High-Resolution Computed Tomography of Lobster (Panulirus interruptus) Stomach

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ABSTRACT We used micro computed tomography to produce a high-resolution density image of the lobster (Panulirus interruptus) stomach and identified on this image the previously defined stomach ossicles. These data are the first coordinate-based, three-dimensional description of the stomach and are a necessary first step for developing biomechanical models of it. They are also interesting for several reasons in their own right. First, the ossicles showed large shape and density variations. These data suggest that different ossicles may serve different functions, with some acting as force-delivering levers, some as spring-like elements, and some as wall-like elements that resist internal stomach pressure or function as two-dimensional force-delivering plates. Second, large intra-ossicle density variations were present in individual ossicles, reminiscent of I-beams, oval bicycle frames, and similar mass-minimizing structural elements, suggesting that evolution has minimized ossicle mass and identifying which ossicle regions are most likely important in force delivery. Third, joints appeared to be either continuous density interconnections without distinct separations between the ossicles or 'floating' joints in which the ends of the two ossicles were separated by relatively large distances spanned by connective tissue. In these latter joints, although the distance between ossicle ends is likely maintained at a relatively constant value, a wide range of ossicle angles would thus be theoretically possible. Notably absent are close-apposition, morphologically specialized joints such as hinge or ball-and-socket joints. J. Morphol. 270:1029–1041, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: stomatogastric; Crustacea; invertebrate; anatomy; μCT

INTRODUCTION

Nervous systems generate movement by activating muscles that move effectors (limbs and the like). Predicting the movements motor neuron activity produces therefore requires understanding both how muscles respond to neural input and the anatomy of the effectors on which the muscles work (Chiel and Beer, 1997). Although making these predictions is never easy, certain effector anatomies are easier to understand than others. Movement of the elbow, in which the hinge joint constrains forearm movement to a single plane and only two muscles must be considered, is easier to understand than movement of the wrist, which is controlled by a larger number of muscles and can move side to side and up and down, and much easier to understand than finger movements, in which highly complex anatomy and shared musculature result in what movements one finger can make depending on the contemporaneous position of other fingers. These problems are increased in small animals because of the increased difficulty in accurately measuring effector anatomy, and for internal organs because it is generally impossible to observe organ movements.

These problems of complex anatomy, small size, and internal location have made predicting behavior from neural activity particularly difficult in the crustacean neuromuscular stomatogastric system. The stomatogastric nervous system generates the movements of crustacean stomachs. On the neural level this is one of the best-understood systems in neurobiology (Harris-Warrick et al., 1992; Marder et al., 1993; Nusbaum and Beenhakker, 2002; Hooper and DiCaprio, 2004; Marder and Bucher, 2007). The system consists of four rhythmic neural networks that cycle with different cycle periods but extensively modify one another’s activity. A great deal is known about the synaptic connectivities that support network rhythmicity and interactions, the active properties of the system’s neurons, and the conductances underlying these properties. A notable feature of the system is neuromodulatory inputs that induce individual networks to produce multiple

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neural outputs, switch neurons between networks, or fuse multiple networks into unified wholes that produce single outputs.

The networks induce the movements of the four regions of the stomach, the esophagus, cardiac sac (in which food is mixed with digestive fluid), gastric mill (in which internal teeth chew the food), and pylorus (which is believed to act as a filter that separates chewed food into three streams, one for absorption, one for excretion, and the third back to the gastric mill for further chewing). Endoscopic work has measured gastric mill teeth movements and associated some movement patterns with different patterns of gastric mill network activity (Heinzel, 1988a,b; Heinzel et al., 1993). The movements of the other stomach regions are completely unknown, and even for the gastric mill the data are only descriptive, not predictive. The functional relevance of the system’s multiple neural output patterns, neuron switching, and network fusions can thus not be predicted from recordings of neural activity. This concern is heightened by measurement of some stomatogastric muscle responses to physiologically relevant motor neuron activity, which shows that these very slow muscles can respond to rhythmic neural activity with tonic contractions or extract from this activity slow modulation imposed on it by other stomatogastric networks, and hence contract in time with these other networks even though none of their neurons innervate the muscles in question (Morris and Hooper, 1997; 1998; 2001; Morris et al., 2000; Thuma et al., 2003).

