Morphological study of the eggs and nymphs of *Triatoma dimidiata* (Latreille, 1811) observed by light and scanning electron microscopy (Hemiptera: Reduviidae: Triatominae)

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Eggs and nymphs of Triatoma dimidiata *were described using both light and scanning electron microscopy. The egg body and operculum have an exochorion formed by irregular juxtaposed polygonal cells; these cells are without sculpture and the majority of them are hexagonal in shape. The five instars of* T. dimidiata *can be distinguished from each other by characteristics of the pre, meso and metanotum. The number of setiferous tubercles increases progressively among instars. The sulcus stridulatorium of 1st instar nymphs is amorphous, showing median parallel grooves; from the 2nd instar on the sulcus is, progressively, elongate, deep and posteriorly pointed with stretched parallel grooves. All instars have a trichobothrium on the apical 1/3 of segment II of the antenna. The opening of the Brindley's gland is on the mesopleura. Fifth instar nymphs have an apical ctenidium on the ventral surface of the fore tibia. Dorsal glabrous patches are found on the lateral 1/3 of abdomen. Bright oval patches are found on the ventral median line of the abdomen, from segment IV-VI; 1st instar nymphs lack these patches. Abdominal dorsal plates are present from the 1st-5th instars; the 1st instar also contains a rectangular plate in segment IX. From the 2nd instar on, variably-shaped plates are present on segments VII to IX. Morphometric data were also obtained and proved to be useful for distinguishing* T. dimidiata *instars.*

Key words: Chagas disease - vector - morphology - systematics

The importance of the knowledge of the Triatominae eggs was emphasised by Pinto (1924) when he described and differentiated the eggs of *Triatoma brasiliensis* Neiva, 1911 and other triatomines found in Brazil. Galliard (1935b) demonstrated that there is a characteristic ornamentation on the external surface of chorion that permits the recognition of different species. The structure and ornamentation of the eggs analysed by light microscopy (LM) are useful in the identification of similar species, i.e., *Triatoma maculata* (Erichson, 1848) and *Triatoma pseudoma-culata* Corrêa & Espínola, 1964 (Gonçalves et al. 1985).

The eggs of 35 species of *Triatoma* were examined using scanning electron microscopy (SEM); this constitutes less than half the species in the genus. In Table I, a summary of papers using SEM to study the morphology and/or morphometry of eggs and an indication to which group they belong to according to the classification of Barata (1998) is presented.

The subfamily Triatominae has 141 species grouped in 15 genera and five tribes (Jurberg et al. 2009, Schofield & Galvão 2009). *Triatoma dimidiata* is found in northwest South America (Peru, Ecuador, Colombia, Venezuela, Guiana), in all of the countries of Central America and in Southern Mexico (Zeledón 1981), with great variability in colour and body proportions according to their source (Fig. 1). *T. dimidiata* is characterised as being between 24.5-35.0 mm long and having extremely diverse coloration varying from piceous to black with connexivum and corium pale yellow to orange yellow, short, inconspicuous hairs, a head that is wrinkled dorsally, a pronotum that is uniformly piceous or black and legs that are uniformly black (Lent & Wygodzinsky 1979). Bargues et al. (2008), in a interpopulational analysis of *T. dimidiata* and its closest relatives within *Triatoma*, combined DNA sequence analyses, phylogenetic reconstruction methods and genetic variation approaches to show that *T. dimidiata* represents a complex of species and subspecies.

The identification of the Triatominae species based only on nymph characteristics is difficult. According to Brewer et al. (1983a), the first keys to separate the 5th instar nymphs were published by Usinger (1944) for some species of Triatominae for North America, by Abalos and Wygodzinsky (1951) for the 1st and 5th instars for some Argentine species and by Ryckman (1962) for the 5th instar of *Triatoma protacta* (Uhler, 1894) complex. Lent and Wygodzinsky (1979) and Galíndez-Girón et al. (1998) offered keys to identifying distinct genera based on 1st and 5th instar nymphs. Twenty two species of *Triatoma* had their nymphs described and/or some morphological aspects analysed in SEM (Lent & Wygodzinsky 1979, Gonçalves et al. 1985, Jurberg et al. 1986, 1991, 1998, 2002, Rosa et al. 1989, 1992a, b, 2005, Rosa 1995, Rocha et al. 1996, 2009, Rosa

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TABLE I

Summary of the articles on morphology of the eggs of *Triatoma* species in scanning electron microscopy and the group they belong according to the classification of Barata (1998)

