ANATOMY AND DEVELOPMENT OF THE BONY INNER EAR IN THE WOOLLY OPOSSUM, *Caluromys philander* (DIDELPHIMORPHIA, MARSUPIALIA)

Marcelo R. Sánchez-Villagra¹ and Thomas Schmelzle²,³

¹ Palaeontologisches Institut und Museum, Karl Schmid-Strasse 4, CH-8006 Zürich, Switzerland, <m.sanchez@pim.uzh.ch> ² Zoologisches Institut, Spezielle Zoologie, Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen. ³ Department of Biology II, University of Munich, D-82152 Germany.

ABSTRACT: The ontogenetic changes of the skeletal tissue of the vestibular and cochlear system of *Caluromys philander* were investigated using CT scans of an adult skull and 3D reconstructions of histological serial sections of three pouch-youngs. The bony labyrinth of *C. philander* differs in several aspects from what has been reported for other marsupials. Some of the proportions of the semicircular canals (SC) are probably characteristic of arboreal as opposed to terrestrial species. Several significant changes in the SCs shape occur postnatally. The most remarkable difference among stages is the different height of the anterior SC and posterior SC in relation to the common crus. In the adult the posterior arm of the lateral SC and the inferior arm of the posterior SC build a common crus, a condition of several basal metatherians and crown-group marsupials. The number of turns of the cochlea in the adult is 2.4.

RESUMEN: Anatomía y desarrollo de los huesos del oído interno de *Caluromys philander* (Didelphimorphia, Marsupialia). Los cambios ontogenéticos en el esqueleto asociado al aparato vestibular y la cochlea de *Caluromys philander* fueron investigados usando CT scans de un cráneo adulto y reconstrucciones 3D de cortes histológicos de tres estadíos tempranos postnatales. El laberinto óseo de *C. philander* difiere en varios aspectos del de otros marsupiales. Algunas de las proporciones de los canales semicirculares (CS) son probablemente características para hábitos arborícolas. Varios cambios significativos en los CSs ocurren luego del nacimiento. La diferencia más notable entre estadíos es la altura diferente del CS anterior y posterior en relación con el ‘common crus’. En el adulto el brazo posterior del CS lateral y el brazo inferior del CS posterior forman un ‘common crus’, una condición característica de varias especies basales de marsupiales. El número de vueltas de la cochlea en el adulto es 2.4.

Key words. Cochlea. CT. Marsupialia. Phylogeny. Semicircular canal.

INTRODUCTION

Didelphids constitute a basal group of marsupials consisting of relatively generalized forms thought to provide a model or at least appropriate comparison in studies of basal therian evolution (Szalay, 1994). This idea has lead to numerous studies of didelphid anatomy, many of them concentrating on the opossums *Didelphis* (e.g., Coues, 1872; Toeplitz, 1920; McClain, 1939) and *Monodelphis* (e.g., Clark and Smith, 1993; Macrini, 2002; Wible, 2003). But the group is diverse, with about 17 genera and 85 species (Voss and Jansa, 2003; Gardner, 2005).

One area of the anatomy of didelphids that has been long neglected because of the invasive nature of its study is the bony labyrinth (Hyrtl, 1845). This structure contains the organs of balance (vestibular system) and of hearing (cochlear system), and can provide functional and phylogenetic information about vertebrates (Spoor and Zonneveld, 1998). With the development of micro-computer tomography (CT) technology, which provides a non-invasive method of skeletal investigations (Rowe et al., 1997; Zollikofer and Ponce de León, 2005), studies of the inner ear of mammals have flourished in recent years (e.g., Spoor and Zonneveld, 1998). The bony labyrinth represents a proxy for the shape and relative size of the internal soft-tissue sensory structures it contains (Spoor et al., 1994). This is particularly important for palaeontological studies that have attempted to find functional correlates of bony inner ear anatomy (e.g. Graybeal et al., 1989; Hurum, 1998).