Because of the stomach’s complex anatomy, small size, and internal location, a predictive link between stomatogastric nervous system activity and stomach behavior is unlikely to be achieved without quantitative simulation of the stomach muscles and effector structures. The anatomies of several palinurid (the infraorder to which Panulirus belongs) stomachs have been described (Patwardhan, 1935; George et al., 1955; Maynard and Dando, 1974; Wolfe and Felgenhauer, 1991), and hypotheses of how the stomachs function based on these observations often advanced. However, these descriptions are only two-dimensional line drawings (see Fig. 1), which are inadequate for quantitative modeling, and the hypotheses of stomach function (except for the endoscope work noted above) have never been supported by physiological data. Given the importance of this system in motor neuroscience, it is important to overcome these deficiencies.

As a first step toward this goal we have used micro computed tomography (μCT) to obtain high-resolution three-dimensional images of stomach density and have associated these densities with previous ossicle identifications. The resolution of previous magnetic resonance imaging work (Heberholz et al., 2004) in the lobster was too low to build such maps. Our study, when coupled with contemporaneous work in our lab to develop muscle models (Geier et al., 2002; Geier, 2002), will allow predictive modeling of motor behavior in this system. The data are also interesting in their own right, because they show that the ossicles have widely varying shapes and densities, suggesting that different ossicles serve different functional roles, because individual ossicles have large, possibly mass-minimizing, intra-ossicle density variations, and because ossicles appear to be either intimately fused or physically well-separated and connected only by connective tissue, but never connected by the close-apposition, morphologically specialized joints often present in other systems.

![Fig. 1. Two-dimensional drawing of P. interruptus stomatogastric ossicles.](image)
MATERIALS AND METHODS

*P. interruptus* were purchased from Don Tomlinson Commercial Fishing (San Diego) and maintained in aquaria with 10–13°C circulating artificial seawater. The photographs in Figures 2 and 3 were taken with a Nikon Coolpix 5400 in macromode. The stomach in Figure 3, from which the views in Figures 4–10 were also made, was prepared as follows. The stomach was removed from the animal using standard techniques (Selverston et al., 1976) except that a longer portion of the intestine was maintained than normal. The intestine was tied shut with fine thread, a rubber cannula was inserted through the esophagus into the cardiac sac, and the esophagus was tied closed around the cannula. The stomach was inflated through the cannula to approximately physiological dimensions with *P. interruptus*

Fig. 2. Photograph of a stomach in situ (A) and drawing labeling the four stomach regions (blue; esophagus, ESO; cardiac sac, CS; gastric mill, GM; pylorus, PYL), some of the muscles (green) and ossicles (black) visible in A and also the hepatopancreatic duct (purple), and showing the nerves and ganglia (red) of the stomatogastric nervous system. Which motor neurons innervate the muscles shown is also indicated. Abbreviations: aln, anterior lateral nerve; COG, commissural ganglion; cpv1b, cardio-pyloric valve muscle 1b; cv2, ventral cardiac muscle 2; IC, inferior cardiac neuron; lvn, lateral ventricular nerve; mvn, median ventricular nerve; OG, esophageal ganglion; p8, pyloric muscle 8; PD, pyloric dilator neuron; PY, pyloric neuron; STG, stomatogastric ganglion; stn, stomatogastric nerve.

Fig. 3. Left (A), dorsal (B), and ventral (C) views of the processed stomach used for the μCT scan. The paper used as a base to support the stomach during the μCT scan, visible ossicles, esophagus, cardiac sac, and cpv 2b muscle are labeled (abbreviations defined in Fig. 1 and 2 legends). Scale bar applies to all panels.

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saline (479 NaCl, 12.9 KCl, 13.7 CaCl₂, 3.9 Na₂SO₄, 10 MgSO₄, 10.9 glucose, 11.1 Tris base, 5.1 maleic acid, pH 7.5–7.6, Sigma, St. Louis, MO, or Fisher Scientific, Pittsburgh, PA) and suspended from the esophagus in a beaker containing 50% alcohol:water solution for 5 min. Ten milliliters of a 50% alcohol solution was then injected into the stomach slowly enough (1–2 min) to not distend the stomach (the prior contents of the stomach exiting through the hepatopancreatic ducts). This procedure was repeated with 75% alcohol:water and two times with 100% alcohol. This procedure made the stomach walls rigid so that stomach shape was maintained even when the stomach was empty. The cannula was then removed, as much as possible of the 100% alcohol drained from the stomach through the esophagus, and the stomach dehydrated overnight in a vacuum chamber. The anterior portion of the cardiac sac was then super-glued to a piece of paper to facilitate handling and to provide a base on which to mount the stomach (most of this paper was cut away to make the photographs in Figure 3, made after the stomach was CT scanned).