Species	Group	Reference
T. arthurneivai Lent & Martins	1	Barata (1998)
T. baratai Carcavallo & Jurberg	1	Rocha et al. (2009)
T. barberi Usinger	2	Barata (1998)
T. brasiliensis Neiva	1	Jurberg et al. (1986), Costa et al. (1997), Barata (1998)
T. brasiliensis macromelanosoma Galvão	1	Costa et al. (1997)
T. breyeri Del Ponte	3	Obara et al. (2007b)
T. circummaculata (Stål)	1	Barata 1998, Rosa et al. (2000)
T. costalimai Verano & Galvão	3	Obara et al. (2007b)
T. delpontei Romaña & Abalos	2	Barata (1998)
T. dimidiata (Latreille)	3	the present paper
T. guazu Lent & Wygodzinsky	1	Silva et al. (2000)
T. infestans Klug	1	Barth and Muth (1958), Barata (1998), Hinton (1981), Jurberg et al. (1998)[b]
T. juazeirensis Costa & Felix[a]	1	Costa et al. (1997)
T. jurbergi Carcavallo, Galvão & Lent	1	Jurberg et al. (2002)
T. lecticularia (Stål)	2	Barata (1998)
T. lenti Sherlock & Serafim	1	Barata (1998)
T. longipennis Usinger[c]	3	Obara et al. (2007a)
T. maculata (Erichson)	1	Hinton (1981), Gonçalves et al. (1985)
T. matogrossensis Leite & Barbosa	1	Obara et al. (2007b)
T. melanica Neiva & Lent[a]	1	Costa et al. (1997)
T. nitida Usinger	3	Jurberg et al. (1991)
T. oliveirai (Neiva, Pinto & Lent)	1	Barata (1998)
T. pallidipennis Stål[c]	3	Obara et al. (2007a)
T. patagonica Del Ponte	1	Visciarelli et al. (2004)
T. phyllosoma (Burmeister)[c]	3	Obara et al. (2007a)
T. picturata Usinger[c]	3	Obara et al. (2007a)
T. platensis Neiva	2	Barata (1998)
T. protracta (Uhler)	2	Barata (1998)
T. pseudomaculata Corrêa & Espínola	1	Gonçalves et al. (1985)
T. rubrofasciata (De Geer)	3	Haridass (1986)
T. rubrovaria (Blanchard)	1	Barata (1998), Rosa et al. (2000)
T. sordida (Stål)	1	Barth & Muth (1958)
T. sherloki Papa, Jurberg, Carcavallo, Cerqueira & Barata	1	Obara et al. (2007b)
T. tibiamaculata (Pinto)	3	Obara et al. (2007b)
T. vandae Carcavallo, Jurberg, Rocha, Galvão, Noireau & Lent	1	Silva et al. (2005)
T. williami Galvão, Souza & Lima	1	Silva et al. (2005), Obara et al. (2007b)

a: cited as populations of *T. brasiliensis* pertaining to the varieties *juazeiro* and *melanica*; *b*: cited as *T. melanosoma*; *c*: cited as belonging to *Meccus;* 1: egg ellipsoid, symmetric, collar present, chorial rim in a very narrow rim, exochorion with hexagonal cells with sparse perforations; 2: egg ellipsoid, without collar, chorial rim in a large rim, exochorium not well defined, hexagonal cells absent; 3: intermediate characteristics of groups 1 and 2.

& Barata 1997, Galíndez-Girón et al. 1998, Silva et al. 1999a, b, 2000, 2002, 2003, 2005, Naegele et al. 2003). In this paper, the morphological description of eggs and nymphs of *T. dimidiata* using both LM and SEM microscopy is shown to contribute to Triatominae systematic and phylogenetic studies.

MATERIALS AND METHODS

The specimens used in this study came from intradomicilary populations from Mexico (Vera Cruz, city of Tuxpan), which are the source of the colonies maintained by the laboratories at the Fundação Oswaldo Cruz-Fiocruz. According to Lent and Wygodzinsky (1979) and Bargues et al. (2008), they belong to *T. dimidiata maculipennis* (Stål, 1859) (Fig. 1). 1st and 2nd instars nymphs were obtained from Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas, Instituto de Pesquisa René Rachou, Belo Horizonte, Minas Gerais, where this species has been maintained since 2002; the nymphs of 3rd, 4th and 5th instars came from Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, where the nymphs have been maintained since 2004 from specimens provided by the first laboratory mentioned above. Illustrations of the five instars were prepared using a *camera lucida* connected to a stereomicroscope. A measuring eyepiece was used for measurements of the morphometric parameters; the measurements are in millimetres. For SEM analysis, the eggs and nymphs were cleaned in distilled water with an ultrasonic cleaner and, after drying, the samples were mounted in stubs and metallised with gold. Thirty eggs were measured. The morphometric parameters used for the eggs were total length, maximum width and diameter of the operculum. We followed Lent and Wygodzinsky (1979) in the morphometric parameters of the nymphs, which are illustrated in Fig. 2, except for abdomen width and total length.

RESULTS

Eggs
(Figs 3-8, Table I)

Egg symmetric and ellipsoid with brilliant white coloration (Fig. 5). The relationship between the diameter of the operculum and the maximum width of the egg was 0.56; between the length and width 1.68. Mean total length was 2.15 ± 0.05. Maximum width of the egg was 1.28 ± 0.02. The diameter of the operculum was 0.72 ± 0.01. The greatest diameter of each cell was about 25 μm

Fig. 1: *Triatoma dimidiata*. Male, dorsal view. Bar = 5 mm.

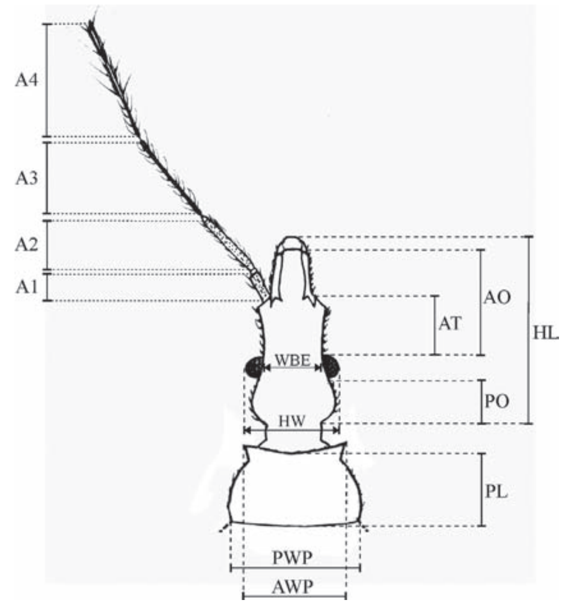
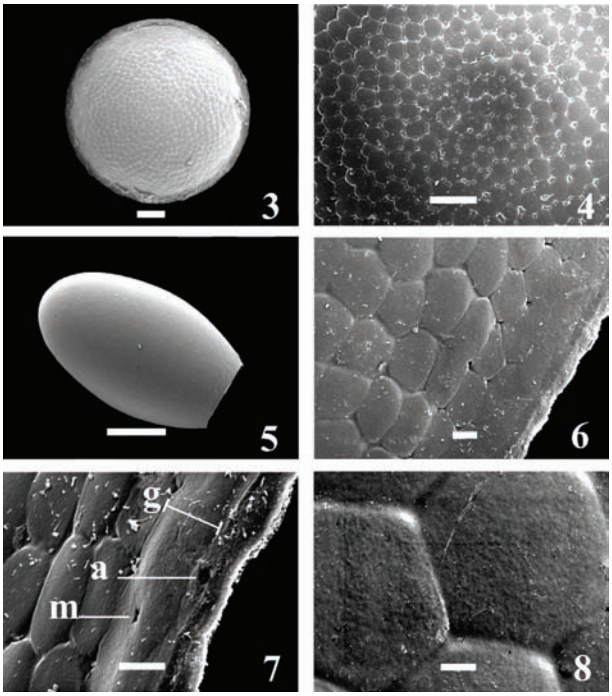


