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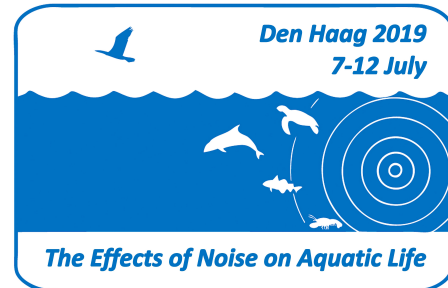
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3D-Visualization of the Ear Morphology of Penguins (Spheniscidae): Implications for Hearing Abilities in Air and Underwater

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The ear morphology of three penguin species (*Aptenodytes forsteri*, *Pygoscelis papua* and *Spheniscus demersus*) was analyzed using diffusible iodine-based contrast-enhanced computed tomography (diceCT). Main aural structures were visualized and the results were animated in 3D videos as open educational resources to facilitate UNESCO's mandate for a free use of these digital models for scholars and teachers. Based on the annotated segmentations, the morphology of main structures of the penguin ear is described. In general, the penguin ear can be regarded as an organ for the reception of air sound with adaptations to the semiaquatic lifestyle of the animals. No alternative pathways for the reception of sound in water, as are known in aquatic vertebrates, were detected by the used methods so far. The significance of missing contralateral connections between the air-filled spaces of the middle ear needs further evaluation in that respect. The low relation of the area of the tympanic membrane to the columella footplate and a potential venous corpus cavernosum in the middle ear are discussed as important factors for pressure regulation and for the protection of the sensitive tympanic membrane. Our results indicate that penguins have at least basic abilities to hear underwater even under high ambient pressures.

1. INTRODUCTION

Penguins (Sphenisciformes: Spheniscidae) are an order of flightless and semiaquatic birds living in cold to temperate waters of the southern hemisphere. While their vocal repertoire and inter-individual recognition have been studied in detail in air (e.g. Lengagne et al. 1997, 2001, Jouventin et al. 1999, Favaro et al. 2015), until now no study has ever investigated the underwater hearing of penguins. In some other sea birds hearing capabilities have been tested using psychophysical testing for cormorants (*Phalacrocorax carbo*) in air (Maxwell et al. 2017) and under water (Hansen et al. 2017). Electrophysiological measurements utilizing the auditory brainstem response have been further developed on diving ducks and diver species (Crowell et al. 2015, 2016), on cormorants (Maxwell et al. 2016) and on the African penguin *Spheniscus demersus* (Wever et al. 1969); with all of these tests being performed in air. Overall, the frequency range of hearing and the shape of audiogram (Hansen et al. 2017) as well as the hearing abilities in general of cormorants (Larsen et al. 2020) align well with known data from testing of related bird species in air, and underwater hearing of cormorants was found to be better than expected and comparable to toothed whale and seal hearing of around 1-4 kHz.

Therefore, our knowledge of how penguins perceive sound in general is very limited and needs improvement to judge effects of anthropogenic noise on the auditory system. Based on other semiaquatic species like seals, which have acute hearing capabilities both in air as well as under water (Reichmuth et al. 2013), it is very likely that penguins have evolved adaptations to cope with pressure differences between air and water to allow for hearing under varying static pressures. This would allow them to perceive sounds to avoid predator attacks and to locate the direction from where a sound is coming from. In the ongoing project “Hearing in penguins” all methods for testing underwater and air hearing of penguins are combined and substantiated using auxiliary data from studies on auks (Mooney et al. 2019). A first result is that penguins react to low levels of frequency centered noise bands by swimming away from the source in captivity (Sørensen et al. 2020). The here presented approach is to scan the inner and middle ears of collection specimens using computed tomography (CT) to describe the morphological structures and to investigate the mechanisms of sound reception. Here, three penguin heads were scanned and segmented to describe the ear morphology as a first step towards understanding which adaptations are present in penguins that differ from other bird species.

2. MATERIAL AND METHODS

For this study, three heads of different penguin species belonging to different phylogenetic lineages and size groups were analyzed; *Aptenodytes forsteri*, *Pygoscelis papua* and *Spheniscus demersus* (Table 1). All specimens belong to the ornithological collection of the Museum für Naturkunde Berlin. Whereas *P. papua* were collected fairly recently, *A. forsteri* and *S. demersus* are historical collection specimens with little information about their origin.