The dried stomach was then placed in a General Electric eXplore Locus in vivo Small Animal MicroCT Scanner with dual CPU operator console and eight-CPU Reconstruction Center and scanned, 720 views with five averaged acquisitions per view, at 46 μm resolution and 70 kVp, 450 μA, and a 2 s exposure time. The Insight Segmentation and Registration Toolkit (ITK), the Visualization Toolkit (VTK) (Schroeder et al., 2006), and ParaView (Sanda National Laboratories, Albuquerque, NM) were used to process the CT data. The Insight Segmentation and Registration Toolkit is an open-source software developed as an initiative of the U.S. National Library of Medicine, available at www.itk.org, and ParaView is an open-source, multi-platform application designed to visualize data sets of size varying from small to very large (paraview.org). The Visualization Toolkit was used to save and load data, clip out volumes of interest, produce volume renderings, remove segmented ossicles from image data, and select seeds for segmentation. ParaView was used to locate volumes of interest, examine segmented ossicles, and produce renderings of the segmented ossicles. The Insight Segmentation and Registration Toolkit was used to segment the ossicles with either the isolated-connected or fast-marching image filters. The programs used are available in the supporting information.

Similar μCT data with respect to ossicle placement, interconnections, surface contours, and density were obtained from a second lobster stomach prepared as above. In both animals similar inter-ossicle variations (e.g., that ossicle 14 is denser than ossicle 8,12) were seen, and the ossicles that show large intra-ossicle density variation (ossicles 24, 6, and 5) showed similar intra-ossicle variations.

In two other animals, ossicle placement and surface contour data were obtained by an alternative physical sectioning method. In this method the stomachs were removed from the animal and dehydrated as above. EPOFIX (a two-part epoxy resin and hardener) colored with Sudan black (both from Electron Microscopy Sciences, Hatfield, PA) was then injected into the stomach through the cannula. The filled stomach was then placed twice in a vacuum chamber for 5 min each time and allowed to cure overnight. The filled stomach was then placed in a plastic box with holes machined in the top and bottom such that wooden dowels placed through the holes would be parallel (the dowels provided fiduciary landmarks for alignment of the plastic box) the box was then filled with the same EPOFIX/Sudan black mixture as above by repeatedly adding 1 cm layers of the mixture (the box had to be filled in this sequential manner to prevent boiling or burning of the epoxy during curing). The resulting epoxy block was then machined to make all opposing faces optically parallel, exactly parallel (adjacent faces parallel to perpendicular (a rectangular box). The bottom of the block was scanned with a flatbed scanner (scan resolution 0.0106 mm) and then 0.127 mm of the bottom of the block milled off with an end mill. The result of the milling bottom was then again scanned. This resulted in an ascending series of images of the portions of the ossicles visible at each level. These images were combined into a three-dimensional image of the stomach using the Insight Segmentation and Registration Toolkit and the Visualization Toolkit software libraries noted above. The ossicle placement and surface contour data obtained by this method were consistent with the μCT data shown here in Figures 4–9. In all four animals, a given ossicle had similar placement and surface contours.

The data presented here are from only one μCT-scanned stomach. Final figures were prepared in Canvas (ACD Systems of America, Miami, FL).

RESULTS

The lobster stomach lies immediately under the dorsal carapace of the thorax (Fig. 2A). The esophagus connects the ventrally located mouth to the cardiac sac. The cardiac sac connects to the gastric mill dorsally and the pylorus ventrally, and the gastric mill also connects to the pylorus (Fig. 2B). Figures 2 and 3 show how few of the ossicles can be seen in the intact stomach. Identifying all stomach ossicles (see Fig. 1) thus requires cutting the stomach (typically one ventral midline cut and two ventral-to-dorsal cuts, one on each side of the stomach, approximately along the dividing line between the cardiac sac and gastric mill) so that it can be flattened and the exterior and interior of the stomach both be seen, and then dissecting away overlying fat, muscle, and connective tissue to reveal the ossicles.

