

Morphobiological aspects of *Rhodnius brethesi* Matta, 1919 (Hemiptera:Reduviidae) from the Upper and Middle Negro River, Amazon region of Brazil. I - Scanning electron microscopy

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The occurrence of autochthonous cases of Chagas disease in the Amazon region of Brazil over recent decades has motivated an intensification of studies in this area. Different species of triatomines have been identified, and ten of these have been proven to be carriers of the parasite Trypanosoma cruzi or "cruzi-like" parasites. Studies conducted in the municipalities of Santa Isabel do Rio Negro and Barcelos, located on the Upper and Middle of the Negro River, microregion of Negro River, state of Amazonas have confirmed not only that Rhodnius brethesi is present in the palm tree Leopoldinia piassaba, but also that this insect was recognized by palm fiber collectors. A morphological study of eyes, inter-ocular and inter-ocellar regions, antennae, buccula, labrum, rostrum, stridulatory sulcus and feet, including the apex of the tibia, spongy fossette and ctenidium was conducted by scanning electron microscopy. The buccula and the stridulatory sulcus presented notable differences in specimens of different genera and also of different species. These data make it possible to suggest that the details presented in these structures can be included as diagnostic characteristics to be used in new dichotomous keys, thereby contributing towards studies of taxonomy and systematics and furnishing backing for comparative analysis of specimens collected from different localities.

Key words: *Rhodnius brethesi* - external morphology - taxonomy - scanning electron microscopy - Amazon region - Brazil

Over recent years, attention has been drawn to Chagas disease infection in the Amazon region of Brazil because of increased numbers of reports of acute cases and the presence of individuals who are serologically positive for this infection (Coura et al. 1995, 1999, 2002a, Fraiha Neto et al. 1995, Valente et al. 1999, Dias et al. 2002). So far, it is unclear whether these growing numbers of human cases are due to increased transmission or whether they are the result from an active search for positive cases. This latter is the case in the state of Pará, where greater numbers of small outbreaks attributed to contamination by oral transmission have been described (Valente et al. 1999).

One of the epidemiological profiles discerned in the Amazon region that has received deserved attention is the one found in the Upper and Middle Negro River region, microregion of Negro River in the state of Amazonas. Initial investigations by Coura et al. (1994, 1995) indicated that the presence of human infection in areas of the Negro River were associated with extractive activities relating to the collection of fiber from the native palm tree *Leopoldinia piassaba* Wallace, 1853. The link in the

transmission cycle was pinpointed as the contact between the fiber gatherer and the species of triatomines present in the extraction areas (Coura et al. 2002a). The data obtained through that investigation showed that two vector species were present in the extraction areas: *Rhodnius brethesi* Matta, 1919, and *Panstrongylus geniculatus* (Latreille, 1811). The first of these was present at a much more significant density than was the second (Junqueira, pers. commun.).

R. brethesi is among the species of triatomines in the Amazon region that have been identified as positive for *Trypanosoma cruzi* (Coura et al. 1999, 2002b). It was first described by Alfredo da Matta in 1919, in specimens collected from an area where extractive activities involving *L. piassaba* had already taken place, in the municipality of Barcelos, state of Amazonas, Brazil. In 1922, Matta drew attention to the fact that the palm fiber gatherers were suffering bites from these insects. This was due to the fact that these insects habitually live among the fibers of these palm trees: they are popularly known in the region as "piassaba lice" (Mascarenhas 1990, 1991). Coura et al. (1994) also conducted field studies in the municipality of Barcelos and verified the attacking behavior of *R. brethesi*. It has been shown that its geographical distribution includes damp areas in the states of Amazonas and Pará, in the Northern region of Brazil, and in drier areas of the state of Maranhão, in the Northeastern region of the country (Rebello et al. 1998). There have also been reports of the presence of this species in Colombia and Venezuela (Carcavallo et al. 1999, Galvão et al. 2003).

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With regard to possible infection by other trypanosomes, D'Alessandro et al. (1971) incriminated this species as a natural vector for *Trypanosoma rangeli*, in Colombia.

All these findings emphasize the importance of studies to promote greater knowledge of this species, and among these, studies of the ultrastructure of specimens coming from the Negro River.

