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On the possibility of introducing X-ray computed microtomography into the practice of biostratigraphic research

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Abstract. Currently, the techniques applied for extraction and study of conodonts from siliceous rocks are associated with a number of problems. This makes it difficult to solve many problems in the areas of development of the volcanic and volcanic-sedimentary rocks, where cherts, jaspers, and phtanites are the only sedimentary formations for dating these deposits.

On X-ray computed microtomography it is possible to avoid some problems to obtain not only excellent 3-D images of conodonts, but sections in any direction too, as well as in video formats. It is shown that similar results are successful under the hollows after the dissolution of the conodonts.

There is no problem in application of X-ray microcomputed tomography when conodonts have been already found on the surface or inside of the sample, or if the content of conodonts in the rock is obviously high. In such a case the scanning without preliminary search is ensured. In cases when conodonts are rare and not obvious, it is proposed the following technique of their discovery.

The rock sample is cut into plates. The conodonts are search for on the surface of the plates, moistened with a mixture of glycerin and water under a binocular microscope. If it is necessary (when the rock is opaque), the result is checked by a chemical reaction: 5–10 % hydrochloric acid plus 1–2 crystals of ammonium molybdate are put on the surface of the sample. The appearance of a yellow sediment means the presence of phosphorus, to indicate the probability the detected object to be a conodont. Next, the sample should be washed from acid, its size should be decreased. Then the microtomographic study should be performed.

Keywords: X-ray computed tomography, μCT, conodonts, biostratigraphy

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Introduction

The object of our research is micropaleontological remains – the conodonts in siliceous rocks. The conodonts were part of the jaw apparatus of the Conodontoforata being extinct at the end of the Triassic period (200 Ma). They are up to 1 mm in size, rarely larger, and consist of a complex calcium phosphate of the apatite group, which ensures their safety in the rock, even under the unfavourable factors. Conodonts are distinguished by very high rates of evolution and are successfully used for detailed stratigraphic subdivisions and global correlations of the Paleozoic and Triassic sections (Clark, 1981; Maslakova et al., 1995). The detailed stratigraphic basis allows solving many regional and global problems of geology, including the reconstruction of the geological history.

Numerous techniques of conodonts extraction from rocks have been developed. However, their use does not always guarantee positive results. In recent years, the technique of X-ray computed microtomography (μCT) has been introduced into the practice of geological research. It is used in visualization and further study of organic remains, the structure of the pore space of oil and gas reservoirs, the structural features of clastic rocks, etc. (Tafforeau et al., 2006; Carlos, 2006; Korost et al., 2010; Yakushina, 2012; Cnudde, Boone, 2013; Yakushina, Khozyainov, 2014; Korolev et al., 2014; Ponomarenko et al., 2014, Yakupov et al., 2014; Savitskiy, 2015; Zavatskiy et al., 2016).

The first most complete publications devoted to the microtomographic study of conodonts most likely are the works of A.V. Zhuravlev (Zhuravlev, 2012, 2013). In these and in subsequent articles A.V. Zhuravlev et al. show that the X-ray μCT method is applicable to the exploration of conodonts. This technique makes it possible to refine the histological model of conodonts, allowing them to be diagnosed as at the genus, and in some cases, as at the species level. The undoubted

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advantage of the tomographic method is the efficiency and ability to obtain a positive result without destroying the sample.

In 2014–2015, the authors attempted to introduce µCT scanning into the technique of the conodonts exploration in cherts (Fazliakhmetov et al., 2014, 2015, 2016).

Review of techniques of extracting conodonts from rocks

The technique to isolate conodonts are diverse (Sergeeva, 1966; Puchkov, 1979; Ivanov, 1987; Artyushkova, 2014). The choice of the right technique depends on the composition of sedimentary rocks and the degree of their lithification.

Traditionally, conodonts are extracted from carbonate and carbonate-containing rocks – limestones, marls, calcareous sandstones, etc. The weak (5–10 %) solutions of methanoic (formic) or ethanoic (acetic) acids are used for this goal. The insoluble residue is looked through under a binocular microscope after its washing out and drying and conodonts are selected.

The low content of carbonate in the rocks or its absence is a problem to disintegrate the rocks, so the probability to obtain a definite conodont is reduced significantly. At the same time, the value of conodonts precisely from siliceous rocks is extremely great, because of the only possible way to subdivide volcanic-sedimentary complexes in folded areas. Phtanites, jaspers, and siliceous mudstones are often the only initial sedimentary formations with organic remains, to be useful to date deposits, correlate them with a regional or general stratigraphic scales. The lack of biostratigraphic data leaves a number of unresolved problems, including the applied tasks, e.g. mapping of volcanic and volcanic-clastic ore-bearing complexes.

The search for conodonts in siliceous rocks can be realized in three ways. First, cherts and siliceous rocks are dissolved in hydrofluoric acid. Then the undissolved residue is viewed and phosphate faunal microremains are selected. The quality of conodonts is poor under this technique; sometimes up to their dissolution (Ivanov, 1987).

The second method is to search for conodonts on polished sections of siliceous rocks or to examine thin flakes of cherts through the light. The shortcoming of this technique is the absence of a full image of the found conodont. In addition, not all siliceous rocks are transparent. An admixture of carbonaceous or clayey material make cherts of untransparent and prevent not only to identify conodonts, but even to find them.

