NON-INVASIVE BIOMETRIC STUDIES ON *SUBERITES DOMUNCULA* BY MULTIDETECTOR X-RAY COMPUTED TOMOGRAPHY

MICHAEL NICKEL*, MARZIA SIDRI*, AXEL KÜTTNER**, ANDREAS KNOPP** & FRANZ BRÜMMER*

*Biologisches Institut, Abteilung Zoologie, Universität Stuttgart, Pfaffenwaldring 57, D 70569 Stuttgart, FRG
**Abteilung für Radiologische Diagnostik, Eberhard-Karls-Universität Tübingen, Hoppe-Seyler-Str. 3, D 72076 Tübingen, FRG
E-mail: michael.nickel@po.uni-stuttgart.de

ABSTRACT

We used *Suberites domuncula* Olivi, 1792 associated with pagurid decapods to evaluate the use of multidetector x-ray computed tomography (MDCT) combined with 3-dimensional reconstruction and virtual measurement techniques for biometric studies of living sponges. Sponges were not damaged in any way. Complete 3D-visualization of the sponge body as well as the incorporated gastropod shell is possible. The method allows the determination of the shell genus or species without dissection of the sponge. We calculated the sponge body volumes in comparison to the shell volume. In this way the comparison of various individuals is more exact than comparison of body diameter, since the internal cavities are cleared. We conclude that MDCT technique is of high potential for various questions in sponge science.

KEYWORDS

Suberites domuncula, computed tomography, sponge volume, non-invasive virtual imaging.

INTRODUCTION

Though X-ray computed tomography (CT) methods have been mainly developed for medical imaging and material science they have also been used in marine ecology to study environmental pollution problems (PEREZ *et al.,* 1999) and in marine biology to determine the structure and age of corals (BOSCHER, 1993) or assess boring activity in corals (HASSAN *et al.,* 1996). Some of us have used CT to characterise the porous structure of a Mediterranean pre-coralligenous structure (NICKEL *et al.,* 1998). For an introduction into CT techniques see JACOBS *et al.* (1995).

In sponge science, CT methods have only been used to determine body volumes of excavating sponges by SCHÖNBERG (2001), who stated the limited use, due to problems to image sponge tissue inside corals.

For the recent work we used multidetector computed tomography (MDCT) to image living specimens of *Suberites domuncula* Olivi, 1792 associated with hermit crabs (Fig. 1). Virtual imaging methods were tested.

MATERIAL AND METHODS

The sponges have been collected with permission in Rovinj (Croatia) and Banyuls-sur-Mer (France) and kept in artificial seawater aquariums in Stuttgart (Germany).

The living sponges were transported to the University of Tübingen (Germany) in batches of 5 - 7 animals in approximately 50 l of seawater. For imaging in MDCT-prototypes (Siemens Somatom Series: Volume Zoom® and Sensation 16®, Siemens AG, Forchheim, Germany) the specimens were transferred to 1 litre plastic containers which were mounted to the head holder of the machines. Spiral x-ray scanning was performed using an 'inner ear mode' at various intensity settings and slice thickness between 0.6 and 1.0 cm.

Serial sections have been reconstructed and stored in the DICOM 3 file format (Fig. 2). Image processing and rendering of 3D volume- and surface-models were performed on a Silicon Graphics or PC-Workstation using 3D-Virtuoso®- or InSpace®-Software (Siemens AG, Forchheim, Germany). Pseudo colour representation of the serial sections as well as the 3D reconstruction allows immediate recognition of differences in density, *e.g.* by canal structures or incorporated foreign material. Real-time 3D-handling at the Workstation using computer controlled shutter glasses enables to view the model of the individuals by any desired angle in the volume rendering mode (VR).

RESULTS

Virtual 3D-reconstructions of the radiograms allow the visualisation from any angle and to do virtual sections in any axis. Therefore, MDCT allowed us to determine *in vivo* the position, size and condition of the shell used by the hermit crab (Fig. 1). In most cases, it is possible a rough identification of the shell, sometimes at the genus level (Tab. I).

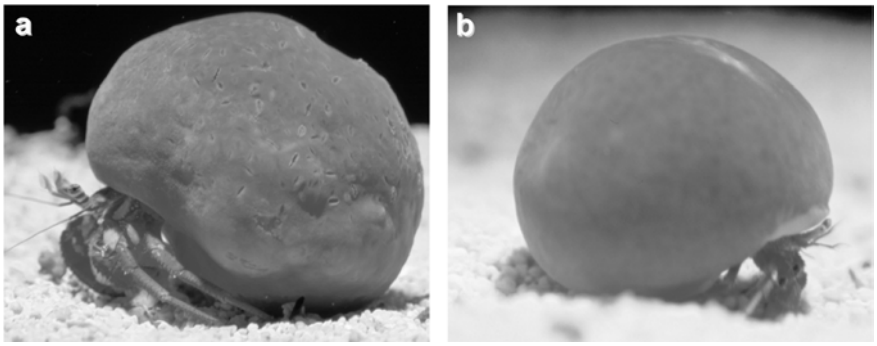

Fig. 1. Two specimens of *Suberites domuncula* used for the experiments. **a,** SD01 from Banyuls-sur-Mer (FR) and **b,** SD02 from Rovinj (HR).

