INITIAL PERIOD OF TOOTH DEVELOPMENT IN DOLPHINS (*Stenella attenuata*, Cetacea) - A PILOT STUDY

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Abstract

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Polyodont dentition in dolphins may contain up to 265 simple conical teeth. In order to analyse prenatal development of this dentition, we chose the spotted dolphin *(Stenella attenuata)*, where 35 - 37 teeth are present in one jaw quadrant. In this present pilot study, tooth development was studied from 32 to 68 days of ontogenesis (DO) using serial histological sections of embryonic heads. Morphometry and three-dimensional (3D) computer-assisted reconstruction of dental epithelium were employed. At 32 DO, a thickening of the oral epithelium was apparent in the area of the future dental lamina. On histological sections, thickening is considered to be an early morphological sign of odontogenesis. The 3D reconstruction revealed the existence of swellings on the dental lamina in embryos at 54 DO. During the next period the number of swellings increased. On frontal sections, the epithelium of the swellings exhibited a tooth-bud shape, described in terrestrial mammals. Approximately 24 morphologically similar buds were observed in one jaw quadrant at 68 DO.

Odontogenesis, homodont dentition, 3D reconstruction, mammals

The evolutionary principle of development of the eutherian dentition has not been clarified until now. Investigating odontogenesis in aquatic mammals should contribute towards elucidation of this problem. Morphologically, dentition in aquatic mammals is often much closer to the functional polyodont homodont dentition of reptiles than to the oligodont heterodont dentition which characterizes terrestrial mammals. In pinnipeds, for example, a tendency towards suppression of morphological differences between individual teeth is evident, even though the increase of functional tooth number is not yet apparent (Watson 1981). In comparison with terrestrial mammals, the tooth number and shape have been extremely modified in toothed cetaceans, where the polyodont and homodont dentition is present. The jaws are covered by up to 265 morphologically similar teeth (e.g. *Stenella longirostris* - Watson 1981). This is the greatest tooth number in vertebrates from reptiles upwards. From a phylogenetic point of view, polyodontia and homodontia in toothed cetaceans are secondary phenomena (Grassé 1955; Špinar 1985), because their ancestors (derived from artiodactyls) still exhibited heterodontia (Peyer 1968). The suborder of toothed cetaceans appears to be a suitable group for investigations of the evolutionarily determined changes in the number and morphology of teeth in mammals. Only Kukenthal has (1889-1893, 1892, 1895) reported a detailed study of aquatic mammalian odontogenesis, and descriptive data are available from the bud stage. For our study of odontogenesis in toothed cetaceans, a member of the family Delphinidae - the spotted dolphin *(Stenella attenuata)* was chosen. An adult animal has 35-37 morphologically similar conical functional teeth per dental quadrant (Watson 1981). The aim of the present pilot study was to obtain fundamental qualitative and quantitative information cocerning the initial period of tooth development using histological sections and 3D computer-assisted reconstructions.

Materials and Methods

We used a set of 11 spotted dolphin (*Stenella attenuata,* Odontoceti, Cetacea) embryos. Their crown-rump length was 10-63 mm and their estimated age ranged between 30-68 days of ontogenesis (DO). These samples were kindly provided by the J. W. Goethe University in Frankfurt am Main. The prenatal specimens had been collected there from the beginning of this century due to incidental catches of fishermen. The age of embryos and fetuses was estimated according to the newly established method by Štěrba (1995) that allows age estimation of prenatal specimens of various species on the basis of morphological criteria. In this way, the embryos of the spotted dolphin were ranked according to the appropriate Štěrba's comparable stage (SCS). The total gestation period in this species is estimated to be about 280 days. The heads of the embryos were processed using a routine histological method and a series of 7 µm frontal sections stained by the modified alcian blue-hematoxylin-eosin method according to Bancroft et al. (1994). Projection drawings of the dental and adjacent oral epithelium were made using an AMPLIVAL microscope (Carl Zeiss) equipped with a drawing chamber. The drawings were memorized on a personal computer connected to a graphic tablet GENIUS 1212, using the best fit procedure (Gaunt and Gaunt 1978) for drawing superposition. The 3D reconstructions of the epithelium were made with help of the special software ANATRECON (anatomical reconstructions). In addition, the height of the tooth epithelium was measured on the drawings (Fig. 1), and changes in the antero-posterior course were plotted.

