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Experiment Report Form

ESRF	**Experiment title:** Reconstructing phylogeny of Early Triassic conodonts using X-ray synchrotron microtomography and 3d morphometrics	**Experiment number**: EC 432
Beamline: ID19	**Date of experiment**: from: 07/03/2009 to: 09/03/2009	**Date of report**: 27/08/2009
Shifts: 6	**Local contact(s)**: Dr. Paul TAFFOREAU	*Received at ESRF:*

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Preliminary Report:

The original aim of the proposed experiment was to image with the best resolution and image quality the outer surfaces as well as the internal structures of a representative range of Early Triassic conodont elements, in order to perform quantitative comparisons among individuals on the basis of their 3d surfaces and ontogeny.

The very small size of our samples implied to use synchrotron microtomography to reach a sufficient level of resolution. The tools and know-how for internal structures imaging based on holotomography and data processing of dental microstructures are available only at the ESRF on the beamline ID19. This designed logically ID19 as the best tool for a high quality microtomographic imaging of our conodonts.

Originally, we planned to use sample holders made of two plastic cones. Each conodont is put in a cone and fixed only by gravity after weak vibrations to ensure that it fits the bottom of the cone. That cone is then put in a second one fixed on the microtomographic column allowing rapid and precise positioning of each new sample. That sample holder system was used successfully several times. It ensures minimal risk for the sample (no glue, very few manipulation), and optimizes data quality since the plastic cones are not creating artefacts in the pictures.

Yet, only very big conodont specimens could be put easily in the plastic cone and then rapidly centered on the beam. So, mostly for timing reasons, we had to glue most of our tiny specimens on small glass rods. About 20 specimens could be attached in a row on one side of the rod using drops of viscous cyano-acrylate. This facilitates and fastens the centering of specimens and a series of scans can be automated too. The specimens can still be retrieved afterwards using acetone.

The main aim being to obtain highly detailed 3D reconstructions, most of the samples were scanned with moderate weak phase contrast in single scans to ensure a high number of scanned specimens (each scan taking between 10 and 20 minutes depending on the sample). Regarding to the sample size, we used the revolver optic of ID19 with voxel sizes ranging from 0.23 to 0.46 microns. We used a nearly monochromatic pink beam at an energy of 17.68 keV using the new ID19 U17.6 undulator. That new insertion device allowed to perform rapid scans nearly free of ring artefacts. It then brough far better data than those usually obtained using multilayer monochromator, with easier processing. About one hundred specimens could be scanned this way.

The most well preserved samples were imaged using high resolution holotomographic approach. That technique is able to bring information on internal growth structure without equivalent. Unfortunately, all high resolution holotomographic attempts failed with these very small specimens. The major problem was that the specimens experienced micrometric displacements during the procedure, apparently due to deformations of the plastic cones during the high-energy exposures. An final attempt using the glue procedure failed similarly due to the growing of originally invisible air bubbles within the relatively fresh glue.

Nevertheless, many specimens could be scanned successfully using propagation phase contrast microtomography. It is still too early to reach conclusions about the success of this part of the project and work is on progress to analyze the huge amount of data obtained during this experiment.

What we can tell though is that this experiment already brought about unexpected results with possibly far-reaching implications. Indeed, we were able to scan a few fused clusters of conodont elements (morphologically different elements pertaining to the same animal that got cemented together post-mortem). One of these helped us deciphering how some of the elements of the Early Triassic *Novispathodus* conodont were positioned and orientated. This led to important revisions of the architectural template of the corresponding superfamily and hence for the taxonomy of most Permian and Triassic conodonts (see abstract 1 below). Further work on this cluster enabled us also to recontruct a virtual 3d feeding apparatus (see Fig. 2) and to propose a new functional model for these conodonts. This model may have in turn important implications for our understanding of the origin of jawed vertebrates (see abstract 2). Two talks related to the ESRF experiment were presented at the last International Conodont Symposium in Calgary this summer. The abstracts are found below (both published in *Permophiles* Vol. **53** supplement 1, pp-16-18). Two corresponding publications are in preparation right now.

Abstract 1

Early Triassic conodont clusters from South China: Revision of the architecture of the 15-element apparatuses of the Gondolelloidea superfamily

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Several fused clusters of conodont elements of the genus *Novispathodus* were recovered from limestone beds at the Smithian-Spathian boundary (Luolou Fm., Early Triassic; Galfetti *et al*., 2008) from several localities in Guangxi province, South China.

Conodont clusters are otherwise extremely rare in the Triassic and these are the first reported for the Early Triassic. The exceptional specimens partially preserve the relative three-dimensional position and orientation of ramiform elements and are therefore extremely important for testing hypotheses on the architecture of apparatuses.

These specimens partially confirm the reconstruction of the *Novispathodus* apparatus by Orchard (2005). Yet, they also demonstrate (see Fig. 1) that the elements previously identified as occupying the S1 and S2 positions occupy in fact the S2 and S1 positions respectively. This affects our interpretation of all apparatuses of Gondolelloidea superfamily, which was based (Orchard, 2005) on bedding plane natural assemblages from the Middle Triassic of Switzerland (Rieber, 1980; Orchard and Rieber, 1999). The same applies to the elements in the S3 and S4 positions, whose positions are actually reversed, at least for the subfamily Novispathodinae (Orchard, 2005).

