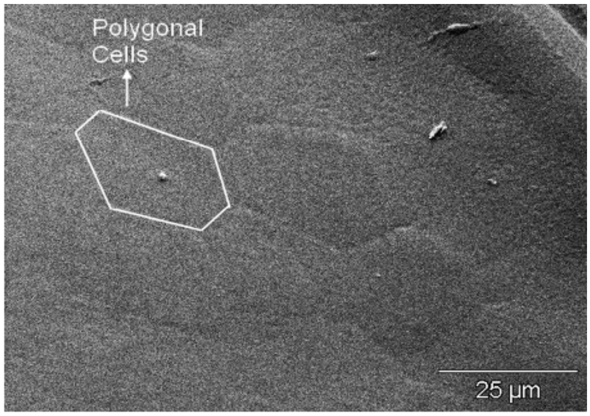

Fig. 10. Detail of polygonal cells on an egg of *Panstrongylus geniculatus.*

Micropyles were observed in the outer region of the chorionic rim above the neck (Figure 11), while aeropyles are located in the spermatic gutter, being smooth, narrow, rounded and adjacent to the operculum and chorionic rim (Figures 11,12). In some regions, it was not possible to observe micropyles and aeropyles around the chorionic rim perimeter, because they were covered with a material maybe from the inner egg. Where it was possible to observe, the distance between each of them were measured, giving an average distance of 0.019 mm between aeropyles and 0.016 mm between micropyles; chorion rim average diameter was 0.72 mm and average perimeter was 2.28 mm. Assuming that micropyles and aeropyles are found at regular intervals, total number of micropyle and aeropyles in a perimeter section was extrapolated to the entire perimeter, obtaining a total of 142 aeropyles and 120 micropyles. In the chorial rim region in touch with operculum there are three layers, two of them in the sealing bar and the third outermost located, where aeropyles are in (Figure 12). In the light microscopy polygonal cells were observed in the exocorion, however in SEM these cells are covered by a layer of smooth cement.

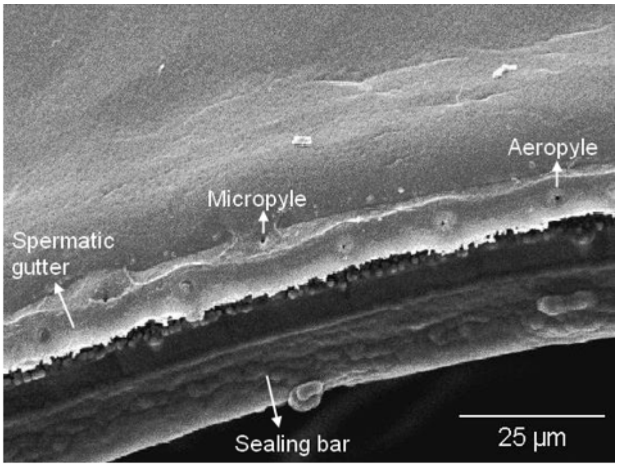

Fig. 11. Detail of the sealing bar (lateral view) of *Panstrongylus geniculatus* displaying the spermatic gutter, aeropyles and micropyles.

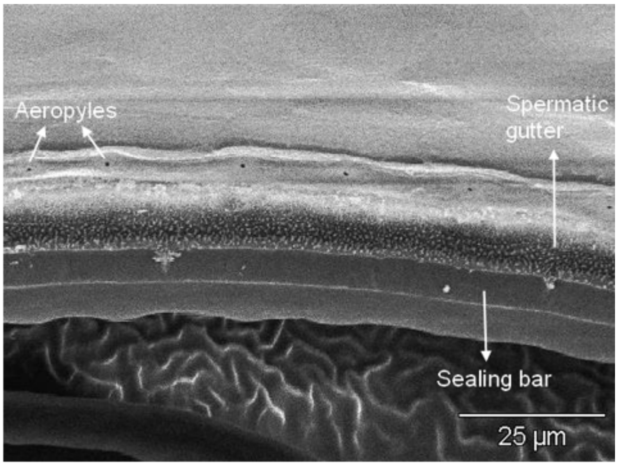

Fig. 12. Detail of the sealing bar (top view) of *Panstrongylus geniculatus* showing the spermatic gutter andaeropyles.