Fig. 2: *Triatoma dimidiata*. Nymph 2nd instar, dorsal view, head and pronotum, indicating 13 morphometric parameters. AO: length of anteocular portion of head; AT: antenniferous tubercle length; AWP: anterior width of pronotum; A1: length of antennal segment I; A2: length of antennal segment II; A3: length of antennal segment III; A4: length of antennal segment IV; HL: head length; HW: head width; PL: pronotum length; PO: length of postocular portion of head; PWP: posterior width of pronotum; WBE: width between eyes.

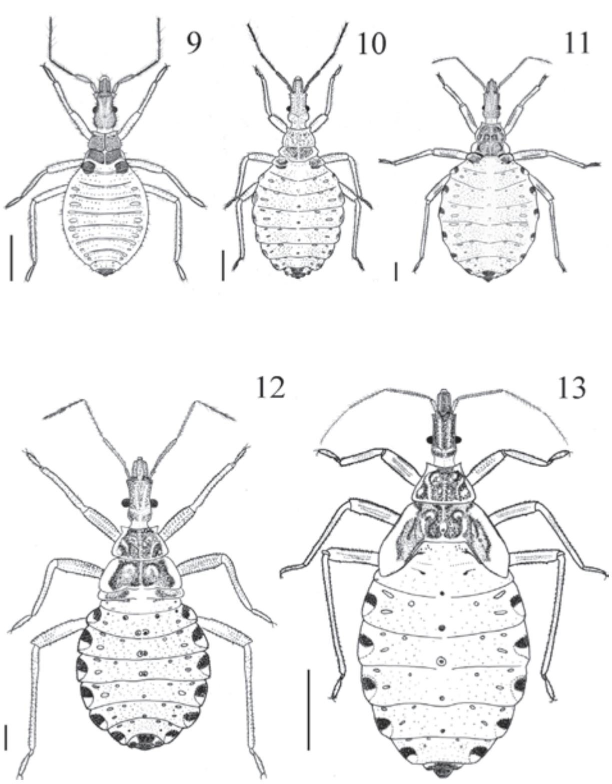
(SEM). Operculum circular, slightly convex, the cells tended to be rounded and apparently not contiguous (Figs 3, 4). Exochorium and operculum with contiguous irregular polygonal cells; the majority being hexagonal, with some pentagonal and, rarely, quadrangular; cells without ornamentation (Figs 4, 6, 8). Chorial and opercular borders in narrow ringed shaped bands without cells (Figs 3, 6, 7); cells of the egg body were lightly padded (Fig. 8). With SEM were observed up to the chorial rim, aeropiles, slightly defined spermatic grooves and micropyles on the external surface (Fig. 7).

Nymphs
(Figs 9-17)

First instar (Figs 9, 14, 15; Tables II, III) - Head, thorax and the two last abdominal segments with medium-brown color, abdomen reddish-brown, legs light-brown. Head, thorax and margins of abdomen covered with conspicuous setiferous tubercles, erect bristles longer than length of tubercle more frequently on the head. Head subcylindrical with bristles inserted in setiferous tubercles concolourous with tegument. Head with its entire surface granular from the clypeus to the neck, with 1 + 1 glabrous longitudinal bands, subparallel, delimiting the central area; clypeus subrectangular surpassing gena. Post-ocular cephalic sutures converging, forming a "Y" and continuing to abdominal segment III in some specimens, to abdominal segment I or II; post-ocular region slightly arched. Anteclypeus almost reaching the middle of antennal segment II. Juga apically narrowed.



Figs 3-8: *Triatoma dimidiata*. Egg. 3: operculum (bar = 100 µm); 4: detail of operculum surface (bar = 50 µm); 5: general aspect of egg (bar = 500 µm); 6: aspect of chorial rim (bar =10 µm); 7: detail of chorial rim (a: aeropyle; g: spermatic groove; m: micropyle) (bar = 10 µm); 8: detail of exochorial surface (bar = 5 µm).



