

# Errata

Below are the corrections as well as further clarifications of issues raised by thesis reviewers:

Contents	Chap. 5.3&8	The chapter title of the manuscript has been changed as follows: Enamel <b>Apatite</b> Crystallinity: a Significant Component of the Mammalian Dental Adaptations.
Page 1	Line 6	references added: 1) Maas M. C. & Dumont, E. R. (1999). Built to last: The structure, function, and evolution of primate dental enamel. <i>Evol. Anthropol.</i> , 8, 133-152. 2) Kohn M. & Cerling T. E. (2002). Stable isotope compositions of biological apatite. <i>Rev. Mineral. Geochem.</i> , 48, 455-488. 3) Thesleff, I. (2003). Epithelial-mesenchymal signalling regulating tooth morphogenesis. <i>J. Cell Sci.</i> , 116(9):1647-1648. 4) Boskey, A. L. & Mendelsohn, R. (2005). Infrared spectroscopic characterization of mineralized tissues. <i>Vib. Spectrosc.</i> , 38(1-2), 107-114. 5) Smith T. M. & Tafforeau P. (2008). New visions of dental tissue research: Tooth development, chemistry, and structure. <i>Evol. Anthropol.</i> , 17, 213-226. 6) Bei M. (2009). Molecular genetics of tooth development. <i>Curr. Opin. Genetics Dev.</i> , 19(5), 504-510. 7) Moradian-Oldak, J. (2012). Protein-mediated enamel mineralization. <i>Front. Biosci.</i> , 17(6):1996-2023. 8) Bartlett, J. D. (2015) Dental enamel development: Proteinases and their enamel matrix substrates. <i>ISRN dentistry</i> , 2013(1): 1-24.
Page 9	Line 31	The differentiation of pre-ameloblasts in the pre-secretory stage is initiated by signalling molecules secreted by enamel knot and underlying odontoblasts. This phenomenon was observed in mouse and human models.
Page 9,11,14&15	Par. 3,2,2&2	The description of all stages of ameloblasts is based on human and mouse models.
Page 11	Line 5	They form a thin layer of prismless enamel directly onto the dentine <i>as documented in the primate, porcine and mouse models.</i>
Page 11	Line 34	The mineralization itself comprises a cooperation of proteins (amelogenin, ameloblastin, enamelin,etc.) of noncollagenous origin, protein-degrading proteinase (enamelysin MMP20) and inorganic components. These observations were shown on human, porcine, rat and/or cow models.
Page 12	Line 1	... biologically <i>controlled</i> mineralization ...

Page 12	Line 6	Beniash et al. (2009) proved that the initial mineral phase in tooth <i>enamel</i> is amorphous calcium phosphate (ACP). This phenomenon was determined using mouse model.
Page 12	Line 31	This model is strongly supported by the observations of Robinson et al. (1981a) using a freeze fracture technique <i>on a rat incisors</i> who proposed ...
Page 13	Line 1	Moreover, the studies of Fincham et al. (1999) and Moradian-Oldak (2012) ... reference added: Yang X., Wang L., Yueling Q., Sun Z., Henneman Z. J., Moradian-Oldak, J. & Nancollas G. H. (2010). How amelogenin orchestrates the organization of hierarchical elongated microstructures of apatite. <i>J. Phys. Chem. B</i> , 114, 2293-2300.
Page 13	Line 3	These findings are based on the observations of rodent model and <i>in vitro</i> experiments.
Page 13	Line 17	... and force the <i>apatite</i> crystals to growth ...
Page 13	Line 26-37	Experiments covering the function of enamelin, amelotin and also enamelysin were performed using porcine, rodent and human models as well as in vitro experiments.
Page 14	Line 1	Preferential formation of interprismatic enamel was observed on extremely derived mammalian taxa such as primates (including humans), elephant, carnivores (dog, cat), etc.
Page 14	Line 18	Documented by experiments in porcine, human and mouse models.
Page 16	Par. 1&2	The changes in pH during secretory and maturation stages were shown on the bovine and rodent models.
Page 17	Par. 2	The final processes of maturation stage were observed in rodent and human models.
Page 20	Line 29	...the highly textured appearance of the <i>enamel</i> .
Page 22	Fig. 2.7	All the images shown were taken on the fully mature first molars of female pigs aged 17-30 month.
Page 40	Section 5.3	The title of the manuscript has been changed as follows: Enamel <b>Apatite</b> Crystallinity: a Significant Component of the Mammalian Dental Adaptations.
Page 85	Chapter title	The title of the manuscript has been changed as follows: Enamel <b>Apatite</b> Crystallinity: a Significant Component of the Mammalian Dental Adaptations.

