Major Article

Checklist and phenetics studies of nymphs of two species of triatomines: *Triatoma lenti* Sherlock & Serafim, 1967 and *Triatoma sherlocki* Papa, Jurberg, Carcavallo, Cerqueira, Barata, 2002 (Hemiptera: Reduviidae: Triatominae)

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Abstract

**Introduction:** *Triatoma lenti* and *Triatoma sherlocki* are endemic species of the State of Bahia, located in northeastern Brazil, where they have records of domiciliation in the human environment. In view of the epidemiological aspect and taxonomic importance of these species for the systematics of the *Triatoma* genus, a study was carried out with nymphs of all five instars. **Methods:** An extensive review of studies on nymphs from the subfamily Triatominae is presented. Morphology was studied using a scanning electron microscope and morphometric analyses. **Results:** The morphological study allowed us to characterize and discriminate species by means of scanning electron microscope of the last abdominal segment. In addition, the results show morphometric variability, with the total size of the head that best discriminates the species. **Conclusions:** Studies on nymphs are fundamental to the ecosystem; however, the literature on the immature forms of certain groups is scarce, difficult to use, or nonexistent. Therefore, this study includes morphological and morphometric data of the nymphal instars of *T. lenti* and *T. sherlocki*, corroborating the specific taxonomy of these species.

Keywords: Chagas disease. Morphometric. Morphology of nymphal. Scanning electron microscope. Taxonomy. *Triatoma* immature instars.

INTRODUCTION

In the Americas, various species of triatomines are vectors of *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae), the etiological agent of Chagas disease. The insects of the subfamily Triatinineae (Jeanneal, 1919) are hematophagous and feed primarily on vertebrate blood. Even though all species of triatomines are hematophagous, species that colonize residential places or are peridomestic have increased chances of transmitting *T. cruzi* to humans. After the successful *Triatoma infestans* (Klug, 1834) control program conducted by the Brazilian National Health Foundation, other triatomines previously considered predominately sylvatic have emerged as potential vectors in several areas of Brazil.

Presently, 157 species (including 3 fossils) within 18 genera are recognized as valid in this subfamily. The genus with the greatest number of species described is *Triatoma* Laporte, 1832, which includes members of the *Triatoma brasiliensis* subcomplex, *Triatoma brasiliensis* Neiva 1911, *Triatoma brasiliensis macromelasoma* Galvão 1956, *Triatoma melanica* Neiva & Lent, 1941, *Triatoma juazeirensis* Costa & Felix 2007, *Triatoma sherlocki* Papa, Jurberg, Carcavallo, Cerqueira, Barata, 2002, *Triatoma lenti* Sherlock & Serafim, 1967, *T. bahiensis* Sherlock & Serafim, 1967 and *Triatoma petrochiae* Pinto & Barreto 1925.

Sherlock and Serafim described *T. lenti*, *T. pessoai*, and *T. bahiensis*. The authors reported that *T. lenti* and *T. pessoai* were naturally infected by *T. cruzi* and were relatively easily maintained in the laboratory by feeding on pigeons. Currently, only *T. pessoai* is not considered a valid species.
Cerqueira et al.\textsuperscript{15} refer to the encounter of wild triatomine, naturally infected by \textit{T. cruzi} in the district of Santo Inácio, municipality of Gentio do Ouro, Bahia. Later in 1982, Cerqueira, in his doctoral dissertation, studied the biological cycle and evaluated the results of crosses of this wild triatomine with \textit{T. brasiliensis}; however, it was not considered a new species. Papa et al.\textsuperscript{14} resumed studies of the triatomines studied by Cerqueira in 1982 and based on consistent morphological characters, such as genital structures, shorter wings, red orange spots on the connexivum and legs, inability to fly, and longer legs, concluded that it is a new species named \textit{T. sherlocki}. \textit{Triatoma sherlocki} was related to \textit{T. lenti} by morphological characteristics, cytogenetics, molecular data, and experimental crosses, and was included as a member of the \textit{Triatoma brasiliensis} complex\textsuperscript{5,9,10,11,14,16,74}.

Morphology and morphometry are tools that contribute to the knowledge of triatomines and generate useful information to establish more effective strategies for vector control\textsuperscript{17}. In Triatominae, biometric studies are used to characterize new species, detect populations, and define structures\textsuperscript{18}. For example, geometric morphometry allows the collection of information about the shape and size of organisms, which helps in systematic and phylogenetic studies\textsuperscript{10,19,20}.

