THREE-DIMENSIONAL MORPHOMETRIC ONTOGENY OF MOLLUSC SHELLS BY MICRO-COMPUTED TOMOGRAPHY AND GEOMETRIC ANALYSIS

Claude Monnet, Christoph Zollikofer, Hugo Bucher, and Nicolas Goudemand

ABSTRACT

As one of the essential components of the fossil record, shells of molluscs provide crucial data for taxonomic, phylogenetic or evolutionary studies. The mollusc shell has often very few discrete morphological characters, and its most important character is its three-dimensional (3-D) geometry, which is well diversified, continuous and highly integrated. However, standard morphometric methods remain mostly two-dimensional (2-D) and do not account for the ontogenetic changes of the shell, which are preserved thanks to its accretionary mode of growth. This study proposes a new non-destructive method enabling acquisition of three-dimensional quantitative morphometric parameters that thoroughly describe the geometry of coiled mollusc shells throughout their ontogeny. First, digital three-dimensional data of a shell is acquired by means of micro-computed tomography, which produces a series of grey-scaled, two-dimensional images. Second, all these stacked images are processed to obtain a three-dimensional reconstruction of the shell, from which a centreline is extracted. Finally, the geometry of shell aperture through ontogeny is extracted by successive cross-sectioning of the shell with a succession of planes, each of these being perpendicular to this centreline. The resulting outlines of the successive apertures can be quantified by elliptic Fourier analysis. These geometric parameters, coupled with the displacement vector of the successive cross-sectioning planes, constitute an *n*-dimensional morphometric space, in which ontogenetic trajectories of different individuals and species can be compared. The approach proposed in this study provides a basis for the quantitative analysis of growth patterns within and across species.

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INTRODUCTION

Gastropods are well diversified, geographically and temporally widespread organisms with a very large range of adaptations. Because of their calcified shell, they are among the most common material in the fossil record. Hence, they constitute an invaluable archive of global and regional biotic and abiotic changes on Earth and can provide important insights into patterns and processes of evolution. However, their contribution remains hampered by the rather simple, highly integrated morphology of their shells, which is not well suited to conventional biometric and phylogenetic methods. Indeed, the gastropod shell consists of a calcified, univalve conch, basically conic and coiled, with well-diversified outlines of the aperture, various colour patterns, and few distinctive and qualitative ornaments (Figure 1). Since colour patterns, mostly expressed as bands of various magnitude and number, are highly variable within the same species, they have little taxonomic value. Moreover, colour patterns are only exceptionally preserved among fossils. Most diagnostic characters of extant gastropod species rely mainly on soft tissues, i.e., sets of characters which are lost in fossil representatives.

The most important set of morphological characters of gastropods resides in the geometry of their shell. However, the shell geometry is difficult to capture by conventional descriptive, qualitative and discrete approaches. For instance, the investigation of the shell geometry has largely been limited to basic two-dimensional measurements such as width of aperture, shell diameter, or apical angle (Figure 1). Such characters poorly capture the subtle differences of shell geometry, and their relevance is thus rather limited. Due to its accretionary mode of growth, the gastropod shell preserves its entire post-hatching ontogeny. The ontogenetic changes of shell geometry constitute a potential additional relevant set of characters. However, to date, ontogenetic studies of molluscs remain rare, qualitative and descriptive; usually the mature stage of the shell is given the greatest emphasis. Hence, the developmental characteristic of mollusc shells remains largely under exploited. The mollusc shell is thus in need of an adequate, quantitative and descriptive method to better take advantage of its excellent fossil record in palaeontological and evolutionary studies.

Significant advances in the quantitative and three-dimensional characterization of the mollusc shell have been first achieved by the pioneer work of Raup (e.g., Raup and Michelson 1965; Raup
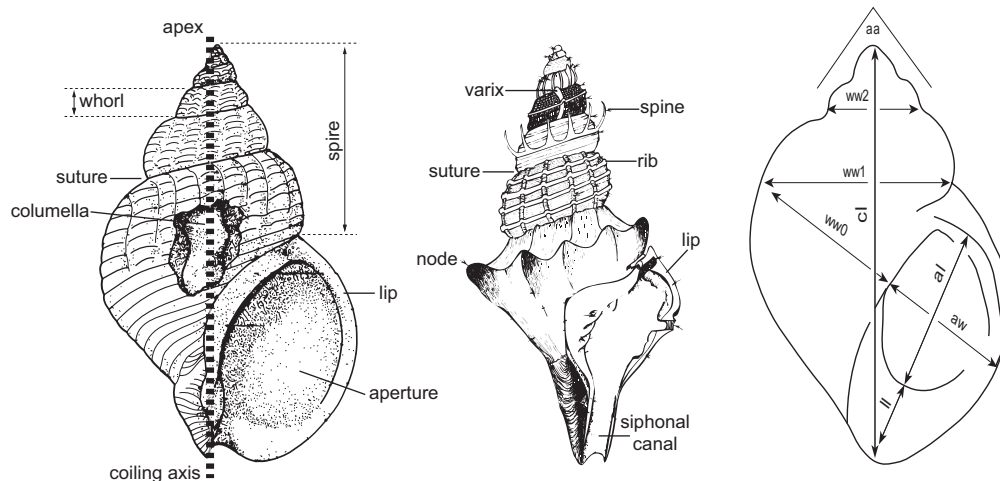

FIGURE 1. Some terminology and standard two-dimensional measurements of the gastropod shell (aa – apical angle; cl – columella length; ll – lip length; al – aperture length; aw – aperture width; ww0 – whorl width 0; ww1 – whorl width 1; ww2 – whorl width 2).