The third technique is the simplest and most effective, but sometimes it requires long field works. It is based on the visual search and identification of conodont elements on the bedding plates of siliceous and siliceous-clayey rocks, and more often their imprints. This method is widely used in the Southern Urals (Puchkov, 1979; Ivanov, 1987; Artyushkova, 2014). It allows to subdivide the sections in detail and correlate the different facies volcanic sections in Magnitogorsk megazone (Maslov and Artyushkova, 2010).

Each of the mentioned techniques of the conodonts study in siliceous rocks has their own features. The disadvantages include 1) an increase of expenditure of time to obtain a result and 2) the inability to see a volumetric image of faunal microremains.

Study of conodonts by µCT

For the first time, we provided a microtomographic study of conodonts in the rocks of the Nyazya strata of the fundamental importance its age. The samples were collected by O.V. Artyushkova in the area of Nyazepetrovsk town on the right bank of the Uraim river, 4.0 km upstream the mouth (Fig. 1). It was impossible to diagnose two specimens of conodonts found visually in cherts because of a risk of destroying them during laboratory preparation. These circumstances were the reason to use the X-ray µCT method.

µCT study was managed in the Institute of Geology and Petroleum Technology of Kazan (Volga Region) Federal University using a nanofocus tube of a Phoenix V|tome|X S 240 X-ray computerized tomography system. The accelerated voltage was 80 kV, the current – 130 mA. The imaging resolution (voxel size) was 2 microns. The volumetric model of the samples was obtained with using the AvizoFire 7.1 software package. The optimal size of studied samples with conodont inclusion should be as 6 × 6 × 5 and 4 × 3 × 3 mm.

Obtained conodont images are of three types: microtomographic sections of the samples, volumetric reconstructions (Figs. 2, 3) and the video materials with conodont rotaiting in different planes. All visualized materials are of good quality. So, morphological elements of conodonts necessary for their diagnosis are clearly visible. It makes it possible to understand their specific identity. Definition of *Pandorinellina miae* (Bultynck) and as well as *Eolinguipolygnathus excavatus* Carls et Gandle are effective to clarify their stratigraphic belonging. For the first time, the rocks of the Nyazya strata containing the above-mentioned conodonts assumed a paleontological characteristic; its age was revised.

It was already noted above that sometimes siliceous rocks are the only sedimentary formations in many Paleozoic sections, formed mainly with volcanic products. The presented results leave no doubt that the X-ray µCT technique has great prospects for the search, visualization, and definition of conodonts enclosed in silicites and other acid-resistant rocks. A.V. Zhuravlev and A.I. Gerasimova (Zhuravlev, Gerasimova, 2016) affirm this method to be rationable in case of conodonts content of 2000 specimens per 1 kg of rock or 5–6 specimens per cm^3. In practice, this is extremely rare. In order to get a positive result, it is enough to have a sample of 0.5–1.0 cm^3 in volume. In the Southern Urals this criterion may closely correspond to jaspers and cherts of the Karamalytash, Yarlykapovo, Mukasovo and Biyagoda formations.

However, the microfossils content in siliceous rocks of different formations and strata varies greatly. For example, the Lochkovian conodonts in Iltibanovo strata cherts (D_1) were found visually in only one outcrop for more than half a century of exploratory history. Until now the duration of the Iltibanovo strata forming is the outstanding question, and the Early Devonian history of the Magnitogorsk island arc system remains without the desired detail, accordingly. The search of the microfossils is still relevant in the Silurian-Lower Devonian sequences, outcropped near the Mansurovo village, in the Ryskuzhino and Uskul' strata, as well as in some Middle-Upper Deonian sections of the Ulutau and Biyagoda formations, etc.

We have a large number of examples pointing the necessity of the conodonts discovery in "silent" siliceous rocks. This implies the urgency of a technique development to be allowed to increase the number and volume of samples under study.

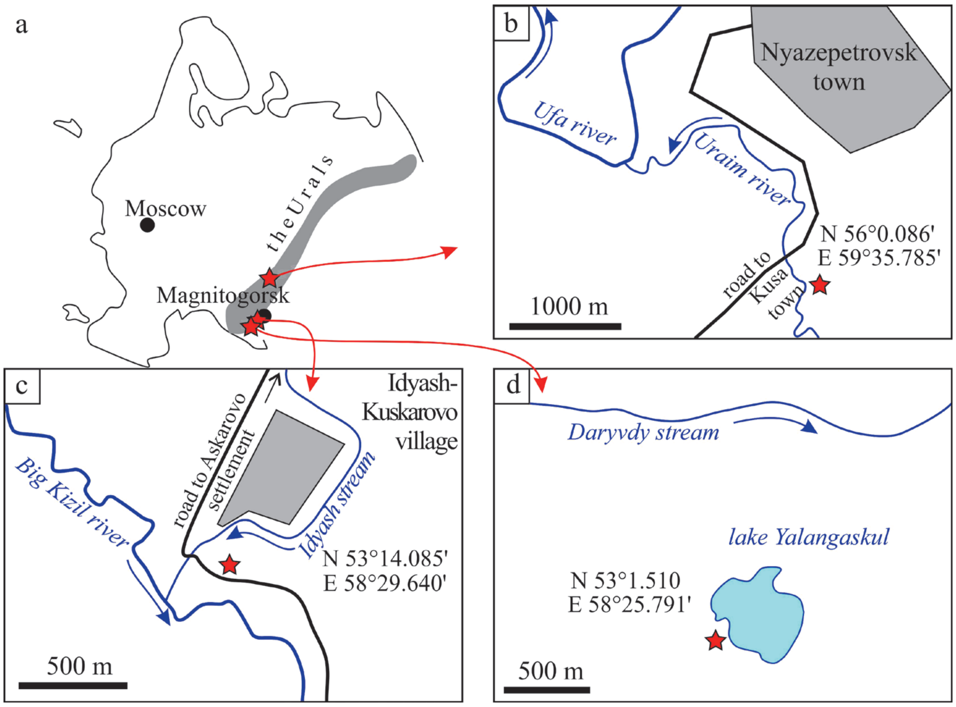

Fig. 1. Sketches of outcrops location: a – overview schematic map; b–d – detailed schemes in Nyazepetrovsk town vicinity (b), Idyash-Kuskarovo village vicinity (c) and the southern shore of the Yalangaskul lake (g)