Several authors have used morphology and morphometry to characterize the species and correlate the known characteristics of the character, isoenzymatic and ecological, and contributed to both systematic analyses. Studies on immature instars of \textit{T. lenti} and \textit{T. sherlock} are scarce; therefore, we evaluated and characterized those species that make up the \textit{T. brasiliensis} subcomplex, a relevant group for the ecoepidemiology of Chagas disease in the northeastern region of Brazil\textsuperscript{17,21-25}, by gathering all information from the literature on the study of immature forms in Triatominae.

\section*{METHODS}

\textbf{Insects}

We used specimens from a \textit{T. lenti} (Figure 1) colony collected on April 9, 2008, which were found in the county of Macaúbas (Mangabeiras and Cana Brava neighborhoods) in the state of Bahia. The specimens were collected at altitudes of 747, 755, 780, and 829 m in the peridomicile and intradomicile. On July 22, 2003, \textit{T. sherlocki} (Figure 1) was collected in Gentio do Outo, Santo Inácio, Bahia state, and later a colony was established in the laboratory. The specimens were kept and deposited at the Triatomine Insectario of the Faculty of Pharmaceutical Sciences, Universidade Estadual Paulista (https://www2.fcfar.unesp.br/#!/triatominae). Approved by the Ethics Committee on the Use of Animals - CEUA, CEUA/FCF/CAr: 15/2017).

\textbf{Morphological analyses}

The fifth instar nymphs from \textit{T. sherlocki} and \textit{T. lenti} (Figure 1) were cleaned using an ultrasound device. Next, the structures were dehydrated in alcohol, dried in an incubator at 45°C for 20 min, and fixed in small aluminum cylinders with transparent glass. Sputtering metallization was then performed on the samples for 2 min at 10 mA in an Edwards sputter coater. After metallization, the samples were studied and photographed using a Topcon SM-300 scanning electron microscope (SEM), according to Rosa et al.\textsuperscript{26}. The images obtained were processed (background, contrast, brightness) using the GNU Image Manipulation Program v2.0.2 (GIMP) software free and open-source image editor, and the structures were described and compared.

\textbf{Morphometric analyses}

For the \textit{T. lenti} and \textit{T. sherlocki} measurements, 15 nymphs specimens in the first, second, third, fourth, and fifth instars were fixed on glass slides using a double-sided tape. Measurements were also taken to determine the thorax, abdomen, and head length, as well as interocular, ante-ocular, and postocular distance, eye diameter, and the three proboscis segments. These distances were defined by Dujardin et al.\textsuperscript{27}. The measurements were taken using a Leica MZ APO stereomicroscope and analyzed using the Motic Advanced 3.2 image analysis software. Descriptive statistics analyses and Welch’s t-test were performed using GraphPad Prism v.5.03.

\textbf{Principal component analysis}

To visualize the general patterns of morphological variation in the multidimensional data obtained with the principal component analysis (PCA) of the references, a factorial map was generated using Past 3.2\textsuperscript{28}. 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Fifth instar nymph of \textit{T. sherlocki} and \textit{T. lenti}. (A) Dorsal view of the fifth instar nymph of \textit{T. sherlocki}. (B) Dorsal view of the fifth instar nymph of \textit{T. lenti}. (C) Ventral view of the fifth instar nymph of \textit{T. sherlocki}, and (D) Ventral view of the fifth instar nymph of \textit{T. lenti}.}
\end{figure}
Checklist of studies on the immature instars of triatomines

The survey of publications that study the immature instars of development had as selection criteria publications with morphology and morphometry of nymphs regardless of the methodological approach. Publications were retrieved from databases such as: National Center for Biotechnology Information - NCBI (available at https://www.ncbi.nlm.nih.gov/), Bibliography of Triatomins - BibTri (available at: https://bibtri.cepave.edu.ar/webbibtri.php), Google Academic (https://scholar.google.com.br/?hl=pt) and Scielo (https://www.scielo.org/). The keywords used for the search were: Nymphs, Triatominae, Hemiptera, Reduviidae, Morphology, Morphometry, Description, Ontogenetic, Instar, Description of nymphs, key, eggs, 1st, 2nd, 3rd, 4th, 5th and instars.

RESULTS

Through an extensive literature survey on immature forms of triatomines, we recovered 115 studies that explored the morphological aspects of nymphs; therefore, we updated the list presented by Galvão 2014 (Table 1).

