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## Phase Contrast X-Ray Synchrotron Microtomography for Virtual Dissection of the Head of *Rhodnius prolixus*

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**Abstract.** Phase Contrast X-Ray Synchrotron Microtomography is a non-destructive technique that allows the microanatomical investigations of *Rhodnius prolixus*, one of the most important insect vectors of *Trypanosoma cruzi*. In this work complete series of virtual thin sections through the heads of selected *Rhodnius prolixus* were obtained. The sections of the head were important to compare the difference in use the spatial resolution of 2 $\mu$ m or 4.5  $\mu$ m and to see anatomical details that couldn't be seen with other technique. Three different groups of *Rhodnius prolixus* were used. One group was fed with defibrinated rabbit blood and after 10 days was sacrificed, other group was sacrificed 4 days after feeding and the last group remained unfed. The results show some differences for each kind of groups and for the different resolutions.

### 1. Introduction

Conventional X-ray computed tomography (CT) is based on the difference in radiation absorption by different materials or tissues. At the same time, a wide range of samples used in materials science, biology, and medicine demonstrate very weak absorption contrast, nevertheless producing significant phase shifts in the X-ray beam. The use of phase information for imaging purposes is therefore a suitable alternative.

Application of X-ray microtomography ( $\mu$ CT) on insects is quite recent [1, 2, 3, 4, 5] and its transposition to use phase-contrast synchrotron X-ray microtomography is even more recent [6, 7, 8, 9]. Phase contrast X-ray imaging is already shown to be useful to study other insects within different matters.

Members of the subfamily *Triatominae*, family *Reduviidae*, are an interesting group to study for comprising a large number of insect species of which some are vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease. *Rhodnius prolixus* is a blood-sucking insect well known by for its



importance as vector of Chagas disease, especially in Latin America. Among the parasitic diseases, Chagas disease is ranked as one of the most important in Latin America in terms of social and economic impact, affecting about 18 million people, with about 100 million people living in what are considered to be high risk zones, and approximately 300,000 new cases occurring every year with around 21,000 deaths annually [10].

Despite the fact that the *R. prolixus* is one of the most well-know model in terms of both physiology and vector-parasite interactions studies, the understanding of how some morphophysiological aspects of mainly the retrocerebral complex in different time of the life cycle are related to the development and reproduction of this insect vector remains remarkably limited. Head morphology is also largely unknown, especially internal features. It was demonstrated in earlier studies that head structures are not only of great importance in terms of coordination of the central nervous system, sensory perception, and food processing, but also provide much phylogenetic information due to their complexity [3].

The demand for this kind of investigation is increasing with the necessity to find new vector control methods. The present study aimed to visualize three-dimensional structures at micrometer scale of the head of *R. prolixus* defining their precise spatial relationships, variations in size, shape and proportion of individual structures.

This work is part of a series of articles that use Synchrotron Radiation Phase Contrast microtomography (SR-PhC- $\mu$ CT) for the study of *R. prolixus* morphology. In the first paper [11] the visualization of various detailed features were highlighted thanks to the edge enhancement effects typical of the phase contrast technique, but the contrast between foreground and background remained poor. In the second study [12] the same data set have been revisited with application of a single distance phase retrieval algorithm for comparison with previous results. The resulting slices showed very high quality images that enable a better visualization of important muscles and neurohemal organs of the central nervous system within the head of *R. prolixus*.

In the present work, the  $\mu$ CT images were obtained using the new experimental set-up which was recently made available at the SYRMEP beamline [13] and these images were compared to those used in the first and second papers. The new set-up uses the polychromatic configuration. In this configuration the outgoing beam from the ring is intercepted before the monochromator and in a dedicated end-station, absorption and phase contrast radiography and tomography set-up are available. A 2  $\mu$ m resolution was achieved in the  $\mu$ CT images with the set-up used for these analyses. The images obtained with the new set-up at SYRMEP beamline provided a better visualization of virtual sections of the head of *R. prolixus*.

