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Cranial Osteogenesis in *Monodelphis domestica* (Didelphidae) and *Macropus eugenii* (Macropodidae)

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ABSTRACT The pattern of onset and general rate of cranial ossification are compared in two marsupials, *Monodelphis domestica* (Didelphidae) and *Macropus eugenii* (Macropodidae). In both species a similar suite of bones is present at birth, specifically those surrounding the oral cavity and the exoccipital, and in both postnatal events follow a similar course. The facial skeleton matures more rapidly than the neurocranium, which is characterized by an extended period of ossification. Most dermal bones begin ossification before most endochondral bones. Endochondral bones of the neurocranium are particularly extended in both the period of onset of ossification and the rate of ossification. These data confirm suggestions that morphology at birth is conservative in marsupials and we hypothesize that the pattern of cranial osteogenesis is related to two distinct demands. Bones that are accelerated in marsupials are correlated with a number of functional adaptations including head movements during migration, attachment to the teat, and suckling. However, the very slow osteogenesis of the neurocranium is probably correlated with the very extended period of neurogenesis. Marsupials appear to be derived relative to both monotreme and placental mammals in the precocious ossification of the bones surrounding the oral cavity, but share with monotremes an extended period of neurocranial osteogenesis.

The most consistent differences between extant metatherian (marsupial) and eutherian (placental) mammals are found in their reproductive anatomy and behavior (Hayssen et al., '85; Kirsch, '77a–c; Lillegraven, '75, '79; Renfree, '83; Russell, '82). Marsupial gestation is relatively short with a limited time taken up by active morphogenesis (range of 6–14 days; Selwood, '80; Lee and Cockburn, '85; Tyndale-Biscoe and Renfree, '87). This consistently short period of active morphogenesis during gestation produces small altricial neonates and litters that do not exceed 1% of maternal adult weight (Lee and Cockburn, '85). Marsupial neonates are generally considered developmentally "equivalent" to eutherian fetuses and are thought to have similar morphology, at least externally, throughout the taxa (Lillegraven, '75).

Although it is well known that the marsupial neonate is altricial relative to the eutherian neonate (Kirsch, '77a,c; Lillegraven, '75, '79; Lillegraven et al., '87; Müller, '67, '68a,b), details on the development of marsupials are relatively poorly understood. General patterns of development have been discussed in a number of papers (e.g., Bancroft, '73; Hartman, '19; Hill, '11; Hill and Hill, '55; McCrady, '38; Selwood, '80; Tyndale-Biscoe and Renfree, '87). The morphology and postnatal maturation of several organ systems have been studied in marsupials, including visceral systems (Buchanan and Fraser, '18; Farber, '78; Farber et al., '84; Krause and Cutts, '84; Krause and Leeson, '73; Krause et al., '85, '86), the central nervous system (Cavalcante et al., '84; Morest, '70; Nelson, '88; Renfree et al., '82; Reynolds and Saunders, '88; Riese, '45; Saunders et al., '89; Ulinski, '71), the peripheral nervous system (Krous et al., '85), and the upper limb (Cheng, '55; Klima, '87). Despite the growing list of contributions, two recent reviews on the adaptations of the marsupial newborn (Hall and Hughes, '87; Hughes and Hall, '88) serve to

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highlight the relative lack of detailed, comparative knowledge of marsupial development.

Few studies have presented details on craniofacial development in marsupials. Most studies of marsupial cranial development have described single or at most a few stages of development (e.g., Broom, '09; Cords, '15; Denison and Terry, '21; Esdaile, '16; Presley, '81; Toeplitz, '20). Only a few have described series of ontogenetic stages (e.g., Clark, '87, '90; Filan, '91; McClain, '46; Maier, '87a,b; Müller, '68a). Many of these studies have concentrated on limited cranial regions. Most studies of cranial development in marsupials (as well as other mammals) focus on the chondrocranium with limited comments on the bony skeleton.

The head of neonatal marsupials is relatively large compared with the rest of the body (e.g., de Beer, '37; Griffiths, '78; Hughes and Hall, '88; Tyndale-Biscoe, '73). It is believed that the primary reason for this is the requirement that the neonate be capable of attaching to and suckling from the teat. This common functional requirement has been suggested as an explanation for the similarity of head morphology in this group (Lee and Cockburn, '85; Lillegraven, '75). Studies of cranial ontogeny in marsupials indicate that the structures associated with suckling appear to differentiate earlier than the rest of the head (Hill and Hill, '55; Renfree et al., '82; Renfree and Tyndale-Biscoe, '73; Sharman, '73; Walker and Rose, '81). In the few marsupials for which data on cranial ossification are available, it has been noted that the bones around the oral cavity are well differentiated in neonates at a time when the remaining cranial bones are undifferentiated (Broom, '09; Clark, '87; de Beer, '37; Esdaile, '16; Gemmell et al., '88; Nesslinger, '56; Sharman, '73) and it is assumed that this pattern of ossification is characteristic of all marsupial neonates. However, only one complete study of cranial ossification in a marsupial is available (Nesslinger, '56) and its value is diminished by relying on many specimens of uncertain age.

In this paper we examine cranial osteogenesis in two marsupials, *Monodelphis domestica* (Didelphidae) and *Macropus eugenii* (Macropodidae). Our primary concern is with three aspects of osteogenesis: 1) the state of ossification at birth, 2) the sequence of onset of ossification, and 3) the relative rate of ossification. Focus in this paper is on the relative timing of ossification, as determined from a finely age-graded series of specimens, rather than on the details of the emergence of specific form in these taxa. In the course of this description we provide basic data on the cranial ontogeny of two increasingly common laboratory marsupials and use these data to test the hypothesis that morphology in marsupial neonates is conservative (Lee and Cockburn, '85; Lillegraven et al., '87). Additionally, we discuss relations between cranial osteogenesis in marsupials and functional requirements associated with their altricial birth. Finally, we place the data from these species in a comparative context through a survey of the literature on osteogenesis in other mammals and non-mammalian tetrapods. This comparative review assists in establishing the primitive pattern of osteogenesis in mammals and in assessing which, if any, aspects of the marsupial pattern are derived.

MATERIALS AND METHODS

Specimens

The specimens used in this study include a total of 21 serially sectioned and 29 cleared and stained specimens of *Monodelphis domestica* at 17 ages of pre- and postnatal development.[1] The youngest age was from a 14-day intrauterine litter, approximately 0.5 day before birth. The oldest specimens examined were 30 days postnatal. Seven serially sectioned and 32 cleared and stained specimens of *Macropus eugenii* from 27 ages of pre- and postnatal development were also studied. The youngest specimen was obtained on its 24th day of gestation, approximately 2 days before birth. The oldest specimen prepared was 52 days postnatal when it was sacrificed. The age of the specimens is indicated as follows: An E following the numerical age indicates the gestational age of prenatal specimens; a P following the numerical age indicates the postnatal age of the specimen. The day of birth is considered day OP.

The animals were obtained from two sources. The specimens of *Monodelphis domestica* were obtained from a breeding colony at Duke University. Female and male adults were mated following the procedure outlined by Fadem et al. ('82). The females were checked daily for the presence of a litter, so that the time of birth was known

[1]Since the original drafting of this paper over 35 additional *M. domestica* specimens and over 10 additional *M. eugenii* specimens have been prepared and used to confirm the results reported here. Complete lists of specimens are available from K.K.S.

within 24 hours. The pups were removed from the teats following anesthesia or restraint of the mother. The pups were sacrificed with an aerosol overdose of Halothane or exposure to cold in the case of very young pups, and fixed in 10% phosphate-buffered formalin. The *Macropus eugenii* specimens were obtained from Dr. Marilyn Renfree of Monash University, Melbourne, Australia. The breeding and harvesting information for her colony has been published previously (see Renfree et al., '82).

Preparation of specimens

Two methods of preparation were used: clearing and differential staining for cartilage and bone (Wassersug, '76), and serially sectioning and staining of paraffin-embedded specimens. The sectioned specimens were cut at a thickness of 10–12 μm. The sections were stained with Milligan's trichrome or Weigert's hematoxylin counterstained with picroponceau (Humason, '72). Some specimens were stained with Bodian's silver stain (Bodian, '36). Both whole and hemisectioned heads of each species were prepared using these techniques. In particular, as the size of the animal increased it became necessary to cut the heads in half for either method to be effective. In some cases one side of a head was cleared and stained while the other was serially sectioned. In cases in which the head was not bisected, we removed the outermost layer of epidermis in order to achieve proper infiltration.

The staining techniques used in this study have different sensitivities in determining the presence of bone. Histological staining of serially sectioned material reveals the presence of bone earlier than the method of clearing and differential staining for cartilage and bone (Hanken and Hall, '88). The difference in detection can be on the order of several days to a week. However, the sequence and pattern of ossification revealed by these two techniques are always the same. Dates given for first ossification generally refer to appearance in serially sectioned material, although much of the description of changing form is derived from examination of cleared and stained material, as well as observations of sectioned material and in some cases computer-assisted three-dimensional reconstructions.

Choice of species

Monodelphis domestica and *Macropus eugenii* represent primitive and derived lineages of metatherians, respectively. The biogeographic history of these two species indicates that their lineages have been evolving separately for at least 40 million years (Woodburne and Zinsmeister, '84). The Didelphidae are considered to resemble most closely the ancestral marsupial condition (Gardner, '82; Kirsch, '77b; Kirsch and Calaby, '77; Lee and Cockburn, '85). They have a diverse representation in the fossil record of the Americas going back to the late Cretaceous of North America (Clemens, '79; Fox, '87). *M. domestica* retains many of the characters expected in a basal didelphid: small adult size (80–140 gm); absence of pouches in both sexes; large mean litter size (~ 8) with relatively small neonates (75–100 gm); short gestation period (14.5 days; Fadem et al., '82; Fadem and Rayve, '85); primitive dentition and didactylous hindfoot morphology (Abbie, '37). *M. domestica* is one of 17 species of the genus *Monodelphis*. Animals of this genus are distributed throughout the northern two-thirds of South America; *M. domestica* is native to eastern and central Brazil, Paraguay, and Bolivia (Nowak, '91; Streilein, '82a–d). Nowak ('91) reports that this genus is apparently the least arboreal of the didelphid marsupials. Streilein ('82a–d) has studied the behavior of *M. domestica* in both the field and the laboratory and reports that it is widespread in all habitats of its native region, including rainforest, cerrado, and the hot, dry rocky environment of the Caatinga region of eastern Brazil. It is an efficient predator of invertebrates and small vertebrates; it also consumes fruit. Streilein found that members of this species are generally solitary and highly intolerant of other individuals regardless of sex. *M. domestica* appears to breed year round in its natural habitat (as well as in the laboratory) with an estimated maximum production of 40–50 young per female per year (Streilein, '82b). The young begin to detach from the teat at about 2 weeks after birth and are weaned about 50 days after birth. Animals reach sexual maturity at 4–6 months (Kraus and Fadem, '87).