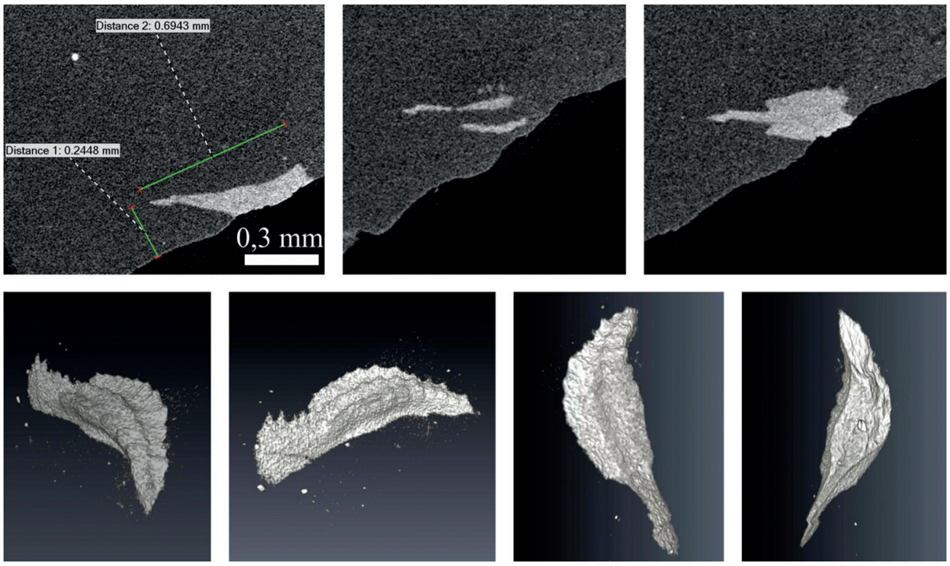

Fig. 2. Conodont Eolinguipolygnathus excavatus Carls et Gandle: top row – tomographic slices, bottom row – volumetric reconstructions

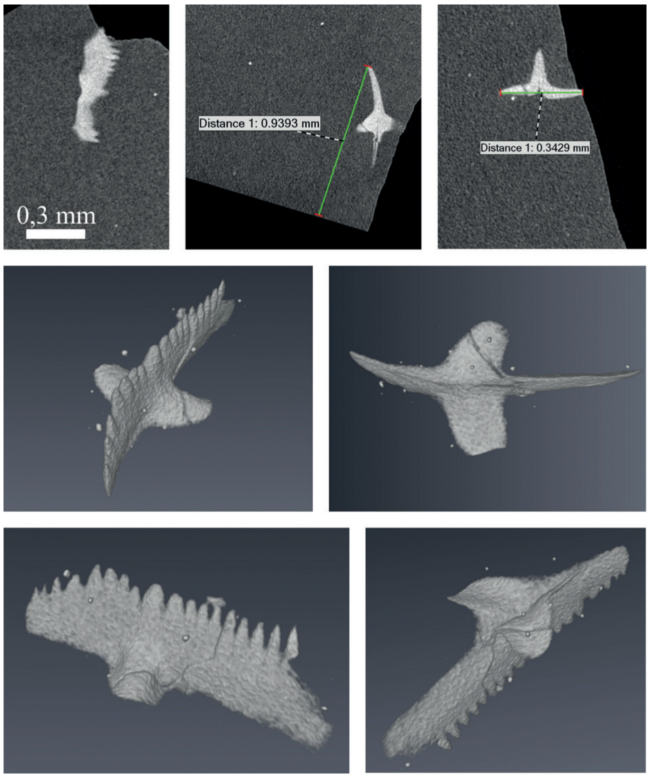

Fig. 3. Conodont Pandorinellina miae (Bultynck). Top row – tomographic sections, the rest – volumetric reconstructions

However, it is not always possible to use an X-ray tomograph for this task and there are two main reasons for this. The first subjective one is the high cost of µCT imaging, which prevents scientific teams, that do not have a free access to CT scannig to use it for biostratigraphic research. Our experience shows that the cost of 4–5 samples using µCT is equivalent to an expedition working 2–3 weeks to collect the same amount of samples or to isolate conodonts from 30–40 kg of limestone. The second reason is objective and connected with the extremely low content of conodonts in the rocks. In addition, conodonts are microscopic objects that require high resolution study. Therefore there is limitation in sample size (the first millimeters across).

One of the simplest solutions allowing the use of X-ray µCT with a guaranteed positive result is the study of a conodont already found in the rock, as it was in the case with cherts of the Nyazya strata. We tried to find and develop a more effective technique of searching for conodonts on chips and cuts of siliceous rocks in comparison with the described above.

Search for conodonts on the surface of siliceous rocks in laboratory conditions

Searching for conodonts on cuts and chips of siliceous rocks is a time-consuming task. In transparent and translucent cherts, it can be carried out under a binocular microscope. But in opaque varieties it is apparently impossible, since it is difficult to identify a conodont by its cut on the rock surface (usually it looks a shapeless speck). In this situation, it seems possible to apply the technique of chemical staining, to determine the presence of phosphate inclusions.