Morphological studies on triatomines, utilizing the resources of scanning electron microscopy, have been performed by several authors on different species and in relation to all stages of development (Lent & Wygodzinsky 1979, Barata 1981, 1998, Gonçalves et al. 1985, Costa et al. 1991, 1997, Galíndez Girón et al. 1994, Silva et al. 2003). These studies have made effective contributions towards the systematics of triatomines, through elucidation of the status of cryptic species and their complexes. The first descriptions of the external morphology of *R. brethesi* using optical microscopy were based on adult specimens (Matta 1919, 1922). The eggs, nymph stages and life cycle were described by Mascarenhas in 1982, 1987, and 1990, respectively. Other studies related to the external morphology, including in relation to the male and female genitalia, were performed using optical microscopy by Lent (1948), Lent and Jurberg (1969), and Lent and Wygodzinsky (1979).

Structures like the stridulatory sulcus, buccula and rostrum were highlighted by Carcavallo et al. (1996), Silva et al. (2003), Ferro et al. (1997, 1998), Andrade et al. (2002), and Silva et al. (2003) as having taxonomic importance in aiding in differentiating between populations existing within the same areas.

Preliminary studies on the external morphology of *R. brethesi* at an ultrastructural level utilizing scanning electron microscopy have been performed on the structures of the head, thorax and feet of nymphs and adults (Ferro et al. 1997, 1998, 1999, Andrade et al. 2002).

With aim of obtaining better knowledge of *R. brethesi*, a morphological analysis was performed on the head (anterior ocular region, ocular-ocellar region, antennae, buccula and rostrum), thorax (stridulatory sulcus) and feet (apex of the tibia, spongy fossette, ctenidium, and tarsus) of adults of this species.

MATERIALS AND METHODS

The specimens of *R. brethesi* were obtained from colonies maintained in the Parasitic Disease Laboratory, Department of Tropical Medicine of Instituto Oswaldo Cruz. They had been collected by means of modified Noireau traps and Shannon-type traps on piassaba palm trees in four rivers located in the left margin of Negro River, in the northern part of the state of Amazonas, Brazil: Acará River, Curuduri River, Preto River, and Padauri River. The first two rivers are situated in the municipality of Barcelos (latitude 68°55'N and longitude 0°10'W), the third in the municipality of Santa Isabel do Rio Negro (latitude 62°55'S and longitude 1°W), and the last is divisor of both municipalities (Fig. 1).

Five male and five female specimens were utilized. The insects were killed using ethyl acetate and were dissected



Fig. 1: district of Barcelos in the microregion of Negro River, state of Amazonas.

to remove the structures. These were mounted on metallic supports suitable for scanning electron microscopy, using double-sided tape. The structures analyzed were the head in dorsal and ventral views, eyes, antennae, buccula, labrum, rostrum, stridulatory sulcus and the feet, to view the apex of the tibia, spongy fossette and ctenidium. Measurements were made in the inter-ocular and inter-ocellar regions.

These structures were covered with gold using an evaporation system known as "sputtering", in which the gold is removed by means of bombardment in high vacuum (Hayat 1970), utilizing Balzers apparatus. The samples were observed at 15-20 kV using the Jeol 5310 scanning electron microscope (Akishima, Tokyo, Japan) belonging to the Carlos Chagas Filho Biophysics Institute of the Federal University of Rio de Janeiro (UFRJ). The images obtained were captured directly onto the computer by utilizing the SemAfore software.

RESULTS

The head of *R. brethesi* presented rugose cuticular features in its dorsal region, with 1+1 longitudinal smooth areas delimiting a central area in which uniformly arranged bristles were seen (Fig. 2). The specimens presented composite eyes that were totally covered with ommatidia, including in the posterior-inferior area of this structure. Between every two or three ommatidia, there were bristles set in protruding buttons that were sometimes visible. These were corrugated and short or long, with apices that were rounded or slightly dilated (Fig. 3). The mean interocular distance measured was 470 µm on the females and 464 µm on the males, while the mean inter-ocular distance was 703.4 µm on the females and 660.3 µm on the males.

The antennae had four segments and presented corrugated integument and sensilla of varied shapes and sizes (Fig. 4). At the base of the second segment of the antenna, there were three trichobotria distributed on the external lateral face (Fig. 5) and two on the dorsal face, and also another four located on the remainder of the segment, thus totaling nine of these structures. At the base of each trichobotrium, the cuticular area was differentiated by presenting lamellar structures and fingerlike prolongations (Fig. 6).