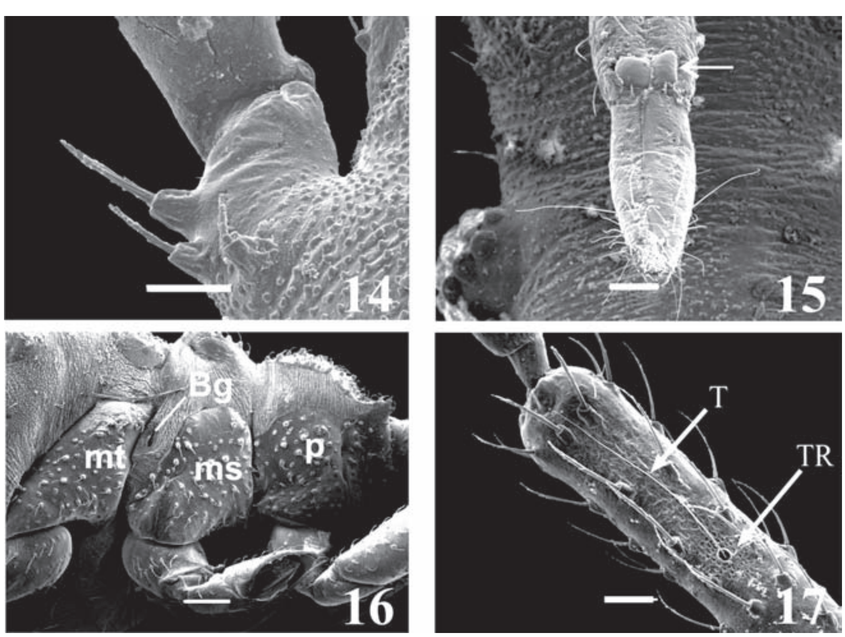
Figs 9-13: *Triatoma dimidiata*. Nymphs, dorsal view. 9: 1st instar; 10: 2nd instar; 11: 3rd instar; 12: 4th instar (bar = 1mm) 13: 5th instar (bar = 5 mm).

Clypeus and gena covered with bristles, two (1 + 1) erect on clypeus, two (1 + 1) curved close the base of the gena and two (1 + 1) erect on each side of the anteclypeus. Antennae four segmented laterally inserted in tubercles at the edge of anterior third of head; antenniferous tubercles with a pair of apicolateral processes with erect bristles (Fig. 14). Antennae with segments I and II light-brown, III and basal third of IV medium-brown, apical 2/3 of IV was whitish. The apex of all segments and intersegmental articulations were also pale. Segment I of antenna not reaching the anterior border of clypeus, with slight pilosity; segment II and III with curved bristles, distributed in parallel rows, on basal half of IV with short bristles and on apical half long and short. Segment II with dorsal trichobothrium and reticulated integument on apical third. Segment III with reticulated integument in a basal straight line. The apical half of segment IV had transversally, finely-striped integument. Segments I and II thicker than segments III and IV. The eyes had globose ommatidia (mulberry-like), with a glabrous ventral postero-inferior area without ommatidia. Ventral region of the head medium-brown with granulosity and a few setaceous tubercles on the lateral margins. The gula were light and glabrous from the anterior-ocular region to the neck; this clear area extends to the intraocular region with 1 + 1 clear, glabrous oval areas. Rostrum triarticulated, rectilinearly touching base of prosternum, light-brown. Segments I and II with short and sparse pilosity; apical third of segment III with a few long bristles and apically with short bristles. Segment II starting at the level of antenniferous tubercles, ending with apex almost reaching the neck; membrane of articulation quadrangular, slightly swollen, dorsally visible between segments II and III (Fig. 15). Thorax medium-brown. Pronotum subtrapezoidal almost as long as meso and metanotum together, with small setaceous tubercles and 1 + 1 discal tubercles; collar with anterolateral angles directed laterally with two (1 + 1) bristles. A stridulatory furrow was visible in SEM, with irregular stripes and flanked by lateral papillae. Mesonotum medium-brown, formed by 1 + 1 subrectangular plates, divided by a membranous transverse stripe circled by areas of lighter colour. Mesonotum length almost equal to half of pronotum longer at the middle than in the lateral margins. Metanotum formed by 1 + 1 sclerotised rounded sublateral plates, with subrectangular borders wider than half of the distance between them; a few median setaceous tubercles. Legs uniformly light-brown, femur and tibia with decumbent bristles bent linearly in parallel rows; tarsi two-segmented with abundant fine bristles, some erect. Coxae with scarce setaceous tubercles. Submedian ventral surface of the femur with a glabrous area, with (1 + 1) subapically prominent tubercles. Ventral region of the thorax light-brown; granulose integument medium-brown around the middle and hind coxae; a row of small setaceous tubercles were present between the meso and metasternum. Pleurae was medium-brown with granular integument posterior region with sparse setaceous tubercles more abundant on mesosternum. Brindley's gland oblong opening placed in the mesopleura. The abdomen was void of connexival markings. Urotergite I with 1 + 1 anterolateral spiracles.

Urotergites I-VIII with two transverse lines of setaceous tubercles arranged linearly. There were glabrous dorsal areas on the lateral third of the abdomen and on segments II-VII, with 1 + 1 on II and 2 + 2 for III-VII; segment VIII with 1 + 1 spots adjoining the anterior margin; segment IX almost completely and X totally medium-brown. Bristles on the edges 3-4 times larger than tubercles. Spiracles between the glabrous lateral areas of ventral segments II-VIII. A rectangular sclerotized plate (chitinous plate) on ventral segment IX. The posterior margins of urosternites VII and VIII with 1 + 1 long erect bristles directed toward the mid-abdomen.

