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# Dinosaur and Crocodile Fossils from the Mesozoic of Portugal: Neutron Tomography and Synchrotron-Radiation Based Micro-Computed Tomography

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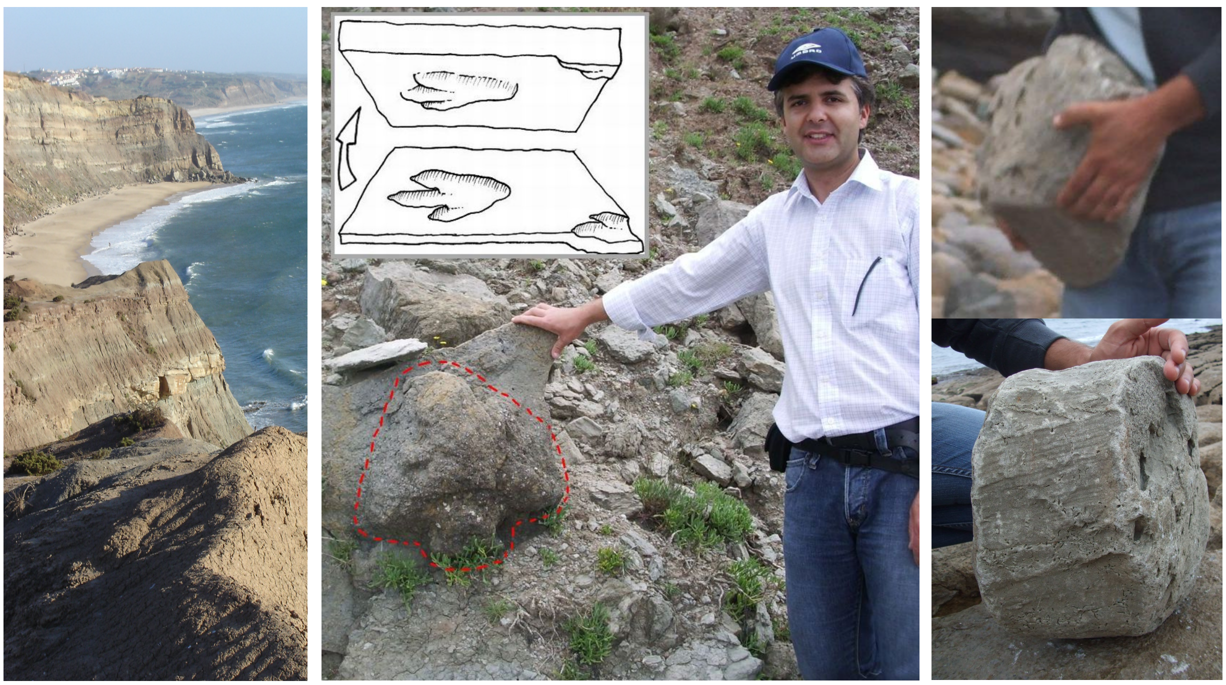
## ABSTRACT

Portugal is ranked within the 10 countries with the most dinosaur taxa and the Lourinhã Formation is known by the Late Jurassic findings of dinosaurs and other fossils. In many cases, studies of the external morphological characteristics of the fossils are not sufficient to extract all the information for a paleontological study and, thus, observations of internal structures, using non-destructive techniques, are required. The fossils studied in the present work belong to the Museum of Lourinhã. The access to the Geesthacht Neutron Facility in Germany allowed us to characterize a jaw of the dinosaur *Baryonyx walkeri* specimen and the jaw of a crocodile (possibly a *Tomistomidae*) by Neutron Tomography. The study allowed us to detect the presence of teeth inside the jaws and it provides valuable information about the development of its dental characteristics. Synchrotron radiation based micro-computed tomography studies on tiny samples have been performed at the beamline HARWI II operated by the Helmholtz-Zentrum Geesthacht at the storage ring DORIS III at the Deutsches Elektronen–Synchrotron DESY in Hamburg, Germany. The first data recorded for eggshells collected in the Lourinhã Formation is shown. It allowed us to visualize the morphology of the pores and their connectivity in the eggshells, providing information that is either exceedingly difficult or impossible to obtain by traditional methods based on section cutting.

## INTRODUCTION

The history of life on earth is generally conceptualized as a series of long-lived eras. The Mesozoic era (251 to 65.5 My) was characterized by the success of coniferous plants and amniote vertebrates on land and cephalopod molluscs (e.g. ammonites and belemnites) in oceans. This era is also known for the appearance and evolution of dinosaurs and by the mass extinction at the end of the Cretaceous period of: non-avian dinosaurs, ammonoid cephalopods and belemnites. This era saw also the rise of angiosperms (flowering plants) [1].