The pyriform labrum that rested on the first segment of the rostrum presented smooth integument with slight depressions and coarse bristles that were generally curving downwards. Under the labrum, there was a triangular depression with regularly distributed granulation that extended laterally and symmetrically as far as the apex of the first segment of the rostrum (Fig. 7).

The third segment of the rostrum presented sensilla of different shapes and sizes (Fig. 8). At the ventral apex, there was an elliptical hairless depression with two perforations located in the basal third. The mouth styli were surrounded by translucent sheaths located in the rostrum. When the rostrum was distended, this made it possible to view the buccula, which was located ventrally between the apex of the head and the anterior-ventral region of the first segment of the rostrum. It was an oval structure, with medial constriction, resembling a figure-of-eight. The basal part had raised borders covered with dispersed tubercles, and in the medial region a slight depression was observed, with corrugated integument. The apical portion had two lateral depressions with longitudinal and transversal striae (Fig. 9).

The stridulatory sulcus was presented in the shape of an amphora (Fig. 10), with transversal striae of appearance varying according to the region observed. In the basal region, they were poorly defined (Fig. 11), becoming delineated from the area of the constriction onwards as far as the apex, which had a rugose appearance (Fig. 12). In the lateral portion of the sulcus, a reticulated area was seen, with granular appearance and close silky tubercles covering its whole extent (Fig. 13).

On the feet, two cuticular structures were prominent at the apex of the tibia: the ctenidium and the spongy fossette (Fig. 14). The ctenidium was found only on the first pair of feet, in both sexes (Fig. 15). The spongy fossette (Fig. 16) was present on the first and second pairs of

feet, in both males and females, and had a pyriform appearance with an external margin covered with short fingerlike projections (Fig. 17). Around the internal margin, bristles were seen, turning inwards; this margin had a laterally flattened appearance and its surface was covered with button-shaped structures (Fig. 18). In the medial region, the bristles were straight, and there was a straight and flattened apex covered with buttons (Fig. 19).

DISCUSSION

Of the species of triatomine found in Amazon Brazil, ten are infected with *T. cruzi* (Almeida 1971, Lent & Wygodzinsky 1979, Miles et al. 1981, 1983, Brazil et al. 1985, Barrett & Guerreiro 1991), among them *R. brethesi* (Coura et al. 1999).

At a recent international meeting on the surveillance and prevention of Chagas disease in the Amazon region, one of the points of consensus was that American trypanosomiasis has high prevalence and wide dispersion as a wild enzootic disease throughout the Amazon region, presenting characteristics favorable towards its expansion as a human endemic disease. Among the particular transmission situations, it was reported that it occurred in areas with extraction activities consisting of the gathering of piassaba palm tree fibers, where the vector species *R. brethesi* has been found (Technical Report 2005).

As stated earlier, few studies have been conducted on this species. These have mainly been restricted to its biology, and no morphological approaches at the ultrastructural level comparable with what has been done for other triatomine species are known of. The present study therefore expands the information available on this vector species coming from the Upper and Middle Negro River, microregion of Negro River. In this first analysis using adult specimens, the presence of some structures already described in the literature for other species of triatomines has been confirmed, and some other, previously unreported structures have been described. These may have taxonomic value, in accordance with the continuation of the comparative study.

Observations on the presence of bristles between the ommatidia in the genera *Alberprozenia*, *Belminus*, *Microtriatoma*, *Cavernicola*, *Rhodnius*, *Psammolestes*, *Dipetalogaster*, *Eratyrus*, *Panstrongylus*, *Paratriatoma*, and *Triatoma*, by means of scanning electron microscopy, were made by Galíndez Girón et al. (1994), thus providing confirmation for the results obtained in the present study for the genus *Rhodnius*.

According to Catalá and Schofield (1994), the cuticle of the four antennae segments of *Rhodnius* is covered with sensilla that have the function of mechanical, chemical and thermal receptors. The trichobotria (the sensilla functioning as mechanical receptors) have taxonomic value among triatomines, and were found only on the second segment of the antennae in *R. brethesi* (Lent & Wygodzinsky 1979). In the adult specimens of the present study, nine trichobotria were observed, and these were distributed from the base to the apex of this segment. This differs from the observations by Lent and Wygodzinsky (1979), who observed seven trichobotria in the second segment of the antennae of adult *R. brethesi*.

