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# **What happens to ear morphology on the way underground?**

## **A case study on the family Spalacidae.**

Ph.D. Thesis

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### **Annotation**

This PhD thesis is concerned with the effect of specialization to the subterranean environment on ear morphology in the rodent family Spalacidae. The outer, middle and inner ear morphology in four species with a different proportion of subterranean activity was described and analyzed. The Chinese bamboo-rat (*Rhizomys sinensis*) was identified as fossorial regarding its ear morphology. The strictly subterranean Gansu zokor (*Eospalax cansus*) possess a highly specialized subterranean ear morphology with a suggested acoustic fovea. Meanwhile, the less subterranean African root-rat (*Tachyoryctes splendens*) shares traits with both strictly subterranean and epigeic rodents. The aboveground characteristics are even more prominent in the fossorial giant root-rat (*T. macrocephalus*). Taken together, the results confirm that the ear morphology of spalacids mirrors their degree of fossoriality.

### **Declaration [in Czech]**

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České Budějovice, 29.11.2020

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## List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

**I. Pleštilová, L.**, Hrouzková, E., Burda, H., Šumbera, R. (2016). Does the morphology of the ear of the Chinese bamboo rat (*Rhizomys sinensis*) show “subterranean” characteristics? *Journal of Morphology*, 277(5), 575-584. (IF=1.62)

*Lucie Pleštilová made the histological analysis and processed the data with help of Ema Hrouzková and Hynek Burda. She wrote the manuscript with the help of all co-authors.*

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*Lucie Pleštilová made the histological analysis and processed the data. She wrote the manuscript with help of Ema Hrouzková, Hynek Burda and Radim Šumbera.*

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# **General introduction**

## **Subterranean and fossorial rodents**

Many mammals have chosen a life strategy that is based on excavation of burrow systems. At the present, more than 300 mammalian species in 54 genera and 10 families are confined to a more-or-less whole life in underground burrows (Nevo, 1999; Burda, 2003). The majority of them (more than 250 species) belong to the order Rodentia (Nevo, 1999; Begall et al., 2007).

Among burrowing mammals, there are at least two groups distinct by the level of specialization to life underground. The larger group uses subterranean burrows as a shelter where they can spend most of the day, but they forage aboveground. These species are denoted as fossorial and within Rodentia, they are found in families Aplodontidae, Sciuridae, Heteromyidae, Ctenomyidae, Octodontidae, Dipodidae, Cricetidae, Muridae, and also in Spalacidae (Nevo, 1999). The smaller group of species spend all their life in a sealed underground burrow system. Such mammals are usually referred to as subterranean, or alternatively, strictly fossorial (Begall et al., 2007; Nevo, 1999). Among rodents, four families contain subterranean representatives, such as Spalacidae, Bathyergidae, Geomyidae, and Cricetidae. However, in nature these lifestyle categories are not so clearly defined as there is a continuum between the subterranean and fossorial way of life (Burda, 2003).

The subterranean way of life evolved primarily to avoid predation, daily and seasonal climatic extremes and to exploit new food resources in the form of storage organs of plants called geophytes. Opportunities for the evolution of this lifestyle probably emerged during middle Eocene (45 mya) as a result of an increased aridity and seasonality of many geographical regions (Nevo, 1999). Subterranean and fossorial rodents inhabit all continents except of Australia (Lacey et al., 2000). Pocket gophers (Geomyidae) occur in North America, fossorial coruros (*Spalacopus cyanus*, Octodontidae) and tuco-tucos (Ctenomyidae) in South America, African mole-rats (Bathyergidae), blind mole rats and root-rats



(Spalacidae) live in Africa. Eurasia is inhabited by family Cricetidae, including voles (Arvicolini), the mole vole (*Ellobius*) and the long-clawed mole vole (*Prometheomys*), as well as by blind mole rats and zokors (Spalacidae) (Nevo, 1999; Lacey et al., 2000).

The tunnel systems burrowed and inhabited by subterranean and fossorial rodents can reach impressive dimensions. They are more than a hundred meters long in the solitarily living Chinese zokor (*Eospalax fontanierii*) and blind mole rat (*Spalax galili*), but they can reach up to 3 km in social mole-rats from the family Bathyergidae (Lövy et al., 2015; Šklíba et al. 2012, Šumbera et al. 2012; Zhang 2007). The burrow systems typically consist of a nest, foraging burrows, foodstores, latrines, deeper burrows and bolt-holes (Davies & Jarvis, 1986, Šklíba et al. 2012, Šumbera et al. 2012). Foraging burrows are the shallowest burrows forming the most extensive part of the burrow system, and their depth is usually 6 – 20 cm (Roper et al., 2001; Jarvis & Sale, 1971). The depth of the nests can be only 15 – 20 cm in the common mole-rat (*Cryptomys hottentotus*) or Cape dune mole-rat (*Bathyergus suillus*), 60 cm in the African root-rat (*Tachyoryctes splendens*) (Jarvis and Sale, 1971), but it can also reach depths of more than one meter in the giant mole-rat (*Fukomys mechowii*) (Lövy et al., 2013) and more than two meters in the Chinese zokor in the cold conditions of the Tibetan plateau (Zhang 2007). The deepest parts of the burrow system are usually the bolt-holes, which probably serve for thermoregulation and as water drainage (Davies & Jarvis, 1986). The underground burrow systems of the studied fossorial species were of a much smaller size, these varied from 5 m to a several hundred meters (Begall and Gallardo, 2000; Bruegger et al., 2010; He, 1984; Schleich and Antenucci, 2009; White, 2009).

Apart from the benefits of a relatively stable temperature (reviewed in Šumbera, 2019), living in the subterranean ecotope also has its drawbacks. These include low food supply, high energy costs of extending the burrows, or permanent darkness (Nevo, 1979; Lacey et al., 2000). As a result of spending entire lives in this environment, various examples of

morphological, physiological, sensory and behavioral convergence have arisen among subterranean mammals across genera, families, and even orders (Nevo, 1999).

### **Sensory capabilities and communication in subterranean rodents**

One of the main challenges faced by mammals in subterranean burrows is the lack of sensory cues and thus restricted navigation and signaling options. Living in a dark ecotope, subterranean mammals do not rely on vision, which has resulted in the degeneration of their eyes (Němec et al., 2007; Nevo, 1979). Although some subterranean rodents such as blind mole-rats possess degenerated eyes covered by skin, the eyes of other species such as African mole-rats and zokors are small, but not of a degenerate structure (Němec et al., 2007; Hetling et al. 2005; Němec et al. 2008). However, a detailed study of these “normally formed” eyes has shown many vestigial traits, especially in their innervation (Němec et al., 2004, 2007), and behavioral experiments too have shown an impaired ability of image forming and no perception of depth (Kott et al., 2016). Therefore, this seriously compromised vision of the subterranean species serves mainly for dark/light discrimination, important for setting the circadian activity and for a quick localization of a breach in their subterranean system as an antipredatory strategy (Hetling et al., 2005; Kott et al., 2010, 2014; Němec et al., 2007).

Due to the lack of optical navigational cues, subterranean mammals are forced to use other ways of orientation when searching for food, digging, and maintaining their burrow systems. It was assumed that finding food sources as plant storage organs is more or less unpredictable, but a series of laboratory experiments has shown that subterranean rodents are able to recognize the plants’ waste products and use them as odor cues for finding the food items (reviewed in Heth and Todrank, 2007).

Regarding the quick and effective navigation through their burrow systems, which can be very extensive (see above), they replaced the landmarks available aboveground with a well-developed tactile sense and, interestingly, also by a magnetic compass for finding directions (Burda et al., 1990; Kimchi et al., 2004). While in some species studied so far, magnetoreception involves specialized photoreceptors, it is light-independent in subterranean mammals and it supposedly implicates magnetite particles (recently reviewed in Burda et al., 2020).

Besides the difficulties in navigation, an almost permanent darkness brings challenges also in communication. Subterranean mammals therefore use other ways of sharing information, such as olfactory or vocal communication. Based on odor, subterranean rodents are able to recognize family members and discriminate between unfamiliar individuals according to their genetic relatedness (Heth and Todrank, 2007). However, olfactory signals do not spread over long distances as the airflow is very low in sealed tunnels (Nevo 1991).

Vocal communication in underground burrows is limited due to special acoustic conditions (see below), yet widely used by all subterranean rodents studied so far (Bednářová et al., 2013; Credner et al., 1997; Dvořáková et al., 2016; Knotková et al., 2009; Vanden Hole et al., 2014; Schleich et al. 2007). This communication channel is used mainly for relatively short distances, but it is not suitable for exchanging information between different burrow systems as the acoustic waves are quickly attenuated by the soil (Francescoli, 2000).

For long-distance communication, the solitarily living subterranean species use seismic signals (Burda et al., 1990; Francescoli, 2000; Schleich et al., 2007). Among different rodent species, the vibrations are produced by several means: African mole-rats use drumming with the hind feet against the ground or chests-beating (Jarvis and Bennet, 1991; Narrins et al., 1992; Bednářová et al., 2013), whereas spalacids use striking the head against the ceiling of the tunnel (Heth et al., 1978, Hrouzková et al., 2013,

Hrouzková et al., 2018). The detection of seismic signals in subterranean rodents is rather understudied, but spalacids, for example, have been reported to exhibit the so called “jaw listening” behavior, which is holding the cheek and lower jaw in a close contact with the burrow wall (Mason et al., 2010; Rado et al., 1989). The vibrations collected by the lower jaw are conducted to the bulla and processed by the auditory system (Mason et al., 2010; Rado et al., 1989).

### **Underground acoustics**

As mentioned above, the acoustic channel is one of the few available ways of gaining information about the surroundings, and hearing and vocalization thus play a major role in intraspecific communication in mammals with a strictly subterranean activity (reviewed in Begall et al., 2007). However, the acoustic conditions in subterranean burrows are quite special.

Airborne sounds spread only over several meters, attenuated or refracted in the subterranean tunnels (Heth et al., 1986; Lange et al., 2007). Communication in subterranean burrows is exacerbated also by a high level of background noise, impairing the ability to recognize true signals (Heth et al., 1986). Experiments carried out in the burrows of African mole-rats (*Fukomys mechowii* and *F. kafuensis*) and blind mole rats showed that signals of lower frequencies are transmitted better than the high frequency ones, with the best spread at frequencies between 200 and 800 Hz (Lange et al., 2007; Heth et al., 1986). Signals of these frequencies are not only the least attenuated, but they can even be also amplified in some cases (Lange et al., 2007). This so-called “stethoscope effect” works best with sounds at 440 Hz; the acoustic pressure at a distance of 1 m from the source can be more than twice as strong as the original (Lange et al., 2007). Similar results were also obtained from experiments carried out in burrows of the fossorial rodent tuco-tuco (*Ctenomys talarum*) (Schleich and Antenucci,

2009). Thus, low frequency signaling seems to be the best form of vocal communication in subterranean ecotope.

### **Hearing abilities of subterranean and fossorial rodents**

Studies focused on hearing biology and vocalization have revealed that mammals living underground cope well with the acoustic conditions in their burrow systems. Subterranean rodents shifted their vocalization to low frequencies 0.5 – 5 kHz to correspond with optimal signal spreading in burrows (Bednářová et al., 2013; Credner et al., 1997; Dvořáková et al., 2016; Knotková et al., 2009; Schleich and Busch, 2002; Vanden Hole et al., 2014; Veitl et al., 2000), as well as the best range for hearing (Brückmann and Burda, 1997; Gerhardt et al., 2017; Heffner and Heffner, 1990; 1992; 1993). They also increased the lowest hearing threshold to a sound pressure of 24-35 dB (Heffner and Heffner, 1992, 1993). Individual behavioral audiograms of subterranean rodents show the hearing range at 60 dB from 0.1 to 12.8 kHz (Gerhardt et al., 2017; Heffner and Heffner, 1990; 1992). It should be mentioned that the hearing range of terrestrial species is much wider, usually exceeding 50 kHz with the threshold at a lower sound pressure (Table 1).

Table 1: Hearing abilities of rodents with subterranean, fossorial and surface activity.

<b>Species</b>	<b>Family</b>	<b>Lifestyle</b>	<b>Hearing range 60 dB (kHz)</b>	<b>Best hearing fr. (kHz)</b>	<b>Lowest treshold (dB)</b>
<i>Heterocephalus glaber</i> <sup>1</sup>	Bathyergidae	subterranean	0.065 – 12,8	4	35
<i>Fukomys anelli</i> <sup>2</sup>	Bathyergidae	subterranean	0.10 – 3.9	1	29
<i>Fukomys micklei</i> <sup>2</sup>	Bathyergidae	subterranean	0.13 – 4.4	1	37
<i>Fukomys mechowii</i> <sup>2</sup>	Bathyergidae	subterranean	0.125 – 6	1 – 1.4	33
<i>Geomys bursarius</i> <sup>3</sup>	Geomyidae	subterranean	0.35 – 8.7	2	24
<i>Spalax ehrenbergi</i> <sup>4</sup>	Spalacidae	subterranean	0.054 – 5.9	1	32
<i>Spalacopus cyanus</i> <sup>5</sup>	Octodontidae	fossorial	0.25 - 20	1.2 – 1.6	7
<i>Cynomys leucurus</i> <sup>6</sup>	Sciuridae	fossorial	0.044 – 26	0.5 – 8	21
<i>Cynomys ludovicianus</i> <sup>6</sup>	Sciuridae	fossorial	0,029 – 26	0.5 – 4	20
<i>Marmota monax</i> <sup>7</sup>	Sciuridae	fossorial	0.040 – 27.5	4 – 8	20
<i>Tamias striatus</i> <sup>7</sup>	Sciuridae	fossorial	0.039 – 52	1 and 8 – 16	16
<i>Phyllotis darwini</i> <sup>7</sup>	Cricetidae	terrestrial	1.055 – 73.5	11	- 4
<i>Neotoma florideana</i> <sup>8</sup>	Cricetidae	terrestrial	0.94 – 56	8	1
<i>Sigmodon hispidus</i> <sup>9</sup>	Cricetidae	terrestrial	1 – 72	8	- 6
<i>Acomys cahirinus</i> <sup>7</sup>	Muridae	terrestrial	2.3 – 71	8	14
<i>Mus musculus</i> <sup>9</sup>	Muridae	terrestrial	2.3 – 92	16	- 10
<i>Rattus norvegicus</i> <sup>10</sup>	Muridae	terrestrial	0.4 – 76	8 and 32	- 10

References: 1 – Heffner and Heffner, 1993, 2 – Gerhardt et al., 2017, 3 – Heffner and Heffner, 1990, 4 – Heffner and Heffner, 1992, 5 – Begall et al., 2004, 6 – Heffner et al., 1994a, 7 – Heffner et al., 2001, 8 – Heffner and Heffner, 1985, 9 – Heffner and Masterton, 1980; 10 – Heffner et al., 1994b

Together with losing the ability to hear high frequencies, subterranean rodents lost also the ability of sound localization, as was demonstrated in the pocket gopher and blind mole-rat (Heffner and Heffner, 1990, 1992). In early 90's, there was a discussion whether the inability to localize the sound, restricted high frequency hearing, and low sensitivity of hearing in subterranean mammals are cases of progressive adaptation, or regression and degeneration. It was suggested that the incapability of hearing certain frequencies in subterranean mammals can be caused by the lack of stimulation, similarly to the degeneration of vision in mammals living in darkness, or the olfactory sense in cetaceans (Heffner and Heffner, 1990 vs. Burda et al., 1992). At the present, the restricted hearing in subterranean mammals is considered more as a convergent adaptation for special sensory environment than degeneration only (Lange et al., 2007).