## 2. Materials and methods

### 2.1. Sample preparation

Fifth-instar nymphs of *R. prolixus* (n=5) were taken from colony in the Laboratory of Biochemistry and Physiology of Insects, Oswaldo Cruz Foundation, Brazil, maintained at 28°C and between 60% and 70% relative humidity. The insects were fed with rabbit blood using a membrane apparatus previously described by Garcia et al. [14]. Three days after blood meal, insects were immobilized at 4°C for 10 min and bounded on a polystyrene table with entomological pins and transversally cut at the junction between prothorax and mesothorax segments of body. The anterior fragments were fixed (gluteraldehyde) and maintained at room temperature.

For the X-ray microtomography ( $\mu$ CT) investigation the head and prothorax were dried to avoid shrinking artifacts caused by water loss during the tomography procedure, which might affect fresh samples exposed to the dry atmosphere in the experimental hutch. This procedure is usual in studies with insects [2, 3, 5, 9]. All specimens (n = 5) were imaged.

## 2.2. Experimental set-up at SYRMEP beamline

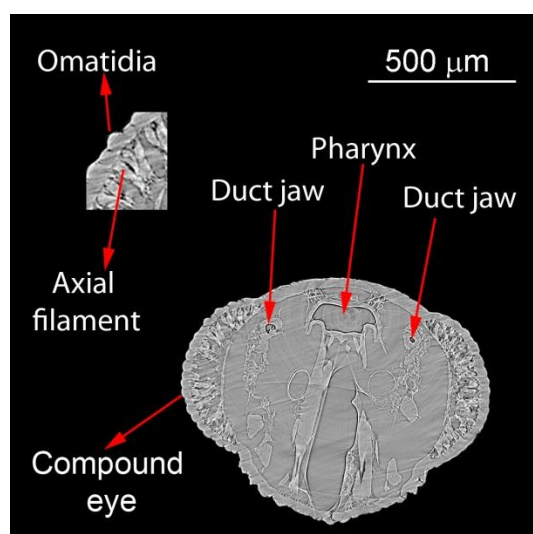
The first microCT images of the entire internal anatomy of the head of specimens of *R. prolixus* were obtained using the microCT setup at the SYRMEP (Synchrotron Radiation on Medical Physics) beamline of the ELETTRA synchrotron radiation facility (Trieste, Italy). The experimental station is placed about 23m and at this distance, the beamline provides a monochromatic laminar-section X-ray with a maximum area of about 160x5 mm<sup>2</sup> at 20 keV. The detector system is comprised of a 12/16-bit CCD camera, with 4008 x 2672 pixels<sup>2</sup>, 4.5 μm pixel size with a field of view of 18x12 mm<sup>2</sup>, coupled to an intensifier screen with no magnification (1:1).

To gather as much information as possible from the specimens of *R. prolixus*, we decided to use the new high-resolution microCT setup which has been recently available at the SYRMEP for this study. In order to optimize the performances of the microCT setup for high resolution experiments in the SYRMEP beamline, a lens-coupled CCD camera system (high-resolution 2048 x 2048 pixels<sup>2</sup>, effective pixel size of 1.03 μm) designed to achieve up to 2 μm spatial resolution was used in white/pink X-ray beam mode that provides a nearly parallel, laminar-section X-ray beam with a maximum area of 100x6 mm<sup>2</sup> at a distance of about 15 m from the source. Although monochromatic beam was not possible to be used in this high-resolution setup at SYRMEP beamline, physics filters of Si (1 mm) and Mo (0.025 mm) were used to minimize beam-hardening. Another advantage of using this new setup was the faster acquisition time achieved. For our experiments, 1440 radiographic projections were acquired over an angular range of 180° with angular step of 0.125°.

Tomographic slices were reconstructed using the conventional filtered backprojection algorithm with Shepp Logan filter using the SYRMEP TOMO PROJECT (STP) software developed by the SYRMEP team. ImageJ® software was used to render the 3D volumes.

