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Synchrotron radiation X-ray tomographic microscopy (SRXTM) of brachiopod shell interiors for taxonomy: preliminary report

NEDA MOTCHUROVA-DEKOVA[1] & DAVID A.T. HARPER[2]

Abstract. Synchrotron radiation X-ray tomographic microscopy (SRXTM) is a non-destructive technique for the investigation and visualization of the internal features of solid opaque objects, which allows reconstruction of a complete three-dimensional image of internal structures by recording of the differences in the effects on the passage of waves of energy reacting with those structures. Contrary to X-rays, produced in a conventional X-ray tube, the intense synchrotron light beams are sharply focused like a laser beam. We report encouraging results from the use of SRXTM for purely taxonomic purposes in brachiopods: an attempt to find a non-destructive and more efficient alternative to serial sectioning and several other methods of dissection together with the non-destructive method of X-ray computerised micro-tomography. Two brachiopod samples were investigated using SRXTM. In "*Rhynchonella" flustracea* it was possible to visualise the 3D shape of the crura and dental plates. In *Terebratulina imbricata* it was possible to reveal the form of the brachidium. It is encouraging that we have obtained such promising results using SRXTM with our very first two fortuitous samples, which had respectively fine-grained limestone and marl as infilling sediment, in contrast to the discouraging results communicated to us by some colleagues who have tested specimens with such infillings using X-ray micro-tomography. In future the holotypes, rare museum specimens or delicate Recent material may be preferentially subjected to this mode of analysis.

Key words: brachiopods, internal morphology, non-destructive technique, SRXTM, tomographic reconstructions, holotypes.

Апстракт. Синхротронска X-зрачна томографска микроскопија (SRXTM) је недеструктивна техника за проучавање и сагледавање унутрашњих особина кад чврстих и непрозрачних објеката. Она омогућава потпуну реконструкцију тродимензијалног изгледа унутрашњих структура на основу снимања разлика у ефекатима енергетских зракова као реакција на те структуре. Насупрот X-зрацима, произведеним у уобичајној X-зрачној цеви, јаки синхротронски светлосни снопови су јасно фокусирани као ласерски сноп. Приказани су охрабрујући резултати добијени употребом SRXTM за таксономске сврхе код брахиопода: начин да нађемо недеструктивну и ефикаснију замену серијским пресецима, неких других метода сечења као и X-зрачној компјутеризованој микро-томографији. Две брахиоподске пробе су проучаване помоћу SRXTM. Код "*Rhynchonella" flustracea* је било могуће сагледи 3Д облик круре и зубних плочица, а код *Terebratulina imbricata* добили смо изглед брахидијума. Насупрот обесхрабрујућим резултатима употребом X-зрачне микро-томографије саопштених од стране неких колега ми смо добили позитивне резултате употребом SRXTM код наше прве две насумичне пробе на примерцима запуњеним финозрним кречњаком и лапорцем. У будућности, холотипови, ретки музејски примерци или деликатан савремен материјал моћи ће се проучавати на овај начин.

Кључне речи: брахиоподи, унутрашња морфологија, недеструктивна техника, SRXTM, томографске реконструкције, холотипови.

[1] Department of Geology and Paleontology, University of Mining and Geology, Sofia, Bulgaria. E-mail: neda_dekova@yahoo.com

[2] Natural History Museum of Denmark (Geological Museum), University of Copenhagen, Copenhagen, Denmark. E-mail: dharper@snm.ku.dk

Introduction

Brachiopods are one of the prime examples among all animal phyla in which the morphology of the shell interior has proved crucial for the classification and understanding of their phylogeny. In order to reveal the internal structures of fossil brachiopods with a consolidated internal matrix, destructive serial sectioning has most commonly been applied. In several cases, this technique is the only way possible to reveal the critical internal morphological features of many fossil taxa. The advantage of such destructive techniques is that they are usually relatively inexpensive and efficient. However, they have several major disadvantages: (i) destruction of the specimen; (ii) providing a poor volumetric (3D) representation of the internal structures; (iii) time and labour consuming. Many of the new brachiopod taxa investigated with this technique were based on a very small number of specimens (sometimes only one), thus only parts of the internal morphology of some species have been sufficiently studied and variability was often neglected and/or open nomenclature was applied more often than necessary.