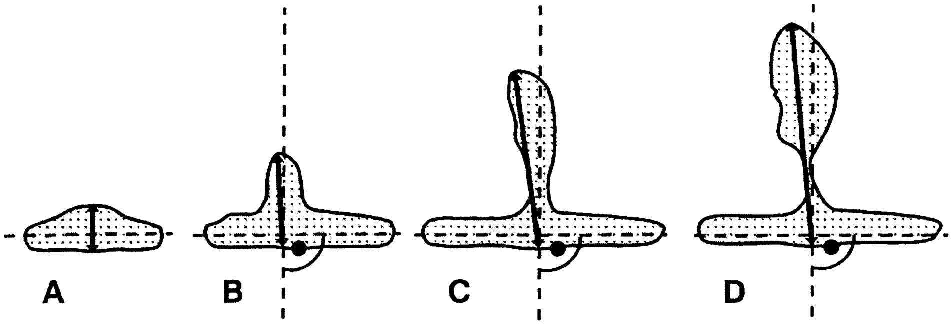

Fig. 1. A schematic diagram of the method used to measure the height of the dental epithelium which was measured as the maximal distance between the point of deepest immersion of the epithelium into the gum mesenchyme and the point where the oral epithelium surface was intersected by a perpendicular line passing through the adjacent part of the dental epithelium. A - incipient dental lamina, B - dental lamina, C - incipient swelling (tooth bud), D - swelling (tooth bud). The greatest height of tooth epithelium was measured between the point of deepest immersion into the gum mesenchyme and the point where the oral epithelial surface was intersected by a vertical line leading through the basal part of the tooth primordium.

Results

The initial morphological stage of tooth development was observed in embryos at DO-32, where the thickening of the oral epithelium was apparent along the jaw margin in the area of the future dental lamina. The height of the dental epithelium was found to increase, such that a well formed dental lamina was present on DO-42. The anterior part of the dental lamina was most deeply buried in the adjacent mesenchyme. The maximal height of the lamina was about 100 µm. The height of the dental epithelium gradually decreased in the posterior direction. It was not possible to distinguish individual tooth primordia during this period (Fig. 2). At DO-54, areas with swellings were detected on the dental lamina in 3-D reconstructions (Fig. 3). On frontal histological sections, the epithelium in these areas