1966). The coiled conic shell of molluscs is a well-known case study of theoretical morphology (e.g., Okamoto 1988; Ackerly 1989a; Cortie 1989; see also review by McGhee 1999). Raup (1966) demonstrated that the nearly self-similar accretionary growth of the mollusc shell allows for simulation of most shell shapes by varying only four geometric parameters. However, Raup's parameters have some important limitations (see review of McGhee 1999): some of them are interdependent (Schindel 1990), the four parameters are constants and apply only to isometric growth (Goodfriend 1983; Gould 1991), and the detailed shape of the growing shell aperture is subsumed to an ellipse. Furthermore, Raup's model assumes the existence of a coiling axis, which is not true for some mollusc shells (e.g., heteromorph ammonoids like *Nipponites* or vermetid gastropods).

Vermeij (1971) and Kohn and Riggs (1975) were the first to study gastropods in the context of the theoretical morphospace of Raup. The theoretical morphology of gastropods has also been investigated in various ways by Rex and Boss (1976), Harasewych (1982), Ekaratne and Crisp (1983) and Schindel (1990), among others. However, they were "forced" to modify Raup's parameters since determining them usually involves axial sectioning of the shell. Measuring the parameters of all these models is usually difficult and requires complicated techniques (e.g., Ackerly 1989b; Schindel 1990). All these models are a simplification of shell geometry, especially of aperture shape, based on 2-D measurements. Few morphometric studies have been devoted to the ontogeny of these accretionary organisms (e.g., Checa and Aguado 1992; Stone 1998). The most complete study is that of Stone (1998), which also compared the results between traditional measurements, parameters of mathematical modelling of shells, and a warp analysis of aperture trajectory centroids. However, gastropod ontogeny is reduced to the relative position of aperture centroid and therefore carries no information about aperture shape. It is also restricted to regularly coiled shells.

In his review about theoretical morphology of mollusc shells, McGhee (1999, p. 111) finally concluded "can a theoretical morphospace of helicospiral form be created that is both morphologically realistic and mensuratively simplistic?" Thanks to continuing improvements in three-dimensional computed image acquisition and analysis, a broad range of non-destructive methods is now available that enable the ontogenetic description and quantification of the gastro-

FIGURE 2. Analyzed specimens: *Cepaea nemoralis* (Linnaeus 1758), family Helicidae. This common European grove snail or brown-lipped snail is a pulmonate land snail, growing to a height of about 20 mm and a width of 25 mm within 4½ to 5½ whorls.

pod shell geometry. The purpose of this study is to propose a method which captures the 3-D morphology of the coiled mollusc shell and exploits quantitative and continuous characters throughout ontogeny.

MATERIAL

The proposed morphometric approach has been established with gastropods of the family Helicidae (Gastropoda, Stylommatophora). Studied specimens are tightly coiled and slightly trochospiraled with a large overlap of successive whorls. All studied specimens come from and are deposited in the Zoological Museum of the University of Zürich. The different steps of the method will be illustrated with the case of one specimen belonging to the species *Cepaea nemoralis* (Linnaeus 1758). This grove snail or brown-lipped snail is a terrestrial pulmonate gastropod. It is one of the most common species of land snails in Europe. It is a relatively small snail, growing to a height of about 20 mm and a width of 25 mm. The semi-glossy shell grows up 4½ to 5½ whorls (Figure 2).

METHOD AND RESULTS

As previously explained, the goal of this study is the development of a method allowing acquisition of quantitative morphometric parameters, which describe the geometry of coiled mollusc shells throughout their ontogeny. The approach
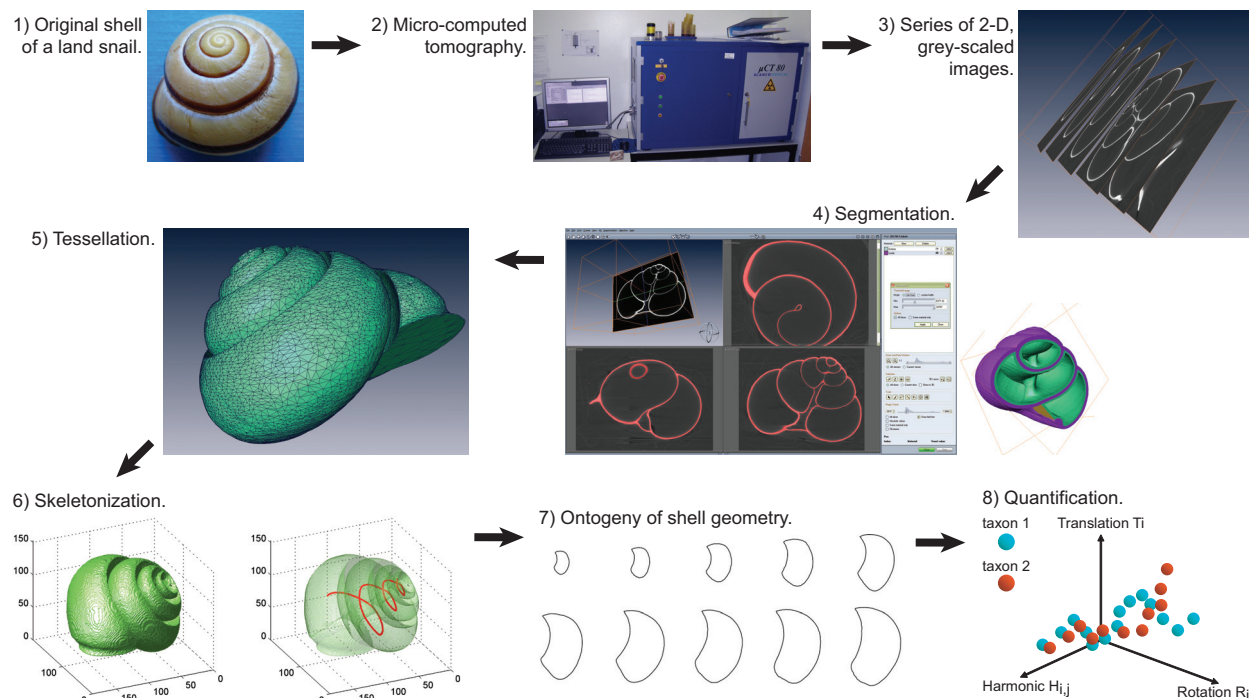
1) Original shell of a land snail.

