



COMPARATIVE CHARACTERISTICS OF INCISOR ENAMEL STRUCTURE IN SELECTED EXTINCT AND EXTANT HORSE FORMS

Vitalii Demeshkant¹ , Leonid Rekovets^{1,2} 

Key words

horses, breeds, teeth, enamel structure, Pleistocene, Holocene

doi

<https://doi.org/10.53452/TU3106>

Article info

submitted 10.06.2026

revised 24.06.2026

accepted 30.06.2026

Language

English, Ukrainian summary

Affiliations

¹ Wrocław University of Environmental and Life Sciences (Wrocław, Poland); ² National Museum of Natural History, NAS of Ukraine (Kyiv, Ukraine)

Correspondence

Leonid Rekovets; National Museum of Natural History, NAS of Ukraine; 15 Bogdan Khmelnytsky Street, Kyiv, 01054 Ukraine; Email: leonid.rekovets@upwr.edu.pl; orcid: 0000-0001-5277-2422

Abstract

The present study reports the results of a comparative analysis of the ultrastructure of incisor enamel in selected extinct species, historical forms, and extant representatives of the genus *Equus*, aimed at identifying evolutionary and functional trends in its structural organisation. The material examined comprised incisors of *Equus suessenbornensis* from the Middle Pleistocene of Germany, *E. latipes* from the Upper Pleistocene of Ukraine, *E. caballus* from different historical periods of the Holocene, as well as modern horse breeds of Central Europe. The study was carried out on horizontal and vertical sections of teeth and enamel using electron microscopy. All examined forms were characterised by a common enamel structural pattern, including Hunter–Schreger Bands (HSB), the *portio interna* (PI) and *portio externa* (PEX) layers, prismatic enamel (PE), and interprismatic matrix (IPM). Differences were revealed in the degree of development and spatial organisation of these structures. Pleistocene forms, particularly *E. latipes*, showed features of a more archaic enamel organisation, including weak differentiation of prism bundles and gradual transitions between structural types. In contrast, *E. suessenbornensis* demonstrated a more complex enamel structure compared with *E. latipes*. Historical and modern horses were characterised by a more complex enamel architecture, a more pronounced undulating arrangement of prisms and structural decussation, as well as considerable variability in the organisation of PI and PEX layers and PE and IPM components. These features may reflect both the evolutionary history of the genus *Equus* and adaptations associated with changes in environmental conditions, feeding patterns, and domestication processes. The study confirmed that Equidae tooth enamel is a morphological feature with a relatively stable structure and reflects functional adaptations. The results indicate that the ultrastructure of incisor enamel retains phylogenetically informative features and may serve as an additional source of data in taxonomic, palaeontological, and palaeoecological studies.

Cite as

Demeshkant, V., L. Rekovets. 2026. Comparative characteristics of incisor enamel structure in selected extinct and extant horse forms. *Theriologia Ukrainica*, 31: 54–68. [In English, with Ukrainian summary]

Порівняльна характеристика структури емалі різців у вибраних вимерлих і сучасних форм коней

Віталій Демешкант, Леонід Рековець

Резюме. Описано результати експериментів порівняльного аналізу ультраструктури емалі різців деяких вимерлих видів, форм історичних часів і сучасних представників роду *Equus* з метою виявлення еволюційних і функціональних тенденцій її структурної організації. Матеріалом дослідження слугували різці видів *Equus suessenbornensis* із середнього плейстоцену Німеччини, *E. latipes* із верхнього плейстоцену України, *E. caballus* різних історичних часів голоцену, а також сучасні породи коней Середньої Європи. Дослідження проводились на горизонтальних і вертикальних зрізах зубів і емалі за допомогою електронного мікроскопу. Встановлено, що всі досліджені форми характеризуються спільним планом будови емалі, який включає системи Hunter–Schreger Bands (HSB), шари *portio interna* (PI) та *portio externa* (PEx), призматичну емаль (PE) і міжпризматичну матрицю (IPM). Виявлено відмінності у ступені розвитку та просторовій організації цих структур. Для плейстоценових форм, особливо *E. latipes*, характерні ознаки більш архаїчної будови емалі, зокрема слабка диференціація призматичних пучків і поступові переходи між структурними типами. В той час як *E. suessenbornensis* має більш складну структуру в порівнянні до *E. latipes*. Історичні та сучасні коні відзначаються складнішою архітектурою емалі, більш вираженою хвилястістю укладів її призм та декусацією структур, а також помітною мінливістю в укладі шарів шарів PI і PEx та PE і IPM. Виявлені особливості можуть відображати як еволюційну історію роду *Equus*, так і адаптації, пов'язані зі змінами умов середовища, живлення та процесом доместикації. Дослідженнями підтверджено, що емаль зубів Equidae є морфологічною ознакою з відносно стабільною структурою та відображає функціональні адаптації. Отримані результати свідчать, що ультраструктура емалі різців зберігає еволюційність морфології і може бути використана як додаткове джерело даних при таксономічних, палеонтологічних, та палеоекологічних дослідженнях.

Ключові слова: коні, породи, зуби, структура емалі, плейстоцен, голоцен.

Introduction

Dental enamel is one of the most highly mineralised tissues in the vertebrate body and is characterised by a high resistance to mechanical wear and post-mortem alteration. Owing to these properties, enamel frequently preserves microstructural features that may provide valuable information regarding taxonomy, phylogeny, functional adaptations, and the evolutionary history of animals.

The ultrastructure of mammalian dental enamel has been the subject of extensive investigation for many decades, resulting in the identification of several structural components of both taxonomic and functional significance. These include Hunter–Schreger bands (HSB), *portio interna* (PI), *portio externa* (PEX), prismatic enamel (PE), interprismatic matrix (IPM), A- and Z-zones, among others. Previous studies have demonstrated that variations in the proportions and spatial organisation of these structures primarily reflect adaptations to specific feeding strategies and environmental conditions, thereby indicating their functional significance. Major contributions to the study of enamel microstructure were made by Korvenkontio [1934], Boyde [1964], Koenigswald and co-workers [1980, 1987, 1997], Pfretzschner [1993], Martin [1999], Kalthoff [2000], and others, whose work established the modern understanding of enamel organisation and its classification.

Most investigations of the family Equidae have focused on the enamel structure of cheek teeth, which play the principal role in food processing. The resulting data have been widely used for reconstructing diet, environmental conditions, and the taxonomic affinities of fossil forms. Considerably less attention has been devoted to incisors, despite their important role in the initial acquisition of food and their potentially high informativeness for evolutionary and systematic studies. Available evidence [Demeshkant & Rekovets 2021] indicates that horse incisor enamel possesses a complex organisation comprising HSB systems and several structural layers; however, comprehensive comparative studies involving fossil, historical, and modern representatives of the genus *Equus* remain scarce.

One of the first such attempts was undertaken for small equid forms traditionally assigned to the ‘tarpan’ group, with an analysis of similarities and differences in enamel structure between extinct and extant forms [Demeshkant *et al.* 2021, 2023].

In recent years, morphological, archaeozoological, and palaeogenetic investigations have substantially expanded our understanding of horse evolution and domestication [Levine 1999; Gaunitz *et al.* 2018]. These findings have been complemented by studies characterising enamel structures specific to perissodactyls [Boyde, 1964; Kilic *et al.* 1997]. Nevertheless, it remains insufficiently understood to what extent the ultrastructure of incisor enamel reflects evolutionary transformations within the genus *Equus*, including Pleistocene forms, historical populations, and modern breeds.

We hypothesise that fossil Pleistocene horses retained more archaic features of enamel organisation, whereas historical and modern forms exhibit a more differentiated enamel architecture shaped by evolutionary functional factors and domestication.

The aim of the present study is to compare the ultrastructure of incisor enamel in fossil, historical, and modern representatives of the genus *Equus* in order to identify evolutionary and functional trends in enamel organisation. To achieve this aim, the following objectives were established: (1) to characterise the microstructure of incisor enamel; (2) to compare the development and spatial organisation of HSB, PI, PEx, PE, and IPM among the investigated groups; (3) to identify similarities and differences in enamel organisation among these forms; and (4) where possible, to evaluate the taxonomic, evolutionary, and functional significance of the observed ultrastructural features.