The procedure includes placing a drop of 10 % hydrochloric acid on the sample surface and 1–2 crystals of ammonium molybdate ($(NH_4)_6Mo_7O_{24}\times4H_2O$). As a result of the reaction, a bright-yellow precipitate appears near the phosphate particle within a few seconds.

Phosphates – implied conodonts, are corroded under reaction with hydrochloric acid. This can make all attempts of search and subsequent X-ray µCT studies in vain. In this regard, it seems important to find out whether it is effective to scan not of a conodont, but of its imprint after its complete dissolution. To clarify this question, the following two experiments were managed.

Experiment 1. The object of the study was conodont-rich, light gray translucent cherts of the Biyagoda Formation (boundary Frasnian-Famenian interval) in the West Magnitogorsk zone (South Urals), sampled in the section near the Idyash-Kuskarovo village (Fig. 1). The characteristics of this section are described in publications (Maslov, Artyushkova, 2010; Fazliakhmetov, 2020).

The experimental tasks are: a) to compare volumetric microtomographic images of conodonts in cherts with cavities after their dissolution; b) to set the minimum threshold for the sensitivity of the technique, i.e. the minimum size of the visualized conodont by CT.

The samples were cut into 3–4 mm thick plates. It is impossible to see anything on the cutting plates because of rough matte surface, so, before viewing under a binocular microscope the plates were moistened with mixture of distilled water with glycerin in a 1: 1 ratio. Glycerin prevents the evaporation of the liquid film and thus eliminates to wet the sample constantly.

Conodonts discovered in this way are numerous. Of this, the two smallest specimens were selected. The size of the original sample was reduced to a cylinder with a diameter of about 5 mm and a height of 6 mm.

Initially, µCT-scanning of the samples was provided before the beginning of reaction with hydrochloric acid. Three-dimensional images were used to identify two specimens of conodonts: the broken Pa-element of *Palmatolepis* sp. (Fig. 4) and a ramiform element with fine teeth (Fig. 5).

Then the samples were placed in 10 % hydrochloric acid for several hours, washed with distilled water, and dried at room temperature. After that, to obtain an image of the hollow space after the dissolution of conodonts, followed by a new µCT-scanning.

The volumetric images of the Pa-element before and after dissolution are almost identical (Fig. 4). The differences are connected with different scanning parameters only, being selected for each sample separately.

Images of the S-element (Fig. 5), obtained before and after processing with hydrochloric acid, differ from each other in the teeth length. In the image of conodont, there are two long teeth and three short ones. The imprint image appears to be sharper, but it has five medium-sized teeth. On tomographic sections of the cavity, five long teeth are visible; two of them have not completely dissolved.

The difference in the images of sample 2 before and after acid processing is caused by the incomplete dissolution of the conodont and also, probably, by the fact that its thin elements merge with the siliceous rock. The fact is that the success of visualization of the internal structure of any object depends on the difference in the linear attenuation coefficient of X-ray radiation of its constituent elements (substances). The larger it is, the clearer the image is, the better small details are visible. The difference in the linear attenuation coefficient of

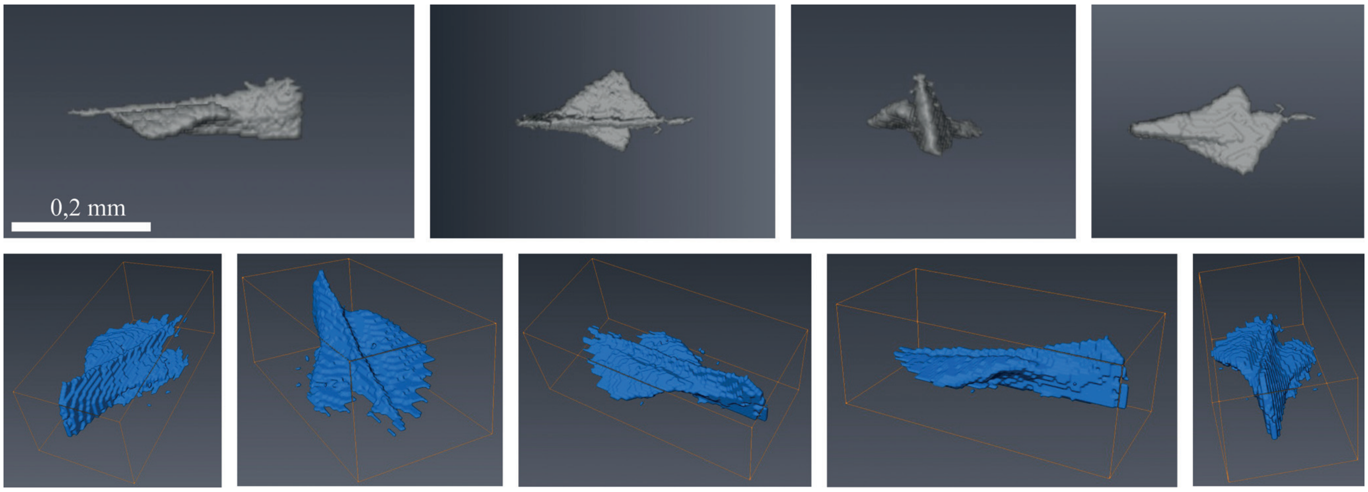

Fig. 4. Volumetric tomographic reconstructions of the conodont fragment Palmatolepis sp. (top row) and the etched print after dissolving (bottom row)