2) Micro-computed tomography.

3) Series of 2-D, grey-scaled images.

4) Segmentation.

5) Tessellation.

6) Skeletonization.

7) Ontogeny of shell geometry.

8) Quantification.

taxon 1
taxon 2

Translation T_i
Harmonic $H_{i,j}$
Rotation R_i

FIGURE 3. Flow diagram of the major steps of the method. See text for explanations.

proposed here includes the following major steps (Figure 3): 1) acquisition of digital three-dimensional data of the shell; 2) quantitative modelling of shell geometry; and 3) extraction of shell geometry through ontogeny.

Data acquisition by micro-computed tomography

In order to reconstruct the three-dimensional geometry of a gastropod shell, appropriate imaging techniques are required. A suitable and robust technique is the micro-computed tomography (micro-CT). This method is well known to allow non-destructive data acquisition of the external and internal geometry of anatomical structures with a high spatial resolution and a relatively fast acquisition time. It is commonly used in medical studies (Morton et al. 1990). In this study, the acquisition of digital three-dimensional data of gastropod shells is performed with a medical Scanco® micro-CT 80 (Figure 4) at the Anthropological Institute of Zürich.

The micro-CT produces a contiguous series of parallel two-dimensional images (Figure 5) obtained by scanning the original shell with X-rays (Ritman 2004). The pixels within each image slice are represented by scalar values that can be interpreted as intensity values (transparency to X-rays). Putting pixels and slices together we obtain a three-dimensional partition of the image space into

volume elements (voxels) forming a 3-D scalar field. Regions of homogeneous intensity values typically represent anatomical structures, whereas strong gradients are indicators of tissue boundaries. Anatomical structures of interest can now be traced between adjacent images. Stacking those sliced structures on top of each other reveals an approximation of their three-dimensional shape.

In this study, gastropod shells are scanned with an isotropic voxel resolution of 0.036 mm. Usually, for a gastropod shell with a diameter of about 2 cm, the scanning process takes roughly 3 hours and results in a file size of about 2 Gb with a standard resolution of 1024 × 1024 pixels. The final size of a gastropod shell can be coded by a 3-D matrix of about 800 × 700 × 600 voxels. A full scan then yields a 3-D scalar field of about 4×10^8 voxels. Medical images are typically stored in DICOM format.

Reconstruction of shell geometry

The next step is to reconstruct a three-dimensional, numerically exact descriptive model of the scanned shell. For this purpose, the series of images resulting from the micro-CT scan of a shell is transferred into a computer visualisation system. In this study, we use the commercial software amira® (http://www.amiravis.com/), which is a con-

FIGURE 4. Micro-computed tomograph Scanco® micro-CT 80 of the Anthropological Institute (University of Zürich) enabling acquisition of digital three-dimensional data of gastropod shells.

venient and interactive system for 3-D data analysis, visualization and geometry reconstruction (Stalling et al. 2005). Algorithms used by amira® for image data analysis and geometry reconstruction are given by Zachow et al. (2007). The 3-D scalar fields resulting from the micro-CT scan of a shell can be visualized, and its structures can be analyzed in many different ways. We propose the following approach.

Many subsequent image analyses require data sets to be stored completely in random access memory (RAM) of the computer. Hence, raw data must fit the available RAM of the computer used for the shell 3-D reconstruction. For this purpose, the series of stacked images is first cropped to remove uninformative parts of the data set. If the data set is still too large with respect to the computer memory, it should be then down sampled.

Raw scanned images may contain additional objects other than the shell (e.g., material to immo-

bilize the shell during scanning, or remaining soft-tissues, or microscopic sand grains), as well as some "noisy" background values (Figure 5). The next step of the method is thus the segmentation of the stacked images. Segmentation is the process of dividing an image data into different segments for 3-D reconstruction, i.e., the process of selecting/identifying voxels belonging only to the shell in our case. The segmentation result is represented in the form of a 3-D label field. From micro-CT, each pixel in an image has a grey-scaled value, which corresponds to the relative density of the material at this spatial position. In our case, the segmentation process is rather simple since shells of living gastropods have a very high density compared to the surrounding air, which leads to highly contrasted images. Raw stacked images are thus threshold-segmented (Figure 6); see Sahoo et al. 1988 for an overview of various thresholding techniques. The result of this segmentation process is a series of 2-D binary images (Figure 7), i.e., black (0) and white (1). Computer memory considerations lead us to perform this step very early in the process. Indeed, segmentation significantly reduces data size by changing data type from signed integer (-32768→32767: 16 bits) to unsigned short integer (0→255: 8 bits).This reduction of size is important because it enables the analysis of larger, better resolved data sets.