A modern variant of serial sectioning is the method used by SUTTON *et al*. (2005) where the morphology of a new Silurian brachiopod was reconstructed digitally by serial grinding at 20-μm intervals.

Here we report some pilot studies using a novel and relatively advanced method to reveal the internal morphology of fossil brachiopods. Synchrotron radiation X-ray tomographic microscopy (SRXTM) is a non-destructive technique for the investigation and visualization of the internal features of solid opaque objects, which allows reconstruction of a complete three-dimensional image of internal structures by recording of the differences in the effects on the passage of waves of energy reacting with those structures. It uses a synchrotron, a form of particle accelerator, as a bright monochromatic X-ray source. Contrary to X-rays, produced in a conventional X-ray tube, the intense synchrotron light beams are sharply focused like a laser beam. The wavelengths range from infrared to hard X-rays. SRXTM can produce tomographic data of exceptional resolution and clarity (SUTTON 2008).

Material and Methodology

The analyses were performed at the Swiss Light Source (SLS), Paul Scherrer Institute, Villigen, Switzerland. The SLS (Fig. 1) is a third-generation synchrotron light source. With energy of 2.4 GeV, it provides photon beams of high brightness for research in materials science, biology and chemistry.

We have applied SRXTM for three-dimensional reconstructions of the internal morphology of two pilot fossil brachiopod samples: (i) the rhynchonellide *"Rhynchonella" flustracea* Schlotheim from Faxe Quarry, Paleogene (Danian) of Denmark; (ii) the terebratulide *Terebratulina imbricata* Owen from the Cretaceous (Lower Cenomanian) in northern Bulgaria.

SRXTM in this study was performed at the TOMCAT beamline (Fig. 2) at SLS (STAMPANONI *et al*.

Fig. 1. Interior view of the experimental hall at the Swiss Light Source SLS (*Photo credit: H.R. Bramaz/PSI, source: http://www.psi.ch/media/MM20071121FruehеVerwandteDE/igp_1024x640%3E_MM071121_sls.jpg).*

Fig. 2. The TOMCAT Beamline at the SLS, *source: http://www.panoramio.com/photo/10865922.*

Fig. 3. External dorsal view of the rhynchonellide brachiopod "*Rhynchonella" flustracea* from the Danian of Denmark.

2006). According to the absorption properties of the samples, monochromatic X-ray beams, respectively 20 keV (for "*Rhynchonella" flustracea*) and 33 keV (for *Terebratulina imbricata*) have been used. The magnification of the X-ray microscope was X4. A scintilator LAG: Ce 20 um was used. The number of projections for both specimens is 1501. Reconstruction was performed on a 32 node Linux PC farm using highly optimized filtered back projection routines.

Slice data derived from the scans were then analyzed and manipulated using Aviso 5.0 on a Dell Precision T 7400 PC with 64 GB DDR SDRAM at the Natural History Museum, Stockholm.

The specific investigative parameters for the two samples were: (i) for "*Rhynchonella" flustracea* the energy was 20 keV; objective - 4X; exposure time 300 ms; voxel size 1,85 µm; (ii) for *Terebratulina imbricata* on-chip binning (2X) was used; energy 33 keV; objective 4X; exposure time 400 ms; voxel size 3,7 µm.

Results

"*Rhynchonella" flustracea* Schlotheim (Fig. 3): The infilling sediment in this species was fine-grained limestone. Using SRXTM it was possible to visualise the 3D shape of the crura, the lack of hinge plates, the orientation and thickness of the dental plates (Figs. 4a, b) that helped to confirm our hypothesis that this species belongs to a new basiliolide genus (to be formally erected elsewhere).

Terebratulina imbricata Owen (Figs. 5, 6): The infilling sediment in *T. imbricata* was marl. It was possible to reveal the form of the brachidium in cross and longitudinal (Fig. 7) sections, especially the details of the tetebratulide loop forming a ring. Some traces of growth of shell material in the umbonal part were also observed (Fig. 5.4). Such growth structures are not usually revealed using X-ray computerised microtomography (Pakhnevich, pers. com. 2010).