With regards to hearing, fossorial species are facing a dichotomic situation. On the one hand they need to cope with the acoustics underground, while on the other hand they need sensitive hearing and particularly the sound localization to be safe from predators when staying aboveground for example during foraging forays. As a result, hearing in the fossorial species is an intermediate between subterranean and aboveground species (Heffner et al., 1994). As can be seen in the Table 1, this includes hearing range, best frequency of hearing, as well as sensitivity.

## **Mammalian ear morphology**

### Outer ear

The hearing apparatus in mammals is usually divided into three sections, the outer, middle and inner ear. The mammalian outer (or external) ear consists of the auricle and the auditory meatus and its auditory function is to collect the air vibrations and couple it to the middle ear. The auricle (or

pinna) is a specific body structure of mammals and it shows remarkable diversity in the shape from complete absence in cetaceans to huge ears in proboscideans (Webster, 1966). Apart from collecting the sound and localizing its source in the space, it serves for thermoregulation in many mammalian species (Rosowski, 1994; Webster, 1966). The auditory meatus (or external ear canal), has also its non-auditory function. Besides the coupling of sound and increasing the level of sound pressure, it protects the middle ear from injuries and infections by the arrangement of hairs and the production of cerumen (Johnson et al., 2001).

### Middle ear

The middle ear carries airborne sound waves from the external ear to the fluid-filled cochlea (Rosowski and Relkin, 2001). Sound energy is collected by the eardrum (or tympanic membrane), which is a thin membrane composed of three layers, bulged inwards with the deepest part termed umbo (Henson, 1974; Rosowski, 1994). In most mammals, the eardrum has two components: the stiffer pars tensa, directly coupled with the ossicular chain, and pars flaccida (or Shrapnell's membrane), which is the thinner and more relaxed part serving probably to maintain equal static pressure on both sides of the eardrum (Rosowski, 1994).

The middle ear of mammals, in contrast with other vertebrates, includes three hearing ossicles, articulated with joints and anchored by two muscles and a few ligaments (Henson, 1974; Rosowski, 1994). The ossicular chain is composed of the malleus ("hammer"), incus ("anvil") and stapes ("stirrup") (Fig. 1), starting with the manubrium of malleus on the eardrum and ending with the stapedial footplate in the oval window of the inner ear (Kaestner, 1995). All ossicles possess central parts called heads and a few processes (or crura) serving as a connection between them.



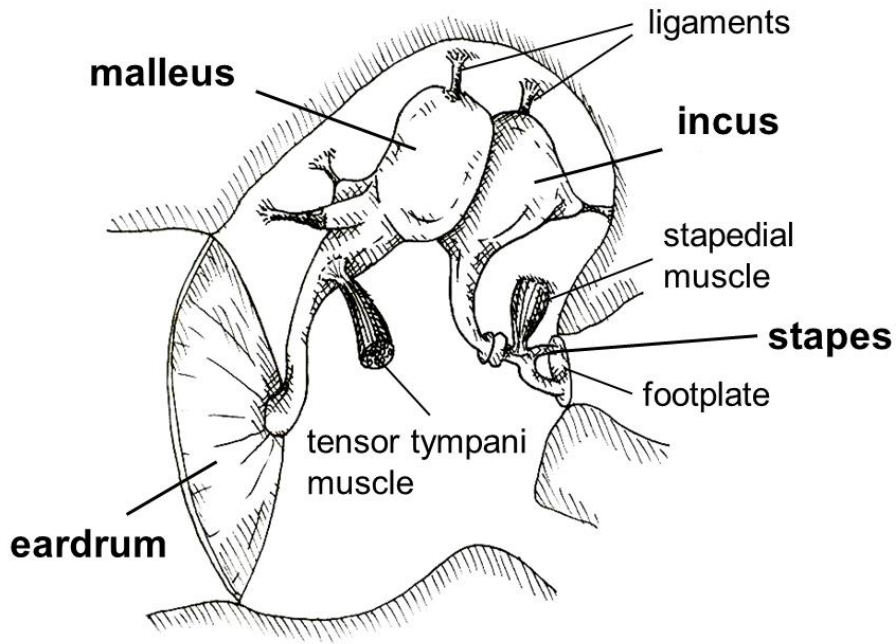


Figure 1. Mammalian middle ear.

The malleus is the biggest hearing ossicle, consisting of two main parts, the head and the manubrium, connected by a relatively long and curved neck. The manubrium is coupled with the eardrum along its whole length, from its tip forming the umbo to the lateral process of malleus (Henson, 1974). The head is connected via the anterior process to the tympanic bone and via the articular facet to the head of the incus. The neck includes the muscular process for the insertion of the tensor tympani muscle (Henson, 1974).

The incus is the middle ossicle of the chain with a simpler constitution, as it includes only the head and two processes. The head is articulated to the malleus as described above. The short process (crus breve) anchors the ossicle to the bullar wall, whereas the long process (crus longum) articulates with the stapes by the lenticular apophysis (Henson, 1974).

The stapes is the last and the thinnest bone of the ossicular chain, typically of a triangular shape. It consists of a small head articulated with the incus, two crura (anterior and posterior) and the footplate fitting into the oval window of the inner ear. The crura are forming a foramen for the stapedial artery and the posterior houses a muscular process for insertion of the stapedial muscle (Henson, 1974).

Different types of middle ear arrangement were described based on the shape, position and attachment of hearing ossicles to the bullar wall (Fleischer, 1978). In rodents, we can find two of them – “microtype” and “freely mobile” (Fig. 2), although Mason (2013) described an additional “Ctenohystrica” type. The microtype is similar to the ancestral type of ear, as it is characterized by a firm attachment of the malleus to the tympanic bone (Fleischer, 1978). The malleus in this type of ear is more complex, with an orbicular apophysis and a well-developed transversal lamina. It is present in small forms of rodents and it is associated with the hearing of higher frequencies (Fleischer, 1978; Mason, 2013). In contrast, the freely mobile type of ear has robust ossicles loosely connected mainly by ligaments and it is considered an adaptation to low frequency hearing (Fleischer, 1978, Mason, 2013).

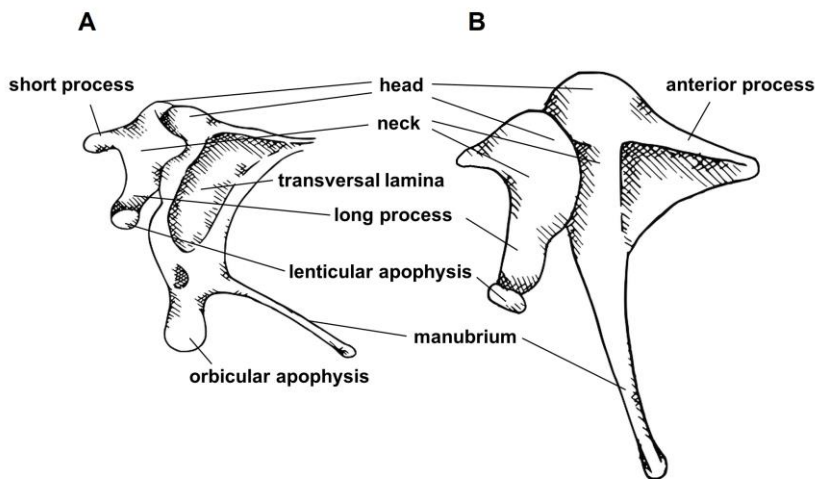


Figure 2. Microtype (A) and freely-mobile (B) type of the middle ear.

As mentioned above, the middle ear serves as the device for matching the difference in impedance between the air and the fluid inside the inner ear (Møller, 1974). Almost all sound energy would be reflected at the interface of the two media without the middle ear apparatus (Webster, 1966). This challenge is accomplished by the ossicular levers and a difference in area size between the eardrum and the stapedial footplate (Henson, 1974; Webster and Webster, 1975).

### Inner ear

The inner ear (or labyrinth) is a system of canals and chambers, divided into the vestibular and auditory section (Echteler et al., 1994). The auditory part is housed in the cochlea, a fluid-filled spiral coiling around the central axis formed by the modiolus, a bony tube that includes the auditory nerves (Slepecky, 1996).

The cochlea consists of two layers, the outer bony and the inner membranous, which is longitudinally divided into three compartments called scalas (Ades and Engström, 1974). The middle one, scala media (or cochlear duct) is filled with endolymph, the side scalas (scala vestibuli and scala tympani) are filled with perilymph (Ades and Engström, 1974). The scala tympani and scala vestibuli are interconnected by a helicotrema at the apical tip of the cochlea. At the base of cochlea, the scala tympani ends with the round window covered by a thin membrane, whereas the scala vestibuli ends with the oval window with an inserted stapedial footplate (Slepecky, 1996). The cochlear duct is separated by a bony spiral lamina with the basilar membrane and by Reissner's membrane (Slepecky, 1996).

The basilar membrane carries the organ of Corti, which is the main sensory structure (Echteler et al., 1994). The organ of Corti is a ridge of sensory hair cells surrounded by supporting cells and covered by an acellular tectorial membrane (Santi and Tsuprun, 2001). The sensory cells with stereocilia are sorted to one row of inner and three rows of outer hair cells separated by the tunnel of Corti (Slepecky, 1996). Both types of hair cells differ not only in their arrangement, but also in their innervation and

function. The inner hair cells are innervated almost exclusively by afferent neurons transmitting the signal to the brain, whereas in the outer hair cells the efferent innervation from the brain prevails (Dallos, 1996). This means that inner hair cells are receptors, while the outer hair cells serve as effector cells actively enhancing the vibrations of the basilar membrane (Dallos, 1996, Brown, 2001).

The function of cochlea is basically to analyze the sound in terms of its intensity, timing, and frequency content. Movement of the stapedial footplate produces pressure fluctuations in the scala vestibuli and scala tympani, resulting in the displacement of the cochlear duct with the sensory cells (Echteler et al., 1994). The cochlea is tonotopically organized along its spiral, which means that the maximum displacement of specific locations along the cochlear duct is determined by the frequency of the acoustic stimuli – high frequencies cause maximum displacement near the base and low frequencies at the apical locations (Békésy, 1960). This feature of displacement is caused by the gradient of width and stiffness of basilar membrane and it results in the characteristic place-frequency map of the basilar membrane (Echteler et al., 1994).

### **Ear morphology in subterranean and fossorial mammals**

The low sensitivity of hearing and low-frequency specialization are reflected in the ear morphology of particular species. Interestingly, there are many features shared in the ear morphology of subterranean mammals and other low-frequency specialized mammals from different groups, such as elephants or humans (Fleischer, 1978; Burda et al., 1992; Mason, 2001).

The outer ear of subterranean species usually consists only of the external ear canal, as the pinna is reduced or even missing (Nevo, 1979). The meatus is narrow compared to epigeic species and it is often filled with cerumen, which is expected to contribute to poor hearing sensitivity (Burda et al., 1992; Begall et al., 2007). It was assumed that the lack of the pinna

cope with the burrowing style of life, as a prevention of injuries caused by abrasion or shoveling the loosened soil to the ear canal, but there are many burrowing fossorial species still possessing pinnae, such as voles, tuco-tucos or root-rats (Begall et al., 2007; Lange et al., 2004). The loss of the pinna results (together with the limited hearing of high-frequency sounds) in a rudimentary ability of sound localization (Heffner and Heffner, 1990; 1992). The sound localization is redundant in subterranean burrows, but it is necessary for their orientation on the surface. Hence, the pinnae in fossorial species are still preserved, although they are smaller relative to body size than in their counterparts living aboveground (Lange et al., 2004; Schleich and Busch, 2004).

The relative size of bullae was assumed to be larger in subterranean mammals compared to aboveground dwellers (Begall et al., 2007; Lange, 2005), as it is in congruence with a good low frequency hearing (Plassmann and Kadel, 1991; Mason, 2016b). Enlarged bullae were observed in fossorial species in Caviomorphs (Begall and Burda, 2006; Schleich and Vassallo, 2003), but they are not particularly large in subterranean mammals generally, as there are exceptions even within Ctenomyidae (Francescoli, 1999; Mason, 2004). The largest bullae among mammals are found in desert species, where this phenomenon is also connected with good low frequency hearing, as it is advantageous for communication in open habitats of an arid environment (Basso et al., 2020; Lay, 1972; Mason, 2016a; Webster, 1962), yet some of the desert species also build large burrows and can thus be labeled as fossorial, such as the Mongolian gerbil (*Meriones unguiculatus*) (Scheibler et al., 2006).

The middle ear of subterranean mammals is characterized by a relatively large eardrum without pars flaccida and by freely-mobile hearing ossicles (Burda et al., 1992; Burda, 2006; Lange et al., 2004). Hearing ossicles are more massive than in species with an aboveground style of life – especially the malleus is clearly distinguishable due to reduction of its anterior process and the position of manubrium roughly parallel with the long process of the incus (Burda et al., 1992; Lange et al., 2004; Mason,

2013). The difference in lengths of malleal and incudial levers (distance of the tips of the manubrium and incudial long process from the rotatory axis) is smaller in subterranean species, which decrease the sound transmission efficiency of the middle ear. A similar function can be attributed to the enlarged stapedial footplate, resulting in a lower ratio to the eardrum area (Burda et al., 1992; Mason, 2001).

Regarding fossorial species, they share middle ear traits with subterranean species, yet, there are some differences related to their surface activity. The functional traits such as the ratios between the eardrum area and stapedial footplate area and those between malleal and incudial levers lie in between the values found in subterranean and epigeic species (Begall and Burda, 2006; Lange et al., 2004).

The auditory part of the inner ear of subterranean mammals also possesses several typical features. The cochlea is higher, more coiled, and it includes a relatively longer basilar membrane compared with species not specialized for staying in burrows (reviewed in Begall et al., 2007), except Caviomorphs, where the more coiled cochlea is present in all species (Begall and Burda, 2006; Pye, 1977; Teudt and Richter, 2007). The width of the basilar membrane and the “triad” (three rows of outer hair cells) in subterranean and fossorial rodents usually increases from the base to the apex as in other mammals (Burda et al., 1988; Lange, 2005; Schleich et al., 2006), but the slope in the apical part is very low in several mole-rats and in the blind mole rat (Bruns et al., 1988; Lange, 2005; Müller et al., 1992). The density of hair cells is higher in the apical part than at the base and the ratio between outer and inner hair cells is higher compared to aboveground rodents (Lange et al., 2004; Begall and Burda, 2006; Schleich et al., 2006).