The Macropodidae represent a derived family of Australian marsupials (Kirsch, '77b; Kirsch and Calaby, '77; Lee and Cockburn, '85). Although they have a fossil record extending only to the middle Miocene, their origin was probably earlier (Archer and Bartholomai, '78). *Macropus eugenii,* like other macropodids, exhibits several derived characters: pouches in females; litter size of 1 with a

neonatal body weight of 500 gm; embryonic diapause; relatively long post-diapause gestation length (25 days; Renfree and Tyndale-Biscoe, '73); an unusual and derived pattern of tooth replacement and derived tooth morphology; bipedal saltatory locomotion; and syndactylous hindfoot morphology (Abbie, '37). *M. eugenii* is a relatively small macropodid (female body weight of approximately 5.5 kg) and like other macropodids is largely herbivorous. It is a seasonal breeder, native to southern Australia where food resources are predictable and good in the spring and early summer and poor in autumn and winter. Birth, for the most part, occurs in late January or early February, followed by a postpartum oestrus. The young are attached to the teat for approximately 100 days and are weaned at the beginning of spring (October–November), approximately 270 days after birth. Females may enter their first oestrus immediately after weaning (Renfree, '83; Tyndale-Biscoe and Renfree, '87). This reproductive cycle is retained in captive animals (Lee and Cockburn, '85).

RESULTS

The first appearance of ossification in each bone is noted for each species, followed by a description of the bone's ontogeny in *Monodelphis domestica* until it reaches adult proportions. Unless specifically noted, the condition for *Macropus eugenii* is similar to that in *M. domestica.* There are some differences in the shape and number of the various ossification centers; the same bones are present in the heads of both species. Unless otherwise specified, we discuss the development unilaterally, i.e., a statement that a single center of ossification exists refers to the condition on one side of the animal. All elements of the head with the exception of the midline vomer, presphenoid, basisphenoid, basioccipital, and supraoccipital are bilaterally symmetrical. The bones of the skull may be divided by a number of criteria: origin of bone (dermal vs. endochondral); embryological region (viscerocranium vs. neurocranium); or functional (facial skeleton, auditory region, cranial cavity). In the presentation of results we divide the bones into viscerocranium vs. neurocranium, but in the discussion compare development within each of these systems of division. The age at which bone first appears in each bone in each species is summarized in Table 1.

Viscerocranium

Dentary

The dentary is well ossified at birth in both *Monodelphis domestica* and *Macropus eugenii,* and in both ossification is present in the earliest available specimen (*M. domestica,* 14E; *M. eugenii,* 24E). On first appearance in both species the dentary is a splint of bone on the lateral surface of Meckel's cartilage and shows no sign of coronoid, condylar, or angular processes. The ossification is confined to the anterior portion of the lower jaw and is most pronounced in the area of the future symphysis (Figs. 1A, 3A). The lower jaw of these two species is initially supported by a

TABLE 1. Time of onset of ossification in skull bones of Monodelphis and Macropus[1]

Dermal bone	*Monodelphis domestica*	*Macropus eugenii*	Endochondral bone	*Monodelphis domestica*	*Macropus eugenii*
Premaxillae	−1	−2	Exoccipitals	0	0
Maxillae	−1	−2	Basioccipital	+3	+3
Palatines	−1	−2	Ala temporalis	+4	+3
Pterygoids	0	−2	Supraoccipital	None	+8
Mandibles	−1	−2	Basisphenoid	+6	+11
Squamosals	+1	0	Malleus	+11	+21
Vomer	+2	0	Periotic	+12	+31
Lacrimal	+2	0	Presphenoid	+13	+33
Tympanic	0	+3	Orbitosphenoid	+14	+33
Prearticular	0	+3	Incus	+17	+42
Nasal	0	+3	Stapes	+25	+52
Jugal	+1	+3			
Parietal	+3	+3			
Frontal	0	+5			
Interparietal	+3	None			
Postparietal	None	+8			

[1]This table summarizes data on osteogenesis of cranial bones, including the type of bone, and its time of ossification onset. The time of onset is standardized with the day of birth defined as day 0. Dermal bones are ordered according to sequence of ossification in *Macropus.* Prenatal (gestation) days are indicated with the prescript −. Postnatal days are indicated with the prescript +.

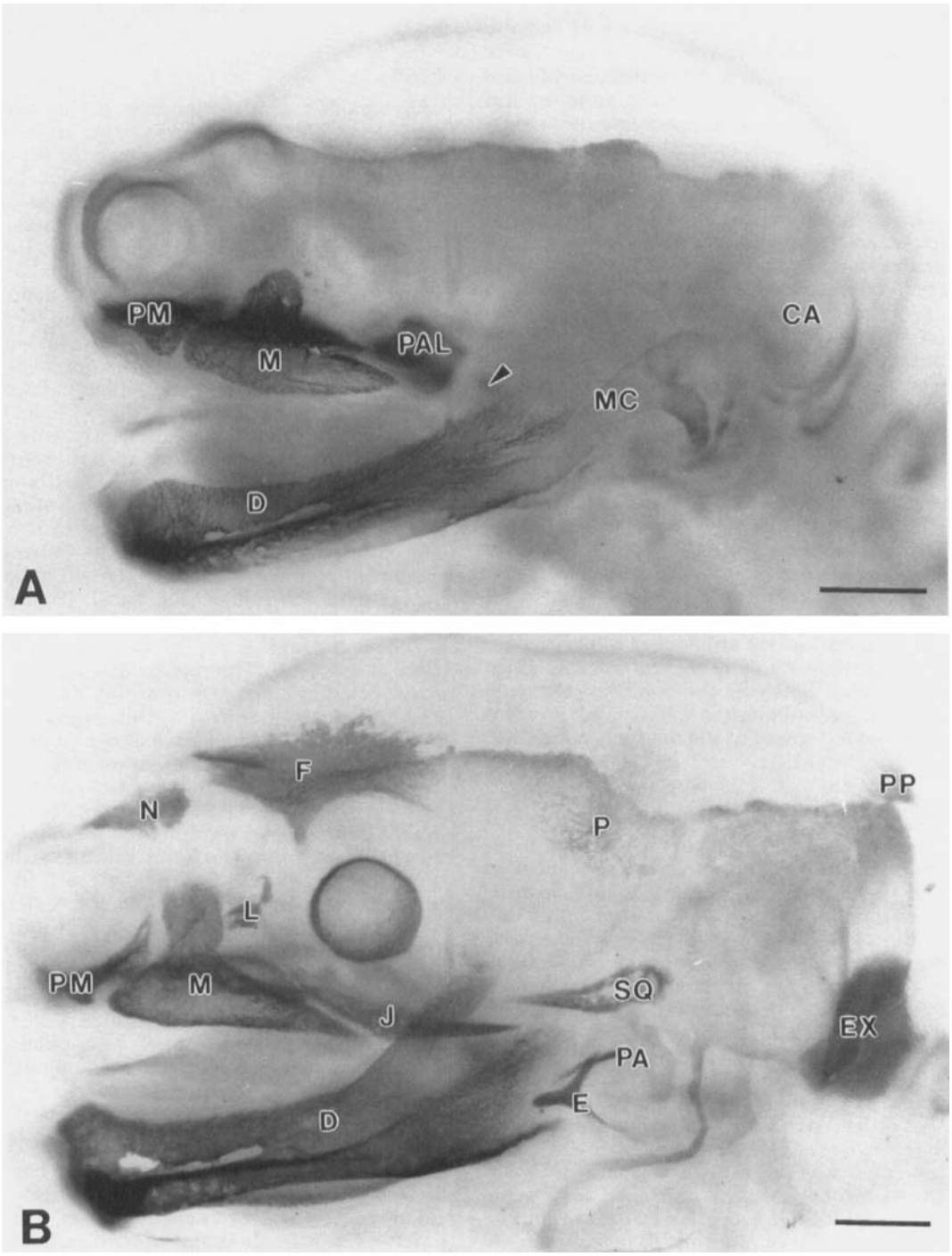

Fig. 1. Photograph of differentially stained and cleared skulls of *M. domestica* specimens. **A:** 2P. **B:** 4P. Note the concentration of ossified tissue around the oral cavity, especially in the youngest specimen, and the robust chondrocranium. CA, canalicular cartilage; D, dentary; E, ectotympanic; EX, exoccipital; F, frontal; J, jugal; L, lacrimal; M, maxilla; MC, Meckel's cartilage; N, nasal; P, parietal; PA, prearticular; PAL, palatine; PM, premaxilla; PP, postparietal; SQ, squamosal; arrowhead in A indicates ossification in pterygoid bone. Scale bars = 0.5 mm.

jaw joint made up of the cartilaginous malleus and incus and their contact with the otic capsule. Growth of the dentary is posteriorly and in *M. domestica* at 2P, trabeculae of bone begin to establish the coronoid, condyle, and angular processes. These processes are recognizable by position only; the condyle shows no signs of secondary cartilage. During the next day (3P specimen) the processes become distinguishable by their morphology (Fig. 1B) and subsequent changes in the mandible are primarily growth related (Figs. 1–4). The condylar cartilage is differentiated by 7P in *M. domestica* and at this time is relatively large (Fig. 5). At this time the condyle does not sit in the glenoid fossa, but abuts a thin wedge of squamosal bone. It is difficult to define a precise date for the formation of a functional dentary-squamosal joint, because for a time the contacts between the condylar cartilage and squamosal and the auditory ossicles and the otic region are equally large (Fig. 6) and probably both serve as buttresses for the lower jaw (Filan, '91). By day 20P in *M. domestica,* although the contact between the auditory bones and the braincase is robust, these bones are no longer connected to the dentary and do not appear to participate in the formation of this joint. At this stage the contact between the condylar cartilage and the glenoid fossa is well established and the synovial cavity of the dentary-squamosal joint is present.