DISCUSSION AND CONCLUSIONS

Ten of 13 studies done about species of the *Panstrongylus* genus, including *P. geniculatus* ([18]) reported that egg's maximum length varies between 2.42 mm in *P. diasi* and 1.53 mm in *P. rufotuberculatus*, diameter between 1.75 mm in *P. diasi* and 1.10 mm in *P. chinai* and laid individually; egg's shape is symmetrical and ranges from ellipsoidal as in *P. megistus* to almost spherical as in *P. rufotuberculatus*. The eggs from animals of this genus show additional characteristics as the absence of lateral groove, neck and collar, and the presence of a chorion border below the sealing bar and spermatic gutter, which is smooth, rounded and it is found adjacent to the operculum and the shell.

Operculum varies from slightly prominent as in *P. rufotuberculatus* to considerable prominent as in *P. tupynambai*; shell is very translucent, bright and white pearl colored. In *P. megistus* exochorion has smooth hexagonal cells and notable and conspicuous limiting lines (cell borders), which are almost imperceptible in *P. herreri* and *P. lignarius*. The study done in *P. geniculatus* specie was based on a sample of 1.55 mm long and 1.16 mm diameter 3 small size eggs, which had perforations at the limiting lines ("LLs") intersections.

P. geniculatus's eggs analyzed in the present study differ from eggs analyzed by Barata [18] on 10 triatomine species, because we were able to observe a prominent lateral groove and a neck, and specifically an absence of perforations at "LLs" intersections. Since other descriptions are not known about ultrastructural features of *P. geniculatus* eggs, we cannot even say whether identified differences correspond to local adaptations or these are a specie characteristics.

Cobben [19] considered about Heteroptera's egg apomorphic conditions a layered chorion and increased number of micropyles displaced from the cephalic region pole. Triatominae chorion invariably consists of several layers ([18]), nevertheless chorion border design shows some variation on the chorionic rim, micropyles and aeropyles present a constant pattern in this subfamily, usually distributed in a variable number on the chorion rim, for example in *T. rubrofasciata*, *Linshcosteus costalis* and *L. confumus* aeropyles are small, numerous (92-177) and located above micropyles ([20]).

Similar results has been described in the *Rhodnius* genus (200) ([21]), in three *Belminus* species ([22]), in *T. barberi* ([23]) and in the present paper, where on the eggs of *P. geniculatus* aeropyles are located in the spermatic gutter at regular intervals and in a number of approximately 120, while Barata [18] and Rosa *et al*. [11] found aeropyles located on the outer edge of the chorion rim in *Rhodnius* and *Triatoma* genera.

Haridass [20] found small numbers of micropyles in the spermatic gutter of *T. rubrofasciata* (12-18), as well as in *L. costalis* (12-16) and in *L. confumus* (9-13), however Evangelista-Martínez *et al*. [23] observed a single micropyle in *T. barberi* eggs. In the present study a higher number of *P. geniculatus* eggs's micropyles were estimated (142), it is difficult to compare micropyles number between species, because these structures have not been enough described in Triatominae.

The egg shape of most insects is the result of force transmitted by follicular epithelium microtubules and desmosomes during oocyte development, when surface development is fast along the polar axis and slow in the circumferential direction at right angles to that axis, follicular resistance to circumferential growth is the main factor responsible for egg ovoid form ([24]); it is only required to give form but it is not necessary to its own maturation ([25]). The *P. geniculatus* eggs described in this paper have a caudal region more circular in shape while the cephalic region is more conical, which, according to Tucker & Meats [24], could be due to the pressure exerted by microtubules and desmosomes of follicular cells- that are greater in the oocyte's region that will constitute the cephalic and middle egg's region.

Egg's shape has proved to be an interesting taxonomic tool, for example between species of Triatomines ([11, 12, 13, 17, 22]), *Drosophila* ([26]), in Embioptera Order ([27]) or Culex ([28]). It has been proposed egg's width ratio at two perpendicular levels to the longitudinal axis as a measure of shape, concluding that variation found in this character indicates their potential taxonomic purpose. Based on this proposal, we calculated a B/D index in eggs of *P. geniculatus* analyzed to provide a new feature that might be of interest to compare *P. geniculatus* from different habitats. And hence, egg's shape index may be useful as a taxonomic tool for higher taxonomic ranks and populational levels in Triatomines.

ACKNOWLEDGEMENTS

The present paper was funded by Fondo Nacional de Ciencia y Tecnología (Fonacit) project N° 2007001425 in the context of social technologic transfer.

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