As mentioned above, the cochlea is tonotopically organized. In most mammals for which tonotopy maps are available, each octave of the hearing range occupies a section of comparable length on the basilar membrane (Lieberman, 1982; Müller, 1996; Ou et al., 2000). In highly

specialized species, the segment of the octave of the best hearing is overrepresented and such expansion can achieve up to one half of the whole cochlear length (Bruns et al., 1988; Vater et al., 1985; Müller et al., 1992). This feature has been called the “acoustic fovea” and it was described both in high frequency specialists as bats (Bruns and Schmieszek, 1980; Russell and Kössl, 1999; Vater et al., 1985) and in low frequency specialists such as Ansell’s mole-rat *Fukomys anseli* (Müller et al., 1992). This fovea can be easily recognizable by its morphology due to the constant width of the basilar membrane and the pattern of hair cells (Bruns et al., 1988; Müller et al., 1992).

### **Specialization of ear morphology in subterranean mammals**

Finding traits which are adaptive to the environment can be tricky, since every pattern has many possible interpretations, as there are only observational data available, rather than controlled experiments (Hansen, 2014). For an evaluation of adaptations, phylogenetically closely related species from a different environment should be compared, as related species tend to resemble each other more than any random species from the phylogenetic tree (Blomberg and Garland, 2002; Harvey and Pagel, 1991). Because the adaptation evolves in response to a selective pressure, it must be a derived character (Harvey and Pagel, 1991).

The assessment of ear morphology adaptations for the subterranean life is complicated in particular groups, as it is dependent on the ancestral state. For example, the freely mobile type of the middle ear can be evaluated as an adaptive trait in talpids where the microtype is ancestral and the freely mobile type is derived (Mason, 2006), whereas in rodents, freely mobile type of ear is probably the ancestral state and the microtype has evolved only in several groups (Argyle and Mason, 2008). The authors comparing the functional morphology of the middle ear were of course aware of this fact, either by simple comparing the groups as Insectivora or Rodentia separately (Burda et al., 1992) or comparing a very large sample

of species distributed evenly across different mammalian groups (Mason, 2001). However, the ear morphology of different main rodent clades differs from each other, as was shown on a squirrel-related clade (Pfaff et al., 2015; Potapova, 2019) or on *Ctenohystrica* (Mason, 2013).

Argyle and Mason (2008) compared several fossorial and aboveground Caviomorphs and found their ear morphology very similar, including traits considered previously as subterranean specializations, such as a large eardrum without pars flaccida, or reduced or missing middle ear muscles (Burda et al., 1992; Mason, 2001; Schleich and Busch, 2004). In that study, fossorial rodents as a group were not recognized, resulting in their assignment to either aboveground or subterranean species, which made the difference between both groups less apparent, as the ear dimensions of fossorial species are intermediate between subterranean and epigeic. However, Argyle and Mason (2008) demonstrated that using the laboratory rat as a “non-specialized” rodent is inappropriate for the *Ctenohystrica* group. Moreover, using mice and rats as the “non-specialized” species is questionable also due to its ability to hear very high frequencies (Table 1).

However, as the colonization of belowground environment occurred independently in different lineages of rodents (Burda, 2006; Nevo 1999) and all the lineages evolved good low frequency hearing (Table 1), shared morphological traits in the ear supporting the low frequency hearing could be considered as an adaptation. For a deeper evaluation of the adaptive traits, it is necessary to analyze closely related species with different lifestyles, a situation we can find in the rodent family Spalacidae.

### **Muroid mole rats (Spalacidae)**

The family Spalacidae consists of small to medium sized solitary rodents living in self constructed underground burrows (Norris, 2017). Spalacidae



are part of the Muroidea superfamily, which separated in the Oligocene in Central Asia and rapidly diversified into three lineages, the subfamilies Rhizomyinae, Spalacinae and Myospalacinae (He et al., 2020; Norris, 2017). All these subfamilies consist of subterranean or fossorial species. However, each lineage entered the subterranean ecotope separately, which is proven by fossil evidence of epigeic representatives of each lineage – *Prokanisamys* and *Kanisamys* for Rhizomyinae, *Debruijnina* and *Heramys* for Spalacinae, and *Plesiodipus* and *Gobicricetodon* for Myospalacinae (Flynn, 1990, 2009; He et al., 2020; Sen and Sarica, 2011). This was probably the reason why these subfamilies had been considered as three phylogenetically distant murid subfamilies until the application of molecular methods (c.f. Norris, 2017).

Rhizomyinae arose in Central Asia and migrated repeatedly into Africa (López-Antoñanzas et al., 2013). They separated into two extant tribes: bamboo-rats (Rhizomyini), represented by four species inhabiting Southeast Asia, and African root-rats (Tachyoricini) in two species (Norris, 2017). However, the situation within the Tachyoricini is more complicated, as it turns out that there are six clades within the genus *Tachyoryctes* (Šumbera et al., 2018). This subfamily contains the most epigeic species among spalacids, as the bamboo-rats are often observed aboveground in bamboo forests (Smith and Xie, 2008, Norris, 2017) and the giant root-rat (*Tachyoryctes macrocephalus*) spends a significant part of the day feeding aboveground in the Ethiopian grasslands (Vlasatá et al. 2017).

Zokors (Myospalacinae) originated in northern China and dispersed to the East Asia, Tibetan plateau and Siberia to the present time (He et al., 2020; Norris, 2017). Living zokors include eleven species in two genera, *Eospalax* and *Myospalax* (Norris, 2017). Zokors are subterranean species with rare occurrence aboveground (Norris, 2017). Their habitat is characterized by a soft and moist soil of the steppes and alpine meadows of the Tibetan plateau (He et al., 2020; Norris, 2017). Apart from the rest of spalacids, zokors dig using their forelimbs with their characteristic large

claws (Norris, 2017). Myospalacinae were formerly considered as cricetids (Nevo, 1999). Interestingly, this was supported also by molecular methods because of mislabeling of a tissue from hamster as “*Myospalax* sp.”. However, later molecular studies classified zokors as Spalacids (Norris et al., 2004, 2017).

Blind mole rats (Spalacinae) originated in south-eastern Europe and the two living genera (*Spalax* and *Nannospalax*) are spread in south-eastern Europe, northern Africa, western Asia and in the Middle East (Norris, 2017). Blind mole rats are living in the sealed tunnels and inhabit dryer habitats than other spalacids, as they occur also in arid zones (Norris, 2017). As their name implies, they differ from all other rodents by having their eyes covered by skin. They are the group most specialized for living underground (Nevo, 1999; Norris, 2017).

### **The aims of this thesis**

While blind mole rats have been studied for several decades and we have a comprehensive view of many aspects of their biology, little attention has been paid to the others.

The ecology of the Chinese bamboo-rat (*Rhizomys sinensis*) has never been studied in the wild. Because some morphological traits can provide us with information on the species' ecology, I tried to estimate to what extent the Chinese bamboo-rat qualifies as a subterranean species, considering the morpho-functional aspects of its ear (Chapter I).

As the level of the subterranean specialization in the lifestyle is more of a continuum than distinctive categories, there was a question if the ear morphology can reflect a small shift within this specialization. Therefore, I studied the ear morphology of the Gansu zokor (*Eospalax cansus*) which has its eyes exposed and thus is less adapted to the subterranean ecotope than the blind mole rat. But at the same time, it shows

more subterranean specializations than Chinese bamboo-rat, such as relatively small eyes or the absence of auricles (Chapter II).

The main problem of many existing studies of ear morphology in subterranean rodents is comparison with inappropriate groups, which may lead to incorrect conclusions. To distinguish between the effect of ecology and phylogeny, only the most closely related species with distinctly different levels of subterranean specialization should be compared. The genus *Tachyoryctes* offers two remarkable forms with a different amount of aboveground activity – the almost strictly subterranean African root-rat and the giant root-rat, active aboveground on an everyday basis (Chapter III).

The comparison of these spalacids between each other, together with their comparison with other subterranean, fossorial and epigeic species from different families can show the distinctive middle and inner ear specializations for the subterranean environment in different genera. We can also estimate to what extent the ear morphology specialization correlates with the amount of time spent underground by each species.

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# Chapter I

## **Does the Morphology of the Ear of the Chinese Bamboo Rat (*Rhizomys sinensis*) Show “Subterranean” Characteristics?**

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# **Does the Morphology of the Ear of the Chinese Bamboo Rat (*Rhizomys sinensis*) Show “Subterranean” Characteristics?**

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## Abstract

In spite of the growing interest in rodents with subterranean activity in general and the spalacids (Spalacidae) in particular, little is known about the biology of most members of this clade, such as the Chinese bamboo rat (*Rhizomys sinensis*). Here, we analyzed the ear morphology of *R. sinensis* with respect to hearing specialization for subterranean or aboveground modes of communication. It is well-known that ecology and style of life of a particular species can be reflected in morphology of its ear, its hearing and vocalization, so we expect that such information could provide us insight into its style of life and its sensory environment. The ratio between the eardrum and stapedial footplate areas, which influences the efficiency of middle ear sound transmission, suggests low hearing sensitivity, as is typical for subterranean species. The cochlea had 3.25 coils and resembled species with good low frequency hearing typical for subterranean mammals. The length of the basilar membrane was  $18.9 \pm 0.8$  mm and its width slowly increased towards the cochlear apex from 60 to 85  $\mu\text{m}$ . The mean density of outer hair cells was  $344 \pm 22$  and of inner hair cells  $114 \pm 7.3$  per 1 mm length of the organ of Corti, and increased apically. These values (except for relatively low hair cell density) usually characterize ears specialized for low frequency hearing. There was no evidence for an acoustic fovea. Apart of low hair cell density which is common in aboveground animals, this species has also relatively large auricles, suggesting the importance of sound localization during surface activity. The ear of the Chinese bamboo rat thus contains features typical for both aboveground and subterranean mammals and suggests that this spalacid has fossorial habits combined with regular aboveground activity.

**Keywords:** ear morphology, hearing, fossorial mammals, Spalacidae

## Introduction

Although living underground provides safety from predators and climatic fluctuations, it is challenging and demanding in terms of locomotion, respiration, thermoregulation, sensory perception and communication (reviewed in Burda et al., 1990b; Nevo, 1999; Begall et al., 2007a). Acquiring sensory information in general as well as sending communication signals via optical and chemical channels is impeded in the sealed, dark burrows with restricted air currents (Nevo, 1999). Similarly, acoustic communication is also limited, because airborne signals are transmitted only for short distances (Heth et al., 1986; Narins et al., 1992). Only sounds with an intensity of more than 100 dB could be distinguishable from background noise at distances above 5 m (Heth et al., 1986). In addition, localization of the sound source is almost useless in underground tunnels, because sound is mostly coming only from the front or back (Burda et al., 1990a,b; Heffner and Heffner, 1990, 1992). Studies on the acoustics of natural burrows of the blind mole rat (*Spalax ehrenbergi*) (Heth et al., 1986), the giant mole-rat (*Fukomys mechowii*) and the Kafue mole-rat (*F. kafuensis*) (Lange et al., 2007) showed that low-frequency sounds of about 440 Hz are transmitted best. Noteworthy, the low frequency sounds are not only the least attenuated, but they may even be amplified due to the so called stethoscope effect (Lange et al., 2007).

At least two types of mammals differing in the level of specializations to the subterranean ecotope can be encountered underground: subterranean and fossorial mammals. In this text, we use “subterranean” for burrowing mammals living and foraging below ground, whereas the term “fossorial” (meaning burrowing) denotes mammals that construct large burrow systems, but forage mainly on the surface (Nevo, 1999; Begall et al., 2007b). Although both terms are sometimes used as synonyms in the literature, for the purpose of this study, it is important to distinguish between both groups in order to reflect the level of specialization to the subterranean ecotope.

Hearing of subterranean mammals is usually tuned to lower frequencies with the best hearing sensitivity occurring between 0.5 and 1 kHz. High-frequency hearing is restricted and the overall hearing sensitivity is reduced (Aitkin et al., 1982; Bruns et al., 1988; Müller and Burda, 1989; Heffner and Heffner, 1990, 1992, 1993; Brückmann and Burda, 1997). Low hearing sensitivity in these mammals has been considered as degeneration (Heffner and Heffner, 1990, 1993) or alternatively an adaptation to avoid overstimulation due to the stethoscope effect and congestion by background noise (Burda, 2006). Because of regular surface activity, hearing in fossorial species is expected to be adapted to the aboveground as well as the underground sensory environment. Thus, their hearing can be considered less specialized for the belowground environment than in subterranean species (Heffner and Heffner, 1992, 1993; Heffner et al., 1994; Wilkins et al., 1999; Lange et al., 2004). In burrowing low frequency-hearing species, remarkable convergent similarities in the ear morphology of different mammalian taxa have been described (Müller et al., 1989; Burda et al., 1992; Mason, 2001; Lange et al., 2004).

Sensitivity of low frequency hearing in small mammals can be enhanced by an enlarged bulla (Webster and Webster, 1975; Fleischer, 1978; Schleich and Vasallo, 2003). Some mammal species possess bullae divided by bony septas, which are reshaping the bullar wall and thus changing the resonance (Fleicher, 1978). Compared to the aboveground dwelling hearing generalists, in some subterranean species the eardrum is rather large, almost round without a distinct pars flaccida and the pinna is reduced or even missing. Auditory ossicles are often heavier and more robust, and the stapedial footplate is relatively large (Müller et al., 1989; Burda et al., 1992; Lange and Burda, 2005; Burda, 2006), although these findings do not characterize all species with subterranean activity (Mason, 2001). The ratio between the areas of the eardrum and the stapedial footplate as well as the ratio between the malleolar and incudal lever arms are lower in subterranean and fossorial mammals than in related

aboveground dwellers (Burda et al., 1992; Mason, 2001). Different middle ear types were recognized in mammals based on shape, position and attachment of hearing ossicles to the bullar wall. In rodents, two main types were described, the “microtype” and “freely-mobile” middle ear (Fleischer, 1978). Recently, a special type of malleo-incudial complex in Ctenohystrica, similar to the “freely-mobile” type, was described (Mason, 2013). The microtype middle ear represents a high-frequency specialization, and is characterized by a malleus with an anterior process firmly attached to the tympanic bone, and an orbicular apophysis, which increases ossicular inertia and moves the ossicular center of mass closer to the manubrium (Fleischer, 1978; Lavender et al., 2011; Mason, 2013). In contrast, the freely mobile type of ear is considered as an adaptation to low-frequency hearing. It is characterized by a ligamentous connection to the skull, heavier ossicles, a relatively large incus and a manubrium roughly perpendicular to the presumed axis of rotation (based on anatomical considerations; Fleischer, 1978; Burda et al., 1992; Mason, 2001, 2013).