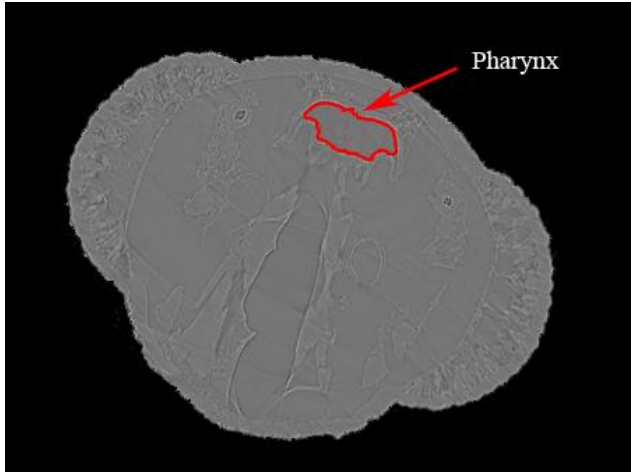
## 3. Results and discussions

Morphological studies on insects often focus on a detailed analysis of the head or parts of it. The insect head shows a broad spectrum of structures, such as sensory organs, endo and exo skeletal features, muscles, brain or mouthparts, whose specific organization can act as an aid to understanding the evolution, development and ecology of the insect under study [15, 16, 17]. In this work, we obtained complete series of virtual thin sections through the heads of selected *Rhodnius prolixus*; these could be used both for the inspection of planar sections and for 3D reconstructions of the entire head. In figure 1, it can be seen anatomical details of the compound eyes such as ommatidia and the axial filament, which could not be revealed from our previous images [11, 12].

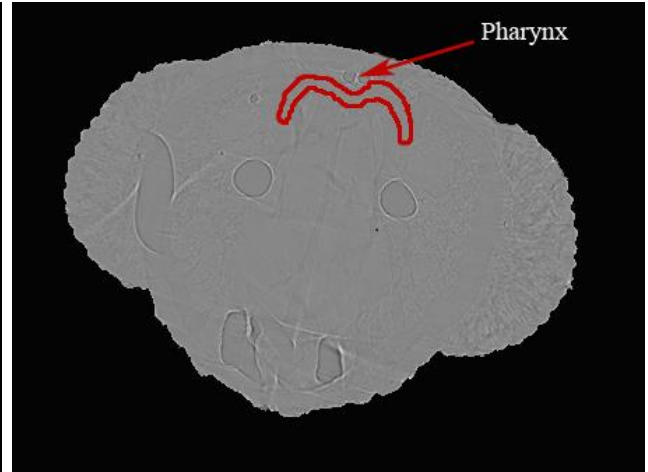


**Figure 1.** Virtual section (transversal view) through the head of *R. prolixus*.

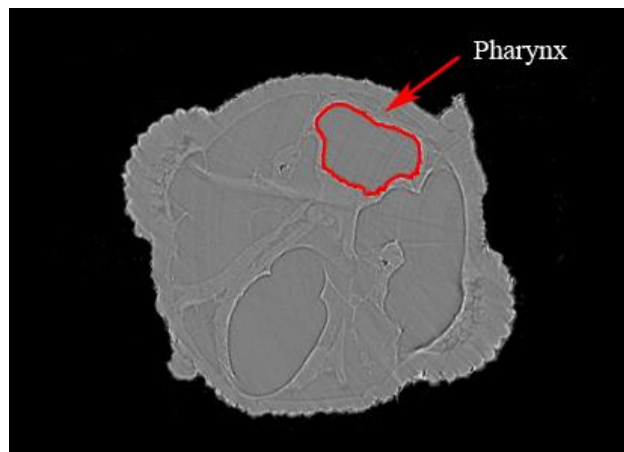
The pharynx and its dilator muscles were recognized in figures 2 to 4. Variation in size of pharynx can be noted in different times after feeding.



**Figure 2.** 3D rendering of the head of *R. prolixus* sacrificed 10 days after feeding.



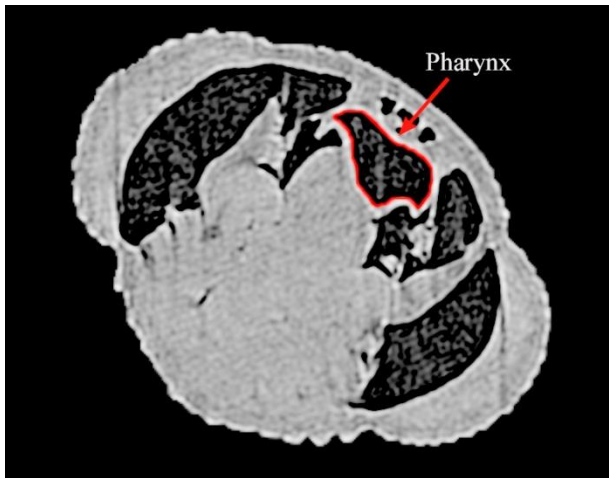
**Figure 3.** 3D rendering of the head of *R. prolixus* sacrificed 4 days after feeding.