Premaxilla

The premaxilla has begun ossification in the 14E specimen of *Monodelphis domestica* and the 24E specimen of *Macropus eugenii.* In both animals there appears to be only one center of ossification. The nasal, palatal, and maxillary processes grow appositionally from this center during the next day, and are well formed at birth in each species (Figs. 1A, 3A). The only subsequent changes are increased size and contact with surrounding bones (Figs. 1–4). In the OP *M. domestica* a condensation of mesenchyme that may be similar to that observed in *Didelphis aurita* and *Caluromys philander* by Hill and de Beer ('49), and interpreted by these authors to be a vestige of the os carunculae of monotremes, is present. In the day 24P *M. eugenii* a structure similar to the vestigial egg tooth of *Trichosurus vulpecula* and *Phascolarctus cinereus* figured by Hill and de Beer ('49) is also seen. The premaxilla, however, is not hypertrophied in early development as observed in monotremes (e.g., de Beer and Fell, '36; Gaupp, '08).

Maxilla

The maxilla is also ossified in the 14E specimen of *Monodelphis domestica* and the 24E specimen of *Macropus eugenii.* In these early stages the palatal, facial, and alveolar processes are recognizable. In *M. domestica* immediately before birth (the 14E specimen) the palatal shelf has not elevated and lies lateral to the tongue (Fig. 7). In the 24E *M. eugenii* the palatal shelves have elevated, but have not met, and lie above the tongue with an open connection between the oral and nasal cavities. In both species the maxilla is well developed at birth with ossified palatal, facial, and alveolar processes (Figs. 1A, 3A). This bone is best developed anteriorly, with a full circle of bone surrounding the oral cavity (Fig. 8A). More posteriorly the maxillary bone is not complete but is composed of splints of bone, connected by connective tissue and undifferentiated mesenchyme (Fig. 9). During its succeeding growth the maxilla will make contact with the premaxilla, nasal, frontal, lacrimal, palatine, and jugal bones as well as its fellow through a suture in the midline palate (Figs. 1, 4). The mid-palatal junction forms secondary cartilage initially, which is later transformed into a suture when the maxillary bones contact each other to complete the hard palate. We cannot date the formation of the suture between the two halves of the palate because cartilage is still present in our oldest specimens of *M. domestica* and *M. eugenii.* The fenestrations of the palate, characteristic of marsupials, are secondary developments. In *M. domestica* they first appear in the palatal process of the maxilla between days 25 and 30 postnatally. They take the form of a keyhole extending anteriorly from the maxilla-palatine contact. A continuation of this fenestration extends posteriorly into the palatine bone. The palatal fenestrations are not yet present in our oldest specimen of *M. eugenii* (52P).

Palatine

The initial ossification of the palatine bone has begun in the 14E *Monodelphis domestica* and in the 24E *Macropus eugenii* (Figs. 1, 3). It appears as a single center of ossification in the unelevated palatal shelf. After palatal shelf fusion just prior to birth, the palatine bone has a well-developed lower horizontal (palatal) plate connected to a vertical plate

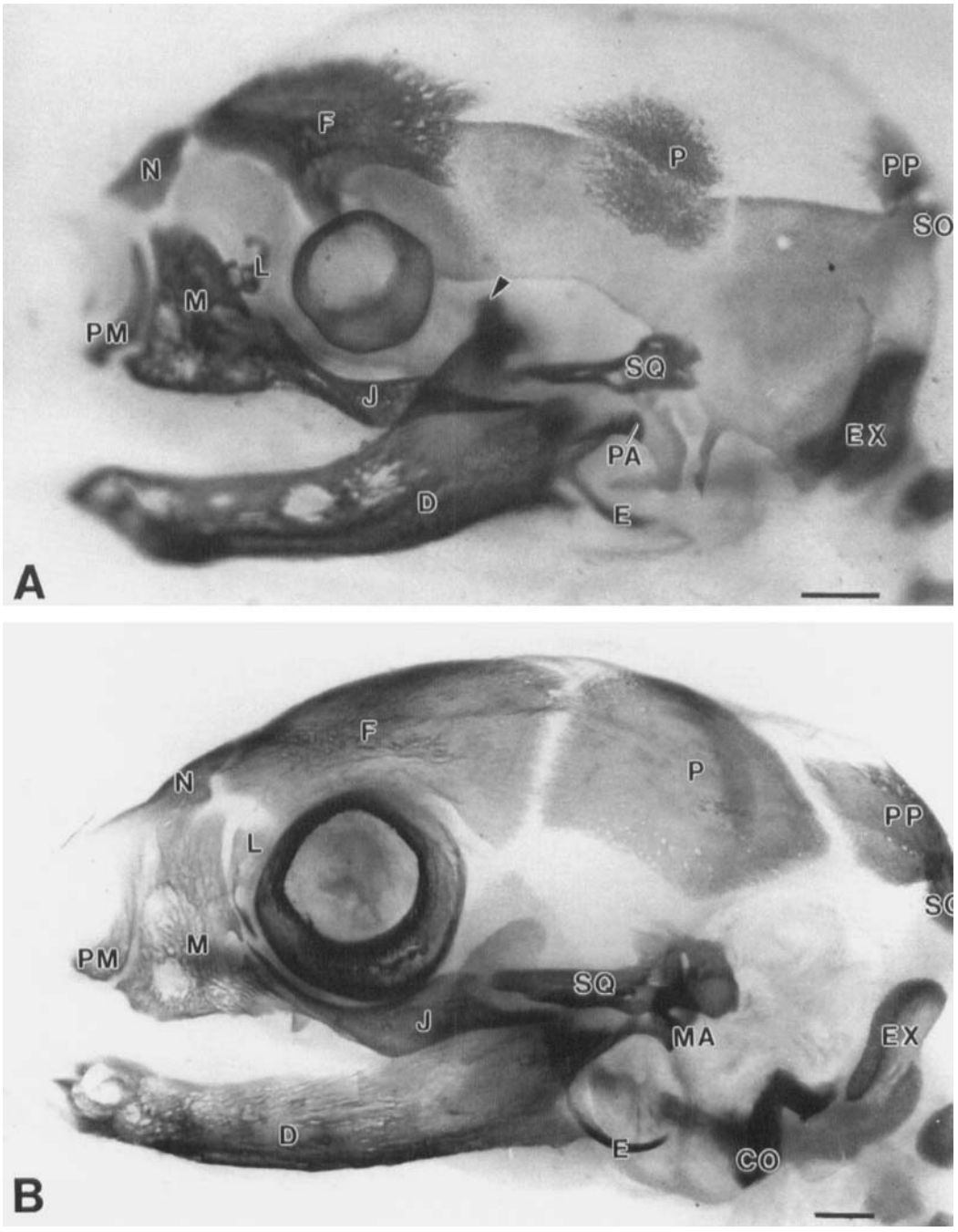

Fig. 2. Photographs of differentially stained and cleared skulls of specimens of *M. domestica*. **A:** 7P. **B:** 13P. CO, cochlear ossification; D, dentary; E, ectotympanic; EX, exoccipital; F, frontal; J, jugal; L, lacrimal; M, maxilla; MA, malleus; N, nasal; P, parietal; PA, prearticular; PM, premaxilla; PP, postparietal; SO, supraoccipital; SQ, squamosal; arrowhead in A indicates ossification of alisphenoid deep to coronoid process of mandible. Scale bars = 1.0 mm.

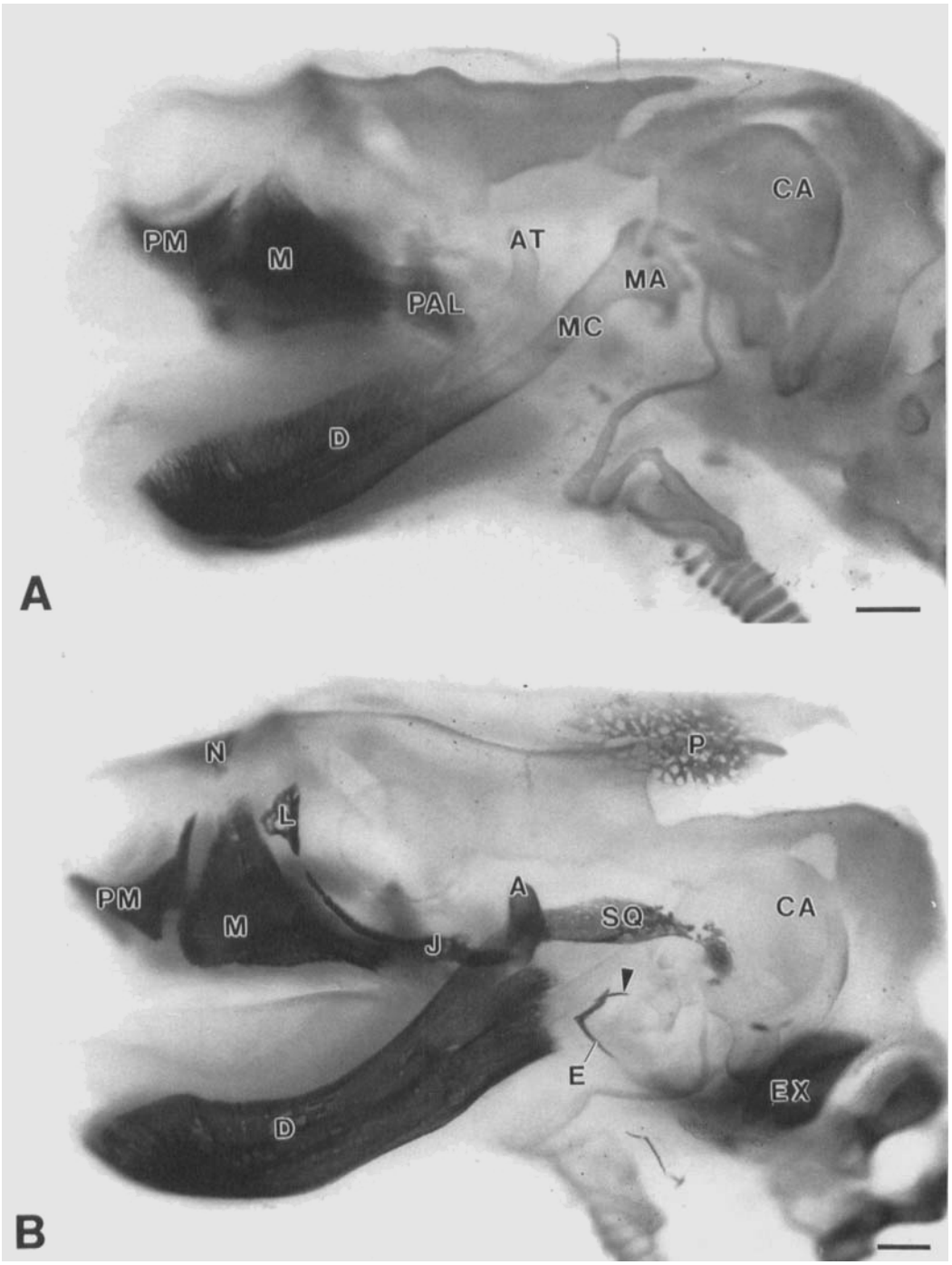

Fig. 3. Photographs of differentially stained and cleared skulls of *M. eugenii* specimens. **A:** 1P. **B:** 6P. A, alisphenoid ossification; AT, ala temporalis (lamina ascendens); CA, canalicular cartilage; D, dentary; E, ectotympanic; EX, exoccipital; J, jugal; L, lacrimal; M, maxilla; MA, malleus; MC, Meckel's cartilage; N, nasal; P, parietal; PAL, palatine; PM, premaxilla; SQ, squamosal; arrowhead in B indicates prearticular. Scale bars = 0.5 mm.