The bony labyrinth morphology of marsupials is poorly documented. There are the references to the American opossum *Didelphis* in older literature (Hyrtl, 1845), but the specific taxonomic allocation of the materials studied in this pioneering study is questionable. The classic and comprehensive study of the labyrinth of mammals by Gray (1907) included eight Australian marsupials, and reported for them some inner ear measurements (see summary in the supplementary information of Spoor et al., 2002). Meng and Fox (1995) reported on the inner ear of isolated petrosals from the Late Cretaceous of Montana belonging potentially to basal metatherians. Ekdale (2005) studied variation in bony inner ear anatomy in six ontogenetic stages of *Monodelphis domestica* using CT scans, and reported in this abstract that “many dimensions of the inner ear are apparently age independent”. Other contributions have either discussed soft-tissue aspects not treated here (e.g., Larsell et al., 1935) or are discussed below. In this paper we describe the bony labyrinth adult anatomy and development of the woolly tailed opossum, *Caluromys philander*, which has been subject of some anatomical, functional morphological and ecological studies by primatologists and other mammalogists, in part because of its arboreal adaptations (e.g. Schmitt and Lemelin, 2002). *Caluromys* is one of the outgroups of Didelphinae, the clade containing the majority of didelphid marsupials (Voss and Jansa, 2003). Therefore, *Caluromys* is one of the most basally-diverging taxon of Didelphidae and extant marsupials.

METHODS AND MATERIALS

CT scanning of adult material

A macerated skull deposited in the Zoologisches Forschungsinstitut und Museum Alexander König, Bonn (ZFMK-unnumbered, foramen magnum width = 7.8 mm) was scanned in the frontal plane with a micro-CT-Scanner RayScan 200 at the Fachhochschule Aalen, Germany. Both sides of the skull were scanned, but only the left side was studied, considering that only small differences exist between sides for the kind of characters studied (Spoor and Zonneveld, 1998). The graphic files produced by the CT scan were used to make a 3D reconstruction of the inner ear using VGStudio Max 1.1®. In order to generate a virtual cast of the inner ear it was necessary to produce a negative of the file. After this change of the CT scan data into a matrix of polygons, the software Cinema 4d® R8 XL (Losch et al., 1999) was used to delete the reconstructed areas of the petrosal bone that cover the bony labyrinth.

Abbreviations used are: ASC anterior semicircular canal; LSC lateral semicircular canal; PSC posterior semicircular canal; ASCl length of the ASC; ASCw width of the ASC; LSCI length of the
SKELETAL INNER EAR OF *Caluromys*

LSC; LSCw width of the LSC; PSCI length of the PSC; PSCw width of the PSC; AAP angle between ASC and PSC; APA ampullar line, connecting the centres of the anterior and posterior ampullae, and projected onto the sagittal plane; ASC-R radius of curvature of the ASC; LSC-R radius of curvature of the LSC; PSC-R radius of curvature of the PSC; SLI sagittal labyrinthine index.

Linear and angular measurements were taken using Amira 4® directly on the 3D-model. Fig. 1 shows the studied adult specimen in different views, including the anatomical terminology used in the text. Our aim was to describe the spatial relations among semicircular canals and obtain data as in our previous publication on marsupials (Schmelzle and Sánchez-Villagra, 2005; Schmelzle et al., 2007) and in that way build a database for future comparative studies. The radius of curvature of each semicircular canal was recorded, measured to the inner border of the canal. The orientation and position of the semicircular canals are reported assuming a horizontal position of the LSC, which may or may not reflect the situation in real life.

The number of turns of the cochlea was quantified using the method of West (1985), and was compared with data obtained from the CT scans studied by Schmelzle and Sánchez-Villagra (2005) and Schmelzle et al. (2007) for eight diprotodontians and reported here for the first time.

**Ontogenetic data**

We studied histological serial sections of three pouch-young specimens deposited in the collections of the Zoologisches Institut, University of Tübingen (ZIUT). The specimens are of unknown age, but represent with certainty the first several days after birth. Photographs of the two smallest ones before sectioning were published by Sánchez-Villagra et al. (2002), who described and modelled several aspects of middle ear anatomy in these specimens. The three specimens have a crown-rump length (CRL, mm) of 11.5, 20 and 25, respectively. The smallest specimen was cut at 10µ section thicknesses and the other two pouch young at 20µ. The three-dimensional models of the serial sections of the cartilaginous/bony labyrinth were produced by using the software Amira 4®. Every section of the smallest specimen was considered for the 3D modelling, whereas only one of every three sections was considered for the other two.