Morphological characteristics

The morphological characteristics of the two species are presented in Figure 2. According to the genital morphology of fifth instar nymphs, the ninth ventral abdominal segment is wider in T. lenti than in T. sherlocki, as well as the presence of a hole in the posterior portion of this segment in T. lenti and its absence in T. sherlocki. The ninth ventral abdominal segment of fifth instar nymphs shows parallel grooves in the posterior region, which are most evident in T. lenti, while their presence in T. sherlocki is poorly visible. The eighth segment was trapezoidal in T. sherlocki and oval in T. lenti. The laterals were irregular at the apex. It was found that the ninth segment had few sensilla, as well as segments 7, 8, and 10. The tenth segment was curved ventrally in the posterior portion. Sexual dimorphisms of the nymphs are characterized by the size of the ninth segment ventrally, in which females have a narrow (Figure 2 A, B) while males have a wide ninth segment (Figure 2 C, D).

| Species | Approach | References |
|---------|----------|------------|
| Alberprosenia goyavargasi | Description of nymphs by SEM* | Carcavallo et al.29 |
| Alberprosenia malheiroi | Description of nymphs of 1st, 2nd, 3rd, 4th, and 5th instars (MO*, SEM) | Carcavallo et al.30 |
| Belminus herreri | Description and geometric morphometry of nymphs | Rocha et al.31 |
| Cavernicola pilosa | Description of nymphs of 1st, 2nd, 3rd, 4th, and 5th instars (MO) | Valderrama & Lizano35 |
| Cavernicola lenti | Description of nymphs (MO), every aspect shown by SEM | Costa et al.33 |
| Dipetalogaster maxima | Description of nymphs of 1st, 2nd, 3rd, 4th, and 5th instars (MO) | Jurberg et al.34 |
| Eratyrus mucronatus | Morphological (MO and SEM) and key | Galíndez-Girón et al.35 |
| Linshcosteus confumus | SEMs and description of eggs | Haridas46 |
| Linshcosteus costalis | SEMs and description of eggs | Haridas56 |
| Linshcosteus karupus | Description of nymphs of 1st, 2nd, 3rd, 4th, and 5th instars by SEM | Galvão et al.37 |
| Triatoma longipennis | Morphological (MO and SEM) and key | Galíndez-Girón et al.35 |
| Triatoma pallidipennis | Ontogenetic morphometrics (MO) | Rodríguez-Sánchez et al.38 |
| Triatoma phyllosoma | Morphological (MO and SEM) and key | Galíndez-Girón et al.35 |
| Mepraia spinolai | Morphological (MO and SEM) and key | Galíndez-Girón et al.35 |
| Microtriatoma trinidadensis | Description of nymphs (MO) | Carcavallo et al.30 |
| Nesotriatoma flavida | Morphometrics | Jiménez and Fuentes,41 |
| Panstrongylus geniculatus | Morphological and key (MO and SEM) | Galíndez-Girón et al.35 |
| Panstrongylus humeralis | Morphological and key (MO and SEM) | Galíndez-Girón et al.35 |