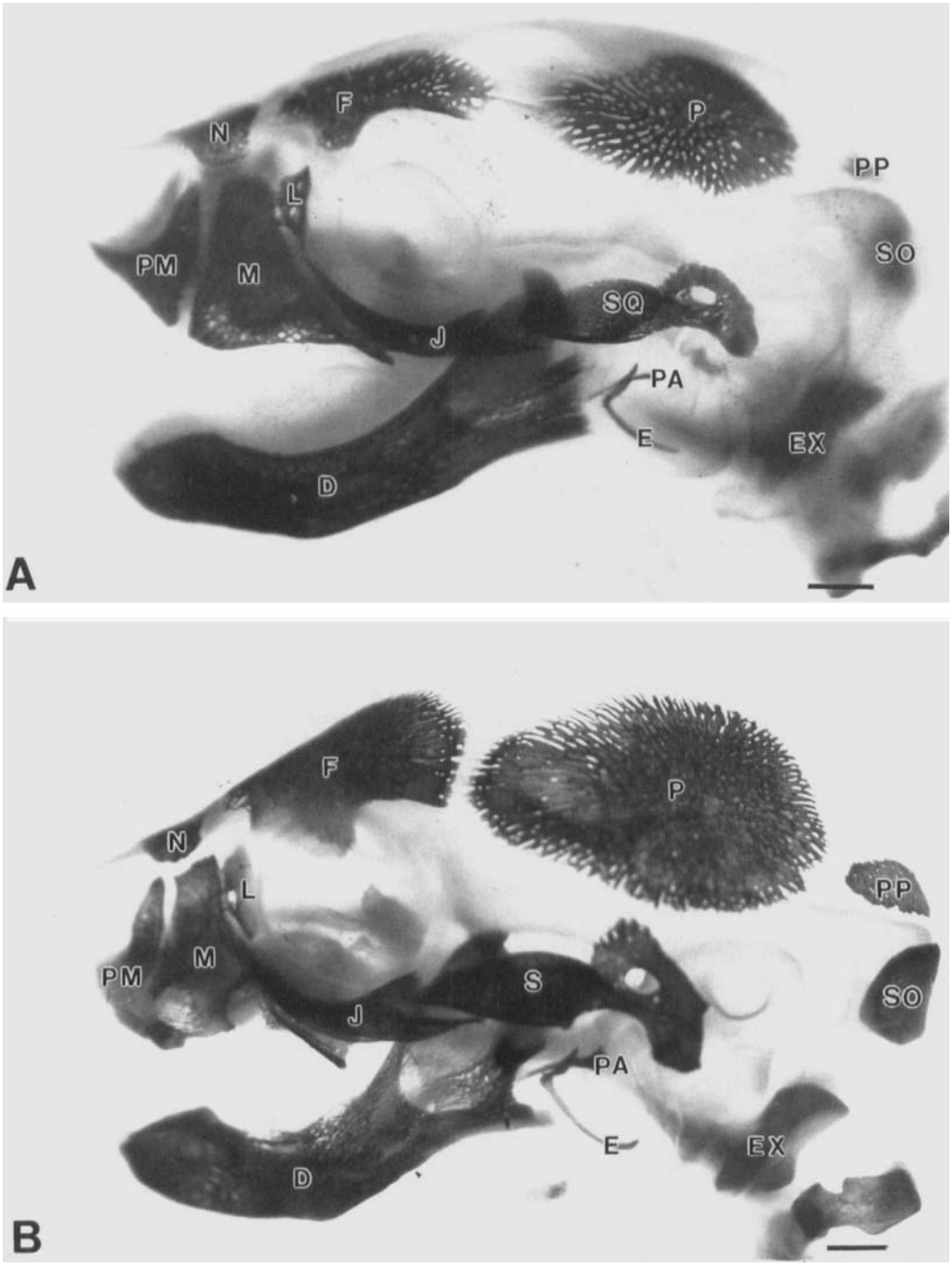

Fig. 4. Photographs of skulls of differentially stained and cleared specimens of *M. eugenii*. **A:** 14P. **B:** 24P. D, dentary; E, ectotympanic; EX, exoccipital; F, frontal; J, jugal; L, lacrimal; M, maxilla; N, nasal; P, parietal; PA, prearticular; PM, premaxilla; PP, postparietal; SO, supraoccipital; SQ, squamosal. Scale bars = 1.0 mm.

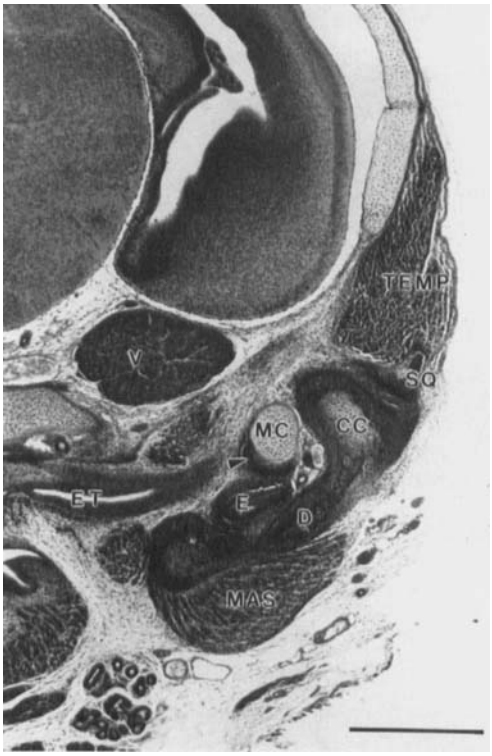

Fig. 5. Photomicrograph of a transverse section through the head of an 8P *M. domestica* (KS130). Note development of condylar cartilage. CC, condylar cartilage; D, dentary; E, ectotympanic; ET, Eustachian tube; MAS, masseter muscle; MC, Meckel's cartilage; SQ, squamosal; TEMP, temporalis muscle; V, trigeminal ganglion; arrowhead points to prearticular ossification. Ten micron paraffin section, stained with Milligan's trichrome. Scale bar = .5 mm.

that recurves toward the midline above the internal choanae of the nasal cavity; it forms the skeletal support for the nasopharyngeal passage (Figs. 8B, 9). This dorsal plate of bone contacts the cartilaginous cranial base in the region of the future presphenoid ossification center. Like the palatal shelf of the maxilla, the shelf of the palatine develops secondary cartilage at the midline.

Pterygoid

The pterygoid bone first appears in 1P specimens of both species. It appears as a single center of ossification on the ventral surface of the pterygoid process of the ala temporalis. In its early stages of ossification the pterygoid is a dermal ossification adjacent to this cartilaginous process (see Fig. 11). The hamulus develops as an outgrowth of this initial center and does not develop any secondary cartilage until the 2nd postnatal week. In *Monodelphis domestica,* the pterygoid becomes intimately associated with the palatine bone and all the divisions of the sphenoid bone later in its ontogeny, but never fuses with these latter bones. Even in the adult the pterygoid is flexibly attached to the cranial base and is easily removed from a macerated skull.

Nasal

The nasal bone first appears in the 1P specimen of *Monodelphis domestica* and in the 3P specimen of *Macropus eugenii.* At this time it is a very thin lamina of bone across the bridge of the nasal capsule (Figs. 1, 3). During further development, the nasal grows in every dimension over the nasal capsule (Figs. 1–4). By 11P in *M. domestica* the nasal bones have met the frontals and by day 16P contact the premaxillae, maxillae, lacrimal, and the nasal of the opposite side. This contact with the other facial bones is present by day 45P in *M. eugenii.*

Jugal

This bone is detectable as a slim bony bar extending from the zygomatic process of the maxillae to the squamosal in the 2P specimen in *Monodelphis domestica* and 3P specimen in *Macropus eugenii* (Figs. 1, 3). It contacts neither of these bones at this stage, but shows some overlap with the zygomatic process of the maxilla. The primary changes in the morphology of the jugal are minor and include forming contacts with the maxilla and squamosal and deepening the midpiece of the bone (Figs. 1–4). The zygomatic arch forms a complete arch extending from the infraorbital canal posterior to the region of the future glenoid cavity by day 11P in *M. domestica* and day 17P in *M. eugenii,* although a gap still exists between the jugal bone and the zygomatic process of the squamosal.