**Figure 4.** 3D rendering of the head of unfed *R. prolixus*.

Comparing this images one can realize that the muscles of unfed *R. prolixus* (figure 5) are thinner than the muscles in figures 3 and 4. Pharynx in unfed insects seems more dilated than in insects that were sacrificed 10 days after feeding.

In figures 5 and 6 it can be seen a virtual thin section of the head of *Rhodnius prolixus* obtained previously with the conventional setup at SYRMEP beamline [ARTIGOS ALMEIDA]. Comparing with figures 3 and 4 it is easy to realize that figures 6 and 7 do not show details seen in figures 2 and 3. The virtual thin sections are approximately in the same region.

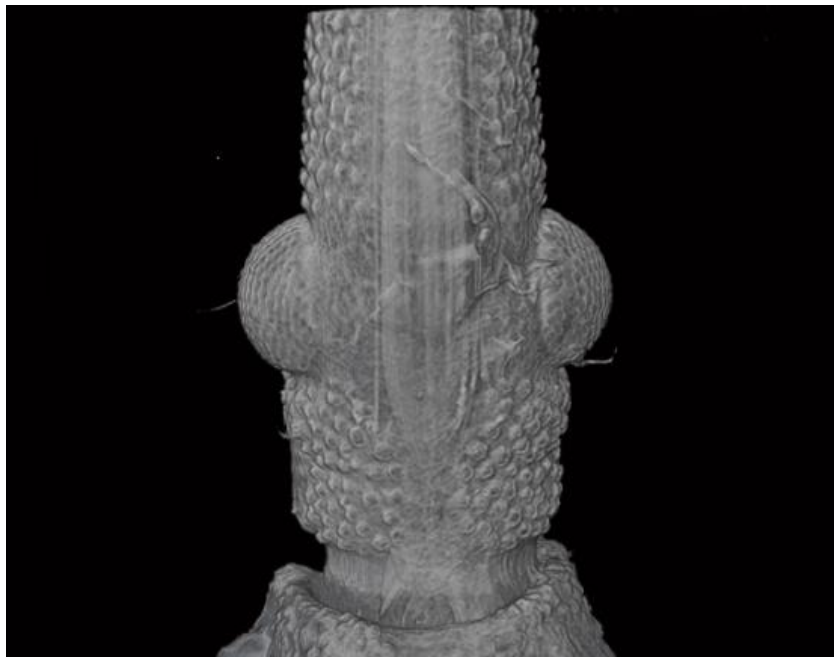


**Figure 5.** 3D rendering of the head of *R. prolixus* sacrificed 10 days after feeding.



**Figure 6.** 3D rendering of the head of *R. prolixus* sacrificed 4 days after feeding.

External morphological features such as individual facets of the composed eyes and the detailed rugose cuticular features of the head capsule could be clearly visualized in figure 7.



**Figure 7.** Volume-rendered SR-PhC-μCT image of *R. Prolixus*.

#### 4. Conclusions

Classical anatomical techniques are costly in terms of time and effort and usually involve the destruction of the specimen under study. The SR-PhC-μCT technique enables an incomparable visualization of internal structures and is a non-destructive technique.

There is not any available vaccine to prevent from Chagas disease, so depending on the region, key tools are vector control, house improvement such as plastered walls, cement floors, corrugated-iron roofs and personal preventive measures. Anyway, controlling the transmission of *Trypanosoma cruzi* by means of its vector insect is much more efficient. In previous studies it has been shown that the control of *Trypanosoma cruzi* relies on the study of the head of *R. prolixus* and its structures. So, high resolution images from internal structures of the head of *R. prolixus* could be of great importance for biology and medicine to understand the main structures where *Trypanosoma cruzi* passes by.

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