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Ontogenetic and Phylogenetic Transformations of the Ear Ossicles in Marsupial Mammals

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ABSTRACT This study is based on the examination of histological sections of specimens of different ages and of adult ossicles from macerated skulls representing a wide range of taxa and aims at addressing several issues concerning the evolution of the ear ossicles in marsupials. Three-dimensional reconstructions of the ear ossicles based on histological series were done for one or more stages of *Monodelphis domestica, Caluromys philander, Sminthopsis virginiae, Trichosurus vulpecula,* and *Macropus rufogriseus*. Several common trends were found. Portions of the ossicles that are phylogenetically older develop earlier than portions representing more recent evolutionary inventions (manubrium of the malleus, crus longum of the incus). The onset of endochondral ossification in the taxa in which this was examined followed the sequence; first malleus, then incus, and finally stapes. In *M. domestica* and *C. philander* at birth the yet precartilaginous ossicles form a supportive strut between the lower jaw and the braincase. The cartilage of Paauw develops relatively late in comparison with the ear ossicles and in close association to the tendon of the stapedial muscle. A feeble artery traverses the stapedial foramen of the stapes in the youngest stages of *M. domestica, C. philander*, and *Sminthopsis virginiae* examined. Presence of a large stapedial foramen is reconstructed in the groundplan of the Didelphidae and of Marsupialia. The stapedial foramen is absent in all adult caenolestids, dasyurids, *Myrmecobius, Notoryctes*, peramelids, vombatids, and phascolarctids. Pouch young of *Perameles* sp. and *Dasyurus viverrinus* show a bicrurate stapes with a sizeable stapedial foramen. Some didelphids examined to date show a double insertion of the Tensor tympani muscle. Some differences exist between *M. domestica* and *C. philander* in adult ossicle form, including the relative length of the incudal crus breve and of the stapes. Several differences exist between the malleus of didelphids and that of some phalangeriforms, the latter showing a short neck, absence of the lamina, and a ventrally directed manubrium. Hearing starts in *M. domestica* at an age in which the external auditory meatus has not yet fully developed, the ossicles are not fully ossified, and the middle ear space is partially filled with loose mesenchyme. The ontogenetic changes in hearing abilities in *M. domestica* between postnatal days 30 and 40 may be at least partially related to changes in middle ear structures. J. Morphol. 251:219–238, 2002.

KEY WORDS: phylogeny; ontogeny; marsupialia; middle ear; ossicles; stapes

The origin of the mammalian ear ossicles is a classic example of gradual evolutionary change and has received considerable attention (Allin, 1975; Maier, 1990; Allin and Hopson, 1992; Rowe, 1996, and references therein). Marsupials have played an important role in this discussion, perhaps because of the vanishing perception that they represent a transitional form between mammalian ancestors and eutherians. Marsupials do not possess a temporomandibular joint at birth (Müller, 1967, 1968; Lillegraven, 1975) and are reported to retain at this stage an operational primary jaw joint (Crompton and Parker, 1978; Maier, 1987; but see Filan, 1991). The development of the ear ossicles in marsupials is used in its general outline as a classic example of recapitulation (Allin, 1975; Maier, 1990).

Most studies about developing ossicles in marsupials have been restricted in the extent of taxa examined (Palmer, 1913; McClain, 1939; Filan, 1991) and have for the most part aimed at addressing issues concerning the origin of these structures in mammals (Maier, 1990; Rowe, 1996). Some aspects of ossicle development are described in works on marsupial craniology (Cords, 1915; Esdaile, 1916; Toeplitz, 1920; Denison and Terry, 1921). McClain (1939) provided the most thorough description of

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developing ossicles in a marsupial, in this case *Didelphis virginiana*. Another didelphid, *Monodelphis domestica*, has also been studied in some detail, by different authors with different goals (Maier, 1987; Filan, 1991; Clark and Smith, 1993; Rowe, 1996). Maier (1987) presented extensive data about middle ear (including ossicles) development aiming at understanding some aspects of marsupial jaw anatomy correlated during development with basicranial structures. Filan's (1991) study concentrated on the earliest postnatal phases of middle ear development. Her goal was to examine functional anatomy around the time of birth. Clark and Smith (1993) provided a comparison between *M. domestica* and *Macropus eugenii* concerning different aspects of skull development and included information on ossicles. Rowe (1996) used *M. domestica* as a model to study integration of development in basicranial structures and the brain and its role in the origin of the mammalian middle ear. None of these works addressed ossicle anatomy and the development of hearing function, for which experimental data for *M. domestica* is now available (Reimer, 1996; Aitkin et al., 1997). In eutherians, some aspects of the middle ear development have been associated with changes in auditory thresholds in development (Woolf and Ryan, 1988; Geal-Dor et al., 1993). To what extent the middle ear is involved in the onset and developmental changes in hearing in marsupials remains unexplored.

Because Marsupialia is taxonomically and ecologically a diverse clade (Springer et al., 1997), it is expected that middle ear structures in this group also show some degree of variation. Concerning the adult anatomy of the ear ossicles, some of the most significant contributions were made in the Nineteenth Century, with the publication of illustrations and descriptions of several taxa by Hyrtl (1845) and Doran (1878). Greater understanding of ossicle diversity and function in mammals was gained after the seminal work of Fleischer (1973, 1978). Fleischer (1973) illustrated and described a few marsupial ossicles, showing a wide spectrum of variation in this group that he correlated with different hearing abilities. Segall (1969a,b, 1970, 1971) provided morphometric data (stapedial ratios, lever arm ratios, etc.) and illustrated ear ossicles in a wide range of marsupial taxa, showing differences that were interpreted functionally and later were incorporated in phylogenetic analyses of the major marsupial Recent clades (Springer et al., 1997). Archer (1976) described the ossicles of several "marsupicarnivores," including representatives of the extant families Didelphidae, Dasyuridae, Thylacinidae, and Myrmecobiidae. Some treatments of single marsupial species have illustrated ossicles (Parker, 1890: *Tarsipes rostratus*; Wood-Jones, 1949: *Dasycercus cristicauda*). Marsupial ossicles have been also treated in discussions on ossicle evolution in mammals, especially concerning the stapes (Novacek and Wyss, 1986; Rose and Emry, 1993; Gaudin et al., 1996).

Much has been written concerning stapes form in mammals, which has played a central role in some discussions on eutherian phylogeny (Novacek and Wyss, 1986; Wyss, 1987; Gaudin et al., 1996). The reconstruction of the marsupial groundplan condition has been very important in this discussion, since it serves as an outgroup comparison. Stapes form in the context of marsupial phylogeny has not been addressed in a modern phylogenetic context (but see Gaudin et al., 1996).

In this study, we examine patterns of developmental change in the ear ossicles of marsupials. We present for the first time for several taxa 3D reconstructions of the ear ossicles at different ontogenetic stages and descriptions of ossicles in adults of taxa not examined to date that reveal interesting patterns of evolutionary change. We consider these data in order to address several issues, the major ones described in what follows.

1) Contradictory statements exist in the literature concerning the state of the ossicles in marsupials at birth (Maier, 1987; Filan, 1991). Clarification of this point is the first requisite in understanding how the masticatory apparatus of the neonate works.
2) What are the most significant ontogenetic changes in middle ear structures after birth and what is their potential functional relation to hearing function?
3) How can stapedial form be best characterized and what is the groundplan condition in Marsupialia? This is a relevant question in discussions on mammalian systematics (Novacek and Wyss, 1986; Rose and Emry, 1993; Gaudin et al., 1996). What are the evolutionary transformations of the stapes within Marsupialia, and do they offer any phylogenetic signal? Is there a relationship between the phylogenetic transformations of the stapes and the stapedial artery (Novacek, 1993)?

MATERIALS AND METHODS

Ontogeny and 3D Reconstructions of Developing Ossicles

Several aspects of the anatomy of the ear ossicles were studied in serial histological sections of at least one representative of five marsupial families. Table 1 presents information concerning the specimens studied. Only in the case of *Monodelphis domestica* is the age of the specimens known. The exact age of the smallest *Caluromys philander* is unknown, but we are certain it is between newborn to no more than 2 days postnatal. Photographs of specimens studied before sectioning are reproduced in Figure 1. Photographs of some of the other sectioned specimens before preparation, as well as descriptions of the basicranial region and nasal region, are presented by Sánchez-Villagra (1998, 2001a). The specimens are from the collections at the Zoologisches

*TABLE 1. Sectioned specimens used for 3D reconstructions of ear ossicles and/or detailed histological observations**

Species	Age	CRL (mm)	HL (mm)
Didelphidae			
Monodelphis domestica			
ZSH-nn	PND-0	ca. 10	ca. 4.5
ZSH-nn	PND-12	ca. 20.5	ca. 8.5
KS-cc60	PND-30	?	?
KS-256	PND-35	?	?
ZSH-nnA	Adult	?	?
Caluromys philander			
ZSH-nn	?Neonate	11.5	6
ZSH-nn	Pouch young	15	7.5
ZSH-nn	Pouch young	20	10
ZSH-nn	Juvenile	49	22
ZSH-nn	Old juvenile	56	26
Peramelidae			
Perameles sp.			
ZSH-nn	Pouch young	39	17.5
Dasyuridae			
Sminthopsis virginiae			
ZSH-nn	Pouch young	7.5	4.5
ZSH-nn	Pouch young	14.5	9
Dasyurus viverrinus			
ZSH-nn	Pouch young	29	12
Phalangeridae			
Trichosurus vulpecula			
ZSH-nn	Pouch young	?	7.5
ZSH-nn	Pouch young	39	18
Macropodidae			
Macropus rufogriseus			
ZSH-AI-7895	Pouch young	37	12

*See text for institutional abbreviations. PND, postnatal day; CRL, crown–rump length; HL, head length.

Institut of the Universität Tübingen (ZSH) and the collection of Dr. K.K. Smith (KS) at Duke University. 3D models of the ear ossicles based on the histological sections were built of several taxa.

Ossicle models of three stages of *Monodelphis domestica* were built manually using the wax-plate method introduced by Born (1883) and later modified by W. Maier. A model of the ossicles of a pouch young *Macropus rufogriseus* was also built using this method. In this case the ectotympanic and the lateral wall of the braincase were also reconstructed to show the spatial relations of the ossicles with neighboring structures.