We report on the ontogenetic changes of the skeletal tissue aspects of the vestibular and cochlear system based on the available data, with the caveat that the 3D models of the pouch-young specimens show artefacts resulting from the shrinking and alignment problems associated with the histological serial sections. As indicated below, the reported measurements for the pouch-younsg are only best approximations.

**RESULTS**

**Description of adult anatomy and comparisons**

Values for several measurements are shown in Table 1. *Caluromys philander* has a rounded ASC. The LSC has its boundary at the lateral (maximal) extension of the PSC instead of being shorter, as is the case in some terrestrial diprotodontian marsupials (Schmelzle and Sánchez-Villagra, 2005; Schmelzle et al., 2007). The LSC is straight in its transition to the lateral ampulla (Fig. 1) as opposed to undulating as in some diprotodontians (Schmelzle and Sánchez-Villagra, 2005; Schmelzle et al., 2007). The PSC is straight when viewed in the plane of the canal, as opposed to curved. The overall shape of the PSC, viewed perpendicular to the plane of the canal, is round as opposed to oval. The posterior arm of the LSC and the inferior arm of the PSC build a common crus (see Discussion). The angle between ASC and PSC is 95°, and the angle between ASC and the sagittal plane is 46°. The angle of the LSC relative to the sagittal plane is less than 81°. The angle going through mediolus of the cochlea and centre of LSC is less than 36°.

The number of turns of the cochlea (sensu West, 1985) in adults are as follows: Didelphimorphia; *Caluromys philander* 2.4, Diprotodontia; *Bettongia penicillata* 2.4, *Dendrolagus* sp. 1.8, *Dorcopsis muelleri* 2.3, *Macropus eugenii* 2.9, *Petauroides volans* 2.8, *Phascolarctos cinereus* 2.8, *Pseudocheirus peregrinus* 2.8, *Vombatus ursinus* 1.4.

**Ontogeny**

The adult proportions of the semicircular canals are more or less present already in the earliest stage examined (Fig. 2). The most remarkable difference among stages is the dif-
ferent height of the ASC and PSC in relation to the common crus.

The s-shape of the LSC in the younger stages is probably an artefact resulting from the alignment of sections during the 3D reconstruction. Some other apparent changes may also be in part the result of this artefact, such as the orientation of the cochlea relative to the LSC. However, several changes clearly occur during ontogeny.

In the adult and in the specimen of CRL=25mm the two angular points of the corresponding semicircular canals reach beyond the level of the common crus. In neither CRL=11.5 mm nor 20 mm is there an elevation of the semicircular canals over the common crus. The common crus itself changes during ontogeny. CRL=11.5 mm has a relatively wide basis in relation to the total length, which becomes smaller later in ontogeny. In the adult and in the pouch youngs examined there is additionally a second common crus, built by the posterior arm of the LSC and the inferior arm of the PSC. The histological sections of the pouch youngs examined show that the membranous labyrinth within the bony

Table 1

Measurements of the vestibular bony labyrinth in three pouch-young and one adult Caluromys philander. As indicated, several of the measurements of the younger specimens are also approximate, due to the artefacts resulting from alignment before the three-dimensional reconstruction of histological sections. See Materials and Methods for a list of abbreviations.

<table>
<thead>
<tr>
<th></th>
<th>ZIUT</th>
<th>ZIUT</th>
<th>ZIUT</th>
<th>Adult</th>
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<tbody>
<tr>
<td></td>
<td>CRL=11.5mm</td>
<td>CRL=20mm</td>
<td>CRL=25mm</td>
<td>(ZFMK)</td>
</tr>
<tr>
<td>ASCII</td>
<td>ca. 0.32</td>
<td>ca. 0.78</td>
<td>ca. 2.13</td>
<td>2.62</td>
</tr>
<tr>
<td>ASCw</td>
<td>ca. 0.5</td>
<td>ca. 1.03</td>
<td>ca. 2.03</td>
<td>2.48</td>
</tr>
<tr>
<td>LSCI</td>
<td>ca. 0.14</td>
<td>ca. 0.36</td>
<td>ca. 1.6</td>
<td>1.64</td>
</tr>
<tr>
<td>LSCw</td>
<td>ca. 0.25</td>
<td>ca. 0.62</td>
<td>ca. 1.7</td>
<td>1.74</td>
</tr>
<tr>
<td>PSCI</td>
<td>ca. 0.28</td>
<td>ca. 0.59</td>
<td>ca. 2</td>
<td>1.75</td>
</tr>
<tr>
<td>PSCw</td>
<td>ca. 0.24</td>
<td>ca. 0.57</td>
<td>ca. 1.64</td>
<td>1.91</td>
</tr>
<tr>
<td>AAP</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>95.3</td>
</tr>
<tr>
<td>APA</td>
<td>ca. 13.9</td>
<td>ca. 9.23</td>
<td>ca. 13.7</td>
<td>9.06</td>
</tr>
<tr>
<td>ASC-R</td>
<td>ca. 0.21</td>
<td>ca. 0.45</td>
<td>ca. 0.45</td>
<td>1.28</td>
</tr>
<tr>
<td>LSC-R</td>
<td>ca. 0.1</td>
<td>ca. 0.25</td>
<td>ca. 0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>PSC-R</td>
<td>ca. 0.13</td>
<td>ca. 0.29</td>
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labyrinth is not connected in the area the bony labyrinth is to form the second common crus, so both canals enter the vestibulum separately.