However, Catalá and Schofield (1994) wrote that, in adult specimens of the genus *Rhodnius*, the total number of trichobotria varied between five and nine, and that this

number could be between species, between individuals of the same species and occasionally between the left and right antennae of the same individual.

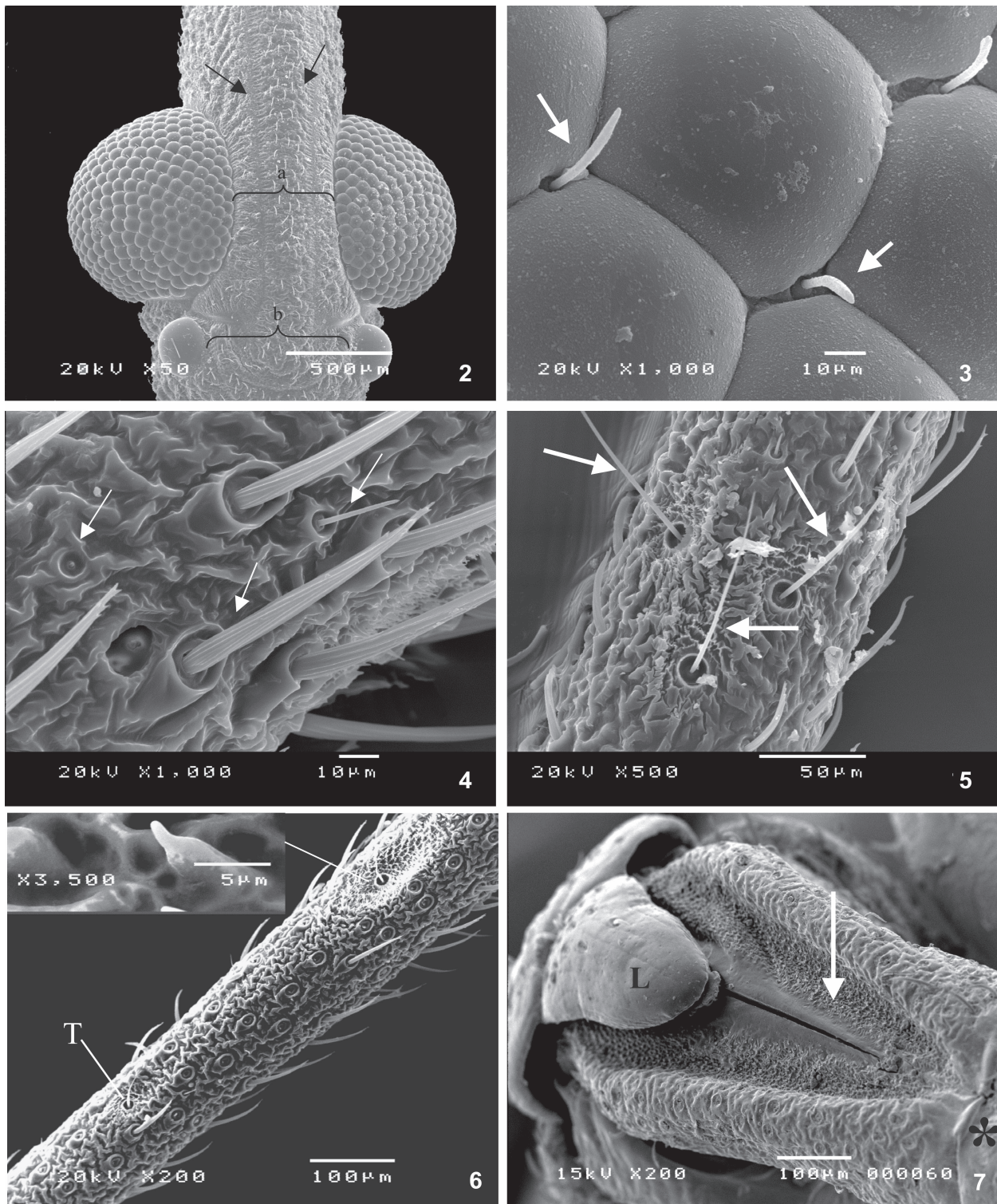


Fig. 2: dorsal region of *Rhodnius brethesi* head with 1+1 longitudinal smooth areas (arrows) showing the inter-ocular (a) and inter-ocular (b) regions where the measurements were made. Fig. 3: bristles between ommatidia (arrow). Fig. 4: sensilla in the antennae with varied shapes and sizes. Fig. 5: three trichobotria on the external lateral face of antennae (arrows). Fig. 6: cuticular area at the base of trichobotrium (T) with lamellar structures and fingerlike prolongations. Fig. 7: the labrum with smooth integument; triangular depression (arrow); first segment of the rostrum (*).

Detailed studies (Andrade et al. 2002) on the principal sensilla found on the antennae of *R. brethesi* (bristle types I, II, and III) are being conducted with the aim of elucidat-

ing their taxonomic value, and also to evaluate the receptor pattern in relation to habitat adaptations. These aspects were also observed by Catalá (1994, 1998) in others