Materials and Methods

The material examined in this study comprised selected extinct, historical, and extant representatives of the genus *Equus*. Fossil remains of *Equus suessenbornensis* originated from the Suessenborn locality (Germany), dated to the Middle Pleistocene, whereas specimens of *Equus latipes* were obtained from the Upper Pleistocene Mizyn locality (Ukraine). Historical specimens of *Equus caballus* were obtained from archaeological and museum collections. In archaeological terminology, the term ‘historical’ refers to periods for which written records are available. The oldest specimen originated from Tyras, an ancient Greek colony on the northern Black Sea coast (ca. 300 BCE), while additional material was derived from collections of Charles University in Prague (13th century CE) and Lviv Museum (18th century CE). These samples were analysed as individual chronological populations positioned between the Pleistocene fossil forms and modern horse breeds. For comparative purposes, modern horse breeds occurring in Central Europe were also investigated, including the Silesian, Hucul, Polish Warmblood, Polish Coldblood, Small Polish Local Horse, and Draft Horse breeds. All studied teeth samples are stored in the Natural History Museum of the University of Life Sciences in Wrocław (Poland).

In total, 28 permanent upper and lower incisors were analysed. All teeth originated from naturally deceased animals or from museum, archaeological, and palaeontological collections. Additionally, canine teeth of modern horses were examined to evaluate the extent to which the structural organisation of enamel is conserved among different tooth types within the equine dentition. Sample preparation followed standard procedures used in studies of mammalian enamel ultrastructure. Tooth sections were prepared in two planes: horizontal sections, parallel to the occlusal surface, and vertical sections, parallel to the long axis of the tooth. Following mechanical grinding and acid etching, the specimen surfaces were coated with a thin layer of gold prior to scanning.

The ultrastructure of enamel was examined using a Zeiss EVO LS15 scanning electron microscope. The resulting digital micrographs were analysed in detail. Particular attention was paid to the organisation of Hunter–Schreger bands (HSB), the development of the *portio interna* (PI) and *portio externa* (PEx) layers, the spatial relationships between prismatic enamel (PE) and interprismatic matrix (IPM), and the nature of transitions between different enamel types.

The comparative analysis was aimed at identifying similarities and differences in the spatial organisation of incisor enamel among fossil, historical, and modern horse forms. For each specimen, the degree of development of the PI and PEx layers, the type of HSB organisation, the structure of

diazones and parazonas, the arrangement of PE and IPM, the presence or absence of prism and prism-bundle undulation, structural decussation, and the characteristics of transitions between enamel types—particularly between Types I and III—were evaluated.

The terminology and interpretation of enamel structures were based on the works of Boyde [1964], Koenigswald and Pfretzschner [1987], Pfretzschner [1993], and other authors. Enamel-type classification followed the scheme proposed by Kilic *et al.* [1997]. A schematic representation of prism arrangement within the enamel structure of horse incisors is presented in Fig. 1.

List of abbreviations (referring to text and Figs. 1–13):

I, II, III—enamel types, A—diazone, EDJ—Enamel-Dentine Junction, HSB—Hunter Schreger Bands (A+Z), IPM—Inter-Prismatic Matrix, OES—Outer Enamel Surface, PE—Prismatic enamel, PEx—*portio externa*, Pi—*portio interna*, TZ—transition zone, Z—parazone.

Results

Extinct Pleistocene species

Equus suessenbornensis. Suessenborn, Middle Pleistocene (Fig. 2)

On vertical sections, the Hunter–Schreger band (HSB) system is clearly discernible and is represented predominantly by a pauserial arrangement of alternating diazones (A) and parazonas (Z) (Fig. 2 *c–d*). The HSB structures occupy most of the enamel thickness and are bordered externally by a well-developed *portio externa* (PEx) layer (Fig. 2 *a–b*).

Compared with the other forms examined, *Equus suessenbornensis* is characterised by a relatively well-developed PEx layer and a dense arrangement of enamel prisms within the diazones and parazonas. The boundaries between individual enamel structural components are clearly defined, resembling the condition observed in historical and modern horses. The prismatic structures constituting the HSB system exhibit a high degree of differentiation, noticeably greater than that observed in *E. latipes*.

The results indicate that the principal enamel types (Types I–III) and their associated structural components characteristic of modern representatives of the genus *Equus*, including HSB, PI, and PEx, were already established by the Middle Pleistocene. Nevertheless, the more complex spatial organisation of enamel prisms and the somewhat weaker differentiation of individual structural components distinguish *E. suessenbornensis* from later historical and modern forms.

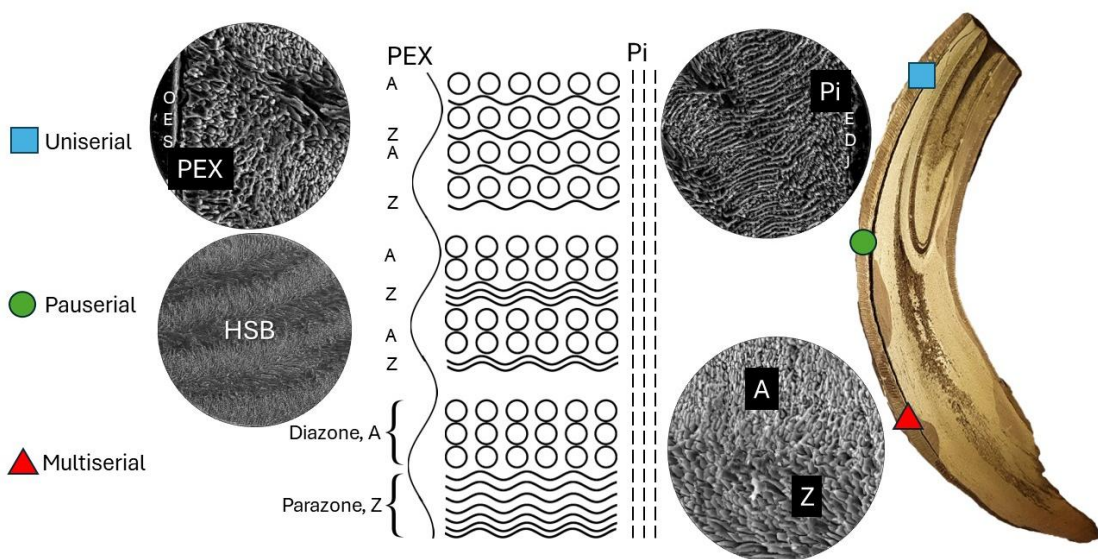


Fig. 1. Schematic structure of incisor enamel in Equidae.

Рис. 1. Схема структури емалі різців у Equidae.

Equus latipes, Mizyn, Upper Pleistocene (Fig. 3)

In the middle of the cross section, the enamel retains the hardly visible HSB structure (Fig. 3 a). The structure of HSB, PI and PEx is visible in the vertical section. Next to the EDJ border, the upper (Fig. 3 b) and lower (Fig. 3 c) teeth have a poorly developed third type of enamel with very elongated prism bundles and relatively thin IPMs.

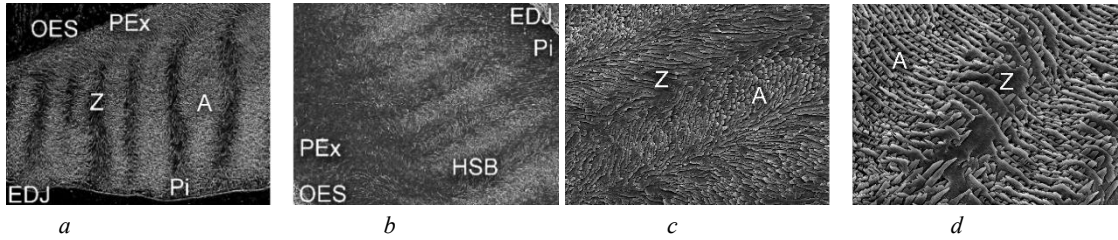


Fig. 2. Enamel structure of incisor of *Equus suessenbornensis*, vertical cross-sections: (a) pauserial, (b) uniserial, (c–d) diazone (A), parazone (Z).

Рис. 2. Структура емалі різців *Equus suessenbornensis*, вертикальний переріз: (a) паусеріал, (b) унісеріал, (c–d) діазона (A), паразона (Z).