Another marked change concerns the position of the anterior ampulla relative to the lateral ampulla. From the youngest stage to the adult there is a rotation at the level of the orifice of the ASC. The pouch young exhibit the orifices posterodorsally, whereas the adult does it anteriorly. This position is related to the high value of the APA, which is higher in the earliest stage than in the adult.

The LSC makes a significant change during ontogeny. In the two youngest stages the LSC is still relatively wide and short and possesses an orientation towards the vestibulum. In the CRL=25 mm stage, in contrast to the nearly round shape of the adult, the LSC is oval. The maximum diameter of the LSC is oriented posterolaterally.

The vestibulum increases in volume in the series studied. The space for the utriculus and saccus is not more than a small connection to the area in which the cochlear duct is located. Already in the CRL=20 mm stage this previously small space becomes wider.

In the earliest stage the cochlea is just a simple structure. The histology shows that the lateral wall of the promontorium is not even fully cartilaginous, which is not shown in the model, in which the inner room is shown independent of the type of surrounding tissue. The CRL=20 mm stage exhibits 2.3 turns of the cochlea, the direction of which is anteroventral. The CRL=25 mm stage exhibits 2.4 turns, as recorded in the adult. The direction of the axis of the cochlea is medioventral.
The only difference in the cochlea between the adult and the CRL=25 mm stage is its orientation. In the adult the axis of the cochlea is more medially orientated than in the CRL=25mm stage.

DISCUSSION

The bony labyrinth shape of *Caluromys philander* differs in several aspects from what has been reported in the few marsupial species studied so far. As expected, a lot of significant changes in the development of this structure occur postnatally. Some of the proportions of the semicircular canals in *C. philander* are probably characteristic of arboreal as opposed to terrestrial species, but this will be established only after data for other didelphids and basal metatherians of different locomotory style become available.

Schmelzle and Sánchez-Villagra (2005) and Schmelzle et al. (2007) found differences between arboreal and terrestrial species examined in a pilot study on diprotodontians, especially in the extension of the LSC and the relation of the anterior to PSC. They found that the LSC is well developed in arboreal species in comparison to terrestrial species. Taking the ASC as reference, the maximal lateral extension (apex) reaches up to the level of the lateral extension of the PSC. This condition of the arboreal diprotodontians is also present in *C. philander*. In contrast, in terrestrial diprotodontians the LSC does not reach the apex of the PSC.

Terrestrial species of the diprotodontians studied by Schmelzle and Sánchez-Villagra (2005) and Schmelzle et al. (2007) are characterized by a relatively larger ASC than in arboreal species. *C. philander* does not follow this pattern, as it exhibits a relatively large ASC. Comparisons with didelphids are of course necessary. Another feature reported by Schmelzle and Sánchez-Villagra (2005) and Schmelzle et al. (2007) for diprotodontians is the caudal view of the posterior arc of the LSC just before the ‘contact’ with the vestibulum, which exhibits a curvature in the case of the arboreal species. *C. philander* lacks this feature, as do the terrestrial diprotodontians studied by Schmelzle and Sánchez-Villagra (2005) and Schmelzle et al. (2007).

