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Reconstructing the auditory apparatus of therapsids by means of neutron tomography

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Abstract

The internal cranial structure of mammalian ancestors, i.e. the therapsids or "mammal-like reptiles", is crucial for understanding the early mammalian evolution. In the past therapsid skulls were investigated by mechanical sectioning or serial grinding, which was a very time-consuming and destructive process and could only be applied to non-valuable or poorly preserved specimens. As most therapsid skulls are embedded in terrestrial iron-rich sediments of Late Permian or Triassic age, i.e. so called "Red beds", a successful investigation with X-Rays is often not possible.
We successfully investigated therapsid skulls by means of neutron tomography at the facility ANTARES at FRM II in Munich using cold neutron radiation. This kind of radiation is able to penetrate iron-rich substances in the range between 5 and 15 cm and produces a good contrast between matrix and bones, which enables segmentation of internal cranial structures such as bones, cavities and canals of nerves and blood vessels. In particular, neutron tomography combined with methods of 3D modeling was used here for the investigation and reconstruction of the auditory apparatus of therapsids.

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1. Introduction

The therapsids were the dominant tetrapods in Late Permian and Early Triassic. Therapsids, pelycosaurs and mammals belong to the same clade, the synapsids, which possess one temporal fenestra in the skull. In contrast, diapsids such as crocodiles, pterosaurs, dinosaurs and birds have two temporal fenestrae.

Therapsids were mouse-like up to hippopotamus-sized animals, which occupied almost all terrestrial habitats. The most abundant and successful herbivorous therapsid clade were the anomodonts due to their unique masticatory apparatus with horny beak, which enabled them to feed resistant plant material (Kemp 2005).

Due to their ancestry to mammals therapsids were formerly also known as "mammal-like reptiles" and are, therefore, of special interest for evolutionary biologists and palaeontologists. Studying therapsid fossils may help to resolve numerous questions regarding the early evolution of mammals, e.g. the origin of the mammalian hearing apparatus, respiration, endothermy, the origin of the mammalian brain, masticatory apparatus and locomotion.

Despite this, little is known about the internal structure of therapsid skulls. Firstly, this is caused by the fact that with the exception of some broken fossils or well preserved natural casts of cranial cavities (Fig. 1), which were exposed by breakage or weathering, the almost complete internal anatomy of therapsid skulls is usually hidden. However, mechanical preparation may help to uncover parts of the outer surface of fossil skulls, but insights into cavities, which are almost completely enclosed by bony walls, is usually impossible without destruction. The problem is further complicated by the fact that therapsid fossils are often rare and valuable, which usually excludes the application of destructive methods for investigation.

Fig. 1. Natural endocast of an inner ear labyrinth of a dicynodont therapsid (from Cox 1962).

Another problem is that most therapsid skulls are relatively large and massive and often filled with iron-rich sediments, so called "Red Beds", which usually absorb X-rays to a large degree. Consequently, non-invasive technologies for investigation of the internal cranial anatomy of therapsids were rarely used.

In particular, the anatomy of the inner ear labyrinth, which is almost completely surrounded by bones of the neurocranium is completely unknown for most therapsid taxa. Although soft tissue structures are usually not preserved, the shape of the bony labyrinth of the inner ear may provide important information about auditory capabilities, the alert posture of the head, behavior and the activity level of extinct animals (e.g. Spoor et al. 1994, Meng and Wyss 1995, Witmer et al. 2003, Alonso et al. 2004, Berlin et al. 2013).

The aim of the article is to give a short overview about methods for the investigation of fossil therapsid skulls with special focus on neutron tomography. Furthermore, it will be demonstrated how neutron tomography may

help to reconstruct the hearing apparatus of therapsids and which information can be inferred from 3D models of the auditory region.

2. Methods for the investigation of therapsid skulls

2.1. Serial grinding and sectioning of therapsid skulls

Sectioning and serial grinding were for a long time the only methods to investigate internal structures of fossil skulls. The serial grinding method was firstly described by Sollas (1903), who built a grinding machine which produces series of parallel surfaces of fossils in small distances with the help of a grinding wheel. The whole procedure is very time consuming and may cost up to two years of working time. As the investigated skulls were completely destroyed, the serial grinding method has only been applied to a few non-valuable, incomplete or poorly preserved specimens (e.g. Sollas and Sollas 1914, 1916; Barry 1967; Keyser 1973; Olson 1944; Fourie 1993; Maier and van den Heever 2002; Sigurdsen 2006).