Squamosal

The squamosal first appears in the 2P specimen in *Monodelphis domestica,* but already exhibits ossification in the 1P specimen of *Macropus eugenii.* In its earliest form it is a dagger-shaped ossification with a sharp anterior process directed toward the jugal (Figs. 1, 3). The posterior portion of the bone is trabeculated and forms the lateral wall of the fossa housing the short crus of the incus

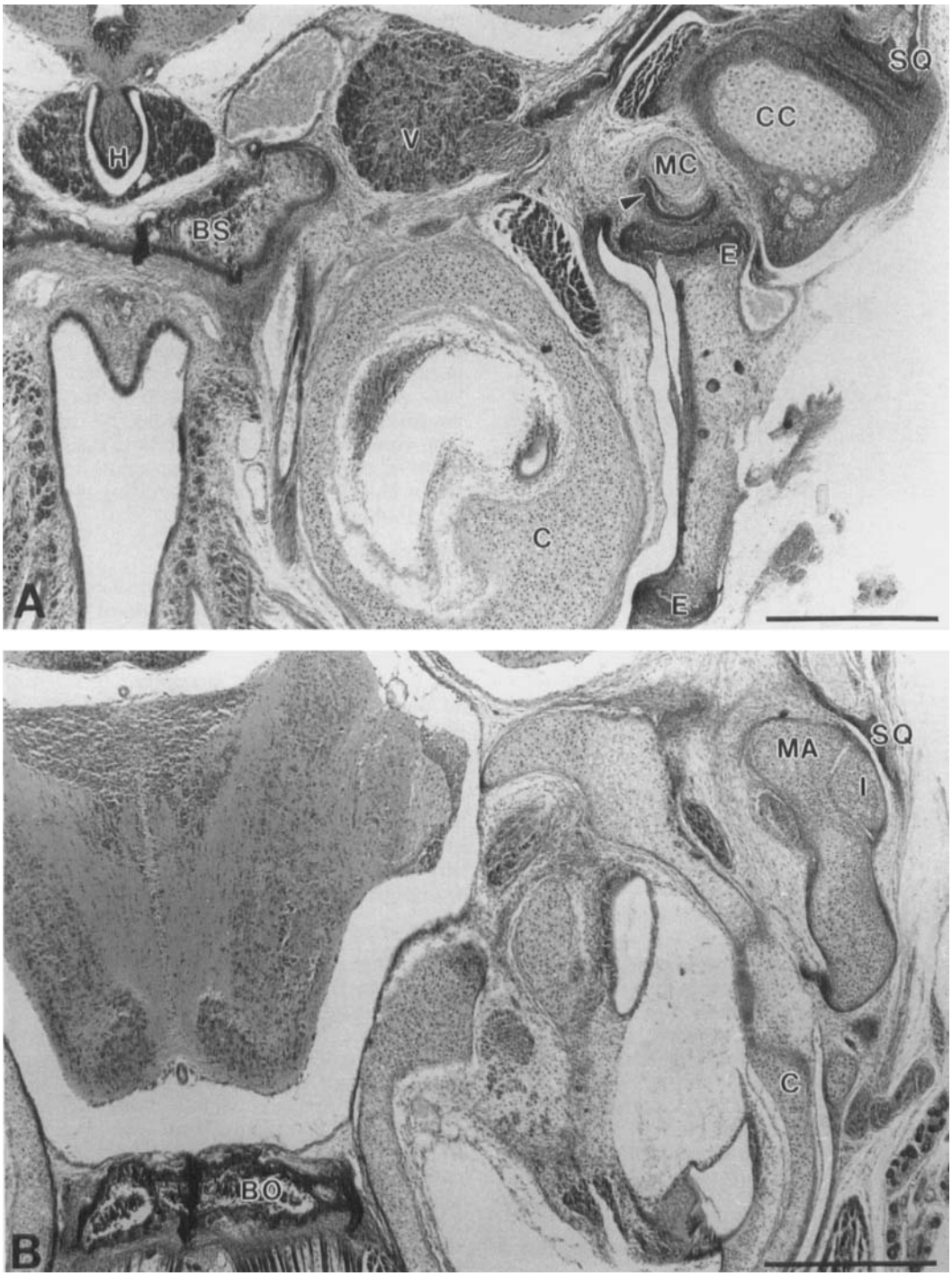

Fig. 6. Photomicrographs of transverse sections through the head of a 15P *M. domestica* (KS184). **A:** Anterior section through the dentary/squamosal joint. **B:** Approximately 800 μm posterior to A. Note the relative size of contacts between the condylar cartilage (CC) and squamosal (SQ) in A and the malleus/incus (MA/I) and squamosal in B. Note also the absence of a synovial cavity at the dentary squamosal joint. Other BO, basioccipital; BS, basisphenoid; C, cochlea; E, ectotympanic; H, hypophysis; MC, Meckel's cartilage; V, trigeminal ganglion; arrowhead in A indicates prearticular. Ten micron paraffin sections stained with Milligan's trichrome. Scale bars = .5 mm.

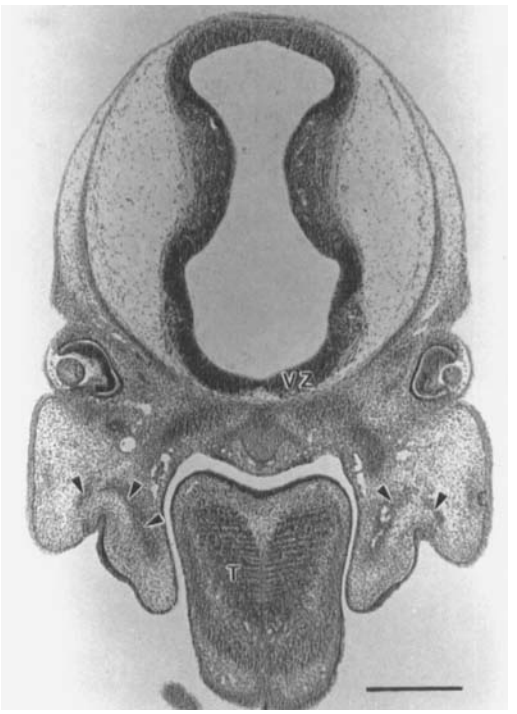

Fig. 7. Photomicrograph of a transverse section through the head of a 14E *M. domestica* (KS108), approximately ½ day before birth. Arrowheads point to the ossification of the maxillary bones in the palatal shelves, which have not yet elevated. Note the organization of muscle fibers in the tongue (T). Note too, that the forebrain consists only of a narrow band of cells at the ventricular zone (VZ). Ten micron paraffin sections, stained with Bodian's silver stain. Scale bar = 0.5 mm.

(fossa incudis). This portion of the squamosal begins ossification at least a week after ossification appears in the zygomatic process, and ossification slowly expands over the canalicular cartilage (although it is not associated with the endochondral ossification of this cartilage). Fusion with the petrosal occurs between days 25P and 30P in *M. domestica,* after ossification of the periotic cartilage is complete. The last portion of the squamosal to form is the part contributing to the lateral wall of the braincase. This is a small triangular plate of bone that grows anteriorly between days 20 and 30 to fill in the space between the alisphenoid and frontal in the lateral wall of the braincase.

Lacrimal

The lacrimal exhibits ossification in the 3P specimen of *Monodelphis domestica,* and in the 1P specimen of *Macropus eugenii.* It almost immediately takes on a complex shape in the medial corner of the orbit (Figs. 1, 3). It is above the facial process of the maxillae but does not contact this process. It does, however, contact the nasal cartilage. In the adult the lacrimal will have both orbital and extraorbital wings. The extraorbital wing contacts the nasal bone preventing the maxilla from touching the frontal, a feature considered primitive for marsupials (Bensley, '03).

Vomer

The vomer first appears in the 3P specimen of *Monodelphis domestica* as a single center of ossification. It appears in the 1P specimen of *Macropus eugenii* as two separate centers below and lateral to the nasal septum, which later fuse across the midline. The vomer almost immediately takes on the form of a posteriorly forked splint of bone below the nasal septum, resembling the adult morphology.

Prearticular (= gonial)

The prearticular begins ossification during the 1st postnatal day of development in *Monodelphis domestica.* However it does not appear in *Macropus eugenii* until the 3rd postnatal day of ontogeny. It appears as a splint of dermal bone on the ventral and medial surface of Meckel's cartilage just anterior to the region of the malleus that first begins ossification (Figs. 1, 3). The prearticular fuses to the malleus during the second postnatal week of ontogeny, becoming the anterior process of the latter. The anterior process is the site of attachment for the malleus to the tympanic ring.

Ectotympanic (= tympanic)

The first sign of ossification in the ectotympanic is in the 1P specimen of *Monodelphis domestica,* and in the 3P specimen of *Macropus eugenii.* In *M. domestica* it appears as a three-pronged bone extending posteriorly from the ascending ramus of the mandible between the angular and condylar processes (Fig. 1). The horizontal limb (also described in *Didelphis* by de Beer, '37; Goodrich, '30) extends forward between Meckel's cartilage and the posterior border of the mandible. The rest of the ectotympanic forms a bony half circle and will later form support of the tympanic membrane. *M. eugenii* lacks the horizontal limb, but the development of the ectotympanic is otherwise very similar to that of *M. domestica* (Figs. 3, 4). During further ontogeny, the latter two processes grow posteriorly until they contact the ventral out-