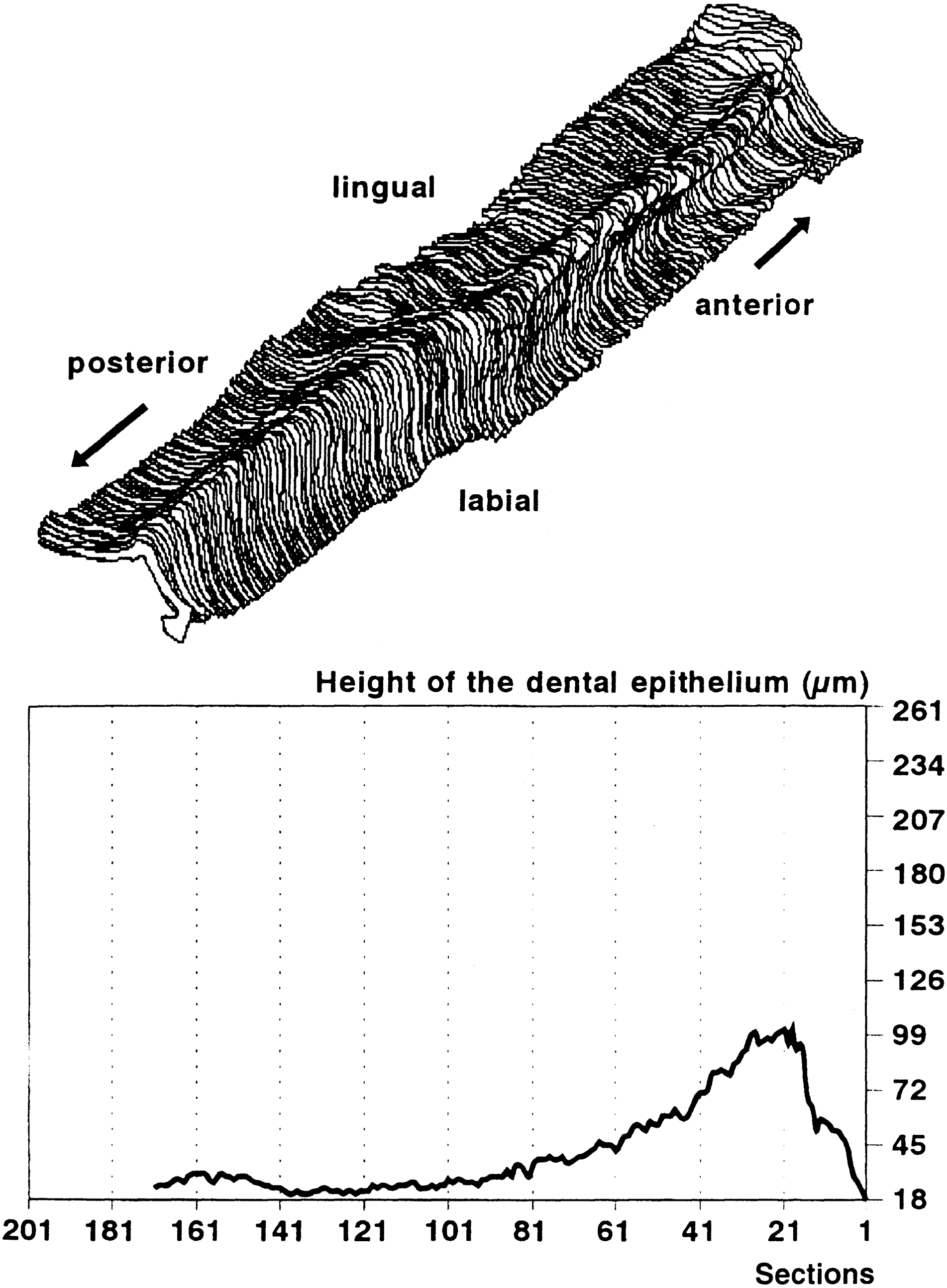

Fig. 2. Computer-assisted 3D reconstruction of the dental and adjacent oral epithelium in the upper jaw quadrant of a spotted dolphin embryo on DO-42 (view upon the mesenchyme side). The 3D morphology of the dental epithelium is compared with the changes of the height of the dental epithelium on the individual sections plotted in the graph in antero-posterior course. Section 1 is the most anterior one.