2.2. X-ray computed tomography

X-rays have been used for investigations of palaeontological objects at least since the 1930s and, more frequently, X-ray computed tomography since the 1970s (Conroy and Vannier 1984; Ketcham and Carlson 2001; Walsh et al. 2013). Because medical CT scanners only achieve a resolution of approximately 0.35-0.5 mm (Schwarz et al. 2005), industrial micro-CT scanners with 1-2 orders of magnitude higher resolution and a better penetration of dense materials (Rowe et al. 2001) were predominantly used for examinations of the internal cranial anatomy of small synapsids such as cynodonts and Mesozoic mammals (e.g. Rowe et al. 1995; Rowe 1996; Rowe et al. 2001; Ketcham and Carlson 2001).

Highest resolutions in the range of only a few µm can be achieved by synchrotron X-rays. The resolution depends from the sample size, which is usually restricted to very small samples in the range of a few millimeters. However, there is a possibility to scan larger samples at some facilities such as the ESRF in Grenoble, which makes synchrotron X-rays also suitable for examinations of fossil synapsid skulls. Examples for examinations of therapsid fossils with synchrotron radiation are the investigation of the small anomodont *Niassodon* from the Late Permian of Mozambique (Castanhinha et al. 2013) and the investigation of a burrow cast with skeletons of the cynodont *Thrinaxodon liorhinus* and the temnospondyl amphibian *Broomistega putterilli* (Fernandez et al. 2013).

2.3. Neutron tomography

Although neutron radiography (NR) is also known since the 1970s, it has rarely been used for investigations of palaeontological samples (Schwarz et al. 2005). Neutron computed tomography (NCT) was introduced in the 1990s at major reactor or spallation neutron sources when digital neutron detectors became available with CCD cameras (Schillinger 1996; Schillinger 1997). Increasing detector sensitivity allowed to sacrifice neutron intensity for higher beam collimation, which in turn allowed for high-resolution tomography images when the first high resolution neutron imaging facilities were built. In former times, spatial resolution in neutron radiography was limited by insufficient beam collimation, which allowed only for very blurred images. Today, samples ranging from a few centimeters up to ca. 30 cm can be examined with highest spatial resolutions similar to microCT.

The main advantage of using neutrons instead of X-Rays is the fact that neutron interaction with matter does, due to the charge neutrality of neutrons, not depend on the number of electrons in the shell of an element, but on the structure of the nuclei of the atoms themselves. This often leads to huge contrast between neighboring elements, even isotopes of the same element. Many heavy elements like iron and other metals can be penetrated easily, while light elements like Li, B and especially hydrogen deliver huge contrast (Laaß et al. 2010, Kardjilov et al. 2006). This might be also the reason that neutron radiation produces usually a good contrast between fossil bones and matrix (Laaß et al. 2010), which is probably caused by hydrogen-containing minerals in fossil bones such as Hydroxyl-Apatite. Neutron tomography was already successfully used, for example, for the examination of

sauropod vertebrae and ribs (Schwarz et al. 2005), archosaurian reptile bones (Cisneros et al. 2010) and therapsid skulls (Laaß et al. 2010; Laaß 2014).

The measurements described below were performed at the ANTARES facility for Neutron Imaging at the FRM II reactor of Technische Universität München. This facility currently delivers the best combination of high neutron flux and high beam collimation world-wide.
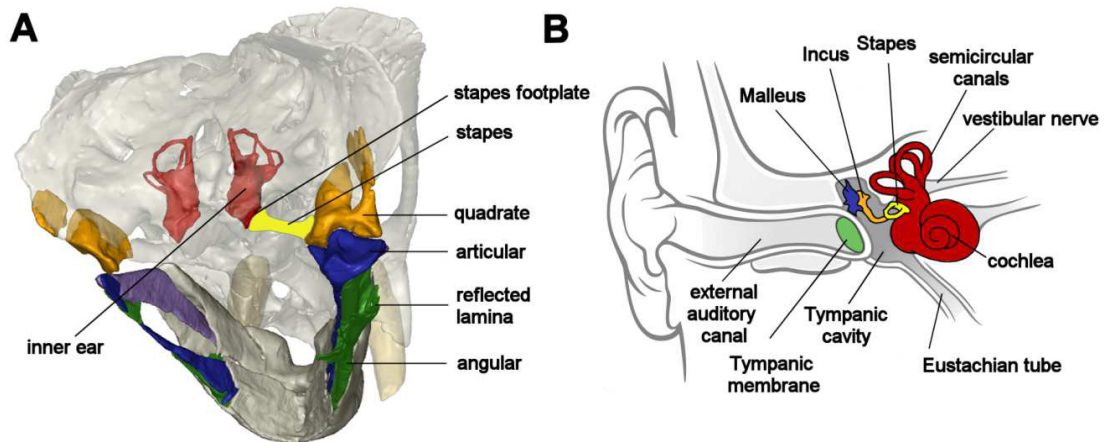

