The species of the subfamily Triatominae (Hemiptera: Heteroptera: Reduviidae) are true bugs specialized in blood-sucking. The subfamily is composed of 151 extant and three fossil species assigned to five tribes and 18 genera (Oxysternon, Triatoma, Rhodnius, Panstrongylus, and Triatominae) in the family Reduviidae (Heteroptera: Reduviidae) are true bugs specialized in blood-sucking. The subfamily is composed of 151 extant and three fossil species assigned to five tribes and 18 genera (Oxysternon, Triatoma, Rhodnius, Panstrongylus, and Triatominae) in the family Reduviidae. The subfamily is composed of 151 extant and three fossil species assigned to five tribes and 18 genera (Oxysternon, Triatoma, Rhodnius, Panstrongylus, and Triatominae) in the family Reduviidae.
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providing species-level descriptions of the five immature stages of *T. ryckmani* including an ontogenetic morphometric study of head development.

**MATERIAL AND METHODS**

The analyzed specimens came from a colony maintained at the Universidad Nacional de Costa Rica. A total of 46 specimens were used for geometric morphometric analysis (10, 13, 9, 7 and 7, from 1st through 5th instars respectively, plus 4 male and 3 female adults). All specimens were preserved in 70% alcohol and deposited in the “Coleção de Triatomíneos do Instituto Oswaldo Cruz”, (CTIOC), Rio de Janeiro, RJ, Brazil, in a single batch. Another specimens were deposited in the CTIOC under voucher numbers 4973 (male), 3079 (male), 3080 (female), 3397 (female) and 3398 (male). The 12 specimens used for scanning electron microscopy (1°), 2 (2°), 2 (3°), 2 (4°), and 4 (5°) were preserved at Faculdade de Ciências Farmacêuticas, Departamento de Ciências Biológicas, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Araraquara, SP, Brazil.

Measurements were made with an ocular micrometer attached to a Zeiss Stemi SV11 stereoscopic microscope and photographs taken with a Nikon Coolpix 990 digital camera. The morphometric parameters of the nymphs was used according to Oliveira et al. (2015).

For ontogenic morphometrics, the dorsal surface of the head of each specimen was assayed from digital images for seven landmarks, as shown in Figure 2, using the TPSdig software, version 1.27 (James Rohlf). Shape variables (x, y coordinates) were subjected to generalized Procrustes superimposition and then, using the TPSrelw software 1.35 (James Rohlf), “partial warps” (weight matrix) were computed to investigate the ontogenetic profile. To quantify the variation among instars, relative warps were analyzed by Principal Component Analysis (PCA) and Canonical Variable Analysis using JMP 3.2.6 (SAS Institute). Additionally, the relationship between size and shape was analyzed using the TPSregr software for multivariate regression to compute the ontogenetic profile (thin-plate spline grid deformations) The geometric morphometry methodology was based on Oliveira et al. (2015).

For the Scanning electron microscopy (SEM), the specimens were dehydrated in a graded series of ethanol from 70% to 100%, cleaned with ultrasound, dried at 60 °C and then attached to metallic stubs using a double-sided stick tape and coated with gold. Specimens were analyzed using Topcon SM 300 (Topcon Corporation, Hasunuma-Cho, Tokyo Itabashiku, Japan) at the Institute of Chemistry of UNESP, Araraquara, São Paulo, Brazil, following Rosa et al. (1999). Digital images were edited with Adobe Photoshop 6.0.

**Figure 1.** *Triatoma ryckmani*, male adult, deposited at CTIOC (n. 4973).

**Figure 2.** Digital image of dorsal surface of the head of *T. ryckmani* showing the seven landmarks captured at TPSdig software.
RESULTS AND DISCUSSION

Overall size and ratios, along with their standard deviations calculated from the body measurements and landmark head measures showed gradual growth from the 1st to the 5th instar in all the morphological characters. Specific details for each instar are given below.