It is noteworthy that most gastropods have a large overlap between successive whorls. The consequence is that it is impossible to reconstruct the shell geometry of covered parts of the shell from the external surface of the shell. To overcome this problem, we do not extract the shell itself, but its internal volume (i.e., the internal mould). Note that the internal surface of gastropod shells is usually
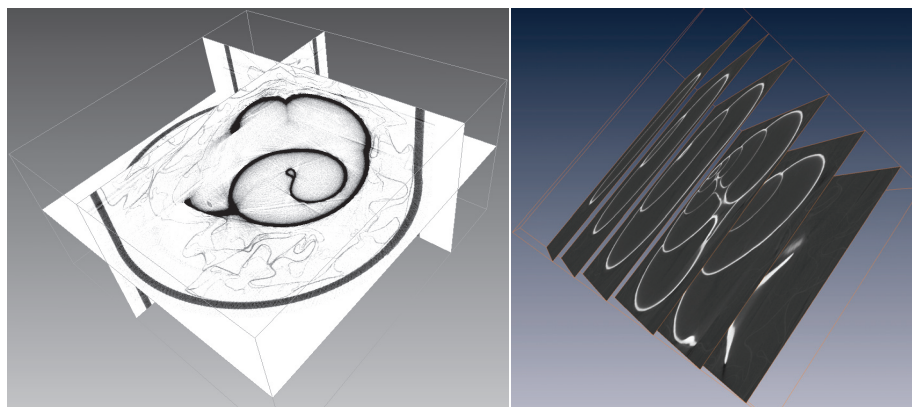

FIGURE 5. Slices of the three-dimensional image stack directly resulting from a scan by micro-computed tomography. The raw data volume of this specimen has a size of 1024 × 1024 × 507 voxels with a resolution of 0.036 mm.
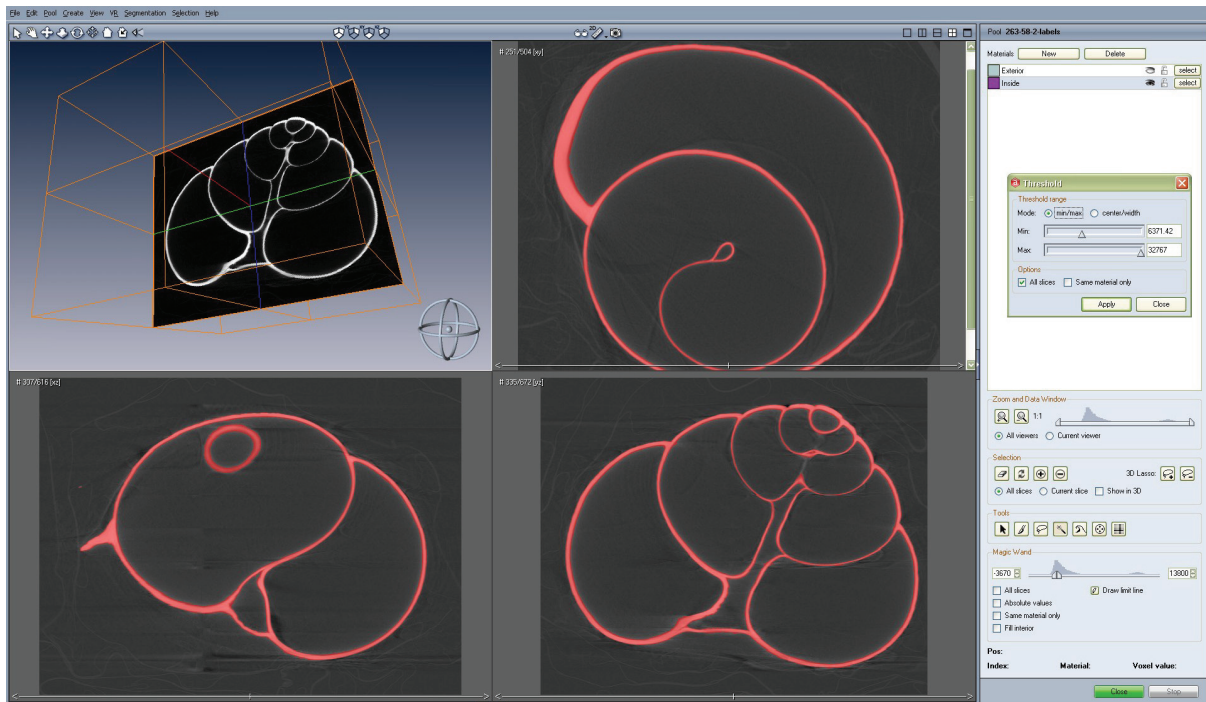
FIGURE 6. Threshold segmentation of the shell with the segmentation module of amira®. The software enables marking and classifying image areas in any of the three orthogonal views with an immediate feedback in all other views including a three-dimensional visualization. Red area in the different slices show voxels selected according to the input threshold.

smoothed compared to its external surface. To extract this internal volume, the shell is numerically bounded at its last aperture. Note that the shell is previously rotated so that the final shell aperture is contained in a plane parallel to the plane O*xy* of the data set. This step is required by the software we used and may be unnecessary with another one. Note also that the real adult margin of the shell aperture is not planar. In order to have a closed outline, the shell aperture is actually bounded at its mature constriction, which is the nearest and most easily identifiable closed outline of the shell aper-
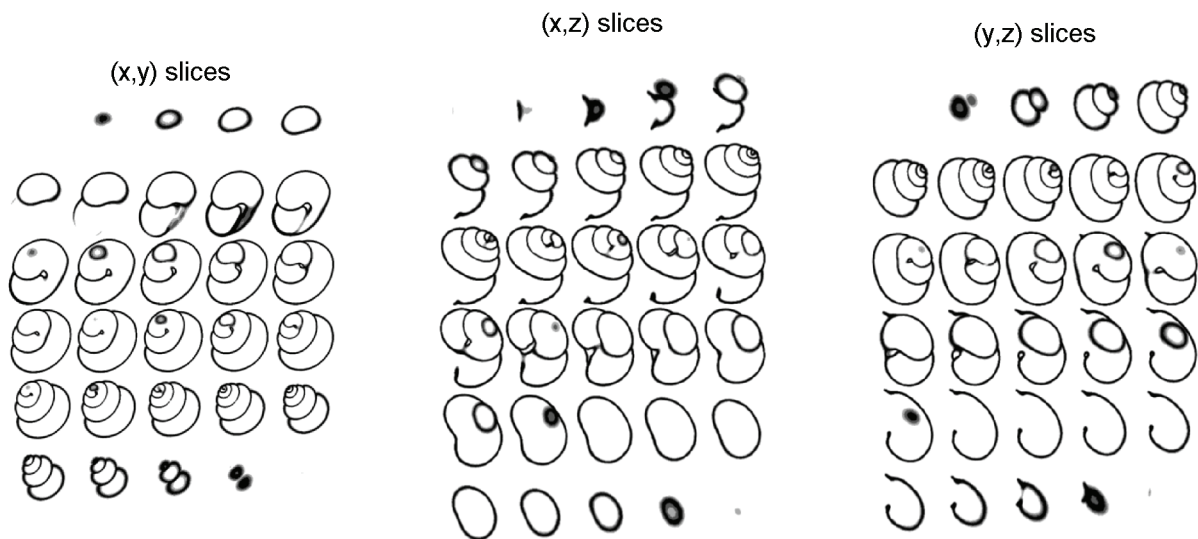