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| Species                          | Approach                                                                 | References         |
|---------------------------------|--------------------------------------------------------------------------|--------------------|
| Panstrongylus lignarius         | Morphological and key (MO and SEM)                                       | Galíndez-Girón et al. 
Panstrongylus megistus          | Morphology of spiracles 5th instar nymphs                               | Rosa et al. 
Panstrongylus megistus          | Sexual distinction between 5th instar nymphs by SEM                     | Rosa et al. 
Panstrongylus megistus          | Morphology of 5th instar nymphs by SEM                                   | Rosa et al. 
Panstrongylus megistus          | Abdominal structures of 5th instar nymphs                               | Rosa et al. 
Panstrongylus megistus          | Morphology of antennae of 1st, 2nd, 3rd, 4th, and 5th instars (SEM)     | Rosa et al. 
Paratriatoma hirsuta hirsuta   | Morphology of 5th instar nymphs (MO)                                     | Ryckman et al. 
Paratriatoma hirsuta kamienstis | Morphological study (MO and SEM)                                        | Galíndez-Girón et al. 
Paratriatoma hirsuta papagoensis | Morphology of 5th instar nymphs (MO)                                     | Ryckman et al. 
Paratriatoma hirsuta pimae      | Morphology of 5th instar nymphs (MO)                                     | Ryckman et al. 
Paratriatoma hirsuta yumanensis | Morphology of 5th instar nymphs (MO)                                     | Ryckman et al. 
Paratriatoma lecitharia          | Description of nymphs (MO) and visualization of structures using SEM.   | Rocha et al. 
Psamolestes arthuri            | Description of nymphs of 1st, 2nd, 3rd, 4th, and 5th instars (MO)        | Carcavallo et al. 
Psamolestes coreodes            | Morphological (MO and SEM) and key                                      | Galíndez-Girón et al. 
Rhodnius brethesi                | Description of nymphs of 1st, 2nd, 3rd, 4th, and 5th instars             | Mascarenhas et al. 
Rhodnius dalessandroi            | Morphological and key (MO and SEM)                                       | Galíndez-Girón et al. 
Rhodnius ecuadoriensis          | Morphological and key (MO and SEM)                                       | Galíndez-Girón et al. 
Rhodnius neglectus              | Morphology of spiracles 5th instar nymphs                               | Rosa et al. 
Rhodnius neglectus              | Sexual distinction between 5th instar nymphs by SEM                     | Rosa et al. 
Rhodnius neglectus              | Morphology of 5th instar nymphs by SEM                                   | Rosa et al. 
Rhodnius neglectus              | Abdominal structures of 5th instar nymphs                               | Rosa et al. 
Rhodnius neglectus              | Morphometry of 1st, 2nd, 3rd, 4th, and 5th instars (MO)                 | Rosa et al. 
Rhodnius neglectus              | Morphology of antennae of 1st, 2nd, 3rd, 4th, and 5th instars (SEM)     | Rosa et al. 
Rhodnius neglectus              | Description of nymphs (MO) and visualization of structures using SEM.   | Mascarenhas et al. 
Rhodnius neglectus              | Morphological and key (MO and SEM)                                       | Galíndez-Girón et al. 
Rhodnius neivai                  | Morphological and key (MO and SEM)                                       | Galíndez-Girón et al. 
Rhodnius pallescens             | Morphological and key (MO and SEM)                                       | Galíndez-Girón et al. 
Rhodnius pallescens             | Morphometric of 1st, 2nd, 3rd, 4th, and 5th instars (MO)                | Marconato et al. 
Rhodnius prolixus               | Morphology of spiracles 5th instar nymphs                               | Rosa et al. 
Rhodnius prolixus               | Sexual distinction between 5th instar nymphs by SEM                     | Rosa et al. 
Rhodnius prolixus               | Abdominal structures of 5th instar nymphs                               | Rosa et al. 
Rhodnius prolixus               | Morphology of antennae of 1st, 2nd, 3rd, 4th, and 5th instars (SEM)     | Rosa et al. 
Rhodnius prolixus               | Morphology and key (MO and SEM)                                         | Galíndez-Girón et al. 
Rhodnius pictipes                | Description of nymphs by MO                                             | Lent & Valderrama 
Triatoma arthurneivai            | Nymphal instars by SEM                                                  | Rosato et al. 
Triatoma arthurneivai            | Description of nymphs (MO) and visualization of structures using SEM    | Rocha et al. 
Triatoma arthurneivai            | Description of nymphs of 1st, 2nd, 3rd, 4th, and 5th instars (MO, SEM)  | Carcavallo et al. 
Triatoma arthurneivai            | Description of nymphs (MO) and visualization of structures using SEM    | Jurberg et al. 
Triatoma breyeri                 | Description and keys for all instars.                                   | Rosa & Barata, et al. 
Triatoma breyeri                 | Morphological and key (MO and SEM)                                       | Galíndez-Girón et al. 
Triatoma caracvallo              | Description of nymphs of 1st, 2nd, 3rd, 4th, and 5th instars (MO)       | Jurberg et al. 
Continuie...
| Species                      | Approach                                                                 | References                          |
|------------------------------|--------------------------------------------------------------------------|-------------------------------------|
| **Triatoma circummaculata**  | Morphology of the head of 1<sup>st</sup> and 5<sup>th</sup> instar nymphs and visualization of some structures by SEM | Rosa et al. [55,56]                 |
| **Triatoma costalimai**      | Description and ontogenetic morphometrics of instars                      | Raigorodschi et al. [57]            |
| **Triatoma deaneorum**       | Description of nymphs of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars (MO) | Galvão & Fuentes [59]               |
| **Triatoma delpontei**       | Morphological and key (MO and SEM)                                       | Galindez-Girón et al. [25,26]       |
| **Triatoma dimidiata**       | Morphological study of nymphs (MO and SEM)                               | Mello et al. [62]                   |
| **Triatoma dispar**          | Morphological and key (MO and SEM)                                       | Galindez-Girón et al. [25,26]       |
| **Triatoma eratyrusiformis** | Morphological and key (MO and SEM)                                       | Galindez-Girón et al. [25,26]       |
| **Triatoma gerstaeckeri**    | Morphological study of nymphs (MO and SEM)                               | Galindez-Girón et al. [25,26]       |
| **Triatoma guasayana**       | Description of nymphs of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars (MO) | Brewer & Garay [63]                 |
| **Triatoma guazu**           | Description of nymphs of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars (MO and SEM) | Silva et al. [64]                   |
| **Triatoma infestans**       | Comparative study of striatalium sulcus, buccula and rostrum             | Galindez-Girón et al. [25,26]       |
| **Triatoma jurbergi**        | Sexual distinction between 5<sup>th</sup> instar nymphs by SEM            | Rosa et al. [65]                    |
| **Triatoma klugi**           | Abdominal structures of 5<sup>th</sup> stage nymphs                      | Rosa et al. [66]                    |
| **Triatoma lenti**           | Description of nymphs of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars (MO, MEV) | Jurberg et al. [67]                 |
| **Triatoma maculata**        | Comparative study of striatalium sulcus, buccula and rostrum             | Silva et al. [68]                   |
| **Triatoma matogrossensis**  | Description of nymphs (MO) and visualization of structures using SEM.    | Jurberg et al. [69,70]               |
| **Triatoma melanocephala**  | Morphological and key (MO and SEM)                                       | Galindez-Girón et al. [35]          |
| **Triatoma melasoma**        | Morphometric characterization of the nymphal instars                      | Oliveira et al. [71]                |
| **Triatoma nitida**          | Morphological study of nymphs (MO and SEM)                               | Galindez-Girón et al. [35]          |
| **Triatoma pintodiasi**      | Abdominal structures of 5<sup>th</sup> instar nymphs                      | Rosa et al. [72]                    |
| **Triatoma platensis**       | Sexual distinction between 5<sup>th</sup> instar nymphs by SEM            | Rosa et al. [73]                    |
| **Triatoma protacta**        | Morphology and key of 5<sup>th</sup> instar nymphs of species and subspecies | Ryckman [74]                        |
| **Triatoma pseudomaculata**  | Morphometric of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars (MO and SEM) | Gonçalves et al. [75]               |
| **Triatoma ryckmani**        | Description of all immature instars based on MO and SEM                  | Rocha et al. [76]                   |
| **Triatoma rubrofasciata**   | SEM                                                                       | Haridass [77]                       |
| **Triatoma rubrovaria**      | Morphology of the head of 1<sup>st</sup> and 5<sup>th</sup> instar nymphs | Rosa et al. [78]                    |
|                              | Antenna morphometry                                                      | Rosa et al. [78]                    |
|                              | Morphological and key (MO and SEM)                                       | Galindez-Girón et al. [25,26]       |