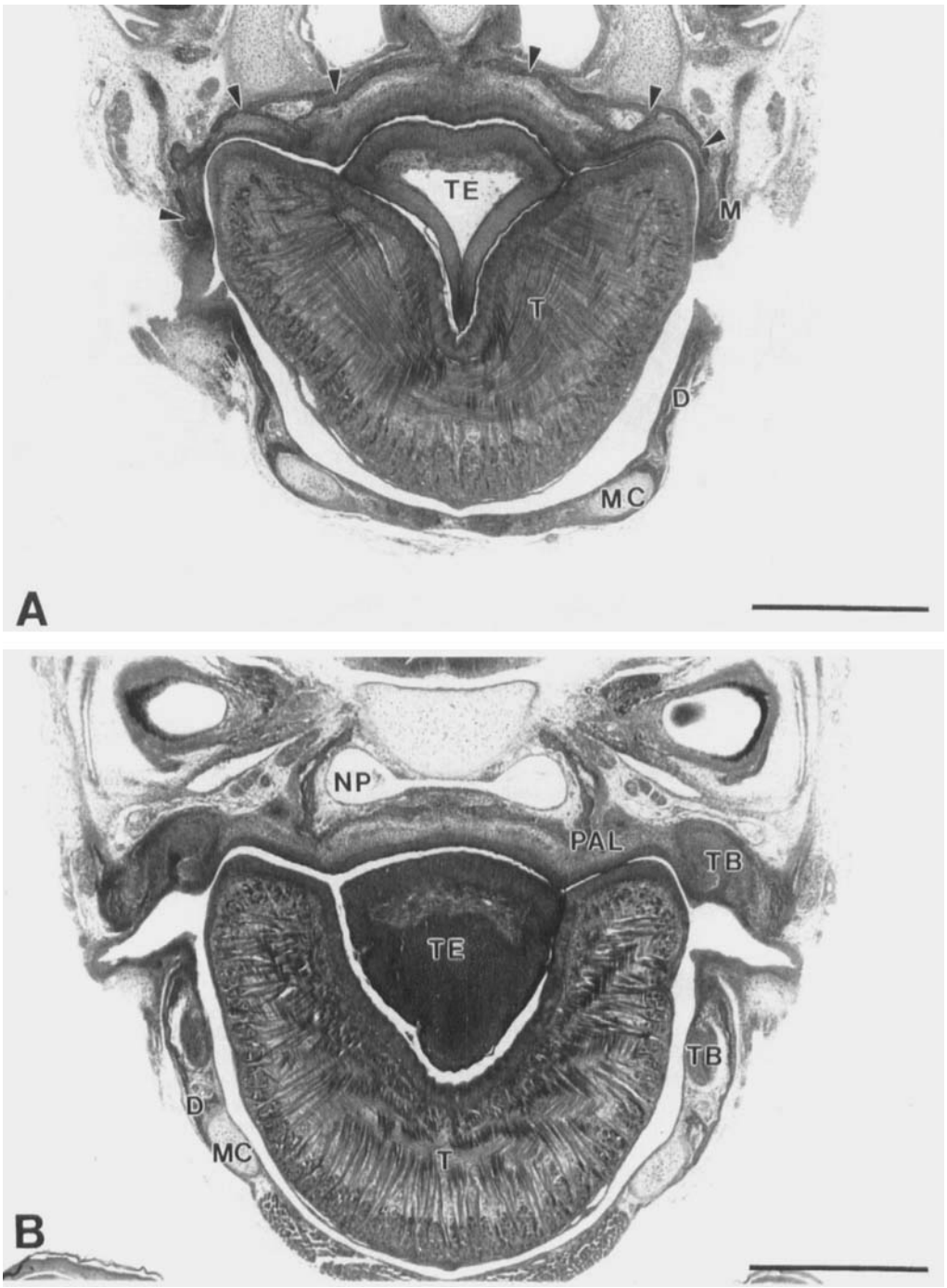

Fig. 8. Photomicrographs of transverse sections through the head of a 3P *M. domestica* (KS145). In this specimen the teat (TE) remains in place. **A:** Anterior section through the maxillary bone (M and arrowheads). **B:** A more posterior section (approximately 430 μm behind A) through the palatine bones (PAL) and the bulb of the teat. Note that the major part of the teat is relatively far posterior in the oral cavity and that the most complete distribution of the bone is anterior to the bulb of the teat. D, dentary; MC, Meckel's cartilage; NP, nasal passage; T, tongue; TB, tooth bud. Ten micron paraffin sections stained with Milligan's trichrome. Scale bars = 0.5 mm.

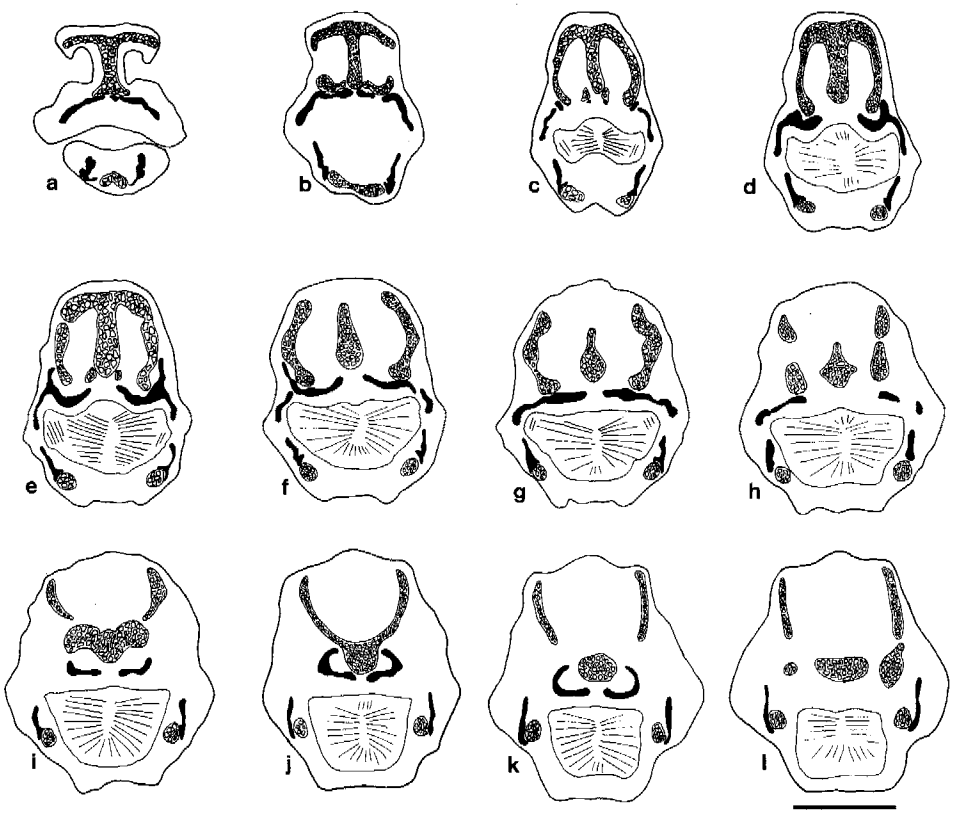

Fig. 9. Camera lucida drawings demonstrating the relative distribution of cartilage and bone in the oral-facial region of a neonatal (<90 minutes old) *M. domestica*. This animal was sacrificed before it had attached to its mother and thus the oral cavity is not modified by contact with the teat. The sections are each separated by 100 μm. Note that the bone (solid black) is best developed anteriorly; in more posterior regions the palate is not complete. Although bone is illustrated as a solid structure, at this time ossification consists of relatively thin spicules of tissue. Cartilage is indicated by irregular polygons; the relative size of the tongue is also indicated. Section g is approximately the same region as in Figure 8A; k is the same region as in Figure 8B. Scale bar = 1.0 mm.

growths of the epitympanic process of the alisphenoid (Maier, '87a,b, '89, '90). During the 3rd week (14–21 days) the anterior process of the ectotympanic is resorbed in *M. domestica* (Fig. 2B), and although the ectotympanic is still in proximity to the mandible, after loss of the anterior process its primary structural association is with the middle ear (see also Filan, '91; Maier, '87b, '90, for detail on the development of the middle ear in *M. domestica*).

Malleus

The malleus has one center of ossification, at the base of what will become the anterior process of the malleus adjacent to the prearticular. In *Monodelphis domestica,* the cartilage of the malleus begins to hypertrophy in the region of the prearticular in the 9P specimen and ossification appears in this area in the 11P specimen. It then extends throughout the malleus from this position (Fig. 2B). The association with the prearticular and the position of the center of ossification is the same in *Macropus eugenii,* but osteogenesis first appears in the 22P specimen (Fig. 4B). The development of the cartilages, bones, and relations of middle ear elements in *M. domestica* is discussed in detail in Filan ('91) and Maier ('87b, '90).

Incus

The first sign of ossification in the incus of *Monodelphis domestica* appears in the 17P specimen as a single center, whereas in *Macropus eugenii* it first appears in the 42P

specimen. Ossification proceeds from the head down the crus longus toward the articulation with the stapes.

Stapes

The onset of ossification in the stapes appears as a single center in the 25P specimen of *Monodelphis domestica,* but not until the 52P specimen of *Macropus eugenii.* This center is located in the footplate in the vestibular window of the cochlea and further ossification proceeds distally toward the articulation with the incus.

Neurocranium

Frontal

The frontal first appears in *Monodelphis domestica* as a single center of ossification in the 1P specimen. It first appears in the 6P specimen of *Macropus eugenii* as a pair of ossification centers on each side. In both species on first appearance, the frontal comprises a few spicules of bone above the anterior portion of the lamina orbitoparietalis (Figs. 1B, 4A). In *M. domestica* the orbital process, which forms the medial wall of the orbit, is recognizable in the 3P specimen. The frontal bone continues to grow until it meets the palatine and maxilla invading the orbit from below, as well as the orbitosphenoid. The growth of the frontal in *M. eugenii* is similar, but occurs later in ontogeny. The bulk of the frontal contributes to the formation of the anterior roof of the braincase. The frontal begins to extend over the braincase immediately following its origination, but it grows into this area very slowly. In *M. domestica,* it has not made sutural contact with its opposite by postnatal day 20, but has contacted the nasal, parietal, and alisphenoid.

Parietal

In both species the first evidence of ossification in the parietal is in the 3P specimens. In *Monodelphis domestica* it appears as a very inconspicuous wisp of bone lateral to the lamina orbitoparietalis just anterior to the otic capsule (Fig. 1B). It grows very slowly in the following days until in the 7P specimen it has expanded evenly above and below the lamina by several millimeters. It continues to grow slowly over the braincase until on day 20P it contacts the frontal anteriorly. At this time it has replaced much of the lamina cartilage, but does not make contact with any bones other than the frontal. It reaches close to the midline, but has not touched the other parietal. By day 25 the parietal has grown to overlap the squamosal and the supraoccipital. It is very close to forming midline contact with the other parietal, but a small gap remains between these paired bones. The only change in the 30P specimen is the overlap with the canalicular ossification center. Growth is similar in *Macropus eugenii* (Figs. 3, 4).