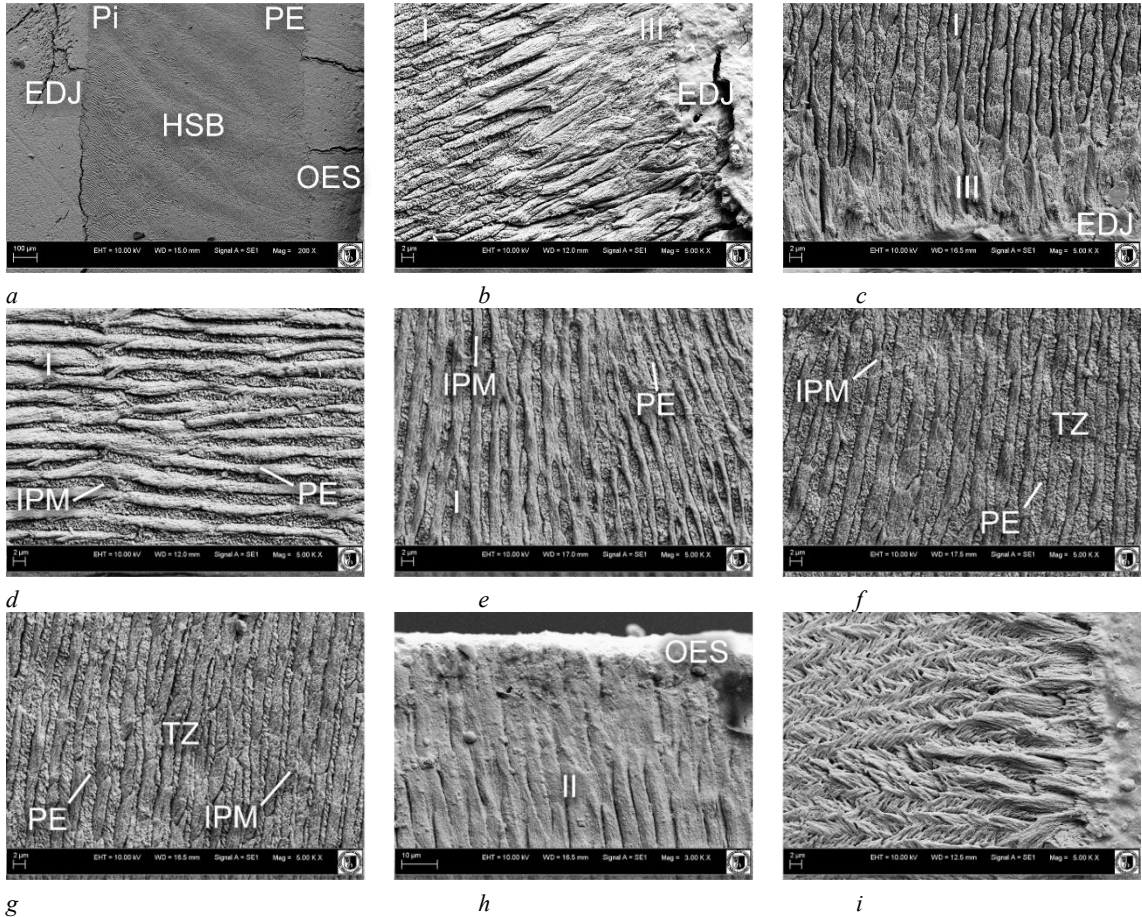


Fig. 3. Vertical (a) and horizontal (b–i) enamel sections of the upper (b, d, f, h) and lower (c, e, g) incisors in *E. latipes* from the Mizyn location.

Рис. 3. Вертикальний (a) та горизонтальний (b–i) зрізи емалі верхніх (b, d, f, h) та нижніх (c, e, g) різців у *E. latipes* із місцезнаходження Мізин.

A rapid transition from type III to type I is clearly visible, with prisms (PE) in the latter scarcely forming bundles. Closer to the centre of the cross-section, the PE strands become equal in thickness to the IPM bands on the upper (Fig. 3 *d*) and lower (Fig. 3 *e*) teeth.

A gradual transition (non-wavelike and decussation) transition of the first type to the second type in the TZ was also observed. The fine PE prisms are not stacked and arranged linearly between the thin IPM structures on the upper (Fig. 3 *f*) and lower (Fig. 3 *g*) teeth. The IPM bands virtually disappear next to the OES boundary, and the PE prisms are very elongated and form a portio externa-like structure (Fig. 3 *h*), inherent in many mammals' incisor [Koenigswald & Pfretzschner 1987; Kalthoff 2000]. No significant difference in the structure of the enamel of the upper and lower incisor in the examined species was observed. However, when the PE prisms were etched with hydrochloric acid, the unusual shape structure of the IPM system was noticeable (pyramid-shaped) (Fig. 3 *i*).

For incisors the elements of archaic morphology are characteristic, expressed in a very poor enamel development of the third type, PE prisms are not arranged in bundles and are very elongated next to the OES border.

Forms of historical times (Holocene)

Equus caballus, ancient form, *Tyras*, (Fig. 4)

In a cross-section, the incisor enamel of this form resembles molar enamel, it includes three types (I, II, III) and has a slightly wavy arrangement of prisms (Fig. 4 *a–c*). The PE prisms of the first and second types are of similar thickness to the IPM prisms and are predominantly rectangular, especially within TZ zone (Fig. 4 *c*). In vertical section, the enamel of both upper and lower incisors displays HSB structures with a ladder-like appearance (Fig. 4 *d*). Strands of prisms, forming the HSB, arranged between the boundaries of the EDJ and the OES with a slight tilt. In these HSB bands, the crystals can be arranged more vertically or more horizontally (acc. [Koenigswald & Pfretzschner 1987], p. 333, Fig. 9 vertical layer is called A or diazone and horizontal—Z or tangential, parazone layer).

These strands alternate along the length of the tooth and have their own specificity. It consists in the fact that closer to the occlusal surface, the HSB strands are narrow and strictly arranged together, and closer to the root, the strands gradually become thicker, sparse and not so close to each other. This means that HSB structures transform from the pauserial type to the multiserial type.

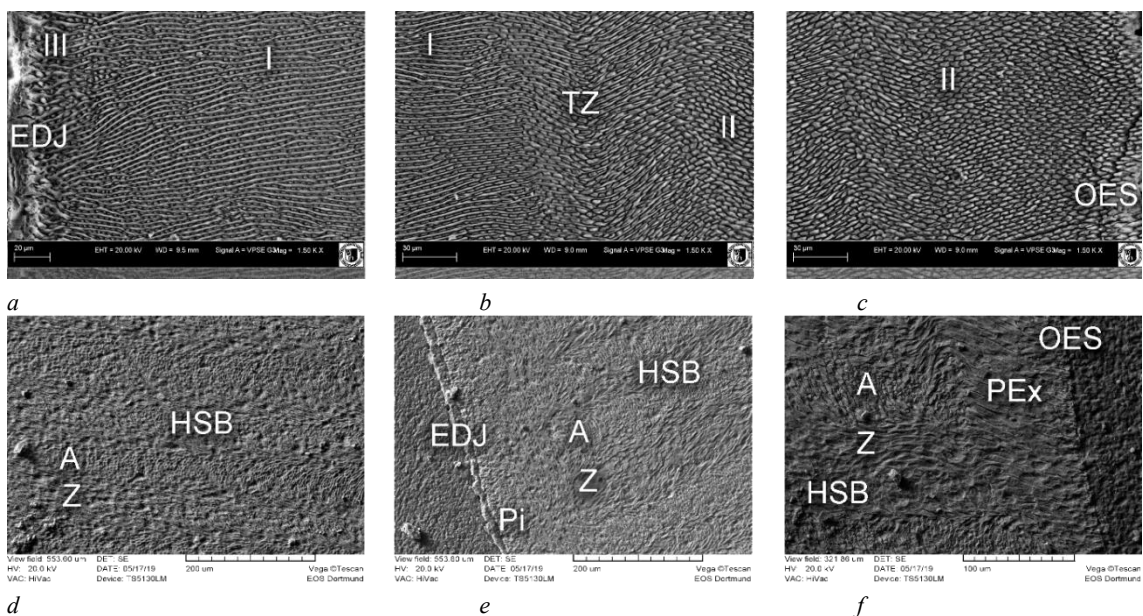


Fig. 4. Horizontal (*a–c*) and vertical (*d–e*) section of the enamel of lower incisors of *Equus caballus* from Tyras.

