Microstructure and geochemical features of hard dental tissues of Deltavjatia vjatkensis and Suminia getmanovi: Permian tetrapods (Kotelnich site, Kirov region, Russia)

O Shilovsky¹², D Kiseleva³

¹Kazan Federal University, Kazan, Russia
²Natural History Museum of Tatarstan, Kazan, Russia
³A.N. Zavaritsky Institute of Geology and Geochemistry, UB RAS, Ekaterinburg, Russia

E-mail: nau@hotmail.ru, kiseleva@igg.uran.ru

Abstract. The Kotelnich pareiasaur site, one of the richest of the Permian period, is characterized by the unique integrity of fossil vertebrate assemblages and the preservation of bone’s internal structure (including fossil cellular structures) due to their burial features. Pareiasaur and suminia are two most widespread and most frequently encountered representatives of fossil fauna at the Kotelnich site, belonging to the herbivorous form of trophic affiliation. In the present work, a high degree of the structural preservation of the hard dental tissues of pareiasaur and suminia is demonstrated using scanning electron microscopy and energy dispersive X-ray spectroscopy. Fluorapatite composition of tooth tissues is indicated with trace rare earth elements and uranium incorporated from the host rock as a result of diagenetic alteration during fossilization. No secondary mineralization has been found in the pulp chamber uncovered by a cleavage. The features of its primary internal structure are observed such as pseudomorphs of iron-containing minerals over the fibrils of collagen fibers connecting odontoblastic processes with the walls of dentin tubules, as well as spherical pseudomorphs over blood-like cells.

1. Introduction

Bone and dental tissues of fossil organisms have a developed, hierarchical structure and complex phase and chemical composition, which requires the use of a comprehensive analytical approach that allows the information to be obtained at all scales, from atomic to macroscopic, as well as their interaction with host rocks to be assessed [1, 2]. Such studies require a qualitative and quantitative analysis of digital data obtained using various analytical methods with high spatial and temporal resolution.

The Kotelnich vertebrate fossil site (extending for more than 30 km along the eastern bank of the Vyatka River, Kirov region, Russia) is considered as a sub-oryctocomplex of the Sokolkovsky oryctocomplex from the paleontological point of view [3]. The unique integrity of vertebrate assemblages [4] as well as the preservation of bone internal structure (including fossil cellular structures) due to the burial features at this location were repeatedly noted by the researchers [1, 2]. Two most widespread and most frequently encountered representatives of the fossil fauna at the Kotelnich site are Deltavjatia vjatkensis and Suminia getmanovi.
pareiasaurs (40% of oryctocenosis) from the Procolophonomorpha clade and Suminia getmanovi anomodonts (25% of oryctocenosis) from the Therapsida clade. Important fact is the trophic affiliation of these species to one type — herbivorous form, where large amphibiotic pareiasaurs form the basis of the coastal association of the complex, and small herbivorous suminia are confined to the low and swampy coastal strip where specific hard vegetation grew [3].

Fossilized dental tissues (in particular, the most mineralized enamel) are characterized by the highest degree of resistance to diagenetic changes [5]. Dentin in modern dental tissues of animals and humans is a calcified intercellular substance penetrated by dentinal tubules [6]. The intercellular substance of dentin is represented by collagen fibers and the main organic substance (containing mainly proteoglycans), which are associated with hydroxyapatite crystals. The latter have the appearance of flattened, hexagonal prisms or plates.

Dentinal tubules are thin, narrowing outside tubules, radially penetrating dentin from the pulp to its peripheral and causing its striation [6]. Odontoblastic processes, nerve fibers surrounded by tissue (dentin) fluid (transudate of peripheral pulp capillaries, which also contains glycoproteins and fibronectin) are situated in dentinal tubules. This fluid fills the periodontoblastic space – the pathway for the transfer of various substances from the pulp to the dentin-enamel junction; it may contain individual uncalcified collagen fibrils [6]. From the inside, the wall of the dentinal tubule is covered with a thin film of organic matter – a boundary plate (Sheath of Neumann), which runs along the entire length of the dentinal tubule [6].

The aim of this work is to assess the preservation degree of the structures of pareiasaur and suminia hard dental tissues at the microscopic scale using scanning electron microscopy (SEM).

