ENAMEL ULTRASTRUCTURE OF LOWER MOLARS OF THE RED SQUIRREL
SCIURUS VULGARIS (MAMMALIA, RODENTIA) FROM DIFFERENT
POPULATIONS IN UKRAINE

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Enamel ultrastructure of lower molars of the red squirrel Sciurus vulgaris (Mammalia, Rodentia) from different populations in Ukraine. — L. Rekovets, O. Kovalchuk, V. Demeshkant, L. Shevchenko. — The second lower molars of the red squirrel Sciurus vulgaris from different regions of Ukraine were examined in order to search for additional features to study the variability and clarify the subspecies status of its individual populations. Generally accepted methods were used to study the enamel ultrastructure. It is established that the tooth enamel of the red squirrel has a layered structure and is represented by different types (radial, HSB and PLEX), which are also characteristic for other rodent species. The arrangement of crystalline prisms of the radial layer in the IPM matrix is the main distinguishing feature of the morphological variability of enamel on main tooth conids. In various regional populations of the species, they act as an indicator of the adaptability of the teeth and their functionality as a single structure. This was reflected in differences in the thickness ratio of the radial layer and HSB, especially between the metaconid and the entoconid. The development of HSB is usually associated with greater functional load. Squirrel populations in the northern part of Ukraine have a relatively thick HSB layer, while those from central and southern parts of the country have a more strongly developed radial layer. According to these features, the Crimean populations are close to those from southern Ukraine and slightly differ from the Altai subspecies Sciurus vulgaris exalbidus, which was introduced into the Crimea in the first half of XX century. It is assumed that the enamel ultrastructure as a morphological character has changed in the process of adaptation of squirrels to a new environment. The distinguishing character of the enamel structure of Altai populations of the Teleut squirrel is that prisms do not fill cells of the radial layer on the hypoconid and protoconid. The tooth enamel ultrastructure of the Eurasian red squirrel cannot be an independent morphological character for their intraspecific differentiation. However, it can be successfully used in combination with other characters (e.g., fur coloration, cranimterical data), as well as the results of special molecular studies.

Key words: Sciurus vulgaris, taxonomy, teeth, enamel, prisms, Ukraine.

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Introduction

The Eurasian red squirrel Sciurus vulgaris Linnaeus, 1758 is a common species in the fauna of Ukraine (Zagorodniuk, 2006, 2009). However, it is still inadequately studied in environmental and taxonomical aspects (Mygulin, 1938; Tsjupka, 2012). Studies concerning the taxonomic structure of the red squirrel were mainly focused on their intraspecific variation (Zizda, 2008).

The opinions about the taxonomic status of geographic forms remain controversial (Sidorowicz, 1971; Lurtz et al., 2005; Amori et al., 2014). From two to six squirrel subspecies are considered for the territory of Ukraine. Among them, three are the most reliable — S. vulgaris vulgaris L. (inhabiting the lowland forests), the Carpathian subspecies S. vulgaris carpathicus Pietruski, 1853 (Ukrainian Carpathians) and the Teleut squirrel S. vulgaris exalbidus Pallas, 1778 brought to the Crimea from the Altai in the 1940s (Puzanov, 1959; Dulitskaya et al., 1990; Zagorodniuk, 2006). The last clearly separated subspecies is of interest in terms of morphological changes during their adaptation to a new environment — mountain forests of the southern Crimea (Dulitsky, Dulitska, 2006).

According to the most recent data (Zizda, 2008, 2018), at least four color morphs (or subspecies) of Sciurus vulgaris were identified for the territory of Ukraine: Sciurus vulgaris carpathicus...
Pietruski, 1853 (the darkest morph from the Carpathian highlands), *S. v. fuscoater* Altman, 1855 (brown morph from the Carpathian region; Barkaszi, Zagorodniuk, 2016), *S. v. kessleri* Migulin, 1928 (red morph of the northern part of Right-Bank Ukraine), *S. v. ukrainicus* Migulin, 1928 (light-red squirrel of Right-Bank Ukraine). The animals from the Crimean population of the Teleut squirrel (*S. v. exalbidus*) are similar in fur coloration to *S. v. ukrainicus* (Zizda, 2018).

Craniometric and genetic studies (Zizda, 2008; Bilokon et al., 2014; Zizda, 2018) did not reveal any significant differences between the studied populations of this species in Ukraine, and molecular data aiming to solve this issue are currently absent. Our study results on the enamel ultrastructure of *Sciurus vulgaris* from different regions, corresponding to different colored morphs or subspecies, were aimed to identify possible local variations in the structure of their teeth enamel.