To serve for comparison, computer models of the ossicles were built in two stages each of three additional phylogenetically distant species: 1) the didelphid *Caluromys philander*, 2) the dasyurid *Sminthopsis virginiae*, and 3) the phalangeriform *Trichosurus vulpecula*. Models were also built of the stapes of pouch young *Perameles* sp. and *Dasyurus viverrinus*. The method works as follows. First, every section (in most cases, 10 μm thick) was digitalized using the Pixera Professional® camera and software. Second, these files were opened with Surfdriver® v. 5.3 (Moody and Lozanoff, 1999), where the contours of the structures of interest were traced, independent of their state of development (blastemous, precartilaginous, cartilaginous, preosseous, bony). That means that in some of the ear-

Fig. 1. Photographs of *Caluromys philander* studied histologically before sectioning. From left to right: CRL = 11.5; HL = 6; CRL = 20; HL = 10; CRL = 49; HL = 22; CRL = 56; HL = 26. Scale = 10 mm.

liest stages examined blastemous congregations of cells were traced, while in later stages cartilaginous and bony structures were traced. Third, three-dimensional models were created with Surfdriver® v. 3.5 and exported to the program Cinema-4D® v. 5.3 (Losch et al., 1999), where they were colored and the desired view for publication produced.

We examine in particular detail the development of the ossicles in *Monodelphis domestica* and *Caluromys philander*, two didelphid marsupials that are phylogenetically quite separate (Jansa and Voss, 2000) and ecologically very different (Rasmussen, 1990). In the case of *M. domestica,* there are experimental data on the development of hearing (Frost and Masterton, 1994; Reimer and Baumann, 1995; Reimer, 1996), much is known about its biology (K.K. Smith, unpublished), and we have excellent developmental series of this animal available for study (Maier, 1987; Clark and Smith, 1993).

The stages of *Monodelphis domestica* examined were selected to document the anatomy of the ossicles at birth, at the time of the onset of hearing (around postnatal day (PND) 30; Reimer, 1996), and at an intermediate stage between the two (PND-12). Although the ages of the specimens of *Caluromys philander* examined are not known, based on the photographs of these animals before sectioning (Fig. 1), we know that they illustrate a similar spectrum of time in postnatal development as that examined for *M. domestica*. The stages of *C. philander* examined are described in detail in order to provide a comparison with *M. domestica.* This will allow us to establish to what extent patterns of development in middle ear structures in *M. domestica* are particular to this species or representative of a didelphid pattern.

Examination of Osteological Specimens of Adults

Ossicles were extracted from the skull in 70 specimens representing 25 species (Sánchez-Villagra and Nummela, 2001); all major Recent marsupial clades (Kirsch et al., 1997) are represented in this sample except for caenolestids and peramelids. Institutions and their abbreviations are listed in the Acknowledgments. Some of the specimens were photographed and some aspects about their form were noted. Particular attention was paid to adult specimens of taxa examined histologically or to close phylogenetic relatives of them, including *Monodelphis domestica, Caluromys philander, Dasyurus viverrinus, Trichosurus vulpecula,* and *Bettongia penicillata*.

Stapedial Foramen

The problems involved in categorizing stapes shape have been carefully discussed previously (Novacek and Wyss, 1986; Meng, 1992; Rose and Emry, 1993; Gaudin et al., 1996). Traditionally, stapes have been characterized as either columellar or stirrup-shaped, but the boundaries between these types are difficult to draw, considering the shape of the distal part of the stapes and the shape and size of the stapedial (=obturator) foramen. Rose and Emry (1993) and Gaudin et al. (1996) presented data, mostly for eutherians, that also show that much of intrataxon variation exists at all levels in stapes shape.

In spite of these caveats, our preliminary examination of osteological specimens and the comparison with reports in the literature (Hyrtl, 1845; Doran, 1878; Winge, 1941; Segall, 1969a,b, 1971; Gaudin et al., 1996) permit a phylogenetically meaningful arrangement of stapes form based simply on the presence of the stapedial foramen. Independent of its size, this foramen is consistently present in some clades and absent in others. The distribution of this trait was plotted in a recent molecular phylogeny (Springer et al., 1998), that in its major outlines agrees with the most recent morphological analysis (Sánchez-Villagra, 2001b) and other recent molecular-based contributions in this field (Burk et al., 1999; Palma and Spotorno, 1999).

RESULTS

Description of Developing Ossicles in *Monodelphis domestica* and *Caluromys philander*

Monodelphis domestica.

PND-0 (Figs. 2, 3). The entire Meckel's cartilage is much larger than the incus and stapes, while the gonial is relatively small. The manubrium of the malleus has not yet formed and only a few blastematous cells are recognized as the anlage of its most proximal portion. The head and the crus breve of the incus, and most of the malleus, are in a young cartilaginous stage. The saddle form of the articular area of the incus with the malleus is already recognizable. The crus longum of the incus (only partially developed in terms of its length) as well as the stapes are precartilaginous. Their articular surfaces have not formed yet, nor has the footplate of the stapes. A small dorsoventrally running artery is seen crossing the obturator foramen of the stapes. The future middle ear space is occupied with mesenchyme; the cavum tympani is just a small space lined by a flat epithelium. Both the incus and the fossa incudis of the crista parotica and malleus and incus are in close proximity to each other, even though no synovial space is present between them.

PND-12 (Figs. 2, 3). The first step in the endochondral ossification of the malleus can be seen at this stage, with the presence of hypertrophic cartilage cells in the portion of the malleus fused with the gonial. The gonial has grown and builds a half-tubal structure running ventrally and partially fused to the endochondral ossification in Meckel's cartilage.

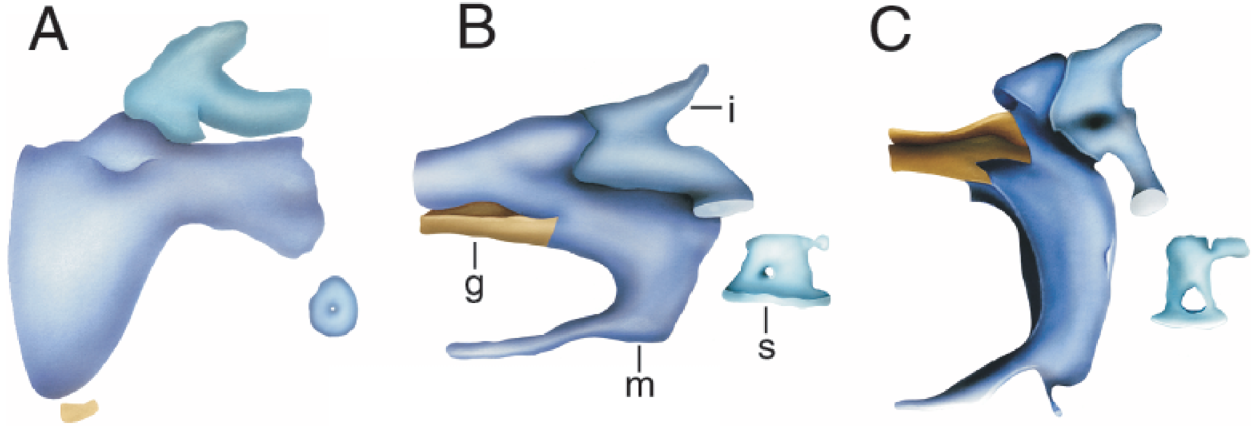

Fig. 2. Medial view of three-dimensional models of the right ear ossicles of *Monodelphis domestica* at (**a**) PND-0, (**b**) PND-12, and (**c**) PND-30. In all cases, only the posteriormost portion of Meckel's cartilage is represented. In this and all other figures of models the stapes is illustrated disarticulated from the incus. Not to scale. g, gonial; i, incus; m, malleus; s, stapes.

In the model (Fig. 2), the border between the gonial and the true malleus is signified by the change of color. The articular contact between malleus and incus is more differentiated; the synovial joint is forming and a few cells are seen in what will become the synovial cavity. The incudal crus breve, proportionally more gracile than in the neonate, is housed within the fossa incudis formed by the ear capsule and sided by the squamosal. The processus lenticularis has not formed yet. No trace of the stapedial artery is to be seen in PND-12 or later stages. The stapedial muscle is feeble and consists of a few fibers that attach to the cartilage of Paauw. The cavum tympani is still relatively small.

PND-30 (Figs. 2, 4). The anterior portion of the head of the malleus in contact with the gonial (in this stage, already transformed into the anterior process) is already ossified. More posteriorly (including the area in contact with the incus), the head is only partially ossified. The manubrium is all cartilaginous and does not show signs of ossification. There is a synovial joint between incus and stapes. The crus longum of the incus and the caput stapedis are cartilaginous. These two structures are in very close contact and in some portions even fused. The lenticular process of the incus, although not fully developed, is recognizable. The posterior portion of the incudal crus breve is attached to the walls of the fossa incudis and the squamosal by a well-defined suspensory ligament. In contrast to the feeble constitution of this structure in PND-12, in PND-30 the cartilage of Paauw is a sizeable structure projecting posteriorly from the stapes. The stapedial muscle is much larger than at PND-12 and is histologically differentiated. There is loose mesenchyme filling portions of what will become the middle ear space; the actual cavum tympani occupies approximately half of the future middle ear space. The tympanic membrane is differentiated. The external acoustic meatus is partially opened.

PND-35 (Fig. 4). The proximal portion of the manubrium is partially ossified, while the anterior portion of the manubrium, although showing the start of ossification, is largely cartilaginous. Concerning the rest of the malleus, the same description as for PND-30 is valid, except that it is more ossified. There is still some loose mesenchyme filling portions of the middle ear space. The Tensor tympani muscle has two heads and two insertion sites that are separated from each other by approximately 0.1 mm. There is no synovial joint between incus and stapes, although both structures are in very close contact (in this area they are both cartilaginous). The processus lenticularis of the incus has formed. The external acoustic meatus is fully formed.

Caluromys philander.