Description of Nymphs

First instar (Figure 3). Total length of the body (average) 2.7 mm. The overall body color light brown and tegument rugose with bristles in setigerous tubercles. Head light brown-yellow and granulose with setigerous tubercles and light bristles. Rostrum attaining the anterior border of prosternum. Ratio of rostral segments 1: 2.2: 1.2. Clypeus light with rounded genae, reaching the apex of antenniferous tubercles, but not attaining the apex of anteclypeus. Postoccipital sutures well delimited, converging in a “Y” shape. The ratio between the width of the anteclypeus region and postoccipital region 1: 0.5, while the ratio between the width of the head at the level of eyes and the synthlipsis region, 1: 1.2. The postoccipital sulcus clearly visible. Antennae pillose, yellow. The first antennal segment not attaining the apex of the head. The ratio of the antennal segments: 1: 1.8: 2.3: 2. Eyes lacking ommatidea in the posterior portion. Thorax rugose with setigerous tubercles, overall color brown. Pronotum larger than meso- and metanotum, with 1 + 1 sclerotized plates. Anterolateral angles rounded with setigerous tubercles. Metanotum dark, formed only by 1 + 1 sublateral sclerotized plates. Legs yellow with setigerous tubercles with small golden bristles in all segments. Abdomen brown, lighter than the head and thorax, with several lighter setigerous tubercles. Urotergites with bristles arranged in transverse rows, external border of connexivum presenting bristles, without connexival spots.

Second instar (Figure 3). Similar to the first instar except total length of the body (average) 4.6 mm. Overall color darker. Head longer. Ratio of the anteclypeus to post-ocular region 1: 0.4 and between the width of the head at the level of eyes and the interocular distance 1: 1.6. Antennal segments darker than with light rings between segments. Ratio of antennal segments 1: 2.6: 2.9: 4.0. Rostrum dark brown. Ratio of rostrum segments: 1: 2.4: 1.2 (Tab I). Pronotum trapezoidal with 1 + 1 light stripe presenting tubercules. Mesonotum with 1 + 1 irregular light-colored areas, setiferous tubercles. Metanotum with 1 + 1 light-colored spots in the external border. Legs yellowish brown. Urotergites with two transverse rows of bristles and abdomen with connexival marks in the middle part of the lateral borders.

Third instar (Figure 3). Similar to the second instar except total length of the body (average) 5.7 mm. Overall color dark brown. Head with light areas from the basal portion of the jugae to the anteclypeus. Ratio between the length of postoccipital and anteclypeus region 1: 0.5 and between the width of the head at the level of eyes and the synthlipsis 1: 1.5. First and second antennal segments lighter than third and fourth, but fourth antennal segment presenting the apical half lighter-colored. Ratio of antennal segments 1: 2.8: 3: 3.4. Ratio of rostrum segments 1: 2.8: 1.4. Thorax with overall color dark brown with setiferous tubercles randomly distributed and bristles on the lateral border. Pronotum with 1 + 1 yellow spots on the hind angles, divided by a longitudinal midline in two equal plates. Mesonotum dark brown with 1 + 1 rectangular plates divided by a lighter longitudinal line, with a well defined 1 + 1 yellow spots and bristles inserted in tubercules. Metanotum dark brown to black with 1 + 1 rectangular plates with bristles. Legs presenting numerous bristles, with the coxae and trochanter lighter in color than the tibia and tarsus. Abdomen granulose with more evident connexival spots.

Fourth instar (Figure 4). Similar to the third instar except total length of the body (average) 8.5 mm. Overall color darker. Head presenting a grainy appearance around the eyes. Ratio between the length of antennocereus region and postoccurrent region 1: 0.4 and between the width of the head at the level of eyes and the synthlipsis is 1: 0.7. First antennal segment with dark spots. The ratio of antennal segments 1: 5.1: 6.7: 5.0. Ratio of rostrum segments 1: 4.8: 1.5. Pronotum with 1 + 1 lighter stripes on the apex with setiferous tubercles and dark glabrous areas. Mesonotum with 1 + 1 lighter stripes at lateral border and glabrous, dark areas. Abdomen with many sensillas and smaller light spots. Connexival spots bigger.