Second instar (Figs 10, 16; Tables II, III) - Head, thorax and abdominal segments VIII-X with variable coloration in tones of brown; the rest of the abdomen was lighter colored legs lighter than the abdomen. The head, thorax, legs and margins of the abdomen covered with tubercles with decumbent hairs more frequently on the head. The integument of the head was granulose covered with short, implanted bristles in tubercles. The margins of the thorax and abdomen were light-colored. The anteclypeus reaching basal third of II antennal segment. The apex of the juga exceeding the antenniferous tubercle. Neck without tubercles with a medium clear line. Antennal segment I slightly exceeding middle of clypeus. The rostrum light-brown, lighter than the head. Segment II of the rostrum beginning at the apex of antenniferous tubercles, ending almost at the neck. Pronotum with 1 + 1 discal tubercles and sparse setaceous tubercles; the glabrous delineated area between tubercles. Mesonotum with 1 + 1 kidney-shaped hairless area occupying 3/4 of the length of the pronotum. The metanotum was formed by 1 + 1 elliptical plates smaller than the distance between them, with an anterior glabrous area, posterior medium margin with a row of rare setaceous tubercles. The thorax was lighter than abdomen ventrally; setaceous tubercles on meso and metasternum. Prosternum with a stridulatory furrow visible with LM. The abdomen with connexival markings on the lateral edges of each segment; urotergites II-VII with apical 2/3 dark and 1/3 light colour. Segments II-VI with a series of five granule aggregations along the mid line. Urotergite I had 1 + 1 linear medium-brown plates at the antero-lateral region, II-VII contained inserted bristles. Segments II-VII contained 3 + 3 glabrous dorsal areas at the lateral thirds of the segments. Segment VIII had 2 + 2 antero and postero-lateral markings and a central sclerotised and tuberculate plate. Segment IX had lateral markings. Curved hairs on the edges pointed backwards. Segments IV-VI had a median shiny oval area. Sclerotised median plates on urosternites VII-IX bell-like; on VII, occupying posterior 2/3 of urosternite; less large than the median third; was oblong on VIII, was wider than the median third of the urosternite; and occupying the basal area of the urosternite on IX.

Third instar (Figs 11, 17; Tables II, III) - The head, thorax and abdominal segments VIII-X with variable coloration in the tones of brown, legs lighter in colour and the abdomen was reddish. Margins of the thorax light brown. Antennal segment I surpassing middle of the clypeus. The pronotum was trapezoidal less longer than the meso and metanotum together; covered with sparse setaceous tubercles among glabrous areas as follows: 1 + 1 hook-like, longitudinal; 2 + 2 oval, posterior, at each side of a



Figs 14-17: *Triatoma dimidiata*. 14: 1st instar, antenniferous tubercle with apicolateral processes, dorsal view (bar = 50 µm); 15: 1st instar, rostrum, detail of membranous articulation between segments II and III, dorsal view (bar = 50 µm); 16: 2nd instar, thoracic pleuron, lateral view (bar = 200 µm) (Bg: Brindley's gland opening; p: propleuron; ms: mesopleuron; mt: metapleuron); 17: 3rd instar, antennal segment II, dorsal view (T: trichobothrium; TR: reticulated integument) (bar = 50 µm).

TABLE II

Morphometric parameters (mm) of *Triatoma dimidiata* (Latreille, 1811) nymphal instars

	1st instar n = 30			2nd instar n = 32			3rd instar n = 17			4th instar n= 32			5th instar n = 34		
	mean ± SD	Min.	Max.	mean ± SD	Min.	Max.	mean ± SD	Min.	Max.	mean ± SD	Min.	Max.	mean ± SD	Min.	Max.
HL	1.18 ± 0.04	1.10	1.28	1.61 ± 0.08	1.44	1.84	2.33 ± 0.09	2.16	2.48	3.12 ± 0.22	2.56	3.72	4.00 ± 0.23	3.56	4.37
AO	0.61 ± 0.02	0.58	0.64	0.91 ± 0.05	0.80	1.00	1.34 ± 0.05	1.28	1.48	1.79 ± 0.13	1.44	2.00	2.35 ± 0.12	2.03	2.59
PO	0.36 ± 0.02	0.32	0.40	0.42 ± 0.03	0.36	0.48	0.57 ± 0.03	0.48	0.60	0.72 ± 0.07	0.60	0.84	0.88 ± 0.07	0.73	0.97
HW	0.56 ± 0.02	0.54	0.60	0.77 ± 0.03	0.68	0.84	1.08 ± 0.03	1.04	1.12	1.52 ± 0.07	1.40	1.60	2.04 ± 0.07	1.86	2.19
WBE	0.40 ± 0.01	0.38	0.44	0.53 ± 0.03	0.48	0.60	0.75 ± 0.03	0.72	0.80	1.03 ± 0.05	0.92	1.12	1.32 ± 0.07	1.13	1.46
AT	0.34 ± 0.02	0.30	0.38	0.48 ± 0.03	0.44	0.52	0.70 ± 0.03	0.64	0.76	0.90 ± 0.07	0.72	1.00	1.26 ± 0.07	1.08	1.36
A1	0.20 ± 0.01	0.18	0.22	0.26 ± 0.02	0.24	0.32	0.37 ± 0.02	0.32	0.40	0.53 ± 0.06	0.44	0.68	0.78 ± 0.05	0.68	0.88
A2	0.41 ± 0.02	0.38	0.44	0.61 ± 0.03	0.52	0.68	0.97 ± 0.07	0.80	1.08	1.55 ± 0.07	1.40	1.68	2.45 ± 0.20	2.12	3.24
A3	0.58 ± 0.01	0.54	0.60	0.77 ± 0.03	0.72	0.80	1.15 ± 0.04	1.08	1.2	1.64 ± 0.08	1.44	1.80	2.31 ± 0.12	2.04	2.52
A4	0.87 ± 0.04	0.78	0.98	1.12 ± 0.06	0.92	1.20	1.49 ± 0.05	1.44	1.60	1.85 ± 0.15	1.20	2.00	2.34 ± 0.22	1.44	2.56
R1	0.30 ± 0.02	0.26	0.34	0.46 ± 0.03	0.40	0.52	0.66 ± 0.03	0.60	0.72	0.96 ± 0.07	0.84	1.08	1.27 ± 0.07	1.08	1.36
R2	0.60 ± 0.03	0.54	0.66	0.87 ± 0.04	0.76	0.92	1.29 ± 0.05	1.20	1.40	1.89 ± 0.09	1.72	2.04	2.45 ± 0.13	2.00	2.72
R3	0.26 ± 0.02	0.22	0.30	0.34 ± 0.02	0.32	0.36	0.45 ± 0.03	0.40	0.48	0.61 ± 0.03	0.52	0.64	0.79 ± 0.05	0.72	1.00
AWP	0.55 ± 0.03	0.48	0.60	0.81 ± 0.04	0.68	0.88	1.17 ± 0.03	1.12	1.24	1.65 ± 0.07	1.56	1.80	2.16 ± 0.12	1.70	2.35
PWP	0.72 ± 0.03	0.66	0.76	1.10 ± 0.08	0.920	1.20	1.69 ± 0.08	1.56	1.88	2.64 ± 0.19	2.36	2.96	3.91 ± 0.23	3.32	4.29
LP	0.40 ± 0.02	0.36	0.44	0.59 ± 0.04	0.52	0.64	0.93 ± 0.04	0.84	1.00	1.38 ± 0.10	1.20	1.56	2.07 ± 0.13	1.78	2.27
AW	1.66 ± 0.28	1.20	2.32	3.00 ± 0.50	1.80	3.61	4.75 ± 0.55	3.44	5.57	6.30 ± 0.83	4.43	7.71	8.35 ± 0.93	5.58	10.17
BL	3.97 ± 0.50	3.08	5.00	6.65 ± 0.69	5.08	7.54	10.37 ± 0.65	9.35	11.32	14.43 ± 1.40	10.99	16.56	20.00 ± 1.70	16.07	22.63