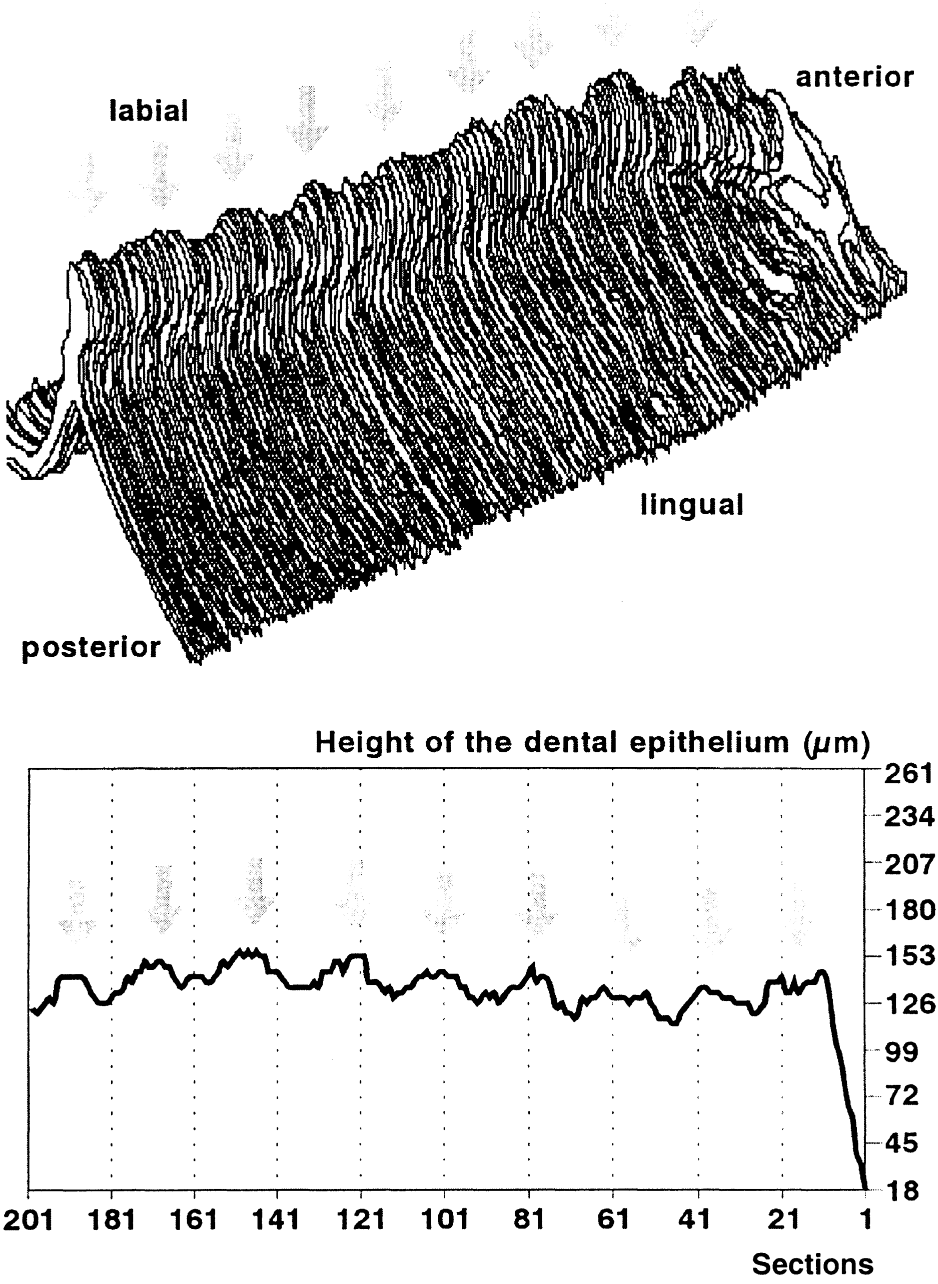

Fig. 3. Computer-assisted 3D reconstruction of the dental and adjacent oral epithelium in the upper jaw quadrant of a spotted dolphin embryo on DO-54 (viewing the mesenchyme side). The 3D morphology of the dental epithelium (above) is compared with changes in the height of the dental epithelium on the individual sections plotted in antero-posterior course (below). For better clarity, only data related to the most anterior 200 histological sections are shown. Arrows indicate presumed loci of the iniciated swellings of tooth epithelium (tooth buds).