Fifth instar (Figure 4). Similar to fourth instar except total length of the body (average) 11.0 mm. Head dark brown with borders presenting a lot of tubercles, giving a grainy aspect. Anteocular region, in the ventral view, with brown color. Ratio of the antennocereus region to postoccurrent region 1: 0.3, and between the width of the head at the level of the eyes and synthlipsis 1: 1.7. Antennae are dark brown with fourth segment lighter. Ratio of antennal segments 1: 4.1: 3.4: 4. Ratio of rostrum segments 1: 5.1: 1.3. Metanotum with 1 + 1 alar thecae, overall color lighter than pro- and metanotum. Abdomen with connexival spots larger with the presence of scattered bright spots.

Ontogenic morphometrics

The first two Principal Components (PC) together represent 85.7% of the variability (PC1 = 71.3% and PC2 = 14.4%). The factorial map of the first two Principle Components show a clear separation between the first through fourth instars, and strong overlap of the fourth and fifth instars.

The Canonical Analysis, based on principal components of each specimen, shows the changes in shape and strong discrimination among all instars. The 1st, 2nd, 3rd and 4th instars show stronger discrimination, while there is overlap of the 4th and 5th, reflecting the PC results.

The ontogenetic profile highlights the allometry of a particular instar, and the transformation grids compare the changes of the shape from 1st to 5th instars nymphs. The analysis shows differences of length, width, as well as the position of antenniferous tubercles and eye size (Figures 5, 6).

Scanning electron microscopy

It was observed that the setigerous granules increase in number and their relative place changes during development, forming distinct outlines for each instar (Figure 7).

The tenth abdominal segment is annular with bristles around the anal opening only in males, and presence of granules and bristles in the internal part. The granules become more evident on the third instar; and the number of bristles increases. In females, the anal opening with has depressions and no bristles (Figure 8).

Stridulatory sulcus is delimited by sensilla and setiferous tubercles in all stages. First and second instars present a triangular shape sulcus with few transversal stripes. Third, fourth and fifth nymphal stages have a cone shaped sulcus, elongated and with a gradual increase on the number of stripes. The border gradually becomes elevated during the postembryonic development (Figure 9).

Relative size between rostrum and stridulatory sulcus was different in the 4th instar, when the third rostrum segment almost reached the final portion of stridulatory sulcus, for the first through third and fifth instars it was shorter.
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Figure 3. Triatoma ryckmani immature stages. First instar (size 2.7mm): A1 - dorsal surface of the head; A2 - dorsal surface of the body; A3 - ventral view. Second instar (size 4.6 mm): B1 - dorsal surface of the body; B2 - ventral view. Third instar (size 5.7 mm): C1 - dorsal surface of the body; C2 - ventral view.

According to Galvão et al. (2005), the study of immature stages is very important because they show epidemiological and systematic importance. Although, the identification of specimens is still centered in morphological traits of the adults and the determination based on nymphs’ characters remains a challenge (Oliveira et al. 2015). In this study, we describe for the first time the five nymphal instars of T. ryckmani, a species previously rare and poorly studied considered as a sylvatic insect capable to move between many habitats (sylvatic, dry forest, peri-domestic and domestic) with high colonization capacity (chicken coops) (Monroy et al. 2004; De la Rua et al. 2014).

According to Lent & Wygodzinsky (1979), T. ryckmani is a "taxonomically isolated species", years later, in a phylogenetic study, Justi et al. (2016) showed that it was related to Triatoma...
One distinguishing nymphal characteristic for this species is the gradual and continuous darkening of the head and thorax, from light brown in the early stages, to dark brown later in the development. Throughout development, the first two antennal segments are darker than the third and fourth antennal segments. The junctions of each segment also have lighter color.

The observation of distinct pilosity patterns in *Triatoma* species (previously studied) suggests this is a taxonomically important character across the genus. The pilosity pattern on the last abdominal segment of *T. ryckmani* can be very useful in the morphological differentiation from other species of *Triatoma* (Jurberg et al. 2002; Ragorodskii et al. 2011; Rocha et al. 2005, 2009; Silva et al. 2005).