AO: anteocular region; AT: antenniferous tubercle length; AW: abdominal width; BL: body length [head length (HL), to antennal segment (A)4, anterior width of pronotum (AWP), posterior width of pronotum (PWP)]; LP: length of pronotum; PO: post-ocular region; R: length of rostral segment (I, II or III); WBE: width between eyes.

TABLE III

Proportions means of some characters of instars of *Triatoma dimidiata* (Latreille, 1811)

	AO/PO	WH/WBE	Antenna	Rostrum	HL/LP	HL/WH
1st instar	1:0.59	1:0.71	1:2.08; 2.94:4.43	1:2.03; 0.87	1:0.34	1:0.48
2nd instar	1:0.46	1:0.69	1:2.37; 2.99:4.31	1:1.88; 0.73	1:0.37	1:0.48
3rd instar	1:0.42	1:0.70	1:2.61; 3.08:4.01	1:1.94; 0.67	1:0.40	1:0.47
4th instar	1:0.40	1:0.68	1:2.91; 3.06:3.47	1:1.97; 0.64	1:0.44	1:0.49
5th instar	1:0.37	1:0.65	1:3.57; 2.99:3.02	1:1.92; 0.62	1:0.52	1:0.51

antenna: ratio of antennal segments, with first segment forming the basic unit of 1; AO/PO: ratio of length of head measured longitudinally from clypeus to anterior border of eye, the clypeus not included and from posterior border of eye to neck; HL/LP: ratio of length of head measured longitudinally from apex of clypeus to neck, the latter not included and pronotum measured midline, from anterior border of collar to posterior margin of hind lobe; HL/WH: ratio of length of head measured longitudinally from apex of clypeus to neck, the latter not included and head measured transversally across eyes; Rostrum: ratio of rostral segments, their relative length, with first segment forming the basic unit of 1; WH/WBE: ratio of head measured transversally across eyes and width between eyes.

sinuous line, one close to the median line and another at the humeral angle; 1 + 1 circular, postero-lateral in relation to discal tubercles and 1 + 1 kidney-like involving discal tubercles. Mesonotum 1/6 smaller than the length of the pronotum, medium-brown and had 1 + 1 kidney-like glabrous areas. The metanotum formed by 1 + 1 elliptical plates slightly smaller than the distance between them. Ventral region of the thorax light-brown; setaceous tubercles abundant on the prosternum, sparse on the meso and metasternum. Abdominal segments II-VII had glabrous areas (3 + 3) on the lateral third. Segment VIII had a central sclerotised plate and many tubercles; segments IX and X were completely medium-brown. Medial margin of spiracles with spicules. Bright oval area, gradually increasing in size from segments IV-VI. Other characteristics were as described for previous instars.

Fourth instar (Fig. 12; Tables II, III) - Head and abdominal segments VIII-X in intense brown tones, abdomen lighter in colour; legs darker than abdomen. Apex of the juga reaching basal third of antennal segment I. Anterior region of the neck with small and scarce tubercles. Segment II of the rostrum beginning at the apex of the antenniferous tubercles ending at the base of the neck. Pronotum trapezoidal equal in length to 3/4 of the meso and metanotum together. Mesonotum less longer than the pronotum. Metanotum formed by 1 + 1 elliptical plates that were slightly larger than the distance between them. Wing pads present, first pair reaching the anterior border of the metathorax and the second reaching urotergite I. Ventral thorax with abundant setaceous tubercles on the prosternum, lateral on meso and metasternum. Other characteristics were as described for previous instars.

Fifth instar (Fig. 13; Tables II, III) - Body brown, tibiae and tarsi lighter in colour. The apex of the juga reaching basal portion of antennal segment I. Antennal segment I reaching 2/3 of the clypeus base. The ventral of head with sparse granules near the eyes. The rostrum was the same colour as the head. Segment II of the rostrum beginning at the apex of the antenniferous tubercles at the apical portion of the neck. The pronotum trapezoidal as long as 3/5 of the meso and metanotum together. Mesonotum in more intense brown at the middle and mid margins of wing pads, with 1 + 1 kidney-like glabrous areas; the lateral margins of the wing pads were light brown; a granulated protuberance bordered with tubercles at the disc. Metanotum scarcely visible. Wing pads well developped first pair covering second both reaching urotergite II. Integument of pleura darkened and tuberculated. Ventral region of the thorax dark-brown. Prosternum and lateral areas of the meso and metasternum with abundant setaceous tubercles. Meso and metasternum at middle with sparse bristles. The tibiae of the forelegs had ctenidia at the apex of the ventral surface. Segments II-VII with glabrous dorsal areas (3 + 3) at the lateral third of abdomen; the anterior region of urotergite II with 1 + 1 medium-brown straight markings and 2 + 2 circular glabrous areas covered by the wing pads. Segments VIII-IX sclerotised and tuberculated. Spiracles on urosternites II-VIII slightly elevated and very close to connexivum between the lateral glabrous areas. Other characteristics were as described for the previous instars.