Discussion

Non destructive techniques for the study of the brachiopod shell interior were applied for the first time by Hagadorn & Nealson (2001), Hagadorn *et al.* (2001) and later by Pakhnevich (2007; 2010a, b),

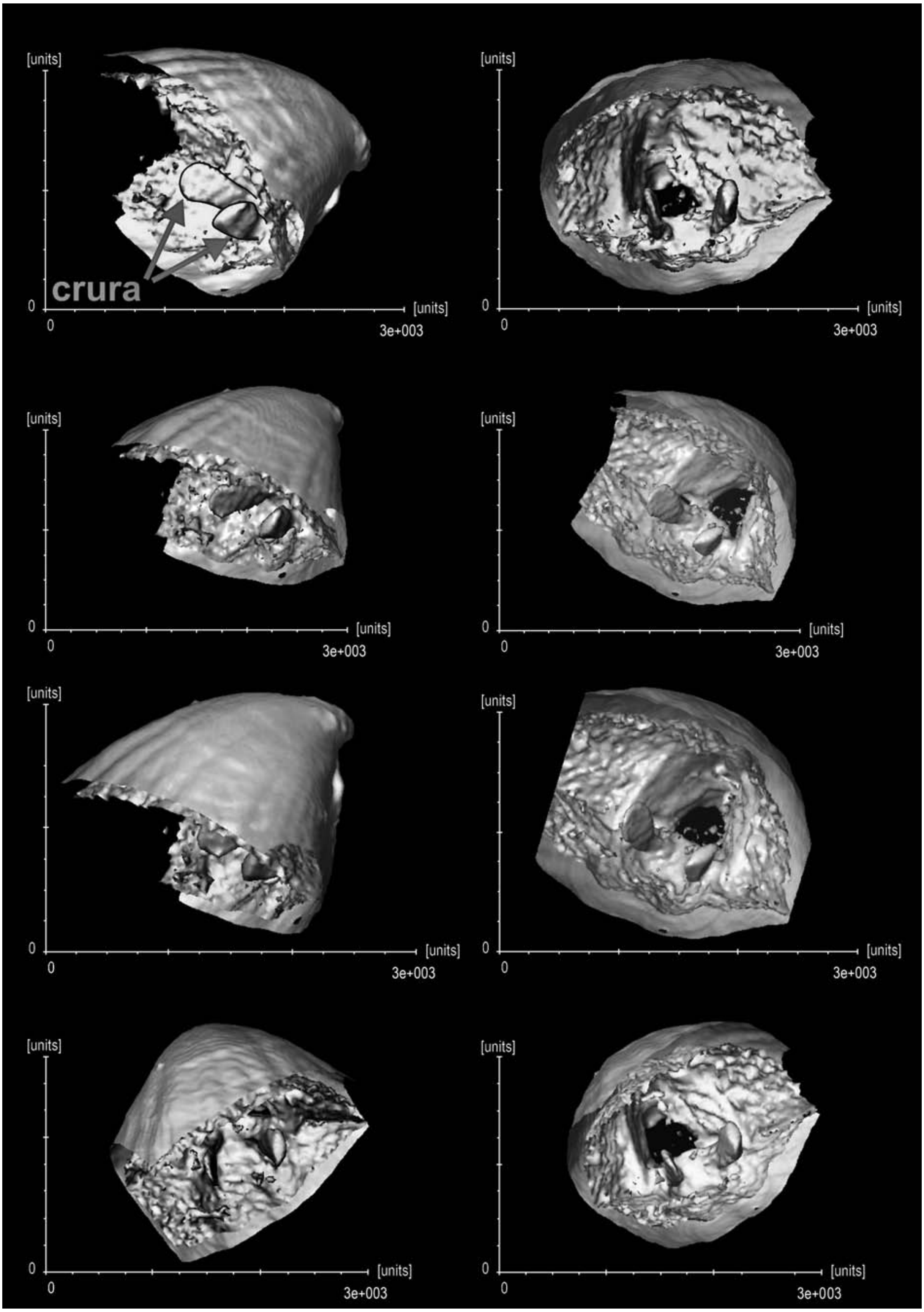

Fig. 4. Several views of tomographic reconstructions of the internal umbonal part of intact "*Rhynchonella" flustracea.* Anterior part of the shell virtually 'removed' to allow visual access to the interior of the umbonal part. Scale: one unit on ***x*** and ***y*** equal to 0.25 mm.