Рис. 4. Горизонтальний (*a–c*) та вертикальний (*d–f*) зрізи емалі нижніх різців *Equus caballus* порода з Тіри.

In addition to the marked specifics in the vertical section, there are also three strands of prism arrangement clearly visible: next to the EDJ border there is a strand with a chaotic arrangement of prisms along the tooth height—this is a poorly developed layer of *portio interna* PI (Fig. 4 e). In the middle there is a wide strand of the HSB type (Fig. 4 d, e), and next to the OES boundary there is a clearly visible band of the *portio externa* PEx layer (Fig. 4 f). In this case, the HSB structure is in the centre of the enamel and is surrounded by PI layers on the EDJ boundary side and a PEx layer on the OES boundary side. The characterised structure of the incisors enamel is to some extent unique because it contains horizontally and vertically three-layered, which differs from the enamel structures of comparable forms of the ‘caballus’ group, which often do not have a distinct PEx layer.

Equus caballus 13th century, Prague University (Fig. 5)

The lower and upper incisors were examined in two projections. The ladder-like HSB structures are clearly visible in the vertical section (Fig. 5 a–b). Next to the dentin-enamel border (EDJ) there is a separate layer of *portio interna* (PI) with an inclined and linear arrangement of the prisms. It takes almost 20% of the total thickness of the enamel. Next to the enamel-cement (OES) boundary, the *portio externa* (PEx) layer is absent or may be weak and sporadic in equine incisors. HSB structures generally have a lattice type IPM matrix filled with crystals. They are arranged at an angle opposite to each other, which looks like a ladder-like structure.

A detailed theoretical framework for the structure of incisor enamel was developed by Wighart von Koenigswald and co-workers [Koenigswald 1980, 1997; Koenigswald & Pfretzschner 1987]. The incisor specimen from the Prague collection shows a pauserial HSB enamel with an uneven order of diazones (A) and parazonies (Z) and the presence of PEx and PI layers of different thickness (Fig. 5 a–b). In the horizontal sections, next to the EDJ border, there is a well visible layer of the third type of enamel (III), which is the PI (Fig. 5 c–d). Then it relatively abruptly changes into structures of the first type with opposite to each other and linearly arranged strands of thick IPM and thinner PE packets. (Fig. 5 c). In the middle zone (TZ), the waviness of the structure is observed with almost equal thickness of IPM and PE with possible decussation (Fig. 5 d).

Equus caballus, 18th century, Lviv Museum (Fig. 6)

In the cross-section of the lower and upper incisors, three types of enamel similar in structure to the cheek teeth are clearly visible.

For the underdeveloped third type close to the EDJ border, is characteristic the relatively strong development of IPM and PE prism bundles (Fig. 6 a). Then they transform into structures of the first type with a linear and dichotomous system of IPM and PE with thick IPM. This type takes up almost 20% of the overall thickness of the enamel strand (Fig. 6 b–c). The transition layer (TZ) is the widest and covers almost 60% of the enamel thickness. According to the nature of the arrangement of IPM and PE, it is most strongly folded with decussation (Fig. 6 b–d). Such a strongly folded structure of the enamel is one of the most characteristic features of this breed of horses.

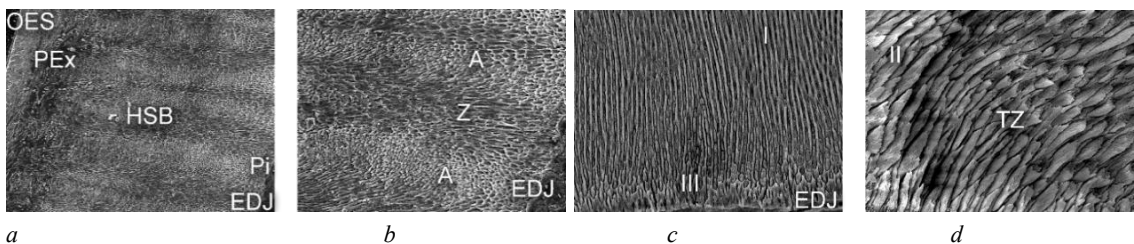


Fig. 5. Vertical (a–b) and horizontal (c–d) section of the enamel of the upper incisor *E. caballus* (Prague University). PEx—*portio externa*, PI—*portio interna*, HSB—Hunter-Schreger-Bands (var. pauserial), A—diazone, Z—parazone.

Рис. 5. Вертикальний (a–b) і горизонтальний (c–d) зрізи емалі верхнього різця *E. caballus* (з колекції університету в Празі). PEx — *portio externa*, PI — *portio interna*, HSB — Hunter-Schreger Bands (var. pauserial), A — diazone, Z — parazone.

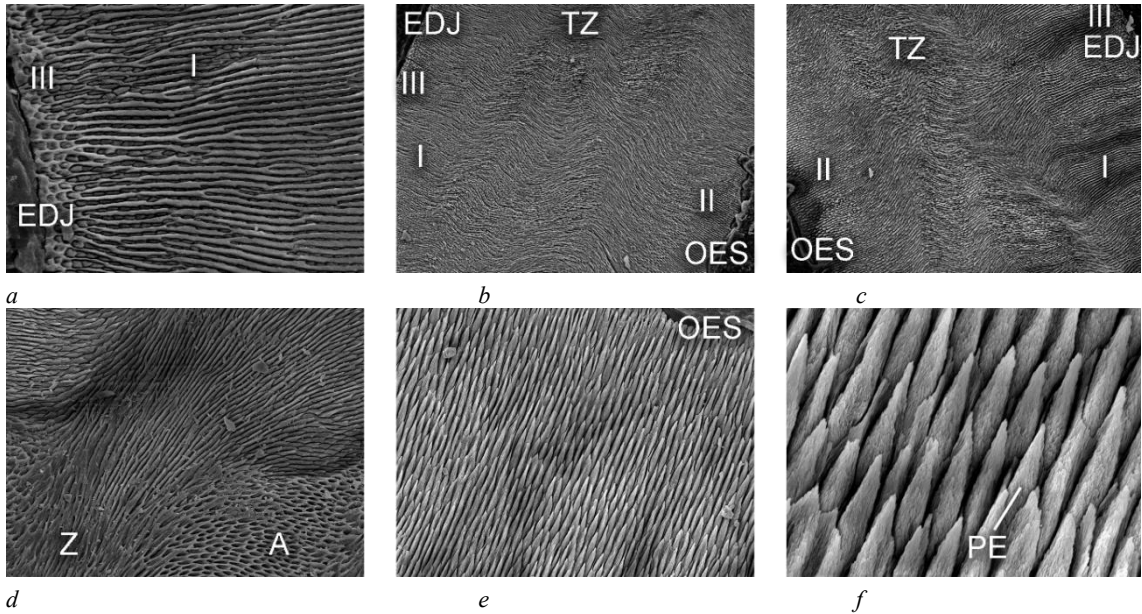


Fig. 6. Enamel cross-section of the upper (a–c) and lower (d–f) incisors of *E. caballus* breed from the Museum in Lviv.
Рис. 6. Поперечний зріз емалі верхніх (a–c) та нижніх (d–f) різців *E. caballus* з колекції Музею у Львові.

The second type of enamel covers 20% of the thickness of the enamel strand, has elongated, sharpened and strongly inclined towards the OES prisms. They are arranged in bundles and there are practically no IPM rows between them (Fig. 6 e–f). The vertical section retains the HSB structure with a PEx layer on the enamel-cement boundaries and a wider PI layer on the dentine-enamel boundaries. The HSB strands are clearly visible and tilted towards the centre of the tooth.

Modern breeds of *Equus caballus*

Equus caballus Silesian breed (Fig. 7)

The first and second maxillar and mandibular incisors were analysed. On the cross-section parallel to the occlusal surface of the tooth, there are clearly visible layers and types of enamel specific to the teeth (Fig. 7 a). Next to the EDJ border is a thin layer of III enamel type with large prisms and weak IPM strands (Fig. 7 b).