CRL 11.5 mm, newborn (Figs. 5, 7). The manubrium of the malleus has not yet developed; just a small condensation of cells represents the most proximal anlage of this structure. The gonial is recognized as a separate ossification, which is not yet fused to Meckel's cartilage, and is proportionally small. The spatial relations typical of the adult are recognizable, but at this stage the crus longum of the incus and stapes are precartilaginous, and there is no contact between these structures, even though they are in close proximity. The rest of the incus and the malleus are already in a young cartilage stage, showing numerous chondroblasts and a well-defined perichondrium. The complex articular area of the incus-malleus of the adult is not yet differentiated. The crus breve of the incus is already clearly identifiable. The stapes is semirounded, with ill-defined contours. A small artery traverses the stapedial foramen. The cartilage of Paauw can be recognized because of its position and spatial relations, but

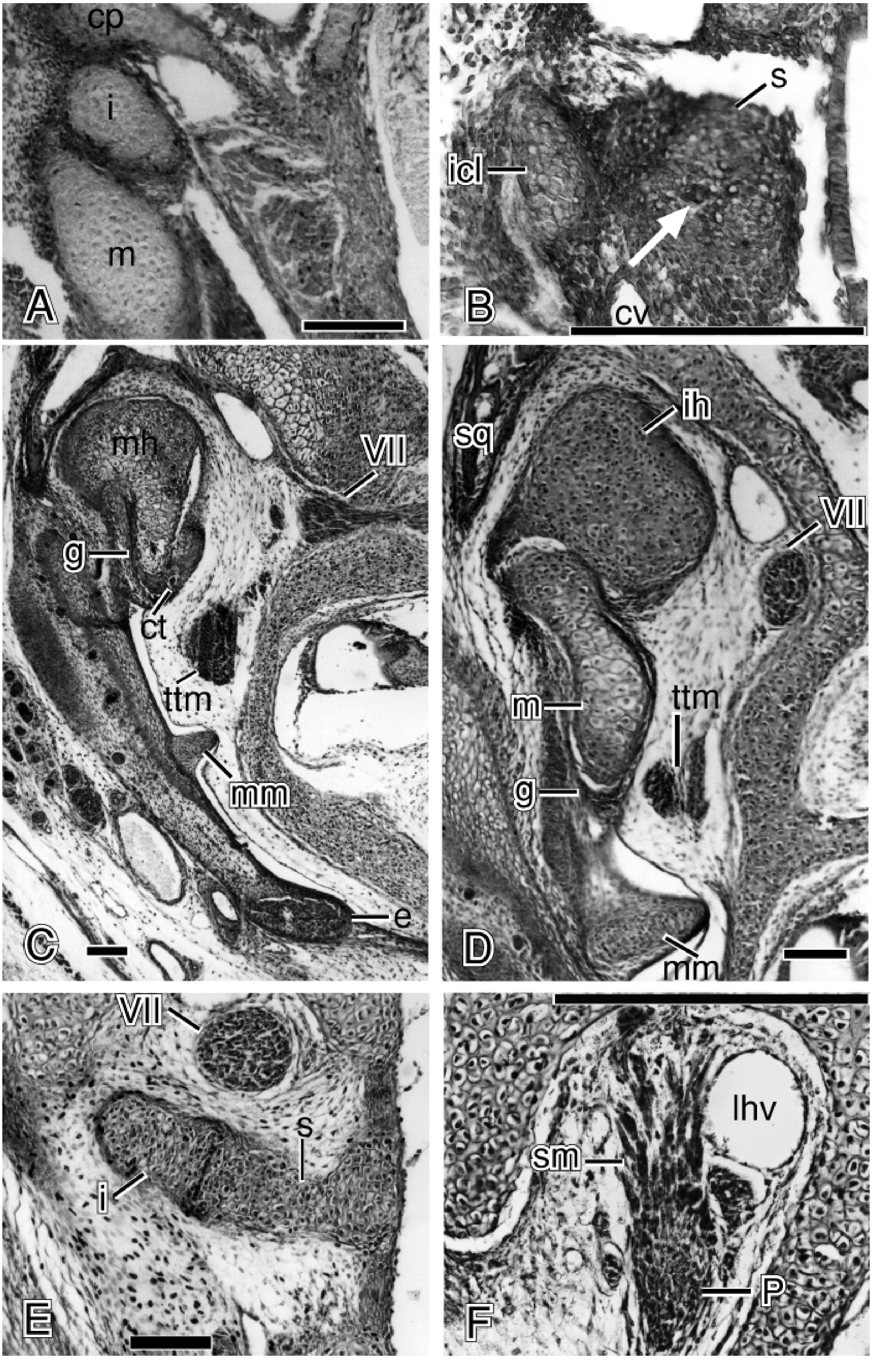

Fig. 3. Photomicrographs of transverse sections through portions of the right middle ear anlage in (**a,b**) neonate and (**c,d,e,f**) PND-12 *Monodelphis domestica*. Arrow in (**b**) points to feeble artery traversing the stapedial foramen. Scale = 0.1 mm. cp, crista parotica; ct, chorda tympani; cv, cavum tympani; e, ectotympanic; g, gonial; i, incus; icl, crus longum of incus; ih, head of incus; lhv, lateral head vein; m, malleus; mh, head of malleus; mm, manubrium of malleus; P, element of Paauw; s, stapes; sm, stapedial muscle; sq, squamosum; ttm, tensor tympani muscle; VII, facial nerve.

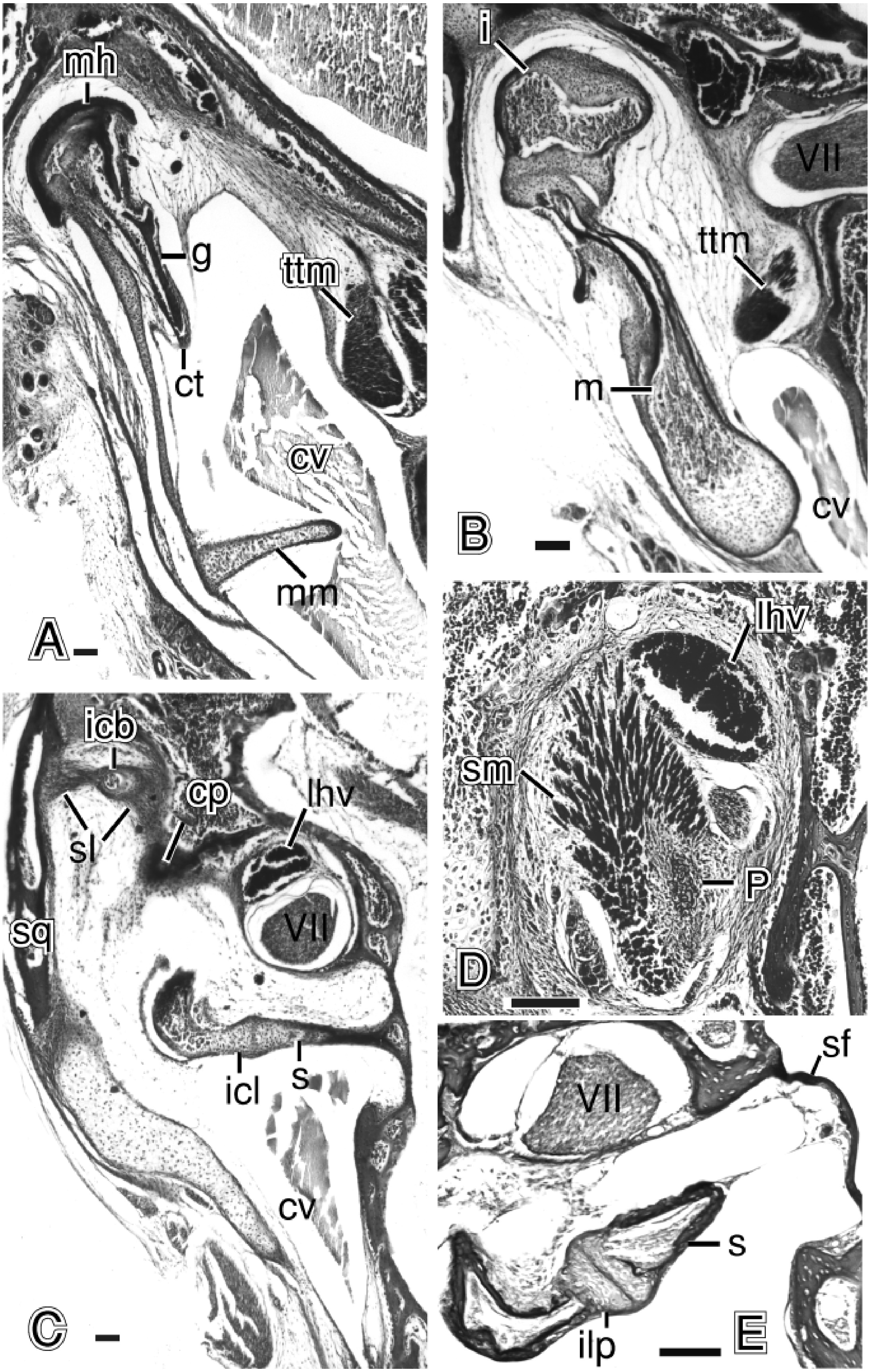

Fig. 4. Photomicrographs of transverse sections through portions of the right middle ear anlage in (**a,b,c,d**) PND-30 and (**e**) PND-35 *Monodelphis domestica*. Scale = 0.1 mm. cp, crista parotica; ct, chorda tympani; cv, cavum tympani; g, gonial; i, incus; icb, crus breve of incus; icl, crus longum of incus; ilp, lenticular process of incus; lhv, lateral head vein; m, malleus; mh, head of malleus; mm, manubrium of malleus; P, element of Paauw; s, stapes; sf, footplate of stapes; sl, suspensory ligament; sm, stapedial muscle; sq, squamosum; ttm, tensor tympani muscle; VII, facial nerve.