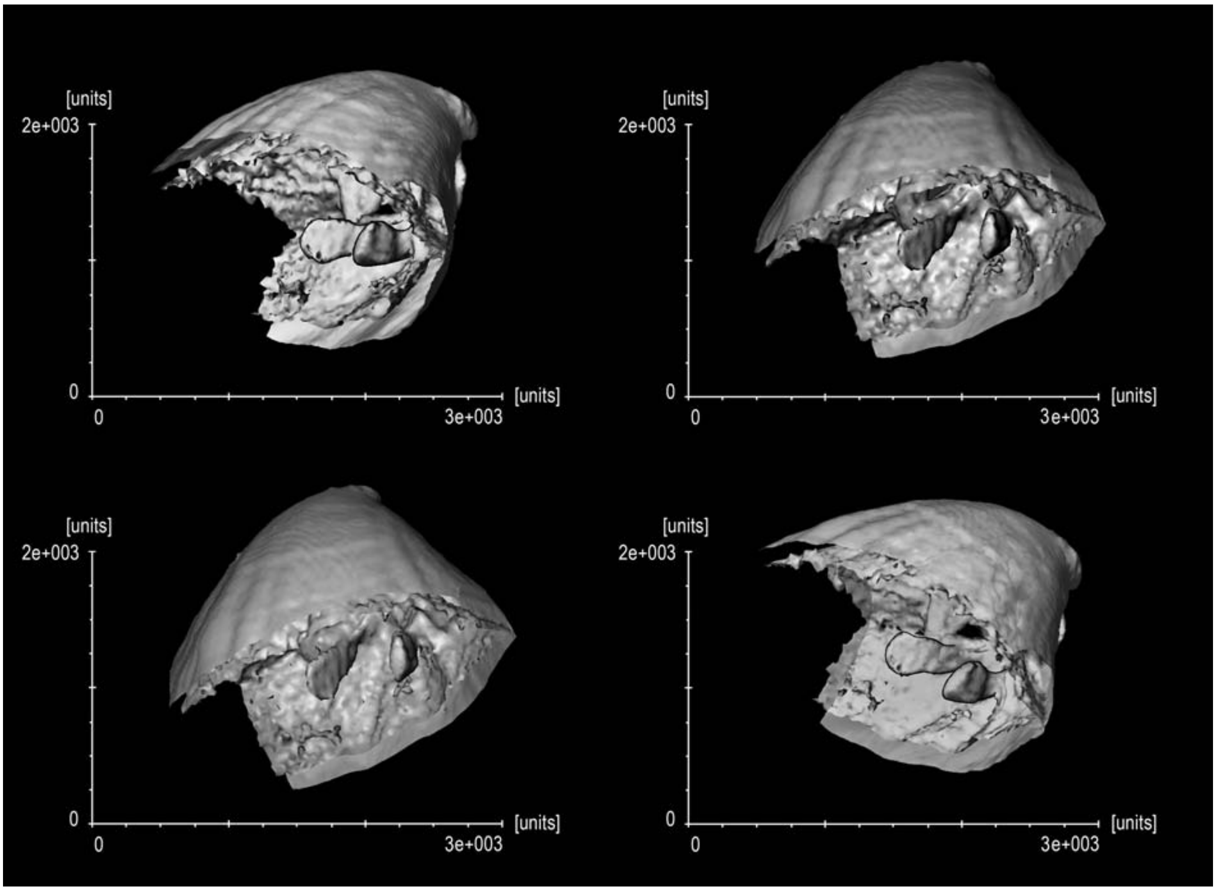

Fig. 4. Continued.