Table 1: Origin and fixation of the specimens

Species	Inventory number	Origin	Fixation	Storage
<i>Aptenodytes forsteri</i>	ZMB 1999.980	unknown	unknown	alcohol
<i>Pygoscelis papua</i>	ZMB 2017.123	King George Island, South Shetland Islands, Antarctica; 1981-1982	formalin	formalin / alcohol
<i>Spheniscus demersus</i>	ZMB 2000.12789	captivity	unknown	alcohol

A. IODINE STAINING

Iodine is the most widely used agent to enhance the contrast of different soft-tissue structures in x-ray images (Gignac et al. 2016). For the present study, an iodine-potassium iodine solution (stock solution from MORPHISTO Evolutionsforschung und Anwendung GmbH, I₂= 13 g/L, KI= 20g/L) was used. The specimens were transferred from the preservative medium into distilled H₂O, and then treated with a 0.57 w/v % aqueous iodine solution. After 44 days of staining, the concentration of the aqueous iodine solution was increased to 1.13 w/v %. The iodine solution was also injected in the head cavities to support the penetration. The staining process was monitored by test scans and terminated after 51 days (*Pygoscelis papua*, *Spheniscus demersus*) and after 145 days (*Aptenodytes forsteri*), respectively.

B. COMPUTED TOMOGRAPHY

Specimens were scanned with cone beam x-rays (expanding from the source towards the detector) at the Museum für Naturkunde Berlin using a FF35 dual-tube system (YXLON International, Hamburg, Comet Group). One scan each per specimen was performed prior to staining to visualize bony structures with the highest possible contrast.

A second scan of each penguin was performed after iodine staining to yield the best results regarding soft tissue visualization. Scan parameters were adapted for each specimen (Table 2).

Table 2: CT scanning parameters

Scan	Voltage [kV]	Current [μA]	Exposure duration [ms]	Projections	Resulting Voxel size [μm]	Scan-Mode
<i>Pygoscelis papua</i>						
Pre-staining	120	110	200	3793	50.70	helical
Post-staining	180	250	330	1440	21.24	integrated circular*
<i>Spheniscus demersus</i>						
Pre-staining	80	150	1000	3606	40.00	integrated helical*
Post-staining	150	190	1000	1500	27.80	circular
<i>Aptenodytes forsteri</i>						
Pre-staining	120	130	200	4086	52.95	integrated helical*
Post-staining	200	220	1000	1400	26.83	circular

*three images per projection, of which one was discarded

C. SEGMENTATION AND MEASUREMENTS

The cone beam reconstructions were performed with the YXLON reconstruction workspace (YXLON International, Hamburg, Comet Group). Relevant structures were segmented using Volume Graphics Studio Max 3.2 (Volume Graphics, Heidelberg). After segmenting the bony elements in the unstained and the soft tissue structures in the stained scans of each specimen, the two scans were combined into one workspace to allow for a composite visualization of bone and soft tissues for each penguin.

Morphometric measurements were taken to validate the transfer of the sound from the tympanic membrane to the inner ear. The area of the tympanic membrane and the columella footplate was calculated as an ellipsoid by measuring the largest diameter and the one perpendicular to it in the CT scans. Therefore, the area of the tympanic membrane is assumed to be a flat structure for the purpose of these calculations. Nomenclature follows Baumel et al. (1993).

3. RESULTS

The segmentations of ear structures were used to compile short videos to demonstrate their morphology. They are available as open educational resources under Bendel et al. (2019), Westphal et al. (2019) and Frahnert et al. (2020).

A. OUTER EAR

As is typical for all birds, penguins have no external auricle. The most lateral part of the outer ear is the opening of the outer ear canal (*Apertura auris externae*) which is situated behind the eye, approximately at the level of its ventral edge. The shape of this opening varies from round to oval. It is completely covered by contour feathers (*Pennae*) which are orientated in a caudal direction. The auditory canal (*Meatus acusticus externus*) runs straight in a caudal direction. Its diameter is only marginally smaller than the tympanic membrane (*Membrana tympanica*) and is largest at its medial end lateral of the tympanic membrane (Fig. 1). It is completely embedded in soft tissue, lacks a bony stabilization but shows a close contact to the quadrate (*Os quadratum*). Among the surrounding tissues of the external ear canal are different muscles. There are no circular muscles, but without their complete reconstruction it cannot be determined whether they have an effect on the opening/closure of the canal. Under scanning conditions and in air the volume of the canal is air-filled. No specializations of the mucosa were observable.