Fig. 2. Comparison of the ear region of therapsids and modern mammals. A. Virtual 3D model of the skull of the anomodont *Diictodon feliceps* (MBR 1000) in posteroventral view. The quadrate (= incus) and articular (= malleus) form the primary jaw articulation. The supposed tympanic membrane was probably situated at the lateral side of the mandible and supported by the reflected lamina. B. Human ear region. Homolog bones are marked by the same colors. Modified from http://commons.wikimedia.org/wiki/File:Anatomy_of_the_Human_Ear.svg

3. Investigation of the hearing apparatus of therapsids by means of neutron tomography and methods of 3D visualisation

3.1. Overview

One of the key innovations of mammals is their ear, which is unique among vertebrates. In contrast to amphibians, reptiles and birds, which have only one ear ossicle, mammals possess even three. Remarkably, it has already been found in the 19th century that the bones, which form the reptilian jaw articulation, the articular and quadrate, are the homologues of the mammalian ear ossicles, the malleus and incus, respectively (Reichert 1837; Gaupp 1913; see Fig. 2). Furthermore, the mammalian tympanic bone, which supports the eardrum, is the homologue of the reptilian angular (Fig. 2). Therefore, it has been assumed that the jaw hinge of therapsids had a dual function for feeding and for hearing (Allin 1975). The eardrum of therapsids has been assumed in the angular region at the lateral side of the mandible, which was supported by a thin bony plate, the reflected lamina. Its function has been controversially discussed as sound receiver (Allin 1975; Maier and van den Heever 2002) or as a surface for muscle attachment (Hopson and Barghusen 1986; Kemp 2007).

However, the fossil record reveals that the bones of the jaw hinge of therapsids miniaturized and became detached from the mandible during the transition from cynodonts to mammals. This coincided with the origin of a secondary squamosal-dentary jaw articulation (Allin 1975) (Fig. 2). It seems very likely that the miniaturization of the ear ossicles resulted in an enhanced capability to hear high-frequency sound, because the upper frequency limit of hearing is restricted by the mass and inertia of the ear ossicles (Hemilä et al. 1995). A further consequence of the detachment of the postdentary bones from the mandible was a higher compliance of this elements and a loss of their stress transmission function from feeding.

Figures 3 and 4 demonstrate how virtual 3D models of therapsid skulls can be reconstructed from neutron tomographic data. Middle and inner ear parameters as well as the volumes of bones and cavities can be determined by using the virtual 3D model in Fig. 4, which is usually not possible on original material.

3.2. What middle and inner ear parameters tell us about the hearing function of therapsids

The hearing capabilities of therapsids can be inferred from the length of the cochlea cavity of the inner ear, which is roughly correlated with the length of the basilar papilla, the auditory sensory organ of the inner ear. As a rule of thumb, the longer the basilar papilla, the wider is usually the hearing range of frequencies.
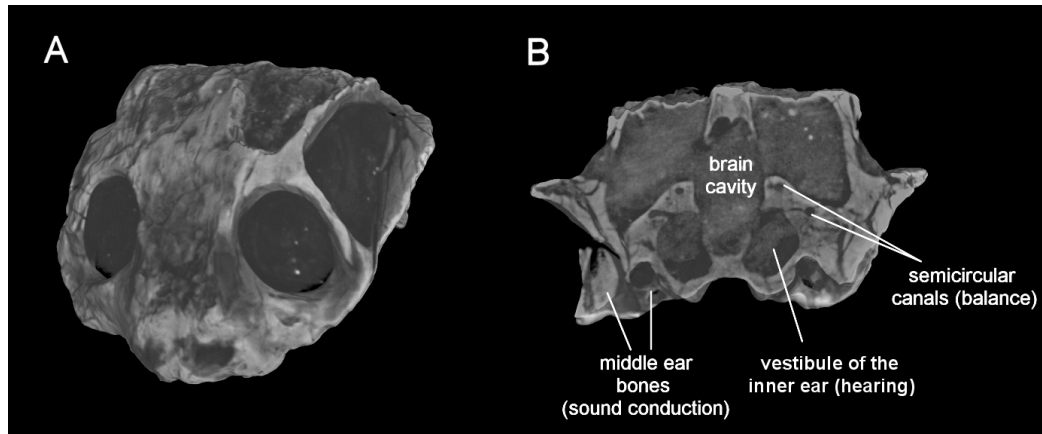