The first enamel type takes up no more than 20% of the total thickness of the enamel, it has well-developed bundles of hydroxyapatite crystals leaning towards the OES boundary (Fig. 7 c–d). The thin strands of IPM almost disappear in the second type of enamel next to the OES border (Fig. 7 e). It is characteristic that the transition zone (TZ) of the first and second type is wide, covers almost 50% of the thickness of the enamel, has a corrugated and decussated model of the IPM and PE bands arrangement (Fig. 7 a–b). The vertical section of incisor has a typical structure of HSB-multiserial prism systems [Koenigswald 1980; Muylle *et al.* 2001; Ferguson *et al.* 2005]. In our work it is called a ladder-like structure (Fig. 7 f–g). The arrangement of the prism strands is orderly-chaotic.

This means that the strands are repeated along the vertical plane of the tooth, but in separate strands the prisms are arranged randomly. Some bands have a more vertical arrangement of the prisms—diazone, other—horizontal, parazone (Fig. 7 h). After the height of the tooth, the width of these bands varies. Closer to the occlusal surface, the strands are relatively narrow and often arranged in line with each other (Fig. 7 g). In this case, the enamel resembles the HSB pauserial structure. Closer to the root exacerbation, the diazone strip and parazones become wide and sparse arranged together (Fig. 7 h–i). In this case, the enamel resembles the structure of a multiseries. Along the boundaries of OES and EDJ, separate layers (PEx and PI, respectively) with a chaotic arrangement of prisms are clearly visible (Fig. 7 f).

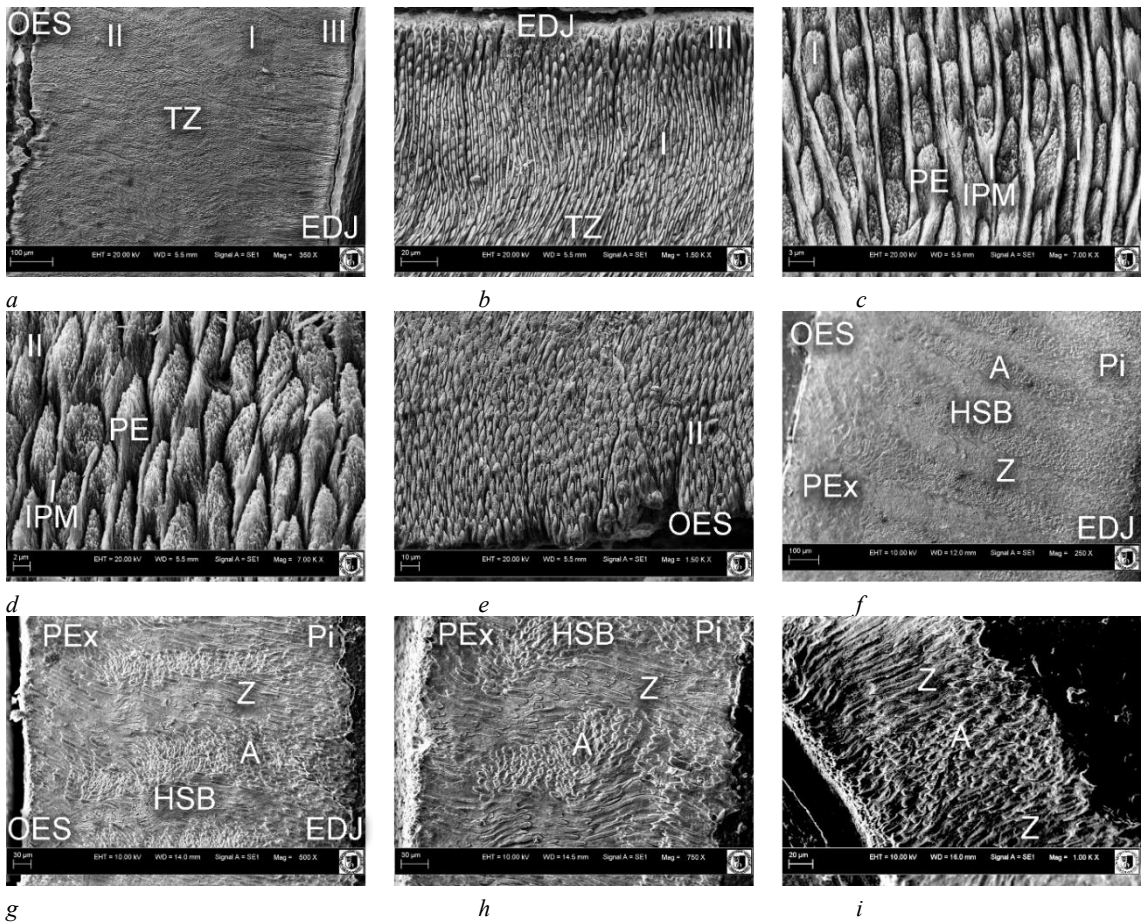


Fig. 7. Cross- (a–e) and vertical (f–i) sections of the enamel of the lower incisor *E. caballus* of the Silesian breed.
Рис. 7. Поперечний (a–e) і вертикальний (f–i) зрізи емалі нижнього різця *E. caballus* Сілезької породи.

Equus caballus cold-blooded breed (Fig. 8)

The enamel of upper and lower incisors was investigated in both deciduous and permanent dentitions. The HSB structure is clearly visible in the vertical section (Fig. 8 a) with PI and PEx bands next to the enamel boundaries. The HSB is poorly visible on deciduous teeth.

In transverse sections, the morphology is like the structures of molars. The third enamel type next to the EDJ border is significantly developed compared to molars (Fig. 8 b–c). Its thick PE prisms and thin IPMs relatively abruptly transform into the first type with parallel strands of prism tufts and thick IPM structures. The transitional (TZ) zone together with the second type demonstrates the thickening of the very inclined PE prisms and the poor development until the disappearance of the IPM next to the OES boundary (Fig. 8 d–f). However, the preserving of the IPM with lenticular PE prisms is also possible (Fig. 8 f).

Equus caballus Hucul breed (Fig. 9)

The structure of the HSB type with poor development of PI and PEx layers was poorly observed in the vertical section (Fig. 9 a). The level cross-section demonstrates the constant nature of the structure arrangements. The third type is underdeveloped with thick IPM, relatively progressively turns into the first type with precisely linear structure of IPM and PE (Fig. 9 b). The transition zone (TZ) is slightly wave-like (Fig. 9 c) with elongated PE prisms and visible IPMs that do not disappear alongside the OES (Fig. 9 d).

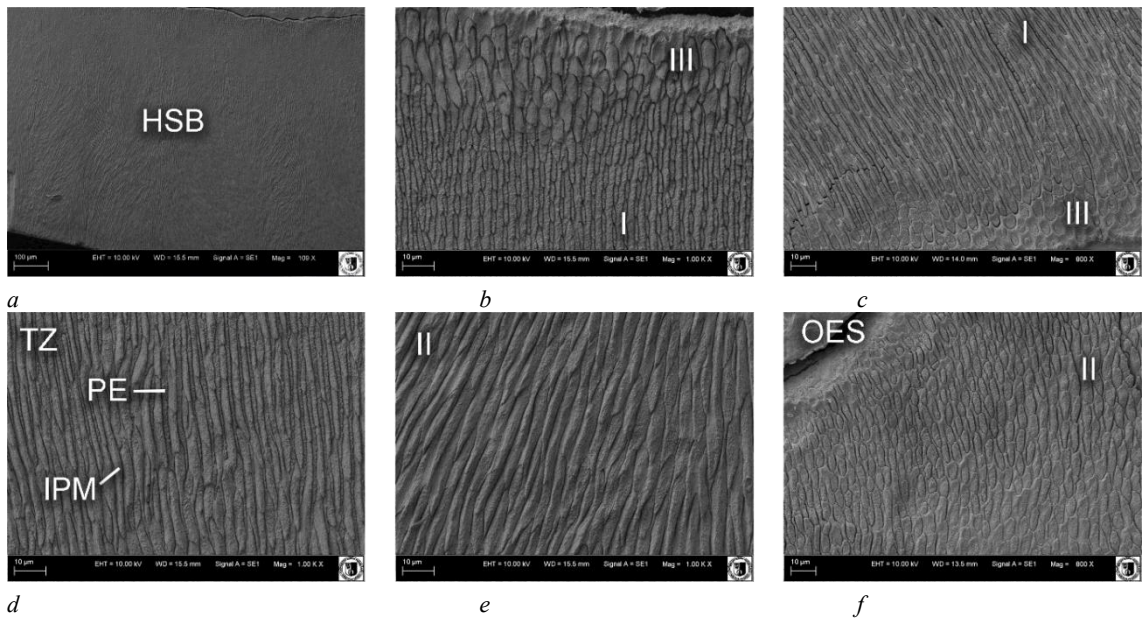


Fig. 8. Horizontal section of enamel in upper deciduous incisors in *E. caballus* cold-blooded breed.