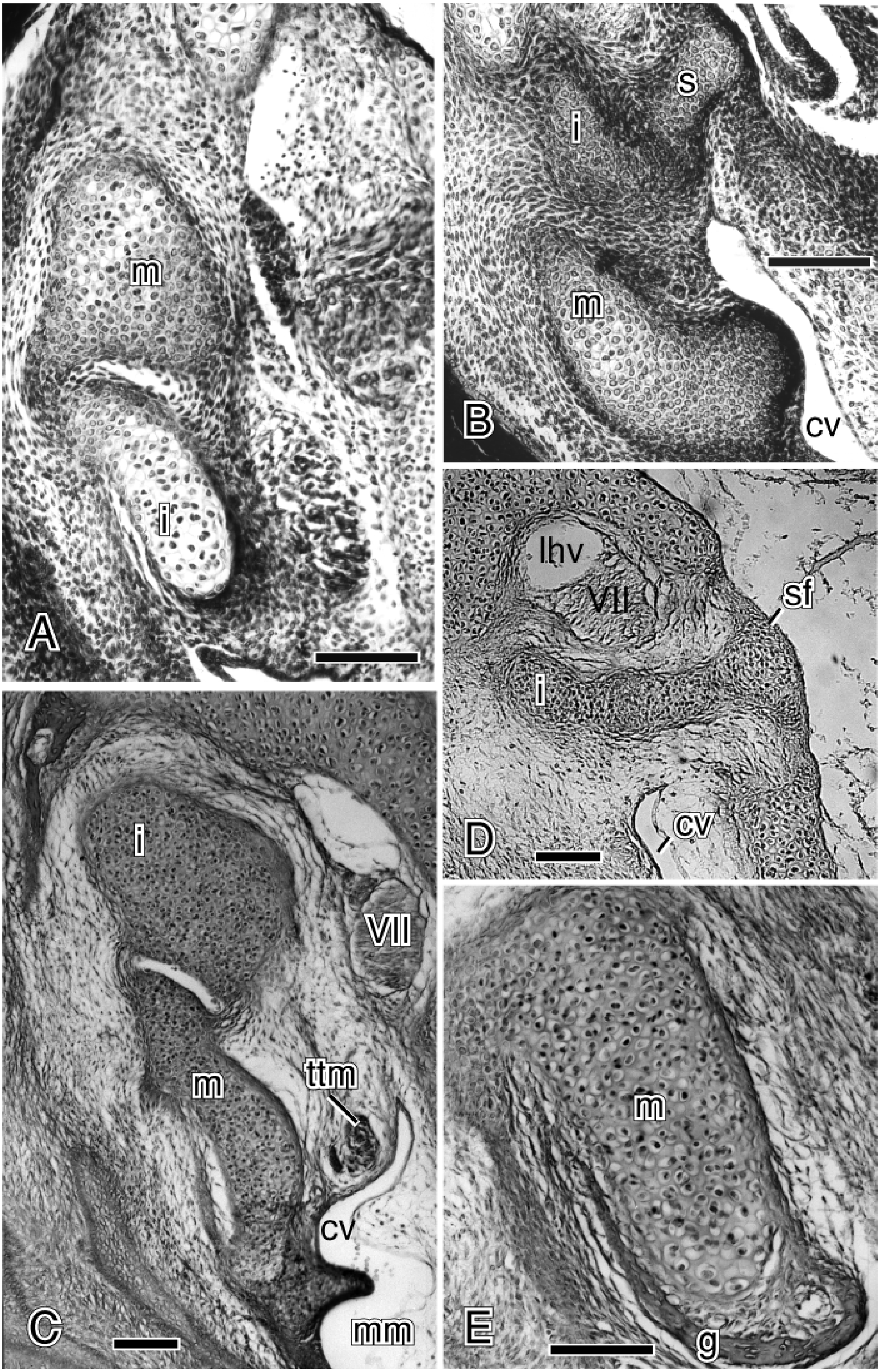

Fig. 5. Photomicrographs of transverse sections through portions of the right middle ear anlage in (**a,b**) CRL = 11.5 mm and (**c,d,e**) CRL = 20 mm *Caluromys philander*. Scale = 0.1 mm. cv, cavum tympani; g, gonial; i, incus; lhv, lateral head vein; m, malleus; mm, manubrium of malleus; s, stapes; sf, footplate of stapes; ttm, tensor tympani muscle; VII, facial nerve.

histologically consist simply of a condensation of cells. The exact boundaries are impossible to define and for this reason it is not reconstructed in the model.

CRL 20 mm (Figs. 5, 7). The malleus is all cartilaginous, except for the gonial, which in this stage is fused to the former, forming the anterior process. The incus and the stapes are all cartilaginous. The articular surfaces between incus and malleus are already formed, as well as the corresponding synovial cavity, even though an adult synovial joint has not yet developed, as the fibrous capsule is still absent. There is a blastematous connection between incus and stapes. Already at this stage, as observed in older specimens as well, the footplate of the stapes sits on the most medial portion of a thick rim of the otic capsule surrounding the Fenestra ovalis, which forms in this way a fossula. The stapedial artery has completely involuted by this stage; no traces of it are recognized. Most of the future middle ear space is filled with mesenchyme.

CRL 49 mm (Fig. 6). The neck and lamina of the malleus are completely ossified, while the head and the manubrium are only partially so. The incus is partially ossified. The incudal crus longum is mostly ossified, except for the articular surface with the stapes.

Stapes and incus are fused and their contact is cartilaginous. The proportionally narrow neck proximal to the lenticular process of the incus, although not fully developed, is distinguished. The incudal crus breve does not contact the crista parotica but is in near proximity to it. A well-defined suspensory ligament attaches to the medial aspect of the posterior portion of the crus breve. Laterally, this portion of the crus breve attaches to the squamosal by a tendinous structure very different histologically from the ligament. The cartilage of Paauw is distinguished. The future air-filled middle ear space is only partially occupied by loose mesenchyme. The external acustic meatus has not opened yet, even though there is a large space lateral to the tympanic membrane that will form the meatus.

CRL 56 mm (Fig. 6). The manubrium is partially ossified. A synovial cavity is present between incus and stapes. The body of the stapes is ossified, while the footplate is partially cartilaginous. The portions of the stapes and incus that articulate with each other are cartilaginous. By this stage the incudal lenticular process has clearly differentiated. The Tensor tympani muscle has two heads but just one site of attachment on the malleus. The posteriormost portion of the cartilage of Paauw is ossified. The middle ear space is mostly free of loose mesenchyme. The external acustic meatus is partially open. Even though relative to other middle ear structures it is very small, Meckel's cartilage is still present and easily recognizable in its posterior portion. In fact, a posterior portion of the dentary shows the meckelian groove.

Observations on the Development of Ossicles in *Sminthopsis virginiae, Trichosurus vulpecula,* and *Macropus rufogriseus*

Sminthopsis virginiae (Fig. 8). In the younger specimen, the gonial is a separate entity from Meckel's cartilage. The malleus is in a precartilaginous stage, showing more signs of differentiation (young cartilage) in the area of contact with the gonial. The manubrium is just a small process. The apophysis orbicularis of the malleus has not yet formed. The cartilage of Paauw consists of just a condensation of cells, and the stapedial muscle has not yet differentiated. The stapes is somewhat pyramidal and has no recognizable footplate. The later stage shows the gonial fused to the malleus, forming its anterior process. The incudal lenticular process is partially developed and the articular surface is recognizable but has not yet fully formed.

Trichosurus vulpecula (Fig. 8). In the earlier stage the stapes shows a foramen absent in the larger sectioned specimen but present in at least some adults of this species. The cartilage of Paauw is recognizable as a condensation of cells; the stapedial muscle is not yet differentiated. The older specimen shows some expected changes: Meckel's cartilage becomes relatively smaller and the manubrium of the malleus develops further. The proportions of the malleus and incus change dramatically between the two specimens examined. In the younger specimen the incus is relatively large in comparison with the malleus. As development progresses, the incus must grow at a slower rate, since the malleus is relatively much larger in the older-sectioned specimen modeled.

Macropus rufogriseus (Fig. 9). The pouch young of *Macropus rufogriseus* examined shows the basic outline of the adult form of the ossicles in this species. The articular surfaces among the ossicles are all formed. The ossified gonial is relatively long. The stapedial foramen typical of adults is already present. The ossicles at this stage are still proportionally very large in comparison with other skull structures.

Ossicle Form in Adult Marsupials

Both *Monodelphis domestica* and *Caluromys philander* show a malleus with a small head, a relatively large lamina, and a long neck (Fig. 10). Some differences exist between the specimens examined of these species. In the malleus, the apophysis orbicularis in *Caluromys philander* is more pointed than that of *Monodelphis domestica*, perhaps just a case of individual variation. The manubrium in *M. domestica* is more ventrally oriented than in *C. philander*. Concerning the incus, the crus breve in *C. philander* is conspicuous and longer than the crus longum. In *M. domestica* the proportion crus longum

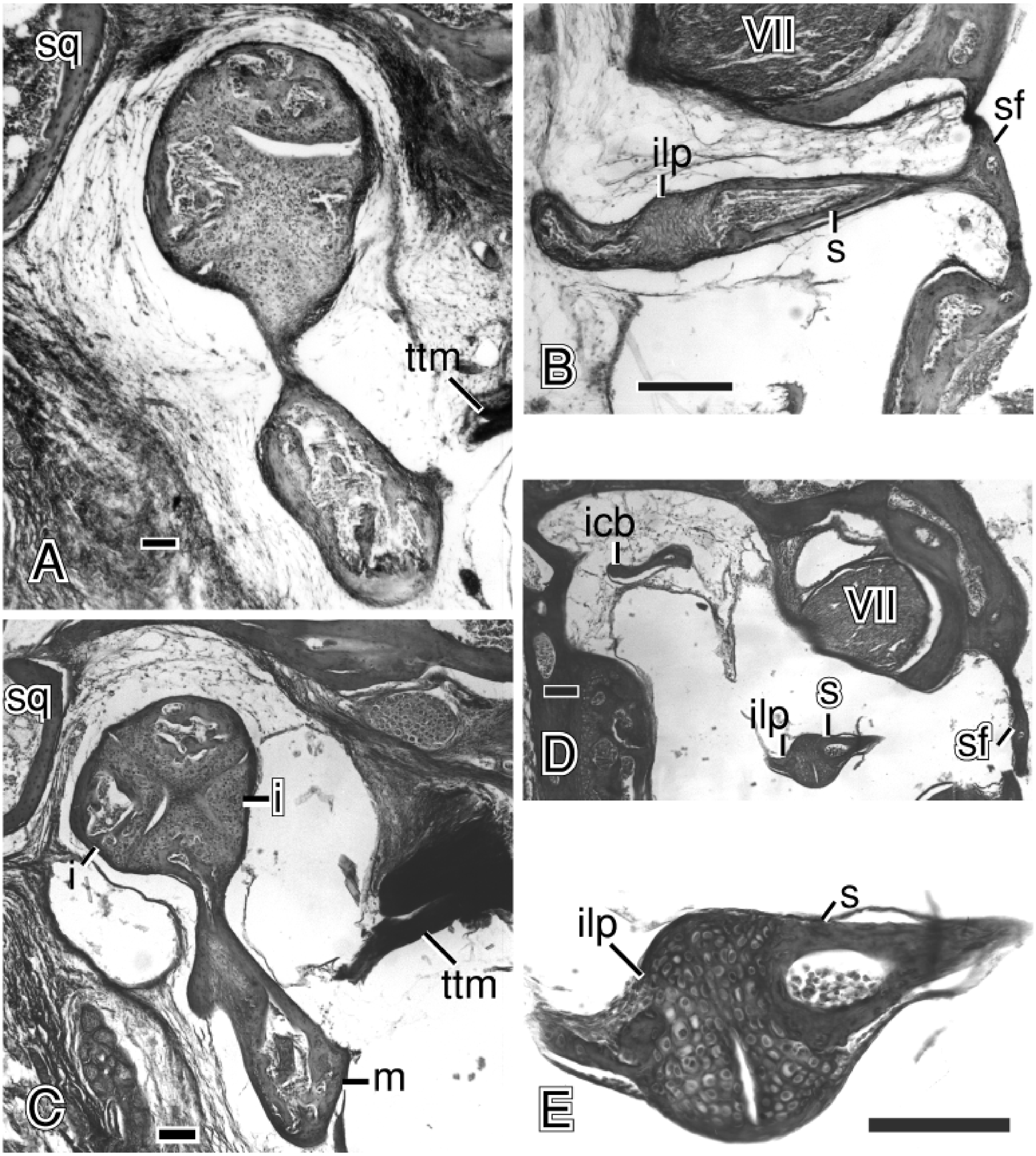

Fig. 6. Photomicrographs of transverse sections through portions of the right middle ear anlage in (**a,b**) CRL = 49 mm and (**c,d,e**) CRL = 56 mm *Caluromys philander*. **e**: A portion of (**d**) in higher magnification. Scale = 0.1 mm. i, incus; icb, crus breve of incus; icl, crus longum of incus; ilp, lenticular process of incus; m, malleus; s, stapes; sf, footplate of stapes; sq, squamosum; ttm, tensor tympani muscle; VII, facial nerve.

