

A microscopic and microanalytical study (Fe, Ca) of the teeth of the larval and juvenile *Ambystoma mexicanum* (Amphibia: Urodela: Ambystomatidae)*

* Dedicated to Prof. Dr. H. Hartwig, Cologne (Germany) on the occasion of his 100th birthday

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> Abstract

We studied the teeth of larvae and one juvenile of the axolotl *Ambystoma mexicanum*, a urodele species that undergoes partial metamorphosis, by light microscopy of ground sections, backscattered electron imaging and semi-quantitative microanalysis in the scanning electron microscope. By applying these techniques it was possible to identify enamel, enameloid and dentin in the teeth. Iron was found to be present in enamel and enameloid, the concentrations being highest in the enamel. A staining indicative of the presence of iron was observed in the inner dental epithelium of tooth germs. Dentinal tubules mostly followed a straight course, but some recurved over a short distance distally. In larval teeth and teeth of “larval type” in the juvenile individual the dentinal tubules ended in the basal portion of the enameloid. Our results show that in the axolotl, monocuspid teeth of the “larval type” that developed after partial transformation still possess an enameloid layer beneath a thin enamel cap. The findings of the present study are consistent with the view that enameloid matrix is secreted by odontoblasts, while enameloid maturation is (largely) controlled by ameloblasts.

> Zusammenfassung

Die Zähne von Larven und einem juvenilen Exemplar von *Ambystoma mexicanum*, einer Urodelenart, die eine partielle Metamorphose durchläuft, wurden mittels Lichtmikroskopie von Dünnschliffen sowie Rückstreuelektronen-Aufnahmen und semiquantitativer Mikroanalyse im Rasterelektronenmikroskop untersucht. Diese Methoden erlaubten die Identifizierung von Dentin, Enameloid und Schmelz in den Zähnen. Eisen wurde im Schmelz und im Enameloid nachgewiesen, mit höchsten Konzentrationen im Schmelz. Eine auf das Vorhandensein von Eisen hindeutende Verfärbung fand sich im inneren Schmelz-epithel von Zahnkeimen. Die Dentinkanälchen verliefen zumeist gerade, waren jedoch in einigen Fällen distal umgebogen. In Larvenzähnen und Zähnen des „larvalen Typus“ des juvenilen Individuums endeten die Dentinkanälchen im basalen Bereich des Enameloids. Unsere Ergebnisse zeigen, dass beim transformierten Axolotl monocuspide Zähne des „larvalen Typs“ weiterhin eine Enameloid-Zone unterhalb einer dünnen Schmelzkappe besitzen. Die Ergebnisse der vorliegenden Untersuchung stehen im Einklang mit der Auffassung, dass die Enameloid-Matrix ein Sekretionsprodukt der Odontoblasten ist, während die Reifung des Enameloids (überwiegend) unter Kontrolle der Ameloblasten erfolgt.

> Key words

Dentition, axolotl, SEM-BSE imaging, EDX-microanalysis, iron, dentin, enamel, enameloid.

Introduction

The dentition of Urodela (= Caudata) undergoes remarkable changes during ontogeny. Typically, teeth of young larvae are monocuspid and non-pedicellate (“early larval type”). Teeth formed later during the larval period are divided by a more or less distinct annular zone of weakness (“late larval type”) located in the crown and the pedicel (“pedicellate condition”). Teeth formed by metamorphosing and post-metamorphic individuals on the remaining or the newly formed dentigerous bones are bicuspid in most species and fully pedicellate, i.e., these teeth show a distinct asymmetrical zone of division (SMITH & MILES 1971, GREVEN 1989, CLEMEN & GREVEN 1994, DAVIT-BÉAL *et al.* 2006, 2007a, b).

A urodele tooth is formed by cells of the (ectomesenchymal) dental papilla and the (ectodermal) enamel organ. Mesenchymal cells of the papilla (odontoblasts) produce dentin, whereas the polarized cells of the inner dental epithelium (ameloblasts) produce enamel. In larval teeth, an extremely thin layer of enamel is underlain by a densely mineralised modified dentin that is referred to as enameloid (SMITH & MILES 1971). Enameloid is a product of joint odontoblast and ameloblast activities. According to recent studies, enameloid matrix is secreted by odontoblasts, while enameloid maturation is controlled by ameloblasts (DAVIT-BÉAL *et al.* 2007a, b). In contrast to dentin, mature enameloid possesses only few collagen fibres (SCHMIDT 1957; ROUX & CHIBON 1973; SMITH & MILES 1971; BOLTE & CLEMEN 1992; KOGAYA *et al.* 1992; KOGAYA 1994, 1999; WISTUBA *et al.* 2002, DAVIT-BÉAL *et al.* 2007a, b).

The contents of calcium and phosphorus, constituting major components of the hydroxyapatite that forms the mineral phase of dental hard tissues, have been studied in functional teeth of post-metamorphic individuals of several urodele species (*Salamandra salamandra*: CLEMEN *et al.* 1980; *Dicamptodon ensatus*, *Onychodactylus japonicus*: SATO *et al.* 1991, 1992, *Ambystoma maculatum*, *Salamandra salamandra*, *Aneides lugubris*: SATO *et al.* 1993) as well as in individuals of paedomorphic species (*Ambystoma mexicanum*: CHIBON & ELOY 1979, BOLTE *et al.* 1996; *Cryptobranchus alleganiensis*, *Amphiuma means*, *Necturus maculosus*, *Andrias davidianus*: SATO *et al.* 1991, 1992; *Megalobatrachus* (now *Andrias*) *japonicus*: SHIMADA *et al.* 1993). To our knowledge, only SATO *et al.* (1991, 1992) and SHIMADA *et al.* (1993) have analysed urodele teeth for trace elements such as fluorine, magnesium and iron.

Similar to several other paedomorphic species (e.g., *Amphiuma means*: CLEMEN & GREVEN 1980; *An-*

drias spp.: GREVEN & CLEMEN 1980; *Cryptobranchus alleganiensis*: GREVEN & CLEMEN 2009), the dentition of the axolotl *Ambystoma mexicanum* undergoes a partial metamorphosis. Juvenile and adult specimens possess a single row of bicuspid fully pedicellate teeth in the upper jaw, a single row of weakly pedicellate monocuspid teeth on the vomeropalatinum (for terminology see CLEMEN 1979) and the coronoids, and a mosaic of mono- and bicuspid on the dentaries (KERR 1960; CLEMEN & GREVEN 1977; BOLTE & CLEMEN 1991). Thus, the axolotl offers the opportunity to compare teeth exhibiting the morphological characteristics of larval and transformed stages in a single individual. However, teeth of larval appearance present in partially metamorphosed individuals may differ in some aspects, e.g. size and shape, type of ankylosis, amount of enameloid, from “true” larval teeth, because the former teeth represent a more advanced developmental stage (CLEMEN *et al.* 2009).

