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Maximum sphere method for shell patency measurements in viviparous land snails based on X-ray microcomputed tomography imaging

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ABSTRACT

This article presents the working principle of an algorithm designed for the purpose of examining a section of the snail shell canal. The procedure of scanning the specimens is described as well as the tests performed using the proposed algorithm. Also, the digital models used for testing the algorithm are described. The article contains a description of the initial processing of the data, including segmentation and detection of the edges of the image. A flowchart of the algorithm is presented together with its implementation. The data obtained in the course of the microtomographic scanning of one of the snails and a digital model of a canal created for this purpose were subjected to the application of the measurement algorithm. This algorithm was aimed at conducting a spatial analysis of the varying dimensions in the canal section. The process of applying the algorithm and the measurement errors are presented and discussed on the basis of the results.

1. Introduction

Measuring physical dimensions of biological structures can be difficult due to their complex shape. While 2-D measurements can be done manually, three-dimensional structures require a novel approach. Microscopic methods can be applied for analysis of 2-dimensional sections; however, this approach requires destructive sectioning of the sample. For objects that can be observed using X-rays, radiography and more advanced methods can be applied, like X-ray tomography and microtomography (high-resolution tomography) [1]. The biggest advantage of X-ray microtomography is its high resolution and its ability to avoid destruction of the inner structure. Utilization of XMT images is usually followed by digital image analysis, including segmentation of the object from images and further structure analysis. What is important here is not only the technical skills required to perform the measurements but, above all, knowledge of the purpose of the measurements.

An example of a complex three-dimensional structure is a snail shell. Shell morphology analysis often provides crucial information used in evolutionary, taxonomic, and paleontological studies; however, very few features of the morphological shell structure occur with a discrete morphology. More frequently, the spatial structure is important as it is used for identifying species [2].

In the past, snail shell measurements were limited mainly to 2-D measurements. It was not until 1966 that a breakthrough study on theoretical shell morphology [3] allowed for simulation of its growth by means of four geometric parameters. The first studies on the theoretical shape of snail shells were performed as late as 1971 [4,5]. Also, other researchers dealt with the theoretical shell model [6–8]; however, all the created models are merely approximations. In particular, the shell aperture is generalized in these models.

In 2009, a method of measuring the snail shell canal was proposed based on its skeletonization and cross-sectioning of the skeleton [2]. In our studies, we tested the skeleton-based approach, but due to the vast number of samples and wide range of shell morphology types, this approach provided poor results. It required numerous parameters to be set before the measurements, and thus could not be applied by untrained personnel. Also, there was a problem with numerous branches appearing as a result of image noise [9–11].

In this study, with the application of a specially designed algorithm [12], the changes in a section of the snail shell were measured. In contrast to the simple external structure, the shape of many snails shells is quite complex in terms of its internal structure [6,12]. It is particularly noticeable in snails of the Clausiliidae family, in which there are rigid projections on the internal walls of the shell canal called "denticles" and a special flexible spoon-shaped plate, which

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closes the shell canal when the snail withdraws and which is ajar while the animal is active. Because of a danger of drying out, or a predator attack, the shell canal is narrow in the Clausiliidae, and the closing mechanism with the spoon-shaped plate closes the shell aperture tightly. Because there was no ready-to-use procedure (e.g., software method or plug-in) for measuring the size of the shell channel, a new algorithm was written, implemented and tested. We could not compare our results with other working solutions without implementing those other methods ourselves. Thus, instead of implementing known methods, we focused on finding one solution that could also be applied to other channel-like systems in the near future.

Among snails from the Clausiliidae family, there are also viviparous species (giving birth to offspring with firm oval or spherical shells); therefore, it was hypothesized that the change of the reproductive strategy in these snails was associated with the necessity to increase shell patency and to adjust it for the passage of offspring during labor [13]. During delivery, the shelled embryos travel along the coiled shell canal, whose width is further limited by the protruding denticles.

Due to the need for a method of measuring shell patency in several Clausiliidae species, a method was proposed that consists of following the route along the shell canal and measuring the maximum radius of a sphere (i.e., a shape resembling a snail embryo) that could be inscribed into this canal at a fixed point. This algorithm solves a problem similar to that which was proposed by Chaudhuri [14]; however, in our approach, the user is able to set parameters in such a way that the algorithm can select a path along the forward direction and omit the local maxima of canals occurring to the side of the main path. In case of our study, we encountered a problem, where, sometimes, depending on how the parameters were set, the algorithm provided us with different paths. In such cases, the proposed approach allowed us to easily change the settings and force the path of the measurement to coincide with the actual path of a nascent snail [15].