Fig. 3. Volume model of the skull of the anomodont *Cistecephalus* (BSPG 1932 I 56) from the Late Permian of South Africa. A Frontolateral view. B. Virtual section through the otic region showing the casts of the inner ears and the sound conducting bones.

In some cases, the vestibule of the inner ear of burrowing animals can be extraordinarily inflated such as in the small anomodont *Kawingasaurus* from the Late Permian of South Africa. *Kawingasaurus* possessed a vestibule volume of the inner ear, which was about 25 times larger than the human one, although its skull was only 4 cm long (Laaß 2014). Vestibule inflation is common among subterranean vertebrates such as amphisbaenids, burrowing frogs, gymnophione amphibians and the extinct multituberculate mammal *Lambdopsalis bulla* and is interpreted as an adaptation to bone-conduction hearing (Luo and Ketten 1992, Meng and Wyss 1995). In the subterranean habitat substrate sound propagates much better through soil than airborne sound, which is cluttered with noise and is usually extremely dampened (Mason and Narins 2001). Furthermore, burrowing animals usually have a close contact between head and substrate. Consequently, fossorial animals mainly hear by bone conduction and often communicate with seismic signals (Mason and Narins 2001).

A number of authors assumed that fossorial mammals show several adaptations to low-frequency hearing in comparison to ground dwelling species and tend to have larger eardrums and middle ear cavities, massive ear ossicles sometimes with extraordinarily enlarged parts, larger stapes footplates and degenerated or completely reduced middle ear muscles. However, a comparison of 155 non-fossorial mammalian genera from 19 orders and 24 mammalian fossorial species showed that this is true for the stapes footplates, but the eardrums and middle ear cavities of fossorial taxa are not significantly enlarged. Enlarged stapes footplates are also interpreted as an adaptation to bone conduction hearing (Webster and Webster, 1975; Burda et al., 1992; Mason, 2001). Interestingly, the same seems to be also true for therapsids, which is supported by measurements and virtual reconstructions of well preserved stapes of therapsids by means of neutron tomography (Laaß 2014).
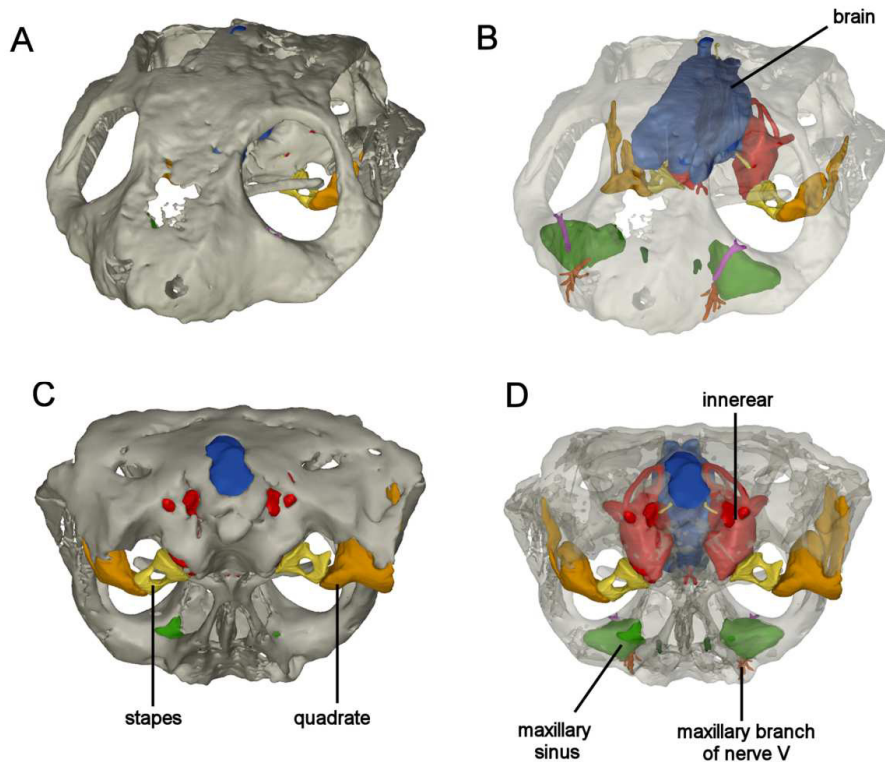
Fig. 4. Virtual 3D model of the *Cistecephalus* specimen in Figure 3. A. Frontolateral view. B. Frontolateral view with transparent bones showing several internal structures of interest such as the brain, the maxillary sinuses, the trigeminal nerves as well as the auditory apparatus, which is marked by different colors.