DISCUSSION

Eggs - Known data on the morphology of eggs of *T. dimidiata* refer to observations made by LM. Barata (1981), Costa et al. (1991) and Jurberg et al. (1993) described generic characteristics, using the morphology of eggs for the genera *Rhodnius*, *Cavernicola* and *Dipetalogaster*, respectively. A great variability is present on the genera *Alberprosenia*, *Cavernicola*, *Dipetalogaster*, *Eratyrus*, *Microtriatoma* and *Psammolestes*, which facilitates the identification of each taxon. In *Triatoma*, at least three patterns could be observed (Barata 1998) (Table I). Exochorion cells void of ornamentation and similar to *T. dimidiata* were found in *Triatoma breyere* Del Ponte, 1929, *Triatoma costalimai* Verano & Galvão, 1958, *Triatoma tibiamaculata* (Pinto, 1926) and *Panstrongylus*. Also the eggs of *T. dimidiata* have form, size and structure (without the ornamentations) very similar to *Triatoma pallidipennis* Stål, 1872, *Triatoma picturata* Usinger, 1939, *Triatoma phyllosoma* (Burmeister, 1835) and *Triatoma longipennis* Usinger, 1939 smooth exochorial structure and slightly separated superficially padded hexagonal cells (Obara et al. 2007a).

Regarding the morphometric data, our median values for the eggs of *T. dimidiata* are similar to the values found by Galliard (1935a) and Lizaraso (1957), if not a little higher; and do not agree with Usinger (1944), who considered the eggs of *T. dimidiata* to be less than 2 mm. Variations can occur and result from both natural and artificial causes, such as colony maintenance and nutrition (Barata et al. 1980). Lizaraso (1957) concluded that populations of the same species, when provided with different laboratory and natural environments, could produce variations that affected the dimensions of the eggs without affecting the characteristics of the chorion, which remained constant. Morphometric studies of 33 *Triatoma* species were published (Galliard 1935b, Lizaraso 1957, Gonçalves et al. 1985, Haridass 1986, Jurberg et al. 1986, 1998, 2002, 2008, Jurberg & Vogel 1994, Jurberg & Campos 1995, Rosa 1995, Rocha et al. 1996, 2009, Costa et al. 1997, Silva et al. 2000, 2005, Visciarelli et al. 2004, Obara et al. 2007a). According to Barata (1998), *Triatoma* possesses the following means: length, 2.05 ± 0.08 and diameter, 1.14 ± 0.05. For the species belonging to the group Rubrofasciata, Phyllosoma complex, Dimidiata subcomplex (Schofield & Galvão 2009), only *T. dimidiata* has the morphology and morphometrics of the eggs known. Of the eggs previously studied, the eggs of *T. dimidiata* are smaller in length and width than the eggs of *T. picturata* (2.37/1.478), *T. phyllosoma* (2.364/1.468), *T. longipennis* (2.202/1.368) and *T. pallidipennis* (2.200/1.418).

Nymphs - Forty three species of *Triatoma* had their nymphs described and/or some morphological aspect presented by LM and/or SEM (Espínola 1966, Galvão & Fuentes 1971, Perlowagora-Szumlewicz & Cruz 1972, Lent & Wygodzinsky 1979, Brewer et al. 1981, 1983a, b, Jiménez & Fuentes 1981, Gonçalves et al. 1985, Haridass 1986, Jurberg et al. 1986, 1991, 1998, 2002, 2009, Brewer & Garay 1989, Rosa et al. 1989, 1992a, b, 2005, Jurberg & Vogel 1994, Jurberg & Campos 1995, Rosa 1995, Rocha et al. 1996, 2009, Rosa & Barata 1997, Galíndez-Girón et al. 1998, Silva et al. 1999a, b, 2000, 2002, 2003, 2005, Naegele et al. 2003). A total of 32 species were shown through illustrations, 31 with photos in LM and 24 using SEM.

Morphology - The five instars of *T. dimidiata* can be distinguished one from the other principally by the dorsal aspect of the thoracic segments (Figs 9-13). The number of setaceous tubercles increases progressively with the development of the nymphs. On the 1st instar, the bristles are erect; the bristles are curved on the 2nd-5th instars. The surface of the body is covered with short bristles imbedded in tubercles, which become more frequent with the development. On the 1st instar, the eyes have globose ommatidia separated so as to appear like the fruit of the mulberry; the later instars increase the number of ommatidia and decrease the space between them.

All of the instars of *T. dimidiata* have trichobothria dorsally on apical third of antennal segment II (Fig. 15).

Lent and Wygodzinsky (1979) highlighted the taxonomic importance of the stridulatory furrow in the characterisation of the species that vary in form, size, number of grooves and the space between them. In *T. dimidiata* the stridulatory furrow varied among instars. It is amorphous with central parallel stripes and a group of papillae on both sides in the 1st instar. The stridulatory furrow becomes progressively longer, deeper and tapered posteriorly, with parallel stripes becoming closer and lateral tubercles becoming more numerous in the later instars.