FIGURE 7. Montage of some slices of the data volume in the three dimensions after the segmentation process.
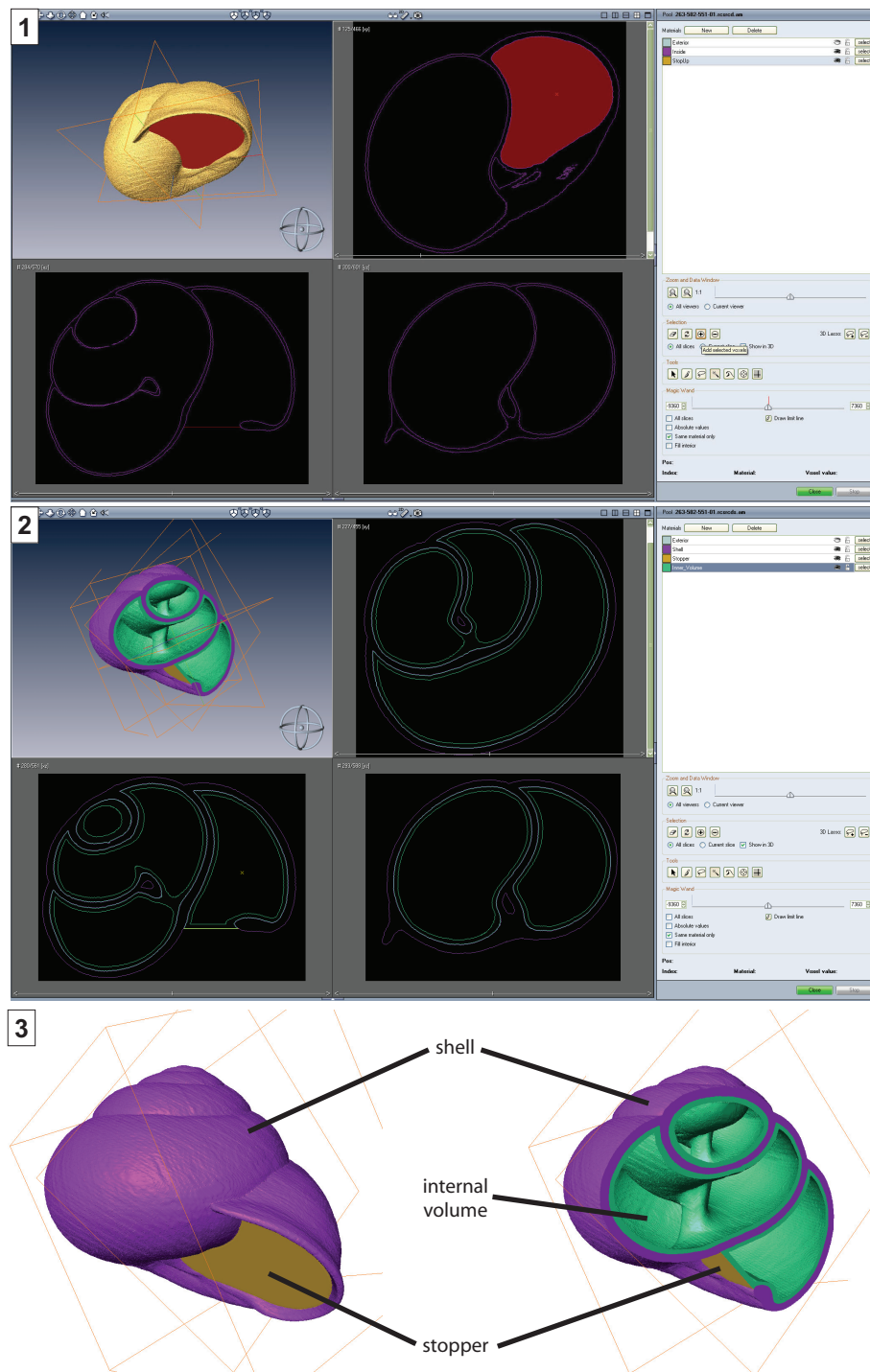
FIGURE 8. Extraction of the internal volume of the shell. **1**. Creation of the stopper at the place of a plane placed at the mature constriction of the shell. This constriction is the nearest closed aperture outline from the final shell aperture. **2**. Selection of the internal volume of the shell, which is constrained by the shell itself and by the numerical stopper. **3**. The data volume is now described by three segments: the shell itself, the stopper, and the internal volume of the shell. The data volume of the shell has now a size of 588 × 561 × 455 voxels. Thickness of the shell is exaggerated on the figure.