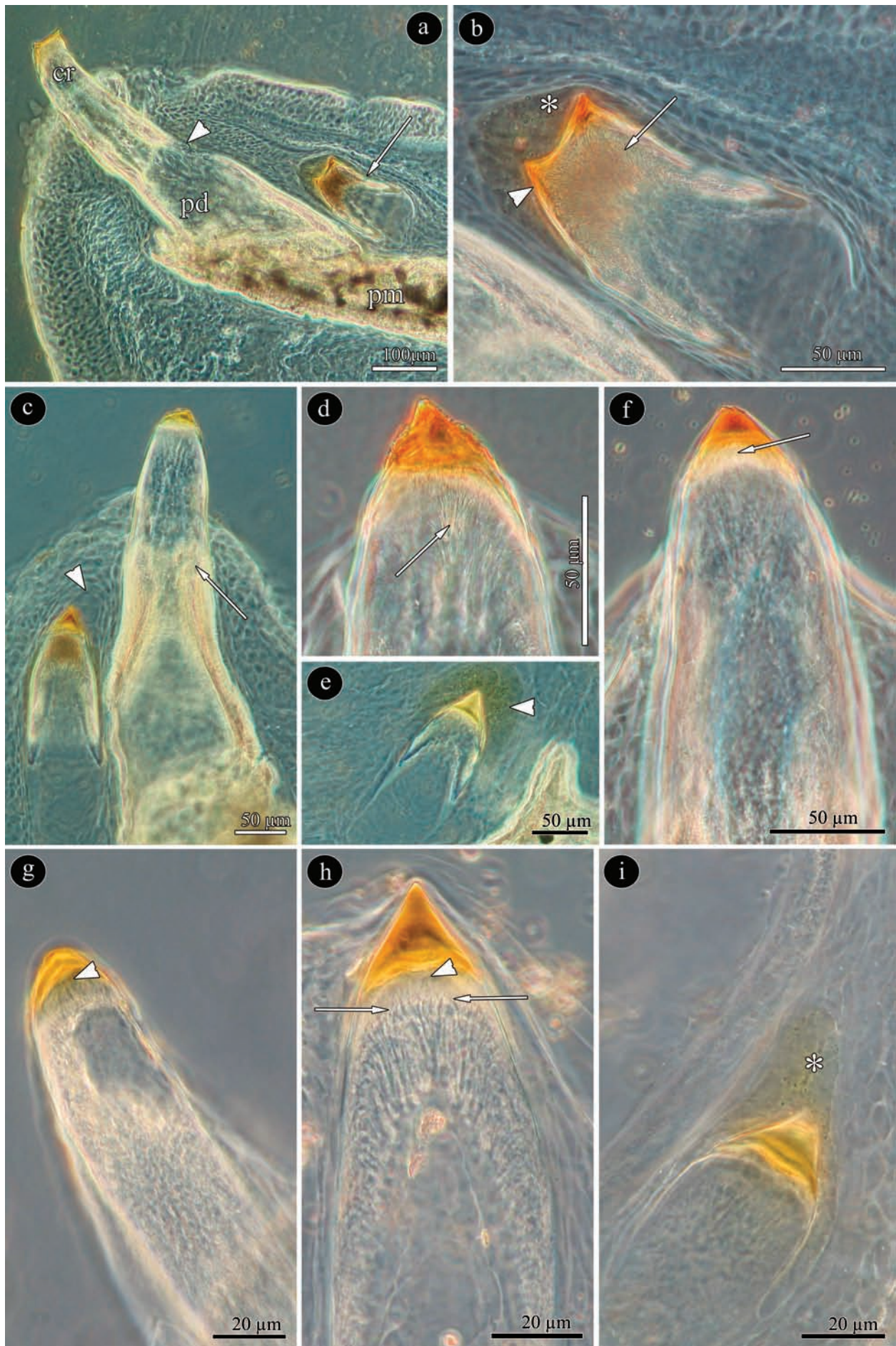
The present paper describes true larval teeth as well as teeth of larval appearance and bicuspid teeth of a transformed individual of the axolotl, using light microscopy of ground sections, backscattered electron (BSE)-imaging in the scanning electron microscope and energy dispersive X-ray analysis of calcium and iron.

Materials and methods

Animals

A juvenile axolotl (length of about 14 cm) and three larvae measuring, respectively, 5.5, 6.5, and 7 cm were obtained from a private breeder. Specimens were killed with an overdose of MS 222 (Sandoz) and de-

Fig. 1 a–i. Unstained ground sections of teeth of a juvenile axolotl (a–f) and of larvae of different lengths (g–i). Note the brownish apices in all teeth (transmitted light, phase contrast). **a:** Bicuspid pedicellate premaxillary tooth and tooth bud (arrow). Zone of division (arrowhead), crown (cr), pedicel (pd), premaxilla (pm). **b:** Tooth bud, detail of (a); note numerous dentinal tubules (arrow), stained enamel layer (arrowhead), and staining of the inner dental epithelium (asterisk). **c:** Established monocuspid coronoid tooth with a rudimentary zone of division (arrow), and a tooth bud (left side of image); note faint staining in the enamel organ of the tooth bud (arrowheads). **d:** Monocuspid apex of an established coronoid tooth with numerous dentinal tubules (arrow). **e:** Tooth bud, dentary; note staining of the enamel organ (arrowhead). **f:** Monocuspid vomerine tooth with enameloid (arrow). **g:** Premaxillary tooth with enameloid (arrowhead). **h:** Vomerine tooth; note the enameloid (arrowhead) and the dentinal tubules (arrows). **i:** Tooth bud from coronoid; note staining of the inner dental epithelium (asterisk).



capitated. Premaxillae, dentaries and vomeres were excised and fixed in 4% neutral buffered formalin for 48 h, and dehydrated in increasing concentrations of ethanol and finally acetone. For embedding, the samples were transferred into dichloromethane. Subsequently they were embedded in epoxy resin (Biodur E12 with hardener E1, Biodur Products, Heidelberg, Germany), evacuated and cured for at least 7d at 30° C. The resulting blocks were sectioned parallel to the long axes of the teeth in a bucco-lingual (vomeres) or mesio-distal plane (premaxillae, dentaries), using a rotary saw with a water-cooled diamond blade (Woco 50, Conrad Apparatebau, Clausthal-Zellerfeld, Germany). One of the two resulting blocks was used for light microscopy, the other for BSE-imaging and microanalysis.