An alternative approach to determine ossicle position is to remove the stomach from the animal but otherwise leave it intact (see Fig. 3), and then to use micro computed tomography (μCT) to scan stomach density. This technique shows stomach connective tissue, dried muscles, and ossicles, with the ossicles being the denser of the three (see Fig. 4), and maintains the three-dimensional arrangement of the ossicles. It also measures ossicle density (the color variations in Fig. 4), and measures both position and density quantitatively (that is, the density of every pixel in the scanned volume has a coordinate-based, identified position in three-space). This three-dimensional representation cannot be fully appreciated in projections such as those shown in Figure 4, and the reader is encouraged to view the movies of the rotating stomach provided in the supporting information. The μCT scans showed that different ossicles have very different densities. For instance, ossicle 14 is denser than ossicle 8,12 (in Panulirus, unlike some other decapod crustaceans, these ossicles are fused and cannot be separately identified; Fig. 4A). The extent to which these density differences correspond to differences in ossicle stiffness was not quantitatively determined in the work reported here, but qualitative determinations by bending various ossicles by hand with forceps showed that, for instance, ossicle 8,12 was indeed more flexible than ossicle 14. These images also show that some individual ossicles had large intra-ossicle density
variations (e.g., ossicle 5), an issue shown in greater detail in Figures 8 and 9.

Comparison of the densities in Figure 4 with the ossicle identifications in Figure 1 allows identification of the individual ossicles (Figure 5; the three-dimensional relationships among the ossicles is again best appreciated by viewing the movies in supporting information). This identification

Fig. 4. Left (A), dorsal (B), ventral (C), anterior (D), and posterior (E) views of the μCT scan of the processed stomach. Visible ossicles, esophagus, cardiac sac, and cpv 2b muscle are labeled (abbreviations defined in Fig. 1 and 2 legends). The paper used to support the stomach during the μCT scan has been removed from the images to allow unblocked view of stomach densities. Note large density differences between different ossicles (e.g., ossicle 8,12 vs. ossicle 14). Scale bar and density scale (black to white increasing density) apply to all panels.
emphasizes the large shape differences among the various ossicles. For instance, ossicles 8,12; 9; 13; and 14 are either strap-like or tubular. Ossicle 30 is U-shaped. Ossicles 1; 2 (both best seen in Figure 4 due to the very low density of parts of the ossicles); 7; 22,23 (again fused in Panulirus); 24; 25; 26; and 31 have extended, plate-like shapes. Ossicles 5; 6; and 32,33 (fused in Panulirus) have complex anatomies that defy easy description.

The high resolution of the µCT scans allows examining the ossicles in fine detail (see supporting information for movies of the data shown in Figures 6–10). Figure 6A–C shows left, dorsal, and anterior views of ossicle 8,12, a thin, strap-like,
low-density ossicle (left panels, surface renderings, right density plots). Figure 6D–F shows left, dorsal, and anterior views (left panels, surface renderings; right panels, density images) of ossicle 14. Although long, the ossicle is throughout its length relatively circular in cross-section (i.e., it forms a tube). If thickness and density correspond to the ability to deliver force, this ossicle could possibly serve as a force-delivering lever.

Complex shape and large intra-ossicle density variations.

Figure 8 shows surface renderings (left images in each panel) and density plots (right images in each panel) of ossicle 6, an ossicle with an even more complex shape and pronounced intra-ossicle density variations. Depending on the view this ossicle is a backwards C (panel A) or a bilaterally symmetrical Y (panels D, E). The anterior surface rendering (left panel D) shows particularly well that the ossicle also has a complex surface...
sub-structure, with a pronounced continuous tubular ridge along the lateral and dorsal edges of the ossicle and a central posterior medial ridge capped with two bilaterally symmetric dorsal interior bulges. This ossicle carries the medial tooth of the gastric mill; the tooth is at the leftmost (anterior) bottom of the C in panel A and the anterior medial unpaired portion of the images in panels B and C. The density images show that the ossicle has pronounced intra-ossicle density differences, with high-density areas corresponding to the exterior ridges of the Y in the surface rendering (panel D). These images also show that not all surface ridges corresponded to high-density regions. In particular, the medial ridge visible in the surface rendering in panel D is not a high-density region (compare left and right images). The tooth is also a low-density region.