Page 85	Line 1*	<i>The monophyodont molar teeth, prismatic enamel and the complexity of enamel microarchitecture are regarded as essential dental apomorphies of mammals. As prominent background factors of feeding efficiency and individual longevity these characters are crucial components of mammalian adaptive dynamics. Little is known, ...</i>
Page 86	Line 12*	<i>... self-assembly processes resulting in supramolecular aggregates (documented by plenty of in vitro experiments, see Ruan &amp; Moradian-Oldak, 2015) are believed to ...</i>
Page 86	Line 15*	<i>The crystallographic properties of enamel apatite and microarchitecture of ...</i>
Page 87	Line 20*	<i>Our results suggest that apatite crystallinity increases ...</i>
Page 88	Fig. 8.1*	<i>... in terms of categories by Grant (1982): 1 - no visible cusp wear (category a-b); 2 - minute wear (category b-c); 3 - pronounced cusp wear, visible small dentine fields (category c-e); 4 - advanced cusp wear, extensive dentine fields (stages e-g); 5 - massive tooth wear, most of the crown enamel is missing (category h-m). The boxplot in wear section covers all individuals in particular stage of tooth wear: the youngest individual is in the most left part of the boxplot; the oldest one is in the most right part of the boxplot and the average age of all individuals in particular wear stage is the middle line inside the rectangle. The plus sign was used when only a single individual of particular stage was analyzed.</i>
Page 90	Fig. 8.3*	<i>Computed volumes of enamel HAp crystallites of (a) miniature pigs. For comparison of changes in crystallite volume with age, the volumes are plotted against the age of each individual. The solid black lines are the average volume for each molar type and the colored areas show a dispersion of data set (<math>1\sigma</math>). Each point in the plot (b) represents the average HAp crystallite volume of individual molar types of red deer.</i>
Page 92	Fig. 8.5 *	<i>The FTIR spectral differences of phosphate vibrations in the enamel apatite of the five fractions (differ in particle size) of three molar types (<math>M_1</math> to <math>M_3</math>) in the series of tested pigs. a) grinding curves the relation of IRSF against FWHM for three different molar types (each point represents the averaged value of five studied pigs.); b) an example of the measured FTIR spectra used for the plotted parameters (FTIR spectra of five different fractions of second molar (<math>M_2</math>) of 26 months old individual showing the variations in FWHM); c) IRSF boxplots of the finest fraction values obtained from all studied individuals.</i>

The whole paragraph has been added at the end of the manuscript: *It should be noted that also other factors, such as the inner architecture of mature enamel or the content of organic residues can attribute to the mechanical behaviour of enamel coat (Koenigswald and Clemens, 1992; He and Swain, 2007, 2008; Xie et al., 2008; Yahyazadehfar and Arola, 2015). In regard to our study the former variable can be considered as an invariant - no posteruptional changes in enamel architecture can be expected (Kallistova et al, 2017). Moreover, the observations of Cuy et al.(2002), Braly et al. (2007) and Fonseca et al. (2008) suggest that the hardness and Young's modulus of enamel apatite are only weakly dependent on the enamel architecture. In contrast, the organic component of the mature enamel (concentrated particularly along EDJ see Robinson et al., 1995) seems to undergo the age-related changes, e.g. aspartic acid racemization (Helfman and Bada, 1975; Park et al., 2008). The scarce data available on that topic suggest that the proteins of mature enamel are capable to affect mechanical quality of enamel coat by dissipating a considerable amount of deformation forces by gradual unfolding of their domain structures (He and Swain,2007, 2008) and reduce deeper propagation of cracks (Yahyazadehfar and Arola, 2015). Hence, the amount of protein may significantly contribute to mechanical qualities of enamel (at least in synergy with other factors) and exhibit certain age-dependent variation as indicated also by our results (see section Results, last paragraph).*

