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3D digital imaging of a concretion-preserved batoid (Chondrichthyes, Elasmobranchii) from the Turonian (Upper Cretaceous) of Morocco

Imagerie numérique 3D d'un batoïde (Chondrichtyens, Elasmobranches) préservé dans un nodule du Turonien (Crétacé supérieur) du Maroc

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ABSTRACT

We describe the manual and digital methods used to prepare an exceptional fossil specimen, as well as the composition of this specimen revealed by these methods. The fossil, a rhinobatoid, is 3-dimensionally preserved in a concretion. Fossils like these are seldom encountered, because flat-bodied animals are traditionally preserved in lithographic beds, or more commonly, are only represented by disassociated dentition. Manual preparation was best conducted with needles and a local application of buffered formic acid and neutralised sodium carbonate. High-resolution computed tomography and post-analysis using the invert ramp option in VGStudio Max 2.0 produced the best results to see the complete skeleton of this specimen. The specimen is distinguishable from the only other known 3D preserved fossil rhinobatoid, the Lower Cretaceous (Albian) genus †*Iansan*, and is probably a member of Platyrhinidae.

RÉSUMÉ

Nous décrivons ici les méthodes manuelles et numériques utilisées pour la préparation, l'observation et l'étude de la composition, d'un spécimen fossile exceptionnel. Ce fossile, un chondrichtyen rhinobatoïde, est préservé en trois dimensions dans une concrétion nodulaire. Une telle préservation est rare, car ces animaux à corps plat sont généralement retrouvés dans des niveaux lithographiques, ou plus communément par leurs restes dentaires isolés. La préparation manuelle de ce spécimen a été optimisée grâce à l'utilisation d'aiguilles et d'une application locale d'acide formique dilué et neutralisé avec du carbonate de sodium. Des acquisitions tomographiques à haute résolution et des analyses de post-traitement utilisant l'option d'histogramme inversé (*invert ramp*) de VGStudio Max 2.0 ont produit les meilleurs résultats pour la visualisation du squelette complet de ce spécimen. Ce batoïde est différent du seul autre fossile de rhinobatoïde conservé en trois dimensions, le genre †*Iansan* du Crétacé inférieur (Albien), et est probablement un membre des Platyrhinidae.

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1. Introduction

Chondrichthyans are an interesting group to study for several reasons, not the least of which is the nature of their skeleton. The chondrichthyan skeleton is composed almost entirely of cartilage, unlike their bony relatives the osteichthyans. The cartilage in the chondrichthyan skeleton does mineralise and harden to a degree, although mineralisation is differential, depending on the skeletal region and the taxon (Dean and Summers, 2006). Preservation of this partially mineralised tissue in the fossil record is, thus, minimal. On certain occasions, however, the chondrichthyan skeleton can be exceptionally preserved, as in the case of the specimen we examine here.

It is important to choose the appropriate methods of specimen preparation, especially when there is such a scarcity of material. Palaeontologists increasingly rely on computed tomography (CT) to examine internal structures or morphology of fossil specimens that run a particular risk of deformation or destruction from conventional preparation (Anderson et al., 2003; Balanoff and Rowe, 2007; Ketcham and Carlson, 2001; Rowe et al., 1997). Its applications reach widely into extant biology and have been a useful tool for studying extant chondrichthyans as well (Claeson, 2008; Dean et al., 2006; Maisey, 2001; Maisey et al., 2007). This technical article is a description of the manual and digital methods used to prepare this specimen, and the composition of the specimen revealed by these methods. We make suggestions on some of the most effective ways to work with similar material.