who reported successful experiments using X-radiographic computed axial tomography in a number of conference abstracts and papers but without providing any illustrations. PAKHNEVICH (2008, 2009a, b) illustrated for the first time some promising results using X-ray computerised microtomography (micro CT) on brachiopods interiors and shell structure. However, the effectiveness of the micro CT technique depends on the mineral composition of both brachiopod shells and host rock. PAKHNEVICH (2009a) performed extensive experiments to test the contrast of the 3D images depending on the mineral and rock compositions of different palaeontological specimens including brachiopods. He developed a scale of contrasts where he divided the studied minerals in 10 groups according to their contrast under micro CT Skyscan 1172. Recently, ANGIOLINI *et al.* (2010) also reported that due to the intrinsic limit of this method based on density differences, only brachiopods filled with sandstone produced valuable CT images showing details of the cardinalia. Out of the many micritic limestone, bioclastic limestone and marlstone infilling the brachiopod interiors, in one case only satisfactory CT images were obtained by these authors due to the presence of a thin void layer (dark grey in the images) between the internal structures and the micritic matrix. The more extensive survey by PAKHNEVICH (2009a; 2010a) on the matrix showed that silicified, ferruginised, or pyritised shells demonstrate clear contrasts in a carbonate rock. Calcite shells in phosphorite rocks and dolomitised shells in carbonate rocks show insufficient contrasts.

In brachiopod research SRXTM was previously used to obtain three-dimensional information from Recent brachiopods to ascertain the function and growth of punctae in the shell and increase our understanding of the role of cell biology in the context of biomineralisation (PÉREZ-HUERTA *et al.* 2009).

Here we report encouraging results from the use of SRXTM for purely taxonomic purposes: an attempt to find a non-destructive and more efficient alternative to serial sectioning and several other methods of dissection together with the non-destructive method of X-ray micro CT. The scans for our pilot attempts were carried out at the lowest available resolution, one that could be accomplished by more easily accessible X-ray micro-tomography scanners that are available at several institutions worldwide. X-ray tomographic microscopy is now a rather a routine method for several other groups of fossils (SUTTON 2008), but not, to date, for brachiopods.

It is encouraging that we have obtained such promising results using SRXTM with our very first two fortuitous samples, which had respectively fine-