Light microscopy

Blocks were ground (silicon carbide paper, grit 1200) until a tooth was exposed at the surface. Under microscopic control it was attempted to produce a longitudinal section running through the tip of the tooth apex (*sensu* SMITH & MILES 1971). The block surface was polished using a series of silicon carbide papers (grits 2400 and 4000) and a section of 2 mm thickness was cut from the block. The polished surface was mounted on a glass slide with Biodur embedding medium. The block was then reduced to a thickness of about 200µm using a face wheel, ground and polished to a final thickness of 70µm with the graded silicon carbide papers, and cover slipped. Ground sections were viewed in transmitted light with phase contrast and photographed, using an Axioskop 2 Plus microscope (Zeiss, Jena, Germany) equipped with a Canon PowerShot G2 digital camera (Canon, Tokyo, Japan). The acquired images were further processed with the software package Photoshop 7.0 (Adobe, San Jose, CA, USA).

Backscattered electron imaging and microanalysis

For BSE-imaging in the scanning electron microscope (SEM), the cut surface of the other block was ground with silicon carbide paper (grit 1200) until a tooth was exposed at the surface. Again care was taken to obtain a longitudinal section through the tip of the tooth apex. The block surface was then polished on a motorized rotor polisher (Labopol-5, Struers, Copenhagen, Denmark) using diamond suspensions (Diapro, Struers) with, respectively, 9 µm and 3 µm particle diameters and a final polishing step (OP-S Colloidal Silica Suspension, Struers). BSE imaging of the polished surfaces was performed with an FEI Quanta 600 FEG SEM (Hillsboro, USA) equipped with a solid-state backscat-

tered electron detector. The SEM was operated in a low-vacuum mode at an accelerating voltage of 20 kV. Semi-quantitative analysis of calcium and iron contents in the teeth was performed with an EDX-microanalysis-detector integrated in the SEM. Line scans of between 40 and 60 µm length were run from the dentin to the enamel of the tooth tip. The data generated with this method should be considered as semi-quantitative.

Results

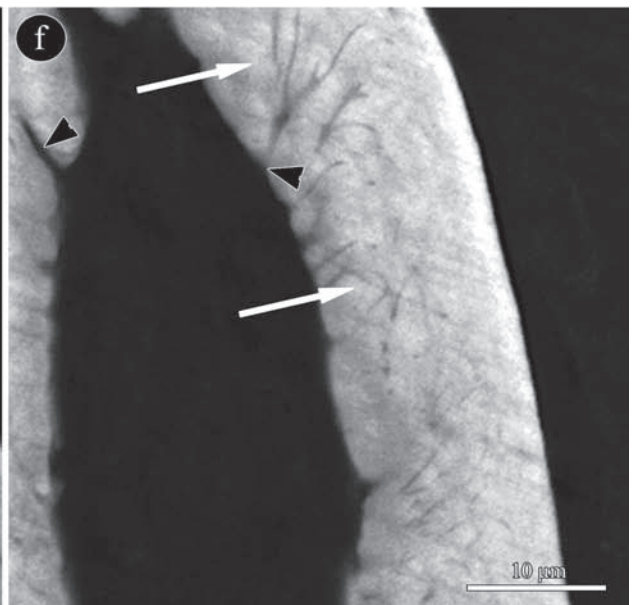
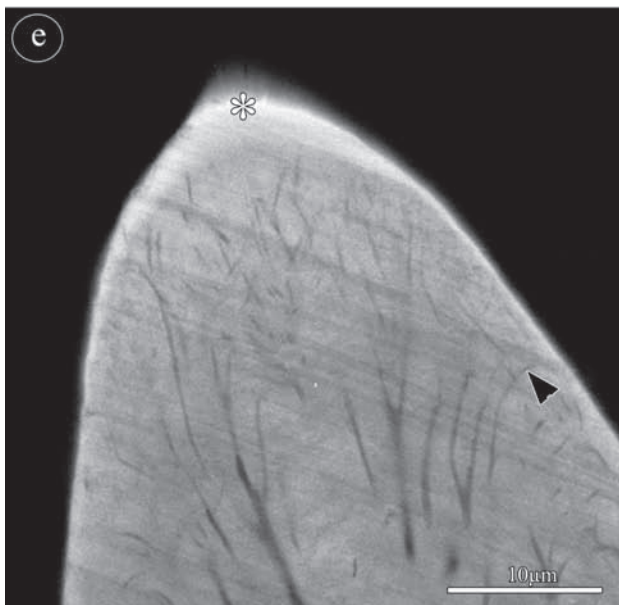
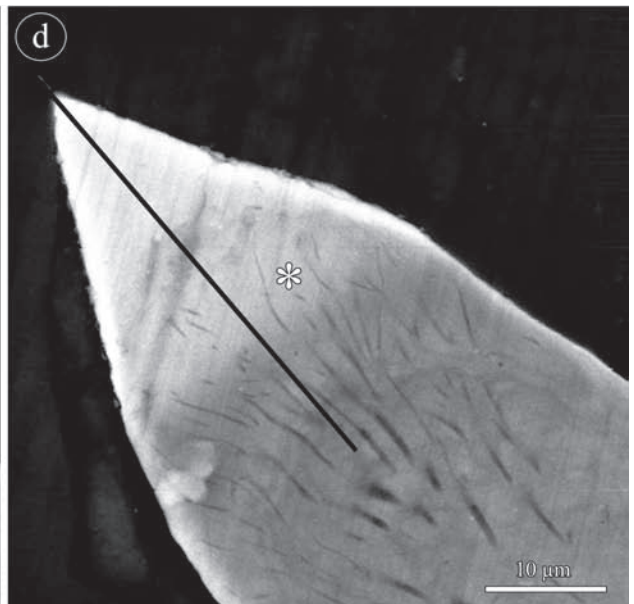
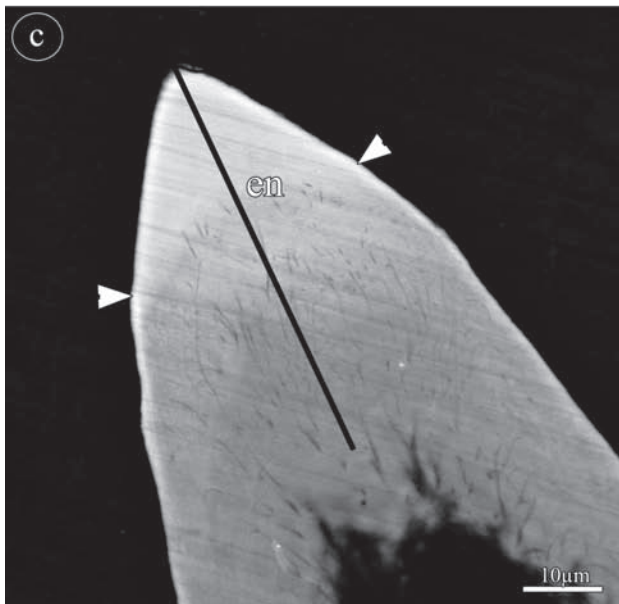
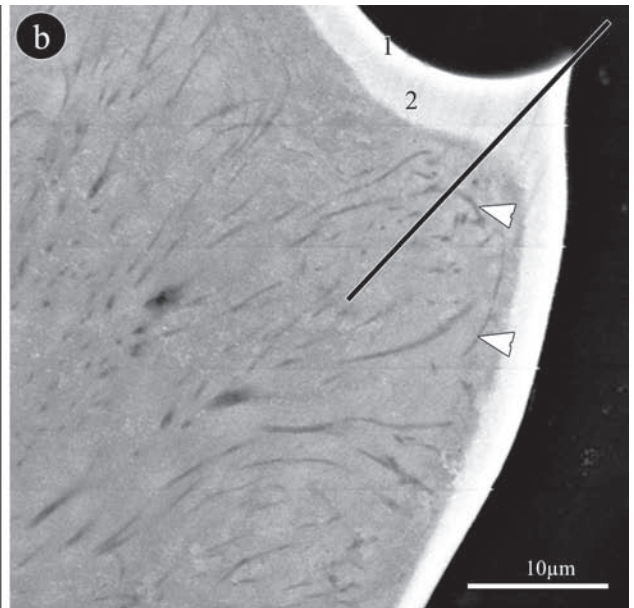
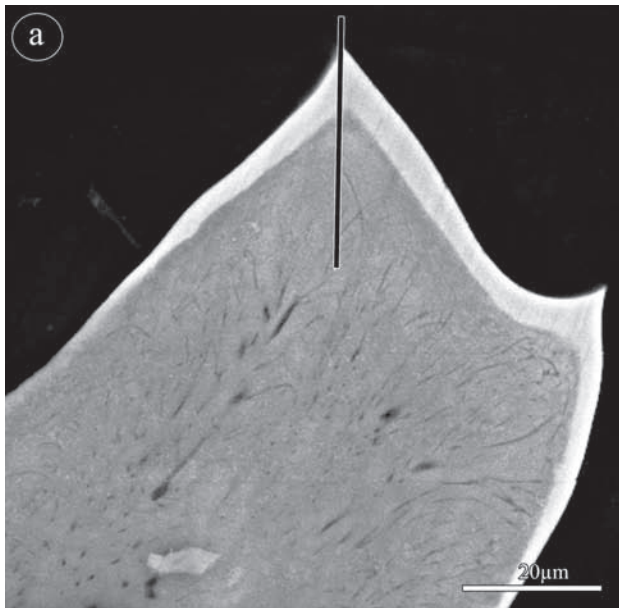
Light microscopy and SEM findings

