

# Software System for Three-Dimensional Volumetric Reconstruction of Histological Sections: A Case Study for the Snake Chondrocranium

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## ABSTRACT

Volumetric digital computer-assisted reconstruction of histological sections is an attractive possibility for developmental studies. Commercial solutions are very expensive for many educational institutions. Therefore, we developed a software system for three-dimensional reconstruction of anatomical virtual models. The input data for the system are the digitized images from the histological samples of the chondrocranium of two crotalines, *Bothrops jararaca* and *Crotalus durissus terrificus*, and one colubrid, *Philodryas olfersii*, using a stereomicroscope connected to a digital camera. These images are then manually registered and segmented. We use computer graphics visualization algorithms such as marching cubes and ray casting to generate three-dimensional visualizations of the volumes. The results show that the digital reconstruction is as good as the manual reconstruction with the advantages of speed of reconstruction, accuracy, and flexibility to handle and study the volume. Compared with commercial options, our system has approximately the same features, and it is available free for the scientific community. © 2005 Wiley-Liss, Inc.

**Key words:** histology; serial sections; 3D reconstruction; snake chondrocranium

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The analysis of organism structures by sectioning techniques has always suffered from the loss of three-dimensional (3D) information of the details visible in microscopic or stereomicroscopic images. This lack of 3D information has led to the development of numerous manual and computer-assisted reconstruction methods. Performing digital volumetric reconstruction has desirable advantages over building laminate models. Three-dimensional virtual reconstruction is applicable to a wide range of investigations, from establishing complex histotopographical relationships to assessment of structural details that can be visualized on the screen. Besides, the structures can be measured and analyzed accurately.

Because of the rapid progress in modern computer hardware, uncomplicated and fast reconstruction commercial systems are available on standard personal computers. Nevertheless, these commercial solutions are either very expensive for the average user and for many educational

institutions in developing countries or not suited for specific reconstruction from histological sections.

Our main goal in this study is to describe and evaluate our own reconstruction software. This system was designed and implemented for our specific needs but can be

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used for reconstruction of three-dimensional models with any structures. Contrary to commercial systems, our results—the software system—are available for the research community for free and represent a valuable model for collaboration among biologists and computer scientists. Haas and Fischer (1997) used, for instance, the product-design package Alias Wavefront software on the Silicon Graphics platform for three-dimensional reconstruction of larval specimens of neotropical frog *Flectonotus goeldii*. Although their results show the viability of using commercial systems, our solution is available for free for the research community, with similar features.

We present the design of our system, the principles of 3D reconstruction from computer graphics techniques, and discuss a case study using our software for reconstruction from histological sections of the nasal capsule of the snakes *Bothrops jararaca*, *Crotalus durissus terrificus*, and *Philodryas olfersii*.

## MATERIALS AND METHODS

### Computer Setup

There are two computer setups, one for the digitalization of the histological sections and one where we run our reconstruction software. For the input setup, we have an Intel Pentium II processor with 128 MB RAM and 6 GB internal disk drive connected to a video camera Olympus Microscope Digital Camera System model DP 10 (resolution of  $1,280 \times 1,024$  pixels) mounted on an Olympus stereomicroscope model SZ 60. The computer system for the visualization consists of an Intel Pentium 4 computer with a 1.6 GHz processor, 256 MB RAM, a graphics video card Nvidia Vanta with 16 MB RAM, a 17" monitor, and 20 GB internal disk drive. This computer is also equipped with Corel Photopaint 9 software, used for registration (relative alignment of sections) and segmentation (identification of different parts in the image) of the images. We used this software for the alignment and segmentation tasks since it was already available, but any other image processing software such as Photoshop or GIMP has similar tools for this task.