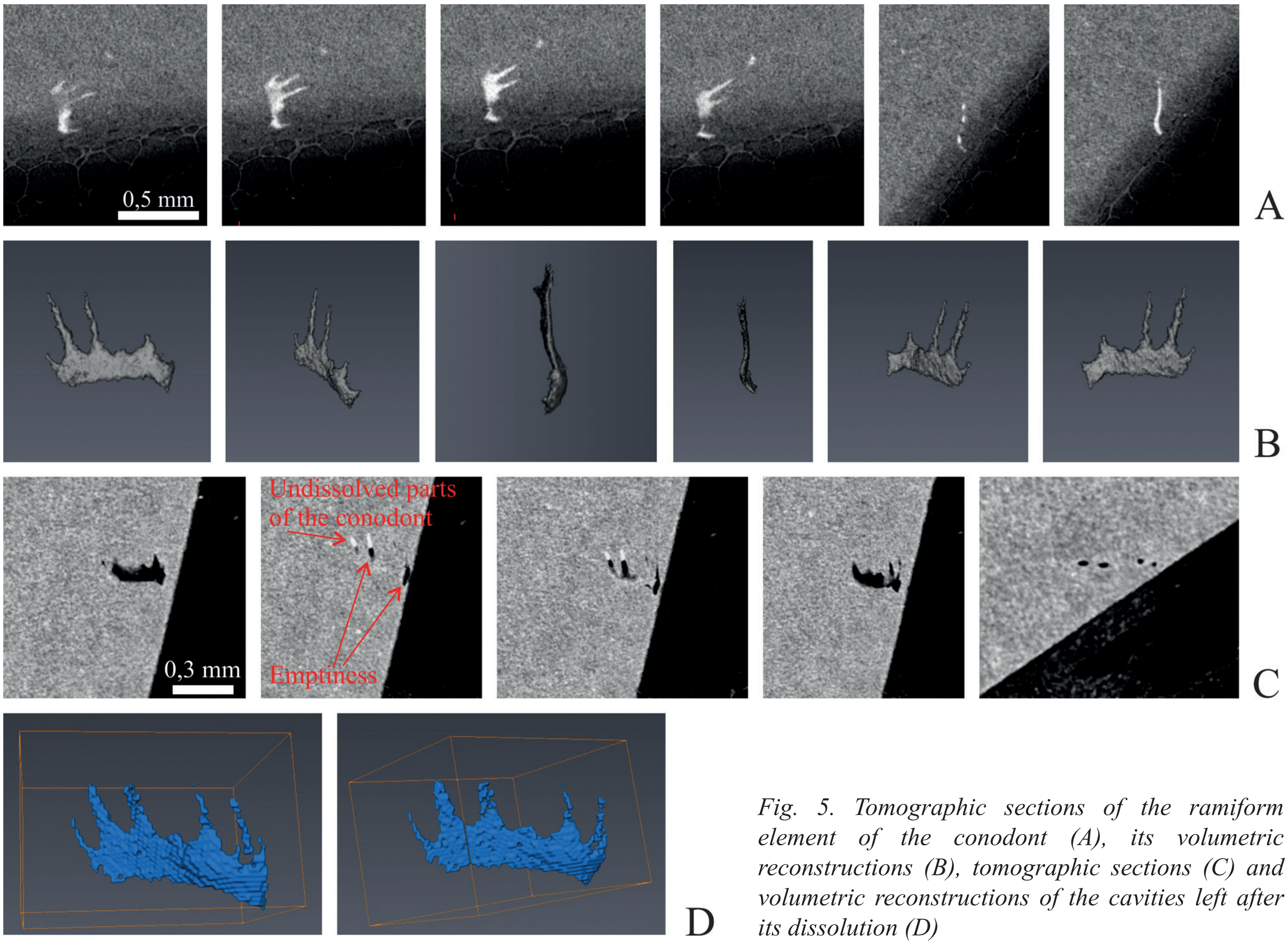

Fig. 5. Tomographic sections of the ramiform element of the conodont (A), its volumetric reconstructions (B), tomographic sections (C) and volumetric reconstructions of the cavities left after its dissolution (D)

cherts and phosphate is less than that of cherts and air; therefore, the image of the conodont imprint is more distinct.

The task of **experiment 2** was how the reaction with hydrochloric acid and ammonium molybdate affects the conodont.

Samples of cherts were collected from the Biyagoda formation section on the southern shore of Yalangaskul Lake (Fig. 1). Six samples were scanned, grouped into two batteries of three samples each (Fig. 6). Thus, the number of scans has been reduced from six to two. Initially samples with intact not affected by acid conodonts were scanned. All morphological elements of the conodonts were clearly observed (Fig. 7–10). The next, a reaction was carried out under the influence by hydrochloric acid and ammonium molybdate. After that, the samples were washed with distilled water, dried at room temperature, and scanned again.