ture (Figure 8.1). The internal volume of the shell is then easily identifiable by being bracketed between the shell itself and this stopper (Figure 8.2). Finally, the studied gastropod shell is segmented into the following parts (Figure 8.3): the shell itself, a stop-
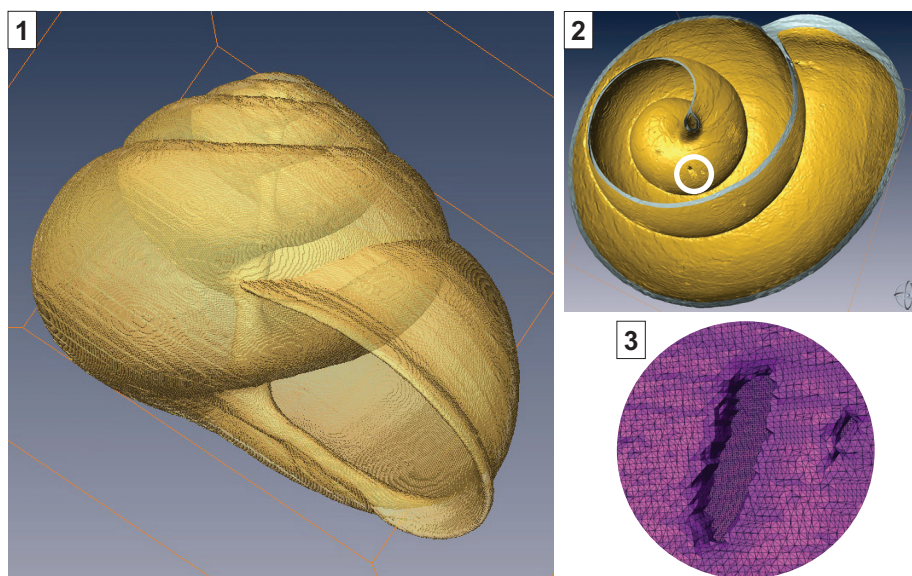
FIGURE 9. Example of perforated inner whorls of a shell. **1**. Translucent view of the gastropod shell after segmentation. **2**. View towards the inner whorls where some holes may occur. **3**. Enlarged view of a hole in the shell.

per in the plane of the mature constriction, and the internal volume of the shell.

The shell may not be thick enough, especially in the innermost whorls, to be accurately detected by the tomograph nor selected during the segmentation process. This may create or artificially enlarge holes in the shell (Figure 9). Such holes complicate the subsequent geometric analyses by connecting successive whorls. Hence, the next step of the method is to apply a dilation operation of two voxels to the stacked images. This dilation enables the removal of (or at least reduce) most of these holes without changing the geometry of the shell.

At this step, the gastropod shell is represented by a 3-D matrix of integer values indicating to which segment each voxel belongs to. This voxel grid will be used later for extraction of a curve-skeleton (see below).

In the current state of data, the different segments of the gastropod shell (i.e., the shell itself, the stopper at the mature constriction, and the internal volume of the shell) can now be accurately reconstructed by tessellating their boundary surfaces from the 3-D image data. Tessellation consists in representing the segmented structure by a rather large set of piecewise linear surface primitives (triangles) that are convenient to render since graphics hardware is optimized for this goal. The internal volume of the gastropod shell is thus described by a triangular mesh, which basically is a connected array of three-dimensional triangles placed at its surface (Figure 10.1). Since the initial

triangulation that is constructed from a segmented data volume occurs on sub-voxel resolution, the number of triangles might amount to several millions very quickly. The surface reconstruction is over-sampled, and a major requirement is the reduction of the high resolution via surface simplification (Figure 10.2). After this step, the user must test that the surface is closed and that the triangles are still consistently oriented and without intersections. Finally, for the following geometric analyses, the 3-D reconstruction (triangular mesh) of the internal volume of the shell is stored in a text file, which lists the three-dimensional coordinates of all vertices of the mesh and the three vertices of each triangle of the mesh.

Ontogenetic extraction of shell geometry

The last part of the method is the extraction of the shell geometry throughout ontogeny. In this study, we propose to recover the ontogenetic changes of the shell geometry by reconstructing and using a curve-skeleton. A curve-skeleton (or centreline, or medial axis, or central path) is a compact 1-D representation of 3-D objects, which is conceptually defined as the locus of centre voxels in the object. In this study, the curve-skeleton is expected to be placed at the centre of the shell aperture at each increment of growth. Due to its compact shape representation, skeletonization has been studied for a long time in pattern recognition (e.g., Trahanias 1992; Baseski et al. 2009), in medicine (e.g., quantification of anatomical structures or virtual navigation for colonoscopy: Pizer et al.
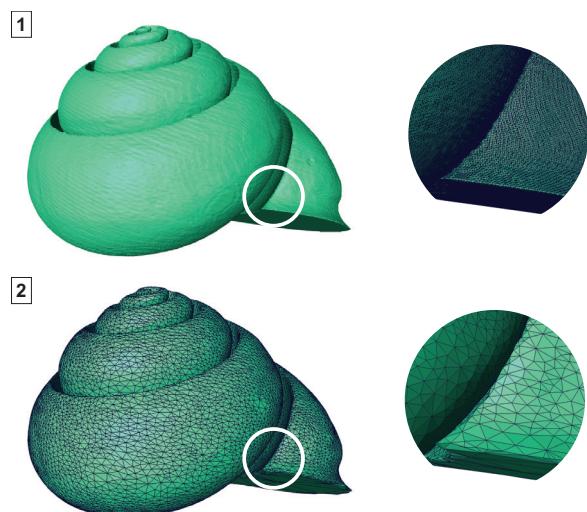
FIGURE 10. Triangular mesh of the internal volume of the gastropod shell. The internal volume of the shell is described as a connected array of triangles placed at the boundary between the shell and its internal mould. **1**. High resolution model with about 4.3 millions of triangles. **2**. Surface simplified to about 0.05 millions of triangles.