The Lourinhã Formation exposed in the area around Lourinhã (figure 1a), central west Portugal, is part of the Lusitanian Basin and consists of terrestrial sediments, deposited during the initial rifting of the Atlantic in the Kimmeridgian to Tithonian [2]. It contains a rich vertebrate fauna comprising fish, amphibians, turtles, mammals, pterosaurs, crocodiles, sauropods, theropods, ornithopods and thyreophorans [3-7] as well as dinosaur nests, eggs and embryos [8-10]. Furthermore, the nature of the deposits with abundant shifts between flood-plain muds and fluvial sands creates a good environment for preservation and fossilization of tracks. Within the last years, several well-preserved tracks of sauropods [11], theropods, thyreophorans and ornithopods have been found, both preserved *in situ* on the sedimentary surfaces and on blocks fallen from the steep coastal cliffs [12]. Although some are now on display at the Museum of Lourinhã other can still be found along the coastline of the area of Lourinhã (figure 1b and figure 1c).



(a) (b) (c)

**Figure 1**. Photographs taken in the area of Lourinhã: (a) Paimogo – view to South; (b) one of the dinosaur "tracks" at the coastline (preserved infill of the original track). The inset shows an illustration of the formation of the vestige (photograph by V. Martins and illustration by S. Mateus); (c) the infill of a vertical-walled track with marks of the striations of the skin scales (see bottom photograph), suggesting that the movement of the dinosaur manus impacting and exiting the mud was vertical with no horizontal component of the stride (photographs by S. Garção).

The area of Lourinhã is indeed very rich in fossils of dinosaurs of the Late Jurassic. It was in this area (at Porto das Barcas) that the first dinosaur fossils were found in Portugal, by Carlos Ribeiro, on 20 July 1863 (two theropod teeth), as noted by Lapparent and Zbyszewski [13]. Furthermore, the only dinosaur embryos known in Europe have also been found in the Lourinhã Formation. One of the most relevant specimens of the Museum of Lourinhã is the nest with embryos of *Lourinhanosaurus antunesi* found in Paimogo [8]. In this nest more than 100 eggs or eggshell concentrations were detected. The discovery of exquisitely preserved dinosaur embryos allows a solid taxonomical identification helping also to understand the ontogeny, evolution and reproductive strategy in this type of dinosaurs [9]. Eggs, eggshells and even embryos from the Lourinhã Formation localities of Peralta and Porto das Barcas are also part of the collections of the museum [10].

Among the fossils of other ages and sources that incorporate the collections of the Museum of Lourinhã, an emphasis is given to the cranial and postcranial material of the theropod dinosaur *Baryonyx walkeri*. Remains of *Baryonyx* (Saurischia, Theropoda) have been found for the first time in clay pits just south of Dorking, England, and later reported from fossils found in northern Spain and in Portugal (at the Barremian of Boca do Chapim, Lisbon and Setubal Province, Portugal) [14]. Its fossils have been collected from formations dating from the Hauterivian to early Barremian stages of the early Cretaceous Period, approximately 130 to 125 My ago. Recently, cranial and postcranial remains of a crocodile discovered in the region of Coimbra, Portugal, integrated as well the collections of the Museum of Lourinhã. The recovered material suggests that it can be associated with a *Tomistomidae*. However, further studies are required in order to draw the final conclusions.

The science of paleontology endeavors to reconstruct life on Earth as exposed by fossil remains. Fossils were known to the ancient Greeks but the experimental study of fossilized organisms can be traced back only a few hundred years [1]. Each fossil represents vestiges of long-extinct organisms, supplying clues about past biotas that can be reconstructed only by carefully examining their fragmentary paleontological remains. In the last two decades, a growing interest in understanding dinosaurs as living animals has generated considerable discussion in the scientific community about their reproductive biology. In modern taxa, the physical characteristics of eggs are closely correlated with the type of incubation environment, thereby providing important insights into physiology and nesting strategy [15,16]. Likewise, fossil eggs and the rocks in which they are found provide valuable information about extinct taxa. Although reproductive behaviors may be difficult to interpret, fossil eggs and nesting horizons are important for understanding dinosaur reproductive biology, their success throughout the Mesozoic Era, and the evolutionary transition from dinosaurs to modern birds.

The collections of the Museum of Lourinhã provide new information for the evolutionary history of development. However, the potential of this material has not been fully realized because of reliance on traditional, non-destructive methods that allow analysis of exposed surfaces only, and destructive methods that preserve only a single two-dimensional view of the interior of the specimen. X-ray imaging techniques are very promising since they are non-destructive. X-ray projection radiography has been the first X-ray technique used historically. In this method, an X-ray beam penetrates the object and is attenuated due to interactions with the electrons of the material and an X-ray-sensitive emulsion or detector behind the object pick up a two-dimensional image of the object. Unfortunately, it is frequently not sufficient to give significant data on a thick or dense specimen. Moreover, the lack of the third dimension largely limits the exploitation of data. Computer Tomography (CT) [17] provides three-dimensional images of fossils and offers an unprecedented insight into their internal morphology [18,19]. By using neutrons [20,21] from nuclear reactors or X-rays generated by synchrotron radiation sources at storage rings [22], hitherto invisible structures can be revealed which are not accessible to conventional tomography based on standard X-ray tube sources. Here, we have applied Neutron Tomography (NT) to study a jaw of the dinosaur *Baryonyx walkeri* and a jaw of a crocodile (possibly a *Tomistomidae*). Additionally, eggshell fragments have been studied by Synchrotron-Radiation based Micro-Computed Tomography (SRμCT). In both cases complete three-dimensional recordings have been obtained using a non-destructive procedure.