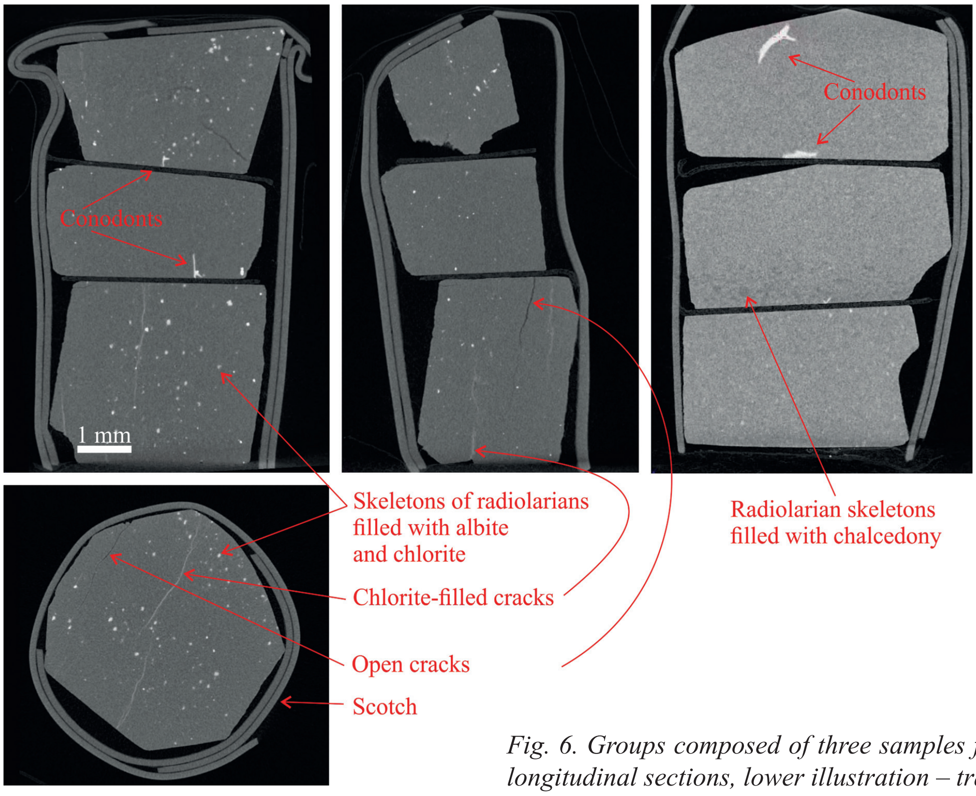

Fig. 6. Groups composed of three samples for tomographic imaging: top row – longitudinal sections, lower illustration – transversal section

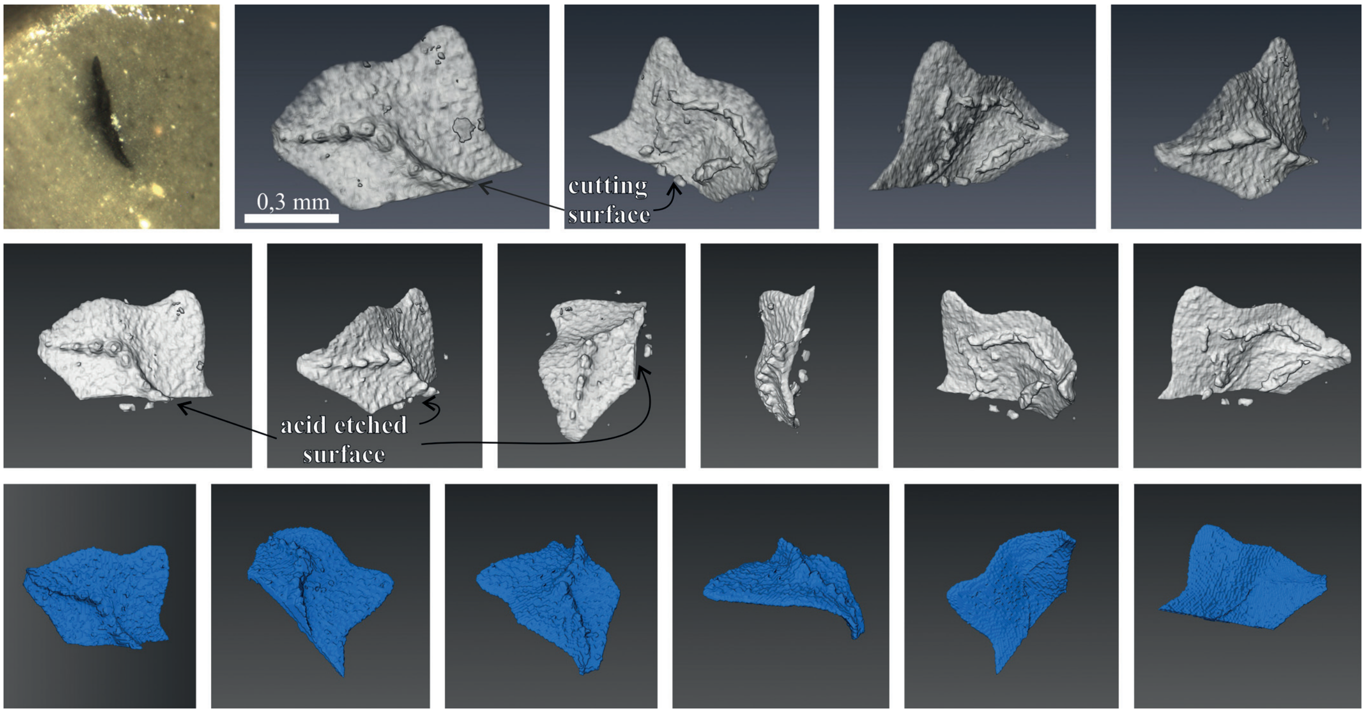

Fig. 7. Photo of a section (top left) and volumetric reconstructions of the conodont Palmatolepis cf. orlovi Khruscheva et Kuzmin before the reaction (top row), after the reaction (middle row) and the cavity after its dissolution (lower row)