2. Materials and Methods

Tooth fragments of Deltavjatia vjatkensis and Suminia getmanovi extracted from the Kotelnich vertebrate fossil site were studied using a Carl Zeiss AURIGA CrossBeam SEM electron microscope with an Oxford instruments Inca X-Max energy dispersive spectrometer (EDS) (Kazan Federal University, Kazan). The fragments were cleaved manually both in transverse and longitudinal directions. Before the study, the samples were carbon-sputtered. Major element contents were normalized to a total sum of 100%.

3. Results and discussion

Fig. 1 shows SEM images of the studied fragments of dental tissues. According to EDS, all fossil dental tissues contain calcium, phosphorus, and fluorine, which may indicate that the dental hydroxyapatite has been converted to fluorapatite during fossilization [7, 8, 9, 10] (Table 1, points 1, 5 and 6). At the same time, in one of the near-surface fragments of pareiasaur tooth, high contents of cerium and uranium are found (Table 1, point 1), which may indicate the incorporation of rare earth elements (REE) and uranium into bioapatite crystal lattice as a result of diagenetic alteration [11].
Table 1. Major element composition of *Deltavjatia vjatkensis* tooth in analytical point 1 and *Suminia getmanovi* tooth in analytical points 5 and 6 (transversal cleavage) and analytical points 7 and 8 (opened pulp chamber)

| Element, Wt. % | Point 1 | Point 5 | Point 6 | Point 7 | Point 8 |
|----------------|---------|---------|---------|---------|---------|
| O              | 54.24   | 54.41   | 42.67   | 50.72   | 45.66   |
| F              | 5.90    | 3.45    | 3.27    | n/d     | n/d     |
| Na             | 0.41    | 1.50    | 1.39    | 1.86    | n/d     |
| Mg             | 0.16    | 1.86    | 0.38    | 0.90    | 0.17    |
| Al             | 0.06    | 4.81    | 1.05    | 1.48    | 1.60    |
| Si             | n/d     | 7.25    | n/d     | n/d     | 3.78    |
| P              | 12.74   | 7.14    | 14.35   | 12.00   | 3.08    |
| S              | 0.33    | 0.73    | 0.94    | 0.98    | 0.29    |
| Cl             | 0.09    | n/d     | n/d     | 0.22    | n/d     |
| K              | n/d     | 0.78    | n/d     | n/d     | n/d     |
| Ca             | 25.58   | 15.39   | 35.95   | 26.91   | 7.02    |
| Ti             | n/d     | n/d     | n/d     | n/d     | 0.29    |
| Fe             | n/d     | 2.68    | n/d     | 4.92    | 38.11   |
| Ce             | 0.34    | n/d     | n/d     | n/d     | n/d     |
| U              | 0.15    | n/d     | n/d     | n/d     | n/d     |

Total 100 100 100 100 100

Note: n/d – not detected

Fig. 1a, b show the dentin of pareiasaur and suminia with a longitudinal structure of dentinal tubules clearly visible, which forms a characteristic striation. Fig. 1c, d shows the holes of the dentinal tubules with transverse cleavage of the teeth of pareiasaur (c) and suminia (d).

Fig. 1e, f show a SEM image of a section along the dentin tubule, inside which fibrous formations are clearly visible, which can be the pseudomorphs of preserved fibrils of collagen fibers that connect odontoblastic processes with the walls of dentin tubules in the structure of the pareiasaur tooth. Their sizes (thickness about 0.2 μm) coincide with the sizes of collagen fibrils found in the bone tissue of the ichthyosaur [12].

The view of suminia tooth with natural cleavage is shown in Fig. 2a. According to EDS, the surface layers of tooth enamel contain Na, Mg, Al, Si, K and Fe, apparently derived from the host rock – clay minerals (Table 1, point 5). In the underlying dentin, these impurities are practically not found (Table 1, point 6).