The aim of the article was to describe the designed algorithm, present the results of its execution on preserved biological material and in model cases, and to analyze its execution time. This algorithm was implemented using the Matlab environment (version 7.02) and its basic functions [16]. The description of the algorithm's implementation is subjects for a future article.

The algorithm was successfully applied for measurements in the study on viviparous snails of the Clausiliidae family [13]. Thanks to this algorithm, results were obtained that proved the proposed hypotheses. The research carried out proved the usefulness of the algorithm in the performance of such measurements.

2. Materials and methods

2.1. Origin of specimens

The shells used in the study were of land snails of the Clausiliidae family from central Europe. The snails, coming both from breeding laboratories and field collection [13], were preserved in 70% alcohol. The preservation was conducted so that the soft part of the snail's body would slide partly out of the shell, resulting in a wide-open shell canal (with the plate ajar) as in the period of a snail's activity (Fig. 1).

2.2. Scanning process

In order to create a set of sections, a microtomography scanner (GE Sensing & Inspection Technologies, Phoenix|X-ray, Wunstorf, Germany) was used. The scanning was performed in the X-ray Microtomography Lab, Department of Biomedical Computer Systems, Institute of Computer and Material Science, University of Silesia (Chorzów, Poland).

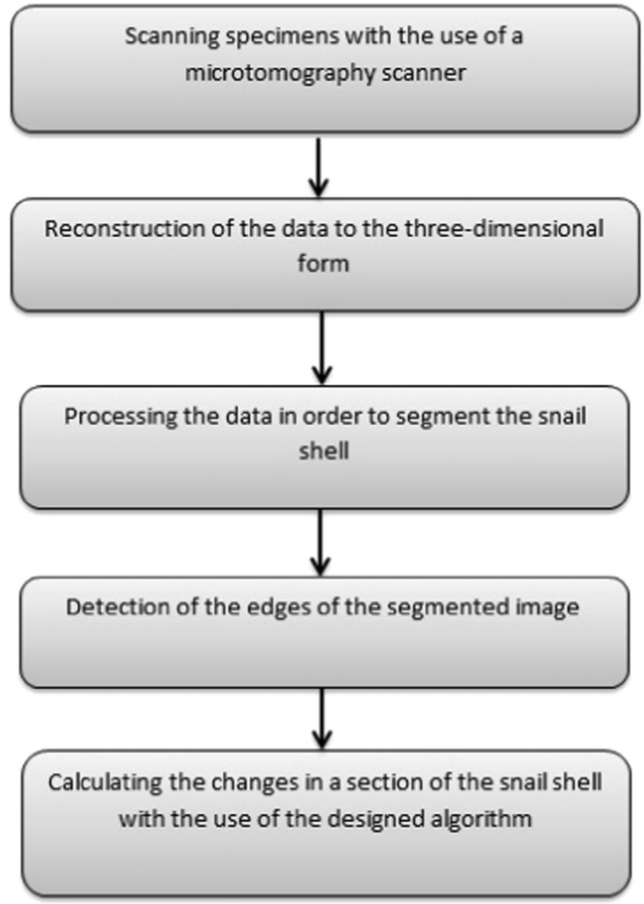

Fig. 1. Flowchart of measurements of the snail shell diameter used in the study.

Each specimen was scanned separately, with the shell aperture directed upwards in a vertical position (Fig. 2). Due to the low absorption of X-ray radiation by the snail shell, the fixation was based on using low-density material so as to reduce artifact occurrence at the site of contact between the specimen and the fixing material.

During the scanning process, 1000 projections were made for each specimen. The outcome of the reconstruction of these projections, performed with the use of the software delivered with the scanner, Datos|x 2.0, were 8-bit data. These data were also recorded on a disc.

The reconstruction algorithm Datos|x 2.0 was used for the reconstruction of cross-sectional images based on the filtered backprojection method. The scanning parameters are presented in Table 1.

Scanning of the specimens was the first of the five main stages of the designed algorithm.

2.3. Data processing

The initial input data for the algorithm is a segmented three-dimensional image – that is, a set of reconstructed sections representing the snail shell canal – which is subject to measurements.

2.3.1. Segmentation

It was assumed that the segmentation process should be separated from the measurement process. This is in accordance with the strategy of designing algorithms so as to ensure their