/ rest of the incus is larger than that of *C. philander*. In this regard *M. domestica* is like *Didelphis virginiana* (McClain, 1939, plate VIII) and the hypothesized groundplan of the Didelphidae. *Caluromys philander* has a more rounded corpus of the incus than *M. domestica*. No other significant differences were noted among the inci of all taxa examined. The stapes of *C. philander* is proportionally longer than that of *M. domestica*. This is undoubtedly correlated with the presence in the former of a fossula (see above) surrounding the oval window.

In dasyurids (e.g., *Dasyurus viverrinus*, SM-1482, Fig. 11) and macropodids (*Bettongia penicillata*, SM-450, Fig. 11), malleolar form is similar to that of

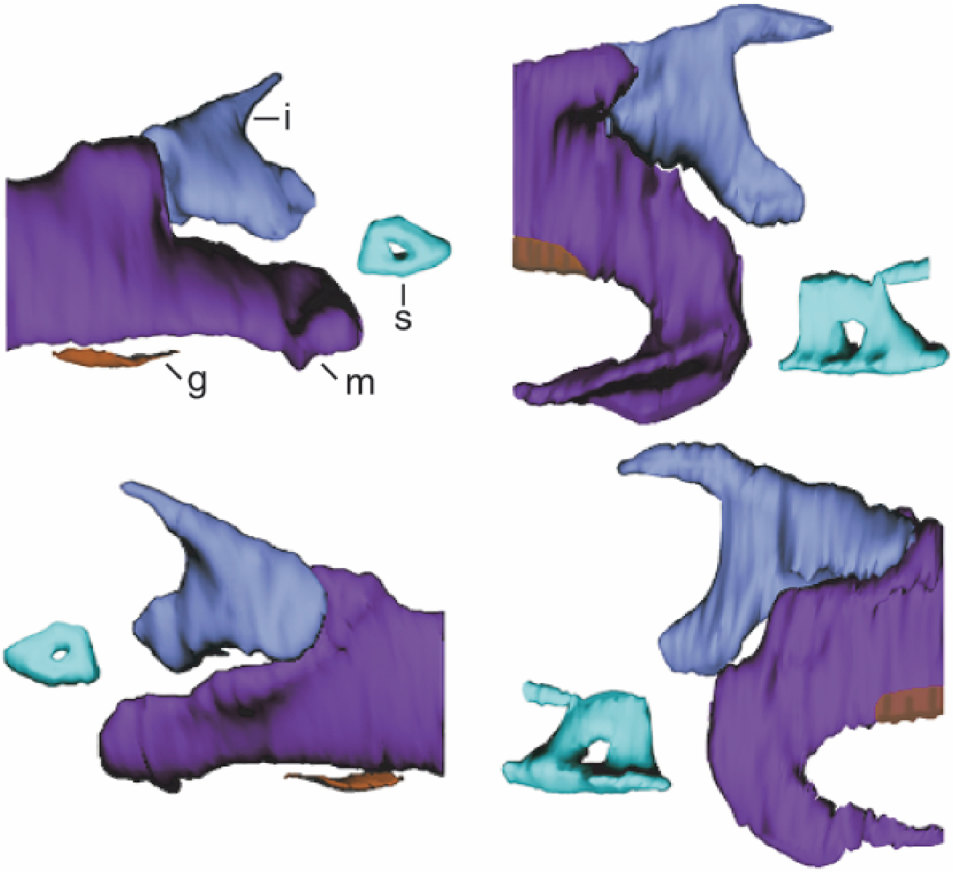

Fig. 7. Computer-reconstructed 3D models of the right ear ossicles of pouch young of *Caluromys philander.* Left: CRL = 11.5 mm; right: CRL = 20 mm. Only the posteriormost portion of Meckel's cartilage is represented. Not to scale. g, gonial; i, incus; m, malleus; s, stapes.

didelphids. *Trichosurus vulpecula* (WM-4, Fig. 11) shows several malleolar characteristics absent in most of the other taxa examined. The head is relatively large, the neck is short, there is no lamina, the apophysis orbicularis is large, the manubrium is directed ventrad, and the most distal portion of the manubrium is relatively wide and robust. *Phascolarctos cinereus* (ZMH-1041, Fig. 11) is similar to *T. vulpecula* in most of these features, except that it shows a more robust neck and a more gracile manubrium. In both *T. vulpecula* and *P. cinereus* the head of the malleus is longitudinally oval. Some aspects of stapes variation among these taxa are discussed in what follows.

Evolution of the Stapedial Foramen

All didelphids examined show a relatively large obturator foramen, including *Thylamys elegans* (AMNH-41711), *Marmosops parvidens* (UWZ-22095), *Marmosops impavidus* (AMNH-47184-62242), *Marmosops* sp. (AMNH-95513), *Gracilinanus agilis* (ZMH-114684-114682), *Gracilinanus* sp. (AMNH-260031), *Lutreolina crassicaudata* (AMNH-210422), *Chironectes minimus* (AMNH-135582), *Metachirus nudicaudatus* (AMNH-73363), *Marmosa murina* (SM-9917), *Marmosa robinsoni* (ZMH-69848), *Micoureus alstoni* (AMNH-131736), *Monodelphis domestica* (KS-AvN-98005, Fig. 10), *Caluromys derbianus* (USNM-3001132), and *Caluromys philander* (ZFMK-unnumbered 2, Fig. 10). Some didelphid specimens show imperforate stapes, in most cases apparently a case of intraspecific variability, in others perhaps representing autapomorphies of certain taxa. Examples of imperforate stapes show specimens of *Monodelphis dimidiata* (Archer, 1976), "some" *Didelphis albiventris* (Gaudin et al., 1996:41), and *Caluromysiops irrupta* (Gaudin et al., 1996; also observed in USNM-397626; see below). A specimen of *C. irrupta* (USNM-396061) shows a right stapes with no foramen and a left one with a small one; a similar case was reported by Gaudin et al. (1996:41) for a specimen of *C. minimus.*

Concerning other marsupials, our observations are in accord with previous reports (Hyrtl, 1845; Doran, 1878; Winge, 1941). The stapedial foramen is absent in all adult caenolestids, dasyurids, *Myrmecobius, Notoryctes*, peramelids, vombatids, and

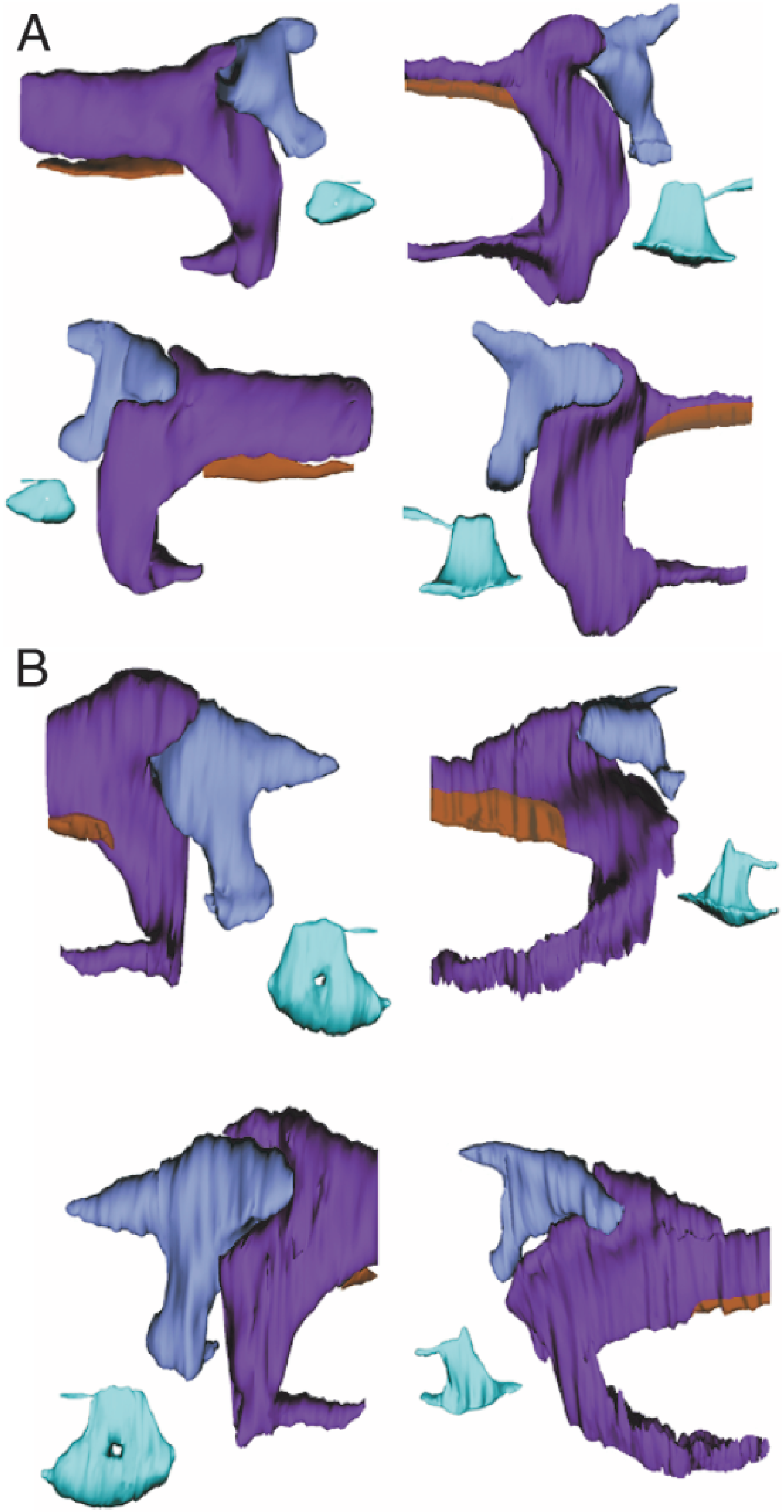

Fig. 8. Computer-reconstructed 3D models of the right ear ossicles of pouch young of (**a**) *Sminthopsis virginiae,* left: HL = 4.5 mm; right: HL = 9 mm, and (**b**) *Trichosurus vulpecula*. Left: HL = 7.5 mm; right: HL = 18 mm. Brown color, the gonial element of the malleus. Only the posteriormost portion of Meckel's cartilage is represented. Not to scale.