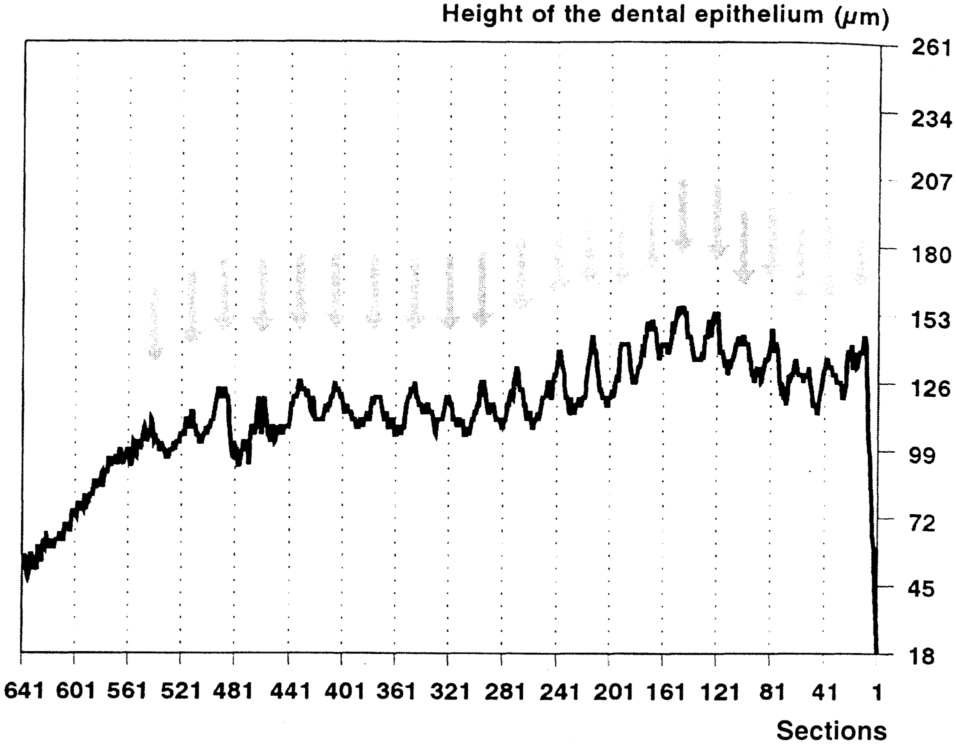

Fig. 4. Graph representing the antero-posterior changes in the height of the dental epithelium in the whole upper jaw quadrant in the embryo on DO-54. Arrows indicate 22 presumed areas of individual tooth bud formation.

achieved the bud shape. The swellings gave rise to small peaks on the curve when the antero-posterior changes in the height of the dental epithelium were measured (Fig. 3 .below). Twenty-two swellings could be recognized per one dental quadrant (Fig. 4). The maximal thickness of epithelium of the swellings achieved 153 μm, however, in the areas between swellings, the thickness of epithelium was 20-30 μm lower. In the posterior direction, the swellings decreased in size and gradually disappeared in the aboral part of the dental lamina, even though the dental lamina was still apparent (Fig. 4). On DO-68, the dental lamina showed 24 elevations exhibiting a bud-shape in frontal sections. The maximal height of the dental epithelium was 240 μm, and decreased by 30-50 μm in the areas separating the individual buds. The development of early tooth primordia proceeded in a comparable way in both the upper and lower jaw. No formation of permanent teeth primordia has been detected up to day 68.

Discussion

The initial steps of tooth primordia morphogenesis in the spotted dolphin were comparable with terrestrial mammals. The thickening of the oral epithelium along the jaw margins gave rise to the dental lamina. The swellings on the dental lamina that appeared during the later period exhibited a bud-shape morphology on frontal histological sections, known in terrestrial mammals. Accordingly, the swellings could be identified with the forming tooth primordia at the bud stage. From the very beginning, however, the number of tooth buds per dentition quadrant in the spotted dolphin significantly exceeded the maximum possible tooth number per quadrant in terrestrial mammals. So far, most of the data concerning mammalian tooth development have been acquired in terrestrial mammals (Weber 1927; Peyer 1968). The original mammalian tooth formula described by Osborne (1907) is 4/4, 1/1, 4/4, 8/8 for the upper and lower incisor, canine, premolars and molars, respectively. Osborne based his evaluations of the class of mammals as a whole on the above-mentioned tooth formula. Actually, the tooth formula characteristic for the so-called insectivorous type of dentition (3/3, 1/1, 4/4, 3/3) is accepted as the general eutherian tooth formula (Scott and Simons 1967), which leads to 44 teeth in adult eutherians. However, the general tooth formula has only a limited validity, as the numbers and shapes of mammalian teeth are highly variable and the fully expressed insectivorous type of dentition is comparatively rare. Contrary to terrestrial mammals, the toothed cetaceans exhibit a significant trend towards an increase in tooth number (polyodontia) associated with a simplification and resemblance of morphology (homodontia) (Peyer 1968).