Lent and Wygodzinsky (1979) did not examine the 1st instar nymphs metatarsi being unable to define the position of *T. dimidiata* into groups and complexes. They considered *T. dimidiata* "...superficially somewhat similar to the species of the *phyllosoma* group...". In the present work, we observed that the 1st instar nymphs presented delicate hairs on the metatarsi of the posterior legs.

The forelegs of 5th instar nymphs of *T. dimidiata* have ctenidia on the apex of the ventral surface of the tibia. Gonçalves et al. (1985), Costa et al. (1991), Rosa (1995) and Naegele et al. (2003) mentioned the presence of ctenidia on the forelegs of Triatominae nymphs. The ventral surface of the femur of *T. dimidiata* has 1 + 1 prominent subapical tubercles with bristles smaller than the tubercles. Tubercles on the submedian surface of the femur were described by Rosa (1995), Galíndez-Girón et al. (1998), Jurberg et al. (2002) and Naegele et al. (2003). Jurberg et al. (2002) described for nymphs of *T. jurbergi* Carcavallo, Galvão & Lent, 1998, 1 + 1 tubercles in the dorsal subapical surface of the femur of metathoracic legs and 2 + 2 in the ventral subapical surface of the meso and metathoracic legs. Naegele et al. (2003) mentioned the presence of 2 + 2 tubercles in the ventral apical surface of the femur of pro and mesothoracic legs of *Triatoma infestans.*

All instars of *T. dimidiata* have an oblong evaporative opening of the Brindley's gland on the mesopleura (Fig. 16). Schofield and Upton (1978) described these glands in adults of *Panstrongylus megistus* (Burmeister, 1835), commenting that nymphs are void of them. Millen et al. (1979) *apud* Rosa et al. (2005) described the development of the Brindley's glands in 5th instar nymphs of *Rhodnius prolixus;* Rosa (1995) observed these glands in 1st and 5th instar nymphs of *Triatoma circummaculata* (Stål, 1859) and *Triatoma rubrovaria* and Rosa et al. (2005) recorded the Brindley's glands in the mesopleura of 5th instar nymphs of *Triatoma arthurneivai* Lent & Martins, 1940.

Lent and Wygodzinsky (1979) described the urotergites of the 5th instar nymphs of *T. dimidiata* with a series of five aggregations of granules along the midline and a finely granulose integument. We observed these aggregations of granules along the longitudinal median line of segments II-VI (Figs 10-13).

The glabrous dorsal and ventral areas of the abdomen of Triatominae nymphs were described as elliptical regions by Del Ponte (1920). These areas are bright and round and are placed on median third of each segment. In nymphs of *T. dimidiata* the glabrous dorsal areas on the lateral third of the abdomen are arranged as follows: 1st instar on segments II-VII, with 1 + 1 on II and 2 + 2 for III-VII (Fig. 9); in later instars, 3 + 3 for II-VII (Figs 10-13). The glabrous areas were mentioned by Gonçalves et al. (1985), Jurberg et al. (1986, 1991, 1998), Rosa (1995) and Rosa and Barata (1997). The bright areas placed in the ventral abdominal region of Triatominae were also described by Del Ponte (1920). In 1997, Rosa and Barata described the abdominal segments of 5th instar nymphs of six species of Triatominae (*P. megistus*, *Rhodnius neglectus* Lent, 1954, *T. brasiliensis*, *T. infestans*, *Triatoma matogrossensis* Leite & Barbosa, 1953 and *T. tibiamaculata*); the presence of bright round areas in the middle of segments IV-VI were registered, with the exception of *R. neglectus*. The bright areas in *T. dimidiata* are oval and coincide with the placement observed by Rosa and Barata (1997). As in *T. dimidiata*, the 1st instar nymphs of *T. circummaculata* and *T. rubrovaria* do not have these bright areas (Rosa 1995).

The chitinous plates were described for 5th instar nymphs of *T. dimidiata;* these structures were named by Galliard (1935a). Rosa and Barata (1997) described the chitinous plate as a black, brilliant, hairy area with an oblong form placed on urosternite VII for the triatomines studied, with the exception of *R. neglectus.* In *T. dimidiata,* the chitinous plates are found in all instars.

Morphometrics - Brewer et al. (1983b) studied *T. infestans*, *Triatoma platensis* Neiva, 1913, *Triatoma delpontei* Romaña y Abalos, 1947, and *Triatoma sordida* (Stål, 1859) and quantitatively analysed which characteristics were the most effective for distinction of the instars of a species and distinction of each one of the instars among different species. Comparing instars of one species, the parameters for which the means differed the most were head length, body length and segment III of the rostrum. These measurements, with the development of nymphs, became extremely useful to distinguish them. Analysing the means of the variables observed in *T. dimidiata*, it was concluded that there is a progressive increase from one to another instar. Considering the range of the following measurements, length of pronotum, posterior width of pronotum, length of segment III of the rostrum and length of antennal segments II and III, they do not overlap along instar development; the posterior width of pronotum was the parameter with the greatest range. On the other hand, the other two parameters suggested by Brewer et al. 1983b, length of the head and total length, did overlap in nymphs of the 4th and 5th instars and the 3rd, 4th and 5th instars, respectively (Table II).

A quantitative analysis of *T. dimidiata* measurements corroborates conclusions made by Brewer et al. (1983b): (i) the anterior ocular region progressively increases in relation to the post-ocular region, (ii) the length of antennal segments I-III is always greater than the length of segment IV, (iii) the length of antennal segment IV progressively increases with development and (iv) the relation between the proportions of the segments I and II of the rostrum and head length/width between eyes are constant through all of the instars.

The means of the variables are progressively higher from the 1st-5th instar. However, when the range of the variables is compared, they overlap. Then, by the analysis of the means, it is not possible to distinguish one instar from the other.

The proportions of the means are presented in Table III.

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