In the Yground sections, a yellowish to brownish staining was observed in the tooth apices. This staining was most intense in the enamel cap at the tip of the tooth crown and faded in proximal direction (Fig. 1). The dentin shaft and the pedicel were unstained (Fig. 1); however, the enamel organ of tooth buds, especially the inner dental epithelium, also showed some staining (Fig. 1b, c, e, i).

The largest specimen available for study was a juvenile individual, possessing bicuspid pedicellate teeth in the upper jaw (Fig. 1a), monocuspid teeth on the coronoid (Fig. 1c, d) monocuspid (Fig. 1e) and bicuspid teeth on the dentaries, and monocuspid teeth on the vomer (Fig. 1f). The dentin exhibited numerous dentinal tubules that in the bicuspid teeth terminated immediately beneath the enamel layer (Fig. 1b, Fig. 2a, b).

All teeth of the three larvae were monocuspid (Fig. 1g–i) and exhibited only traces of a dividing zone. Dentinal tubules terminated at a certain distance from the enamel cap, with some of the tubules appearing to

Fig. 2 a–f. SEM-BSE images of teeth of a juvenile axolotl (a–c) and of larvae of different lengths (d–f). The courses of the EDX line scans (Fig. 3) are indicated. **a:** Bicuspid premaxillary tooth with the lingual cusp on the left side; note presence of numerous dentinal tubules. **b:** Detail of (a), labial cusp; note the division of the enamel layer into two sublayers (1, 2) of different brightness (mineral density) and the recurved dentinal tubules (arrowheads) beneath the enamel cap. **c:** Monocuspid vomerine tooth; note thin enamel layer (arrowheads) and the enameloid (en). Dentinal tubules can be seen to reach into the basal portion of the enameloid. **d:** Monocuspid dentary tooth (7 cm larva), the approximate position of the enameloid-dentin junction is indicated by an asterisk. **e:** Monocuspid, tangentially sectioned vomerine tooth (6.5 cm larva); note numerous, partly recurved dentinal tubules (arrowhead) and thin enamel cap (asterisk). **f:** Monocuspid vomerine tooth (5.5 cm larva); note cloudy pattern of mineralization in juxtapulpal dentin (arrows) and openings of dentinal tubules at the dentin-pulp interface (arrowheads).



follow a recurved course distally (Fig. 1h, Fig. 2d–f). SEM-BSE images of the polished cut surfaces of the teeth showed a variation in brightness of the dental hard tissues, ranging from the bright (highly mineralized) enamel layer to the grey of the less mineralized dentin (Fig. 2). In the bicuspid teeth, two enamel portions could be distinguished by their brightness. A thin outermost rim of highly mineralized enamel (appearing very bright on SEM-BSE images), overlaid a slightly less bright (= less mineralized) inner enamel layer (Fig. 2b). The entire enamel layer was clearly delimited from the underlying dentin.