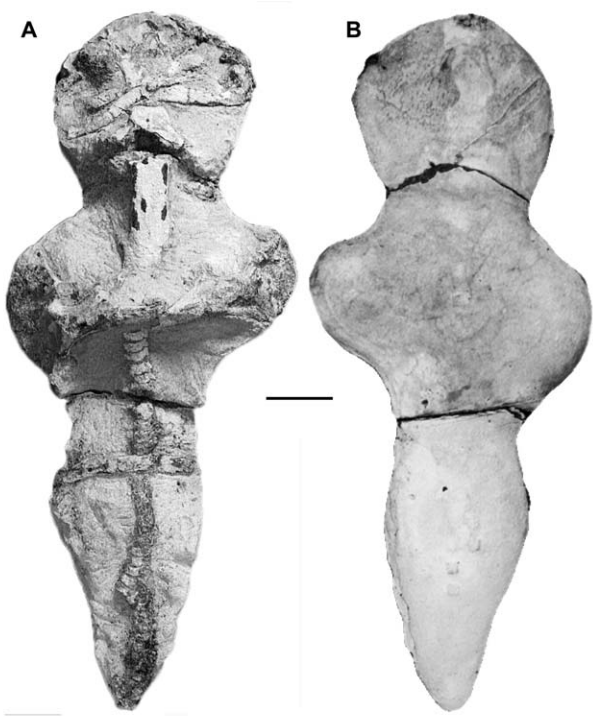

Fig. 1. Specimen NHM P. 66857. A. Ventral view. B. Dorsal view. Scale bar = 1 cm.
Fig. 1. Spécimen NHM P. 66857. A. Vue ventrale. B. Vue dorsale. Barre d'échelle = 1 cm.

2. Materials and methods

The specimen comes from the Middle Turonian of the Goulmima region, South East Morocco (Kennedy et al., 2008) and is housed at the Natural History Museum in London (NHM P.66857). This region yields abundant and exceptionally well-preserved fossils from at least two levels of concretions in a marl unit within an otherwise shallow water to peritidal carbonate succession (Cavin and Dutheil, 1999). Most of the concretions contain ammonites, occasionally teleost fish and rarely marine reptiles. Skeletal remains of batoids are present but rare (Cavin et al., 2010), and this specimen represents one of the most completely preserved chondrichthyans known from the Mesozoic.

The specimen was preserved in a hard carbonate concretion (Fig. 1) and the ventral surface of the specimen was partially exposed. Mechanical preparation using needles and pneumatic tools expose and reveal large portions of the ventral surface of the specimen. The specimen was subsequently placed in a bath of buffered 7.5% volume/volume formic acid at a pH of 3.3. The reaction was halted after only a few minutes because it proceeded more rapidly than expected when compared with similarly preserved bony fish from the same locality. This exposed a further portion of the ventral surface, including in situ teeth and dermal denticles (Fig. 2). Acid preparation also detached some prismatic cartilage tesserae from the exposed skeleton and its use was suspended in favour of more sensitive preparation techniques.

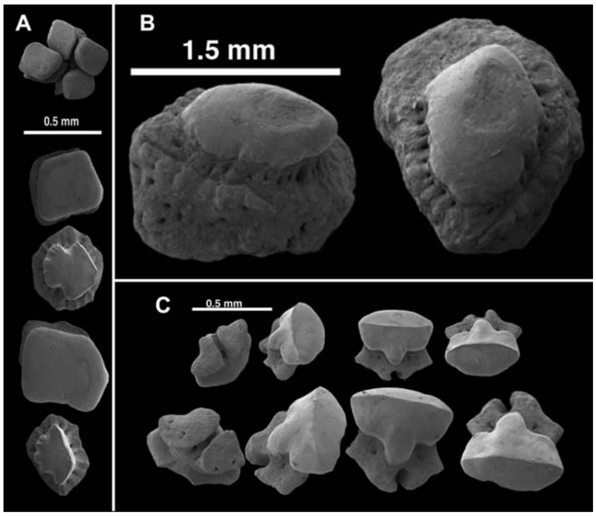

Fig. 2. Dentition and denticles. A. Smallest and moderate sized denticles. B. Largest denticles. C. Dentition, small and large.
Fig. 2. Dentition et denticules. A. Denticules de tailles moyenne et petite. B. Denticules les plus grands. C. Dents isolées de petites et grandes tailles.