Рис. 8. Горизонтальний переріз емалі верхніх молочних різців холоднокрової породи *E. caballus*.

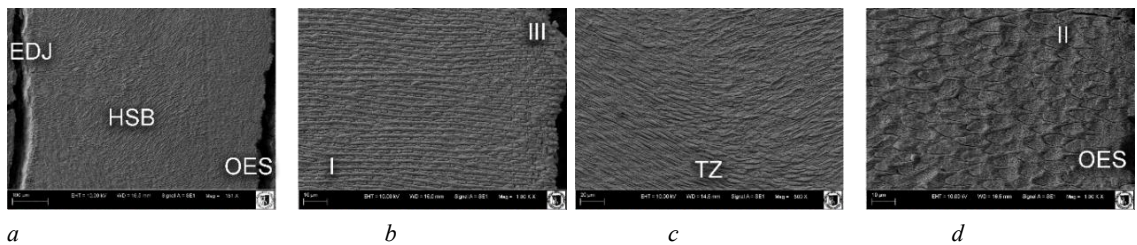


Fig. 9. Vertical (a) and horizontal section (b–d) of lower incisors enamel of *Equus caballus* of Hucul breed.

Рис. 9. Вертикальний (a) та горизонтальний зріз (b–d) емалі нижніх різців *Equus caballus* гуцульської породи.

Equus caballus Polish warmblood breed (Fig. 10)

The HSB structures and the PEx layer in the vertical section are hardly visible and the PI layer next to the EDJ is more pronounced. In the horizontal section, the first and third types of enamel are clearly visible and with a characteristic crack between them with (Fig. 10 a). In the central part of the TZ, a dichotomy of elongated IPM structures of the second type is observed (Fig. 10 b–c).

Equus caballus small breed horse (Fig. 11)

The front labial part of the lower incisor retains the faint HSB structure with parallel and elongated PE prisms (Fig. 11 a–b). The third type of enamel is most visible on the lingual part of the tooth enamel (Fig. 11 c) with strong IPM. The central part of the TZ is composed of thick elongated PE prisms and weak IPMs (Fig. 11 d). PI and PEx layers are barely visible.

Equus caballus draft cross horse (Fig. 12)

The teeth demonstrate a clear HSB structure (Fig. 12 a–b), which can be assessed as a pauserial variation with slightly tilted diazone prisms (A) and practically horizontally arranged parazone prisms (Z) (Fig. 12 c). A similar organisation was also observed in several comparable form. We admit that it is a specificity, appropriate for this breed. The PI layer is hardly visible, and the PEx layer has at an angle but parallel arranged PE and IPM prisms (Fig. 12 c–d).

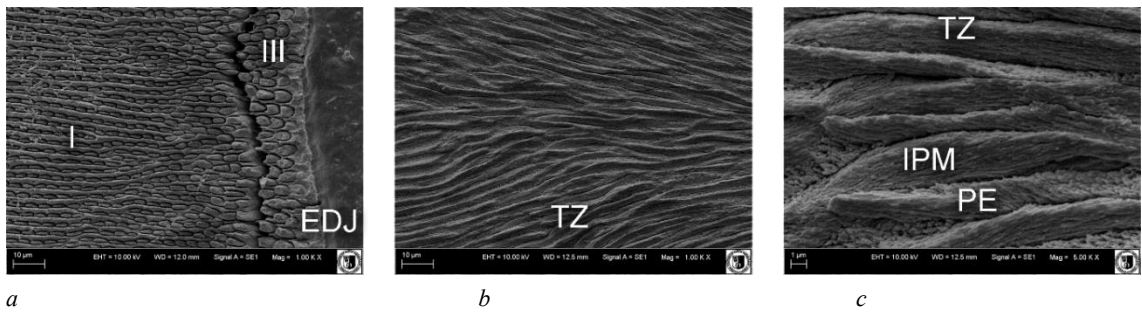


Fig. 10. Horizontal section of the lower incisor enamel of Polish warmblood *E. caballus*.

Рис. 10. Горизонтальний зріз емалі нижнього різця *E. caballus* польської теплокровної породи.

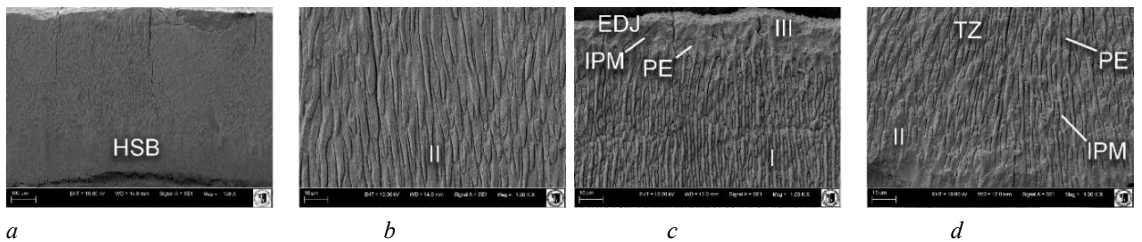


Fig. 11. Vertical (*a*) and horizontal (*b–d*) enamel sections of incisors in small breed *E. caballus*.

Рис. 11. Вертикальний (*a*) і горизонтальний (*b–d*) зрізи емалі різців у малої породи *E. caballus*.

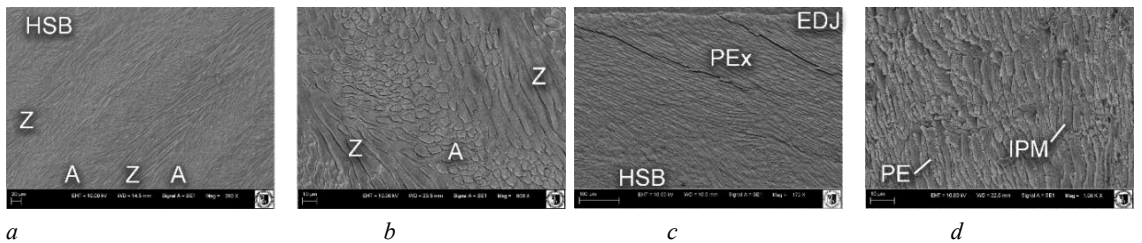


Fig. 12. Vertical (*a–c*) and horizontal (*d*) enamel section of upper incisors in draft cross *E. caballus*.

Рис. 12. Вертикальний (*a–c*) і горизонтальний (*d*) зріз емалі верхніх різців у ваговозної помісі *E. caballus*.

Ultrastructure of canine teeth enamel (canini) in horses (Fig. 13)

The enamel structure of the upper and lower canines, which is like that of the incisors, was also examined (Fig. 13 *a*). In the vertical section of the canine, three basic structures were distinguished. The PI layer, next to the EDJ, is well developed and composed of parallel strands of prisms (Fig. 13 *b*). The PEx layer is hardly visible next to the OES boundary (Fig. 13 *c*). HSB structures include strands of diazone prisms (A) and parazone (Z) arranged after the uniserial type (Fig. 13 *a, c–d*). Closer to the root base, HSB adopts a shape like the multiserial type with a chaotic arrangement of structures (Fig. 13 *e*). In the horizontal section, the arrangement of PE and IPM prisms is uniform across the enamel width (Fig. 13 *f*). It can be concluded that the systems of enamel structures in incisors and canine teeth are very similar, although they have their own specificity, for example in the HSB multi-series system. This may reflect taxonomic and functional differences.