It is known that the structure of the tooth enamel is strongly correlated with their function and, therefore, with environmental conditions. The enamel structure has evolved in parallel with the teeth function and morphology (Koenigswald, 1997; Rekovets, Kovalchuk, 2017). An approach for the study of enamel ultrastructure was successfully used to establish family relationships between the relatively close groups of animals and for the creation of phylogenetic schemes (Koenigswald, 1996; Martin, 1997). It is believed that the tooth enamel has a complicated structure and consists of individual layers. Its basis is a relatively primitive (radial) enamel type; other layers are represented by the tangential and lamellar types (Koenigswald, 1980). Besides, additional enamel types (e.g., Hunter-Schreger bands in various modifications, lemming enamel, PLEX, etc.) were also identified (Koenigswald, 1990; Koenigswald, Sander, 1997). Each enamel layer is composed of prisms, laying in various directions on the interprismatic matrix (IPM).

Relative position and inclination of prisms, and the IPM (linear, reticular, weave, tilted) indicates the level of evolutionary advancement of each enamel layer (progressive and primitive enamel types), as well as the nature of adaptation and the role of enamel as a functional structure (Rekovets, Kovalchuk, 2017). It allowed us to characterize the enamel structure in teeth of different taxa in connection with their teeth function (Rekovets, Nowakowski, 2013; Popova, 2016; Rabiniak et al., 2017) as well as to justify its taxonomic significance as a diagnostic character (Koenigswald, 1990).

**Material and methods**

Thirteen second lower molars (m2) of adult, wild-caught squirrels from different regions of Ukraine were used for this study: Volyn (n = 1), Zhytomyr (1), Poltava (1), Sumy (1), Cherkasy (1), Chernihiv regions (2), as well as the Ukrainian Carpathians (2), Crimea (2), and Altai (n = 2). Our previous studies (L. Rekovets, unpublished data) showed the absence of any reliable differences in the enamel structure of other molars and incisors of the Eurasian red squirrel from different populations; therefore, we focused on a detailed investigation of m2 ultrastructure.

The preparation of the teeth for the analysis follows von Koenigswald (1980). The method was slightly modified by omitting the cut with a diamond saw (IsoMet® Low Speed Saw), which can damage the tooth. The specimens were ground immediately after embedding them in epoxy resin (Epoxy Embedding Medium kit — 45359 Sigma-Aldrich GmbH). The quality of grinding was controlled under stereomicroscope. The samples were rinsed for 10 seconds in a 5% HCl solution followed by ethanol, and placed for 30 seconds in ultrasonic bath to remove the dust. The samples were analyzed using a scanning electron microscope (Zeiss LEO 435) in the Laboratory of Electron Microscopy, Wrocław University of Environmental and Life Sciences (Poland).

The number of examined tooth areas depends on the regions of interest and the quality of grinding. The images were taken at different magnifications. Preparation and photography of examined samples were performed in Wrocław University of Environmental and Life Sciences. The paper presents the figures that are compared. In some cases, depending on the level of the teeth wear, the enamel at the talonid was also investigated (Fig. 1). The enamel ultrastructure was studied on standardized cross sections, i.e. in parallel to the occlusal surface, which is usually taken in such studies for other groups of animals. It should be noted that Koenigswald (2004) used the vertical (longitudinal) sections of the teeth. The scheme and the names of all elements for the squirrel teeth were essentially taken from the specified work (Fig. 1).
Results of the study

There are no significant differences in the enamel ultrastructure of molars in the Eurasian red squirrel on their horizontal and vertical sections. The tooth enamel of this species has a layered structure and consists of IPM, crystalline prisms, radial layer R (= portio externa according to Koenigswald, 1990, 2004) and relatively coarse multiserial HSB layer (= portio interna after Koenigswald, 1990, 2004). This enamel type was called as S-type (Koenigswald, Sander, 1997). It was formed from the more primitive enamel, which is typical for Paleogene rodents. The S-type enamel of Sciurus vulgaris is represented mainly by the radial type. The HSB layer is coarse and can be placed in different places with a total thickness of the enamel layer. The enamel ultrastructure of lower molars (m2) of Sciurus vulgaris from different parts of Ukraine is described in detail below.

Ukrainian Carpathians

The HSB layer of m2 consists of coarse structures (except those at the mesostylid), in contrast to other comparable populations. It takes 30 % (up to 90 % at the entoconid-mesostylid) of the total enamel thickness (Fig. 2 c, e, f) along the tooth perimeter. Cells of the radial layer are not densely filled by small prisms (Fig. 2 d). The talonid enamel consists only of the reticular IPM with prisms. The boundary between enamel layers is somewhat blurred. Moreover, the transitional layer is composed by the IPM with ellipsoid cells filled by prisms. This character is common for all studied populations. The enamel structure at the entoconid of m2 in squirrels from the Carpathian population resembles the pauciserial type, i.e. alternation of prisms in the radial and HSB layers (Fig. 2 a, b, e). It is similar to those in individuals from Zhytomyr and Sumy regions, as well as those from Altai.