In the bicuspid teeth, the maximum thickness of the enamel layer (at the cusp tips) was between 9 and 10 μm . Between the tips, enamel thickness was slightly less, further decreasing towards the pedicel. The monocuspid teeth of the juvenile individual showed thin, bright enamel caps that measured approximately 3 μm in thickness (Fig. 2c). In these teeth, a layer of enameloid was intercalated between the enamel cap and the dentin. SEM-BSE imaging revealed that the degree of mineralization of the enameloid was intermediate between enamel and dentin, gradually increasing towards the enamel (Fig. 2c). Dentinal tubules were present in the basal portion of the enameloid, but did not reach up to the enamel-enameloid junction (Fig. 1d, h). The junction between enameloid and dentin was indistinct (Fig. 1d, f, 2c).

The teeth of the larvae resembled the monocuspid teeth of the juvenile individual insofar as they also possessed a very thin enamel cap and an enameloid layer interposed between enamel and dentin (Fig. 2d). In both ground sections and SEM-BSE images, the junction between enameloid and underlying dentin was indistinct (Fig. 1h, 2d). At the dentin-pulp interface, openings of dentinal tubules were visible, and the juxtapulpal dentin was characterized by a cloudy pattern of mineralization (Fig. 2f).

Microanalysis

Line scans through bicuspid teeth revealed the presence of iron in the enamel, with concentrations increasing towards the tip of the enamel cap (Fig. 3a, b). The low Fe-counts in the dentin are regarded to represent a non-specific background. In the monocuspid teeth of the juvenile individual (Fig. 3c) and one of the larvae (Fig. 3d), iron was observed to be present in both enameloid and enamel. Fe-concentrations progressively increased throughout the enameloid layer towards the enamel and further in the enamel cap towards the tip of the tooth apex.

In all analyzed teeth, calcium concentration in enamel and enameloid tended to be inversely related to that of iron (Fig. 3a–d).

Discussion

SEM-BSE images are a quick means of determining the relative degree of mineralization of dental hard tissues. The brighter a tissue appears, the more highly mineralized it is. Presence of an enamel cap covering the tooth apex was demonstrated in all studied teeth, the enamel being discernable as a bright layer of variable thickness. Enamel thickness was highest at the cusp tips and gradually decreased towards the pedicel. The bicuspid teeth of the transformed individual possessed the thickest enamel, while the enamel was thinnest in true larval teeth. In the bicuspid teeth, an inner and an outer enamel layer could be distinguished on SEM-BSE images. It is assumed that this subdivision corresponds to the findings in other urodele species, in which an inner and an outer enamel layer were distinguished that differed in crystal arrangement and element distribution (SATO *et al.* 1991, 1992, 1993).

In the present study, presence of enameloid was observed in true larval teeth. The tissue was identified by its location, its higher mineral content (greater brightness in SEM-BSE images) compared to dentin, and the lack of dentinal tubules in its more apical portions. Using the above criteria it is concluded that enameloid is also present in the monocuspid teeth of the juvenile, which have retained their larval appearance, but is obviously absent from the bicuspid teeth of the same individual.

There is consensus that the teeth of larval urodeles possess a cap of dentin-like enameloid covered by a very thin layer of enamel (ROUX & CHIBON 1973; BOLTE & CLEMEN 1992; BOLTE *et al.* 1996; KOGAYA 1994, 1999; WISTUBA *et al.* 2002; DAVIT-BÉAL *et al.* 2007a, b). The present study demonstrated that the same is also the case for the “larval-type” teeth of the partially metamorphosed axolotl. The microscopic and microanalytical findings of the present study are in accordance with the view that enameloid matrix is secreted by odontoblasts, while enameloid maturation is (largely) controlled by ameloblasts (DAVIT-BÉAL *et al.* 2007a, b).

In accordance with the findings of the present study, also transmission electron microscopic (TEM) studies on teeth of *A. mexicanum* (WISTUBA *et al.* 2002) and *A. maculatum* (SATO *et al.* 1993) suggest absence of enameloid in bicuspid axolotl teeth. In contrast, KAWASAKI & FEARNHEAD (1983) described a highly mineralized tissue containing collagen beneath the enamel layer of teeth in adult *Hynobius nigrescens* and *Cynops pyrrhogaster*, for which they used the term enameloid. Results of TEM-studies and microprobe analysis indicated the presence of true enamel in the teeth of different adult pedomorphic and metamorphosing