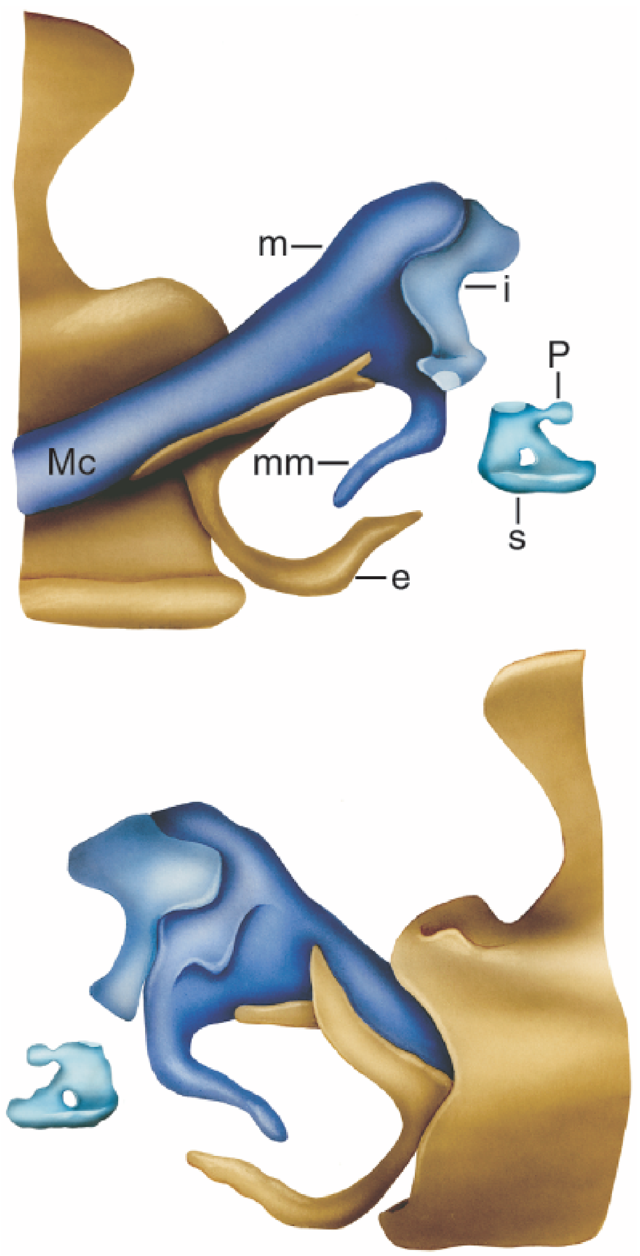

Fig. 9. Medial (top) and lateral (bottom) views of a manually made 3D model of the right ear ossicles, ectotympanic, and a portion of the alisphenoid of a pouch young *Macropus rufogriseus* (ZSH, HL = 12 mm). Only the posteriormost portion of Meckel's cartilage is represented. In this, as in all other figures, the stapes is depicted disarticulated from the incus. Brown color, the dermal bones (gonial, ectotympanic) and the lateral wall of the braincase. e, ectotympanic; g, gonial; i, incus; m, malleus; Mc, Meckel's cartilage; mm, manubrium of malleus; P, element of Paauw; s, stapes.

phascolarctids. A very small basal perforation might be present in *Notoryctes*, thylacinids (Gaudin et al., 1996, and references therein), and some phalangeriformes (Segall, 1969a). The groundplan of all other marsupial clades, in contrast, do posses a large obturator foramen.

Pouch young *Perameles* sp. and *Dasyurus viverrinus* (Fig. 12) show a bicrurate stapes with a sizable obturator foramen, as opposed to the adult of these species, which show the derived columellar condition.

DISCUSSION

Developing Ossicles

In all cases examined, and in agreement with previous reports in the literature (Palmer, 1913; Toeplitz, 1920), we found that the portions of the ossicles that are phylogenetically older (Allin, 1975) develop earlier than portions that represent more recent evolutionary inventions. The crus breve of the incus (=dorsal plate; Luo and Crompton, 1994), phylogenetically part of the oldest portion of the incus, develops earlier than the distal portion of the crus longum (=stapedial process; Luo and Crompton, 1994), phylogenetically younger. Similarly, the manubrium is the last portion of the malleus to develop (Fleischer, 1973:165) and represents a neomorphic outgrowth of mammals (Allin and Hopson, 1992). The base of the manubrium is homologous to a portion of the retroarticular process of the articular (Allin and Hopson, 1992, and references therein), and develops earlier, at the same time as the portion of the malleus homologous with the articular.

The presence of a "joint" (see below) between the incus and the crista parotica in developing marsupials mirrors the condition found in early Mammaliaformes (Rowe, 1988) such as *Morganucodon, Dinnetherium* and *Megazostrodon* (Luo, 1994; Luo and Crompton, 1994: fig. 13). During the evolution of Mammaliaformes, the incudal articulation to the crista parotica preceded the detachment of the middle ear ossicles from the mandible (Luo, 1994; Luo and Crompton, 1994). In this regard, the same sequence of events were recorded in the ontogenetic transformations of *Monodelphis domestica* and *Caluromys philander*.

In all cases examined the gonial, a dermal bone that becomes the anterior process of the malleus, appears before any of the ossicles has started to ossify. The onset of endochondral ossification examined follows the same sequence in all cases: first malleus, then incus, and finally stapes. Our observations concerning ossification timing in *Monodelphis domestica* are in accordance with those reported by Clark and Smith (1993). They reported the following times of onset of ossification = gonial, PND-0; malleus, PND-11; incus, PND-17; stapes, PND-25.

Even though we did not quantify this trend, we noticed that the ear ossicles show negative allometric growth during development in comparison with other structures in the skull (Maier, 1987; Rowe, 1996).

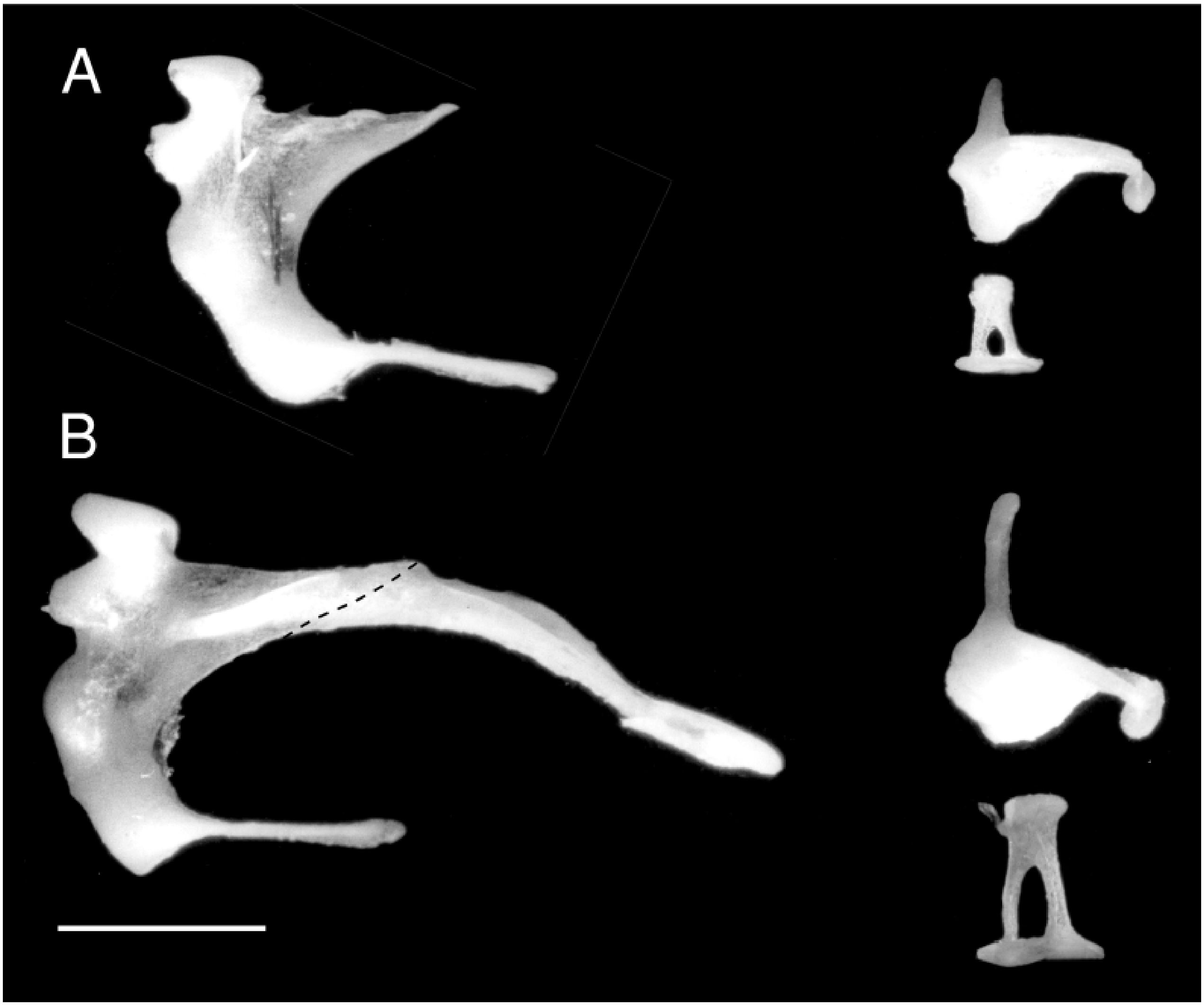

Fig. 10. Photographs of adult left ossicles of (**a**) *Monodelphis domestica* (KS-AvN-98005 stapes, KS-AvN-98002 malleus and incus), and (**b**) *Caluromys philander* (ZFMK-unnumbered 2). In the latter the ectotympanic has not been separated from the processus gracilis of the malleus. The approximate border between these two elements is marked by a dotted line. The stapes of *M. domestica* illustrated here is the mirror image of the right one. Scale = 1 mm.

Condition in Neonates

Based on our observations of *Monodelphis domestica* and *Caluromys philander*, we conclude that at birth the relatively large but little-differentiated ear ossicles are probably important in buttressing the mandible against the skull. At birth there is no differentiated joint or synovial cavity between malleus and incus or between incus and braincase through the crista parotica. The term "joint" describing the condition between these structures is used (as in Maier, 1987) in a general sense to refer to movable contacts, not to diarthroses only (contra Filan, 1991). These contacts are best characterized as immature syndesmoses, forming a supportive strut between the lower jaw and the braincase, with no articular surfaces involved. It seems quite safe to hypothesize that jaw mechanics in marsupial neonates are different than that of adults of mammalian ancestors (Crompton and Hylander, 1986). As stated by Kuhn (1971) and Maier (1987:128), in marsupial neonates the elasticity of the proximal portion of Meckel's cartilage most likely plays a role in the mechanics associated with the small movements and stresses involved in the act of suckling.