1999; Sorantin et al. 2002; Perchet et al. 2004) and in computer graphics (e.g., body animation or morphing: Blanding et al. 2000; Wade and Parent 2002).

Although straightforward in 2-D, the extraction of a curve-skeleton in 3-D remains a difficult task. There exists a large number of methods, based on different kind of data (cloud points, polygonal mesh, or voxel grid); each one with advantages and drawbacks. For a review of major methods see Kirbas and Quek (2004) or Cornea et al. (2007). Briefly, the choice of the method is guided by the following aspects: preservation of the original object's topology and connectivity, centeredness of the curve, its thickness (one voxel width), its branching, its computing time and its noise sensitivity. In this study, we selected the potential field method (Chuang et al. 2000; Cornea et al. 2005), which can produce directly a smooth and single-branched skeleton. Briefly, the potential field method is basically a distance transform. It means that for each voxel of the shell, we calculate the Euclidean distance between this voxel and the nearest boundary voxel (i.e., a voxel placed on the surface of the shell). The raw skeleton is then derived by identifying the "sinks" of the distance field (i.e., the locally maximal distances), which are locally centred within the object, and by connecting them using a force following algorithm (see Cornea et al. 2005). In the potential field method, instead of using the shortest distance to the region border, a scalar function (the potential) is used (see Ahuja and Chuang 1997; Chuang et al. 2000). Practically the result of the extraction of a curve-skeleton is a series of 3-D points (Figure 11), which are extracted from the voxel grid.

The curve-skeleton of a gastropod shell is a very useful tool. It can be compared with the aperture trajectory (Stone 1995), although defined and calculated in a different way. It can be used as a guide to extract the geometry of a succession of whorl sections in an automated navigation throughout ontogeny of the shell. The same idea is widely applied in medicine such as in virtual colonoscopy (e.g., Deschamps and Cohen 2001; Chaudhuri et al. 2004). For this purpose, successive cross-sections, centred on and perpendicular to the curve-skeleton, are computed along the curve-skeleton (Figure 12), by calculating the intersection of the corresponding cutting plane with the triangular mesh (Figure 12). The result is an ontogenetic
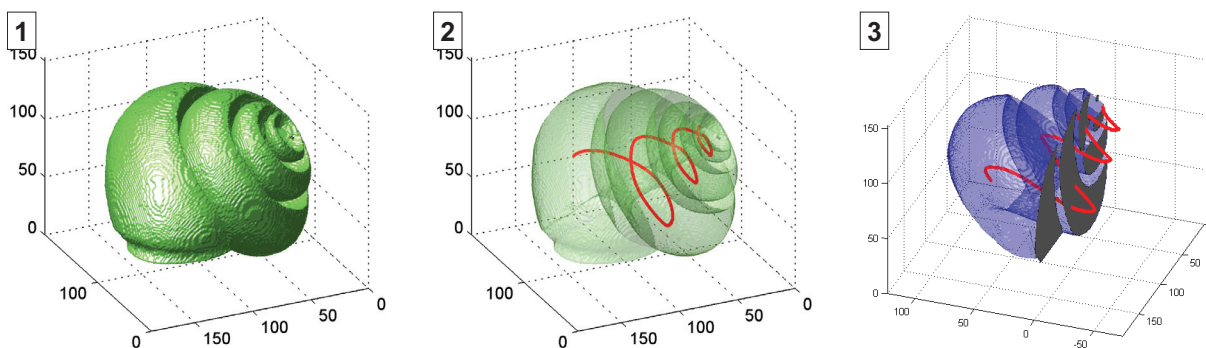

FIGURE 11. Curve-skeleton of the internal volume of the gastropod shell reconstructed by means of the potential field method. **1**. Voxel grid of the internal volume of the shell. **2**. Translucent view of the internal volume of the shell with the curve-skeleton. **3**. Cut shell with the curve-skeleton.
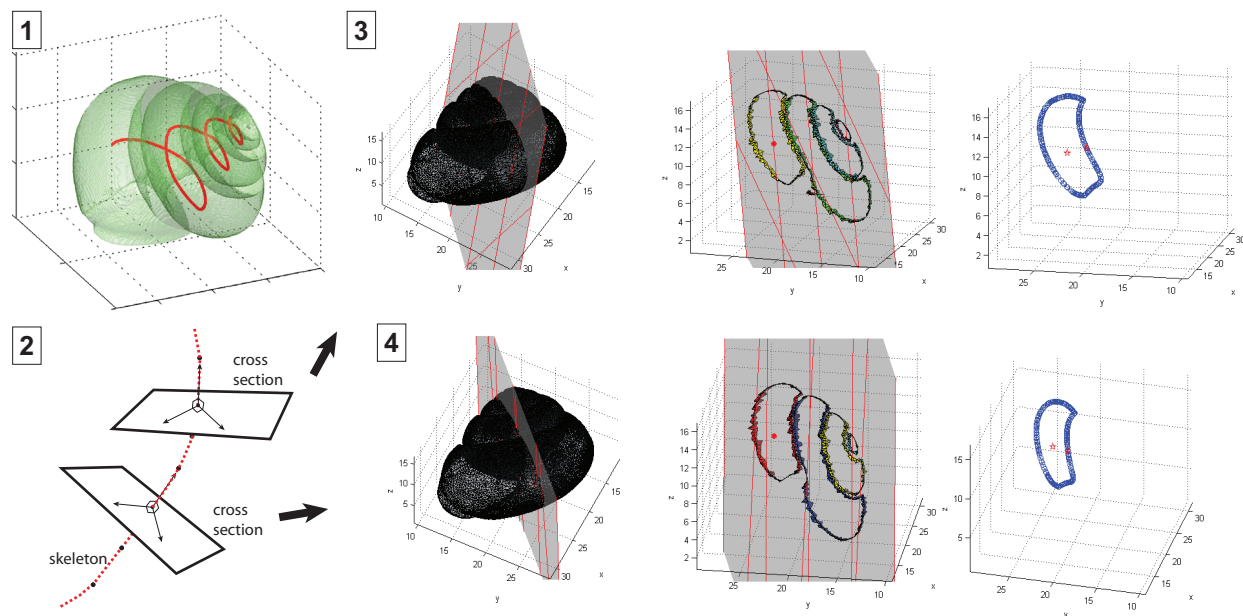
FIGURE 12. Extraction of whorl section through ontogeny by successive sections of the triangular mesh with planes perpendicular to the curve-skeleton. **1**. Translucent view of the internal volume of the shell with its curve-skeleton. **2**. Scheme illustrating two successive cross-sectioning planes perpendicular to and placed along the curve-skeleton. **3, 4**. The results of the two cross-sectioning planes, respectively: left figure shows the triangular mesh of the shell and the cutting plane; middle figure shows the triangles of the mesh which intersect the cutting plane; and right figure shows the resulting outline of the whorl section at each examined ontogenetic stage.