The inner ear in species with dominant subterranean activity is characterized by a rather coiled, tower-like cochlea, with a long and wide basilar membrane and a wide organ of Corti (Müller et al., 1989; Burda et al., 1989b; Begall and Burda, 2006; Schleich et al., 2006). The ratio between the numbers of outer and inner hair cells tends to be higher in species with low-frequency hearing (Müller et al., 1989; Schleich et al., 2006). Mammals specialized for hearing of a narrow frequency range may possess the so-called “acoustic fovea”, which means that part of the basilar membrane dedicated to registration of a certain frequency range is longer than expected. This overrepresentation is reflected also in the anatomy, where there is a plateau of high hair cell density, constant thickness and constant width of the basilar membrane or a constant width of the organ of Corti (Schuller and Pollak, 1979). Acoustic fovea was found also in the Ansell's mole-rat (*F. anseli*) (Müller et al., 1992), it shows high specialization for underground acoustic environment.

Members of the rodent family Spalacidae are of interest in terms of comparative and functional ear morphology and adaptations to belowground life, because this family includes highly specialized subterranean species, such as the genera *Spalax/Nannospalax* or *Myospalax/Eospalax*, the east African root rat (*Tachyoryctes splendens*) as well as less specialized species such as the fossorial genus *Rhizomys*, the giant root rat (*Tachyoryctes macrocephalus*), and the lesser bamboo rat (*Cannomys badius*; Nowak, 1991). The studies on the ear of subterranean spalacids such as the plateau zokor (*Eospalax fontanierii*), *S. ehrenbergi* and *T. splendens* show that their subterranean style of life is reflected in some morphological characteristics of the middle and inner ears (Burda et al., 1989a, 1990a; Burda, 2006; Mason et al., 2010). The studies of the ear in spalacids assumed to be active also aboveground are still missing. The analysis of an earlier description of the middle ear ossicles of *C. badius* (Doran, 1878) indicates that this species is less specialized for a belowground way of life than the greater mole rat (*Spalax microphthalmus*). Unfortunately, no measurements of the middle ear ossicles of *C. badius* were provided.

The Chinese bamboo rat is one of the three species of the genus *Rhizomys* (Musser and Carleton, 2005). This is a large solitary living rodent with weight 1 – 4 kg, body length of 216 – 380 mm and tail length of 50 – 96 mm (Smith and Xie, 2008). It usually occurs in tropical-subtropical zones with annual average temperature of 18 °C and precipitation over 1,100 mm. *Rhizomys sinensis* inhabits bamboo thickets, pine forest or evergreen broad-leaved forests at altitudes between 1,500 – 2,800 meters (He, 1984; Smith and Xie, 2008). The diet of bamboo rats of the genus *Rhizomys* includes shoots and roots of bamboo. In cultivated lands, they eat also sugar cane or tapioca (Nevo, 1999). The living habits and especially activity of *R. sinensis* are largely unknown. It was reported to live in sealed underground burrow systems with total length up to 45 m at a depth of about 20 – 30 cm, to forage underground and to leave burrow systems only during the short mating period (He, 1984; Nowak, 1991),

meaning that this species can be classified as subterranean. On the other hand, the Chinese bamboo rat was also reported to forage on the surface (Smith and Xie, 2008). Moreover, the closely related hoary bamboo rat (*Rhizomys pruinosus*) feeds principally on the surface and some *Rhizomys* species are even able to climb the bamboo, according to Medway (1978).

A particular way of life is usually well mirrored in the sensory biology of the respective species (e.g. Burda et al., 1990b; Peichl, 2005; Begall and Burda, 2006; Burda, 2006; Pfaff et al., 2015). This is especially the case for hearing and vocalization parameters, which may perfectly reflect the acoustic environment and thus the ecotope. Importantly, hearing parameters are reflected in the anatomy of the ear. Thus, morpho-functional aspects of the ear are considered to reflect adaptations to the species' environment (e.g. von Békésy, 1974; Webster and Webster, 1975; Burda et al., 1990b, 1992; Mason, 2001). The morpho-functional study of the ear is a useful approach especially in those cases when audiological and sensory ecological studies are difficult or impossible (Gleich et al., 2005; Coleman et al., 2010). A subterranean style of life can be deduced from hearing biology, as demonstrated in the convergent evolution of ear morphology (Burda et al., 1990b).

In this study, we analyzed the ear morphology of the Chinese bamboo rat (*Rhizomys sinensis*) with the intention to learn more about its hearing ecology and thus indirectly about the autecology of this little known species.

## **Materials and methods**

Five adult specimens, originating from Zhangjiajie, Hunan, China, were examined. Specimens were collected in 1982 and were deposited in the National Museum in Prague (NMP 95333 – 95337). According museum records, the heads were preserved by immersion in 10% formalin within



few minutes *post mortem*. Prior to immersion, the fixative was injected also into the jaw muscles, mouth and in the ear canals. The results presented here are based on the examination of the left ear from each specimen. Only four ears were used for the inner ear histological examination due to an improper preservation in one individual (R4).

The condylobasal length and the length of the pinna were measured by calipers with an accuracy of 0.1 mm. The jaw musculature of the skinned head was cut through and the lower jaw was removed. The skull base was cut from the foramen magnum to the palatinum and the bulla was extracted. Subsequently, the length of the meatus was measured. The extracted bulla was cleaned of soft tissues and its length, width and height were measured.

Afterwards, the bulla was dissected and examined under a binocular light microscope (Nikon SMZ 1500) using magnification of  $\times 20-40$ . A cross-section of the bony meatus was photographed and then the bulla was broken through its lateral wall, in the place where the bony meatus enters the bulla. After removing part of the bullar wall, the eardrum was exposed and carefully removed so that the ossicular chain remained in situ. The ossicles were photographed in situ and, after being extracted in toto, they were weighed using analytical scales (A&D GR-202).

Cross-sections of the bony meatus, eardrum, stapedial footplate and levers of ossicles were measured on photos taken by a digital camera (Nikon DMX 1200) and processed using GIMP 2 (the GNU Image Manipulation Program). The areas of the eardrum and the stapedial footplate were each calculated as the area of an ellipse. As lever arms, the shortest distances (perpendiculars) between tips of the longest ossicular process and the anatomical axis of the malleus and incus were taken (Lange et al., 2004). The area ratio was calculated as the eardrum area divided by the area of the stapedial footplate, while the lever ratio was

calculated as the length of the malleus lever arm divided by the length of the incus lever arm.

The stapes was removed from the oval window and the inner ear was dissected under magnification of  $\times 117.5$ . The bony shell on the cochlear apex was carefully broken and peeled off to expose the scala vestibuli. Toluidin blue was then dropped into the cochlea through the hole thus created. After staining, Reissner's membrane, the spiral ligament, and the tectorial membrane were better distinguishable and were subsequently removed, and the basilar membrane with the organ of Corti was exposed and stained by Ehrlich's hematoxylin (Burda et al., 1988). The basilar membrane was released from the lateral wall, extracted and placed as a surface specimen into a drop of anhydrous  $>99\%$  glycerin on a microscope slide (Lange et al., 2004). Fine watchmakers' tweezers and ophthalmological scalpels were used for the preparation.

The whole basilar membrane spiral was prepared turn by turn from the apex to the base and examined under a light microscope (Lambda DN45) under magnification of  $\times 400$ . The total length of the basilar membrane was measured and divided into ten segments of equal length. The basilar membrane width was taken from the inner edge of the tectorial membrane (spiral limb) to the rest of the spiral ligament at the outer side of the cochlea. The width of the organ of Corti and the "OHC triad" (i.e. radial width of cuticular plates of the three rows of outer hair cells, as observed on the reticular lamina) were then measured and the mean was calculated for each segment (Fig. 1). The part of the basilar membrane length occupied by ten hair cells of the inner and each outer row was measured and the hair cell density of all the three rows per 1 mm was calculated (Burda, 1985).

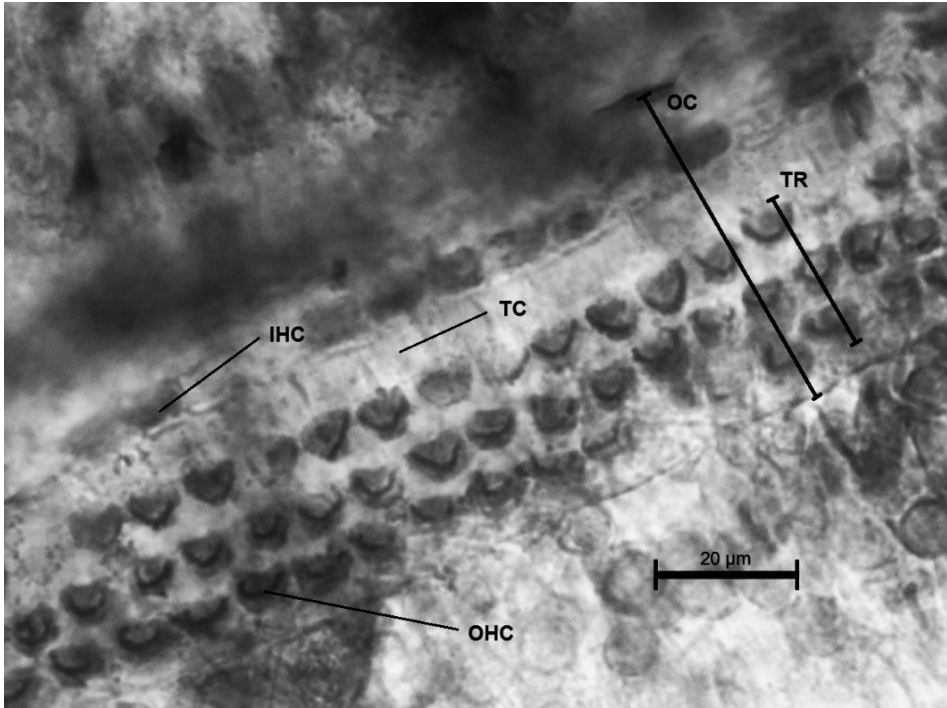


Fig. 1. *Rhizomys sinensis*, organ of Corti: IHC, inner hair cells; OHC, outer hair cells; TC, tunnel of Corti; TR, width of triad; OC, width of the organ of Corti.

## Results

The mean lengths of the pinna and the meatus of *R. sinensis* were  $16.5 \pm 1.5$  mm and  $18.7 \pm 0.9$  mm, respectively ( $n = 5$ ). The bony meatus entered the tympanic bulla at an acute angle from the superior direction. The bullar dimensions and middle ear parameters and some additional measurements are given in Table 1.

Table 1. Middle ear parameters and additional measurements in five *Rhizomys sinensis* individuals. ID – identification of specimen, CB – condylobasal length, P – pinna length, BL – bullar length, BW – bullar width, BH – bullar height, MCS – area of cross section of the bony meatus, M – length of the meatus, ED – area of the eardrum, SF – area of the stapedial footplate, ML - mallear lever, IL – incudial lever, MM – mallear mass, IM – incudial mass, SM – stapedial mass.

ID	CB	P	BL	BW	BH	MCS	M	ED	SF	ML	IL	MM	IM	SM
	(mm)	(mm)	(mm)	(mm)	(mm)	(mm <sup>2</sup> )	(mm)	(mm <sup>2</sup> )	(mm <sup>2</sup> )	(mm)	(mm)	(mg)	(mg)	(mg)
R1	79.9	19.2	15.3	11.7	6.8	4.9	19.1	18.45	0.93	3.54	1.75	3.5	2.4	0.5
R2	65.6	16.3	15.7	11.7	7.3	4.9	18.9	17.10	0.92	3.53	1.62	4.1	2.7	0.3
R3	64.2	15.9	15.4	11.9	7.5	4.5	18.9	16.96	0.90	3.69	1.61	4.0	2.7	0.4
R4	64.3	15.2	16.4	11.2	7.6	4.6	19.6	17.11	0.87	3.71	1.65	3.6	2.8	0.3
R5	69.4	15.9	15.7	11.1	7.3	4.2	17.0	16.33	0.96	3.84	2.02	3.1	2.6	0.3
Mean	70.2	16.2	15.7	11.5	7.3	4.6	18.7	17.19	0.92	3.66	1.73	3.7	2.6	0.4
	± 6.6	± 1.6	± 0.43	± 0.35	± 0.31	± 0.29	± 0.99	± 0.77	± 0.03	± 0.13	± 0.17	± 0.40	± 0.15	± 0.09

The tympanic membrane was nearly round, flat and without apparent pars flaccida. Its area was  $17.6 \pm 1.1 \text{ mm}^2$ . The middle ear ossicles (Fig. 2) were robust and loosely attached to the bullar wall by ligaments, which means this species has a freely mobile middle ear. The rigid manubrium had a triangular shape at the cross-section and was connected to the eardrum by a flat and relatively large surface. The malleus was attached to the bullar wall at its anterior process and at the posterior side of the proximal part of the manubrium. The tensor tympani muscle was present, but very thin. The malleus was firmly joined with the incus and the separation of both ossicles from each other was very difficult in some specimens.

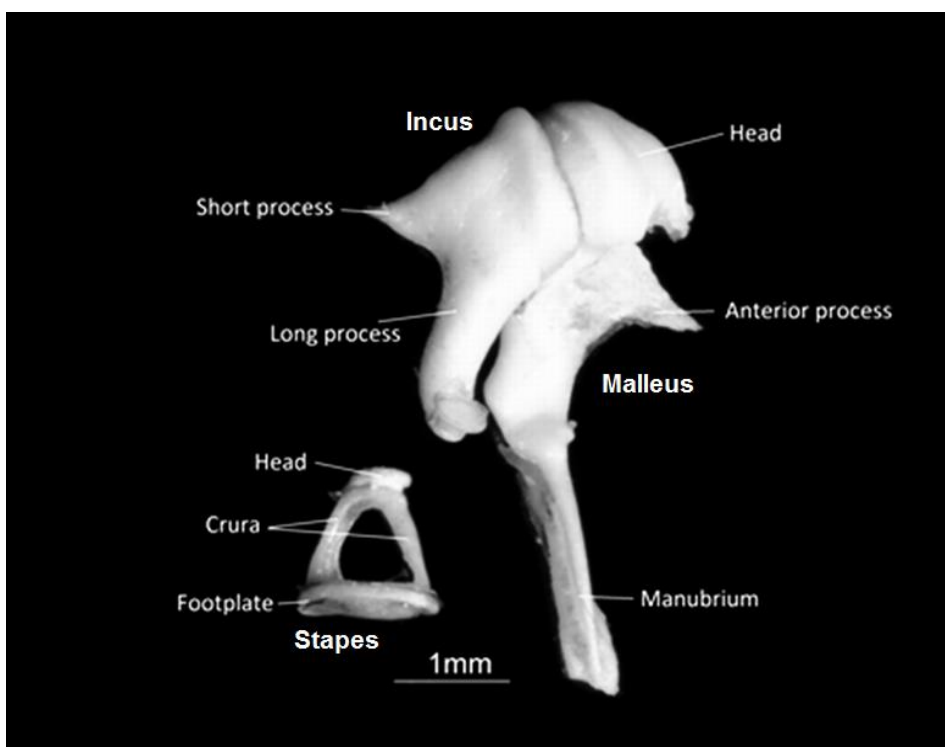


Fig. 2. *Rhizomys sinensis*, left middle ear ossicles. Malleus and incus are from the internal view, stapes from the ventral view.