Whether the condition of the marsupial neonate with regard to the evolution of the ossicles and the mammalian masticatory apparatus can be cited as an example of recapitulation or not depends on how recapitulation is defined. The marsupial condition around the time of birth is neither anatomically nor functionally like that of any living adult sauropsid or purported adult mammalian ancestor (Luo, 1994). What is usually called Haeckelian recapitulation (the accuracy of the claim about what Haeckel

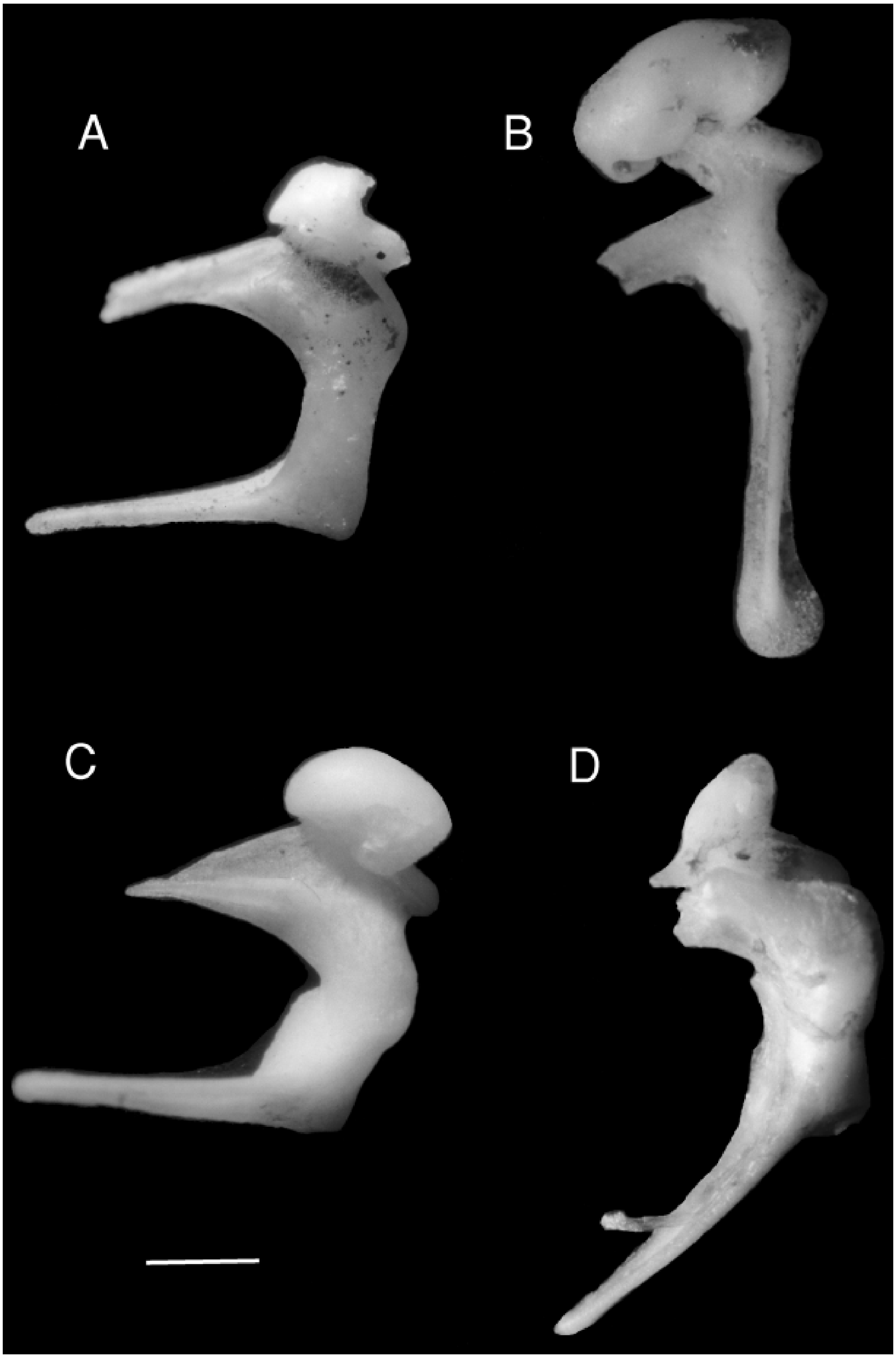

Fig. 11. Mallei of (**a**) *Dasyurus viverrinus* (SM-1482), (**b**) *Trichosurus vulpecula* (WM-4), (**c**) *Bettongia penicillata* (SM-450), (**d**) *Phascolarctos cinereus* (ZM-1041). Scale = 1 mm.

meant aside) involving the "repetition of adult stages of the ancestor during the ontogeny of the descendant" (e.g., Lovejoy, 2000:356), clearly does not take place. It seems more appropriate in this case to talk about "von Baer's recapitulation" (Lovejoy, 2000, and references therein), since we hypothesize that the condition of the marsupial neonate resembles that of the embryonic condition of mammalian ancestors.

Malleus and Incus Form in Adults

Malleus and incus form in the two didelphids examined (*Monodelphis domestica* and *Caluromys phi-*

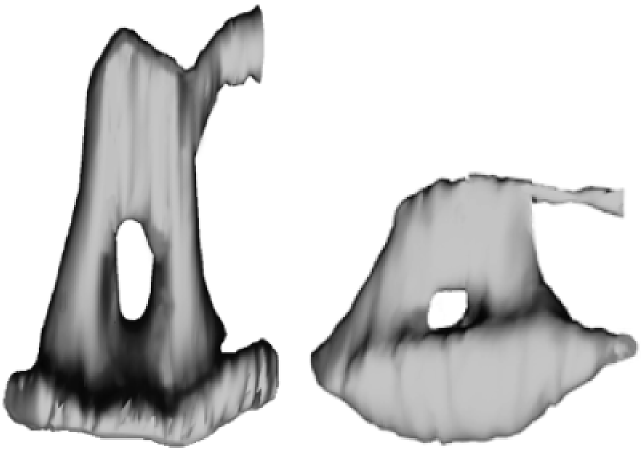

Fig. 12. Lateral views of computer-reconstructed 3D models of the right stapes in pouch young (left) *Perameles* sp. (ZSH, HL = 17.5 mm) and (right) *Dasyurus viverrinus* (ZSH, HL = 12 mm). Not to scale.

lander) is similar in general to that described by Segall (1969a,b) for *Didelphis*. In contrast to *M. domestica* and other didelphids examined (*Marmosa, Didelphis*), *Caluromys philander* shows a specially long incudal crus breve (see also Segall, 1969b: fig. 9), the functional role of this specialization being unknown (Bruns et al., 1989). Other caluromyines and didelphines should be examined to check the potential systematic value of this feature. Even though our sampling of didelphids was limited, we predict that some of the differences we found between *M. domestica* and *C. philander* can be of phylogenetic significance. Furthermore, ossicle form could provide useful characters for intrageneric phylogeny. The stapes of other species of the "genus" *Monodelphis* besides *M. dimidiata* (Archer, 1976), showing an imperforate stapes, and *M. domestica*, showing the perforate condition, should be examined.

The comparison across taxa that are phylogenetically and functionally different shows that while some variation is found in malleolar and stapedial form, that of the incus is very uniform (see also Fleischer, 1973; Archer, 1976). A similar observation was made by Nummela (1995) during her study of ossicles in eutherians. The only marsupial that significantly departs in incudal form is *Notoryctes typhlops*, which as described by Segall (1973) shows in this regard similarities to other fossorial mammals.

The specializations of the malleus in *Trichosurus vulpecula* are not universal among phalangeriformes (sensu Kirsch et al., 1997). *Pseudocheirus herbertensis* (cf. Segall, 1971: fig. 6) has a lamina and the manubrium is oriented like that of didelphids. Absence of a lamina of the malleus has evolved independently several times among marsupials. A similar malleus to that of *T. vulpecula* is present in some phalangeriformes (e.g., *Petauroides volans*, Segall, 1971: fig. 4) and in *Dromiciops gliroides* (Segall, 1969b). Several similarities are noted between the malleus of *T. vulpecula* and that of *D. gliroides* as described by Segall (1969b). We did not sample the adult mallei and inci of caenolestids and peramelids.

Cartilage of Paauw

We found that the development of the cartilage of Paauw is closely associated with that of the stapedial muscle. The relatively late development of the cartilage of Paauw in comparison with the ossicles, also found in this work, has been mentioned (Allin, 1975:406; Maier, 1987:130) as a fact that is not in accordance with the hypothesis that this element is the homolog of a portion of the extracolumella of other tetrapods (van der Klaauw, 1923; Goodrich, 1930; Westoll, 1944). Hinchcliffe and Pye (1969:286) discussed the functional role of the cartilage of Paauw in mammals and suggested (as did McCrady, 1938) a functional equivalence with a sesamoid bone. The cartilage of Paauw clearly plays a functional role, concentrating and transmitting forces between the stapedial muscle and the stapes. But the comparison with a sesamoid bone does not seem justified. Sesamoid bones are partially covered with articular cartilage that slides over a bone, and this is obviously not the case with the cartilage of Paauw. Hinchcliffe and Pye (1969:286) also pointed out that the functional role of the cartilage of Paauw does not preclude the homologization of this element with a portion of the extracolummella. As mentioned by Allin and Hopson (1992), the homology of the cartilage of Paauw is questionable.

The nature of the tissue connecting the cartilage of Paauw with the stapes is variable, depending on the stage considered. In the older histological specimens examined the connection of the cartilage of Paauw with the stapes is slender and tendinous. There is some variation as well concerning the cartilage of Paauw itself. It can be similar to hyaline cartilage (see Maier, 1987: fig. 15) or, as in the oldest stage of *Caluromys philander* examined, even ossified

Stapedial Foramen and Stapedial Artery (Fig. 13)

The presence of a large stapedial foramen is almost universal for adult didelphids and is reconstructed in the groundplan of the group. Some taxa show an imperforate stapes (*Monodelphis dimidiata, Caluromysiops irrupta*), and this condition might represent autapomorphies at different lower levels of the didelphid phylogeny.