Small areas, particularly the rostrum and the olfactory capsules, were cleared of matrix using a combination of acid and mechanical preparation. Buffered formic acid was applied locally, in single drops by pipette and neutralised by 10% weight/volume sodium carbonate solution. Partially prepared areas of cartilage, thus exposed, were cleaned of matrix using a mounted needle under a binocular microscope before the cycle was repeated.

When further acid and mechanical preparation were no longer practical, computed tomography methods were used to further examine this specimen. The specimen was scanned at the High-Resolution Computed Tomography Laboratory at The University of Texas of Austin (UTCT) using their highest energy scanner. This particular scanner was used because it exceeds details attainable by conventional medical scanners. Also, it is capable of scanning large specimens of up to 30 cm in diameter. This specimen was not a candidate for neutron scans, because too much natural background radiation was detected during preliminary assessment at the Helmholtz-Zentrum Berlin, in Berlin, Germany (Hilger et al., 2006). Background radiation due to trace levels of uranium (Parker and Toots, 1970) is often present in fossilized specimens. During neutron scans, atoms become temporarily radioactive and must go through a decay period before they may be safely returned. With background levels marginally high, it was deemed unadvisable to scan.

The ultimate scan was conducted at UTCT using the P250D detector set to a small spot size, at 450 kV and 1.3 mA. There was no filter and the specimen was not wedged in any material, although care was taken during pre-scan mounting, because the specimen was partially exposed. The scan was conducted with the long axis of the body perpendicular to the scanning plane. This allowed for maximum transmission of X-rays and maximum number of cross-sectional slices generated during reconstruction.

Slice data were further analyzed using VGStudio MAX 2.0 and Adobe Photoshop in the University of Texas Digital Methods Lab. Slices were imported into VGStudio MAX 2.0 using normal ramp settings on unaltered cross-sectional slice data, using the inverse ramp setting on unaltered cross-sectional slide data, and using normal ramp settings on cross-sectional slices previously inverted in Adobe Photoshop. Once in VGStudio MAX 2.0, skeletal material was digitally prepared from the matrix using contrast thresholding, opacity optimization, and region segmentation.

3. Results

The use of computed tomography was highly successful. Reconstruction resulted in 906 cross-sectional slices, each 0.25 mm thick. Surface skeletal morphology was partially sacrificed during post-scan processing in all methods in order to evaluate internal morphology. The exposed skeletal material was best examined on the actual specimen.

Initial cross-sectional images had a negative contrast of skeletal element to matrix. That is, the cartilage skeleton was less X-ray attenuating, and therefore darker than the surrounding matrix (Fig. 3A). This complicated modelling in VGStudio Max 2.0 because contrast thresholding selects for lighter voxels on greyscale images. As a result of this negative contrast, we could only render a surface model when working with unaltered cross-sectional images, which was little different than examining the specimen as it was (Fig. 3B, C).