B. MIDDLE EAR

The tympanic membrane is orientated ventrolaterally, slightly caudally directed. It is connected with the neurocranium (*Os squamosum*, *Os exoccipitale*, *Os basisphenoidale*) at the distal aperture of the tympanic cavity (*Cavitas tympanica*) and most probably with the quadrate. Due to the fact that the boundaries between specific soft tissues were not always identifiable in the scans, it was not possible to pinpoint to what extent the tympanic membrane is connected to the otic process (*Processus oticus*) of the quadrate (Fig. 4). In any case, the quadrate has a very close relation to the tympanic membrane. The avian tympanic membrane is typically conically shaped with the tip nearly in the center pointing laterally (Kühne and Lewis 1985).

Medial to the tympanic membrane follows the tympanic cavity. Here the single ear ossicle, the columella (*Columella*), connects the tympanic membrane with the oval window (*Fenestra vestibuli*) of the inner ear. The three processes of the extracolumella (*Cartilago extracolumellaris*) connect the tympanic membrane to the shaft of the columella (*Scapus columellae*). Due to remnants of the staining medium it was not possible to distinguish the single processes in all specimens clearly. The main part of the extracolumella lies under the tip of the tympanic membrane and its processes extend laterally in different directions. At its medial end the shaft of the columella continues into its footplate, which is connected to the oval window by a ligament (*Ligamentum anulare*, segmented in *P. papua* only). The shape of the columella shaft of each penguin species differs slightly. It is straight in *A. forsteri* and *P. papua* but is slightly curved in *S. demersus*. The largest penguin in the study, *A. forsteri*, has the columella with the largest dimensions, although its footplate area is the smallest in relation to its shaft length compared to the other two species. The ratio between the area of the tympanic membrane and the area of the footplate of the columella is given in Table 3. It ranges between 12.88:1 and 13.65:1 for the three measured species.

The tympanic cavity is connected to pneumatic recesses in the skull and the pharyngotympanic tube (*Tuba auditiva*). The pharyngotympanic tube links the ventral portion of the tympanic cavity to the oral cavity (Fig. 1). On both sides of the skull, these components unite as the *Tuba auditiva communis* and join the oral cavity at the palate via a single porus (*Ostium pharyn-*

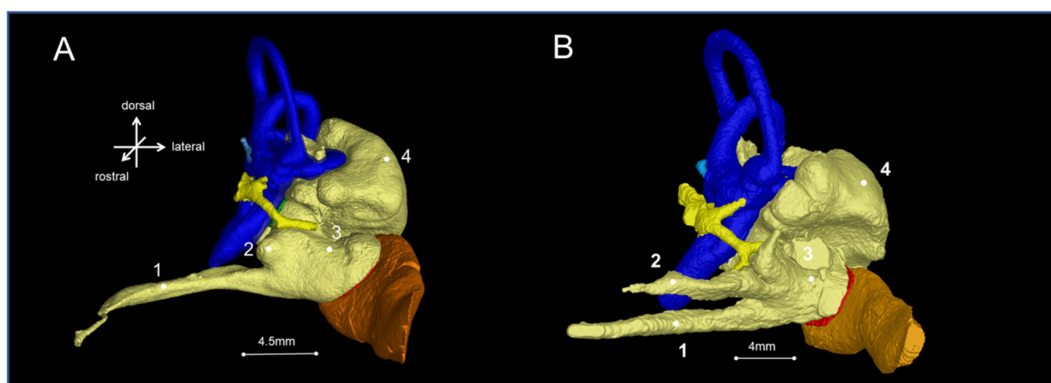


Fig. 1: 3D reconstruction of the ear structures of A) the African Penguin *Spheniscus demersus* B) the Gentoo Penguin *Pygoscelis papua*; left side, frontal view; **colors:** blue inner ear, yellow Nervus vestibulocochlearis and N. facialis, golden tympanic cavity with recesses and pharyngotympanic tube, red tympanic membrane, orange external meatus; **numbers:** 1 Tuba auditiva, 2 Recessus tympanicus rostralis, 3 Cavitas tympanica, 4 Recessus tympanicus caudalis