Ossicle 5 is another very complexly shaped ossicle with pronounced intra-ossicle density variations (Fig. 9, left images surface renderings, right images density plots). The shape of this ossicle defies easy description. It contains the lateral tooth, marked with an asterisk in panels B-E. The surface rendering again shows prominent ridges (particularly well seen in panel A). This ossicle shows large intra-ossicle density variations, with the surface bulges being denser than the interior of the anterior portion, and the posterior dorsal region, of the ossicle (panel A).

The high resolution of the μCT scans and their ability to show density differences also allows the joints between ossicles to be examined in greater detail than has been previously possible. The most salient characteristics of these joints is that they either consist of fusions of ossicles in which it is difficult or impossible to discern ossicle interconnections, or consist of connective-tissue-based connections lacking morphologically specialized high-density structure. The top row of Figure 10 shows
examples of both types of interconnections. These images contain four ossicles, ossicles 1 (red in middle panel) and 7 (yellow in middle panel), both of which are present as a single copy in the stomach, and the two ossicle 2s (green in middle panel). The segmented images (middle panel) show only the high density portions of the ossicles. The density images (left panels) show that these high-density regions are continuous with lower-density regions that are, however, more dense than the unchitinized stomach wall (regions of the image immediately above and below ossicle 7). Examination of these moderate-density regions of ossicles 1 and 2 (the regions anterior to the high-density portions of the ossicles) show no low-density lines that would allow identifying a joint between these three ossicles, and thus these ossicles appear to form a single fused entity with one highly chitinized edge and the rest of which forms a sheet of continuous lower density. A low-density region does clearly demarcate the boundary between ossicles 1 and 7.

However, note that there is no close apposition of high-density, morphologically specialized structures between the ossicles, but rather that where the two ossicles join ossicle 7's density simply smoothly decreases, eventually becoming very similar to the density of the unchitinized stomach lining.

A similar lack of close-apposition, morphologically specialized joint structure is also seen between ossicles 5 and 14 (Fig. 10, middle row). In this case both ossicles have clearly defined high-density boundaries separating them from the unchitinized stomach wall tissues. However, although ossicle 14 has a well-defined, tubular end that one might imagine could well fit into a socket, no such socket exists in ossicle 5 (the large indentation at this location in ossicle 5 being much too large and flat to serve such a purpose). Views at other angles (data not shown but see movies in supporting information) confirm this lack of a specialized socket in ossicle 5 into which the end of
ossicle 14 might fit, and show instead, as with the connection between ossicle 1 and ossicle 7 in the top row, that the end of ossicle 14 and the surface of ossicle 5 are separated by a considerable physical distance filled with low-density, presumably unchitinized, connective tissue.

The bottom row of Figure 10 shows an example of ossicle fusion, but again without any sign of a specialized morphology that would allow, for instance, rotation of the two ossicles relative to one another. In this case the ends of the two ossicles (ossicle 8, 12 and ossicle 13) instead appear simply to fuse smoothly one onto the other, with no sign of morphological specialization such as is seen in a hinge joint (e.g., human elbow) or ball-and-socket connection. In examining the other stomatogastric ossicle joints we have never seen morphologically specialized joints, instead seeing only 1) smooth fusions of joints into unified plates, often in which it is impossible to discern any clear dividing line between the ossicles (as in ossicles 1 and 2 in Fig. 10, top row); 2) joints where ossicle ends approach one another but are connected simply by connective tissue (as in the joints between ossicles 1 and 7 in Fig. 10, top row, and ossicles 5 and 14 in Fig. 10, middle row); or 3) joints where ossicles simply fuse without any specialized structures that would allow rotation (as in the joint between ossicle 8, 12 and ossicle 13 in Fig. 10, bottom row).