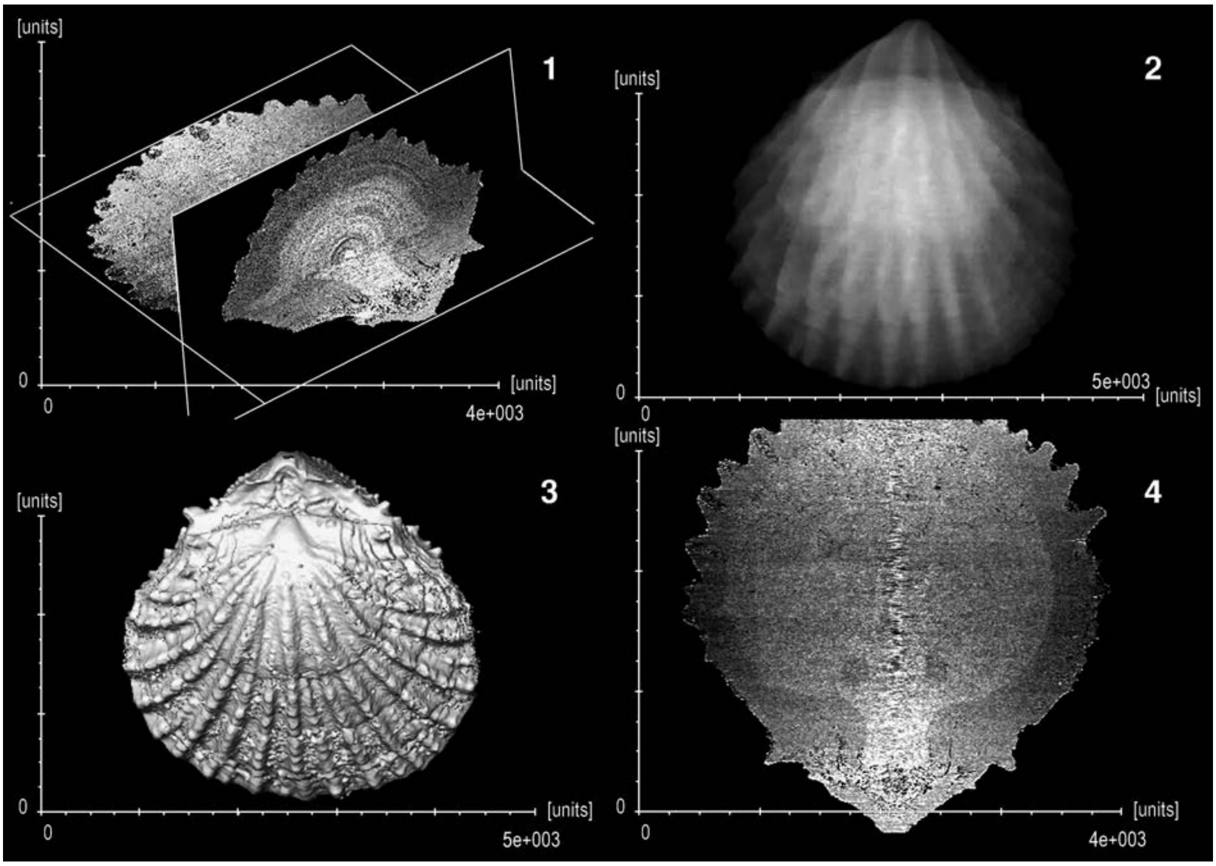

Fig. 5. Tomographic reconstructions of *Terebratulina imbricata*, 3.5 mm long. **1,** Orthogonal slices in *xy* and *xz* directions – general view; **2,** Volume rendering of the specimen (Voltex); **3,** Shape and external morphology of the specimen (isosurface) – dorsal view; **4,** Orthogonal slice in ***xz*** direction (longitudinal section). Note the traces of growth of shell material in the umbonal part.

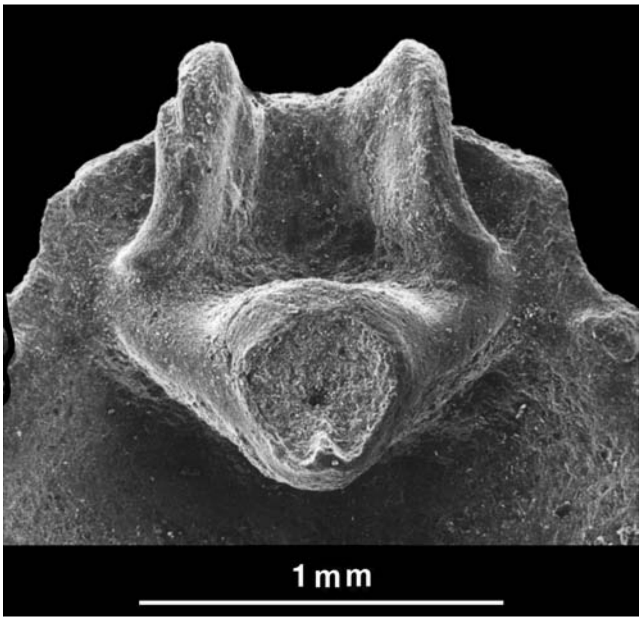

Fig. 6. A SEM micrograph of a prepared dorsal valve of another specimen of *Terebratulina imbricata* showing the shape of the cardinalium which can be revealed on the longitudinal sections obtained by the SRXTM (see Fig. 7). Courtesy M.A. BITNER.

grained limestone and marl as infilling sediment, in contrast to the discouraging results communicated to us by some colleagues who have tested specimens with such matrices using X-ray micro-tomography.

A major disadvantage of SRXTM, however, is that it is quite expensive and not readily accessible to taxonomists.

ALVAREZ & BRUNTON (2008) noted that very often, due to the scarcity of well-preserved specimens and the destructive nature of the technique, some authors chose the worst specimens for serial sectioning, keeping the best preserved (frequently only one) for the holotype. In future the holotypes may be preferentially subjected to this mode of analysis in order to check their conspecificity with the sectioned paratypes. Rare museum specimens or delicate Recent material can also be subjected to non destructive analyses (PAKHNEVICH, 2010). SRXTM and X-ray micro-tomography are also useful for the investigation of shell microstructure, microtexture and punctation (PÉREZ-HUERTA *et al.* 2009; PAKHNEVICH 2008, 2010a).