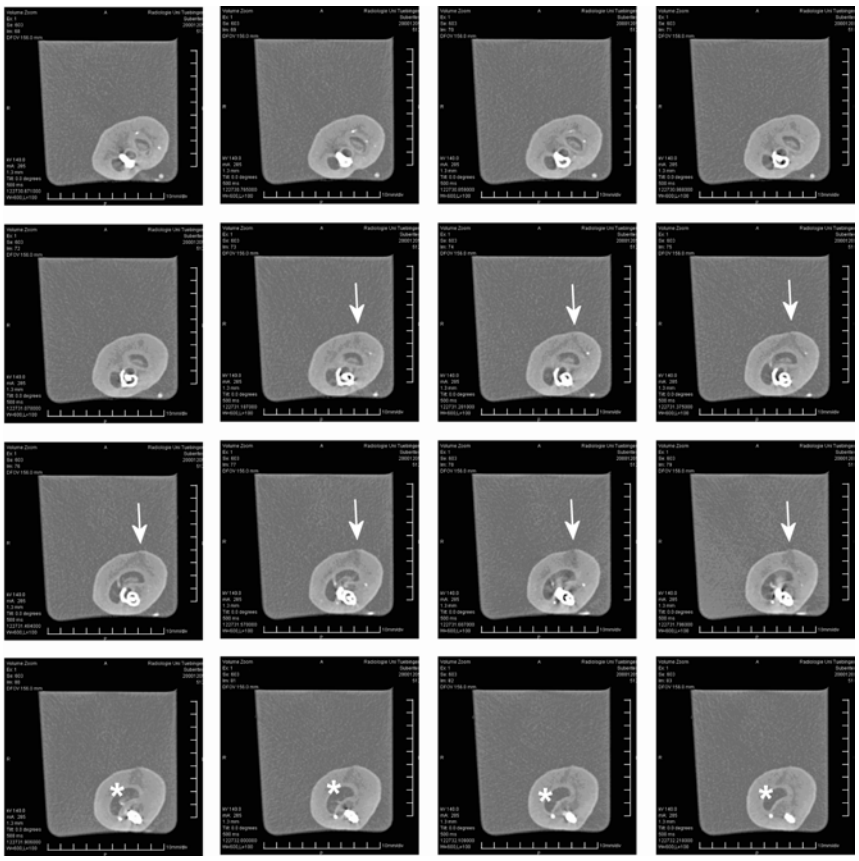

Fig. 2. Example for a reconstructed MDCT section series of specimen SD01 kept in a plastic container for scanning (clipping of a total of 133 images). Asterisk: helical living tube of the hermit crab; arrows: exhalant canals and oscule of the sponge. Section thickness: 1.3 mm; scale bars: 10 mm/div.

Tab. I. Volume analysis of *S. domuncula* specimens using InSpace®-Software-package. Calculation has been done by choosing density rates which correlate to sponge tissue or gastropod shell material in the reconstructed sections.

Specimen	volume sponge (sp) mm³	volume shell (sh) mm³	ratio sp:sh	shell species
SD01*	70091	1107	63.3:1	*Fusinus rostratus*
SD02*	16821	1682	10.0:1	*Gibbula* sp.
SD03	5338	783	6.8:1	*Coralliophyla* sp.
SD04	12179	1249	9.8:1	*Coralliophyla* sp.
SD06	7650	451	17.0:1	*Fusinus* sp.

* Specimens shown in Figures 1 to 4.