### Input Procedures

The reconstruction software uses as input data a sequence of images obtained from the histological sections. For our case study, we used histological sections from skulls of embryos of the neotropical snakes *Bothrops jararaca*, *Crotalus durissus terrificus*, and *Philodryas olfersii*. The first two were obtained from female killed for embryo removal at the Butantan Institute in São Paulo, Brazil, and the third one was obtained from a female killed at Museu de Ciências Naturais from Fundação Zoobotânica of the Rio Grande do Sul state in Brazil. All embryos have a fully formed chondrocranium and were prepared for histology (Fig. 1a). They were first decalcified in a solution of  $\text{HNO}_3$  (6.5%) and then prepared using standard procedures for dehydration and Paraplast Plus as the embedding medium, using xylene as the clearing agent. The serial transverse sections (at  $10 \mu\text{m}$  on a Microm microtome) of the nasal capsule were stained with alcian blue and alizarin red modified from procedures of Domagk (Romeis, 1968). The sections on the glass plate are organized into five columns and six rows (Fig. 1b), with a total of 300 sections for the *B. jararaca*, 175 sections for the *C. durissus terrificus*, and 210 sections for the *P. olfersii*. In

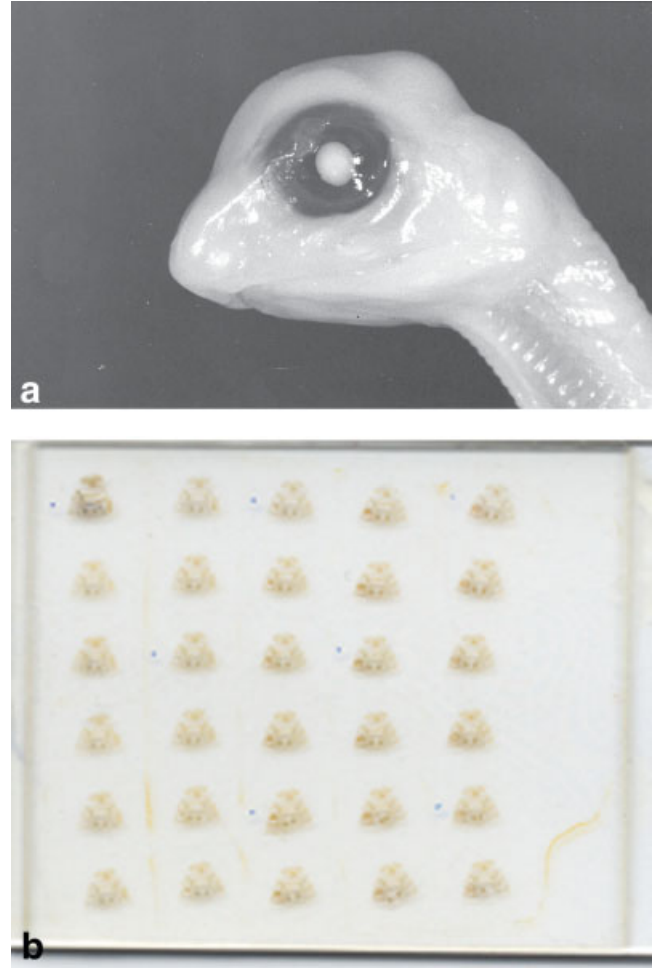


Fig. 1. Input data. **a:** Snake embryo. **b:** Sections mounted on glass plate.

order to identify the sections properly, we name them by the glass plate number, followed by the column and row numbers. For instance, “2,3,1” means the section on the third column and first row of the second glass plate.

The images from the histological sections were scanned in using the stereomicroscope connected to the digital camera. The alignment of the glass plate under the stereomicroscope is done manually and therefore the digitized images have to go through an alignment procedure (registration) later. Since the biological material is fragile and small, it is difficult to add internal landmarks for alignment purposes. Moreover, since the histological sections are very thin, they do not differ much from one section to the next and therefore manual alignment is possible. We used a 1.2 increase (zoom) in the stereomicroscope, which allowed us to scan even the large histological sections of the nasal capsule that ranges in areas from 1 to 5 mm. The image files were then imported as background images into the Olympus Camedia Master 1.0 and saved with resolution of  $1,280 \times 1,024$  pixels in jpeg format. The jpeg files are smaller than similar image formats, such as tiff, and are better suited therefore for this task, since we had a great number of files to handle.