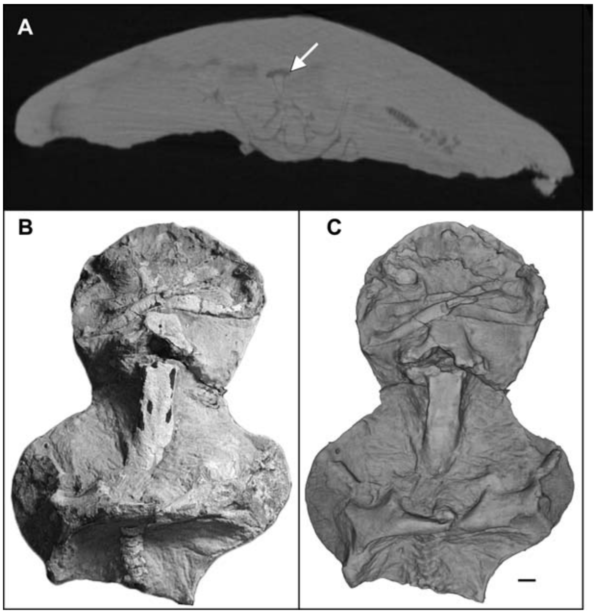

Fig. 3. Results when data were loaded as unaltered, ramp option of VGStudio Max 2.0. A. Cross-sectional slice number 557. B. Ventral view of anterior portion of original specimen. C. Ventral view of CT model. Arrow is pointing to calcified cartilage layer that is less dense than surrounding matrix. Scale bar = 1 cm.

Fig. 3. Résultats obtenus avec les données tomographiques non modifiées, option « ramp » de VGStudio Max 2.0. A. Coupe transversale n° 557. B. Vue ventrale de la portion antérieure du spécimen original. C. Vue ventrale du modèle 3D. La flèche indique la couche de cartilage calcifié, moins dense que la matrice rocheuse. Barre d'échelle = 1 cm.

When cross-sectional images were imported using the inverse ramp option, the matrix was darker than the skeletal cartilage, however, the entire specimen was surrounded by a mass of white space (Fig. 4A). It was possible to subtract much of the white space from the model (Fig. 4B, C) with the exception of a thin layer around the specimen. This thin layer of high contrasting voxels was likely due to beam hardening (Ketcham and Carlson, 2001). The most detail for these models was found when the background was turned black, because the model was slightly translucent (Fig. 4C).

Finally, when we inverted cross-sectional images in Adobe Photoshop and adjusted contrast levels prior to opening them in VGStudio Max 2.0, the specimen rendered relatively opaque. The Adobe Photoshop procedure left a much brighter layer of voxels that needed to be segmented away from the model (Fig. 5A). This was conducted by two methods of segmentation. The first used the region-draw tool, to select the bright layer of voxels on the dorsal surface of the specimen manually (Fig. 5B). The second used the 3D propagation tool to select the 'blank space' around the specimen. Once selected, the dilate tool was implemented to expand the region uniformly, to include the bright layer that surrounded the specimen (Fig. 5C).

4. Specimen description

The specimen is relatively complete in the unexposed areas of the concretion. There is a natural fracture at the approximate joint between the craniovertebral articula-

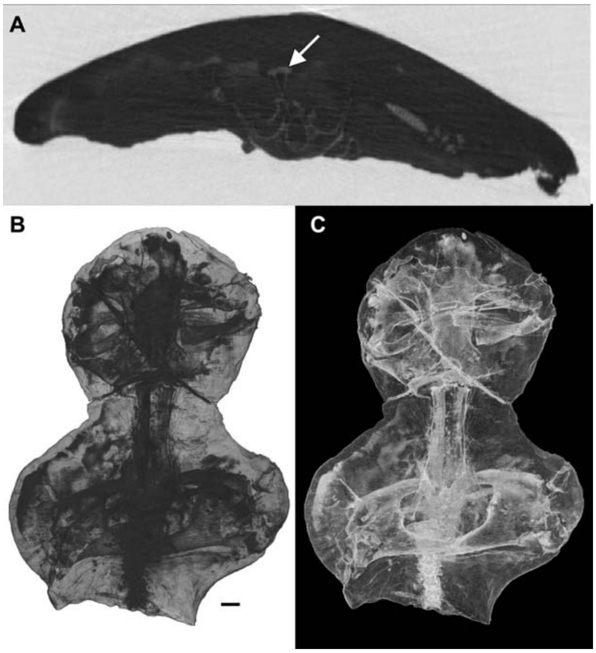

Fig. 4. Results when data were loaded as unaltered, inverse ramp option of VGStudio Max 2.0. A. Inverted cross-sectional slice number 557. B. Ventral view of anterior portion of CT model over white background. C. Ventral view of anterior portion of CT model over black background, most detail. Arrow is pointing to calcified cartilage layer that is less dense than surrounding matrix. Scale bar = 1 cm.