The short process of the incus was attached to the bullar wall by short ligaments. The distal part of the long process was curved towards the lenticular process, thus the stapes was perpendicular to other parts of the ossicular chain. The stapes was attached by a distinguishable lenticular apophysis.

The stapes had a triangular shape with slightly asymmetric position of the head. The stapedia muscle was very fine and was attached to the shorter and thicker posterior crus. The stapedia artery was missing. The footplate was of a slightly asymmetrical oval shape and its area was  $0.92 \pm 0.03 \text{ mm}^2$ . The mean value of the lever ratio in *R. sinensis* was  $2.13 \pm 0.11$ ; the area ratio was  $18.79 \pm 0.82$ .

The cochlea had three and a quarter turns. The morphometric characteristics of the organ of Corti are given in Table 2. The length of the basilar membrane was  $18.9 \pm 0.8 \text{ mm}$  and the width increased from  $60.5 \mu\text{m}$  at the base to  $81.3 \mu\text{m}$  at the apex (Table 2, Fig. 3a). The OHC triad width increased from  $23.7 \pm 1.5 \mu\text{m}$  at the base to  $34.0 \pm 0.8 \mu\text{m}$  at 80 % of the length from the base (Fig. 3b). The width of the basilar membrane as well as the width of the OHC triad demonstrated similar trends, increasing linearly from the base to the apex, with a lower slope at 60 % of the length from the base. The hair cells were arranged in a geometrically regular pattern and their density increased from base to apex. Their mean density was  $114 \pm 7.3 \text{ cells per mm}$  for the inner hair cells (Fig. 3c), and  $344 \pm 22$  in the outer hair cells (Fig. 3d). At 60 % of the basilar membrane length from the base, the density of both hair cells types was slightly higher than was expected from the baso-apical trend. The ratio between the outer and inner hair cells slightly decreased from base to apex; there was a decrease of the ratio at around 60 % of the basilar membrane length (Fig. 4).

Table 2. Parameters of inner ear measured along basilar membrane (mean  $\pm$  SD): BM – basilar membrane, OHC triad – radial width of three rows of outer hair cells, IHC – inner hair cells, OHC – outer hair cells.

Distance from basis	10%	20%	30%	40%	50%	60%	70%	80%	90%
BM width	60.50	62.75	70.00	74.75	77.50	80.75	83.00	85.33	81.33
( $\mu\text{m}$ )	( $\pm 2.69$ )	( $\pm 4.38$ )	( $\pm 2.24$ )	( $\pm 2.59$ )	( $\pm 0.87$ )	( $\pm 2.17$ )	( $\pm 2.55$ )	( $\pm 2.05$ )	( $\pm 1.25$ )
OHC triad width	23.75	25.00	26.50	29.25	31.00	31.75	33.00	34.00	31.33
( $\mu\text{m}$ )	( $\pm 1.48$ )	( $\pm 2.12$ )	( $\pm 2.50$ )	( $\pm 3.77$ )	( $\pm 2.45$ )	( $\pm 2.17$ )	( $\pm 1.22$ )	( $\pm 0.82$ )	( $\pm 2.62$ )
IHC density	102.31	102.35	105.09	109.80	114.04	120.97	118.56	122.89	128.79
/mm	( $\pm 5.26$ )	( $\pm 5.53$ )	( $\pm 3.19$ )	( $\pm 4.73$ )	( $\pm 2.97$ )	( $\pm 3.84$ )	( $\pm 5.04$ )	( $\pm 7.34$ )	( $\pm 2.08$ )
OHC density	325.11	321.56	328.53	331.97	333.90	350.67	348.58	366.64	387.24
/mm	( $\pm 14.86$ )	( $\pm 2.04$ )	( $\pm 10.34$ )	( $\pm 10.17$ )	( $\pm 2.94$ )	( $\pm 7.76$ )	( $\pm 5.54$ )	( $\pm 13.37$ )	( $\pm 9.49$ )

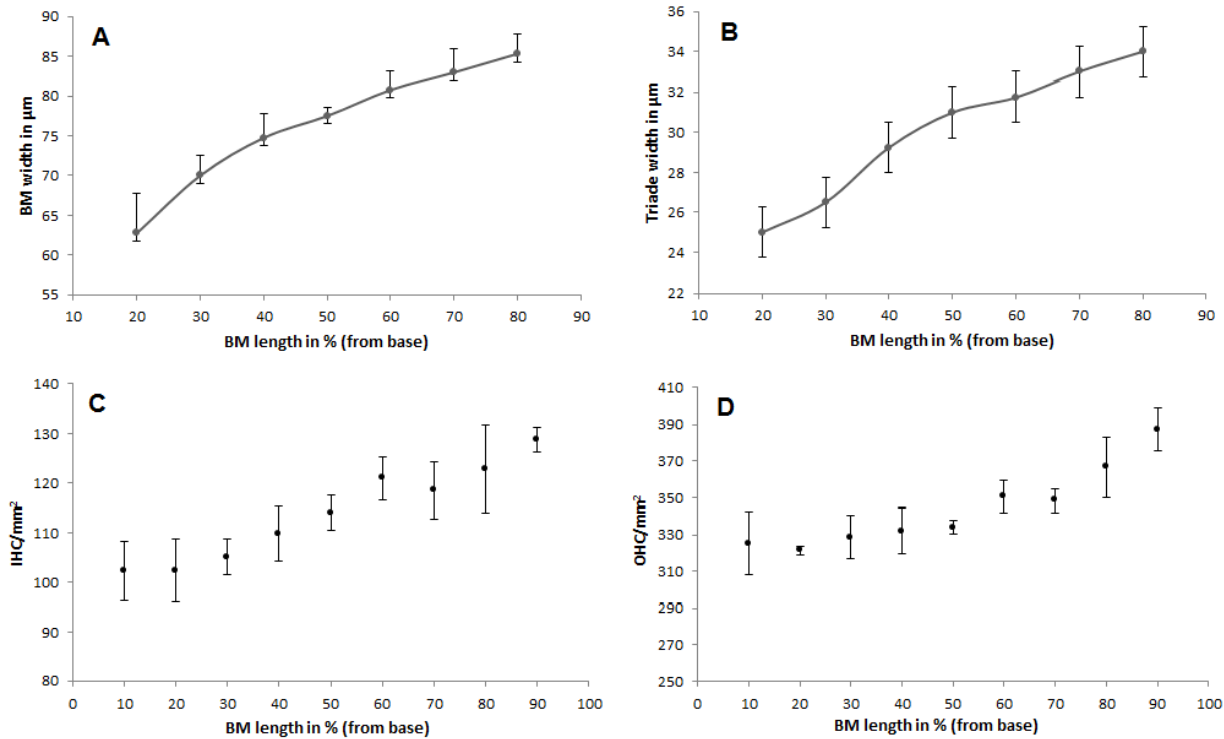


Fig. 3. Courses of a) the width of the basilar membrane, b) the width of three rows of outer hair cells, c) the density of inner hair cells (IHC), d) the density of outer hair cells (OHC; mean, SD) along the basilar membrane (BM) from base to apex.



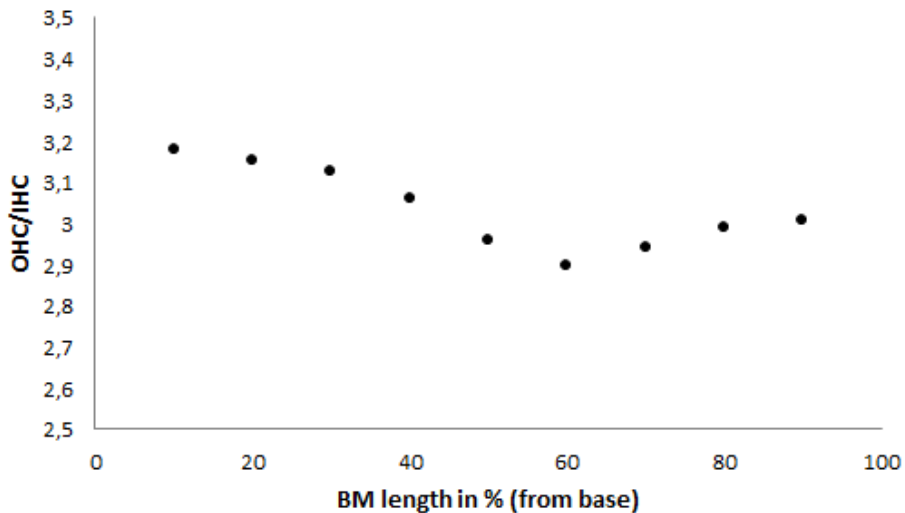


Fig. 4. Course of the ratio of mean outer hair cells and inner hair cells (OHC/IHC) along the basilar membrane.

## Discussion

We studied the hearing apparatus of a species for which ambiguous information about style of life and activity is available. According to some authors *R. sinensis* could be classified as subterranean, however according to some anecdotal report and some features such as body size, relatively well developed eyes, or a relatively long tail this species has aboveground activity. Because ear morphology and hearing abilities mirror the species lifestyle (cf. Burda et al., 1992; Pfaff et al., 2015), especially if it lives in a challenging sensory environment such as in underground burrows, we can obtain some information about its lifestyle and activity based on parameters of ear morphology. Due to fact that colonization of belowground environment occurred independently in different lineages of mammals and at different historical times, mammals with subterranean activity are not uniform in their biology, but they represent rather a mosaic of species with different level of adaptations to subterranean environment

(Nevo, 1999). From the point of diversity of adaptations to subterranean life in mammals, genus *Rhizomys* could be of interest because of its large body size, which is expected to preclude them from strict subterranean way of life.

The ear morphology and hearing abilities of particular species could be influenced not only by function (Békésy, 1974) and ecology (Webster and Webster, 1975), but also by phylogeny (Lavocat and Parent, 1985). This is clearly visible in the hystricomorphs, which are characterized by a specific type of middle ear, the so-called “ctenohystrica type” (Mason, 2013). This type of ear is characterized e.g. by larger middle ear structures tuned to low frequency hearing usually considered to be associated with a fossorial style of life. Such traits can be found in the fossorial tucotuco (*Ctenomys talarum*) and the coruro (*Spalacopus cyanus*) as well as in surface dwellers such as the guinea pig (*Cavia porcellus*) or the chinchilla (*Chinchilla lanigera*) (Schleich and Busch, 2004; Begall and Burda, 2006; Argyle and Mason, 2008). Low frequency hearing together with the associated morphological traits was found in many other hystricomorphs irrespective of their style of life (Miller, 1970; Heffner et al, 1971; Begall et al., 2004) suggesting its plesiomorphic state for the hystricomorphs (Begall and Burda, 2006; Argyle and Mason, 2008).

There is a higher diversity in hearing specialization within the myomorphs than in hystricomorphs. Myomorpha contains species specialized on high sensitive low frequency hearing, such as desert rodents, e.g. the fat-tailed gerbil (*Pachyuromys duprasi*), the Mongolian gerbil (*Meriones unguiculatus*) (Ryan, 1976; Plassmann and Kadel, 1991), low sensitive low frequency hearing specialists, such as the subterranean *S. ehrenbergi* (Bruns et al., 1988; Heffner and Heffner, 1992), as well as species with high sensitive high frequency hearing as the Norway rat (*Rattus norvegicus*; Heffner et al., 1994), the wood rat (*Neotoma floridiana*), and the grasshopper mouse (*Onychomys leucogaster*; Heffner

and Heffner, 1985). Concerning the middle ear ossicles morphology, “freely-mobile” as well as “microtype” are widespread within muroid rodents (Burda et al., 1992; Lange et al., 2004; Lavender et al., 2011; Mohammadpour, 2011). According to some authors, the “microtype” middle ear is probably the ancestral condition in muroid rodents (Lavender et al., 2011). If *R. sinensis* hear mainly low frequencies, we can expect that it will possess the “freely mobile” type.

The middle ear ossicles in *R. sinensis* are loosely attached to the bulla by ligaments and middle ear muscles. There was no evidence of a bony cup as described by Burda et al. (1989), Mason et al. (2010), and Rado et al. (1989) in *S. ehrenbergi* and *T. splendens* as a way of incudial articulation to the bullar wall. Both middle ear muscles are present, but they are very slender. The orbicular apophysis of the malleus is missing and the incus is relatively large in comparison with the malleus. This "freely mobile" type is found in many rodents with subterranean way of life as well as in many aboveground species with low frequency hearing (Fleischer, 1973; Burda et al., 1992; Mason, 2013). Subterranean mammals, as low sensitivity and low frequency specialists, are assumed to have relatively larger tympanic membranes than high sensitivity and high frequency hearers, though smaller than high sensitivity and low frequency specialists (Burda et al., 1992; Lange et al., 2004, but see Mason, 2001). The area of the eardrum in *R. sinensis* and the area of the stapedial footplate were comparable with the respective values reported for the subterranean *T. splendens* and *E. fontainierii* (see Table 3). However, *R. sinensis* is much larger than *T. splendens*, so it means that, related to the body and skull size, these structures are not enlarged. Yet on the other hand, this can be also interpreted as an indication for the same absolute frequency tuning of the middle ear in *R. sinensis* as in *T. splendens*.

Table 3. Middle ear parameters in selected spalacids. CB – condylobasal length, ML - malleal lever, IL – incudial lever, LR – lever ratio, ED – area of eardrum, SF – area of stapedial footplate, AR area ratio. (1) Mason, et al., 2010, (2) present study.

Species	CB (mm)	ML (mm)	IL (mm)	LR	ED (mm <sup>2</sup> )	SF (mm <sup>2</sup> )	AR
<i>Spalax ehrenbergi</i> <sup>1</sup>	40.6	2.44	1.13	2.16	12.38	0.62	19.97
<i>Eospalax fontanierii</i> <sup>1</sup>	43.2	2.87	1.37	2.09	16.96	0.97	17.56
<i>Tachyoryctes splendens</i> <sup>1</sup>	44.5	2.94	1.41	2.09	16.81	0.82	20.50
<i>Rhizomys sinensis</i> <sup>2</sup>	70.2	3.66	1.73	2.12	17.19	0.92	18.68

The ratio between the eardrum and the stapedial footplate areas, and the ossicular lever ratio reflect the sensitivity of hearing and thus indirectly the sensory ecology of a particular species. In *R. sinensis*, the ratio between the eardrum and stapedial footplate areas (AR) was 18.7, which is comparable to values found in other spalacids (Table 3). Such a low ratio is associated with low hearing sensitivity and life underground (Burda et al., 1992; Burda, 2006), while the aboveground species have a higher ratio, about 30 (Burda et al., 1992; Mason, 2001). The lever ratio in *R. sinensis* is comparable with values found in *S. ehrenbergi*, *T. splendens* and *E. fontanierii* (Mason et al., 2010). Interestingly, the lever ratio in this family is closer to the mean ratio in the aboveground species than to the mean ratio in other subterranean species (Mason, 2001). However, it is known that the difference in the lever ratio between subterranean and non-specialized species is generally not as distinctive as the difference in the area ratio (Mason, 2001).