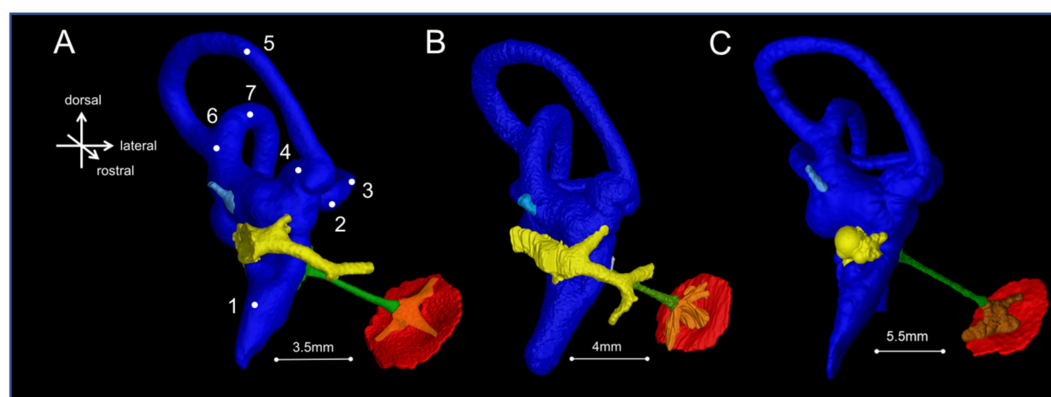


Fig. 2: 3D reconstruction of the bony inner ear, columella, and tympanic membrane of A) the African Penguin *Spheniscus demersus* B) the Gentoo Penguin *Pygoscelis papua* C) the Emperor Penguin *Aptenodytes forsteri*; left side, mediofrontal view; **colors:** dark blue inner ear, yellow Nervus vestibulocochlearis and N. facialis, light blue Ductus endolymphaticus, green columella, red tympanic membrane, orange Cartilago extracolumellaris; **numbers:** 1 Cochlea, 2 Ampulla lateralis, 3 Ductus semicircularis lateralis, 4 Ampulla anterior, 5 Ductus semicircularis anterior, 6 Crus commune, 7 Ductus semicircularis posterior

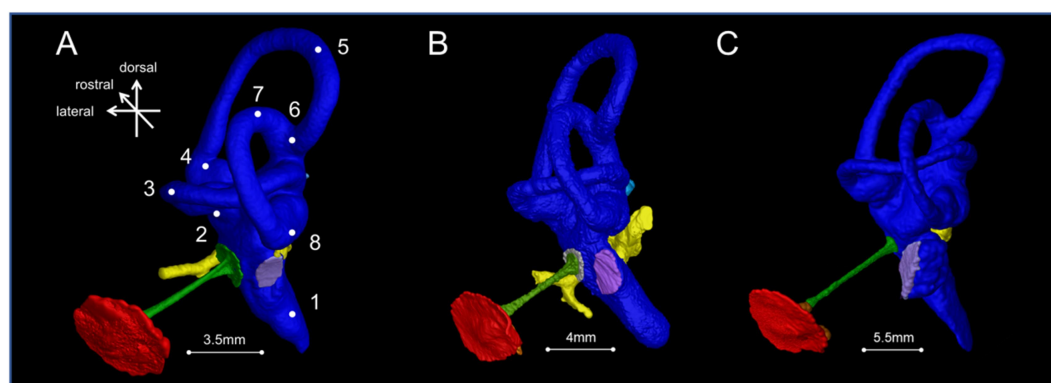


Fig. 3: 3D reconstruction of the bony inner ear, columella, and tympanic membrane of A) the African Penguin *Spheniscus demersus* B) the Gentoo Penguin *Pygoscelis papua* C) the Emperor Penguin *Aptenodytes forsteri*; left side, caudolateral view; **colors:** dark blue inner ear, yellow Nervus vestibulocochlearis and N. facialis, light blue Ductus endolymphaticus, green columella, red tympanic membrane, orange Cartilago extracolumellaris, white Ligamentum anulare, violet Membrana tympanica secundaria; **numbers:** 1 Cochlea, 2 Ampulla lateralis, 3 Ductus semicircularis lateralis, 4 Ampulla anterior, 5 Ductus semicircularis anterior, 6 Crus commune, 7 Ductus semicircularis posterior, 8 Ampulla posterior

geale) caudally of the pterygoid (Os pterygoideum) (see visualization in Bendel et al. 2019, Westphal et al. 2019 and Frahnert et al. 2020). The pharyngotympanic tube is enclosed by a bony canal in its caudal part. In *P. papua* and *S. demersus* the canal for the tube is opened laterally at its rostral end (Fig. 6).