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TABLE 1: Continuation.

| Species               | Approach                                                                 | References                                      |
|-----------------------|--------------------------------------------------------------------------|-------------------------------------------------|
| **Triatoma sordida**  | Morphometry of 1st, 2nd, 3rd, 4th, and 5th instars                      | Brewer et al. 92                                |
|                       | Description of nymphs of 1st, 2nd, 3rd, 4th, and 5th instars (MO)       | Brewer et al. 61                                |
|                       | and keys for all instars                                                | Brewer & Garay 53                               |
|                       | Morphology and key (MO and MEV)                                         | Galíndez-Girón et al. 36                         |
| **Triatoma tibiamaculata** | Abdominal structures of 5-instar nymphs                                 | Rosa & Barata 46                                |
| **Triatoma vandae**   | Description of nymphs (MO) and visualization of structures using SEM.   | Silva et al. 72                                 |
|                       | Comparative study of the stridulatorium sulcus, buccula and rostrum of nymphs | Silva et al. 57                                 |
| **Triatoma vitticeps**| Antenna morphometry and morphology                                       | Rosa et al. 45                                  |
|                       | Morphology compared to other Reduviidae                                 | Weirauch 73                                     |
| **Triatoma williami** | Description of nymphs (MO) and visualization of structures using SEM.   | Silva et al. 72                                 |
|                       | Comparative study of the stridulatorium sulcus, buccula and rostrum of nymphs | Silva et al. 57                                 |
|                       | Morphology and key (MO and SEM)                                         | Galíndez-Girón et al. 36                         |
| Diverse species       | Some structures and key for Triatominae (MO and SEM)                    | Galíndez-Girón et al. 35                         |

MO: optical microscope, SEM: Scanning electron microscope.

FIGURE 2: Ventral view of the terminal segments of the male and female fifth instar nymph of *T. sherlock* and *T. lenti*. (A) Female of *T. sherlock*, (B) Female of *T. lenti*, (C) Male of *T. sherlock*, (D) Male of *T. lenti*. X, IX, VIII: tenth, ninth, eighth ventral abdominal segment.
Morphometric characteristics

Morphometric characteristics of the two species are presented in Table 2. The averages of the total length of heads in *T. sherlocki* and *T. lenti* were 1.34 and 1.42 mm, 1.84 and 1.83 mm, 2.65 and 2.93 mm, 3.61 and 3.86 mm, and 4.56 and 4.53 mm in the first, second, third, fourth, and fifth instar, respectively.

The averages of the total length of thoraxes in *T. sherlocki* and *T. lenti* were 0.98 and 0.99 mm, 1.43 and 1.43 mm, 2.08 and 2.02 mm, 2.93 and 3.26 mm, and 4.68 and 5.29 mm in the first, second, third, fourth, and fifth instar, respectively.