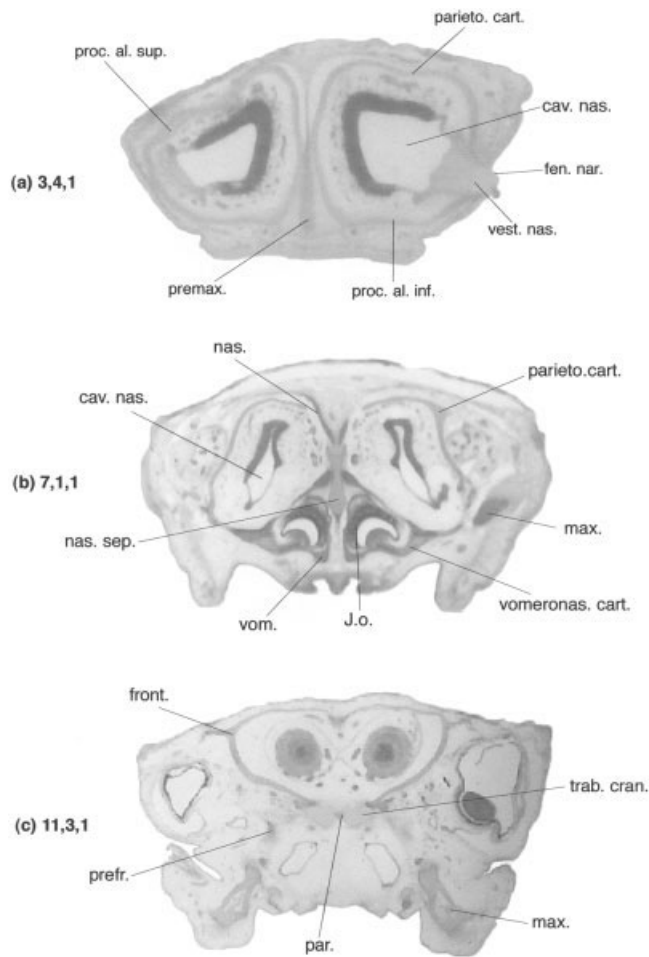


Fig. 2. Samples of digitized sections. Cross-section in the level of the nasal vestibule of *B. jararaca* (a); cross-section in the level of Jacobson's organ of *B. jararaca* (b); and cross-section in the posterior region of the nasal capsule of *B. jararaca* (c).

The pictures of the sections were then imported into Corel Photopaint 9 as background pictures for registration. For the segmentation task, the contours of structures were drawn interactively on screen with the mouse. In Figure 2, we show three sections from the collection we used.

### Registration

During the scanning of sections, we cannot guarantee a perfect alignment from section to section, since this process is done individually for each section. Manual alignment of the digitized sections was also done in Haas and Fischer (1997). We used Corel's Photopaint for the alignment task, but any other image processing software such as Photoshop or GIMP has similar tools for this task. We begin the alignment process loading a reference image first. This first image will guide the alignment and it is the image where the structures cover the largest area; in our case, it was the last section. We then load the second image with a given transparency (usually 50%). The transparency allows us to rotate and translate the second image using the reference image as a visual guide in the

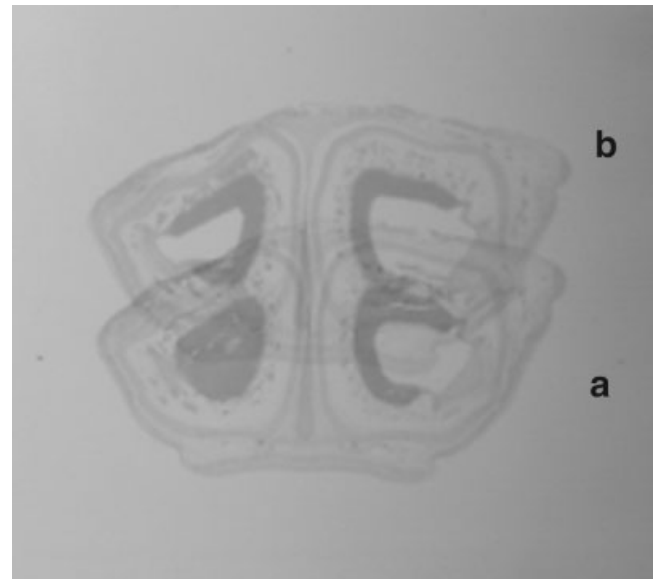


Fig. 3. Alignment of cross-sections in the level of the nasal capsule of *B. jararaca*. a: 3,2,6. b: 3,3,4.

process (Fig. 3). We then proceed by making the second image the new reference image and continue the process for all sections. This manual alignment requires someone familiar with the structures in order to align the images properly. Although it is a manual process, in our experience it has proved adequate, considering that we do not have markers to help in the registration procedures, which can be used for bigger mammals (personal communication, Maier, 1995).