Penguins only have two recesses connected to the tympanic cavity: The caudal recess (Recessus tympanicus caudalis) is an extended pneumatized cavity which is well developed in the three species of penguins (not segmented for *A. forsteri* (Westphal et al. 2019)). It is mostly orientated laterally and caudally in relation to the inner ear but does not extend as far dorsally as the posterior semicircular canal (Canalis semicircularis posterior) (Fig. 1). The rostral recess (Recessus tympanicus rostralis), a frontal extension of the tympanic cavity, is situated dorsally of the pharyngotympanic tube. The extension of this recess differs in the three investigated species: it reaches far rostrally and medially in *A. forsteri* and *P. papua*, nearly until the rostral end of the bony canal for the pharyngotympanic tube. However, it is much shorter in *S. demersus*. In none of the studied species, the contralateral recesses communicate (Fig. 1).

The tympanic cavity as well as its recesses is air-filled. The caudal recess of *A. forsteri* appears to be lined by a thicker layer of soft tissue in the scans, but the resolution of the scans does not allow for a specification to what kind of tissue it is (Fig. 5). Similar findings could be made for *S. demersus* (smaller portions of such a tissue). In *P. papua*, however, the recess was completely air-filled.

The quadrate lacks a pneumatic foramen.

Table 3: Middle ear measurements

	<i>Pygoscelis papua</i>	<i>Aptenodytes forsteri</i>	<i>Spheniscus demersus</i>
Membrana tympanica area (mm ²)	18.93	27.30	20.67
Basis columellae area (mm ²)	1.47	2.00	1.55
Area of M. tympanica : Area of B. columellae	12.88:1	13.65:1	13.33:1

C. THE INNER EAR

The inner ear is composed of the cochlear organ (Labyrinthus cochlearis) as well as the vestibular organ (Labyrinthus vestibularis) and is enclosed in the bony cavities. The conservation status and the staining of the three specimens was not sufficient to study the anatomy of the membranous inner ear (Labyrinthus membranaceus), only in *S. demersus* it was partially visible. Therefore, the description only contains information about the bony inner ear (Labyrinthus osseus). The anatomy of this complex is very similar in all three analyzed species (Fig. 2, 3).

There are two windows to the cochlea: the round window (Fenestra cochleae) and the oval window (Fenestra vestibuli) rostradorsal of the former. Dorsally to the cochlea lies the vestibule (Vestibulum) which extends in the common crus (Crus commune). There are three osseous semicircular canals (Canalis semicircularis anterior, Canalis semicircularis posterior and Canalis semicircularis lateralis). The anterior semicircular canal lies in the medial plain, arising caudally from the common crus and bends into a rostral direction to the anterior ampulla (Ampulla ossea anterior). The posterior and lateral canal cross each other diagonally and are arranged laterally. The posterior semicircular canal arises rostrally from the common crus and bends into a ventral direction to the posterior ampulla (Ampulla ossea posterior). The lateral semicircular canal emerges caudally between the common crus and the posterior ampulla and bends into a rostroventral direction to the lateral ampulla (Ampulla ossea lateralis). At the lateral connection with the posterior canal, the lateral semicircular canal is slightly indented.

Fig. 4: CT slice of the otic region of the **Gentoo Penguin *Pygoscelis papua***; left side, frontal plain at the level of the columella, **numbers:** 1 Labyrinthus osseus, 2 Basis columellae, 3 Cavitas tympanica, 4 Scapus columellae, 5 Cartilago extracolumellaris, 6 Membrana tympanica, 7 Meatus acusticus externus, 8 Quadratum, 9 Recessus tympanicus caudalis, 10 Cavitas cranialis, 11 Cavum pharyngis

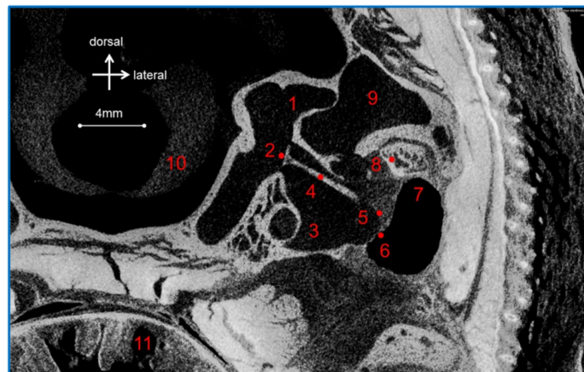


Fig. 5: CT slice of the otic region of the **Emperor Penguin *Aptenodytes forsteri***; left side, frontal plain at the level of the pharyngotympanic tubes, **numbers:** 1 Recessus tympanicus caudalis, 2 soft tissue resp. Corpus cavernosum (Sadé 2008) 3 air-filled lumen, 4 Tuba auditiva, 5 Canalis caroticus, 6 Cavitas cranialis