Discussion

The present study demonstrates that incisor enamel exhibits a high degree of structural conservatism while simultaneously displaying gradual evolutionary and functional modifications within the genus *Equus* throughout the Late Pleistocene and Holocene. Representatives of the genus *Equus*, irrespective of geological age, share a common structural organisation of incisor enamel.

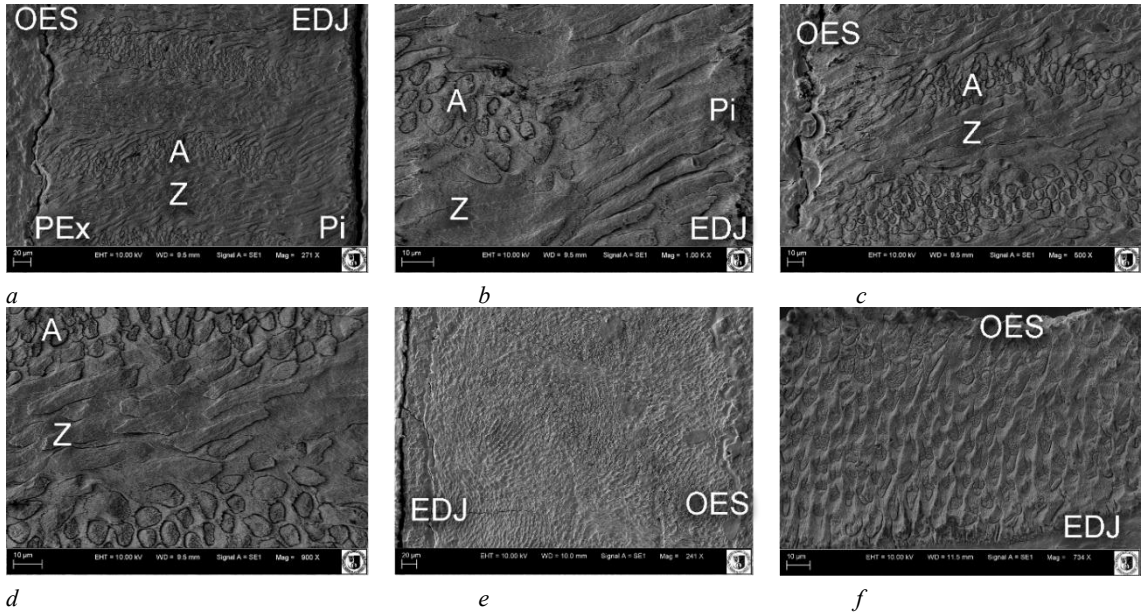


Fig. 13. Enamel structure of canine tooth of modern *Equus caballus*: (a–d) vertical section, (e–f) horizontal section.

Рис. 13. Будова емалі ікла сучасного *Equus caballus*: (a–d) вертикальний переріз, (e–f) горизонтальний переріз.

In all examined forms, Hunter–Schreger bands (HSB), *portio interna* (PI), and *portio externa* (PEx) layers were identified, together with diazones and parazones, as well as the three principal enamel types (Types I–III), which are defined by different proportions of prismatic enamel (PE) and interprismatic matrix (IPM). These findings are consistent with previous studies of the enamel structure of both incisors and cheek teeth in Equidae, while also revealing differences in prism arrangement and organisation [Kilic *et al.* 1997; Demeshkant & Rekovets 2021].

The most primitive structural features were observed in *Equus latipes*. This species is characterised by weak differentiation of Type III enamel, the absence of clearly defined prism bundles, the persistence of interprismatic matrix near the external enamel surface, and gradual transitions between individual structural zones. Such an organisation indicates a relatively low degree of enamel specialisation and resembles the more archaic condition described for some early perissodactyls [Boydé 1964; Pfretzschner 1993]. In contrast, the Middle Pleistocene species *Equus suessenbornensis* exhibits a well-developed HSB system with a highly organised arrangement of prisms within diazones and parazones, as well as a distinct PEx layer adjacent to the enamel–dentine junction (EDJ). These features suggest that the principal mechanisms responsible for enamel reinforcement had already evolved at an early stage in the evolutionary history of horses.

According to Koenigswald [1980] and Koenigswald & Pfretzschner [1987], the HSB system represents one of the most important structural components of incisor enamel and plays a key role in enhancing its mechanical resistance, particularly against wear. Our results support these conclusions and further allow the evolutionary development and variability of this structure to be traced through both time and morphology. The Pleistocene forms, especially *E. latipes*, possess a comparatively simple enamel organisation, whereas in historical and modern horses the HSB system becomes increasingly complex and differentiated. This trend is particularly evident in the eighteenth-century specimen from the Lviv Museum and in the Silesian breed, where well-developed diazones and parazones, complex undulating structural arrangements, and localised decussation were observed. Despite the considerable temporal interval separating the investigated forms, the principal structural elements of incisor enamel remained largely unchanged, whereas differences were expressed mainly in their spatial organisation and degree of development.

The most distinctive feature of the incisor enamel in the examined Equidae is the presence of a well-developed Hunter–Schreger band (HSB) layer located between the PI and PEX enamel layers. This layer consists of decussating enamel prisms arranged in alternating nearly vertical diazones (A) and nearly horizontal parazonies (Z). This structural organisation was consistently observed in all investigated taxa.

The HSB layer exhibited marked structural variation along the entire length of both the lingual and labial enamel surfaces of the incisors. Away from the occlusal surface, the A and Z bands were numerous, regularly arranged, and closely resembled the uniserial HSB pattern. Towards the tooth root, the arrangement of the A and Z bands gradually changed, becoming like the pauserial HSB type. Near the root apex, the HSB layer was invariably multiserial, characterised by broad and structurally less ordered A and Z bands. This gradual transition in HSB organisation along the longitudinal axis of the incisor, documented here for the first time in the examined *Equus* forms, represents a previously undescribed pattern whose functional and developmental significance remains unclear.

In transverse sections, the incisors of all examined taxa exhibited a well-developed Type III enamel corresponding to the PI layer adjacent to the enamel–dentine junction (EDJ). A pronounced undulation of the prism enamel (PE) and interprismatic matrix (IPM) was observed within the transition zone (TZ), accompanied by prism decussation. This feature was particularly well developed in the historical horse from Lviv and in the canines, whereas it was absent or considerably less pronounced in the remaining forms. The undulating architecture of the TZ is likely to have functional significance by enhancing resistance to mechanical stress. Its weak development, especially in modern domestic horses, may reflect adaptation to a softer diet. Furthermore, in all examined incisors the PE and IPM exhibited nearly equal thickness within Type I enamel, in contrast to the condition observed in molars, where these components differ substantially in thickness [Demeshkant *et al.* 2021].

Comparative morphological analysis indicates that enamel evolution did not proceed through a simple, linear increase in structural complexity over time. The most complex enamel architecture was not observed in modern breeds but rather in *E. suessenbornensis* and the eighteenth-century specimen from the Lviv Museum. These forms are characterised by pronounced undulation of PE and IPM structures, a complex organisation of the transitional zone, and well-developed A and Z zones. This observation suggests that enamel complexity was determined not only by evolutionary age [Kilic *et al.* 1997a, b, c; Demeshkant *et al.* 2021], but also by functional factors associated with feeding behaviour and husbandry conditions [Seetah *et al.* 2016].

It is well established that horse domestication was accompanied by substantial changes in diet, patterns of use, and selective pressures [Seetah *et al.* 2016; Orlando *et al.* 2021]. The present results suggest that these processes may also have influenced the microstructural organisation of enamel. Modern breeds exhibit considerable variability in the development of individual structural components. For example, draft horses display the most highly organised HSB system, whereas Hucul horses and small local breeds show simplification of certain structural elements, including reduced prism undulation and weak development of A and Z zones. Modern forms are further characterised by well-developed prism bundles, elongated prisms of Type II enamel, and relatively weak development of IPM and PEX near the external enamel surface.

The differences identified between Pleistocene, historical, and modern representatives of the genus *Equus* indicate gradual modifications in the organisation of incisor enamel during the last million years. These changes primarily involved the degree of differentiation of HSB, PI, and PEX structures, whereas the overall structural framework of enamel remained relatively stable.

The results of this study further suggest that enamel microstructure may represent an important source of phylogenetic information and may reflect long-term processes of transformation within the equine dentition over extended evolutionary timescales. Particularly informative features include the degree of HSB development, the relationship between PE and IPM, the nature of transitions between enamel types, and the organisation of the PI and PEX layers [Kalthoff 2000].