A further consequence of an enlarged stapes footplate is a lower impedance matching function of the middle ear. It should be noted that the middle ear functions is a leverage system and amplifies sound waves before they reach the cochlea of the inner ear. This is particularly important for animals which mainly hear airborne sound, because airborne sound is considerably weaker than sound in water or in substrate (Tumarkin 1968). The impedance matching of mammalian middle ears is in the range between 10 and 40 (Rosowski and Graybeal, 1991; Rosowski, 1992). It can roughly be estimated from the area ratio between the tympanic membrane and the stapes footplate. The larger the stapes footplate, the lower is the impedance matching of the middle ear. In other words, larger stapes footplates may be an indication for an adaptation of the middle ear to hear substrate sound by bone-conduction, because bone-conduction hearing is not much affected by the problem of impedance matching (Tonndorf 1972).

Furthermore, the 3D reconstructions of the hearing apparatus of therapsids as well as studies of well preserved therapsid fossils revealed that in species, which are believed to have been fossorial and which have enlarged stapes footplates, the long axis of the stapes points lateroventrally to the ground (Laaß 2014). In contrast, therapsids such as cynodonts and therocephalians, which were usually ground-dwelling animals, often have horizontally oriented stapes. Consequently, the long axis of their stapes points to the supposed eardrum at the lateral side of the mandible, which might be an indication that these species were adapted to hear airborne sound. At the same time this supports the hypothesis that the reflected lamina served as a sound receiver.

Consequently, the downward sloping stapes combined with a relatively large footplate of the small anomodont *Cistecephalus* from the Late Permian of South Africa (Figs. 3, 4) is an indication that this animal heard substrate sound by bone-conduction. Probably, *Cistecephalus* also pressed its head and the mandible against the walls of its

burrow to hear seismic vibrations as it is known from some modern fossorial mammals such as the blind-mole rat *Spalax ehrenbergii* (Rado et al. 1989).

4. Summary and Conclusions

Neutron tomography is a useful tool for investigations of the auditory region of therapsids. Our previous results show that cold neutron radiation penetrates iron-rich fossils well in the range between ca. 5 to 15 cm and produces a good contrast between fossil bones and matrix. In the examined therapsid specimens it was possible to reconstruct three-dimensionally the cast of the bony labyrinth of the inner ear as well as the middle ear apparatus. The 3D models provided several middle and inner ear parameters such as the stapes footplate area and the inclination of the stapes, the volumes of the middle ear bones, the vestibule volume and the length of the cochlear cavity, which may be suggestive for the auditory capabilities of extinct animals, their behavior and lifestyle.

Acknowledgements

References

Allin, E.F. 1975. Evolution of the mammalian middle ear. Journal of Morphology 147, 403-438.

Alonso, P.D., Milner, A.C., Ketcham, R.A., Cookson, M.J., Rowe, T.B. 2004. The avian nature of the brain and inner ear of *Archaeopteryx*. Nature 430, 666–669.

Barry, T. H. 1967. The cranial morphology of the Permo-Triassic anomodont *Pristerodon buffaloensis* with special reference to the neural endocranium and visceral arch skeleton. Annals of the South African Museum 50, 131-161.