Hyrtl (1845) investigated the number of openings at the vestibulum that lead to the semicircular canals. With a few exceptions, the number of openings is usually five. They are located at the anterior arm of the ASC, the anterior arm of the LSC, the posterior arm of the LSC, the inferior arm of the PSC, and the so-called common crus, the meeting point of the posterior arm of the ASC and the superior arm of the PSC. In some species, there is a deviation to this general pattern, because the posterior arm of the LSC and the inferior arm of the PSC build a second common crus. This condition is found indeed in *C. philander*, as well as in *Dasyurus, Monodelphis, Isoodon, Herpetotherium* from the Oligocene of Wyoming (Sánchez-Villagra et al., 2007), and in a palaeothentid caenolestoid from the early Miocene of Patagonia (Sánchez-Villagra et al., unpublished). Meng and Fox (1995) described the inner ear in fossil eutherians in which there is a second common crus resulting from the fusion of the posterior arm of the LSC and the inferior arm of the PSC. According to these authors, this condition is found amongst placentals only in Canidae, but Hyrtl (1845) had already illustrated it in *Felis leo, F. tigris, F. pardus, Canis lupus, C. aureus, Paradoxurus* and *Herpestes*. This second common crus is absent in a Late Cretaceous metatherian from Montana (Meng and Fox, 1995).

The relative exactitude of the kind of data produced with CT scans for the inner ear has already been explored by Spoor and Zonneveld (1995) in a comparison with histological sections. The latter can reveal detail in both bony and soft tissue structures that is simply unchallenged, with the caveat that artefacts of preservation and fixation (e.g. shrinking) can affect morphometric studies based on such materials.

Jeffery and Spoor (2004) showed that in humans the labyrinth does not change after its complete ossification. It is to be expected that in adult marsupials there are also no changes
in the labyrinth once this is completely ossified. But the ossification of the labyrinth in humans is completed before birth, whereas in marsupials much of organogenesis takes place outside the uterus, posing the question of what the influence of the external environment may have on labyrinth development. To test this, future studies could take a similar morphometric approach to that of Jeffery and Spoor (2004) in humans to study labyrinth development in marsupials showing differing degrees of altriciality at birth (Hughes and Hall, 1988). Gemmel and Rose (1989) reported differences in the morphology of the utricle, cochlea and semicircular canals at birth among different Australian marsupial species.

We have no information on intra-specific adult variation in *C. philander* for the features examined, but we hypothesize based on previous studies of primates by F. Spoor and collaborators (e.g. Spoor and Zonneveld, 1998 and references therein), that many if not most features reported here are not variable within species. Ekdale (2005) also found little variation among quantitative characters of the inner ear measured from a sample of *Monodelphis domestica*.

*Caluromys philander* exhibits 2.4 cochlear turns, almost the same as the two and a half turns reported for *Didelphis* (McCray, 1938; Fernández and Schmidt, 1963). As is the case for *Didelphis* (McCray, 1938), the adult number of cochlear turns in *C. philander* is attained postnatally. The one-week postnatal *Didelphis* exhibits still one and a half cochlear turns (McCray, 1938).

Meng and Fox (1995) reported on basal metatherian petrosals from the Late Cretaceous of Montana, with only one and half turns of the cochlea. *Didelphis* and *Caluromys* are derived in the turns of the cochlea, in comparison to the condition of the metatherian stem-group. Meng and Fox (1995, p.61) also stated based on literature review (e.g., Gray, 1907) that “in living therians, only the marsupial mole, the hedgehog, and the sea-cow posses a cochlea with as few as one and a half turns.” To this list we would add *Vombatus ursinus*, which also exhibits less than 1.5 complete turns of the cochlea. All other Australidelphian marsupial taxa have more than two and half turns of the cochlea (Gray, 1907).

Once the evolutionary transformations of the cochlea and semicircular canals in marsupial phylogeny are understood, the ontogenetic changes of individual species could be compared to the phylogenetic changes, as in Macrini (2002).

The data provided here could be used as a basis of comparison with other living and extinct didelphimorphians. Future work on this group could not only further test the phylogenetic value of bony labyrinth anatomy, but also explore issues of allometry and function. Even the comparisons with just the few data available for *Didelphis* permits an appreciation of the morphological diversity of living South American opossums. The exponential growth in the use of computer tomography technology (CT scans) will certainly increase the sampling of this and other mammalian groups in a non-destructive way.

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