The absorption profile of X-rays and neutrons is very distinct. X-rays interact mainly with the electron shell of atoms. X-ray attenuation coefficients increase with atomic number Z and, thus, the tomographic images display differences in density and material Z composition within an object. Neutrons interact mainly with atomic nuclei and, contrary to X-rays, they interact significantly with some light materials (e.g., materials containing large amounts of hydrogen, boron or lithium) and penetrate heavy materials with minimal attenuation [21]. NT is hence appropriate for the imaging of organically preserved fossils (e.g. plants) but it may also be very useful for the study of larger fossils since the maximum cross-section of rocks and fossilized bones that can be penetrated by neutrons is higher than by X-rays. However, NT resolution is normally lower than that of X-ray CT in terms of minimum voxel size. Furthermore, high-intensity neutron bombardment can induce hazardous levels of radioactivity in some geological materials, for example, those containing cobalt or europium.

Synchrotron-radiation facilities producing hard X-rays provide high quality microtomographic images when compared to conventional X-ray tube sources [23,24]. High intensity and strong collimation of the synchrotron-radiation beam allow rapid data acquisition at very high spatial resolutions and the application of simple reconstruction techniques (parallel beam). The photon energy can be optimized for the examination of each sample using monochromators, and due to the small bandwidth of the resulting photon spectrum, non-linear effects ("beam hardening"), that are observed in conventional CT, can be avoid in SRμCT. However, limits exist in the technique, for example in the size of the specimens investigated. Therefore, the decision of which technique to use (X-ray CT or NT) should be dictated by the research questions, the preservation and material properties of the fossil.

## EXPERIMENTAL DETAILS

The set-up for NT was installed in the irradiation chamber of GENRA-3 of the Geesthacht Neutron Facility. GENRA-3 was designed for industrial applications. For this purpose a special collimator has been mounted in GENRA-3 which allows a very homogeneous illumination of the sample combined with a very large cross section up to 45×45 $cm^2$. A detailed description of the tomographic apparatus can be found in Ref. [25]. It consists of a two-dimensional neutron detector and a high precision sample manipulator that can be fixed at different distances to the source. Therefore, an optimal value of L/D with respect to the size of the sample can be selected. This is required to obtain a good spatial resolution in the tomogram. The distance L is the distance between the neutron aperture with diameter D = 2 cm and the object (collimation ratio: 100 to 300). Thus, the L/D fraction represents the divergence of the beam and is used as a quality value. More information about high-resolution neutron imaging experiments with very compact and efficient neutron collimators can be found in Ref. [26]. The experiments were run with a L/D of 150, a beam size of 24×24 $cm^2$ and a flux of $3.2\times10^6$ n $cm^{-2}$ $s^{-1}$. During the experiments the samples were exposed to the neutron beam at different sample rotation equally stepped (0.25°) between –180 and 180°. The exposure time of each image was 16 seconds. The samples studied were a *Baryonyx walkeri* jaw and a jaw of a crocodile, probably a *Tomistomidae*. A spatial resolution of about 400 μm was achieved.

For the evaluation of the NT data, as in the case of the SRµCT studies, slices perpendicular to the rotation axis were reconstructed from the single projections by a tomographic reconstruction algorithm using "filtered backprojection." The slices were then collected in an image stack that can be visualized, edited and exported into different file formats using a three-dimensional rendering software. In this work, the visualization of the morphology of the samples has been carried out by means of the software VGStudio Max 1.2.1 (Volume Graphics, Heidelberg, Germany).

The SRµCT measurements were carried out at the DORIS III storage ring (at beam line HARWI II) of the Hamburger Synchrotronstrahlungslabor (HASYLAB) at the Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany. The technical details of the beamline have been published by Beckmann et al. [27,28] and in the book by Reimers, Pyzalla, Schreyer, and Clemens [29]. Recent information can be found in J. Herzen Ph.D. thesis work [30]. The tomography set-up consists of a high precision rotation stage, which allows us to mount the sample hanging from above, and an X-ray detector. In the detector a fluorescent screen converts the X-rays into visible light that is magnified by a lens and detected by a CCD camera. The specimens were imaged by microtomography in absorption mode with photon energy of 37 keV. More information about the modes to achieve good contrast between different materials inside an object can be found in Ref. [30]. For acquiring the X-ray attenuation projections the sample was rotated between 0 and 180° in equidistant steps of 0.25°. Here, the effective pixel size corresponds to 6.4 µm, which allows a direct, non-destructive visualization of the morphology of the pores and their connectivity in the eggshell fragments.