The integration of these data with morphometric, archaeozoological, and palaeogenetic evidence may contribute to a more comprehensive understanding of phylogenetic relationships, domestication processes, and ecological adaptations within the genus *Equus*.

Conclusion

The conservative nature of enamel structure and its relatively limited variability indicate a common pattern of prism arrangement despite the differing functional roles of incisors and cheek teeth in both extinct and extant Equidae. Functionally, incisor enamel is more homogeneous than molar enamel and exhibits greater morphological stability, with the principal mechanical load being borne by the Hunter–Schreger band (HSB) system. Consequently, structural modifications observed among extinct species, historical forms, and modern horses mainly involved minor qualitative refinements in prism arrangement rather than fundamental changes in enamel architecture. Given the relatively short geological timescale represented by the investigated material, these modifications remain subtle. The similarities and differences identified in the present study reflect both the shared phylogenetic heritage of the examined forms and the influence of environmental conditions, including diet, as well as management practices and selective breeding in the modern species *E. caballus*.

A preliminary synthesis of our findings concerning the enamel structure of all tooth categories in horses—incisors, canines, premolars, and molars [Demeshkant *et al.* 2021, 2024]—indicates the presence of a common, although highly conservative, structural framework. This framework is determined by a relatively limited set of morphological components, namely hydroxyapatite prisms and their spatial arrangement within distinct enamel layers. During phylogeny and ontogeny, the relative organisation of these components gives rise to integrated structural systems, such as Hunter–Schreger bands, which are established during embryogenesis through the activity of enamel-forming cells [Currey 1999]. Through evolutionary processes, these interactions have resulted in the emergence of an exceptionally efficient and mechanically resilient biomaterial characterised by a unified structural plan. This organisational pattern is shared not only by all tooth types within Equidae but also, to varying degrees, by many other vertebrate taxa [Anisimov 2014].

The remarkable hardness and durability of enamel arise from the hierarchical organisation of its constituent elements rather than from the properties of individual prisms alone. From a broader systems perspective, enamel structure may represent one of the clearest examples of an integrated biological system in which the properties of the whole emerge from the spatial arrangement and interaction of relatively simple structural units. In this context, the organisation of enamel reflects the synergistic effects of developmental, functional, and evolutionary processes acting upon its constituent elements—in this case, hydroxyapatite prisms [Ponomarev 2004].

Particularly noteworthy is the dual significance of enamel as both a functional component of living organisms and a highly stable material that preserves structural information long after the death of the organism. These considerations may also be extended to other mineralised tissues and hard biological structures, including dentine, bone, shells, otoliths, and related biomineralised systems. A broader systems-based analysis of their structure, properties, and functions may contribute to a deeper understanding of the general principles governing the organisation and evolution of biological materials.

Acknowledgements

We are grateful to Prof. Ivan Horáček, Prof. Ralf-Dietrich Kahlke, and Prof. Dariusz Nowakowski as well as Drs Andriy Starkin, Igor Zagorodniuk, and Zoltán Barkaszi for their help in work with materials and manuscript.

Declarations

Funding. No funding was allocated for this study.

Conflict of interests. The authors have no conflicts of interest to declare that are relevant to the content of this article.

Handling of materials. The study was conducted in compliance with the requirements of the current legislation regarding work with live and collection materials.

Use of artificial intelligence. No generative artificial intelligence systems were used in the preparation of this manuscript. All scientific results, interpretations, and text are original contributions of the authors.

References

- Anisimov, I. O. 2014. *Synergetyka: Textbook*. Kyiv University Press, Kyiv, 1–511. [Ukrainian]
- Currey, J. D. 1999. The design of mineralised hard tissues for their mechanical functions. *Journal of Experimental Biology*, **202**: 3285–3294. DOI: 10.1242/jeb.202.23.3285
- Demeshkant, V., L. Rekovets. 2021. The ultrastructure of the tooth enamel of small Equus of the ‘tarpan’ group and their possible phylogenetic connections. *Fossil imprint*, **77** (1): 55–72. DOI: 10.37520/fi.2021.007
- Demeshkant, V., M. Biegalski, L. Rekovets. 2023. Teeth Enamel Ultrastructural Analysis of Selected Equidae Taxa. *Diversity*, **15**: 1141. DOI: 10.3390/d15111141
- Gaunitz, C., A. Fages, K. Hanghøj, et al. 2018. Ancient genomes revisit the ancestry of domestic and Przewalski’s horses. *Science*, **360** (6384): 111–114. DOI: 10.1126/science.aao3297
- Kalthoff, D. C. 2000. Die Schmelzmikrostruktur in den Incisiven der hamsterartigen Nagetiere und anderer Myomorpha (Rodentia, Mammalia). *Palaeontographica Abteilung A*, 1–193.
- Kilic, S., P. M. Dixon, S. A. Kempson. 1997a. A light microscopic and ultrastructural examination of calcified dental tissues of horses: 1. The occlusal surface and enamel thickness. *Equine Veterinary Journal*, **29** (3): 190–197. DOI: 10.1111/j.2042-3306.1997.tb01668.x
- Kilic, S., P. M. Dixon, S. A. Kempson. 1997b. A light microscopic and ultrastructural examination of calcified dental tissues of horses: 2. Ultrastructural enamel findings. *Equine Veterinary Journal*, **29** (3): 198–205. DOI: 10.1111/j.2042-3306.1997.tb01669.x
- Kilic, S., P. M. Dixon, S. A. Kempson. 1997c. A light microscopic and ultrastructural examination of calcified dental tissues of horses: 3. Dentine. *Equine Veterinary Journal*, **29** (3): 206–212. DOI: 10.1111/j.2042-3306.1997.tb01670.x
- Koenigswald, W. V. 1980. Schmelzmuster und Morphologie in den Molaren der Arvicolidae (Rodentia). *Abhandlungen der Senckenbergisch naturforschenden Gesellschaft*, **539**: 1–129.
- Koenigswald, W., H. U. Pfretzschner. 1987. Hunter-Schreger-Bänder im Zahnschmelz von Säugetieren (Mammalia). *Zoomorphology*, **106** (6): 329–338. DOI: 10.1007/BF00312255
- Koenigswald, W. V., P. M. Sander. 1997. Schmelzmuster differentiation in leading and trailing edges, a specific biomechanical adaptation in rodents. In: *Tooth enamel microstructure*. CRC Press, 259–266. DOI: 10.1201/9781003077930-14
- Korvenkontio, V. A. 1934. Mikroskopische Untersuchungen an Nagerincisiven unter Hinweis auf die Schmelzstruktur des Backenzahnes. In: *Annales Zoologici Societatis Zoologicae-Botanicae Fennicae*. Vanamo, Helsinki, **2**: 1–274.
- Levine, M. A. 1999. Botai and the origins of horse domestication. *Journal of Anthropological Archaeology*, **18** (1): 29–78. DOI: 10.1006/jaar.1998.0332
- Martin, T. 1999. Phylogenetic implications of Glires (Eurymyliidae, Mimotonidae, Rodentia, Lagomorpha) incisor enamel microstructure. *Zoosystematics and Evolution*, **75** (2): 257–273. DOI: 10.1002/mmnz.19990750207
- Muylle, S., P. Simoons, H. Lauwers. 2001. The distribution of intratubular dentine in equine incisors: a scanning electron microscopic study. *Equine Veterinary Journal*, **33** (1): 65–69. DOI: 10.2746/042516401776767395
- Pfretzschner, H. U. 1993. Enamel microstructure in the phylogeny of the Equidae. *Journal of Vertebrate Paleontology*, **13** (3): 342–349. DOI: 10.1080/02724634.1993.10011514
- Ponomarev, A. A. 2004. *Synergetics of Living Systems*. Scientific and Educational Center of Applied Informatics. Kyiv, 1–77. [Russian]
- Seetah, K., Cardini, A., & Barker, G. 2016. A ‘long-fuse domestication’ of the horse? Tooth shape suggests explosive change in modern breeds compared with extinct populations and living Przewalski’s horses. *The Holocene*, **26** (8): 1326–1333. DOI: 10.1177/0959683616638436