The inner ear of *R. sinensis* possesses some characteristics which are connected with low frequency hearing and subterranean way of life. A long and highly coiled cochlea (3.25 turns) is considered a trait which enhances low frequency sensitivity (Crumpton et al., 2015). A high number of cochlear coils is typical for species with prevalent subterranean activity (Müller et al., 1989, 1992; Burda et al., 1990a; Burda, 2006) but also for caviomorphs with different hearing ecologies (Pye, 1977; Begall and Burda, 2006; Teudt and Richter, 2007). As already mentioned, in the *Ctenohystrica*, low frequency hearing and the related morphological traits are assumed to be plesiomorph traits (Begall and Burda, 2006; Argyle and Mason, 2008). In muroids, a high number of cochlear coils (3.25 turns) is known in the Mongolian gerbil (*Meriones unguiculatus*), which is a desert specialist with good low frequency sensibility (Plassmann et al., 1987; Plassmann and Kadel, 1991), whereas the number of coils is 1.75 – 2.3 in other muroid species studied so far (Ehret and Frankenreiter, 1977; Burda, 1988; Lange et al., 2004).

The width of the basilar membrane and the OHC triad in *R. sinensis* were lower at the base and increased toward the apex, similar to other mammals (Burda et al., 1988). In the basal half of the cochlea, the width increased relatively rapidly, while in the apical part of the spiral (around 50 - 60% of the length from base in particular) the increase was not steep (Figs. 3a and 3b). This change in the slope of the width of the basilar membrane and the OHC triad was not as obvious as in the subterranean Ansell's mole-rat (*Fukomys anelli*) (denoted as *Cryptomys hottentotus* in the original study), a hearing specialist possessing an acoustic fovea (Müller et al., 1992; Fig. 5). Nevertheless, the fact that the parameters changed only slightly means that a longer part of the organ of Corti is devoted to registration of frequencies within a narrow frequency range indicating higher hearing sensitivity and/or frequency resolution within the given frequency range (Burda et al., 1989b).

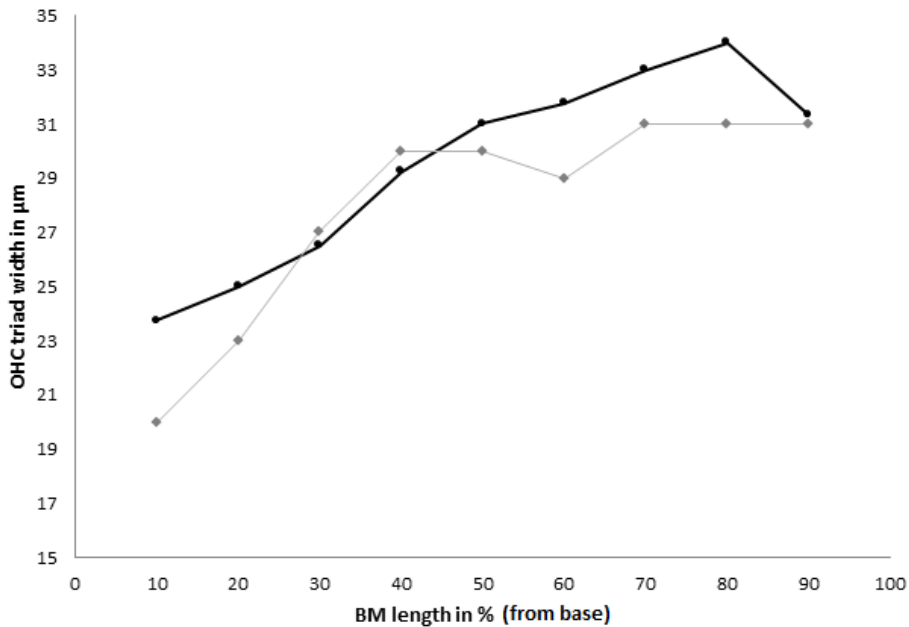


Fig. 5. Comparison of the slope in the width of the OHC triad. Black circles – *Rhizomys sinensis*, gray diamonds – *Fukomys anelli*. In *F. anelli*, acoustic fovea can be seen between 40% and 80% of the BM width (Lange, 2005).

The inner hair cell density in the studied species was similar to that in other subterranean and fossorial rodent species studied so far. Nevertheless, the density of the outer hair cells was relatively low, which corresponds more to the pattern observed in aboveground mammals than in subterranean species (Burda et al., 1988; Müller et al., 1989; Lange et al., 2004). In the rodent species studied so far, the curve of the frequency discrimination thresholds is reflected in the pattern of hair cell density (Burda and Voldřich, 1980; Burda, 1984; Burda et al., 1989a; Schleich et al., 2006). The density of both types of hair cells in the studied species increased toward the apex, which is probably positively correlated with good low frequency hearing as reported also for *C. talarum* (Schleich et al., 2006). The maximum density of hair cells in *R. sinensis* occurred in the apical part of the organ of Corti, whereas in species with good high

frequency hearing, such as in mice and rats, the maximum density was at 65 % of the length from the base (Burda et al., 1988).

## **Conclusions**

Our study of the ears of the Chinese bamboo rat revealed a combination of traits characteristic for rodents with both aboveground and subterranean activity. Its auricle is prominent like in aboveground or fossorial species indicating the necessity to localize sound and thus regular aboveground activity. Aboveground activity is also supported by the full-developed eyes, a relatively long tail and low hair cell density in inner ear. Some characteristics of the middle ear, such as a freely mobile type of ear and the absence of pars flaccida, suggest specialization for low frequency hearing. A low ratio between the areas of the eardrum and stapedial footplate suggests active reduction of hearing sensitivity as reported for subterranean mammals. The values of the ratios between the levers and the areas are comparable to all the subterranean spalacids studied so far. There are also some low-frequency specializations in the inner ear, such as the high number of cochlear coils, the length and width of the basilar membrane and increasing density of hair cells towards the cochlear apex. The overall hair cell density is more typical for aboveground dwellers. Therefore, we suppose that this species is specialized to subterranean acoustic conditions to some extent, but it is probably still able to use higher frequencies for sound localization aboveground.

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# Chapter II

## **Additional row of outer hair cells - the unique pattern of the Corti organ in a subterranean rodent, the Gansu zokor (*Eospalax cansus*)**

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**Additional row of outer hair cells - the unique pattern of  
the Corti organ in a subterranean rodent, the Gansu zokor  
(*Eospalax cansus*).**

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## **Abstract**

Acoustic conditions in burrows are different from those aboveground and restrict hearing of subterranean mammals to low frequencies, which is reflected in the ear morphology. While low-frequency adaptations of the middle ear attracted more attention of researches, the inner ear remained rather understudied. Here, we examined the cochlea of the inner ear of the Gansu zokor (*Eospalax cansus*), a subterranean rodent from the Tibetan Plateau. We focused on the quantitative parameters of the organ of Corti, which are assumed to determine hearing sensitivity and frequency tuning. Apart from the morphological traits common to the ear of subterranean rodents studied thus far, the Gansu zokor shows two unique features: the presence of a fourth row of outer hair cells along 20 % to 50 % of the basilar membrane length and almost constant width of the organ of Corti over more than 10 % of its spiral length. Both these anomalies occur in the middle of the cochlear spiral. These features are unusual in comparative morphology of the organ of Corti and presumably are reflected in the functional specialization. They are expected to affect sensitivity and /or resolution of hearing in the frequency range registered in the given cochlear segment. The Gansu zokor thus profiles to an interesting candidate for hearing research which might provide further insight not only into morpho-functional adaptations in subterranean mammals in particular but also in the function of outer hair cells in general.

**Keywords:** Inner ear, cochlea, Corti organ, outer hair cells, *Eospalax*, zokor, subterranean rodent

## **Introduction**

Across the globe, representatives of several rodent families have adapted to life in self-constructed extensive underground burrow systems, where they forage and spend virtually all their lives. The subterranean environment restricts communicative signals and lacks most spatial and temporal orientation cues available aboveground. Perception, communication, and orientation of subterranean mammals have attracted much attention of sensory biologists, neurobiologists and ethologists over the past decades (Nevo, 1979; for further reviews see Begall et al., 2007; Burda, 2006; Burda et al., 1990a; Nevo, 1999).

For communication and orientation in the darkness, acoustic signals seem to be suitable, as exemplified by bats. Correspondingly, audition, the auditory system and vocalization are expected to be well developed, and have become one of the most studied topics of sensory ecology in subterranean mammals. Generally, since the sensory environment of subterranean rodents appears to be uniform, either convergent evolution due to the exertion of the same selection pressures or random degeneration due to a lack of stimulation were expected (Burda et al., 1990a; Nevo, 1979, 1999).

Sound propagation in burrows is limited. The level of background noise is high and higher frequencies are attenuated, while lower frequencies in the range of about 200-800 Hz are best propagated (Heth et al., 1986; Lange et al., 2007). Accordingly, the hearing range in subterranean rodents is restricted, and, at the same time, best hearing is shifted to the given low frequency range as demonstrated by behavioral testing (Begall et al., 2004; Brückmann and Burda, 1997; Heffner and Heffner, 1990, 1992, 1993). The properties of tunnel acoustics and hearing are reflected also in vocalization characteristics, which also are tuned to lower frequencies (Bednářová et al., 2013; Credner et al., 1997; Dvořáková

et al., 2016; Heth et al., 1986; Knotková et al., 2009; Pepper et al., 1991; Vanden Hole et al., 2014; Veitl et al., 2000).

Logically, restriction and tuning of hearing to low frequencies is reflected in the morphology of the hearing apparatus (cf. Begall and Burda, 2006; Burda, 2006; Burda et al., 1989, 1992; Lange et al., 2004, 2005; Mason, 2001; Mason et al., 2010, 2016; Pleštilová et al., 2016; Schleich and Busch, 2004; Schleich et al., 2006; Wannaprasert, 2016). Indeed, the ear (like the dentition or the limb skeleton) is an example par excellence of morphology reflecting function and ecology. As György von Békésy (1974) stated: “Assuming that during the evolution, the most efficient system always survived, we expect that the physical laws served as guidelines to the evolution of the structures and functions of the middle and inner ear”. In other words, we expect that any convergence in hearing parameters will be reflected in (and will be due to) convergence in ear morphologies.

However, while most of the cited studies dealt with middle ear specializations, the inner ear has been understudied. Since the studies by G. v. Békésy (reviewed in Békésy, 1960), many parameters of the cochlea of the inner ear (such as the width and thickness of the basilar membrane, width of the secondary spiral osseous lamina, width of the spiral ligament, height of the scalae, height of the organ of Corti, width of the reticular lamina, and density of the hair cells) have been suggested to play a role in cochlear mechanics and to affect the sensitivity and resolution capability of hearing (cf. Burda and Voldřich, 1984; Burda et al., 1988a, 1988b).

Cochlear hair cells play a major role with respect to hearing sensitivity and tuning. The hair cells are of two types – inner hair cells (IHC) and outer hair cells (OHC) – and they differ in their morphology, innervation and function (reviewed in Dallos, 1992; Echteler et al., 1994; Ekdale, 2016). IHCs are synapsed with many afferent nerves and work primarily as sensors, while OHCs mainly modify the mechanical

properties of the organ of Corti and the basilar membrane (Dallos, 1992; Slepecky, 1996). The OHCs enhance the vibration of a narrow segment of the basilar membrane depending on transmitted frequency and act as a “cochlear amplifier” leading to the extraordinary sensitivity, frequency selectivity, and wide dynamic range of the auditory system (Chen et al., 2008; Dallos, 1992; Davis, 1983; Dong and Olson, 2013; Ren and Gillespie, 2007; Wang et al., 2016).

Due to the tonotopic organization of the cochlea (i.e. each particular place along the cochlea registers and processes a different frequency, unique for a given place) frequency- place maps can be constructed (cf. Liberman, 1982; Müller, 1996; Ou et al., 2000; Vater et al. 1985; Vater and Kössl, 2011). In most mammals for which tonotopy maps are available, segments of about the same length of the cochlear duct are allocated to the processing of particular octaves of the respective hearing range of a given species. The dimensions of the basilar membrane, which plays a role in cochlear mechanics (see above), change continuously along the cochlear duct. In a few species, however, the segment where the octave (frequency range) of the best hearing is represented is apparently expanded in comparison to (and thus on cost of) other octaves (comparable frequency ranges). This feature was called "acoustic fovea" in analogy to the visual fovea in the human eye. The acoustic fovea was so far described in the greater mustached bat (*Pteronotus parnelli*) (Russell and Kössl, 1999), the greater horseshoe bat (*Rhinolophus ferrumequinum*) (Bruns and Schmieszek, 1980; Vater et al., 1985), and in the Ansell's mole-rat (*Fukomys anelli*, previously named *Cryptomys hottentotus*) (Müller et al., 1992), a subterranean rodent specialized for low frequency hearing.

Of particular interest for studying the functional and comparative morphology of the ear is the rodent family Spalacidae, which includes three subfamilies: Spalacinae (blind mole rats), Myospalacinae (zokors), and Rhizomyinae (bamboo rats and root rats). Among them, the Spalacinae are the most specialized for life underground, their miniscule eyes are

covered by skin, and they shear the ground exclusively with their incisors while digging. The Rhizomyinae are fossorial, i.e. they are underground dwellers, which, however, forage also aboveground. They have small, yet prominent eyes and small pinnae. Myospalacinae are less adapted to the subterranean ecotope than the spalacines but show more subterranean specializations than rhizomyines. They have no auricles, their eyes are exposed, yet are relatively smaller than those of the Rhizomyinae. They dig primarily with their forefeet. Their middle ear morphology reveals typical specializations encountered in subterranean and fossorial rodents with some common but also some different traits (Mason et al., 2010). Whereas the cochlea with the Corti organ in *Spalax* mole rats (Bruns et al., 1988; Burda et al., 1989) and in the bamboo rat (Pleštilová et al., 2016) have been studied, no information is available on the inner ear in any representative of the Myospalacinae. In this study, we examined the cochlear morphology of the Gansu zokor (Plateau zokor) *Eospalax cansus*. This name was formerly used as a synonym to *E. fontanierii* or as a name of its subspecies (Musser and Carleton, 2005). We are following the new Mammal Species of the World (Wilson et al., 2017), where *E. cansus* is a separate species. Our aim was to identify the otomorphological traits which it shares with fossorial and subterranean rodents studied thus far as well as traits unique for spalacids.