The X-ray tomographic microscopy and specifically SRXTM provides an opportunity to study the interiors of many taxa established only on exteriors, con-

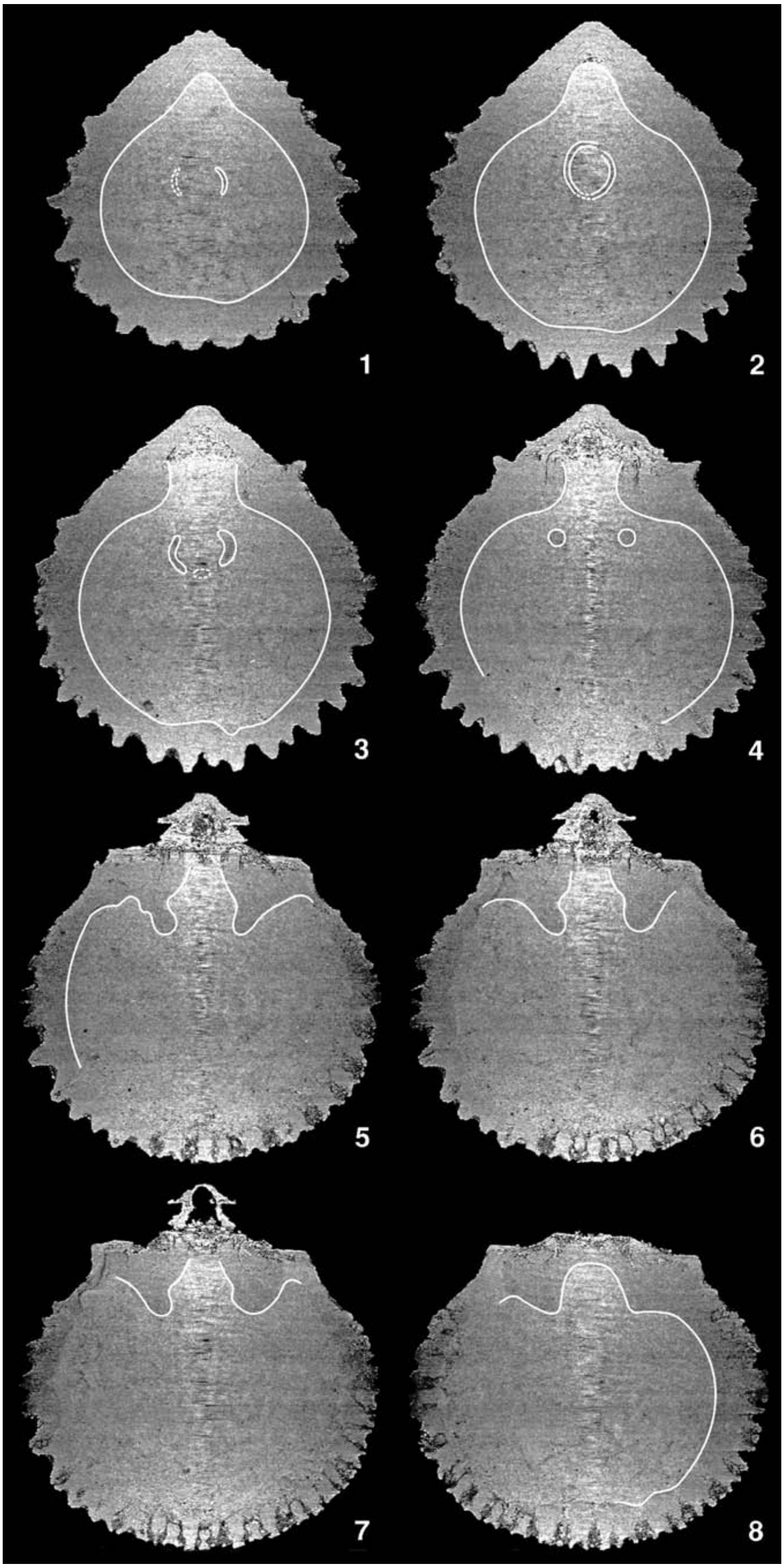

Fig. 7. Eight longitudinal sections of *Terebratulina imbricata* – tomographic reconstructions of the intact specimen on Fig. 5 obtained by the SRXTM (the most important outlines highlighted in white). Note the almost complete section of the ring in the second section.

firming or otherwise their taxonomic placement. We hope that this new methodology will also stimulate the study of intraspecific and intrageneric variability when enough material is available and help avoiding unjustified splitting or lumping in brachiopod taxonomy due to insufficient data on internal structures.