### Segmentation

The segmentation process is the last preprocessing step before we can load the images into the reconstruction software. In Figure 4, we show the same sections presented in Figure 2 with the background removed. Another use for segmentation is to isolate specific parts of the volume, such as bones and muscles. We have performed segmentation also with the Photopaint program. In this program, we used the advanced cut features for selecting and removing the background.

### Visualization

There are two classes of algorithms for visualizing a series of images as a volume: direct volume rendering (Levoy, 1988, 1990) and the marching cubes approach (Lorenson and Cline, 1987). These algorithms are known as volume visualization techniques and were developed to explore the availability of tomographic images. They are extensively used in commercial medical imaging systems and are responsible for the fast advancements in the field of volumetric reconstructions of digital volume data. In our system, the user selects which visualization method he or she wants to use. The marching cubes approach builds a geometric spatial model from the volumetric data. This model can then be explored and stored as a graphics file. The direct volume rendering approach is implemented using ray casting techniques, where rays (one for each



Fig. 4. Samples of segmented digitized sections. Cross-section in the level of the nasal vestibule of *B. jararaca* (a); cross-section in the level of Jacobson's organ of *B. jararaca* (b); and cross-section in the posterior region of the nasal capsule of *B. jararaca* (c). a: 3,4,1. b: 7,1,1. c: 11,3,1.

pixel of the final image) are sent through the volume and accumulate density as they travel inside the volume. The final density for each pixel is mapped to a gray-scale color to produce the image. Whereas marching cubes provide better frame rates when doing interactive investigations, the ray casting technique allows more advanced operations, such as the definition of transparent parts of the volume, and arbitrary orientation cut view planes, which are useful tools for inspecting the volume. We have used the VTK software library to build the system (Schroeder et al., 1998).

## RESULTS

In this section, we present the results of using our system for virtual reconstruction of the chondrocranium of two crotalines, *Bothrops jararaca* and *Crotalus durissus terrificus*, and one colubrid, *Philodryas olfersii*. The use of three different species of snakes with one of them being of another family stresses the utility and sensitivity of our method.

A full view of the developed system is given in Figure 5. Here, we can see a reconstructed volume (on the upper left corner) and the parameters window (bottom right), where the user sets the color and opacity values for gray-scale ranges. In this figure and the following, the colors are just for illustrative purposes and do not follow any standard color information for manual 3D reconstructions. Due to the high number of sections, we estimate that was enough to scan the sections in steps of six. In total, we digitized 30 images for the *B. jararaca*, 28 sections for the *C. durissus terrificus*, and 22 sections for the *P. olfersii*.

In Figure 6, we show two reconstructions of the nasal region of the *B. jararaca*, using marching cubes and ray casting techniques. We can see that the ray casting result is visually slightly better than the result with the marching cubes technique. The sequence of images in Figure 7 shows the same volume with three different visualization options: original gray-scale image, gray-scale image with light, and with the color applied.

In Figure 8a, we show one reconstruction of the nasal region of the *C. durissus terrificus*, and in Figure 8b, one reconstruction of the nasal region of the *P. olfersii*, using the ray casting technique.

## DISCUSSION

The technique of three-dimensional reconstruction from histological samples represents a valuable tool for functional-morphological, developmental, and phylogenetic investigations. Although we have used the system only for reconstructions of three skulls of snakes, it can be applied to any other similar studies with other animals, being enough to have histological sections to work with. In this respect, we plan to apply the system to reconstructions of skulls of lizards from the Amazon region, trying to elucidate the relationship between the anatomical structures and the microhabitat.