Fig. 4. Résultats obtenus à partir des données tomographiques non modifiées, avec option « inverse ramp » de VGStudio Max 2.0. A. Coupe transversale inversée n° 557. B. Vue ventrale de la portion antérieure du modèle 3D sur fond blanc. C. Vue ventrale de la portion antérieure du modèle 3D sur fond noir (couleurs inversées), présentant plus de détails qu'en B. La flèche indique la couche de cartilage calcifié, moins dense que la matrice rocheuse. Barre d'échelle = 1 cm.

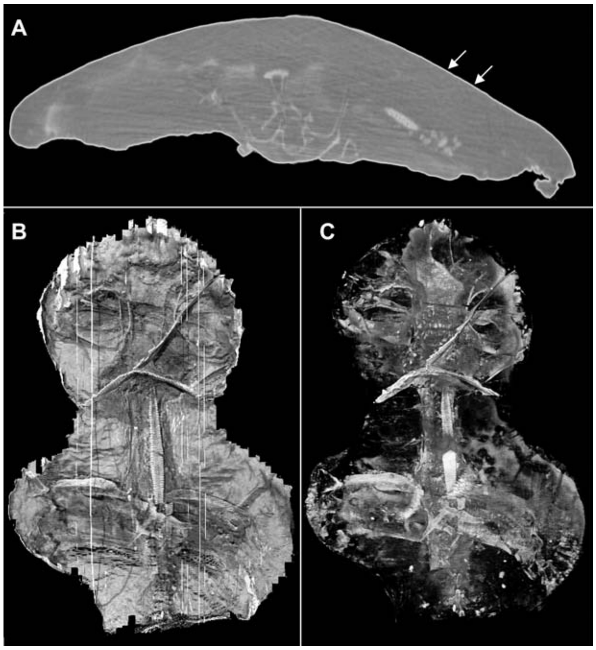

Fig. 5. Results when data were loaded as Adobe Photoshop Inverted, ramp. A. Adobe inverted cross-sectional slice number 557. B. Dorsal view of anterior portion of CT model after using regional-draw tool to remove "beam-hardened" dorsal aspect of matrix away. C. Dorsal view of anterior portion of CT model after using 3D propagation and dilate tools to remove total "beam-hardened" surface area of matrix, most detail. Arrows point to "beam-hardened" whitened surface. Scale bar = 1 cm.

Fig. 5. Résultats obtenus à partir des données tomographiques traitées sous Adobe Photoshop, option histogramme inversé. A. Coupe transversale n° 557 inversée sous Adobe. B. Vue dorsale de la portion antérieure du modèle 3D après utilisation de l'outil de retouche locale pour retirer les artéfacts de dureté du faisceau (*beam hardening*), dus à la gangue rocheuse présente en région dorsale. C. Vue dorsale de la portion antérieure du modèle 3D après utilisation des outils de « *propagation 3D* » et « *dilatation* » pour retirer virtuellement l'ensemble des artéfacts dus à la gangue rocheuse ; le fossile apparaît alors très détaillé. Les flèches pointent la surface blanche de l'artéfact de dureté du faisceau. Barre d'échelle = 1 cm.