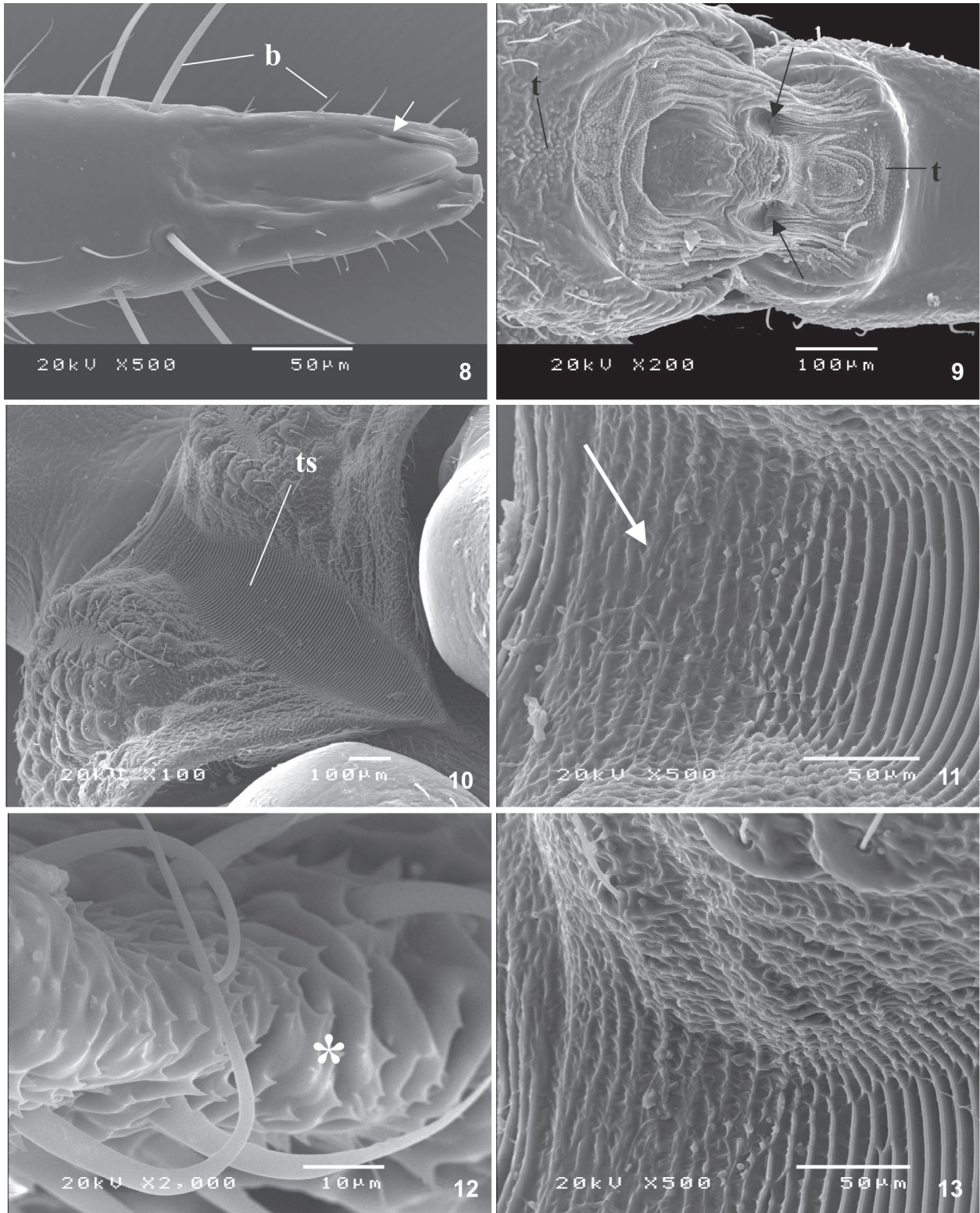


Fig. 8: rostrum with bristles (b) and elliptical hairless depression (arrow). Fig. 9: general view of the buccula. Lateral depressions (arrows) t = tubercles. Fig. 10: stridulatory sulcus with amphora shape showing transversal striae (ts). Fig. 11: basal region of stridulatory sulcus (arrow). Fig. 12: rugose and granular appearance (*) of the apex of stridulatory sulcus. Fig. 13: reticulated area in the lateral portion of the sulcus with tubercles covering its whole extent.

species of *Rhodnius* genus. Recently, Catalá et al. (2004) demonstrated that specimens kept in a laboratory for long periods of time can present modifications to the sensilla,

thus suggesting that the behavioral and physiological results obtained with insects from laboratories might be compromised.