series of successive outlines representing shell geometry (Figure 13). Note that this approach assumes that the shell aperture is within a 2-D plane, which is actually not the case. Even if the resolution of the micro-CT is enough to distinguish the growth lines, the whorl overlap of the shell prevents their use throughout ontogeny. Nevertheless, this 2-D approximation of the shell aperture remains sufficient to capture morphometric changes of the shell through ontogeny.

We now have a 3-D reconstruction of the gastropod shell throughout its ontogeny. The geometry of the shell can then be quantified by two sets of parameters. The first set is the displacement vector between two successive cross-sectioning planes. This vector records the translation and rotation coefficients between the origins of two successive cross-sectioning planes. The second set is the successive outlines of whorl sections through ontogeny. Since the whorl shape of gastropods may not have a sufficient number of landmarks to capture the shape (see Johnston et al. 1991), these data can be analyzed by outline analysis instead of landmark-based methods (see Adams et al. 2004). Three major approaches exist: eigenshape analysis (Lohmann 1983), elliptic Fourier analysis (Ferson et al. 1985) and the more recent sliding semi-

landmark approach (Bookstein 1997; MacLeod 1999). Each outline is here quantified by elliptic Fourier analysis (EFA). This method is well suited to quantitatively model the complex outline of a shape in two or three spatial dimensions (Lestrel 1997; Haines and Crampton 1998; McLellan and Endler 1998). In general terms, Fourier analysis can be thought of as supplying the coefficients of a trigonometric function that reproduces as closely as possible a sample curve. As more terms (harmonics) are added to the function, the fit to the sample curve (in our case the aperture outline) becomes better. In EFA, the outline is characterized by a series of four coefficients for each selected harmonics. The number of harmonics necessary to reconstruct an outline depends on the
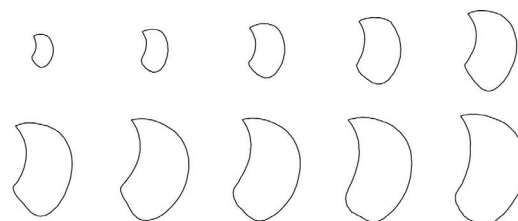

FIGURE 13. Series of some outlines of whorl sections of the studied gastropod shell through ontogeny.

complexity of this outline but usually does not exceed 20. The series of coefficients of the selected harmonics are then used as a mathematical characterization of the geometry of whorl sections.

The calculated coefficients of harmonics of a whorl section of the gastropod shell, coupled with the displacement vector (translation and rotation) between two successive cross-sectioning planes, constitute an *n*-dimensional morphometric space. The successive values of these harmonics and of the displacement vector of a single gastropod shell through ontogeny constitute a morphometric ontogenetic trajectory in this *n*-D space. The ontogenetic trajectories of each specimen and species can thus be analysed and compared quantitatively within this *n*-D space. The geometry of a shell is thus quantified by a set of multivariate data. The analysis of such data sets is standard in morphometrics, and one can find methodological developments and examples in Bookstein (1991), Marcus et al. (1996) and Zelditch et al. (2004), among others.

CONCLUSIONS

This study proposes a quantitative, non-destructive and semi-automatic method to quantify the geometry of the mollusc shell through its ontogeny. The proposed approach consists to acquire digital 3-D data of a gastropod shell by micro-computed tomography. The resulting series of grey-scaled 2-D images is then processed to obtain a model of the shell both as a grid of voxels and as a triangular mesh. Next, a centreline of the shell is extracted and serves as a guide to slice the shell throughout ontogeny and extract the successive outlines of the whorl sections. The series of outlines are then quantified by means of elliptic Fourier analysis. The resulting coefficients of the harmonics, coupled with the displacement vector of the successive cross-sectioning planes, constitute an *n*-dimensional morphometric space, in which ontogenetic trajectories of different individuals and species can be compared. This approach adds a novel set of continuous and quantitative characters on the geometry of the mollusc shell. The approach proposed in this study produces a relatively detailed, accurate and realistic representation of the shell of gastropods. It makes very few assumptions about the shell characteristics by assuming only that the shell is basically conic, coiled and characterized by an accretionary growth. This method also does not assume any coiling axis, which makes it possible to apply to irregularly coiled mollusc shells compared to existing methods. It thus provides a quantitative basis for the analysis of growth patterns and ontogeny within and across species. The analysis of a complete family of snails to illustrate this point will be dealt with in another forthcoming paper.

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