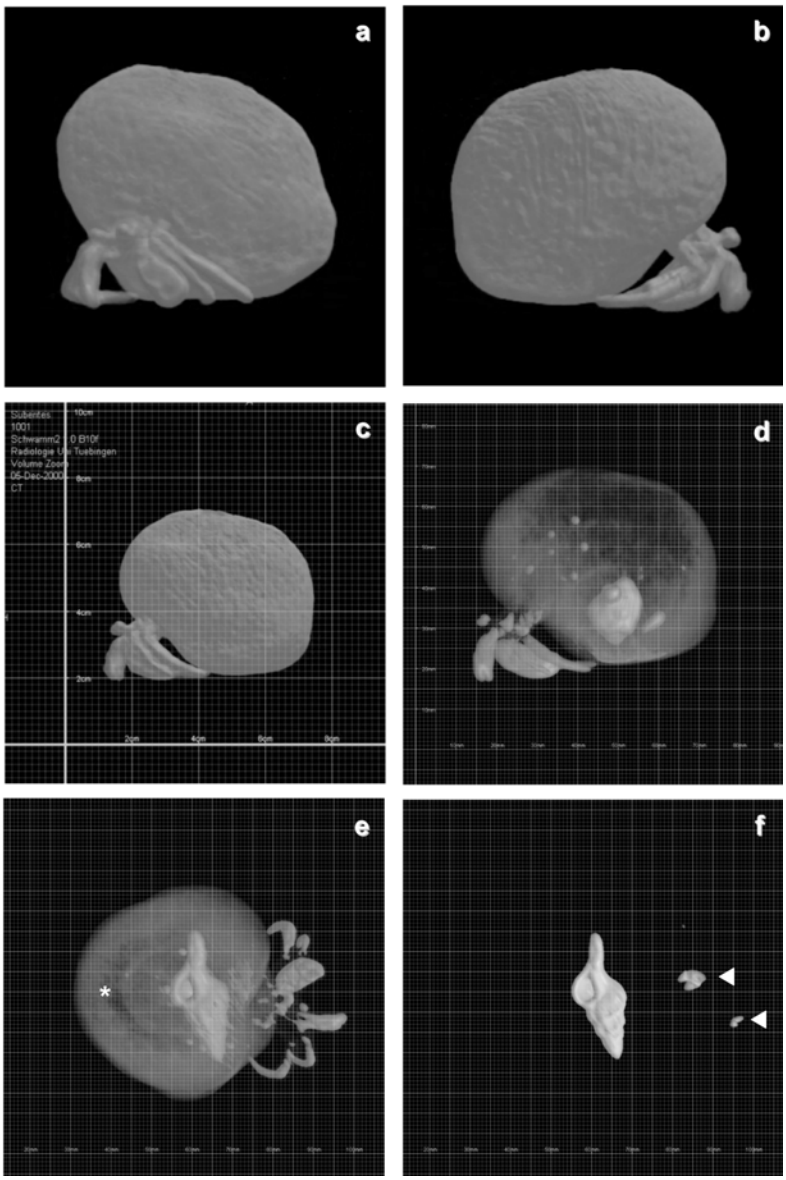

Fig. 3. 3D-reconstruction of specimen SD01 with a diameter of 58 mm, based on MDCT-data. Surface (**a** and **b**; **c** with measuring grid) and complete structures in semi-transparency (**d** and **e**, different viewing angles) as well as the virtually isolated view of the coring gastropod shell of a *Fusinus rostratus* **(f),** with the two claw tips of the *Pagurus* crab (arrowheads) also visible. Asterisk **(e)**: helical living tube of the hermit crab inside the sponge.

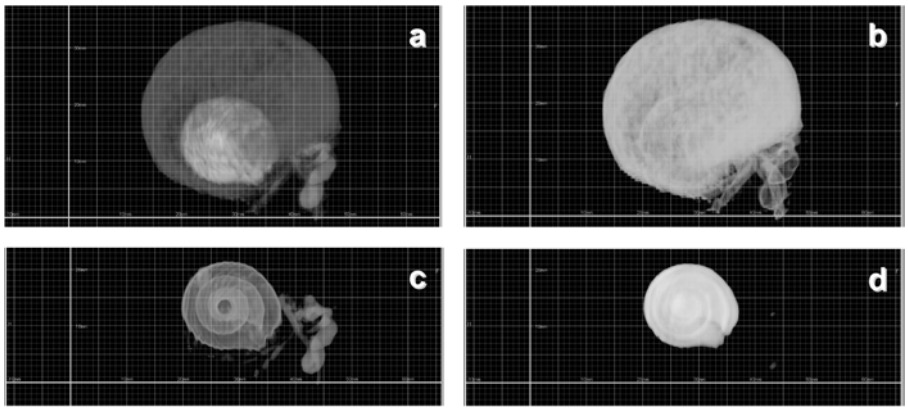

Fig. 4. 3D-reconstruction of specimen SD02 with a diameter of 35 mm, based on MDCT-data. Complete structures in semi-transparency **(a)** as well as the virtually isolated views of the sponge tissue **(b),** the harder organic material of the shell and the hermit crab **(c)** and the coring gastropod shell of a *Gibbula* sp. **(d)**.