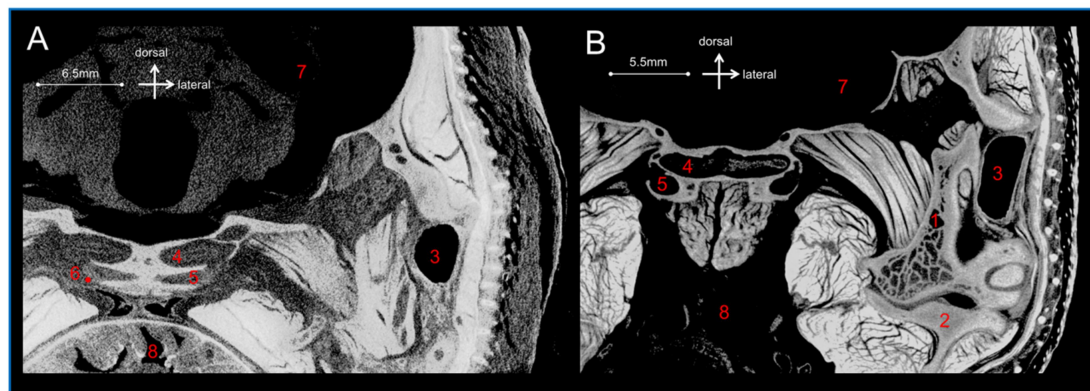
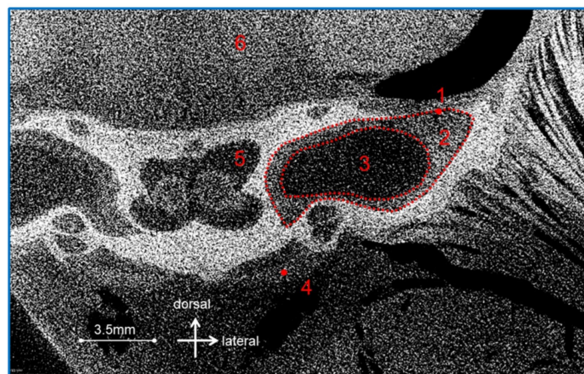


Fig. 6: CT slice of the otic region of **A) the Gentoo Penguin *Pygoscelis papua* B) the African Penguin *Spheniscus demersus***; left side, frontal plain at the level of the pharyngotympanic tube, **numbers:** 1 Quadratum, 2 Os articulare, 3 Meatus acusticus externus, 4 Canalis caroticus 5 Canalis tubae auditivae, 6 Tuba auditiva, 7 Cavitas cranialis 8 Cavum pharyngis

The inner ear of the different penguin species is very similar in shape and shows only minor variations. The anterior canal of the three penguins is shaped like an elongated, upside-down “U” and is oblique towards its dorsocaudal end. In *S. demersus* the superior portion of the rostral canal is slightly bent towards the medial. Another variation can also be recognized at the tip of the common crus: In *P. papua*, the common crus seems to be elongated and the canals appear relatively thick compared to the other species (Fig. 2, 3).

In lateral view the posterior canal appears vertical in *S. demersus* while it is slightly oblique in different directions in *A. forsteri* and *P. papua* (Fig. 3). The lateral and posterior canal of *S. demersus* almost cross perpendicularly, while in *A. forsteri* and *P. papua* a ventrolateral curvature of the distal portion of the lateral canal leads to the impression that angles are more deviating. The semicircular canals of all species are compressed.

4. DISCUSSION

A. STAINING QUALITY

There was no set protocol for iodine staining for bird heads of penguin size available. Although the staining duration was adapted to the individual object, the staining result was not optimal for tissue differentiation. The best differentiation was reached in *S. demersus* and the least useful in *P. papua* (Fig. 6). As already reported, the maximum staining is dependent on the conservation status and time as well as the kind of the fixation medium of the specimens (Gignac et al. 2016). The formalin fixation as well as the storage in the same medium and the subsequent transfer to another storage medium (alcohol) of *P. papua* evidently prevented the binding of iodine to the tissues (see also Gignac et al. 2016). As the specimens of *S. demersus* and *A. forsteri* originated from the historic bird collection of the Museum für Naturkunde Berlin, there is no information about the fixation medium and the accession date available. Therefore, the obvious differences of the staining results of both individuals cannot easily be interpreted. The good staining result of *S. demersus* in combination with the notice “captivity” might be a hint that the specimen came in good condition from the Zoological Garden Berlin in the second half of the 20th century and was not fixated in formalin.