**DISCUSSION**

We have shown here that μCT scans have sufficient resolution to construct accurate three-dimensional maps of lobster stomach density in which the individual ossicles and their relative positions can be identified. These data represent a significant addition to the previously available anatomical data on the stomach, which consisted of two-dimensional line-drawings lacking quantitative density information (Patwardhan, 1935; George et al., 1955; Maynard and Dando, 1974; Wolfe and Felgenhauer, 1991). The data presented here are from a μCT scan of only one stomach, but data from a second μCT scan and from an alternative physical sectioning technique from two animals showed that in all cases the data were similar with respect to ossicle positioning and ossicle surface contours (both techniques), and the presence
Panulirus ossicles show large inter-ossicle shape and density variation, some of the ossicles have large intra-ossicle density variation, and the ossicles do not appear to be interconnected by morphologically specialized joints. For instance, the joint between ossicles 1 and 7 would appear to allow ossicle 7 to rotate throughout an extremely wide range of angles into and out of the plane of the figure in Figure 10, to rotate to a limited degree up and down (laterally) in the plane of the figure, or even to rotate about the ossicle’s midline, with one edge descending below, and the other above, the plane of the figure. The joint between ossicles 5 and 14 would appear to allow ossicle 14 to rotate at will, limited only by the physical constraint of being blocked by contact with other ossicles. The ‘joints’ between ossicles 1 and 2, and between ossicle 8,12 and ossicle 13, alternatively,
raise the question of whether these joints can rotate at all. That is, ossicles 1 and 2 are connected by a continuous sheet of relatively low-density (but still denser than the surrounding flexible stomach wall) material. As such, whether these ossicles can move relative to each other depends on how flexible this connecting material is, with the extremes being that the material is like a sheet of paper reinforced along two of its edges, but which can bow and flex in the rest of its area into a large variety of shapes, or being a rigid plate. The joint between ossicle 8,12 and ossicle 13, alternatively, appears to be a true fusion, and thus likely serves as a connecting point across which force could be delivered, but around which rotation would not occur.

The highly varying density of the stomach ossicles raises the related question of to what extent some of them serve as force-delivering elements. First, it is essential to note that density does not necessarily equate with rigidity, and hence the ability to act as a force-delivering lever (lead, after all, is denser but more flexible than aluminum). Nonetheless, the combination of low density and thinness of many of the stomach ossicles (e.g., ossicle 8,12, Fig. 6; ossicle 7, Fig. 7), particularly coupled with preliminary by-hand experiments testing the resistance of these ossicles to bending, suggests that these ossicles are highly flexible. They are therefore unlikely to be able to deliver force along their lengths, instead likely acting as springs that would bend to store force delivered by muscle contraction and being able to flex in response to changing positions of stomach contents or other stomach ossicles. This latter interpretation may be particularly relevant to ossicle 7, which is associated with the much-more rigid (see next paragraph) medial tooth. This rasp-like ossicle could thus drag over the surface of food held in place by the closed lateral teeth, moving up and down to closely follow irregularities on the food moiety’s surface and in so doing remove from it loosely attached bits of food.

Other ossicles, particularly 5 (see Fig. 9) and 6 (see Fig. 8), which bear the food-chewing teeth of the gastric mill (Maynard and Dando, 1974; Heinzel and Selverston, 1988; Heinzel, 1988a, 1993), almost certainly function in large part as force-delivering levers. A notable aspect of these ossicles, and also of ossicle 24 of the pylorus, is their complex internal morphology, with prominent surface ridges and grooves and pronounced intra-ossicle density variations (similar complex morphology is also present in many other ossicles, data not shown in ossicle-specific figures, but visible in the whole stomach views in Figs. 4 and 5 and in the movies). These complex anatomies are interesting for two reasons. First, assuming that increased mass and density are associated with increased rigidity, these morphologies presumably indicate along which directions the ossicles deliver force. Second, these complex morphologies resemble the mass-reducing principles used in much modern structural design, such as the use of oval supporting struts in bicycle frames. Although it is axiomatic that evolution works on all size scales, if mass-minimization is indeed an explanation for these complex morphologies, we nonetheless find it striking that it would occur on ossicles that are such a small percentage of total animal mass.

Although the anatomical data presented here are not adequate to predict stomach movement from stomatogastric neural activity, they do have two important implications with respect to stomach movement. First, the stomach ossicles are embedded in the stomach wall, and indeed some of the lower-density ossicles blend smoothly into the stomach wall without distinct boundaries. The stomach wall is flexible but has little elasticity, which may limit the range of possible ossicle movements. Second, the complex ossicle interconnection patterns may make ossicle movement dependent on the contemporaneous positions of other stomach ossicles. The physical separation of the stomach regions suggests that these issues are unlikely to be so extreme that predicting the movements of every ossicle requires considering the simultaneous position of every other ossicle. Nonetheless, these considerations do suggest that predicting ossicle movements cannot be achieved by considering the ossicles pair by pair, but instead requires considering at least groups of related ossicles (e.g., the gastric mill or pyloric ossicles) as an interconnected and interacting system. The data presented here, providing as they do a three-dimensional mapping of ossicle position, density, and connectivity, provide a necessary first step in this analysis, and thus predicting whole stomach movement.

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