## **Materials and methods**

The Gansu zokor (*Eospalax cansus*) is a solitarily living subterranean rodent inhabiting grasslands, shrubs and pastures of the Tibetan plateau (Zhang et al., 2003). It feeds mainly on roots and stems. Foraging tunnels are at a depth of 8 – 13 cm and its burrow systems extend for up to 100 meters (Smith and Xie, 2008; Zhang, 2007). This species is considered an important agricultural pest and is not protected under any local, regional, national, or international decree.

Three adult males (weight 174 g, 212 g and 231 g) and three adult females (162 g, 200 g and 217 g) were trapped in June and July 2015 at the Tianzhu field station of the Gansu Agricultural University, Tianzhu Tibetan Autonomous Country, Gansu, China (37°12' N, 102°46' E, altitude 2900 m a.s.l.). The animals were euthanized by an overdose of diethyl-ether and immediately decapitated, dissected and fixed in a 10% solution of formaldehyde. We measured the condylobasal length (CBL) of the skull by calipers with an accuracy of 0.1 mm. The lower jaws were removed and the bullae were extracted and opened laterally from the bony meatus. The eardrum and the ossicular chain were removed and the cochlea was dissected.

The cochlear partition of each ear was prepared by the surface specimen technique (cf. Burda, 1978, 1979, 1985; Burda and Braniš, 1988). The cochlea was opened from the apex, the bony shell was subsequently removed, and the cochlear duct was stained in toto with toluidine blue and Ehrlich hematoxylin. The cochlear partition in each coil was released from the cochlear outer wall and the modiolus, and then mounted as a surface specimen on a microscope slide in water, which was subsequently replaced by glycerin, so that it floated under the cover-slip. Each organ of Corti was examined along its entire course and each segment was measured using an ocular micrometer under an Olympus CX41 light microscope (magnification x 1000). We measured the length of the basilar membrane along the tunnel of Corti, the radial width of the three (or four) rows of cuticular plates of the outer hair cells (see Fig. 1) and the density of outer and inner hair cells. The density was counted indirectly from the length of a segment occupied by ten hair cells in a row. About 60 to 70 (according to the actual length of the basilar membrane) visual fields were examined in each ear. For the sake of comparison, each cochlear partition was then divided into ten segments of equal length, and the results were expressed as the average width of the OHC triad and average densities of OHC and IHC per mm per segment, i.e. the values of particular parameters



were related to the relative and not the absolute distances along the cochlear spiral (Burda, 1978, 1979, 1985; Burda and Braniš, 1988).

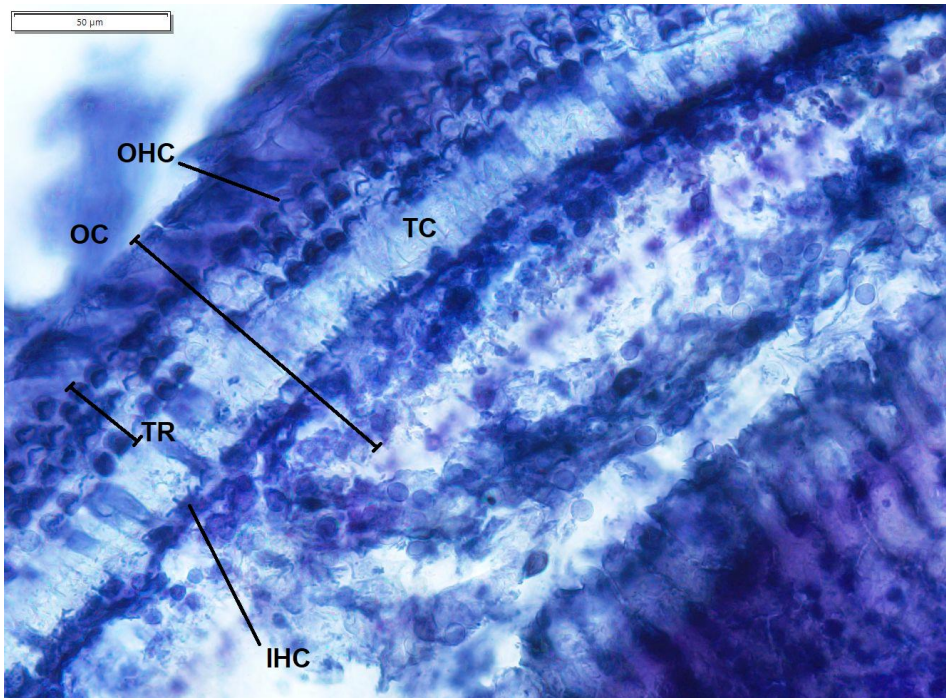


Fig. 1. Organ of Corti: IHC, inner hair cells; OHC, outer hair cells; TC, tunnel of Corti; TR, width of triad; OC, width of the organ of Corti.

## Results

The mean condylobasal length of the *E. cansus* skull was  $39.7 \pm 1.0$  mm ( $n = 6$ ). The cochlea had 3.5 coils and the length of the basilar membrane (BM) was  $13.3 \pm 0.8$  mm (12.0 – 14.0 mm). The mean width of the reticular lamina of the organ of Corti (OC) (Fig. 1) increased from  $104.5 \pm 5.1$  μm at the base to  $160.0 \pm 20.7$  μm at the apex with only a slight change in the central part of the BM length, and a plateau of constant width between 60 and 70 % of the total BM length (Fig. 2a). OHC were organized in triads but an additional fourth row was present in all the specimens in the middle

part of the BM length (Fig. 3), between about 30 and 70% BM length from the base, and stretching along the organ of Corti for about 20 to 50 % of its length (Fig. 2 b, c). The OHC in the additional row were organized in a less regular pattern compared to the "normal" three OHC rows. Mean width of the stripe of OHC cuticular plates on the reticular lamina was  $31.4 \pm 5.1 \mu\text{m}$  and reached its maximum at about the 60 % length from the base, i.e. in the region where the fourth OHC row contributed to its width (Fig. 2c). The mean density of IHC was  $110.4 \pm 8.2$  cells per mm and, with a few exceptions, it slowly rose towards the apex (Fig. 2d). The mean density of OHC was  $433.0 \pm 58.9$  with the maximum in the middle of the BM length (Table 1, Fig. 2c). The total number of cochlear hair cells was  $1463 \pm 108$  IHC and  $5733 \pm 222$  OHC (Table 1). The mean ratio between outer and inner hair cells was thus 3.94, with a minimum value of 3.63 at the base and a maximum of 4.84 in 60 % of the spiral length of the organ of Corti (Fig. 4).

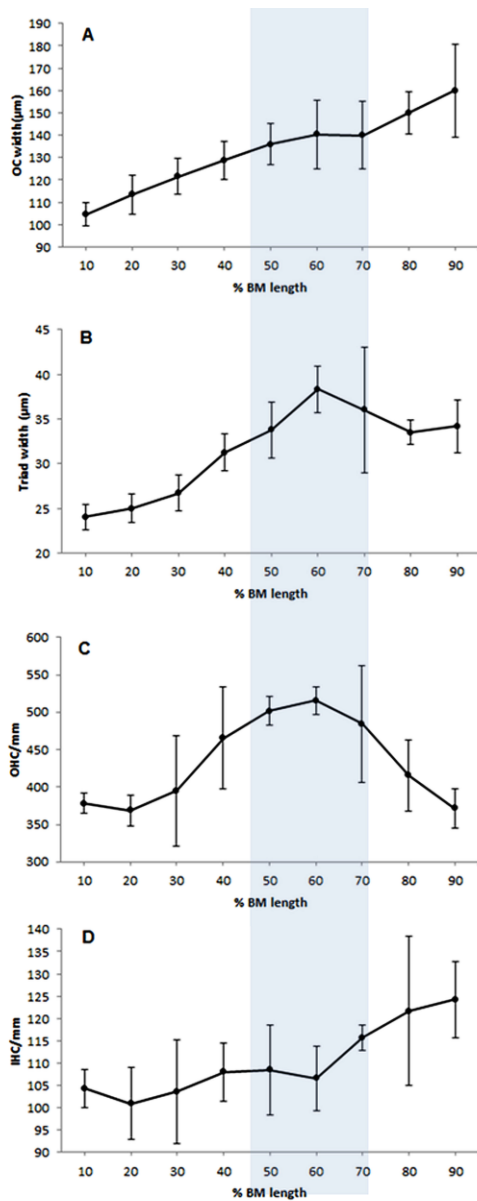


Fig. 2. Courses of a) the width of the organ of Corti, b) the width of three rows of outer hair cells, c) the density of outer hair cells (OHC), d) the density of inner hair cells (IHC) along the basilar membrane (BM). BM length at x axis is oriented from base to apex (0–100%), all graphs display mean and SD. The BM part including the fourth OHC row is coloured.

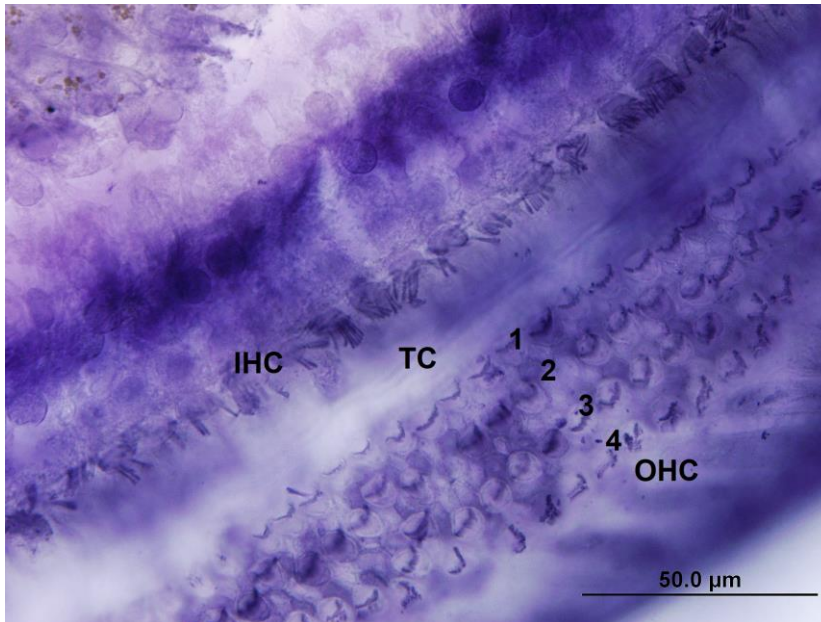


Fig. 3. Organ of Corti: four rows of OHC. IHC, inner hair cells; TC, tunnel of Corti; 1, OHC – outer hair cell rows.

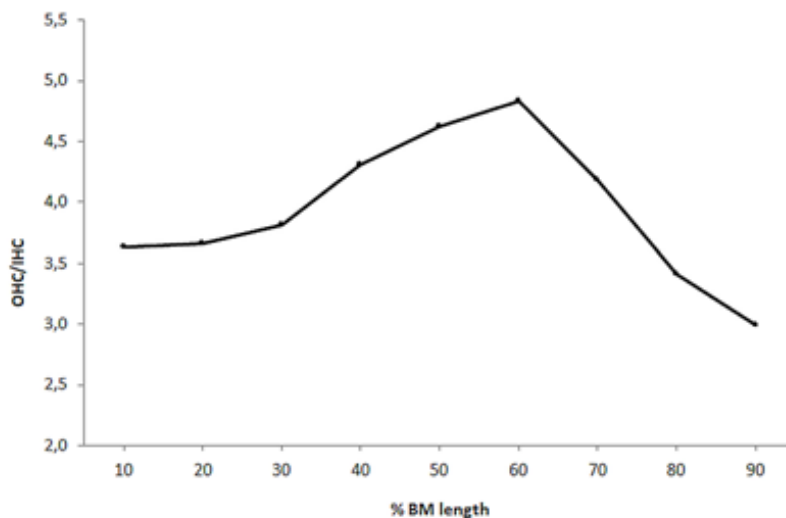


Fig. 4. Course of the ratio of mean between outer and inner hair cells. BM length atx axis is oriented from base to apex (0–100%).

Table 1. Parameters of the inner ear measured along the basilar membrane (mean,  $\pm$  SD): OC, organ of Corti; OHC strip width, radial width of three or four rows of outer hair cells; IHC, inner hair cells; OHC, outer hair cells including the additional row.

Inner ear parameters	Distance from base (%)									mean
	10	20	30	40	50	60	70	80	90	
OC width ( $\mu\text{m}$ )	104.5	113.5	121.5	128.8	135.8	140.4	140.0	150.0	160.0	132.7
SD	5.1	8.8	8.0	8.6	9.2	15.4	15.3	9.6	20.7	17.6
OHC strip width ( $\mu\text{m}$ )	24.0	25.0	26.7	31.3	33.8	38.3	36.0	33.5	34.2	31.4
SD	1.4	1.6	2.0	2.1	3.1	2.6	7.0	1.4	3.0	5.1
IHC density/mm	104	101	104	108	108	107	116	122	124	110
SD	4.2	8.0	11.6	6.6	10.1	7.2	2.9	16.7	8.5	8.2
OHC density/mm	378	369	395	466	501	516	484	416	372	433
SD	13.6	20.6	74.1	67.7	18.9	18.3	78.2	47.9	26.3	58.9
OHC/IHC	3.6	3.7	3.8	4.3	4.6	4.8	4.2	3.4	3.0	3.9

## Discussion

Within the spalacids, the inner ear was first described in the Middle East blind mole rat, *Spalax ehrenbergi* (Bruns et al., 1988; Burda et al., 1989), a species with extreme adaptations for subterranean life, and, recently, in the bamboo rats, *Rhizomys sinensis* and *Cannomys badius*, both species with fossorial lifestyles (Pleštilová et al., 2016; Wannaprasert, 2016).

The Gansu zokor represents a transitional form between blind mole rats and bamboo rats in respect to the degree of adaptations to the subterranean environment. The number of cochlear coils (3.5) in the zokor is similar to that found in other spalacids (Bruns et al., 1988; Burda et al., 1990b; Pleštilová et al., 2016; Wannaprasert, 2016) and is higher than in epigeic "non- specialized" muroid rodents which possess only about two cochlear turns (e.g. Burda et al., 1988a). Among rodents, a highly coiled cochlea characterizes subterranean rodents in particular (see also Begall and Burda, 2006; Burda et al., 1988b; Lange et al., 2004; Schleich et al., 2006) and hystricognath rodents in general (Pye, 1977; Teudt and Richter, 2007). A higher number of cochlear coils is usually related to a broader frequency range of hearing and to good low frequency hearing (Crumpton et al., 2015; West, 1985).