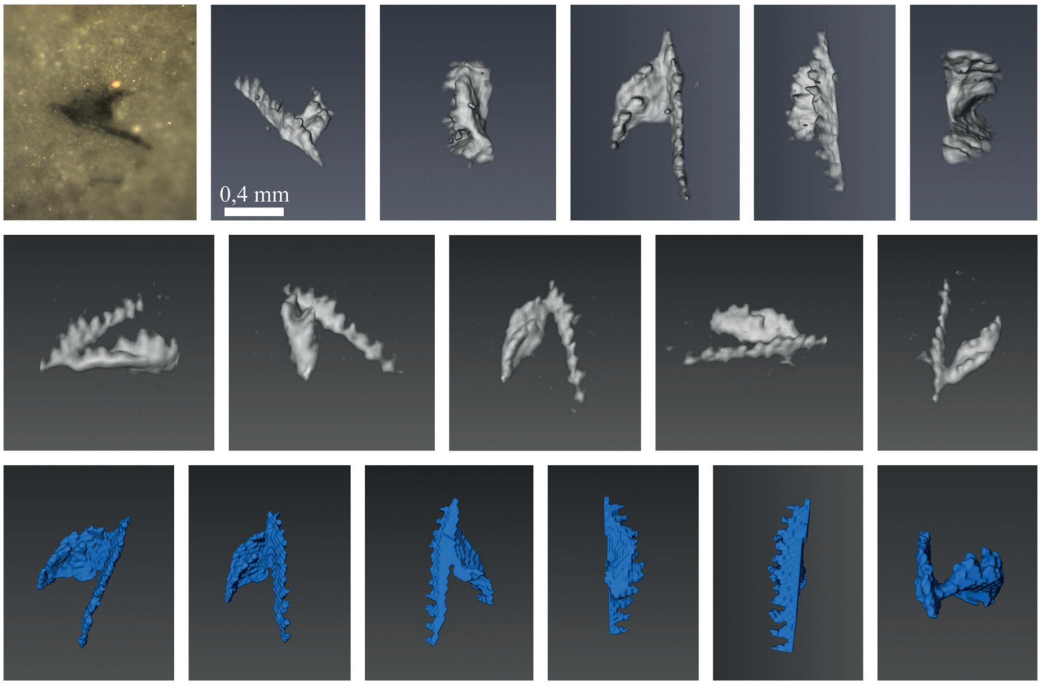

Fig. 8. Photo of the section (top left) and volumetric reconstructions of the conodont Ancyrodella sp. before the reaction (top row), after the reaction (middle row) and the cavity after dissolution (lower row)

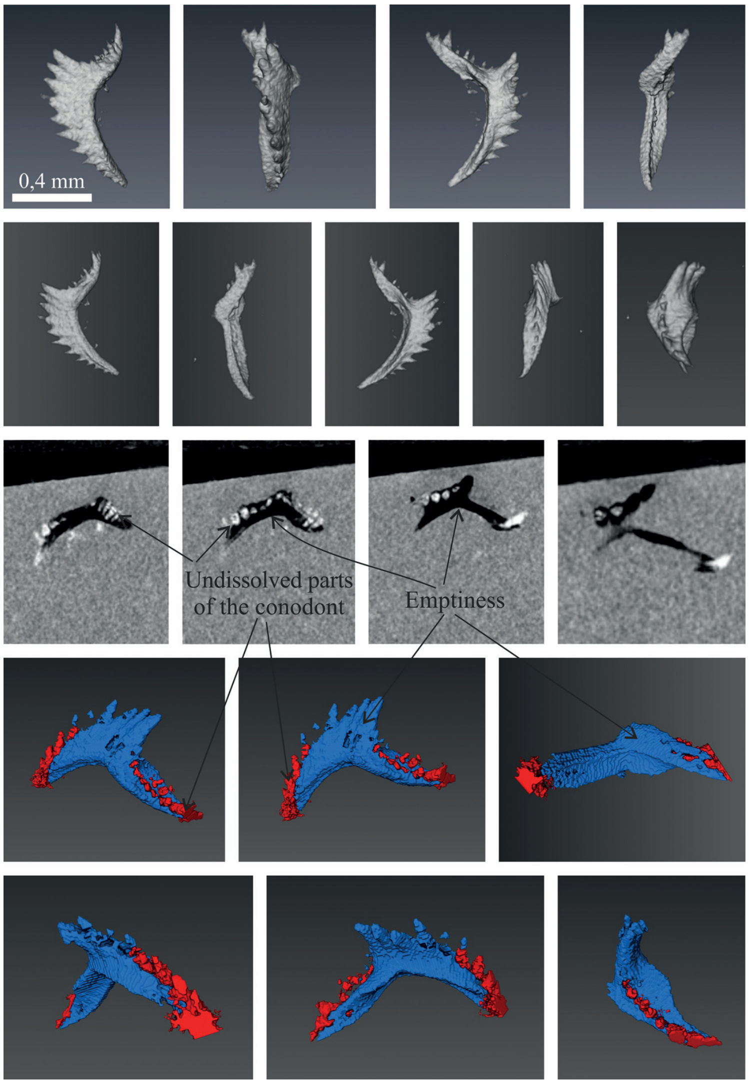

Fig. 9. Volumetric reconstructions of the conodont Nothognathella sp. before the reaction (top row), after the reaction (second row from the top), tomographic sections (middle row) and volumetric reconstructions (two lower rows) of the void left after dissolution of the conodont