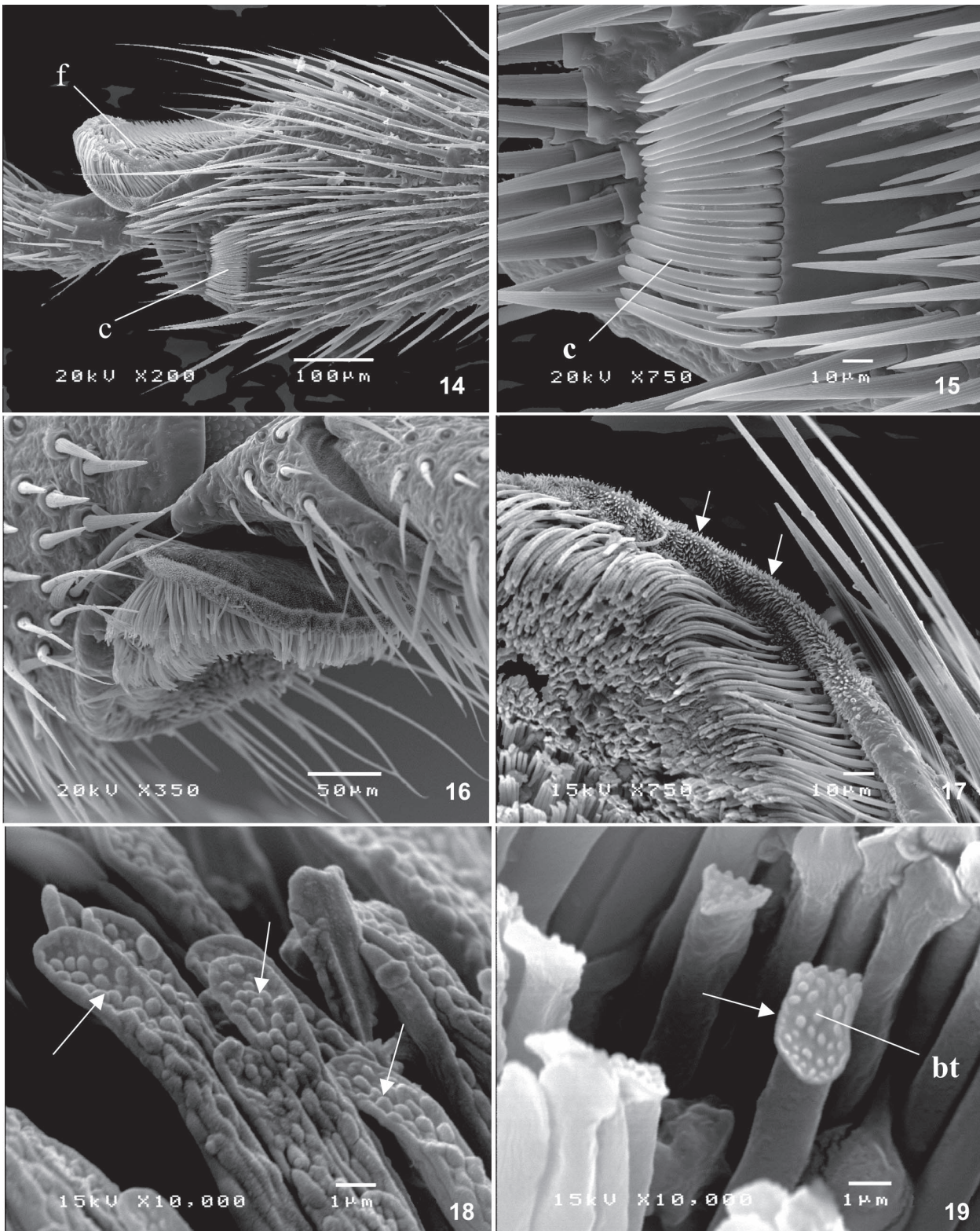


Fig. 14: the apex of the tibia: the ctenidium (c) and the spongy fossette (f). Fig. 15: ctenidium (c). Fig. 16: spongy fossette. Fig. 17: fingerlike projections (arrow) in the spongy fossette. Fig. 18: button-shaped structures (arrows) in bristles presents in the margin of the spongy fossette. Fig. 19: buttons(bt) in the bristles (arrow) of medial region of spongy fossette.

In the present study, the third segment of the rostrum presented sensilla of different shapes and sizes. The importance of the rostrum for characterizing the genus as *Rhodnius* was made by Pinto (1931) using light microscopy. Likewise, Catalá (1996) emphasized this in an analysis by scanning electron microscopy of the rostrum of eight species of the genus *Triatoma*. This latter author concluded that the numbers and distribution of the sensilla did not differ between nymph and adult forms, but did differ between the species of triatomines.

The buccula is a structure that has been demonstrated to have taxonomic value, and in the present study on *R. brethesi* it was found to have the format of a figure-of-eight. In this, it differs from the species *Triatoma williamsi* and *Triatoma gerstaeckeri* (Ferro et al. 1997) and also from *Triatoma guazu* and *Triatoma jurbergi* (Silva et al. 2003), which have a U-shaped format.

The stridulatory sulcus is another structure presenting features of taxonomic value, in relation to shape, number of striae and lateral ornamentation of the integument. In the present study on *R. brethesi*, it was found to have the shape of an amphora. In this, it differs from what has been observed for other genera, and for other species of the same genus, as demonstrated by Lent and Wygodzinsky (1979) and Silva et al. (2003).