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Резиме

Синхротронска X-зрачна томографска микроскопија (SRXTM) унутрашњости брахиоподске љуштуре значајне за таксономију: прелиминарни извештај

Брахиоподи су један од најбољих примера у животињском царству у коме је унутрашња морфологија љуштуре од кључног значаја за класификацију и разумевање њихове филогеније. У циљу откривања унутрашњих структура фосилних брахиопода са консолидованим унутрашњим матриксом, најчешће се примењује деструктивна серија пресека. У неколика случајева ова техника је једини начин да откријемо веома важне унутрашње морфолошке особине фосилних таксона. Предност овакве деструктивне технике је у томе што је она обично релативно јефтина и ефикасна. Међутим она има неколико недостатака: (1) уништавање примерка, (2) дају лошу просторну слику унутрашњих карактеристика и (3) захтева време и рад. Многи до сада проучавани таксони са овом техником су засновани на веома малом броју примерака (понекад на само једном примерку), тако да је само делимично проучавана унутрашња морфологија, док је варијабилност често занемаривана, тако да је отворена номенклатура често примењивана.

Овом приликом, употребом нове и сасвим напредне методе, приказана је пробна студија за

проучавање кардиналијума фосилних брахиопода. Синхротронска X-зрачна томографска микроскопија (SRXTM) је недеструктивна техника за проучавање и сагледавање унутрашњих особина кад чврстих и непрозрачних објеката, која омогућава потпуну реконструкцију тродимензијалног изгледа унутрашних структура на основу снимања разлика у ефекатима енергетских зракова као реакција тих структура. Она користи синхротрон (врста акцелератора) као извор монохроматских X-зрака. SRXTM може да производи томографске податке изузетне резолуције и јасноће (SUTTON 2008). Употребом SRXTM добили смо тродимензионалне податке о унутрашњој морфологији два фосилна брахиоподска таксона: (1) ринхонелид "*Rhynchonella*" *flustracea* SCHLOTHEIM из палеогена Faxe Quarry у Данској и (2) теребратулид *Terebratulina imbricata* OWEN из доњег ценомана северне Бугарске. Ове анализе су обављене код Swiss Light Source (SLS), Институт Паул Шерер, Вилиген, Швајцарска. Код "*Rh.*" *flustracea* mogli смо да посматрамо облик крура (види стрелице код сл. 4) која је помогла да докажемо нашу предпоставку да ова врста припада новом басилиолидном роду (који ће бити ускоро уведен), док код *T. imbricata* било је могуће да добијемо детаље теребратулидске петље која формира прстен. SRXTM је раније била употребљивана за добијање тродимензионалних података, упознавање функције и раста пункти љуштуре и бољег разумевања улоге цитологије у контексту биоминерализације рецентних брахиопода (PÉREZ-HUERTA *et al.* 2009). Приказани су охрабрујући резултати употребом SRXTM за основне таксономске сврхе: покушај да се нађе замена за методе серијских пресека као и других метода сечења. Скенови су урађени у најнижој резолуцији тако да се ова метода може користити и са више прихваћеним X-зрачним микротомографским скенерима које поседују неколико институција у свету. X-зрачна томографска микроскопија је сада рутинска метода за неколико других фосилних група (SUTTON 2008).

Веома често, због недостатака добро очуваних примерака и деструктивне технике, неки аутори користе оштећене примерке за серије пресека, чувајући најбоље очуван примерак за холотип. У будуће холотипови могу бити приоритетни предмет оваквог начина проучавања у циљу провере њихове конспецифичности са сеченим паратиповима. X-зрачна томографска мокроскопија пружа могућност за проучавање унутрашњости многих таксона уведених само на основу спољашњости, потврђујући или оспоравајући њихову таксономску припадност.