The stridulatory sulcus of the nymphs acquires a shape of V, with papillae, tubercles and sensilla delimiting it, as
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Figure 5. Factorial map of the first two Principal Components (PC1 and PC2) for each nymphal stage (1st-I, 2nd-II, 3rd-III, 4th-IV, 5th-V, M-adult).

Figure 6. Transformation grids of the ontogenetic profile comparing the development of the deformations with the extreme values of the axis x and y (indicated by narrows).

Figure 7. Scanning electron micrographs showing changes in the integument of the cephalic capsule and thorax at various instars of development. A - 1st instar: (A1) integument with scattered granules, (A2) scattered granules and sensilla in the thorax region, (A3) large and irregular sensilla; B - 2nd instar: (B1) integument head with granules presenting a regularity in positioning, (B2) pronotum with organized granules, (B3) smaller, down-turned sensilla; C - 3rd instar: (C1) granules at the center of the head capsule, (C2) granules forming ornamentation in the thorax; D - 4th instar, (D1) and (D2) integument with granules forming ornamentation; E - 5th instar: (E1) and (E2) ornamentation in the integument resulting from organization of the granules; F - the wing bud (F1) which characterizes the 5th instar.

Figure 8. Scanning electron micrographs of 5th instar nymphs: A - fine bristles inside the anal disk 200X magnification: (1) - bristles, (2) fine bristles inside the anal disk. B - X abdominal segment of a nymph 200X magnification: (1) absence of bristles, (2) increased roughness on edge; C - detail of bristles on the 8th abdominal segment 300X magnification; D - detail of single bristle on the 8th abdominal segment 500X magnification.
previously observed in other nymphs of Triatominae, such as Linshcosteus karupus Galvão, Patterson, Rocha & Jurberg, 2002. Belminus hereri Len & Wygodzinsky, 1979, Triatoma vandae Carcavallo et al., 2002, Triatoma williamsi Galvão, Souza & Lima, 1965, Triatoma jurbergi Carcavallo, Galvão & Lent, 1998 and Triatoma guazu Lent & Wygodzinsky, 1979 (Galvão et al. 2002; Galvão et al. 2005; Rocha et al. 2005; Silva et al. 2005). Head shows many setigerous tubercles unevenly distributed in the first instar. From the second to fifth instar there is a gradual organization of such tubercles, resulting in a different ornamentation for each instar (Silva et al. 2003; Rocha et al. 2005; Silva et al. 2005). The rostrum segments shows a unique and curious development in relation to the stridulatory sulcus. The apex of third rostral segment changes the position only in the third instar, reaching the anterior border of stridulatory sulcus (in other studies species of Triatoma in the first instar the apex reach the prosternum).

The geometric morphometrics is widely used in different approaches in Triatominae. This tool is useful to sylvatic and domestic differentiation, to discriminate field and laboratory populations, or between geographic regions, and to taxonomic or phylogenetic inferences (DuJardin et al. 1997; Jaramillo et al. 2002; Monroy et al. 2003). Geometric morphometrics studies among nymphal instar, could be a useful tool for the characterization during the species development, however, only two studies, in different genera have been published so far, L. karupus (Galvão et al. 2005) and B. hereri (Rocha et al. 2005). Our study agreement with both previous studies that conclude the geometric morphometrics is an informative tool for the detection of anatomical variation, on the other side, the analysis in a comparative way of three unrelated genera is not easy. Our data about geometric morphometry provide information about the ontogenetic profile of each instar, showing that the biggest conformational and size changes are observed in early development. SEM has revealed structures that differentiate each stage, for example: the area of stridulatory sulcus, shape of the pronotum, the distance between setiferous tubercles, and other structures such as the position of the rostrum relative to the stridulatory sulcus or the structure of the abdominal segments and sensilla.

Because important vectors have, amongst other characteristics, the ability to colonize human dwelling, finding immature stages inside houses could be suggestive of the colonization process. In addition, often only immature stages are found inside houses, and being able to rapidly identify the taxon is important for the design of control strategies. For this reason, systematic and phylogenetic studies are necessary for efficient control. The description of morphological and morphometric data on immature stages can be greatly aided if new characters can be found in immature forms, increasing the identification process, the speed and efficiency of control strategies of Chagas’ disease vectors.

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