Many examples can qualify the use of the development in the phylogenetic analysis and in the functional morphology: patterns of the ossification of the orbitosphenoid have been used to differentiate the phylogeny of the amphisbaenians, the squamates, the lizards, and the snakes (Bellairs and Gans, 1983); the extracapsular course of the lateral branch of ethmoidal nerve and the absence of the foramen epiphaniale in the nasal capsule of *B. jararaca*

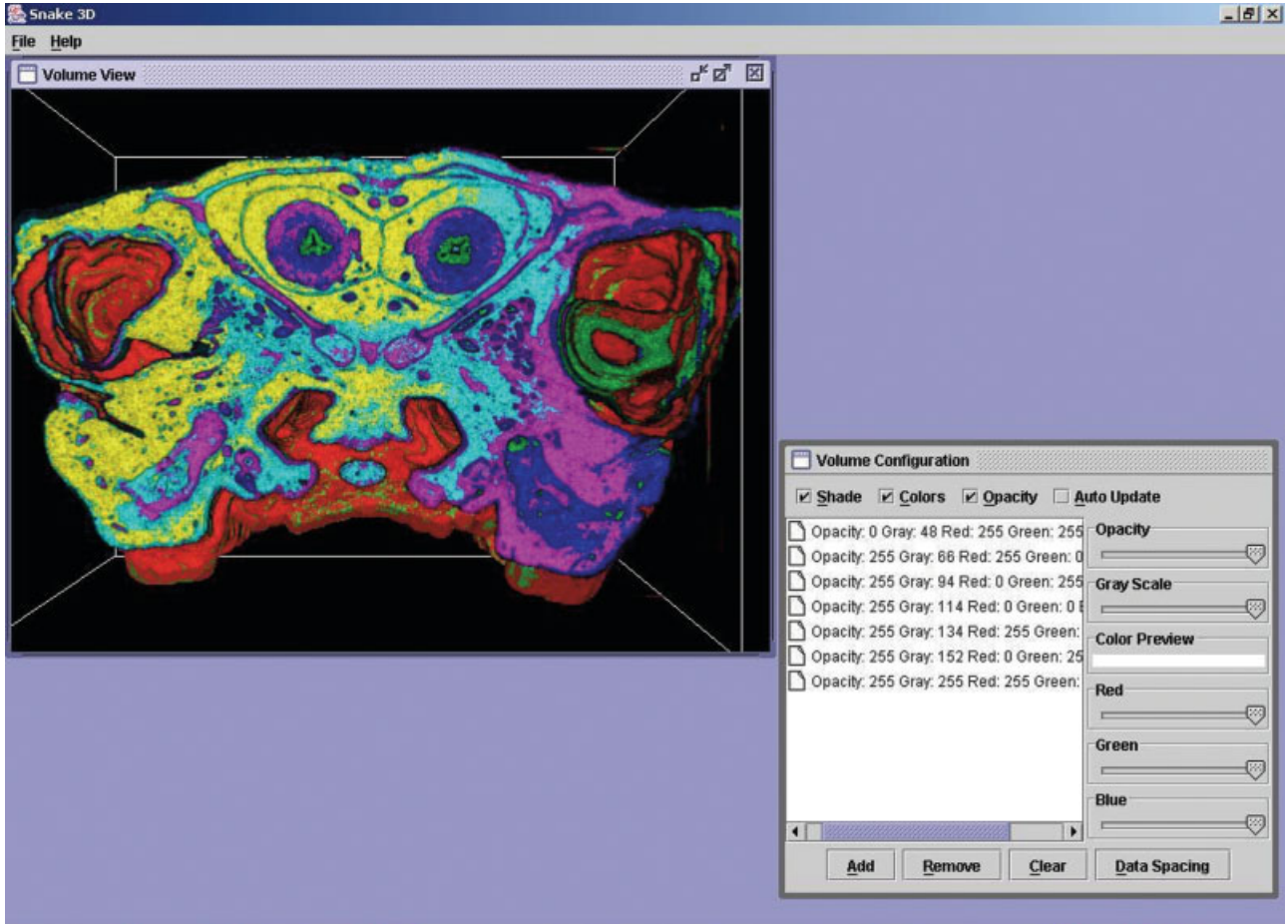


Fig. 5. Interface of the system. Cross-section in the posterior region of the nasal capsule of *B. jararaca* (11,5,1).

