

X-ray phase technology shed new light on Weberian apparatus evolution: context and aim of the case study of †*Chanoïdes* (Otophysi, *incertae sedis*)

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Abstract - Otophysan fish are identified as a natural group and have been studied as such since the first half of the 19th century. This very speciose group largely dominates the vertebrate diversity of inland waters today. In the 1980's passionate debates concerned the otophysan radiation and notably their primary habitat: are they primitively freshwaters or marine animals? To answer we need to better understand the phylogenetic relationships including those of the fossils and thus we need to enhance our knowledge on key bony structures such as the Weberian Apparatus. In this context we propose a CT scan approach, for a specimen of †*Chanoïdes macropoma*. In the last years, palaeontology benefited from the developments of phase contrast X-ray microtomography. We expect great advances to investigate the Weberian apparatus and its evolution in Otophysan fishes.

Keywords: Weberian apparatus, evolution, stem otophysan, fossil

1. Introduction

Otophysan fish are identified as a natural group and have been studied as such since the first half of the 19th century. This very speciose group largely dominates the vertebrate diversity of inland waters today. Its diversification starts in the Cretaceous in the fossil record (Mayrinck *et al.*, 2015b; DM and OO current studies) while last fossil calibrated molecular studies give for the clade a Jurassic age, or a Jurassic to Early Cretaceous age, and even an early Cretaceous age when excluding saturated partition for nucleotide substitution (respectively Near *et al.*, 2012, Broughton *et al.*, 2013, Dornburg *et al.*, 2014). The success of these fishes in continental freshwaters is generally imputed to the great development in their auditory ability in relation with the presence of an original structure called Weberian Apparatus. Since the first definition of the group, Sagemehl (1885) recognised Ostariophysi (= Otophysi according to current taxonomy) based on the presence of this unique morphological and osteological feature. First described by Ernst Heinrich Weber (Weber, 1820), it consists in a deep modification of at least the first four vertebrae and associated bones and tissues and allows the transmission and amplification of pressure variation from the swimbladder to the inner ear. It includes a series of small paired bony elements (claustrum, scaphium, intercalarium, tripus) linked by the interossicular ligament (e.g. Rosen and Greenwood, 1970). Its presence is now recognised as the apomorphy of the clade Otophysi and lacks at

such in the sister ostariophysan group Gonorynchiformes (e.g. Rosen and Greenwood, 1970; Grande and de Pinna, 2004). Moreover, the Weberian structures present a wide diversity in their relative dimension, shape and organisation which allow distinguishing the members of the five otophysan orders, i.e. Characiformes (including piranhas, tiger fish and tetras), Cypriniformes (including carps, barbs and minnows), Siluriformes (catfishes) and Gymnotiformes (electric eels). Otophysan fish are mostly freshwaters and include most of the specific diversity of the Ostariophysi while Gonorynchiformes (including milkfish and shellers) is a marine and comparatively depauperate clade.

In the 1980's passionate debates concerned the otophysan radiation and notably their primary habitat: are they primitively freshwaters or marine animals? How to explain that Siluriformes and Characiformes originate onto Western Gondwana dependences (South America and/ or Africa) while Cypriniformes are primitively Laurasian animals? To resume in the main lines, several marine fossil fish were described as stem otophysan which pleads for a marine and late Mesozoic origin of the clade. Conversely, a freshwater origin is assumed based on their modern dominant ecology and supports an earlier origin on Pangea at the very beginning of the Mesozoic, or later rafting between the landmasses of the dislocating Pangea. The different scenarios for their diversification oppose vicariance and dispersion models (see for instance Briggs, 2005; Otero *et al.*, 2008 for details). In the background, there were also debates about the phylogenetic position of

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extant and fossil taxa compared to major clades, and even about the relevance of fossils to reconstruct evolutionary process due either to a low confidence in morphological phylogenies or in the partial information yielded by fossils. We are convinced that fossils are relevant to retrace early stages of ancient clade diversification, and that dedicated studies may even allow to resolve remaining discrepancies between molecular and morphological studies as recently demonstrated (Davesne *et al.*, 2016).