(a) (b)

**Figure 2.** Photographs of dinosaur eggshell fragments: (a) collected in Peralta focusing the internal surface of the eggshell (bottom) and the external surface of the eggshell (top); (b) collected in three different localities of the Lourinhã Formation mounted on the sample holder used for SRµCT.

The eggshell fragments have been collected in three different sites of the Lourinhã Formation, namely: Peralta, Paimogo and Porto das Barcas. Figure 2a shows a photograph of two eggshell fragments collected in Peralta focusing the inner surface of the eggshell (bottom) and the outer surface of the eggshell (top). The photograph presented in figure 2b shows eggshell pieces collected in the three different localities mounted on the sample holder used for SRμCT. The eggshell type detected in Paimogo, of thickness of about 0.92 mm [31], is ascribed to the theropod *Lourinhanosaurus antunesi*. This specimen belongs to the nest found in Paimogo, which contains embryos. The external morphology and the size of the eggshells of Paimogo and Peralta sites are comparable. The Peralta site is less than 800 m from the type locality of *Lourinhanosaurus* and within the same stratigraphic range. They can be from the same species or a closely related taxon. The eggshell fragment from Porto das Barcas has been detached from the clutch of approximately 65 cm diameter that was found south of Porto das Barcas presenting bones and teeth of a saurischian dinosaur. The eggshell fragments collected in this site exhibit a thickness of about 1.23 mm and a different type of morphology.

## DISCUSSION

The first results obtained by NT for a jaw fragment of the *Baryonyx walkeri* remains from the Museum of Lourinhã as well as for a crocodile, possibly a *Tomistomidae*, are presented in this section. Furthermore, the first data obtained for eggshells, recorded using the SRμCT technique, of the Lourinhã Formation is shown. The results demonstrate that this technique is an invaluable investigation tool for paleontologists.

### Neutron Tomography (NT)

*Baryonyx* lived in the Early Cretaceous within other groups of dinosaurs in the Iberian region, which may have formed a biogeographical "stepping-stone" for baryonychine dispersal between Europe and Africa [14]. It was known for its long low snout with narrow jaws filled with finely serrated teeth and for the gaffe-hook-like claws. Due to its teeth and snout morphology *Baryonix* presents strong analogies with crocodiles [32]. The upper jaw had a sharp angle near the snout, a characteristic seen in crocodiles, which helps to prevent its preys from escaping. This dinosaur had much more teeth than its relatives. Thirty-two large ones were placed in the upper jaw (maxilla) and sixty-four in the lower jaw (mandible).

Figure 3 shows results obtained for a fragment of a jaw of the *Baryonyx* remains from the Museum of Lourinhã. The specimen ML 1190 has a length of roughly 162 mm (figure 3a), which is the main reason for the selection of NT as the appropriated technique for its study in order to obtain a complete overview of the fossil. NT allowed imaging of the internal structures providing valuable information concerning the presence of teeth, their shape and development. The three-dimensional view shown in figure 3b of the front part of the jaw shows two of the small teeth that are still inside. In figure 3d it is possible to distinguish a large tooth that it is also still inside the jaw and visualize its shape. This last image has also been selected in order to show that it is important the users understand that artifacts can occur. Several ring artifacts are perceptible in this tomographic reconstruction. The users should be able to identify and separate them from the real morphology of the sample. Streaking, shading, rings and distortion are artifacts that can occur in these image techniques. To optimize image quality, it is necessary to understand why artifacts occur and how they can be prevented or suppressed. Detailed information on this topic can be found in Ref. [17] and Ref. [33]. Figure 3e shows as well a slice through the jaw, which reveals several teeth in the jaw.