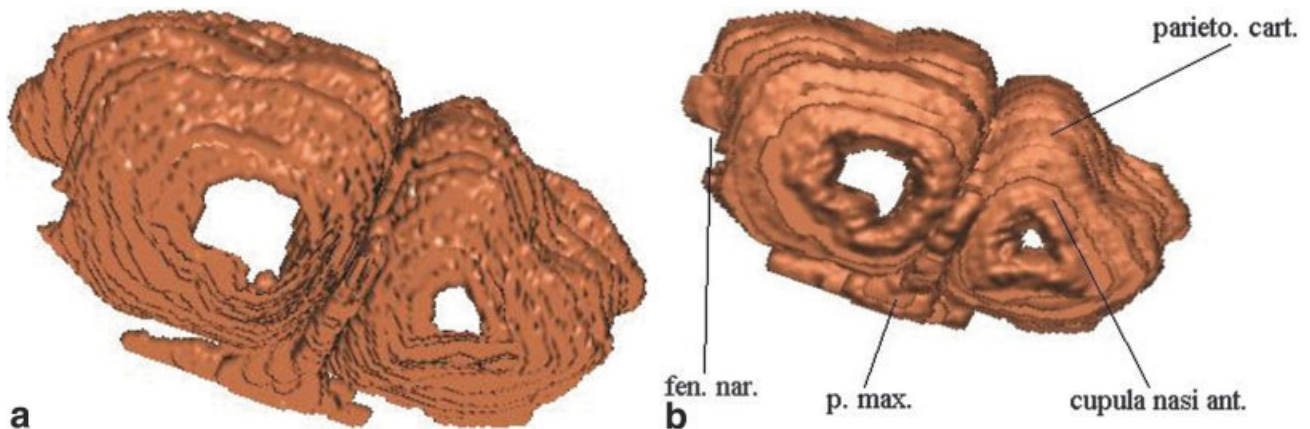


Fig. 6. Examples of reconstruction in 3D of the nasal capsule of *B. jararaca*. **a**: Reconstruction using marching cubes. **b**: Reconstruction using ray casting.

and *C. durissus terificus* are functionally linked to the pit organ representing a new synapomorphy of the crotalines (Hofstadler-Deiques, 2002). We can elucidate aspects of the development in response to selection for adaptive fea-

tures, for example, the presence of the subnasal muscle and the cavernous tissue of the nasal capsule of aquatic snake *Helicops infrataeniatus* (Santos-Costa and Hofstadler-Deiques, 2002).