The averages of the total length of abdomens in *T. sherlocki* and *T. lenti* were 1.75 and 1.57 mm, 3.17 and 3.04 mm, 5.24 and 5.60 mm, 6.68 and 6.63 mm, and 11.29 and 11.14 mm in the first, second, third, fourth, and fifth instar, respectively. The mean lengths of the abdomen were larger than those of the head, which were larger than those of the thorax in the first stage nymphs in both *T. lenti* and *T. sherlocki*. As in the first stage nymphs, the average abdominal length in second instar nymphs was longer than those of the head and the thorax nymphs for both species.

In *T. lenti* and *T. sherlocki*, the highest measurement observed was the total length of the abdomen that was longer than the head as well as the thorax. The average lengths of the abdomen were higher than those of the head as well as those of the thorax for *T. lenti* and *T. sherlocki*. Abdomen and eye diameter measurements showed no significant difference between *T. lenti* and *T. sherlocki* (Table 2: p<0.001). Analyzing the fifth instar nymphs of the two species, we found that the abdomen was the largest segment, and unlike the other nymphal instars, the thorax was larger than the head in the fifth stage nymphs of both species.

The PO, IE, and AO followed an ascending order: first instar > second instar > third instar > fourth instar > fifth instar, for both species (Table 2). Among these parameters, the largest length was

| Parameters | Triatoma sherlocki | Triatoma lenti |
|------------|--------------------|----------------|
|            | 1<sup>st</sup>      | 2<sup>nd</sup>  | 3<sup>rd</sup> | 4<sup>th</sup> | 5<sup>th</sup> | 1<sup>st</sup> | 2<sup>nd</sup> | 3<sup>rd</sup> | 4<sup>th</sup> | 5<sup>th</sup> |
| Total length of head (mm) | $1.34 \pm 0.03$ | $1.84 \pm 0.05$ | $2.65 \pm 0.06$ | $3.61 \pm 0.17$ | $4.56 \pm 0.14$ | $1.42 \pm 0.08$ | $1.83 \pm 0.05$ | $2.93 \pm 0.06$ | $3.86 \pm 0.12$ | $4.39 \pm 0.16$ |
| Outer distance between the eyes (OE) (mm) | $0.25 \pm 0.01$ | $0.39 \pm 0.03$ | $0.53 \pm 0.03$ | $0.69 \pm 0.04$ | $0.94 \pm 0.04$ | $0.24 \pm 0.01$ | $0.36 \pm 0.03$ | $0.53 \pm 0.04$ | $0.70 \pm 0.03$ | $0.93 \pm 0.03$ |
| Inner distance between the eyes (IE) (mm) | $0.51 \pm 0.01$ | $0.66 \pm 0.02$ | $0.92 \pm 0.03$ | $1.23 \pm 0.07$ | $1.54 \pm 0.05$ | $0.55 \pm 0.02$ | $0.68 \pm 0.02$ | $1.01 \pm 0.03$ | $1.29 \pm 0.03$ | $1.69 \pm 0.07$ |
| Postocular distance (PO) (mm) | $0.24 \pm 0.02$ | $0.31 \pm 0.03$ | $0.41 \pm 0.02$ | $0.53 \pm 0.04$ | $0.60 \pm 0.04$ | $0.28 \pm 0.03$ | $0.30 \pm 0.02$ | $0.48 \pm 0.03$ | $0.60 \pm 0.04$ | $0.88 \pm 0.04$ |
| Ante-Ocular distance (AO) (mm) | $0.83 \pm 0.02$ | $1.20 \pm 0.04$ | $1.81 \pm 0.04$ | $2.50 \pm 0.01$ | $3.21 \pm 0.12$ | $0.89 \pm 0.04$ | $1.22 \pm 0.04$ | $1.95 \pm 0.05$ | $2.66 \pm 0.07$ | $3.65 \pm 0.15$ |
| Total thorax length (mm) | $0.98 \pm 0.03$ | $1.43 \pm 0.05$ | $2.08 \pm 0.06$ | $2.93 \pm 0.15$ | $4.68 \pm 0.18$ | $0.99 \pm 0.05$ | $1.43 \pm 0.05$ | $2.02 \pm 0.05$ | $3.26 \pm 0.11$ | $5.29 \pm 0.15$ |
| Total abdomen length (mm) | $1.75 \pm 0.11$ | $3.17 \pm 0.24$ | $5.24 \pm 0.23$ | $6.68 \pm 0.42$ | $11.29 \pm 0.39$ | $1.57 \pm 0.10$ | $3.04 \pm 0.13$ | $5.60 \pm 0.22$ | $6.33 \pm 0.33$ | $11.14 \pm 0.75$ |
| Proboscis 1<sup>st</sup> segment (1S) (mm) | $0.29 \pm 0.03$ | $0.43 \pm 0.02$ | $0.58 \pm 0.04$ | $0.84 \pm 0.04$ | $1.18 \pm 0.07$ | $0.29 \pm 0.03$ | $0.40 \pm 0.02$ | $0.6 \pm 0.04$ | $0.85 \pm 0.03$ | $1.19 \pm 0.06$ |
| Proboscis 2<sup>nd</sup> segment (2S) (mm) | $0.65 \pm 0.02$ | $1.0 \pm 0.04$ | $1.35 \pm 0.04$ | $1.85 \pm 0.07$ | $2.50 \pm 0.08$ | $0.64 \pm 0.02$ | $0.98 \pm 0.03$ | $1.48 \pm 0.03$ | $1.94 \pm 0.06$ | $2.58 \pm 0.09$ |
| Proboscis 3<sup>rd</sup> segment (3S) (mm) | $0.35 \pm 0.02$ | $0.54 \pm 0.01$ | $0.69 \pm 0.02$ | $0.93 \pm 0.03$ | $1.26 \pm 0.05$ | $0.36 \pm 0.02$ | $0.52 \pm 0.02$ | $0.72 \pm 0.02$ | $0.97 \pm 0.02$ | $1.22 \pm 0.06$ |
the distance before the eye, and the smallest was the diameter of
the eyes and the distance between both *T. lenti* and *T. sherlocki*.