Subterranean rodents in particular and hystricognath rodents in general exhibit also relatively (with respect to body size) longer basilar membranes than their respective aboveground counterparts (Burda et al., 1988b). The relative length of the BM (related to the length of the skull) in the zokor is within the range of expected values based on data estimated for other spalacid and hystricognath species (including subterranean ones). It is surely relatively and absolutely longer than the BM in the larger, non-subterranean muroid wild Norway rat and it is very similar to the subterranean blind mole rat, which is also muroid rodent (Table 2, Fig. 5). Consequently, this parameter reflects either subterranean hearing ecology of the zokor. The alternative interpretation that the longer BM in the zokor

would be a plesiomorph ancestral trait in spalacids is less probable because the clades representing the three subfamilies had an epigeic ancestor and occupied the subterranean ecotope independently (Flynn 2009).

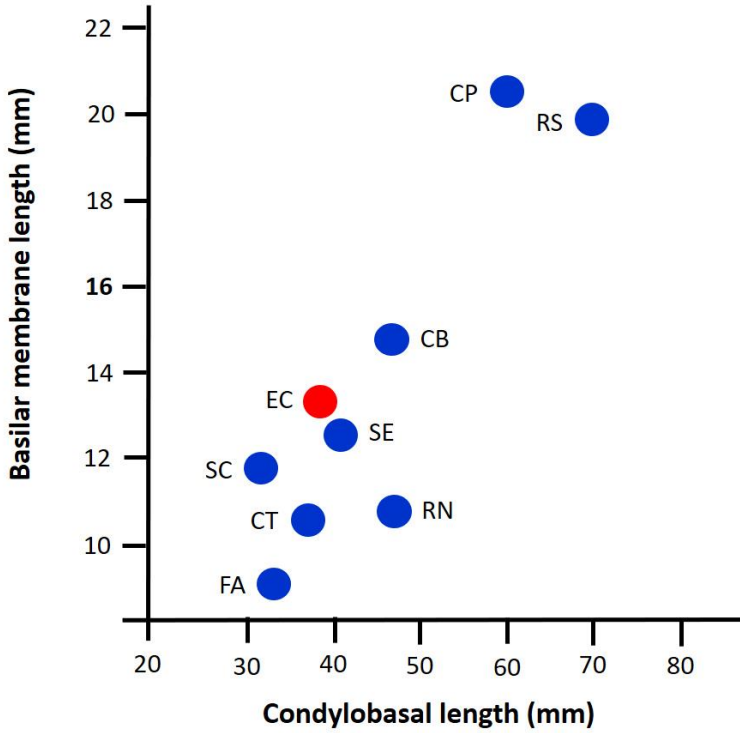


Fig. 5. Relationship between condylobasal length and the length of basilar membrane. CB, *Cannomys badius*; CP, *Cavia porcellus*; CT, *Ctenomys talarum*; EC, *Eospalax cansus*; FA, *Fukomys anelli*; RN, *Rattus norvegicus*; RS, *Rhizomys sinensis*; SC, *Spalacopus cyanus*; SE, *Spalax ehrenbergi*.

Table 2. Inner ear parameters from earlier studies on other rodent species. CBL, condylobasal length; BM, basilar membrane; OHC, outer hair cells; IHC, inner hair cells. Given width of OHC triad was measured in 10%, respectively 90% of BM length.

Rodent species	Body weight (g)	CBL (mm)	Cochlear coils	BM length (mm)	OHC/mm	IHC/mm	OHC triad width base ( $\mu\text{m}$ )	OHC triad width apex ( $\mu\text{m}$ )	Reference
<i>Arvicola terrestris</i>	130	33.8	2.3	10.5	405	116	x	x	Lange (2004)
<i>Cavia porcellus</i>	406	60	4.25	20.5	361	100	x	x	Fernandez (1952); Burda (1984)
<i>Ctenomys talarum</i>	140	37.4	3.25	10.6	400	101	18.6	29.9	Schleich et al. (2006)
<i>Eospalax cansus</i>	196	39.7	3.5	13.3	433	110	24	34.2	This study
<i>Fukomys anelli</i>	80	33.1	3.5	9.3	448	118	19	31	Lange (2005)
<i>Microtus arvalis</i>	27	24.3	2.3	8.5	442	121	x	x	Lange (2004)
<i>Mus musculus</i>	22	20.5	1.95	6.0	411	121	12	24.2	Burda et al. (1988)
<i>Rattus norvegicus</i>	325	47.0	2.2	10.7	364	98	12.6	33.8	Burda et al. (1988)
<i>Rhizomys sinensis</i>	1900	70.2	3.25	18.9	344	114	23.8	34	Pleštilová et al. (2016)
<i>Spalacopus cyanus</i>	90	30.8	3.5	11.7	397	109	x	x	Begall and Burda (2006)
<i>Spalax ehrenbergi</i>	135	41.3	3.5	12.6	386	93.3	18	25	Burda (2006); Burda et al. (1989)



The organ of Corti in the zokor widens from the base towards the apex, as typical in mammals. Nevertheless, the width remains unchanged between 60 and 70 % of the BM length. The width of the OHC triad is indicative of the width of the reticular lamina composed of cuticular plates (cf. Burda, 1985; Burda and Braniš, 1988). It widens markedly from the base towards the apex, reaches its maximum at 60 % of the BM length and then, unusually among mammals, narrows again towards the apex. The overall hair cell density is relatively high, but still within the values found in some other subterranean rodents (Table 2). Short irregular segments of a fourth OHC row were occasionally found in the most apical regions of the organ of Corti in diverse mammalian species, e.g. the European water vole (*Arvicola terrestris*) (Lange et al., 2004), domestic pig (Lovell and Harper, 2007), or human (Glueckert et al., 2005). The presence of additional OHC rows along parts of the Corti organ was reported in the blind mole rat (Bruns et al. 1988), with up to six rows of OHC in the apical parts (Bruns et al., 1988; Raphael et al., 1991). The regular occurrence of a fourth row of OHC over a long segment of the organ of Corti, and notably in its middle part, is however, an exceptional trait among mammals.

The presence of the fourth OHC row influences the total hair cell number and density (Fig. 2c). The mean OHC density in *E. cansus* is higher than in most rodents studied so far with the exception of the subterranean Ansell's mole-rat, *F. anelli* and fossorial field vole, *Microtus arvalis* (Table 2). Notably, the higher OHC density in the zokor is achieved through an additional row of OHC, not through decreasing the size of the cells and/or the distance between them, i.e. through compressing the cells in a row. Generally, the region on the BM, which represents the frequency range of the best hearing, is characterized also by the highest hair cell density (Burda, 1984; Burda and Voldřich, 1980; Ehret and Frankenreiter, 1977). Studies based on the selective loss or dysfunction of hair cells show that OHC influence the hearing threshold, especially in characteristic frequencies (Chen et al., 2008; Dallos and Harris, 1978; Ryan and Dallos,

1975; Wu et al., 2004). This frequency-dependent sensitizing influence of OHC is most apparent in low input level, such as 10 dB SPL (Wang et al., 2016). It means that low intensity sounds are amplified relatively more than loud sounds (Robles and Ruggero, 2001; Hudspeth, 2008). These experiments have also shown that hair cell loss must be extensive, at least one-third of the OHC population, to have a significant effect on hearing (Chen et al., 2008; Nienhuys and Clark, 1978; Salvi et al., 2017). We expect that the increase of the OHC population caused by the fourth row in the zokor for more than one third should have a significantly enhance hearing sensitivity within the frequency range registered in the given cochlear segment. These features are unusual in comparative morphology of the organ of Corti and presumably are reflected in the functional specialization. The Gansu zokor thus profiles to an interesting candidate for hearing research which might provide further insight not only into morpho-functional adaptations in subterranean mammals in particular but also in the function of outer hair cells in general.

Whereas *S. ehrenbergi* and *R. sinensis* possess the OHC maximum in the apical part of the cochlea (Burda et al., 1989; Pleštilová et al., 2016), the maximum OHC density in the zokor was shifted towards the middle of the cochlea. Interestingly, if we consider the OHC density only in the three rows, the maximum would also be in the apical part. The IHC density in the zokor was within the range of values found in other rodent species (Table 2).

Wang and Li (2011) studied the hearing thresholds in zokors in the frequency range of 0.5-32 kHz electrophysiologically, by recording auditory brainstem response. These authors found the lowest auditory threshold at 12 kHz. This is an unexpectedly high frequency, as the lowest threshold in all the subterranean and fossorial rodent species studied so far occurs at much lower frequencies, mainly 0.5-4 kHz (Brückmann and Burda, 1997; Bruns et al., 1988; Gerhardt et al., 2017; Heffner and Heffner, 1990, 1992, 1993) and 4-8 kHz respectively (Heffner et al., 1994a, 2001).

On the contrary, mice, rats and hamsters have their best hearing at about 10 kHz (Heffner and Heffner, 1985; Heffner et al., 1994b, 2001). Although the electrophysiological audiogram differs from the behavioral one regarding sensitivity (it appears generally lower in electrophysiological audiograms) and also in high frequency hearing limits (see Gerhardt et al., 2017) it still provides relevant information about the hearing abilities of species in which the behavioral audiogram is not available (cf. the audiograms obtained by diverse techniques in *F. anselli*: Brückmann and Burda, 1997; Kössl et al., 1996; Müller and Burda, 1989; Müller et al., 1992).

If the hearing range covers at least the eight octaves as common in other rodents and if, following the tonotopy principle, the segments dedicated to processing of particular octaves are comparably long, we expect that each octave stretches over less than 12.5 % of the BM length. Furthermore, if the best hearing in the Gansu zokor is centered at 12 kHz (Wang and Li, 2011), and hence within the octave 8-16 kHz, and assuming that the maximum HC density is found in the region processing the best hearing octave (Burda and Voldřich, 1980; Burda, 1984, 1985), then this best octave stretches over more than 12.5% of the basilar membrane length (Fig. 2).

Since the change of BM and OHC triad width along the cochlear partition is related to frequency representation (tonotopicity), the constant width indicates representation of the same frequencies along the given segment. Such a segment with a nearly constant BM and reticular lamina width spreads in *F. anselli* along 45 % of the BM length and is dedicated to registration of just one octave and was designated as the acoustic fovea (Müller et al., 1992).

Although such a segment with a rather constant width is much shorter in the zokor, we believe that together with the extra OHC row it indicates the existence of an acoustic fovea in the zokor. Of course, a

morphological study can work only with correlates and assumptions. To prove acoustic fovea directly we would have to work experimentally (neurophysiologically and neuroanatomically) with live animals and to have sacrificed many more individuals, which was not possible. Nevertheless, the experimental and morphological studies done thus far on other species provide us a great bulk of evidence that the selected morphological correlates are valid.

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# Chapter III

## **Ear morphology in two root-rat species (genus *Tachyoryctes*) differing in the degree of fossoriality**

Lucie Pleštilová, Ema Hrouzková, Hynek Burda,  
Yonas Meheretu & Radim Šumbera

**Ear morphology in two root-rat species (genus *Tachyoryctes*) differing in the degree of fossoriality.**

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## Abstract

Subterranean way of life is supposed to be reflected in the ear morphology and tuning of hearing to low frequencies. We studied two root-rat species, genus *Tachyoryctes*, to see if the ear morphology reflects the difference in the amount of their surface activity. *T. splendens* possess shorter pinna as typical for subterranean species, but on the contrary, it possess smaller bulla. In both species, the ratio between eardrum and stapedial footplate area and ratio between malleal and incudial lever are typical for subterranean mammals, but both values were lower in *T. splendens* ( $19.3\pm 0.3$  and  $1.9\pm 0.0$ ) than in *T. macrocephalus* ( $21.8\pm 0.6$  and  $2.1\pm 0.1$ ) reflecting probably higher surface activity of latter species. Although, the cochlea has 3.5 coils in both species, the basilar membrane is longer in smaller *T. splendens* ( $13.0\pm 0.5$  versus  $11.4\pm 0.7$  mm, respectively) what indicates its wider hearing range and/or higher sensitivity to some frequencies. In both species, the highest density of outer hair cells (OHC) was in the apical part of cochlea, while the highest density of inner hair cells (IHC) in its middle part. The pattern of the OHC density corresponds with good low frequency hearing in both species, whereas the IHC pattern suggests their sensitivity to higher frequencies.

**Keywords:** Ear morphology, hearing, subterranean mammals, *Tachyoryctes*, Spalacidae.

# **Conclusions and future perspectives**

In my thesis, I described the ear morphology of four species from the family Spalacidae representing different phylogenetic clades within this family, as well as a whole continuum from a strictly subterranean to a fossorial lifestyle.

The objective of the first study was to estimate the degree of fossoriality in *Rhizomys sinensis*, based on its ear morphology. This species was denoted as “subterranean” in the literature, while its appearance suggests at least some aboveground activity and thus a fossorial lifestyle. The ear morphology exhibits a combination of traits typical for subterranean specialists as well as for epigeic forms and proved to be a good predictor of the ratio between subterranean and aboveground activity.

In the second study we described the ear morphology of *Eospalax cansus*, with an aim to identify if even only a small shift in subterranean specialization is mirrored in the ear morphology. Based on its inner ear morphology, we suggested the acoustic fovea as was previously found in other subterranean species. However, the acoustic fovea in the studied species had a lower extent, which can be related to a lower level of its specialization to the subterranean ecotope.

In the last study we used two species of the genus *Tachyoryctes* which spend different amounts of time aboveground, to eliminate phylogenetically caused differences from the comparison of the ear morphology focused on the ecology. The difference in surface activity pattern was found to be reflected in the ear morphology of studied species.

This thesis can bring a new insight on how ear morphology is shaped by the subterranean ecotope and it can answer the question if and to what extent are the subterranean rodents specialized to the environment they inhabit. For an even deeper insight into how ear morphology translates into hearing abilities, it would be certainly worthy to establish the audiograms of studied species.

# Curriculum Vitae

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### **Study stays**

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**Pleštilová, L.,** Valesky, E.M., Burda, H., Šumbera, R. (2019). Skin morphology in context of thermoregulation and heat dissipation in the social African mole-rat *Fukomys mechowii*. The 12<sup>th</sup> International Congress of Vertebrate Morphology, Prague, Czech Republic, July 21-25.

**Pleštilová, L.,** Hrouzková, E., Burda, H., Šumbera, R. (2018). Ear morphology in two root-rats (genus *Tachyoryctes*) with a different ecology. The 7<sup>th</sup> European Ground Squirrel Meeting & Subterranean Rodents Workshop, Budapest, Hungary, October 1-5.

**Pleštilová, L.,** Hrouzková, E., Hua, L., Burda, H., Šumbera, R. (2016). Subterranean specialization of the ear morphology in *Eospalax fontanierii* (Rodentia: Spalacidae). The 11<sup>th</sup> International Congress of Vertebrate Morphology, Washington DC, USA, June 29-July 3.

## **Publications**

**Pleštilová, L.**, Okrouhlík, J., Burda, H., Sehadová, H., Valesky, E. M., Šumbera, R. (2020). Functional histology of the skin in the subterranean African giant mole-rat: thermal windows are determined solely by pelage characteristics. *PeerJ*, 8, e8883.

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