Postparietal (= interparietal)

In *Monodelphis domestica* a single (midline) postparietal first appears in the 3P specimen as a strand of spicules along the dorsal surface of a ligament that occupies the position of the tectum posterius (i.e., it runs between the two postero-dorsal corners of the otic capsules; Figs. 1B, 2). The postparietal expands rostrally over the posterior surface of the brain during the next few days. It also encroaches on the pars canaliculae. As this encroachment continues, endochondral ossification as well as resorption of cartilage appears in this region of the canaliculae. Additionally, by day 8P endochondral ossification is proceeding at the supraoccipital ossification (Fig. 2) and the fused postparietal-supraoccipital forms a single unit. During this period the postparietal also continues to grow anteriorly, but even at day 20P it has not contacted the parietal or any other bone of the skull roof. By postnatal day 30 the postparietal contacts the parietal bone anteriorly. The postparietal in *Macropus eugenii* appears as a bilateral structure in the 8P specimen (Fig. 4A). These two structures are originally bilateral and separate from each other and the median supraoccipital, but by day 14P these bones fuse into one complex of dermal and endochondral bone. Following fusion with the supraoccipital, the pattern of growth is much the same as that of *M. domestica.*

Supraoccipital

The ossification of the supraoccipital is closely tied to the ossification of the postparietal in *Monodelphis domestica* and never appears as a separate ossification center (Fig. 2). As noted above, when the dermal ossification in the postparietal contacts the posterior margin of the chondrocranium, endochondral ossification of the supraoccipital is initiated. This contact occurs at approximately day 8P. In *Macropus eugenii* distinct supraoccipital and postparietal ossification centers are present (Figs. 3, 4). The supraoccipital is

first present in the 8P specimen. It grows as an independent endochondral ossification center, meeting the postparietal by day 14P. The major difference between *M. eugenii* and *M. domestica* is that in the former the supraoccipital is a distinct center that provides at least as much bone as the postparietal at the fusion of these two bones, while in *M. domestica* the supraoccipital is much smaller than the postparietal and does not appear as a distinct center before the contact between the spreading postparietal and the chondrocranium.

Exoccipital

This bone appears in the OP specimens in both *Monodelphis domestica* and *Macropus eugenii*. As with all of the endochondral centers in these two species, the first evidence of ossification is the presence of perichondral ossification. The exoccipital center appears in the angle between the basal plate and the pars canalicularis just lateral to the pair of hypoglossal foramina. During the 1st postnatal week of development in *M. domestica* it expands in this area, incorporating the hypoglossal canals, until it forms a plate of bone between the foramen magnum and the pars canalicularis (Figs. 1, 2). At this time (around day 8P) it is joined to the basioccipital medially through a synchondrosis. Laterally the exoccipital is joined to the pars canalicularis through a plate of hypertrophied cartilage. This cartilage becomes a synchondrosis between these two elements after the onset of ossification in the pars canalicularis (8P specimen). The course of development is similar in *M. eugenii* (Figs. 3, 4).

Basioccipital

The basioccipital ossification center appears as an oval center of ossification in the 3P specimens of *Monodelphis domestica* and *Macropus eugenii*. It extends from near the level of the hypoglossal canals to just in front of the otic capsules (Fig. 10). During the next week, synchondroses are established between the basioccipital and the basisphenoid and exoccipital. These relations are maintained throughout the period for which we have specimens. In later stages the basioccipital becomes a Y-shaped bone with the fork directed posteriorly and its synchondroses with the basisphenoid and exoccipital forming its contacts with the surrounding skeleton. Laterally the basioccipital is bordered by the basicochlear fissure, a slit that separates the cochlea from the basal plate. The foramen magnum forms the posterior boundary of the bone.

Alisphenoid (= ala temporalis)

There is a single center of ossification in the sidewall of the braincase in *Macropus eugenii*, whereas there are two centers in *Monodelphis domestica*. In the 3P specimen of *M. eugenii*, the alisphenoid originates as a perichondral sheath of bone around the lamina ascendens of the ala temporalis (Fig. 3). In *M. domestica* the two centers of ossification first appear in the 4P specimen. Both of these ossification centers are perichondral and are associated with two distinct cartilaginous processes of the ala temporalis (Fig. 11A). The anterior center of ossification in *M. domestica* appears around a cartilaginous process between the first and second branches of the trigeminal (processes ascendens) and provides the majority of bone for the alisphenoid (Fig. 12A). The posterior center appears around a cartilaginous process between the second and third branches of the trigeminal (a process that shares the same relations as the lamina ascendens; Fig. 12B). In the early stages of ossification, the centers are independent of each other, appearing on opposite sides of V_2 and the foramen rotundum (Fig. 11B). The two centers of ossification subsequently expand into the sphenobturator membrane and also spread along the surface of the ala temporalis to meet medial to V_2 forming the medial border of the foramen rotundum (Fig. 11C). At the same time that perichondral bone forms around the cartilage of the processus ascendens, this process exhibits chondrogenic hypertrophy (Fig. 12A). The cartilage of the processus ascendens is replaced by endochondral bone in a dorsal to ventral progression, and has been completely ossified by 12 days postnatal. The lamina ascendens ossifies by a combination of cartilage resorption, and backward extension of the perichondral bone. This results in the appearance of a rod of bone in the position of the lamina ascendens that abuts the alar cartilage directly. The remaining ossification of the alisphenoid occurs by appositional growth into the sphenobturator membrane from both centers of ossification. The second center (the lamina ascendens) was not described by Maier ('89) in his study of the development of the sidewall of the brain case of *M. domestica*. There is no evidence of an independent intra-membranous center of os-

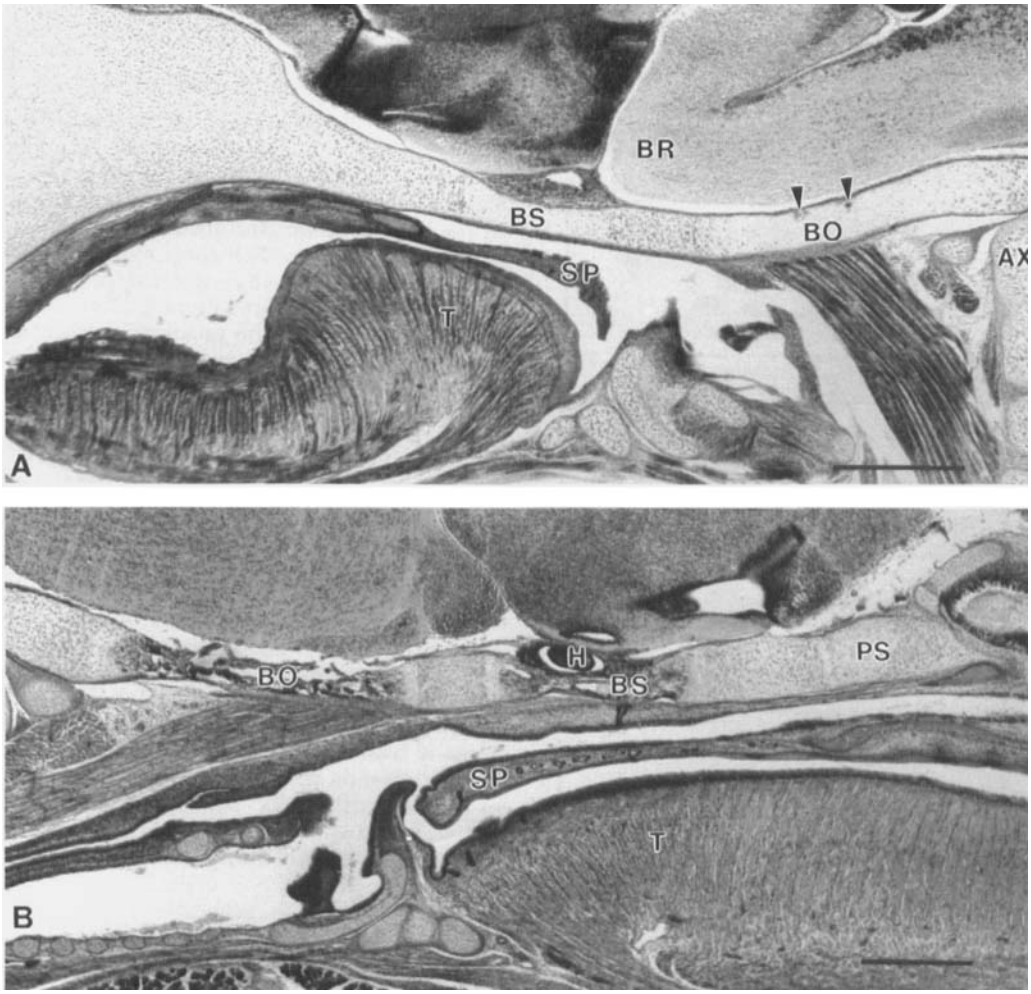

Fig. 10. Photomicrographs of parasagittal sections through the cranial base of *M. domestica*. These sections illustrate the caudal to rostral pattern of cranial base ossification. **A:** 3P specimen (CC24). Vascular buds (arrowheads) penetrating the cartilage indicate early ossification of the basioccipital (BO). The basisphenoid (BS) is made up of hypertrophied cartilage at this age. **B:** 13P (CC28) specimen. Ossification of the basioccipital and the basisphenoid has proceeded and the cartilage of the presphenoid (PS) is hypertrophied in the first stage of ossification. AX, axis; BR, brain; H, hypophysis; SP, soft palate; T, tongue; TR, trachea. Ten micron paraffin sections stained with hematoxylin and picroponceau. Scale bars = 0.5 mm.

sification in either species (Presley and Steel, '76).

Basisphenoid

The onset of ossification in this bone is signaled by the presence of a perichondral sheath of bone around the basisphenoid region of the cartilaginous cranial base (Fig. 10). It is apparent in the 5P specimen of *Monodelphis domestica,* but not until the 11P specimen of *Macropus eugenii.* The primordium is almost rectangular with a straight anterior and posterior border and inwardly curved lateral borders in the vicinity of the carotid foramina. The endochondral ossification of the basisphenoid proceeds rapidly so that in the 11P specimen it is well ossified and surrounded by four cartilaginous growth plates. The intersphenoidal and sphenoccipital synchondroses are in front and behind, respectively. Laterally, paired synchondroses join the basisphenoid to the ali-