From the aspect of the way of feeding in the toothed cetaceans, the simple tooth shape combined with their increased number represents a suitable capturing apparatus, while allowing for the chewing and crushing of food which is not necessary due to anatomy and physiology of the digestive tract in these animals. The polyodont homodont dentition of toothed cetaceans is a phylogenetically new character amongst mammals. It appeared secondarily in this taxonomical group, related to its transfer to aquatic environment. Strong feeding specialization probably played a role during this process. The ancestors of recent dolphin had a greater number of teeth than recent species of terrestrial mammals, but their teeth still showed differences in shape and size (Grassé 1955; Peyer 1968). The paleontological records document (Špinar 1985) that the transfer from a terrestrial to an aquatic way of life gradually led to a morphological simplification of dentition and probably also to monofyodontia.

In recent pinnipeds (mammals closely related to dolphins, as their environment and way of feeding are concerned), the deciduous dentition is already resorbed before birth (Bryden 1972); only the permanent dentition becomes functional. In whales (toothless cetaceans), a rudimentary dentition was found by Geoffroy St. Hillaire in the year 1806. However, the rudimentary teeth are also resorbed before birth. There is not a consensus whether the rudimentary dentition in whales is deciduous (Kukenthal 1889-93) or permanent (Peyer 1968). The functional dentition in toothed cetaceans is considered persisting deciduous dentition (eg. Schlosser 1890-1891ab; Kukenthal 1891ab; Terra 1911). We did not observe signs of permanent dentition development in the spotted dolphin during the first quarter of its prenatal development. Such finding is in accordance with data on the terrestrial mammals, where the permanent dentition does not develop before the bell stage (Luckett and Maier 1982; Luckett 1993ab). An integration of the existing study of odontogenesis in the spotted dolphin with later developmental stages, and improvement in the technical quality of the 3D reconstructions may contribute to the elucidation of origin of the specific features of dentition in toothed cetaceans.

Počáteční perioda vývoje zubů u delfínů (*Stenella attenuata*, Cetacea) - pilotní studie

Polyodontní dentice delfínů může obsahovat celkem až 265 tvarově stejných (homodontních) zubů. Pro analýzu prenatálního vývoje tohoto typu dentice jsme zvolili delfína pobřežního *(Stenella attenuata)*, který má 35-37 zubů v jednom čelistním kvadrantu. V prezentované pilotní studii jsme metodou seriových histologických řezů hlav v kombinaci s trojrozměrnými počítačovými rekonstrukcemi a morfometrií zubního epitelu studovali vývoj dentice u embryí ve stáří od 32 do 68 dní ontogeneze - t.j. přibližně do konce první čtvrtiny gestační periody (280 dní). Zesílení ústního epitelu v oblasti budoucí zubní lišty, považované za časnou morfologickou známku vývoje dentice na histologických řezech, bylo patrné od 32. dne ontogeneze. Pomocí 3D rekonstrukcí jsme u zárodku ve stáří 54 dní ontogeneze zaznamenali nástup vývoje ztluštěnin na zubní liště, jejichž počet se během dalšího období zvětšoval v antero-posteriorním směru. V místě těchto ztluštěnin měl epitel na frontálních histologických řezech podobu zubního pupenu popisovaného u suchozemských savců. Na konci sledovaného období (68 dní ontogeneze) bylo možné identifikovat přibližně 24 morfologicky podobných pupenů v jednom zubním kvadrantu.