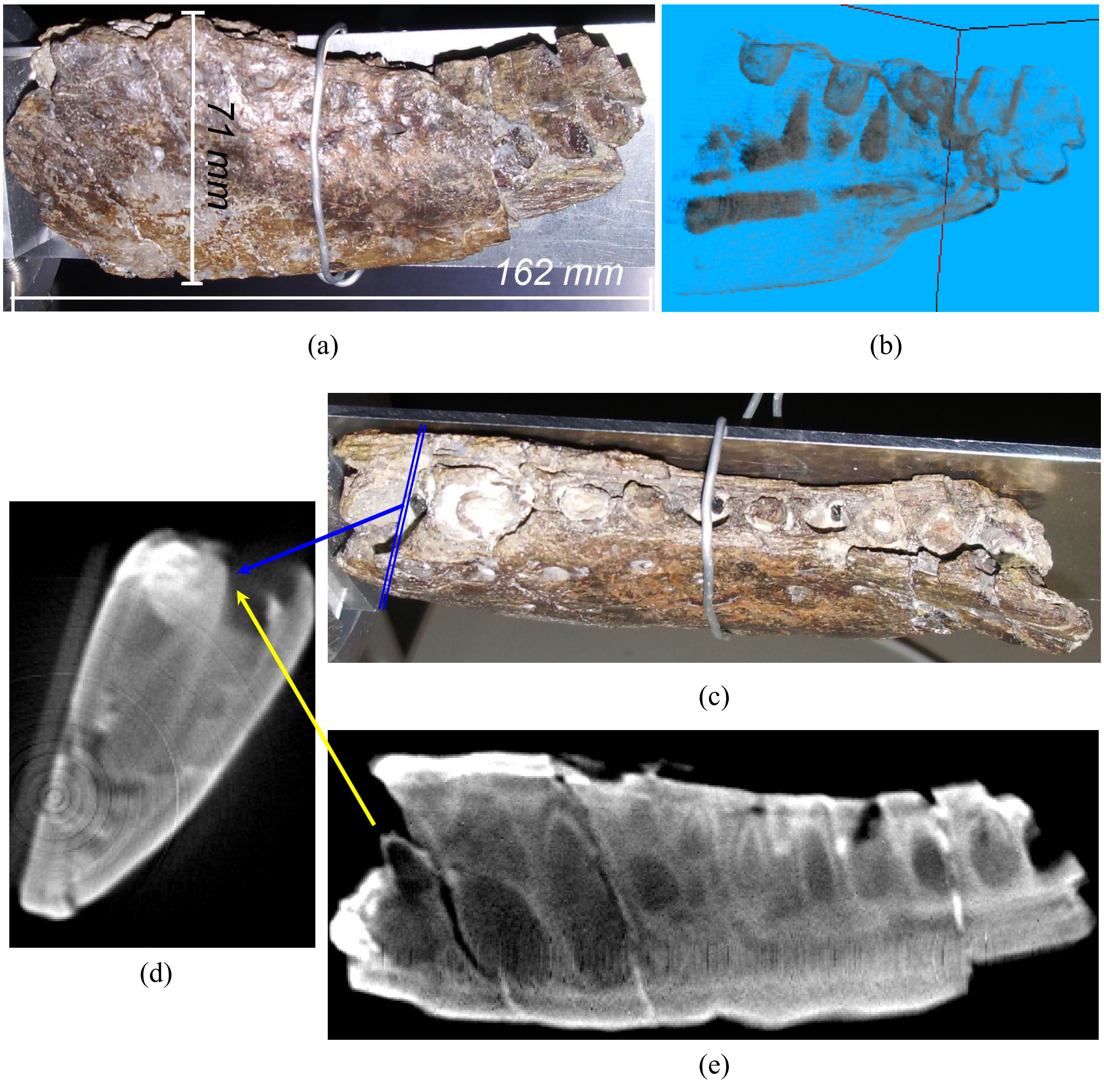
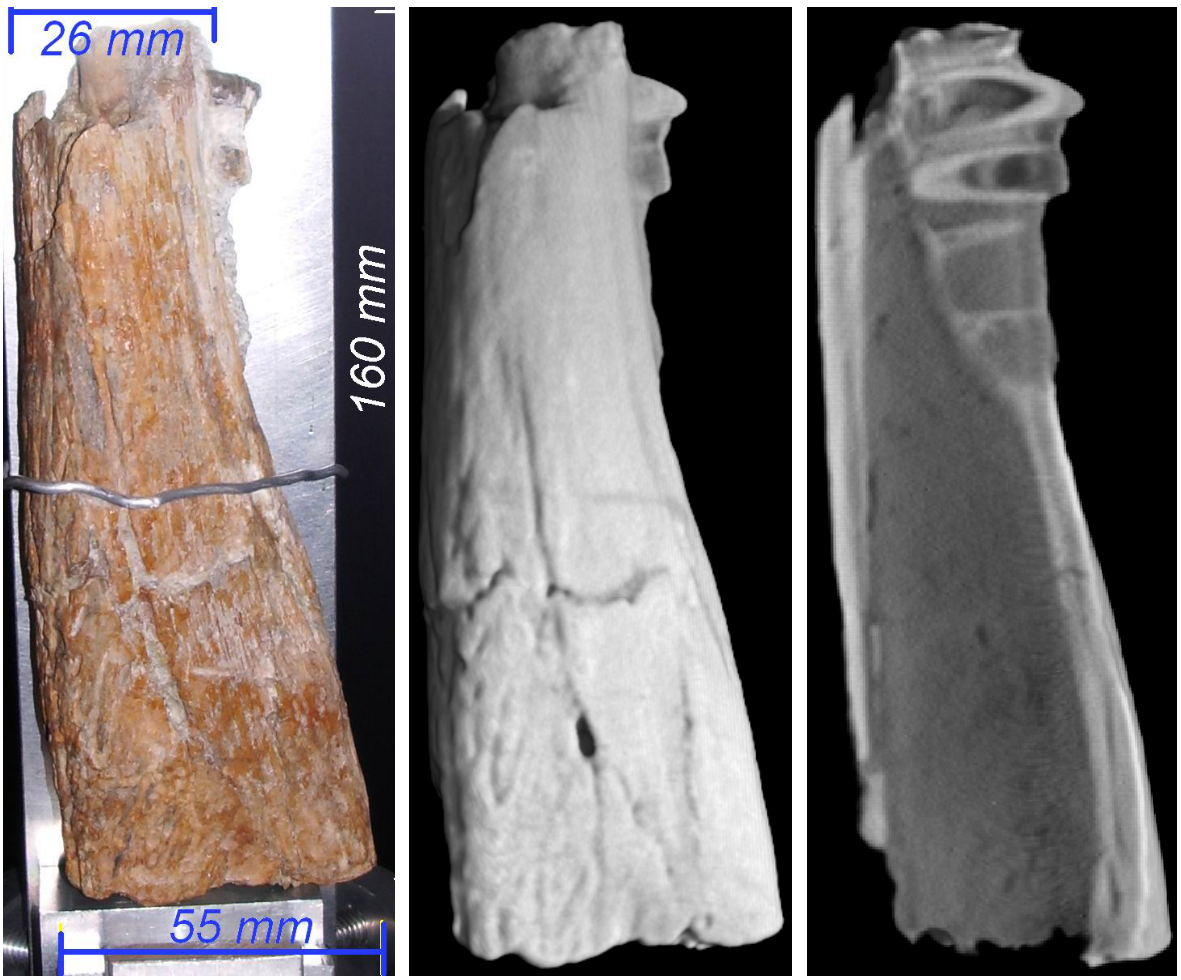