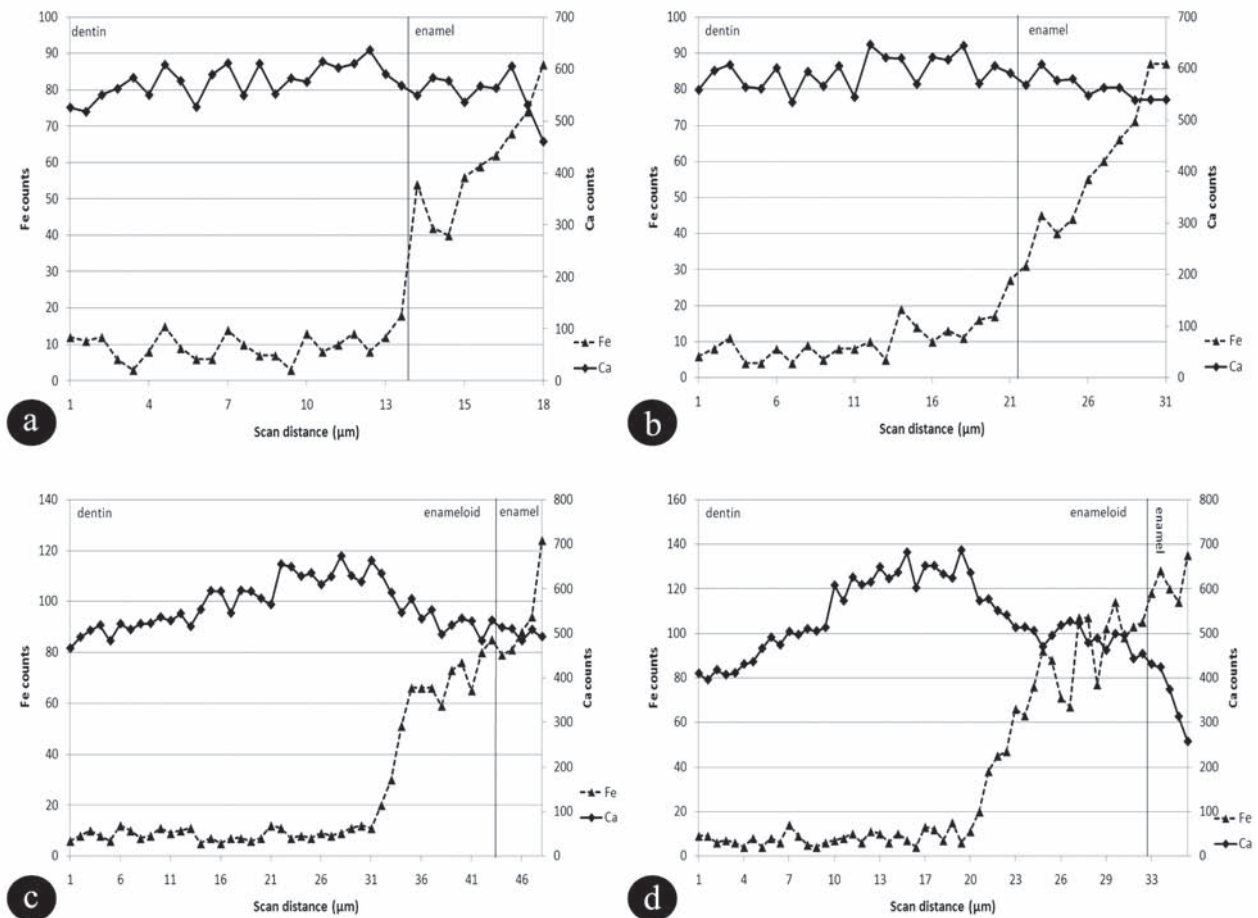


Fig. 3 a–d. EDX line scans of calcium and iron in axolotl teeth. **a, b:** Scans through lingual (**a**, cf. Fig. 2a) and labial (**b**, cf. Fig. 2b) cusps of a bicuspoid premaxillary tooth of the juvenile individual. **c:** Scan through a monocuspid vomerine tooth of the juvenile individual (cf. Fig. 2c). **d:** Scan through a monocuspid dentary tooth of a larva of 7 cm length (cf. Fig. 2d).

urodeles (SATO *et al.* 1991, 1992, 1993). The authors distinguished an outer from an inner layer covering the tooth apex, which differed in the concentration of certain elements. In neither of these two layers collagen was detected, thereby confirming their nature as enamel. This condition has so far been demonstrated in fully transformed species (*Dicamptodon ensatus*, *Onychodactylus fischeri*, *Salamandra salamandra*, *Aneides lugubris*, *Ambystoma maculatum*), in partially transformed species with bicuspoid or otherwise modified teeth (*Amphiuma means*: CLEMEN & GREVEN 1980, *Andrias* spp.: GREVEN & CLEMEN 1980; SHIMADA *et al.* 1993), and in the monocuspid teeth of the “late larval stage” of adult paedomorphic *Necturus maculosus* (GREVEN & CLEMEN 1979).

However, collagen was found to be present in the inner portion of the covering layer of the tooth apex in the paedomorphic *Cryptobranchus alleganiensis*, a species with a very early occurrence of teeth of the transformed type during ontogenesis (GREVEN & CLEMEN 2009) and in the caecilian *Dermophis* sp. (SATO *et al.* 1992). SATO *et al.* (1992) concluded that the inner part of the covering layer of the tooth apex

of these species is enameloid rather than true enamel. Also KOGAYA *et al.* (1992) observed two zones in the covering layer of the tooth apex of *Triturus* (now *Cynops*) *pyrrhogaster* considering the outer zone as true enamel and the inner as mixture of dentin and enamel matrices.

It is believed that heterochronic shifts in ameloblast differentiation have caused the evolutionary change from enameloid to enamel in vertebrates (e.g., SLAVKIN & DIEKWISCH 1996). We suggest that also timing and duration of the formation of mono- and bicuspoid teeth in paedomorphic urodele species are affected by heterochronic shifts, and that similar (species-specific) shifts may be responsible for the presence of enameloid in paedomorphic and transformed urodele taxa.

Many urodeles possess teeth capped by an iron-rich covering layer (SCHMIDT 1958; KERR 1960). The present study has demonstrated that teeth of larval and transformed axolotls contain iron in their enamel and, if present, also in the enameloid. RANDALL (1966) and SMITH & MILES (1971) have demonstrated ferritin-containing vesicles in the cells of the inner dental epithelium (ameloblasts) of developing teeth of *A. mexicanum*

and *Triturus* (now *Lissotriton*) *vulgaris*. The brownish to yellowish staining of tooth crowns and parts of the enamel organ seen in the ground sections in combination with the results of the EDX-analysis demonstrate the presence of iron in our studied specimens.

It is presently unclear in which form(s) iron is present in the enamel and enameloid of axolotl teeth. KOZAWA *et al.* (1988), who studied the pigmented enamel of shrew teeth (genus *Sorex*), observed three different types of iron in the enamel, viz. amorphous ferritin at the surface of the apatite crystals, iron atoms incorporated into the apatite lattice, and iron oxide crystals deposited onto the apatite. The hitherto presented data for mammalian teeth (SÖDERLUND *et al.* 1992) and for the axolotl (BOLTE *et al.* 1996) suggest that some iron may substitute for calcium in the apatite lattice. The higher degree of mineralization recorded in the outer enamel layer of the bicuspid teeth in our study can probably be related to the increased iron concentration of this layer that was demonstrated by EDX-analysis.

Certainly, iron is taken up from the environment, but the route of uptake has to our knowledge not yet been studied in amphibians. Iron has frequently been found in mammalian dental enamel and it has been suggested that iron increases enamel hardness and thereby wear resistance of the teeth (SELVIG & HALSE 1975; KOZAWA *et al.* 1988). However, SÖDERLUND *et al.* (1992), studying the hardness of shrew incisors, found unpigmented to enamel be slightly harder than pigmented enamel.

A role of iron in increasing the wear resistance of teeth was also suggested by MOTTA (1987), who found a positive relationship between the iron content of enameloid in the teeth of butterfly fish (Chaetodontidae) and the "hardness" of their prey species. In contrast, SUGA *et al.* (1989, 1992) related the presence or absence of iron in the enameloid of tetraodontiform and perciform fish to their phylogeny rather than to their mode of feeding. Clearly the biological significance of the iron content of amphibian enamel and enameloid needs further study.