And the last paragraph has been rewritten as follows: *Nevertheless, the content of protein in the mature enamel takes about 1% of enamel weight only (0.5-3% Robinson et al., 1995; Nanci, 2008) and for obvious reasons it hardly can play a role of prevailing factor of enamel hardness. It would be beyond scope of this paper to hypothesize further on patterns of synergy and roles of particular factors causing the extraordinary mechanic qualities of mammalian mature enamel. We focused just on one of them and convincingly demonstrated that the basic crystallographic properties of enamel crystallites can significantly contribute to the mechanical quality of enamel and are scaled by duration of enamel calcification. For comparative and functional analyses of mammalian dentitions, traditionally operating with teeth shapes, prism arrangements and schmelzmusters (Koenigswald, 1992; Koenigswald and Sander, 1997; Koenigswald and Clemens, 1992), it provides a new variable worth serious analysis.*

References added: 1) He, L. H. and Swain, M. V. (2007). Influence of environment on the mechanical behaviour of mature human enamel. *Biomater.*, 28, 45124520. 2) Yahyazadehfar, M. and Arola, D. (2015). The role of organic proteins on the crack growth resistance of human enamel. *Acta Biomater.*, 19, 3345. 3) Fonseca, R. B. et al. (2008). Radiodensity and hardness of enamel and dentin of human and bovine teeth, varying bovine teeth age. *Arch. Oral. Biol.*, 53, 10231029. 4) Robinson, C., Kirkham, J., Brookes, S. J. and Shore, R. C. (1995). Chemistry of mature enamel in Dental enamel: formation to destruction (eds. Robinson, C., Kirkham, J. and Shore, R.), CRC press, 167191. 5) Helfman, P. M. and Bada, J. L. (1975). Aspartic acid racemization in tooth enamel from living humans. *P. Natl. Acad. Sci.*, 72(8), 28912894.

Page 94      Line 37\*

A more detailed explanation has been added to the paragraph: *As the crystallite is considered to be of elongated ribbon shape (Rönholm, 1962; Daculsi and Kerebel, 1978; Cuisinier et al., 1993) its size was computed for each individual reflection in order to take into account anisotropic broadening (for details see Kallistova et al., 2015). In the present paper, the term length is denoted to the size of crystallite in [00.1] direction (i.e. parallel to the c-axis) and the term thickness represent the shortest dimension of crystallite cross-section (i.e. perpendicular to the c-axis). Compared to alternative computation methods (Popa, 1998; Scardi and Leoni, 1999; Stephens, 1999; Popa and Balzar, 2008) the present one exhibited far the best statistic resolution. Unfortunately it did not enable to easily discriminate between the anisotropy related to a-axes (thickness) and that of b-axis (width). Consequently, the comparative analyses are preferably based on the variable of crystallite volume which covers sizes computed in all crystallographic directions.*

Reference added: Rönholm, E. (1962). The amelogenesis of human teeth as revealed by electron microscopy. II - The development of the enamel crystallites. *J. Ultrastruct. Res.*, 6, 249–3003.

A more detailed explanation has been added to the paragraph: *The structure of enamel apatite is disordered at the atomic level by the various ions (mainly anions) substitutions. These anion substitutions of enamel apatite lead not only to the variation in physical properties such as stability and solubility but also to the changes of the shape and width of the characteristic  $\nu_4$  phosphate vibrational band (originally three times degenerate -  $F_2$  symmetry). The volume of the atomic disorder can be separated from the other significant contribution (particle size, which also significantly influenced the vibrational band character) by the infrared splitting factor (IRSF) plotted (Asscher et al., 2011) against the full width in half maximum (FWHM) of the strongest infrared peak ( $\nu_4$  phosphate anion). Crystallinity index of splitting factor (IRSF) (Weiner and Bar-Yosef, 1990; Asscher et al., 2011; Poduska et al., 2011) is mathematically defined as a quotient of a sum of intensity of the two infrared bands originated from the  $\nu_4$  phosphate vibration with maxima at 565 and 605  $\text{cm}^{-1}$  ( $I_{565}$  and  $I_{605}$ ) divided by intensity of saddle-point ( $I_{590}$ ) between these bands, see equation:*

$$IRSF = \frac{I_{565} + I_{605}}{I_{590}} \quad (1)$$

*The maxima of the bands and position of the saddle-point between these maxima were obtained by the bands fitting by Lorentzian function using the spectroscopic OMNIC software. Full width in half maximum (FWHM) is the parameter of the envelope curve of the split  $\nu_3$  (originally three time degenerate vibration;  $F_2$  symmetry) and  $\nu_1$  (non-degenerate vibration;  $A_1$  symmetry) phosphate vibrations obtained by fitting in OMNIC software.*

... mainly in the  $[001]$  direction.