Additionally, the long storage in the water based staining solution seems to cause some changes in the tissue structure (e.g. loss of feathers). Furthermore, remains of the storage medium or staining liquids usually in air-filled organs and structures caused problems in the analysis, as the tissues then proved to be difficult to distinguish in the scan data. Accordingly, due to these obstacles as well as the natural limits of resolution of CT scanning it was not possible to analyze very small structures such as small vessels and nerves as well as the membranous inner ear. Moreover, the staining solution did not always penetrate into the skull as far as the designated soft tissues and therefore some structures could not be interpreted with certainty.

B. PROTECTION AGAINST PENETRATING WATER

Penetration by water in the external ear canal might potentially interfere with a hearing process adapted more to hearing in air. Since the external ear opening is completely covered by dense feathers, these prevent water from entering into the ear cavity (Dooling and Therrien 2012) but it is not certain whether this mechanism is sufficient to completely protect the external ear canal from water intrusion, especially under the influence of strong and changing ambient water pressure.

In the CT scans the external ear canal is air-filled and has a large diameter to receive and transfer incoming sound. The course of the ear canal is straight. This further complicates the prevention of entering water. No curvature of the outer ear canal was detected in the observed penguin species, excluding the possibility of curvature acting as a potential closing mechanism as was proposed for whales and auks (de Vreese et al. 2014, Fraser and Purves 1960, Kartaschew and Iljitschow 1964). Moreover, no circular muscles around the ear opening could be identified in the scan data (contra Sade et al. 2008). It might be the case that muscles near the ear canal can pull the round meatus into a slit, which could explain the different shape of the ear openings in the three penguin species. For this, a segmentation of muscles around the meatus needs to be performed. Furthermore, by studying the tissues surrounding the outer ear canal in detail it could be determined whether a venous corpus cavernosum around the ear canal is present which could constrict the lumen of the cutaneous canal (see Sade et al. 2008).

C. ADAPTATIONS TO STRONG PRESSURE CHANGES

Due to their semiaquatic lifestyle, especially jumping into water and diving rapidly in deep water (more than 400 m depth for *Aptenodytes forsteri* (Kooyman and Kooyman 1995)); penguins have to cope with rapid and strong ambient pressure changes. Negative pressure differences due to insufficient pressure equilibration might be problematic for thin structures such as membranes as well as filigree bones such as the columella (e.g. Kringlebotn 2000). In the ear region the air-filled cavities of the outer and the middle ear are especially affected and an effective pressure equilibration mechanism is necessary to prevent baro-traumata.

In mammals, this compensation is often achieved via the pharyngotympanic tube and the oral cavity (Heldmaier and Neuweiler 2003, Odend'hal and Poulter 1966). This connection is present in penguins as well. However, it is not yet proven whether this mechanism also functions in birds under water. On the one hand, it needs to be clarified whether the remaining air reservoirs in the mouth of penguins during diving facilitate pressure equilibration. On the other hand, a possible opening-closure mechanism of the pharyngotympanic tube on its rostral end has yet to be described in detail.

Sade et al. (2008) described the presence of a venous corpus cavernosum in the middle ear and surrounding the external ear canal of *Aptenodytes patagonicus*. It expands when the external pressure rises (see also Odend'hal and Poulter 1966). This way it replaces the relative air volume loss with an incompressible medium and protects the tympanic membrane from injury. In this CT study, soft tissues were found in *A. forsteri* at the wall of the caudal tympanic recess. It could potentially be the same structure as that mentioned by Sade et al. (2008). By comparison, in *A. forsteri* it does not fill the whole recess. In *S. demersus* there are some remaining tissues (and maybe liquids) in this recess. In *P. papua*, the recess was completely air-filled, no soft tissue was detectable. Assuming that the tissue found in this study is a corpus cavernosum, its distribution in the different taxa is possibly correlated with varying diving depth of the three species with *S. demersus* reaching around 130 m (Wilson 1985) and *P. papua* reaching 210 m (Bost et al. 1994).

D. ADAPTATIONS TO UNDERWATER HEARING

It was analyzed whether morphological traits could be observed like those already discussed as adaptations to underwater hearing in other vertebrates. The general morphology of all parts of the ear in penguins is the same as in land birds specialized to hearing in air (Stresemann 1927, Kühne and Lewis 1985). The external air sound is transmitted via the tympanic membrane and transferred by the columella to the oval window and the inner ear (Heldmaier and Neuweiler 2003, Kühne and Lewis 1985). No other mechanism of sound transmission as for instance via the lower jaw and tympanic bulla could be interpreted on the base of this morphology (see Ladich and Winkler 2017). There is also no evidence for sheathed fat in the external ear canal for penguins so far (see also Ketten 1999 for similar investigations on auks and murre). Furthermore, the bony ear capsule is fully connected to the skull and no sound isolation between both structures is visible in the scans. This makes it unlikely that penguins have specialized sound producing organs in the skull as for instance toothed whales developed for ultrasound detection (e.g. Mooney et al. 2014, Nummela et al. 2007, Reidenberg 2007).