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References

BANCROFT, J. D., COOK, H. C., STIRLING, R. W., TURNER, D. R. 1994: Manual of Histological Techniques and their Diagnostic Application. Churchill Livingstone, Edinburg-Tokyo

BRYDEN, M. M. 1972: Growth and development of marine mammals. In: Functional Anatomy of Marine Mammals. Academic Press, London-New York

EVANS, P. G. H. 1987: The Natural History of Whales and Dolphins. Facts on File Publ., New York-Oxford

GAUNT, P. N., GAUNT, W.A. 1978: Three Dimensional Reconstruction in Biology. Pitman Medical, Kent

GRASSÉ, P. P. 1955: Traité de Zoologie. XVII. Mammiféres. Masson et Cie, Paris

GEOFFROY St. HILLAIRE, E. 1806: Cited in Peyer, B. 1968: Comparative Odontology. The University of Chicago Press, Chicago-London, p. 270

KÜKENTHAL, W. 1889-1893: Vergleichend-anatomische und entwicklungsgeschichtliche Untersuchungen an Walthieren. Jenaische Denkschr. I-III

KÜKENTHAL, W. 1891a: Einige Bemerkungen über die Säugetierbezahnung. Anat. Anz. **6**:364-371

KÜKENTHAL, W. 1891b: Das Gebiss des Didelphys. Anat. Anz. **6**: 658-666.

KÜKENTHAL, W. 1892: Über den Ursprung und die Entwicklung der Säugetierzähne. Jen. Z. f. Naturwiss. **26**:469-489

KÜKENTHAL, W. 1895: Zur Dentitionsfrage. Anat. Anz. **12**:653-692

OSBORN, H. F. 1907: Evolution of Mammalian Molar Teeth. MacMillan, New York

LEATHERWOOD, S., REEVES, R. R. 1983: Whales and Dolphins. Sierra Club, San Francisco

LUCKETT, W. P. 1993a: Ontogenetic staging of the mammalian dentiton, and its value for assessment of homology and heterochrony. J. Mammal. Evol. **1**:269-282

LUCKETT, W. P. 1993b: An ontogenetic assessment of dental homologies in therian mammals. In: Mammal Phylogeny: Mesozoic Differentiation, Multituberculates, Monotremes, Early Eutherians, and Marsupials (Ed. by F. S. SZALAY, M. J. NOVACEK, M. C. McKENNA), pp. 182-204. Springer Verlag, New York

LUCKETT, W. P. and MAIER, W. 1982: Development of deciduous and permanent dentitions in Tarsius and its phylogenetic significance. Folia primatol. **37**:1-36

MÜLLER, A. H. 1970: Lehrbuch der Paläozoologie. Band III - vertebraten. G. Fischer, Jena

OSBORN, H. F. 1907: Evolution of Mammalian Molar Teeth. MacMillan, New York

PEYER, B. 1968: Comparative Odontology. The University of Chicago Press, Chicago-London

SCHLOSSER, M. 1890-1891a: Über die Deutung des Milchgebisses der Säugetiere. Biol. Zentbl. **10**:81-92

SCHLOSSER, M. 1890-1891b: Die Differenzierung des Säugetiergebisses. Biol. Zentbl. **10**:238-252.

SCOTT, J. H. and SIMONS, N. B. B. 1967: Introduction to Dental Anatomy. Livingstone Ltd., Edinburgh

ŠPINAR, Z. V. 1984: Paleontologie obratlovců [Paleontology of Vertebrates]. Academia, Praha (in Czech)

ŠTĚRBA, O. 1995: Staging and ageing of mammalian embryos and fetuses. Acta vet. Brno **64**:83-89

TERRA, P. de 1911: Vergleichende Anatomie des menschlichen Gebisses und der Zähne der Vertebraten. Fischer, Jena

WATSON, L. 1981: Whales of the World. Hutchinson, London-Johannesburg

WEBER, M. 1927: Die Säugetiere. 1. Bd. Anatomischer Teil. G. Fischer, Jena

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