**Figure 3.** *Baryonyx walkeri* jaw (ML1190): (a) photograph with the indication of the dimensions of the jaw; (b) three-dimensional view of the front part of the jaw showing two of the small teeth that are still inside; (c) photograph of the area where the teeth appear; (d) slice focusing a large tooth still inside the jaw (several ring artifacts are visible in the figure); (e) slice showing the overall internal morphology. The tomography data allow virtually cutting the jaw in any desired direction.

The researchers in charge of this work, at the Museum of Lourinhã, will continue the evaluation of the data collected for this sample. The study of dental development and their microstructure provide relevant information: life history, ageing, growth, dentition process, diet, mechanical adaptations etc. Thus, it is most likely that this work will supply very useful information about this type of dinosaur and, therefore, it will be disseminated in future publications. It is not in the scope of the present paper to describe any fossil specimen in detail.

A photograph of the overall fragment of the jaw of the crocodile that has been examined during the present work is shown in figure 4a. As in the case of the jaw of the *Baryonyx* it required the use of the NT technique for the visualization of the morphology of the whole sample (length of about 160 mm).




(a) (b) (c)

**Figure 4.** *Tomistomidae* (?) jaw: (a) photograph with the indication of the dimensions of the jaw; (b) three-dimensional-representation by VGStudio Max 1.2.1; (c) sagittal section.

Figure 4b shows a three-dimensional image of the jaw of the crocodile obtained with the software VGStudio Max 1.2.1 and a selected tomogram is presented in figure 4c. Tomography has been as well in this case an effective tool for imaging the internal structure of the fossil. The collected data will allow us to extract important information about the dental details of this species. Together with the data record for other anatomical parts (data not shown) it will be a precious source of information for helping the paleontologists to correctly identify the species and to prepare a detailed description of the species.

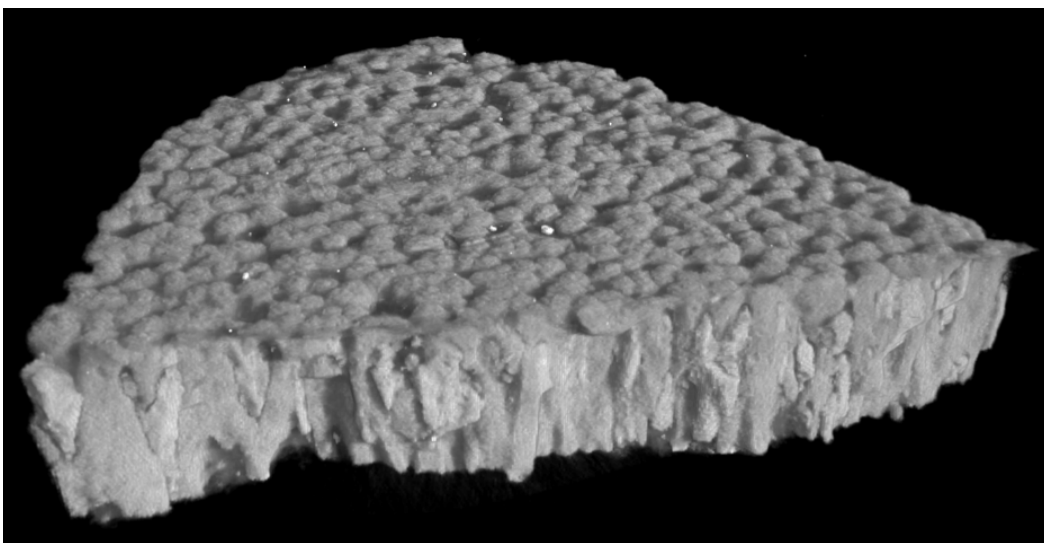
To finalize this subsection dedicated to results obtained by NT, the authors would like to make a few statements based on the experience acquired during the preparation and execution of this type of experiments dedicated to the study of fossils:

- NT is a technique suitable for the study of larger fossils since the maximum cross-section of rocks and fossilized bones that can be penetrated by neutrons is higher than by X-rays;
- a detailed identification of the elements present in the specimen should be performed prior to neutron bombardment since it can induce hazardous levels of radioactivity in some geological materials. The researches aiming to perform such type of tomography should contact the staff of the nuclear reactor to discuss the elements present in the fossil and the feasibility of the experiment. The instrumental neutron activation analysis technique is a valuable tool when a very small portion of the sample can be removed (normally as solid ranging from mg to several grams). In this technique the small sample is irradiated with neutrons. The characteristic gamma rays emitted by the decaying radionuclides of the irradiated sample are quantitatively counted by high-resolution radiation detectors allowing the identification of the elements as well as their concentration;
- NT is sensitive to differences in the concentration of some light materials like hydrogen, boron and lithium. Therefore, the image quality is strongly influenced by the resin materials used for the reconstruction and conservation of the objects. However, this is not always a disadvantage. It can be used to investigate the nature of historical preparation of a fossil found in a museum. This technique would allow us to identify parts that have been glued or even modeled with resin materials;
- metal inclusions in fossils lead to a strong scattering of X-rays, whereas these inclusions can be penetrated and therefore lead to less disturbing contrasts in NT. Highly X-rays attenuating metal objects often lead to under-range in the data acquisition electronics. Image artifacts similar to photon starvation appear which is dominated by high-intensity streaks. Together with beam hardening artifacts originated by the metal, both shading and streaking artifacts are visible in the reconstructed image [17];
- this method can be used prior to specimen's preparation to identify fine and delicate bone structures, which can be destroyed through mechanically preparation before they are even discovered.

## Synchrotron-Radiation based Micro-Computed Tomography (SRμCT)

The study of fossilized eggshells is a very important topic for the Museum of Lourinhã [10]. This type of fossil can provide precious information about the organisms that laid the eggs and the depositional environment in which eggshell has been preserved. Although the eggshells fragments are subject to alteration, associated with some processes of transport and destruction due to its physical and chemical composition, unique features can be preserved. Their internal eggshell structure is often still marked, despite mineralogical alteration. The structure of the eggshells (shape, size, porosity) may be related not only to the environment of incubation but also to climatic conditions. The eggshells pores pattern is related to the gas exchange ratios between nest environment and embryo, in which a very porous eggshell is an indicator of a humid environment [34]. Eggshells exhibiting lower porosity and water vapor conductance are usually associated with a more bird-like uncovered incubation of eggs. Since for embryo survival in arid climates minimal water loss would be essential, low porosity has also been linked to these types of climates. In conclusion, the histostructure of the eggshell provides information with biological and paleoenvironmental implications. Therefore, it is very important to gain insight into the pore structure of the fossilized eggshells through non-destructive techniques.

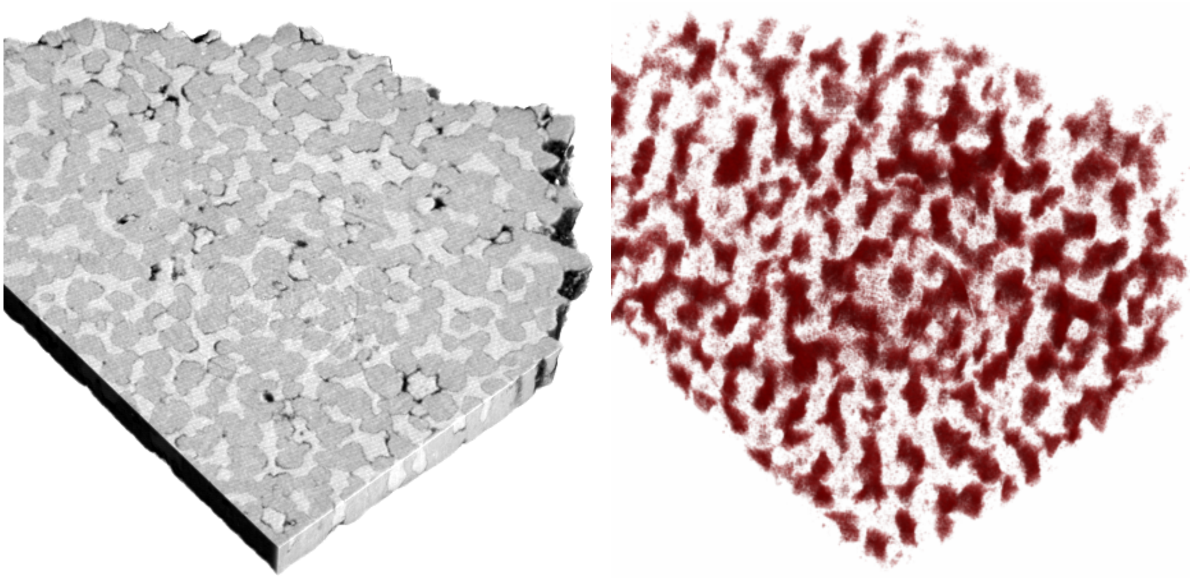
To date, the techniques used for the study of eggshells of the collections of the Museum of Lourinhã were based on thin sectioning and high resolution optical or electron microscopy. These methods are destructive and have the potential for introducing artifacts. Based on these traditional destructive methods, preliminary results on the eggshells from Paimogo show that they correspond to obliquiprismatic morphotype [31], similar to those from Morrison Formation from Colorado and Utah (USA). The eggshells from Peralta are likely ascribed as well to obliquiprismatic morphotype (column: 0.56 mm and mammilla: 0.21 mm), possibly related to Paimogo's nest taxon (*Lourinhanosaurus*). It presents an ornamentation, with a pore diameter similar to Paimogo (0.16 mm). The eggshells from Porto das Barcas possibly represent another theropod, and belong to discretispherulitic morphotype. They present prolatocanalicute pore system and ramotuberculate ornamentation.