As a result of natural cleavage, pulp chamber was opened (Fig. 2b); it should be noted that it was not filled with secondary mineralization, and its internal structure could be preserved in the form of pseudomorphs over cellular structures. Numerous spherical structures (Fig. 2f) with a diameter of about 8 μm, with a high iron content (38%), low calcium content (7%), and the presence of Si and Ti (Table 1, point 8) as compared with the elemental composition of the peritubular dentin of the pulp chamber wall (Table 1, point 7), were revealed inside the pulp chamber on its walls. The size of the holes in the wall of the pulp chamber (Fig. 2d, e) corresponds to the size of the dentinal tubules (0.5-1.5 μm). Based on the size and elemental composition, these structures can be interpreted as hematite or goethite pseudomorphs over blood-like cells. In [12], the detection of red and white blood cells in the bone tissue of an ichthyosaur (~ 180 Ma) was reported. It should be noted that, similarly to the cellular structures that we previously discovered in the bone tissue of the pareiasaur [1, 2], the fact that these structures
were found in the dentin on the inner wall of the cavity of the pulp channel, at the location of the blood and lymphatic vessels, speaks in favor of their non-bacterial origin. With bacterial colonization, these structures would not be so clearly localized, but would be found everywhere, including various parts of the dental tissue, which in our case was not found. In addition, coccoid-shaped bacteria usually have smaller sizes (0.5–2 μm) [12] than the cell structures we identified (~ 8 μm).

![SEM images of dentin. A cleavage along dentinal tubules, striation is visible: pareiasaur (a) and suminia (b); a cleavage across the dentinal tubules: pareiasaur (c), suminia (d); dentinal tubules of pareiasaur tooth (e); a section along the dentin tubule with pseudomorphs along the collagen fibrils (f). Analytical points 5 and 6 correspond to those given in Table 1](image-url)
Figure 2. SEM images of suminia tooth: general view of a tooth (a); a section cleaved along the tooth uncovering the pulp chamber (b); an enlarged image of the walls of pulp chamber with spherical formations (c); close-ups of spherical formations and the holes of the dentinal tubules (d, e, f).
Analytical points 7 and 8 correspond to those given in Table 1

4. Conclusions
As a result of the microscopic studies of fossilized dental tissues of Permian Deltavjatia vjatkensis and Suminia getmanovi, a high degree of their structural preservation is demonstrated. The presence of fluorine and apatitic composition of enamel and dentin indicate hydroxyapatite conversion into fluorapatite during fossilization. At the same time, the near-surface layers of enamel are enriched in REE and uranium derived from the host rock due to their incorporation into apatite crystal lattice as a result of diagenetic alteration. Due to the studies of fresh cleavages, a pulp chamber has been uncovered and found free of secondary mineralization, providing the possibility to observe the features of its intact internal structure. For example, the pseudomorphs of iron-containing minerals over preserved fibrils of collagen fibers connecting odontoblastic processes with the walls of dentin tubules are observed. Spherical structures are discovered in the pulp chamber opened by a cleavage, which can be interpreted as pseudomorphs over blood-like cells.
Acknowledgments

The authors are grateful to B.M. Galiullin (Kazan Federal University) for SEM-EDS analyses.

References

[1] Shilovsky O P and Kiseleva D V 2018 *Metallogenia drewnikh i sovremennykh okeanov (Miass)* vol 25 (Miass: IMin UrO RAN) pp 241-245 (in Russ.)
[2] Kiseleva D et al 2019 *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 526 28–42
[3] Benton M J et al 2012 *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 319–320 58–83
[4] Ivakhnenko M F 2001 *Tetrapody Vostochno-Evropeyskogo plakkata – pozddepaleooyoyskogo territorialno-prirodnoogo kompleksa* (Moscow: Paleontol. inst. RAN) (in Russ.)
[5] Bentley R A 2006 *J. Archaeol. Method Th.* 13 135–187
[6] Tjäderhane L et al 2009 *Endod. Topics* 20(1) 3–29
[7] Ryanskaya A et al 2019 *Powder Diffr.* 34(S1) S14–S17
[8] Elorza J et al 1999 *Cretac. Res.* 20 169–187
[9] Hubert J F et al 1996 *J. Sediment. Res.* 66 531–547
[10] Chipera S J and Bish D L 1990 *Adv. X-Ray Anal.* 34 473–482
[11] Trueeman C N and Tuross N 2002 *Rev. mineral. geochem.* 48 427–453
[12] Plet C et al 2017 *Sci. Rep.* 7 13776