Volyn region

Near 90 % of the enamel thickness around the tooth perimeter is composed of coarse HSB structures. The presence of rare prisms in small IPM cells of the radial layer and uncertain structures such as PLEX is noted at the posterior tooth wall, near the EDJ (Fig. 3 a). These characters distinguish this population from the others and may be associated with greater tooth wear. The radial layer at the mesostylid consists of structures, sloping to the EDJ and resembling the HSB (Fig. 3 b).

Zhytomyr region

The radial layer with prisms in the IPM cells is well-developed. The cells are more ellipsoid in shape toward the middle part of the enamel layer. The HSB is located near the OES and forms a ridge structure together with the radial enamel. This feature relates the population from Zhytomyr region to those from Sumy region and the Carpathians, and distinguishes it from the others (Fig. 4 a). The enamel of the lingual tooth wall is composed only of coarse HSB, especially at the mesostylid (Fig. 4 b). Unusual ribbed HSB structure at the hypoconid and protoconid is associated with irregular vertical deflection of the radial structures to the OES. It is visible in cross-sections as rings (near the EDJ) or ellipses (closer to the middle layer). This ribbed structure is not characteristic for other populations, except those from the Carpathians and Sumy region.
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Fig. 2. Ultrastructure of the m2 enamel of Sciurus vulgaris from the Ukrainian Carpathians: a — protoconid, b — entoconid, c — mesostylid, d — posterolophid, e — hypoconid, f — anterolophid.

Рис. 2. Ультраструктура емалі m2 Sciurus vulgaris з регіону Українських Карпат: a — протоконід, b — ентоконід, c — мезостилід, d — постеролофід, e — гіпоконід, f — антеролофід.

Fig. 3. Ultrastructure of the m2 enamel of Sciurus vulgaris from Volyn region: a — protoconid, b — mesostylid.

Рис. 3. Ультраструктура емалі m2 Sciurus vulgaris з Волинської області: a — протоконід, b — мезостилід.

Fig. 4. Ultrastructure of the m2 enamel of Sciurus vulgaris from Zhytomyr region: a — protoconid, b — mesostylid.

Рис. 4. Ультраструктура емалі m2 Sciurus vulgaris з Житомирської області: a — протоконід, b — мезостилід.
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**Sumy region**

The structure of the tooth enamel is identical to those from the above-mentioned regions. Some differences (except the ribbing) are in the radial layer that is filled by prisms at the anterior and posterior surfaces (anterolophid, posterolophid), as well as on the lingual side (entoconid — metaconid). The HSB structure is wavy. It occupies near 50 % of the total thickness of the tooth enamel (Fig. 5 a). For comparison, the HSB layer of m2 in the specimens from Zhytomyr comprises from 80 to 100 % of the enamel thickness. The structure of the radial layer consists of densely packed fine prisms in the elongated IPM cells. The boundaries between cells are not clearly visible (Fig. 5 b).

**Chernihiv region**

The enamel has a structure similar to the previous one, but ribbing (i.e. interlace of the R and HSB) is absent. The radial enamel is well-developed at the hypoconid (Fig. 6 a). Cells of this layer form the prisms disposed at an acute angle to the EDJ. The HSB is undulating in structure (Fig. 6 b). The inner enamel ring consists predominantly of the radial layer with prisms in the IPM cells. The HSB layer is very poorly represented.

**Cherkasy region**

The enamel is similar to the previous one. It is represented by the radial layer with prisms at the hypoconid. The HSB has an undulating structure and occupies about 50 % of the total enamel thickness. On other parts of the tooth, including the lingual one, the HSB comprises up to 80 %. Fine prisms of the radial layer resemble those in squirrels from Sumy region. They densely fill distinct ellipsoid cells of the IPM (Fig. 7).
Poltava region

The tooth enamel in representatives of this population somewhat differs from those in other studied populations. The HSB at the hypoconid occupies only 20% (radial layer — 80%). At the same time, this layer is up to 80% of the total enamel thickness at the protoconid (Fig. 8a), anterolophid and posterolophid. The HSB is up to 100% at the mesostylid, where the presence of PLEX enamel is possible (Fig. 8b). It should be noted that squirrels from this region (and the adjacent part of the Sumy region) differ considerably in color, and some morphological characters from other lowland populations within Ukraine. This was the basis for Mygulin (1938) to identify this population as a separate subspecies S. v. ukrainicus.

Fig. 7. Ultrastructure of the m2 enamel of *Sciurus vulgaris* from Cherkasy region: a — hypoconid, b — metaconid.

Fig. 8. Ultrastructure of the m2 enamel of *Sciurus vulgaris* from Poltava region: a — posterolophid, b — mesostylid.