The helical living tube of the hermit crab built by *S. domuncula* can be visualised (Figs 2, 3e) as well as the detailed body structures of the hermit crab itself (not shown here). In the case of large hermit crab-sponge association, the hermit crab was no longer inhabiting any part of the shell, but only the helical tube, since the opening of the shell was too small. In case of younger hermit crabs the helical tube was not that extended and the cavity of the shell was still used by the hermit crab. Volume comparisons are presented in Tab. I.

The surface structure of *S. domuncula* with its oscula as well as its internal morphology of the aquiferous system can partly be visualised in grey scale (Fig. 2) or pseudo colour. None of the specimens of *S. domuncula* was damaged by the procedure and all of them were brought back to the aquarium.

DISCUSSION AND CONCLUSIONS

The use of MDCT in combination with 3-dimensional reconstruction and virtual measurements allowed us to perform non-invasive studies on the association of *S. domuncula* and hermit crabs. In all samples examined it is evident that the gastropod shell is positioned asymmetrically. The length of the helical living tube which extends from the gastropod shell opening depends on the age of the association between sponge and hermit crab. Comparing specimens of various size indicates that growth of *S. domuncula* is strongest in the area of the opening of the living tube of the hermit crab, which explains the asymmetric position of the shell. The reason for unequal growth of the sponge traces back to the feeding behaviour of the hermit crab. Like other decapods hermit crabs tear their food to tatters, which can be easily observed in the aquarium. During feeding a lot of organic material is released either as dissolved or as particulate matter. Hence the food supply for the sponge is increased locally around the crab, a conclusion which dates back to OLIVI (1792). Also the excrements of the crab may be used by the sponge. This is another increase of food locally around the living tube. Alternatively or in addition it is possible that the

hermit crab releases substances that act as mitogens in the sponge. No evidence has been shown for this yet, but the possibility should not be excluded.

The results of ratio comparisons between sponge body volume and shell volume (including inner space), which are summarized in Tab. I, demonstrate the difficulty to conclude the sponge body mass from size, since the sponge diameter does neither harbour information on the coring shell, which can widely vary in size depending on the species, nor on the open spaces used by the hermit crab or the exhausting canals of the sponge. The calculation of the sponge body volume from the density profiles of the reconstructed sections is much more precise. However, the method has also limitations, since the relative density of the sponge tissue varies in dependence of the settings of the MDCT machine and has to be defined for every image series. Defining the thresholds is a source of error. In addition the volume calculator of the InSpace® Software package is not powerful enough to define the right density area in inhomogeneous tissue automatically. For sponge tissue a lot of corrections by hand have to be made after automatic detection.

On the other hand the MDCT system in combination with the software-package used allows virtual presentation of the examined object from any angle. Also virtual sections in any direction and length measurements are possible. For the visualisation of the canal system, however, the method is limited to the larger canals and cannot visualise neither single ostia nor canals of smallest diameter. Apart from the present study MDCT has a wide range of possible applications in sponge science. We have imaged and reconstructed many specimens of various sponge groups, either the complete alive animals or dead skeletons of sponges (data not shown). This survey perfectly defined the strengths and limitations of MDCT combined with virtual imaging for sponge science. Two examples of possible usage: for two *Cliona* species we reconstructed their excavated networks inside two rocks and found substantial differences in the network patterns (unpubl. data). For *Tethya aurantium* we were able to visualize and reconstruct the general skeletal morphology of the style bundles *in vivo*. But the resolution lies in the mm-range and is not high enough to visualise the megasters of this species (unpubl. data). Therefore, the limitation by resolution is the main problem for MDCT. This can be overcome by micro computed tomography which basically uses the same principles like MDCT, but provides a resolution up to 1μm, depending on the sample size. For the visualisation of the mineral skeletons of a complete specimen of *Tethya wilhelma* and an oscule of *Clathrina* sp. a resolution of 4.2 μm and 3.6μm respectively was reached, providing data on single spicules (NICKEL & BECKMANN, 2002, 2003). Eventually this method could be used also for the study of skeletal ontogeny in sponges.

We have shown that MDCT technology is of substantial use for biometric studies in *S. domuncula*. We have also applied the method to other sponge species (data not shown) and found a high potential to address questions of growth and general morphology. Even exhalant canals of larger size can be visualised and complex 3-dimensional growth forms can be analysed by means of surface area, volume, and other parameters. The limitations of the technique are few. The MDCT cannot be moved to the field. For the study of living material a working aquarium system for sponge maintenance is indispensable. The 3-dimensional reconstruction and virtual data analysis is time consuming and deserves high end computer hardware and software for comfortable data handling.

Further studies will be performed to obtain more data on the biometry of *S. domuncula* and on other sponge species for structural and growth analysis.

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