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References

- BOLTE, M. & CLEMEN, G. (1991): Die Zahnformen des Unterkiefers beim adulten *Ambystoma mexicanum* Shaw (Urodela: Ambystomatidae). – *Acta Biologica Benrodis*, **3**: 171–177.
- BOLTE, M. & CLEMEN, G. (1992): The enamel of larval and adult teeth of *Ambystoma mexicanum* Shaw (Urodela: Ambystomatidae) – a SEM study. – *Zoologischer Anzeiger*, **228**: 167–173.
- BOLTE, M., KREFTING, E.-R. & CLEMEN, G. (1996): Hard tissue of teeth and their calcium and phosphate content in *Ambystoma mexicanum* (Urodela: Ambystomatidae). – *Annals of Anatomy*, **178**: 71–80.
- CHIBON, P. & ELOY, J.F. (1979): Local analysis of the composition of amphibian and shark teeth using laser probe mass spectrometry. – *Journal de Biologie Buccale*, **7**: 263–283.
- CLEMEN, G. (1979): Experimentelle Veränderungen am knöchernen Gaumenbogen der Axolotl-Larve und ihre Auswirkungen während der Metamorphose. – *Zoologischer Anzeiger*, **203**: 23–34.
- CLEMEN, G. & GREVEN, H. (1977): Morphologische Untersuchungen an der Mundhöhle von Urodelen. III. Die Munddachbezeichnung von *Ambystoma mexicanum* Cope (Ambystomatidae: Amphibia). – *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenie der Tiere*, **98**: 95–136.
- CLEMEN, G. & GREVEN, H. (1980): Morphologische Untersuchungen an der Mundhöhle von Urodelen. VII. Die Munddachbezeichnung von *Amphiuma* (Amphiumidae: Amphibia). – *Bonner Zoologische Beiträge*, **31**: 357–362.
- CLEMEN, G. & GREVEN, H. (1994): The buccal cavity of larval and metamorphosed *Salamandra salamandra*: Structural and developmental aspects. – *Mertensiella*, **4**: 83–109.
- CLEMEN, G., GREVEN, H. & SCHRÖDER, B. (1980): Ca and P concentrations of a urodele tooth as revealed by electron microprobe analysis. – *Anatomischer Anzeiger*, **148**: 422–427.
- CLEMEN, G., SEVER, D. & GREVEN, H. (2009): Notes on the cranium of the paedomorphic *Eurycea rathbuni* (STEINER, 1896) (Urodela: Plethodontidae) with special regard to the dentition. – *Vertebrate Zoology*, **59**: 157–168.
- DAVIT-BÉAL, T., ALLIZARD, F. & SIRE, J.-Y. (2006): Morphological variations in a tooth family through ontogeny in *Pleurodeles waltl* (Lissamphibia, Caudata). – *Journal of Morphology*, **267**: 1048–1065.
- DAVIT-BÉAL, T., ALLIZARD, F. & SIRE, J.-Y. (2007a): Enameloid/enamel transition through successive tooth replacements in *Pleurodeles waltl* (Lissamphibia, Caudata). – *Cell and Tissue Research*, **328**: 167–183.
- DAVIT-BÉAL, T., CHISAKA, H., DELGADO, S. & SIRE, J.-Y. (2007b): Amphibian teeth: current knowledge, unanswered questions, and some directions for future research. – *Biological Reviews*, **82**: 49–81.
- GREVEN, H. (1989): Teeth of extant amphibian: morphology and some implications. – *Fortschritte der Zoologie*, **35**: 451–455.

- GREVEN, H. & CLEMEN, G. (1979): Morphological studies on the mouth cavity of urodeles. IV. The teeth of the upper jaw and the palate in *Necturus maculosus* (RAFINESQUE) (Proteidae: Amphibia). – Archivum histologicum japonicum, **42**: 445–457.
- GREVEN, H. & CLEMEN, G. 1980: Morphological studies on the mouth cavity of urodeles VI. The teeth of the upper jaw and palate in *Andrias davidianus* (Blanchard) and *A. japonicus* (Temminck) (Cryptobranchidae: Amphibia). – Amphibia-Reptilia, **1**: 49–59.
- GREVEN, H. & CLEMEN, G. (2009): Early tooth transformation in the paedomorphic hellbender *Cryptobranchus alleganiensis* (Amphibia: Urodela). – Vertebrate Zoology, **59**: 71–79.
- KAWASAKI, K. & FEARNHEAD, R.W. (1983): Comparative histology of tooth enamel and enameloid. In: SUGA, S. (ed.): Mechanisms of tooth enamel formation. – Quintessence Publishing, Berlin, Chicago, pp. 285–303.
- KERR, T. (1960): Development and structure of some actinopterygian and urodele teeth. – Proceedings of the Zoological Society of London, **133**: 401–422.
- KOGAYA, Y. (1994): Sulfated glycoconjugates in amelogenesis. – Progress in Histochemistry and Cytochemistry, **29**: 1–110.
- KOGAYA, Y. (1999): Immunohistochemical localisation of amelogenin like proteins and type I collagen and histochemical demonstration of sulphated glycoconjugates in developing enameloid and enamel matrices of the larval urodele *Triturus pyrrhogaster* teeth. – Journal of Anatomy, **195**: 455–464.
- KOGAYA, Y., KIM, S., YOSHIDA, H., SHIGA, H. & AKISAKA, T. (1992): The enamel matrix of the newt, *Triturus pyrrhogaster* contains no sulphated glycoconjugates. – Cell and Tissue Research, **270**: 249–256.
- KOZAWA, Y., SAKAE, T., MISHIMA, H., BARCKHAUS, R.H., KREFTING, E. -R., SCHMIDT, P.F. & HÖHLING, H.J. (1988): Electron-microscopic and microprobe analyses on the pigmented and unpigmented enamel of *Sorex* (Insectivora) – Histochemistry, **90**: 61–65.
- MOTTA, P.J. (1987): A quantitative analysis of ferric iron in butterfly fish teeth (Chaetodontidae, Perciformes) and the relationship to feeding ecology. – Canadian Journal of Zoology, **65**: 106–112.
- RANDALL, M. (1966): Electron microscopical demonstration of ferritin in the dental epithelial cells of urodeles. – Nature, London, **210**: 1325–1362.
- ROUX, J.P. & CHIBON, P. (1973): Etude ultrastructurale de l'amélogenèse chez la larve du triton *Pleurodeles waltlii*. (Amphibien Urodèle). – Journal de Biologie Buccale, **1**: 33–44.
- SATO, I., SHIMADA, K. & SATO, T. (1992): Morphology of the teeth of adult Caudata and Apoda: Fine structure and chemistry of enamel. – Journal of Morphology, **214**: 341–350.
- SATO, I., SHIMADA, K., SATO, T., ETHERIGE, R., (1993): Morphological study of the teeth of *Ambystoma maculatum*, *Salamandra salamandra* and *Aneides lugubis*: Fine structure and chemistry of enamel. – Okajimas Folia Anatomica Japonica, **70**: 157–164.
- SATO, I., SHIMADA, K., YOKOI, A., SATO, T. & KITAGAWA, T. (1991): Structure and elemental analysis of the enamel in *Andrias davidianus* (Cryptobranchidae) and *Onychodactylus japonicus* (Hynobiidae). – Journal of Herpetology, **25**: 141–146.
- SCHMIDT, W.J. (1957): Zur Durodentinbildung bei Urodelen-Zähnen. – Zeitschrift für Zellforschung und mikroskopische Anatomie, **46**: 281–285.
- SCHMIDT, W.J. (1958): Zur Histologie und Färbung der Zähne des japanischen Riesensalamanders. – Zeitschrift für Zellforschung und mikroskopische Anatomie, **49**: 46–57.
- SELVIG, K.A. & HALSE, A. (1975): The ultrastructural localization of iron in rat incisor enamel. – Scandinavian Journal of Dental Research, **83**: 88–95.
- SHIMADA, K., SATO, I., MURAKAMI, G. & IKEDA, T. (1993): Fine structure and elemental analysis of the enamel in the cryptobranchid salamander *Megalobatrachus japonicus*. – Okajimas Folia Anatomica Japonica, **69**: 345–351.
- SLAVKIN, H.C. & DIEKWISCH, T. (1996): Evolution in tooth developmental biology: of morphology and molecules. – The Anatomical Record, **245**: 131–150.
- SMITH, M.M. & MILES, A.E.W. (1971): The ultrastructure of odontogenesis in larval and adult urodeles; Differentiation of the dental epithelial cells. – Zeitschrift für Zellforschung und mikroskopische Anatomie, **121**: 470–498.
- SÖDERLUND, E., DANNEID, E. & ROWCLIFFE, D.J. (1992): On the hardness of pigmented and unpigmented enamel in teeth of shrews of the genera *Sorex* and *Crocidura* (Mammalia, Soricidae). – Zeitschrift für Säugetierkunde, **57**: 321–329.
- SUGA, S., TAKI, Y. & OGAWA, M. (1992): Iron in the enameloid of perciform fish. – Journal of Dental Research, **71**: 1316–1325.
- SUGA, S., WADA, K., TAKI, Y. & OGAWA, M. (1989): Iron concentration in teeth of tetra-odontiform fishes and its phylogenetic significance. – Journal of Dental Research, **68**: 1115–1123.
- WISTUBA, J., GREVEN, H. & CLEMEN, G. (2002): Development of larval and transformed teeth in *Ambystoma mexicanum* (Urodela, Amphibia): an ultrastructural study. – Tissue & Cell, **34**: 14–27.