(a)

(b)

(c)



(d)

**Figure 5**. Eggshell fragment collected in the locality of Porto das Barcas: (a) a three-dimensional image of the complete sample used for the present study; (b) a microtomographic slice through the eggshell; (c) sectioning of the three-dimensional image of the sample of figure 5a providing information about pore connectivity in the eggshell; (d) three-dimensional representations of a fraction of thickness of about 0.615 mm of the sample (total thickness of roughly 1.23 mm). Pore canals run through the eggshell, which permit gas exchange between the embryo and atmosphere.

Here, SRµCT has been applied to eggshell fragments of dinosaurs collected in Peralta, Paimogo, and Porto das Barcas (see figure 2). The high quality data show that this is an extremely useful technique for non-destructive imaging of the morphology of eggshells. It proved to be very effective for the acquisition of three-dimensional images with high spatial resolution, revealing otherwise inaccessible structures such as the three-dimensional internal structure of pore (see figure 5). The visualization of the connectivity of the pore structure is also a plus for a complete and accurate characterization of this precious material found in the Lourinhã Formation, in Portugal. The data collected at the beam line HARWI II is now in evaluation and a detailed description of the eggshells and a comparison between the results obtained for the eggshells of the different localities will be published elsewhere.

The aim of the paleontologists is to obtain the maximum knowledge about the fossils they are working on. However, the decision of which technique to use should be dictated by the research questions, the preservation and material properties of the fossil, as well as the costs associated with the technique. At the end of this subsection, reserved for the presentation of results recorded by SRµCT, the authors would like as well to make a few remarks:

- medical CT scanners are broadly available in many medical institutions, whereas SRµCT is available only in few places. Nevertheless, medical CT is mainly optimized for human-scale objects (the scanners have minimum voxel sizes of several hundred microns) although it can be used to study large vertebrate fossils in order to visualize the material within the matrix or to create three-dimensional models that are required for functional studies. Medical CT scanners are thus not appropriated to study small objects such as most remarkably conserved invertebrate fossils;
- synchrotron-radiation facilities sources offer very high flux, the access to monochromatic or white radiation, and a high degree of coherence. They have led to the very highest spatial resolution and highest density resolution achieved to date [35]. However, the potential, convenience and economy of laboratory X-ray tube sources should not be neglected [19]. The scanners developed in the last decade, initially designed for engineering and materials applications, are also a very useful tool for scanning small fossils. The spatial resolution achieved by some of these sources can often compete with that achieved by synchrotron facilities and can be very suitable for the study of some of the fossils [24];
- millimeter sized specimens, too dense to be penetrated by the conventional X-ray sources, or with very low X-ray attenuation contrast, most likely require a synchrotron source. Again, the authors suggest the paleontologists to contact the staff working on the different types of X-rays sources to discuss the experiments most feasible for studying their important fossils.

## CONCLUSIONS

The methods applied here for carrying out tomography are non-destructive and allow us to visualize the internal morphology of a large variety of fossils. The high-quality tomographic datasets can be effectively studied through interactive digital visualization. Although there are some limitations, these types of studies provide a direct window into the evolutionary history of organisms.

With a vast array of fossilized bone, tracks, eggs and embryonic remains, the Museum of Lourinhã has been able to do cutting-edge research in vertebrate paleontology. The Museum of

Lourinhã started this project on tomography because this “virtual paleontology” approach is a powerful tool to share information of precious well-preserved fossils within the scientific community, which augment the ability of researchers to extract maximal data from our most rich resources. At the moment the authors of the present study are working on the characterization of the first dinosaur embryonic remains reported in Europe, which have been found at the Paimogo site (Lourinhã Formation), using SRμCT.

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