The first, second, and third proboscis segment lengths were
in the following order: first segment > third segment > second
(*Table 2*). In fifth stage nymphs, the second and third segments
showed significant differences in their length for both species. In
both cases, it was observed that the second segment was larger
than the third and this was larger than the first for all nymphal
instars (*Table 2*). After measuring and performing statistical analysis
on the three segments of the proboscis in first stage nymphs, it
was observed that only the second segment showed a significant
difference, while the first and third segments did not show
significant differences between *T. lenti* and *T. sherlocki* (*Table 3*).

Comparisons between proboscis segments and head and
abdomen lengths of the two species are presented in *Table 3*. In the
second instar nymphs, the three segments of the proboscis revealed
measurements that showed significant differences, according to the
statistical analysis, for the two species. In the third and fourth instar
nymphs, measurements of the second and third proboscis segments
showed a significant difference between *T. lenti* and *T. sherlocki* (*Table 3*).

In the first instar nymphs, statistical analyses revealed significant
differences in total head and abdominal length measurements. Thorax
measurements were not different between the two species. Regarding
the measurements of head parameters of the first stage nymphs,
the distance between the anterior, postocular, interocular, and eye
diameters were significantly different when comparing *T. lenti* and
*T. sherlocki*. Statistical analysis showed significant differences only
for interocular distance and eye diameter in second instar nymphs.

Measurements of the thorax, abdomen, ante-ocular distance,
postocular distance, and total head length revealed no significant
differences between the two species (*Table 3*). For the third instar
nymphs, all measurements except for the eye diameter and first
proboscis segment measurements, showed significant differences
between both species, (*Table 3*). For the fourth instar nymphs, all
parameters showed statistically significant differences, except for
the eye diameter and first segment of the proboscis, as was also
observed for the third instar nymphs (*Table 3*). The measurements
total head length, ante-ocular distance, postocular distance,
and interocular and thorax distance of fifth instar nymphs were
significantly different between the two species.

### Principal component analysis

The main components (PCA1 and PCA2) are presented through
biplot graphics showing the morphometric variability between *T.
lenti* and *T. sherlocki*. The total size of the head was responsible
for greater discrimination between the studied specimens.
Alternatively, PC1 and PC2 were 99.569% and 0.431% for the
first stage nymphs (*Supp. Figure 1*), 99.966% and 0.034% for the
second stage (*Supp. Figure 2*), 99.937% and 0.062% for the
third stage (*Supp. Figure 3*), 99.791% and 0.208% for the fourth
(*Supp. Figure 4*), and 99.84 and 0.15% for the fifth (*Supp. Figure 5*).