It was possible to reach these conclusions thanks to a X-ray synchrotron microtomography (Tafforeau *et al*., 2006). In this particular case a pink beam setup at 17.6 keV, very recently developed at the ESRF on the ID19 beamline, and allowing submicron

resolution (0.23µm) with a speed and an overall quality never reached before, has been successfully tested on these fossils. This technique appears as very successful for high quality and high resolution imaging of microfossils. It will certainly allow the non-destructive study or restudy of other known specimens, particularly of fused clusters, for which only exposed surfaces are otherwise accessible.

Co-occurring isolated elements from the same sample and pertaining to the same multi-element species were also scanned using this technique and it allowed us to reconstruct a virtual apparatus.

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Acknowledgements

We acknowledge the European Synchrotron Radiation Facility for provision of synchrotron radiation facilities and for granting access to beamline ID19.

Abstract 2

A animated functional model of the Lower Triassic *Novispathodus* apparatus based on X-ray synchrotron microtomography and computer graphics.

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The starting point of this study was the discovery (Goudemand et al., 2009, this volume) in South China of several fused clusters of conodont elements of the genus *Novispathodus* (Orchard, 2005). Some of them were scanned at the ESRF on the ID19 beamline using propagation phase contrast microtomography (Tafforeau et al., 2006), as were also isolated elements from the same sample. The high quality and high resolution (voxel size 0.23 µm) imaging obtained using a newly developed pink beam setup at 17.6 keV, allowed us to reconstruct a virtual 3d apparatus.

The clusters impose constraints on the position and orientation of the S1-4 ramiform elements. Observing a striking resemblance between the posterior process of the S0 element and the S1 element (as revised by Goudemand *et al.* 2009), we added further constraints on the position of the S0 element relative to the others. We have assumed that this position corresponds to the arrangement at rest, and thereafter considered their relative positions during feeding movement. New observations of geometrical correspondences between elements allowed us to make new hypotheses and to derive a new functional model of the conodonts' feeding apparatus, presented in animated form.

In our view, the best solution implies the presence of a presumably cartilaginous 'copula' upon which the conodont elements are moving independently, more or less as do dental plates in extant lampreys, but not in the manner that was suggested in the pioneering work of Purnell and Donoghue (1997). This solution suggests that during retraction towards the caudally located platform elements, the S0 element first had a closing, rotating movement, most probably synchronized with the closure of the M elements, with which it would have performed a pinching, seizing function; followed by a sub-straight, dorso-caudally directed translation, by which it would have torn off the prey's 'flesh' and brought it towards the platform elements. The latter movement is accompanied by the closure of the other S elements, constraining the food in the desired direction.

Contrary to known Palaeozoic apparatuses where all S elements seem to be oriented in the same direction, this apparatus shows (as previous reconstructions suggested, see Orchard and Rieber, 1999) that within at least some Triassic apparatuses, both S1 and S2 were oriented with the cusps pointing caudally, which in our interpretation suggest an evolutionary re-assignment of teeth from the 'palate/cheecks' to the 'tongue'.

Finally, considering that the presence of a cartilaginous copula associated with tongue protractor and retractor muscles has been asserted only for myxinoidea (hagfishes) and petromyzontida (lampreys) (see Donoghue, Forey and Aldridge, 2000, for further details), it led some authors (Yalden, 1985) to argue it would be a synapomorphous feature of cyclostomes and evidence of their monophyly. The debate is going on but our new model may shed new light and consequently hold important implications for the affinity of conodonts and for our general understanding of the origin of vertebrates.

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Acknowledgements
We acknowledge the European Synchrotron Radiation Facility for provision of synchrotron radiation facilities and for granting access to beamline ID19.
We wish to thank also S. Urdy and T. Brühwiler for their help with the scans, C. Monnet, R. Lebrun and C. Zollikofer for their expertise on Amira and Formit softwares, as well as T. Galfetti, A. Brayard and H. Bucher for their assistance on the field.

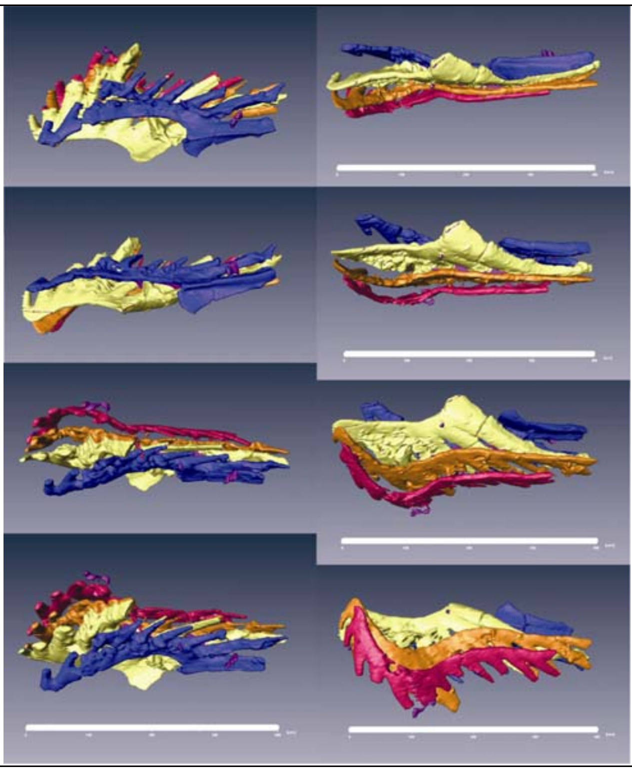

Figure 1: fused cluster with four elements (S1 to S4). Scale bar is 400 microns.

Figure 2: animated 3d reconstruction of the feeding apparatus of *Novispathodus*.