Gaudin et al. (1996) plotted the character stapedial foramen in three marsupial phylogenies and found that in two of them a large stapedial foramen

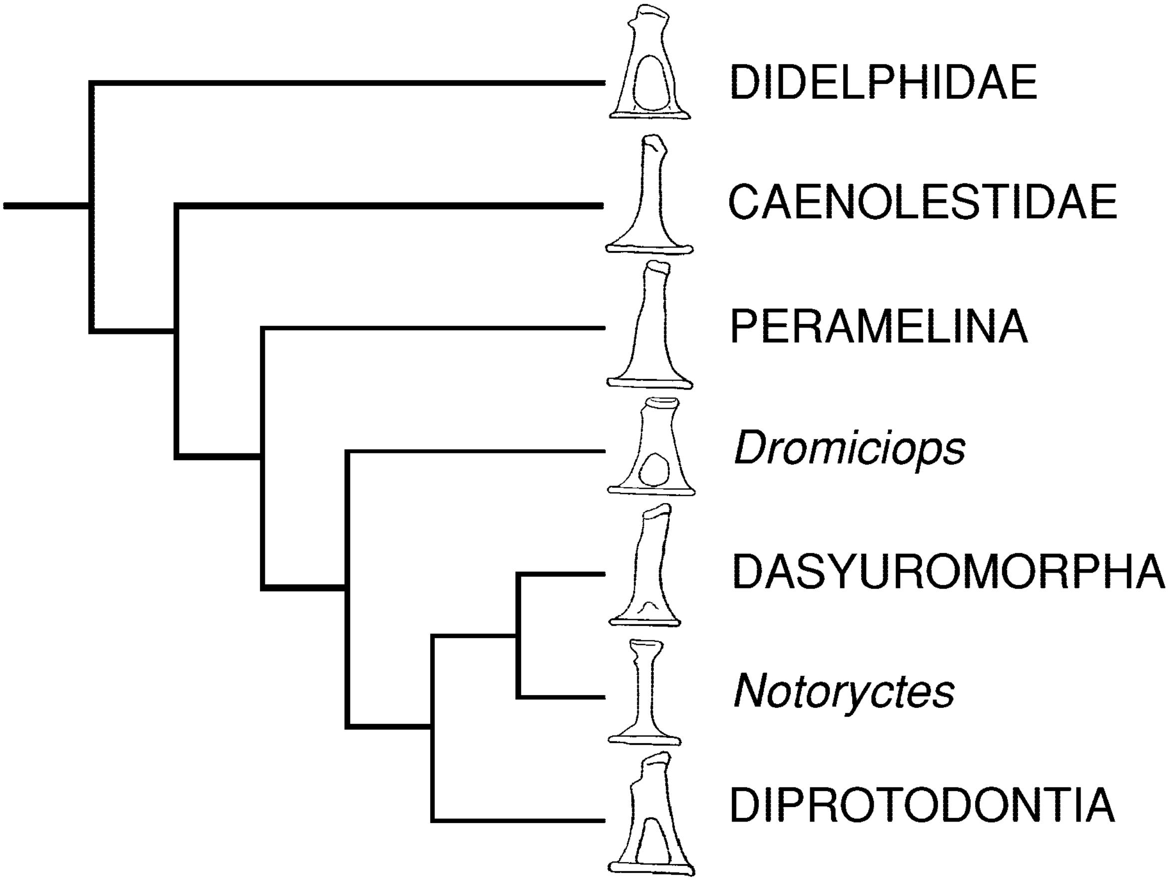

Fig. 13. Groundplan condition of the adult shape of the stapes in the major Recent marsupial clades plotted in a marsupial phylogeny based on the interordinal relationships hypothesized by Springer et al. (1998). The didelphid condition is hypothesized as plesiomorphic for Marsupialia, while that of caenolestids, peramelinans, dasyurids, and *Notoryctes* is hypothesized as derived. Sketches are modified from Doran (1878), Segall (1970), Fleischer (1973), and Novacek and Wyss (1986).

was reconstructed as present in the groundplan. In the context of the most current phylogenetic hypotheses on marsupial interordinal relationships (see Methods), a large stapedial foramen in the marsupial groundplan is also the most parsimonious reconstruction (as in Szalay, 1994). The didelphid condition is hypothesized as plesiomorphic for Marsupialia, while that of caenolestids, peramelinans, dasyurids, and *Notoryctes* is derived. The study of developing stapes in *Perameles* sp. and *Dasyurus viverrinus* provides support for the hypothesis that the imperforate rod-like stapes is secondarily derived in peramelids and dasyurids with respect to a stapes with a large stapedial foramen.

The adult didelphid condition (large stapedial foramen) is also shared by *Dromiciops* and the diprotodontian groundplan, which provides support for the association of this clade (Kirsch et al., 1997; but see Szalay, 1994) under the interpretation that the large stapedial foramen in these taxa represents a common reversal to the marsupial groundplan condition.

Presence of a stapedial foramen is not correlated with a stapedial artery. All adult marsupials examined to date lack a stapedial artery (Tandler, 1899; Archer, 1976; Wible, 1984; Aplin, 1990; Sánchez-Villagra, 1998), yet some marsupials possess a bicrurate stapes. The degeneration of the stapedial artery is not followed by the development of a columelliform imperforate stapes (contra Novacek, 1993: 522). In this work, a very feeble artery was seen traversing the obturator foramen of the stapes in the youngest stages of *Monodelphis domestica, Caluromys philander*, and *Sminthopsis virginiae*, the first two showing a bicrurate stapes as adults. There are other reports of the stapedial artery in early stages of some marsupial species, a case of recapitulation. Wible (1984:300-301) found the stapedial artery in prenatal stages of *Dasyurus viverrinus* leaving the internal carotid artery beneath the otic capsule. Maier (1987) reported a small artery traversing the stapes in a neonate *M. domestica*. Clark (1990:114) also reported the stapedial artery in a 6-day-old of *M. domestica*, but in a 10-day-old specimen the artery shows "signs of degeneration." Cords (1915) reported the stapedial artery in a 14 mm total length *Marmosa robinsoni* (="*M. chapmani*"). McCrady (1938) described a proximal stapedial artery in the newborn *Didelphis marsupialis*, arising from the internal carotid artery and piercing the stapes. Toeplitz (1920) reported a pouch young of *D. marsupialis* (greatest length = 45.5 mm) showing the presence of a stapedial artery piercing the stapes, but in this case arising from the external carotid artery. According to Wible (1984:302), the timing of disappearance of the stapedial artery "must be highly variable for *Didelphis marsupialis*," since he found no trace of this vessel in a smaller opossum pouch young (HL-H-Ms.14, greatest length = 18 mm) he examined.

The young specimens of *Trichosurus vulpecula* examined (histologically) lack a stapedial foramen, even though this structure is present in adults and Goodrich (1930) had pointed out that this foramen is present in young specimens of *T. vulpecula*. Palmer (1913) and Cords (1915) examined young specimens of *Perameles* sp. (total length 23 mm and 42 mm, respectively) showing imperforate stapes, while the pouch young of this genus examined in this work does show the foramen. Denison and Terry (1921) reported an imperforate stapes in a 17 mm pouch young *Caluromys philander*, while we found the foramen present in the pouch young examined. We found a perforated stapes in a pouch young *Dasyurus viverrinus*, while Cords (1915) reported an imperforate stapes in *Dasyurus* sp. (total length 50 mm). These results simply point out that single specimens should not be taken to represent the whole ontogenetic process. Based on our own results and those of McClain (1939), who examined a fine ontogenetic series of *Didelphis virginiana*, it appears that the pattern of development concerning the stapedial foramen is as follows. Very early in ontogeny (around birth), a feeble artery is seen traversing the foramen of a precartilaginous stapes. With the involution of the artery a few days later, and the growth of the stapes, the foramen disappears from stapes that has not yet attained its adult shape. The foramen reappears in later stages, to disappear as development continues in the case of species showing an imperforate stapes as adults and to persist in others.

Ontogeny of Hearing and the Development of the Ossicles in *Monodelphis domestica*

In recent years experimental work has been conducted concerning the onset and development of hearing in *Monodelphis domestica* (Reimer, 1996; Aitkin et al., 1997). Reimer (1996:147) reported significant changes in the audiograms between PND-29 and PND-40. Based on brainstem auditory evoked potentials (BAEP), the onset of hearing was recorded at PND-29, "as judged from consistent BAEP recordings." She (1996:147) stated that "adult-like BAEPs are reached about 10 days later. Initially, only frequencies in the lower mid-range of the adult audiogram evoke responses. During postnatal development the response range expands towards both lower and higher frequencies." Slightly different results concerning the onset of hearing were obtained by Aitkin et al. (1997), probably related to methodological procedures, laboratory conditions, and biological variability. Aitkin et al. (1997) measured evoked potentials to click stimuli from the vertex of the skull immediately over the inferior colliculus. Aitkin et al. (1997) recorded responses in one 24-day-old individual, and only a couple of other individuals of age less than 28–30 days old also showed responses. In any event, it appears that most *M. domestica* are able to hear around postnatal days 28–30.

Is the middle ear involved during the onset of hearing in *Monodelphis domestica*? Aitkin et al. (1997:73) reported that "the middle ear cavity was clear of fluid from approximately 26 days and the ear canal was clearly patent at 28–30 days." We did observe loose mesenchyme occupying portions of the middle ear in animals 30 and 35 days old (Fig. 4). Concerning the ear canal, in the PND-30 specimen examined the external auditory meatus is open, but only partially so. By PND-35 the meatus is much wider. The opening of the external auditory meatus had been reported at PND-32 (in Cant, 1998). The ossicles are not fully ossified by PND-30. In summary, by PND-30, around the time when the onset of hearing occurs, middle ear structures are not mature, but they are in a stage in which it is conceivable that they participate in sound transmission. The ability to perceive air-conducted auditory stimuli is not necessarily correlated with mature middle ear structures. Response to this kind of stimuli has been recorded in laboratory rats at stages in which the meatus is still occluded (Geal-Dor et al., 1993: 240).

It is worth mentioning that in all species of eutherians examined to date that show a postnatal onset of hearing, there is a time gap of usually several days between the earlier first auditory responses to bone-conducted stimuli and later to air-conducted stimuli (Woolf and Ryan, 1988: gerbil; Wolfson et al., 1990: sheep; Geal-Dor et al., 1993: rat). Perhaps future experiments examining onset of hearing through bone conduction could test if this is the case also in *Monodelphis domestica*.

It is interesting to note that during the time in which significant changes in the audiograms have been recorded (between PND-29 and PND-40), changes occur as well in middle ear anatomy. However, concerning ear ossicle anatomy and the ontogeny of hearing, it is practically impossible to establish a significant quantitative relationship. Changes in the inner ear during growth perhaps also play a role in the ontogenetic changes of hearing. Geal-Dor et al. (1993:241), based on experimental and anatomical data on the rat, concluded that "immaturity of the impedance matching mechanism of the middle ear is not a significant conductive factor responsible for elevated neonatal auditory thresholds." Resorption of middle ear mesenchyme and/or ossicular ossification probably affects impedance matching (Woolf and Ryan, 1988; Geal-Dor et al., 1993:241). These factors probably also affect (perhaps even more significantly) the absorption of the sound energy in the middle ear. It is expected that less energy is being absorbed as a consequence of ossification, as bone is harder than cartilage and absorbs less energy.

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