Fig. 9. Ultrastructure of the m2 enamel of *Sciurus vulgaris* from the Crimean Peninsula: a — hypoconid, b — talonid.
The Crimean Peninsula

The radial enamel at the hypoconid has very small IPM cells filled by prisms ellipsoid in the middle part. It depends on the degree of vertical deviation of the IPM structures (Fig. 9 a). The HSB has an undulating structure and comprises up to 70 % of the total enamel thickness. On the other tooth surfaces this layer can take up to 50 % (Fig. 9 b). The HSB of the second, more erased, m2 has a wavy structure and occupies 80 % of the enamel thickness, and up to 100 % at the lingual side.

Altai

Aboriginal population of S. v. exalbidus is of interest for comparison. On the labial side of the tooth (hypoconid — protoconid) the radial enamel comprises up to 30 % of the total enamel thickness (HSB layer — 70 %) and small cells in the IPM are filled by prisms only near the EDJ.

Other cells of the radial layer, particularly at the hypoconid and protoconid, lack prisms (Fig. 10 a, b). The anterior and posterior tooth walls (antero- and posterolophids) have about 50 % thickness of the HSB and the radial layer. The talonid enamel has not yielded the HSB. Small cells of the radial enamel at the tooth perimeter (except the hypo- and protoconids) are filled by prisms. The enamel at the lingual side (metaconid–entoconid section) is composed only of HSB (Fig. 10 c, d).

Discussion and conclusions

Obtained data on the tooth enamel ultrastructure for Sciurus vulgaris from different populations of Ukraine and the Altai territory of Russia, as well as the results of their comparison with previously published data (Koenigsfeld, 1990, 2004) indicate that there is no significant difference among these populations. The enamel ultrastructure is almost identical to each other.
It is established that the entire perimeter of the tooth enamel is mainly composed of two enamel types — radial (R) and HSB. They are formed mostly on the IPM whose cells are almost always filled by crystalline prisms. According to Koenigswald (2004), this structure has the S-type enamel. It is characteristic for the representatives of Sciuromorpha and Sciuravidae with low tooth crowns. It is characterized by a relatively strong development of the HSB. The latter may be located near the EDJ or the OES, or inside the radial layer. In the samples studied here, the HSB layer is always located near the OES. Other distinguishing characters include the different thickness of the HSB around the tooth perimeter (from 30 to 100 %) and its location near the OES.

This character probably depends on the individual age, i.e. from the level of the teeth wear. In addition, the enamel of talonid structures consists only of the radial layer and its IPM is always filled with prisms, except for the samples from the Ukrainian Carpathians. More undulating structure of the HSB is characteristic for the populations from Cherkasy region, the Crimea and the Carpathians. The specificity of alternating layers of the radial enamel and HSB (grooved structure) in populations from Zhytomyr, Sumy, as well as the Carpathians should be emphasized.

The squirrels of all studied populations could be divided into three groups based on their enamel ultrastructure: (1) Carpathians, Zhytomyr and Sumy — characterized by a distinct ridge structures (alternation of the R and HSB); (2) Volyn, Chernihiv, Cherkasy, Poltava and the Crimea — the radial layer is located near the EDJ, and HSB near the OES; (3) Altai — the absence of prisms in the radial layer, especially on the protoconid and hypoconid.

The proportion of HSB can be increased up to 100 % of the enamel layer during the teeth wear in this species (Figs 6 b, 8 b). The same applies to the undulation and proportion of the radial enamel. Zhytomyr, Sumy and Carpathian populations are the most different among the compared groups. These populations have a ribbed HSB structure (alternating of the radial layer and HSB).

The Teleut squirrels (S. v. exalbidus) of the Crimean population seem to be closer in their enamel structure to the populations of the Ukrainian plain than to those from the Altai. Such conclusion is further supported by the complex of craniometric characters (L. Shevchenko, unpubl. data). A significant increase in skull height at the level of M1 in the Teleut squirrels of the Crimean population was reported by Dulitsky and Dulitska (2006). Such an increase of this feature is beyond the variability range of S. v. exalbidus, and even was a basis for the recognition of a new subspecies — S. v. puzanovi (Dulitskaya et al., 1990). As it was noted by Dulitsky and Dulitska (2006: 73), “the ecological status of the squirrel in the Crimea has changed, but the systematic status requires further clarification”. The enamel ultrastructure as a morphological character has also changed in the process of adaptation of these squirrels to new conditions in the Crimea. The distinguishing character of the enamel structure of Altai populations of the Teleut squirrel (from the Crimean Peninsula) is that the cells of the radial layer on the hypoconid and protoconid are not filled with prisms.

It should be noted that the tooth enamel ultrastructure of the Eurasian red squirrel cannot be a reliable morphological character for their intraspecific differentiation. However, it can be successfully used in combination with other data — fur coloration, craniometrical data as well as special molecular studies.

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