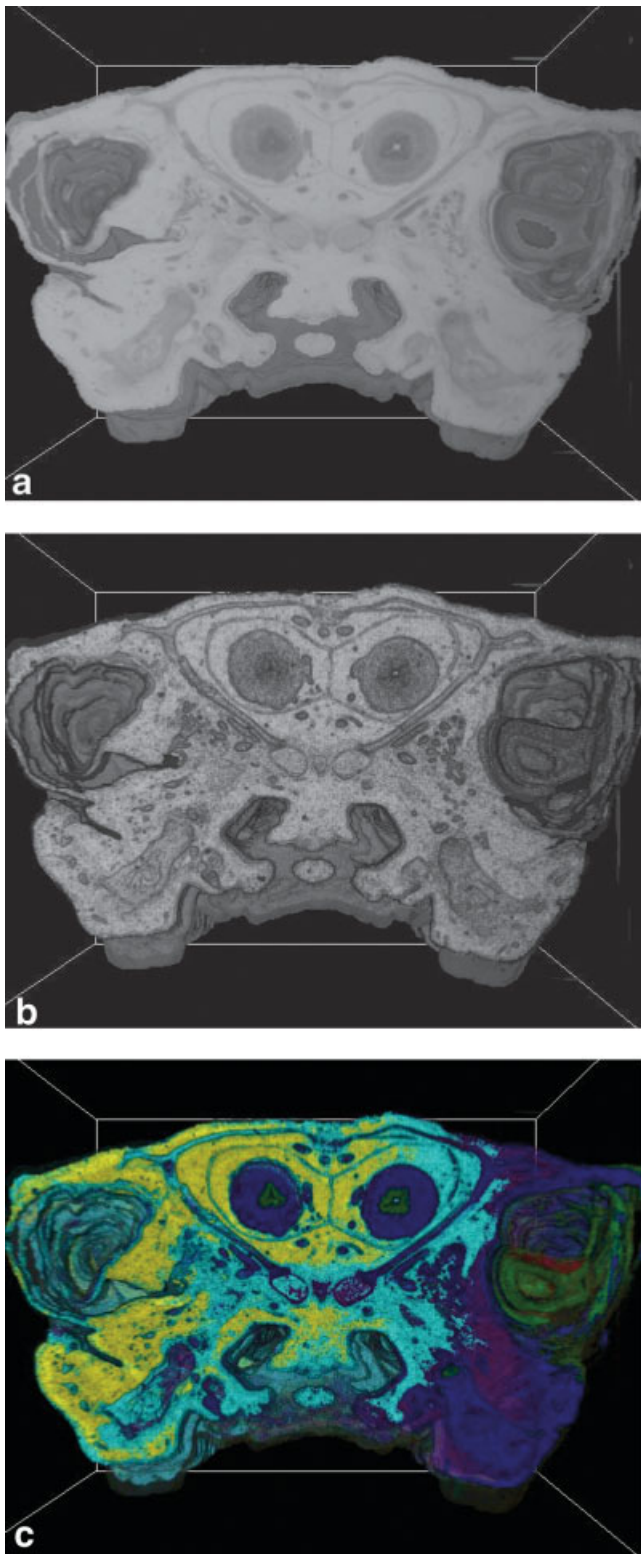


Fig. 7. Visualization options for the volumes. Cross-section in the posterior region of the nasal capsule of *B. jararaca* (11,5,1). **a:** Gray-scale reconstruction. **b:** Gray-scale reconstruction with light. **c:** Color reconstruction.

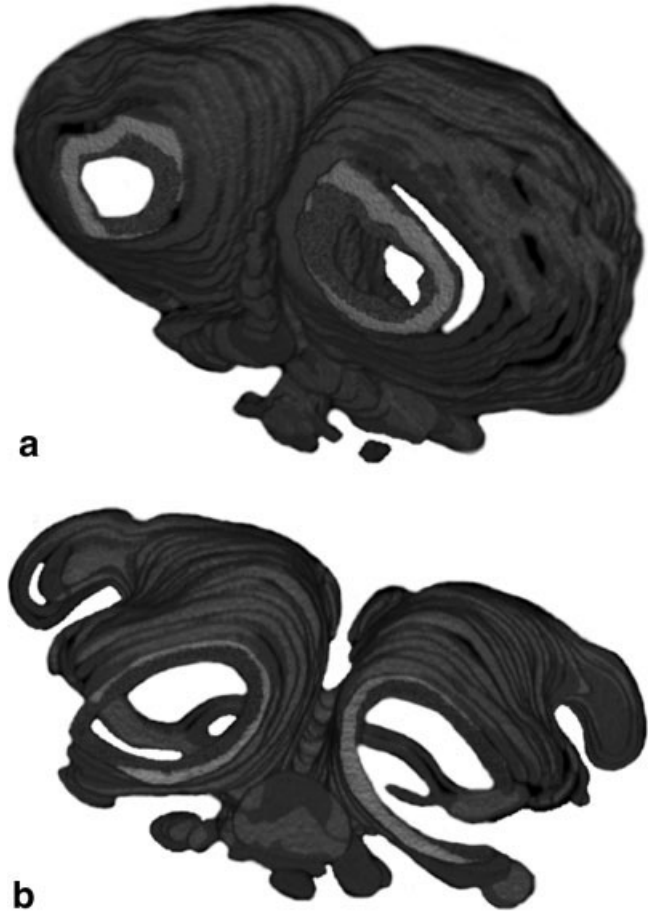


Fig. 8. **a:** 3D model of *C. durissus terrificus*. **b:** 3D model of *P. olfersii*.

With respect to the registration and segmentation steps, it is still very hard to perform these operations automatically. Manual segmentation guarantees that only the relevant structures are included for reconstruction. In our experience, these steps can be performed from 3 to 4 days of work. In purely operational aspects, our system has the advantage that it is freely available for researchers and institutions and allows specific operations on the volume, such as the abilities to shift, rotate, and spin a model, to zoom in and out, and to switch between parts of models. The models can be cut or dissected electronically in any arbitrary plane, and parts can be selected for combined viewing. We plan to explore enhancements in the current version of the system, including the possibility of automatic or at least semiautomatic alignment and segmentation procedures. The system is available for free download on the Web (<http://www.saude.unisinos.br/laboratorios/embriologia/>).

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