Book review

JOSEPH S. NELSON, HANS-PETER SCHULTZE &
MARK H-V WILSON (editors)

Origin and Phylogenetic Interrelationships of Teleosts – Honoring Gloria Arratia Proceedings of the international symposium at the ASIH Annual Meeting in St. Louis, Missouri, 2007

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Im Rahmen der Jahrestagung der American Society of Ichthyologists and Herpetologists (ASIH) 2007 organisierten die drei Herausgeber dieses Buches ein Symposium über „Origin and phylogenetic interrelationships of teleosts“. Dieses Buch, mit dem gleichen Titel wie das Symposium, beinhaltet Beiträge dieser Veranstaltung und ist GLORIA ARRATIA gewidmet. Frau ARRATIA wurde auf derselben Jahrestagung mit dem Robert H. Gibbs, Jr. Award geehrt und ist eine der herausragendsten und erfolgreichsten Forscher der letzten Jahrzehnte auf dem Gebiet der Phylogenie der Teleostier. Die Beiträge in diesem Buch sind durchweg von führenden Forschern (Paläontologie und Neoontologie) der letzten Jahre auf dem Gebiet der Knochenfische geschrieben worden und geben den aktuellen Stand der Forschung bei den einzelnen basalen Teleostiergruppen (Osteoglossomorpha, Clupeiformes, Gonorhynchiformes, Cypriniformes, Characiformes, Siluriformes, Salmoniformes, Esociformes) wieder.

Das erste Kapitel ist GLORIA ARRATIA gewidmet und erzählt von ihren wichtigsten Lebensstationen, ihren großen Erfolgen und gibt ebenso Einblicke in ihr Privatleben. Im zweiten Kapitel wird die schon sehr lange geführte Diskussion um die Schwestergruppe der Teleostier besprochen. Dabei gibt der Autor eine kurze Einleitung in die Problematik und stellt einige gängige Theorien zu der möglichen Schwestergruppe da. Er selber beschäftigt sich dann mit den pycnodonten Fischen und stellt die von ihm neu benannte Gruppe der Pycnodontomorpha als Schwestergruppe der Teleostier dar. Die weiteren 18 Kapitel beschäftigen sich dann hauptsächlich mit den oben genannten Gruppen

der basalen Teleostier. Dabei vereint das Buch Biologie und Paläontologie sowie Ergebnisse und Erkenntnisse aus molekularen und morphologischen Studien. Jede einzelne Arbeit gibt eine ausführliche Einleitung in die jeweilige Gruppe und Problematik, mit der sich die Arbeit beschäftigt.

Alle Kapitel sind auch für „Laien“ auf dem Gebiet der Entstehung und Phylogenie der modernen Knochenfische verständlich und nachvollziehbar. Allgemeine Vorkenntnisse auf dem Gebiet der Ichthyologie oder Evolution der niederen Wirbeltiere sind jedoch Voraussetzung, denn dieses Buch ist vor allem an Wissenschaftler und Wissenschaftsinteressierte gerichtet. Insgesamt ist es ein gelungenes Buch, in welchem Wissenschaftler aus den verschiedenen Gruppen der basalen Teleostier einen sehr guten Einblick in den aktuellen Wissenstand und die aktuelle Forschung geben.

Ein kleiner Wehmutstropfen ist jedoch der Preis mit 120 Euro, insbesondere für „nicht professionelle“ Wissenschaftsinteressierte. Allerdings im Vergleich mit anderen aktuellen wissenschaftlichen Büchern noch vertretbar.

Martin Licht