Berlin, J.C., Kirk, E.C., Rowe, T.B. 2013. Functional implications of ubiquitous semicircular canal non-orthogonality in mammals. PLoS ONE 8(11): e79585.

Burda, H., Bruns, V., Hickman, G.C. 1992. The ear in Subterranean lnsectivora and Rodentia in comparison with grounddwelling representatives. I. Sound Conducting System of the Middle Ear. Journal of Morphology 214, 49–61.

Castanhinha, R., Araújo, R., Júnior, L.C., Angielczyk, K.D., Martins, G.G., Martins, R.M.S., Chaouiya, C., Beckmann, F. and Wilde, F. 2013. Bringing dicynodonts back to life: Paleobiology and anatomy of a new emydopoid genus from the Upper Permian of Mozambique. PLoS ONE 8, e80974.

Cisneros, J.C., Gomes Cabral, U., de Beer, F., Damiani, R., Costa Fortier, D. 2010. Spondarthritis in the Triassic. PLoS ONE 5(10): e13425. doi:10.1371/journal.pone.0013425

Conroy, G.C., Vannier, M.W. 1984. Noninvasive three-dimensional computer imaging of matrix-filled fossil skulls by high-resolution computed tomography. Science 226, 1236-1239.

Fernandez, V., Abdala, F., Carlson, K.J., Cook, D.C., Rubidge, B.S., et al. (2013). Synchrotron Reveals Early Triassic Odd Couple: Injured Amphibian and Aestivating Therapsid Share Burrow. PLoS ONE 8(6): e64978. doi:10.1371/journal.pone.0064978.

Gaupp, E. 1913. Die Reichertsche Theorie. Archiv für Anatomie und Entwicklungsgeschichte 1912, 1-426.

Hemilä, S., Nummela, S. and Reuter, T. 1995. What middle ear parameters tell about impedance matching and high frequency hearing. Hearing Research 85, 31–44.

Hopson, J.A., Barghusen, H.R. 1986. An analysis of therapsid relationships, in "*The Ecology and Biology of Mammal-like Reptiles*". In: Hotton, N., McLean, P.D., Roth, J.J. and Roth, E.C. (Eds.). Smithsonian Institution Press, Washington D.C., 83-106.

Kardjilov, N., Fiori, F., Giunta, G., Hilger, A., Rustichelli, F., Strobl, M., Banhart, J., Triolo, R. 2006. Neutron tomography for archaeological investigations. Journal of Neutron Research 14(1), 29–36.

Kemp, T.S. 2005. The origin and evolution of mammals. Oxford University Press, New York, 331pp.

Kemp, T.S. 2007. Acoustic transformer function of the postdentary bones and quadrate of a nonmammalian cynodont. Journal of Vertebrate Paleontology 27, 431-441.

Ketcham, R.A., Carlson, W.D. 2001. Acquisition, optimization and interpretation of X-ray computed tomographic imagery: applications to the geosciences. Computers and Geosciences 27, 381-400.

Keyser, A.W. 1973. A preliminary study of the type area of the *Cistecephalus* zone of the Beaufort Series, and a revision of the anomodont family Cistecephalidae. South African Geological Survey Memoirs 62, 1-71.

Laaß, M., Hampe, O., Schudack, M., Hoff, C., Kardjilov, N. and Hilger, A. 2010. New insights into the respiration and metabolic physiology of *Lystrosaurus*. Acta Zoologica 92, 363-371.

Laaß, M. 2014. Bone-conduction hearing and seismic sensitivity of the Permian anomodont *Kawingasaurus fossilis*. Journal of Morphology 276:121-143.

Lehmann, E.H., Vontobel, P., Schillinger, B., Bücherl, T., Baechler, S., Jolie, J., Treimer, W., Rosa, R., Chirco, P., Bayon, G., Legoupil, S., Körner, S., Böck, H., Micherov, V., Balasko, M., and Kuba, A. 2000. Status and Prospect of Neutron Tomography in Europe. Abstracts 15th World Conference on Nondestructive Testing, Roma, 1-12.

Luo, Z.-X., Ketten, D.R. 1991. CT Scanning and computerized reconstructions of the inner ear of multituberculate mammals. Journal of Vertebrate Paleontology 11, 220–228.

Maier, W., van den Heever, J. 2002. Middle ear structures in the Permian *Glanosuchus sp.* (Therocephalia, Therapsida), based on thin sections. Mitteilungen des Museums für Naturkunde Berlin, Geowissenschaftliche Reihe 5, 309-318.