Progress in the reliability of phylogenetic assumption based on both morphological (e.g. Fink and Fink, 1981, 1996) and molecular data (Calcagnotto *et al.*, 2005; Peng *et al.*, 2006; Sullivan *et al.*, 2006; Saitoh *et al.*, 2011; Chen *et al.*, 2013) and progress in understanding developmental aspects of some ostariophysan phylogenetic key bony features (Coburn and Futey, 1996; Bird and Mabee, 2003; Britz and Hoffman, 2006; Hoffman and Britz, 2006; Britz and Moritz, 2007) led us (DM and OO) to undertake a review of fossil ostariophysan fish (Mayrinck *et al.*, 2015a, b, in review). The final aim is to be able to include confidently fossil data in comprehensive reconstruction of the ostariophysan diversification. So far we were able to confirm a probable stem-otophysan status solely to †*Chanoides macropoma*, a fossil fish from marine Middle Eocene deposits of the Monte Bolca (Mayrinck *et al.*, 2015a, b, in review). The detailed anatomical studies of its Weberian apparatus by Patterson (1984) and by Mayrinck *et al.* (2015b) lead to conclude that †*Chanoides macropoma* presents certainly an Otophysan anatomy but certainly not one of the modern patterns displayed by the members of extant orders.

In that frame, investigating as far as possible the Weberian Apparatus of †*Chanoides macropoma* appears critical, and was the subject of a dedicated 3D-imaging study based on one of the most powerful X-ray microtomography available today, i.e. the Synchrotron in Grenoble (ESRF). Methodological details and preliminary results on the study of the fossil in propagation-based phase-contrast X-ray microtomography (PBPC) are given below in the paper.

2. Material and methods

The specimen NHM P. 51216, housed in the Natural History Museum, London was imaged in PBPC. It has been prepared by transfer method (Toombs and Rison, 1959) and was studied by Patterson (1984) and Mayrinck *et al.* (2015b). They provided a detailed and mainly convergent description of the species and notably of its Weberian Apparatus, including information on NHM P. 51216.

2.1. Microtomography acquisition, reconstruction and visualization

The microtomogram was obtained by propagation-based phase-contrast X-ray synchrotron microtomography at the ID19 beamline of the European Synchrotron Radiation Facility (Boistel *et al.*, 2001; Tafforeau *et al.*, 2006; Sutton, 2008; Zanette *et al.*, 2013). The energy of pink beam was tuned to 41 keV to obtain a suitable signal/noise ratio with this kind of sample. The X-ray detector was an in-house FReLoN (Fast Readout Low Noise) camera (Labiche *et al.*, 2007). Images of 5 levels were taken in the phase contrast mode with pixels size of 7.46 μm . For the tomographic reconstruction, a phase retrieval approach was used from a single long propagation distance using the Paganin

filter of the PyHST2 algorithm (Paganin *et al.*, 2002; Mirone *et al.*, 2014). Finally, volume renderings were obtained after semi-automatic segmentation of the volumes of interest and iso-surfaces were produced using Avizo 7.1 (VSG, Visualization Sciences Group).

2.2. Natural light observation and photographs

The specimen was observed with a binocular (Leica MZ 75) and photographs were realised with a grazing light with a NIKON Coolpix S9900.

3. Results

3.1. Observation of the Weberian apparatus of NHM P. 51216 observed in natural light

The specimen is probably the best available Weberian apparatus for †*Chanoides macropoma* (Fig. 1A). Most of the information presented for the species in the papers of Patterson (1984) and Mayrinck *et al.* (2015b) is easily recovered. In brief we were able to recognise: the claustrum, scaphium and intercalarium; the third centrum (which is the shortest); the small process which is interpreted as a tripus by Patterson (1984) but not by Grande and Pinna (2004) and Mayrinck *et al.* (2015b); an os suspensorium that projects backward; the contiguous supraneurals 2 and 3 (Fig. 1).

3.2. Volume rendering of Weberian area in NHM P. 51216

On an iso-surface of the Weberian area obtained by a threshold (Fig. 2) we recognize the presence of the Weberian apparatus. Further segmentation will allow isolating the various Weberian elements and produce a 3D mesh. We will also be able to discuss further the modern and archaic aspects of its anatomy when compared with the Weberian apparatus displayed by the members of the four extant otophysan orders.



Fig. 1. Detail photograph of the Weberian apparatus of †*Chanoides macropoma* NHM P. 51216 in natural light. Scale bar is 5 mm.



Fig. 2. Volume rendering of the Weberian apparatus of †*Chanooides macropoma* NHM P. 51216.

4. Conclusive Discussion

In the last years, palaeontology benefited from the developments of phase contrast X-ray microtomography. For instance, the methodologies developed at the ID 19 at the ESRF shed new light on evolution through the study of emblematic fossils (e.g. <http://paleo.esrf.eu>). We expect great advances to investigate the Weberian Apparatus and its evolution in Otophysan fishes.

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