The ctenidium, which has the function of removing impurities from the insect's cuticle, was found only on the first pair of feet in the adult form. The spongy fossette has an adhesive function that allows adult specimens to move across smooth surfaces and allows the male to seize the female during copulation (Lent & Wygodzinsky 1979). It was only found on the first and second pairs of feet of both the male and female adults, compatible with species of the genus *Rhodnius*. These authors found the spongy fossette in all the nymph stages of *Parabelminus* and at least in the fifth nymph stage of *Microtriatoma*. Campannuci et al. (1997) stated that the presence of the spongy fossette on the first and second pairs of feet of *Triatoma infestans* served to keep the female in a position that enabled successful copulation. They also suggested that some type of secretion might be released in relation to sexual interactions and/or the spongy fossette might furnish sensory information associated with sexual behavior. In the present study, the differences in the bristles found in the spongy fossette suggest the need for comparative studies within and between species.

The present work has expanded the morphological knowledge of *R. brethesi*, since previous descriptions of specimens were basically produced from optical microscopy. Among the structures analyzed, it is suggested that detailed features of the buccula and the stridulatory sulcus could be included as diagnostic characteristics to be utilized in new dichotomous keys, because of the notable differences in these structures that are presented in comparisons with species in different genera and also with different species. In addition to these two characteristics, ultrastructural study of the antennae, rostrum and spongy fossette of adults could be included in these new keys, to complement the information existing in the literature.

The results obtained from this work, allied with the observations made by various authors on other species

utilizing scanning electron microscopy, furnish data that will contribute towards establishing specific diagnostic characteristics, especially with regard to differentiating between cryptic species, and also in the analysis of specimens from different localities, either with or without making associations with their habitats.

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