### DISCUSSION

Studies on immature forms of triatomines are relevant to
taxonomy and provide important information for the understanding
of several biological aspects of these vectors. In this study, a list of
works with immature forms were presented and a morphological
characterization of five nymphal instars of *T. lenti* and *T. sherlocki*,
species that are closely related phylogenetically, were described.
Triatoma lenti and T. sherlocki have reproductive compatibility with other members of the species T. brasiliensis subcomplex, which are frequently found in dwellings and infected with T. cruzi; therefore, they are potential vectors of Chagas disease. Costa et al. conducted a comparative morphological analysis of the external genital structures and eggs of T. brasiliensis to differentiate chromatic forms. Gonçalves et al. used classic and geometric morphometry as a tool to distinguish T. jatari from other species. Mendonça et al. used morphological, morphometric, molecular, and cytogenetic approaches as well as experimental crosses to revalidate the specific status of T. bahiensis and differentiate it from T. lenti. Combining morphometric and molecular approaches has provided important clues about the T. brasiliensis complex, which includes the species and subspecies T. lenti, T. petrocchiae, T. b. brasiliensis, T. b. macromelasoma, T. juazeirensis, T. sherlocki, T. melanica, and T. bahiensis.

In the present study, using SEM images, morphological differences were observed in the ninth ventral abdominal segment of female and male nymphs of the fifth instar. Comparing the morphology of the ninth ventral abdominal segment of male and female nymphs in the fifth instar of the species T. melanocephala Neiva & Pinto, 1923, T. brasiliensis, T. infestans, T. matogrossensis Leite and Barbosa, 1953, T. tibiamiculata (Pinto, 1926), T. lenti, and T. sherlocki, it can be seen that seven of these species differ by this character. This indicates that the shape and size of the ninth abdominal segment in fifth instar nymphs may be taxonomically valid.

The measurements of the head, thorax, and abdomen served to better characterize and distinguish T. lenti and T. sherlocki across their evolutionary instars, as well as in the comparative analysis of nymphal instars of other species of the Triatoma genus. The combination of morphometric and morphological approaches provides important clues about the delimitation of the complex. Oliveira et al. morphometrically analyzed the species of the T. brasiliensis complex and showed that the variations in the shape of the head were statistically significant. The wings showed sexual dimorphism in shape, while the heads were not dimorphic as expected.

In this study, as in all other nymphal instars, we found that the largest measurement among the head measurements was the anocular distance and the smallest was the postocular distance. In the morphometry, all parameters in the first instar, except the average eye diameter and the first and third proboscis segments, were significantly different between T. lenti and T. sherlocki. Measurements of interocular distance, eye diameter, and the three segments of the proboscis revealed significant differences between the second instar nymphs of T. lenti and T. sherlocki. The third and fourth instar nymphs showed significant differences in the measurements of the abdomen, head, thorax, ante-ocular, interocular, postocular, and second and third proboscis segments. In the fifth instar, measurements of thorax length, head length, ante-ocular, interocular, postocular, and second and third proboscis segments showed significant differences in taxonomic differentiation between T. lenti and T. sherlocki. In all nymphal instars, the total length measurement ratio were in the following order: abdomen > head > thorax. In Triatoma melanocephala Neiva & Pinto, 1923, the nymphal instars presented the following length pattern: in the first instar, thorax > abdomen > head; in the second instar, abdomen > head > thorax; and in the third, fourth, and fifth instars, abdomen > thorax > head.

In all nymphal instars of T. lenti and T. sherlocki, it was observed that the second segment of the proboscis was larger than the third which was larger than the first segment. In T. melanocephala nymphs, it was found that specimens in the first three nymphs presented the same length order (2 > 3 > 1), while those in the fourth and fifth instars, along with the adults, possessed mouthpart segments of the same order (2 > 1 > 3). The main components (PC1 and PC2) illustrated the differences between the studied parameters and showed that the total size of the head is or that it discriminates against T. lenti and T. sherlocki.

Studies on nymphs are crucial for the systematic development of certain groups. However, the literature on immature forms of certain groups is scarce, difficult to use, or nonexistent. Epidemiological studies and control measures require precise taxonomic determination of T. brasiliensis subcomplex. Therefore, this study provides morphological and morphometric data on the nymphal instars of T. lenti and T. sherlocki, corroborating the specific taxonomy of these species.

ACKNOWLEDGMENTS

The authors are thanks to Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). Thanks to the São Paulo State University (Unesp), Faculty of Pharmaceutical Sciences. The authors also thank the researchers and students of the Parasitology Laboratory at the same institution. We thank the members of the Institute of Biology at the State University of Campinas (Unicamp).

FINANCIAL SUPPORT

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, process numbers 2008/55000-7). Coordenação de Pessoal de Nível Superior (Capes) - Finance Code 001. Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq, PQ-2, process 307 398/2018-8.

AUTHORS’ CONTRIBUTION

JAR: Conception, design study and data acquisition. VFP: Writing, Formal analysis and investigation, review, and editing. TB: Writing, Methodology, review, editing, Formal analysis, and investigation. JO: Writing, data acquisition, review, and editing. VJM: Review, data acquisition and editing. LAR: Supervision, Conception, and design study.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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