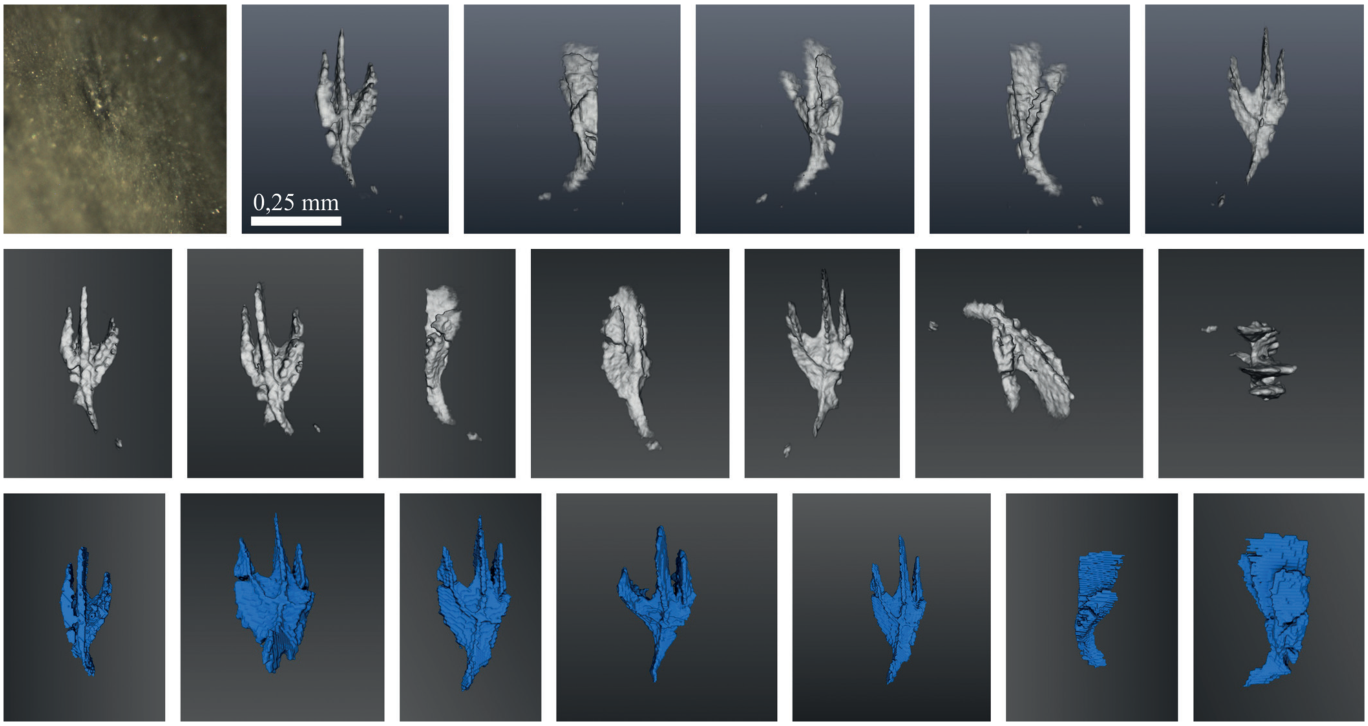

Fig. 10. Photo of a section (top left) and volumetric reconstructions of the Ancyrodella nodosa Ulrich et Bassler conodont before the reaction (top row), after the reaction (middle row) and the void left after its dissolution (lower row)

The resulting images (Fig. 7–10) are practically indistinguishable from the initial ones, except as the surface of sample under reaction became uneven and etched. Before the third scanning, the samples, as in experiment 1, were set in 10 % hydrochloric acid for several hours to dissolve the conodonts completely. Volumetric tomograms of the cavities after the conodonts dissolution look rather worse, but sufficient for determination the majority of the morphological elements of microfossils.

It should be noted, that in addition to the conodonts found visually on the sections, one specimen was found inside the sample (Fig. 9). In parallel, microtomographic studies made it possible to pay attention to some features of the siliceous rocks themselves, in particular, to the presence of open cracks, chlorite veins, and radiolarian shells filled with chalcedony and albite (the mineralogical composition was established from thin sections). Modern software makes it possible to fully study their distribution in the rock and make measurements, for example, of the volumetric content of radiolarians or the angles between cracks and veins of different orientations.

Conclusion

Experiments have shown that the reaction with ammonium molybdate and hydrochloric acid does not cause significant damage to conodonts, being mostly enclosed in siliceous rocks. Therefore, this technique can be used to find and identify conodonts. However, it is necessary to take into account the probability of the presence of the other phosphate fragments in the studied samples.

Tomograms of the cavities after complete dissolution of the conodonts are also informative and in some cases can help identify the conodont or clarify the definition of conodont images made before processing.

The suggested technique is possessed of the indisputable advantage because the participation of qualified specialists on tomography and palaeontology is necessary only at the final stages of study of an already found conodont. The performance of the preceding work can be provided by technical personnel.

The proposed method makes it possible to reduce the duration of days-long searching for conodonts in the field works, thereby to set free a specialist-conodontologist from routine work and transfer the search partially or completely to the laboratoty stage.

It is especially important for a palaeontologist to obtain a volumetric image of the object under study. The undoubted advantage of the X-ray μCT method is the fact that the obtained images are stored in electronic form and can be sent (for example, for consultation) anywhere in the world in a matter of seconds, via e-mail. In addition, electronic documentation, unlike rock samples, does not require numerous cabinets for its storage.

The presented data allow us to conclude about the efficiensy of the proposed technique. But in view of its high price at the moment, in biostratigraphy it can be used only to solve the most important and fundamental problems.

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