E. RATIO OF THE TYMPANIC MEMBRANE AREA TO THE COLUMELLA FOOTPLATE AREA

The ratio of the tympanic membrane area to the columella footplate area is a crucial aspect for the function of sound amplification in the middle ear (Kühne and Lewis 1985, Schwartzkopff 1955). The columella shaft, which is connected to the eardrum via the extracolumella, transfers

the force of the pressure wave acting on the large tympanic membrane to the relatively small columella footplate. This causes the main portion of the amplification of sound pressure.

All three penguin species have a ratio of circa 13:1. Most birds have area ratios of 15:1 up to 40:1 (Schwartzkopff 1955, 1957), but Kartascheff and Iljitschow (1964) gave ranges from 3:1 to 13:1 for Alcidae and Kühne and Lewis (1985) 11:1 to 15:1 for Podicipedidae. That means that the ratio in penguins is to be found at the lower end of the scale of Schwartzkopff (1955), but in between the semiaquatic Alcidae and Podicipedidae. This low ratio may be an effect of the semi-aquatic lifestyle to protect the tympanic membrane since it is comparatively small. By contrast, it is well known that penguins have a well-functioning hearing organ that allows social communication, at least on land (Jouventin et al. 1999). It still needs to be studied whether other effects play a role in this respect. Especially the long scapus could be effective in tilting, a mechanism that is known for modulation of sensitivity (Kühne and Lewis 1985). Finally, it cannot be excluded that the differences in the measuring methods influence deviations from the data in the literature.

F. RELATIONSHIP OF THE TYMPANIC MEMBRANE TO THE QUADRATE

The quadrate has a very close relation to the tympanic membrane. However, it is not clearly visible in the scans to what extent the membrane is fixed to the otic process of the quadrate. The literature gives contradictory evidence regarding the exact relationship of the quadrate and the tympanic membrane as well as about the influence of the movement of the quadrate on the hearing process in birds (Pohlmann 1921, Stresemann 1927, Kühne and Lewis 1985, contra Saiff 1976, Claes et al. 2017), but both structures are definitely situated side by side. The quadrate is involved in the craniokinesis in birds: if the beak is opened, the quadrate is dislocated backwards with the pivot point at the otic process. Therefore, the backward shift should be minimal in the contact region to the tympanic membrane but the membrane should be minimally released if the beak is opened (see detailed study for the chicken in Claes et al. 2017). Further studies are necessary to estimate the impact of this effect on the hearing abilities.

G. INTERAURAL PATHWAYS

Nearly all birds have air-filled connections between the tympanic recesses of both sides of the body, the interaural pathways (Ksepka et al. 2012). Such connections could not be traced in the analyzed penguins. Only *Aptenodytes forsteri* seem to preserve remains of such a structure in the rostral tympanic recess. As those connections are described for fossil penguins as well (*Paraptenodytes patagonicus*, Ksepka et al. 2012), it seems that these became lost during reduction of the pneumatized spaces in the skull during penguin evolution (Ksepka et al. 2012, Smith 2011). Ksepka et al. (2012) interpreted this as an effect of paedomorphosis. The interaural pathways are often discussed as support for the directional hearing in small birds (Calford and Piddington 1988), but the abilities of directional hearing in penguins and its functional background in relation to body and head size needs to be studied in more detail in the future.

5. CONCLUSIONS

The analyzed morphological structures of the penguin ear give no hint on specific adaptations for analyzing sound in water so far. However, in vertebrates, the transition from living on land exclusively to the ability to dwell in water occurred several times with different adaptations to underwater hearing (Ladich and Winkler 2017). It might therefore be possible that penguins developed so far unknown other mechanisms. Possible mechanisms to prevent baro-traumata under rapidly changing ambient pressure are discussed but their exact function has to be

analyzed in more detail. In these respects, studies on the lever function of the columella as well as on the musculature and venous structures in the region of the external ear canal, the rostral opening of the pharyngotympanic tube and the middle ear recesses are proposed.

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