Mason, M.J. 2001. Middle ear structures in fossorial mammals: A comparison with non-fossorial species. Journal of Zoology 255, 467–486.

Mason, M.J., Narins, P.M. 2001. Seismic Signal Use by Fossorial Mammals. American Zoologist 41, 1171–1184.

Meng, J., Wyss, A.R. 1995. Monotreme affinities and low-frequency hearing suggested by multituberculate ear. Nature 377, 141–144.

Reichert, C. 1837. Über die Visceralbogen der Wirbeltiere im Allgemeinen und deren Metamorphosen bei den Vögeln und Säugetieren. Archiv für Anatomie, Physiologie und wissenschaftliche Medicin 1837, 120-222.

Rosowski, J.J. 1992. Hearing in transitional mammals: Predictions from the middle-ear anatomy and hearing capabilities of extant mammals, in: "*The Evolutionary Biology of Hearing*". In: Webster, D.B., Fay, R.R., Popper, A.N. (Eds.). Springer, New York, pp. 615–631.

Rosowski, J.J., Graybeal, A. 1991. What did *Morganucodon* hear? Zoological Journal of the Linnean Society 101, 131–168.

Rowe, T., Carlson, W., Bottorff, W. 1995. *Thrinaxodon*, Digital Atlas of the Skull (Second edition). CD–ROM. University of Texas Press, Austin.

Rowe, T. 1996. Coevolution of the mammalian middle ear and neocortex. Science 273, 651–654.

Rowe, T., Colbert, M., Ketcham, R., Maisano, J., Owen, P. 2001. High-Resolution X-ray Computed Tomography in Vertebrate Morphology. Journal of Morphology 248, 277-278.

Schillinger, B. 1996. 3D Computer tomography with thermal neutrons at FRM Garching. Journal of Neutron Research 4, 57-63.

Schillinger, B., Gebhard, R., Haas, B., Ludwig, W., Rausch, C., Wagner, U. 1997. 3D Neutron Tomography in Material Testing and Archaeology. Proceedings of the 5th World Conference of Neutron Radiography Berlin 1996, 688-693.

Schwarz, D., Meyer, C. A., Lehmann, E. H., Vontobel, P., Bongartz, G. 2005. Testing Neutron tomography in comparison with X-ray computed tomography as a technique for the investigation of the internal structure of sauropod vertebrae and ribs. Palaeontologia Electronica 8, 1-11.

Sigurdsen, T. 2006. New features of the snout and orbit of a therocephalian therapsid from South Africa. Acta Palaeontologica Polonica 51, 63-75.

Sollas, W.J. 1903. A method for the investigation of fossils by serial sections. Philosophical Transactions of the Royal Society of London B 196, 259-265.

Sollas, I.B.J., Sollas, W.J. 1914. A study of a *Dicynodon* by means of Serial Sections. Philosophical Transactions of the Royal Society of London B 204, 201-225.

Spoor, F., Wood, B., Zonneveld, F. 1994. Implications of early hominid labyrinthine morphology for evolution of human bipedal locomotion. Nature 369, 645–648.

Tonndorf, J. 1972. Bone conduction, in "*Foundations of Modern Auditory Theory*". In: Tobias, J.V. (Ed.). Academic Press, London, Vol. 2, pp. 197–237.

Tumarkin, A. 1968. The evolution of the auditory conducting apparatus in terrestrial vertebrates, in *"Ciba foundation symposium on hearing mechanisms in vertebrates"*. In: de Reuck, A.S., Knight, J. (Eds.). Churchill, London, 18-37.

Walsh, S.A., Luo, Z.-X., Barrett, P.M. 2013. Modern Imaging Techniques as a Window to Prehistoric Auditory Worlds, in "*Insights from Comparative Hearing Research*". In: Köppl, C. et al. (Eds.). Springer Handbook of Auditory Research 49, DOI 10.1007/2506_2013_32.

Webster, D.B., Webster, M. 1975. Auditory systems of Heteromyidae: functional morphology and evolution of the middle ear. Journal of Morphology 146, 343–376.

Witmer, L.M., Chatterjee, S., Franzosa, J., Rowe, T. 2003. Neuroanatomy of flying reptiles and implications for flight, posture and behaviour. Nature 425, 950-953.