tion. A second fracture traverses the right side of the skull. A section of the abdominal vertebral column was disarticulated from the axis at an intervertebral disc, probably post-mortem, and is shifted anteriorly, so that it runs parallel to the main vertebral axis between the pectoral girdle and pelvic girdle. The exposed skeletal elements are composed of a layer of small tesserae, prismatic cartilage, which surrounds an inner region of uncalcified cartilage. This inner region is of the same X-ray attenuation as the surrounding matrix. Without petrographic thin sections, we cannot say for certain if that is due to primary cementation or secondary replacement. Tesserae are visible around all skeletal elements, even in 3D models of elements obscured by matrix. This contributes to the hollow appearance of skeletal elements in cross-section (Figs. 3A, 4A, 5A) and volume rendered models (Fig. 4C). In volume models, the tesserae layer is rendered as most X-ray attenuating along the median crest of the synarcual cartilage and around the posterior portion of the chondrocranium. The areolar cartilage of the vertebral centra renders the brightest of all aspects of the skeleton and reflects its denser calcification nature. A great deal of the chondrocranium and pectoral skeleton appears weakly calcified, especially the anteriormost portion of the rostrum and the pterygial cartilages. This is consistent with the typical pattern of cartilage density observed in volume rendered models of extant elasmobranch taxa.

Three denticle morphologies, which are all similar to the denticles of the guitarfish *Aptychotrema*, were recognized (Fig. 2A–C). During acid preparation, an articulated section of a ventral covering of polygonal denticles was exposed and it is possible that there would have been a fairly complete covering of the ventral body with denticles. It is more common to have denticles on the dorsal surface and a naked ventral surface in extant taxa. The teeth are of underived "rhinobatid" form (Fig. 2D). The shape of the central uvula and absence of well-formed lateral uvulae are similar to those of *Platyrhinoides*. This is different from other modern rhinobatids and *Platyrhina*, but is more developed than in *Zanobatus*. Although the overall shape of the teeth is very different, the uvulae are not unlike those of *Rhynchobatus* and pristids. The largest teeth barely exceed 0.5 mm and the smaller ones, which are the most abundant, are under 0.35 mm. The root is variable with a wide groove in some, closed over in others.

Compared to other extinct and extant guitarfishes and skates, this specimen possesses a short rostral cartilage and small precerebral fontanelle, the nasal capsule is large and anteriorly directed and the mandibular arch cartilages are slender and laterally elongate. The synarcual cartilage is narrow in dorsal view, and in cross-section, the lateral stays are strongly curved anterolaterally. The median crest is wide and tapers slightly at the anterior aspect. The first vertebral centrum of the synarcual is positioned far posteriorly. The pectoral girdle is loosely connected to the vertebral column and the suprascapula is dislocated and fragmentary. The scapulocoracoid comprises a gracile, fused, coracoid bar and a shallow and rectangular scapular process. A robust propterygium is preserved proximally with a few thick proximal radials in articulation. The mesopterygia and metapterygia are difficult to discern in the fossil.

5. Discussion and conclusions

By combining traditional preparation with high-resolution X-ray computed tomography and digital preparation, we were able to produce imagery for this unique specimen that can serve as a voucher during comparative analysis. The cross-sectional images generated correspond closely to serial sections of modern taxa and when those cross-sections are compiled, they produce 3-dimensional representations that match the original specimen (Fig. 3). These data can be digitally altered by using image processing packages like VGStudio Max 2.0 or Adobe Photoshop to enhance efficiency when visualizing the skeleton (Figs. 4 and 5). By performing digital preparation, we could maintain the integrity of the fossil and get an accurate representation of the relative position of skeletal elements. In our opinion, the results of using the invert ramp option in VGStudio Max 2.0 produced the best results to see the complete skeleton of this specimen (Fig. 4C).

Three-dimensional preservation of chondrichthyans is rare, but under the right circumstances, concretions may preserve a skeleton close to its original shape. This specimen shows exceptional detail for a fossil elasmobranch and is potentially only the second 3-dimensionally preserved fossil batoid taxon discovered. The first 3D Cretaceous batoid was †*Iansan*, Brito and Seret, 1993. Our results indicate that this specimen differs substantially from †*Iansan*, as well as most other Mesozoic batoids and modern taxa such as *Rhinobatos*. It is most like members of the Platyrhinidae, and may prove instrumental in understanding the radiation of that lineage.

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