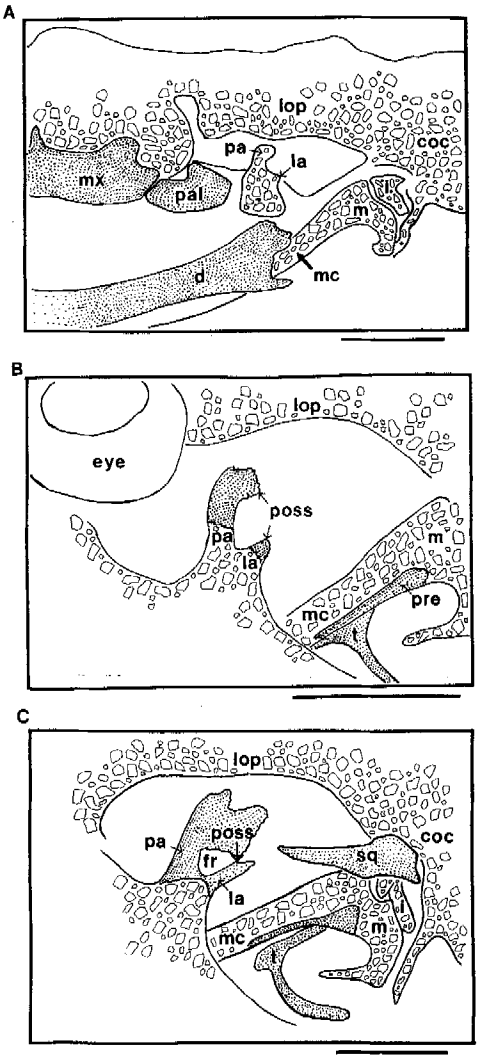

Fig. 11. Drawings of development and ossification of the alisphenoid in *M. domestica* taken from cleared and stained specimens. In all cases anterior is to the left and dorsal is to the top. **A:** 2P specimen in lateral view. Both the processus ascendens (pa) and lamina ascendens (la) of the cartilaginous ala temporalis are present. **B:** 6P specimen in medial view. Perichondral ossification (poss) is seen around both the processus ascendens and lamina ascendens (pa, la). The two ossification centers are independent, but are both perichondral. **C:** 7P specimen, shown from medial view. Ossification (poss) continues to expand from the processus ascendens and lamina ascendens (pa, la). The foramen rotundum (fr) is almost encircled by the bone. Irregular polygons indicate cartilage, stippling indicates bone. coc, cochlea; d, dentary; i, incus; lop, orbitoparietal commissure; m, malleus; mc, Meckel's cartilage; mx, maxilla; pal, palatine; pre, prearticular; sq, squamosal; t, ectotympanic. Scale bars = 1.0 mm.

sphenoids. These lateral growth plates have disappeared by the 25P specimen.

Presphenoid and orbitosphenoid

Although these centers arise separately, they fuse almost immediately. In *Monodelphis domestica* the cartilaginous precursor of the presphenoid first begins to hypertrophy in the 11P specimen and shows the first signs of ossification in the 13P specimen. The orbitosphenoids are paired centers of ossification anterolateral to the presphenoid first seen in the 14P specimen. The onset of ossification of these elements in *Macropus eugenii* is first observed in the 33P specimen. Although in *M. eugenii* the presphenoid and orbitosphenoid are considerably delayed in their onset of ossification relative to *M. domestica*, they follow much the same pattern of ossification and fusion. In *M. domestica* the presphenoid and orbitosphenoid centers have fused by day 16P into a T-shaped complex of bone that borders on several regions of the skull. Laterally, the complex contributes a wing of bone to the posterior wall of the orbit, lying between the orbital process of the frontal and the anterior margin of the alisphenoid. Posteriorly the intersphenoidal synchondrosis divides it from the basisphenoid while anteriorly it extends into the nasal septum. This extension will ossify the nasal septum as ontogeny proceeds, there being no independent ethmoid ossification center in marsupials (Broom, '26).

Periotic

The periotic is made up of the cochlear cartilage, in which there are four ossification centers, and the canalicular cartilage, which ossifies from two ossification centers (Fig. 13). These chondrocranial structures chondrify independently and also begin ossification at different times, but because of their proximity they will be described together. In *Monodelphis domestica* ossification begins in the cochlea as three separate centers (12P specimen). Centers 1 and 2 are located above and below the foramen rotundum, and do not contact each other at this time (Fig. 2B). Center 3 is located in the anterolateral corner of the cochlea near the ampullae of the anterior and lateral semicircular canals lying just medial to the head of the malleus. During the next 2 days centers 1 and 2 fuse around the foramen rotundum and ossification extends anteriorly and medially toward the basioccipital. This wing of ossification

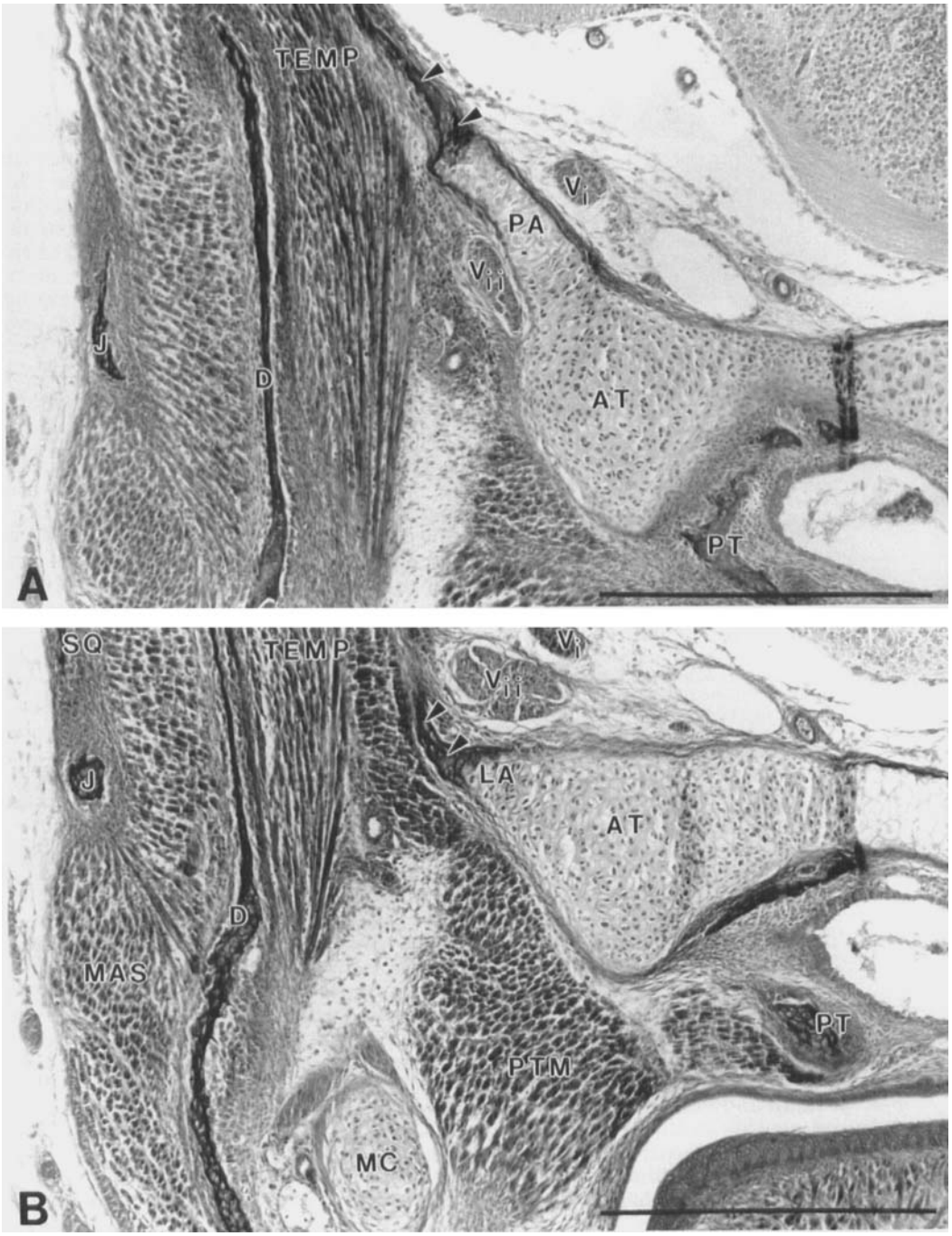

Fig. 12. Photomicrographs of transverse sections through the ala temporalis in a 7P *M. domestica* (KS182) demonstrating the two independent centers of perichondral ossification (arrowheads). **A:** Anterior section through the processus ascendens (PA) extending between the first (V_i) and second (V_{ii}) branches of the trigeminal. **B:** More posterior section, approximately 160 μm behind section A. Note the lamina ascendens (LA) lateral to both the first and second branches of the trigeminal nerve (V_i & V_{ii}). The third branch of the trigeminal is out of the section plane. AT, ala temporalis; D, dentary; J, jugal; MAS, masseter muscle; MC, Meckel's cartilage; PT, pterygoid bone; PTM, pterygoideus muscle; SQ, zygomatic process of the squamosal; TEMP, temporalis muscle. Ten micron paraffin sections stained with Milligan's trichrome. Scale bars = 0.5 mm.

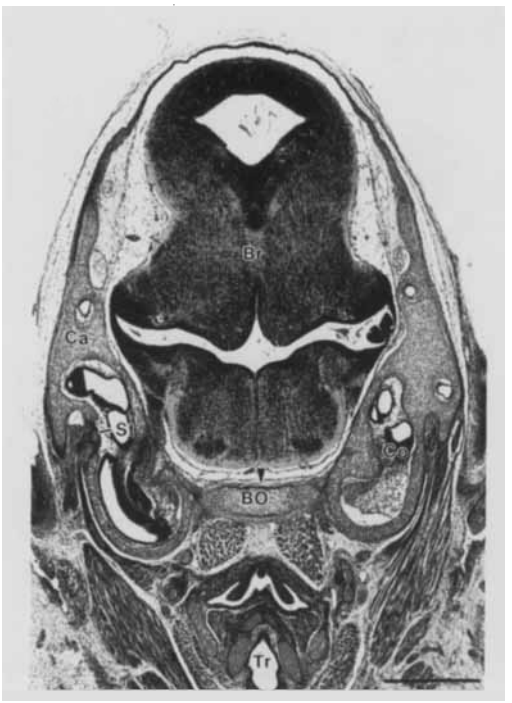

Fig. 13. Photomicrograph of a transverse section through the cochlear region of a 4P *M. domestica* specimen (KS165). This specimen is 1 day older than that shown in Figure 8, illustrating that while the facial region is ossified, there is only slight perichondral ossification of the basioccipital (BO, arrowhead) in the neurocranium. The remainder of the neurocranium is composed of cartilage and membrane and houses a minimally differentiated brain (Br). Ca, canalicular cartilage; Co, cochlea; St, stapes; Tr, trachea. Ten micron paraffin section stained with hematoxylin and picroponceau. Scale bar = 0.5 mm.

appears to follow the coil of the cochlea during its progression so that in the 16P specimen it curls back laterally underneath the cochlea. Center 3 does not spread as quickly through the cartilage, but in the 16P specimen it has extended anteriorly and laterally along the apex of the cochlea in front of the foramen vestibuli and has fused with center 4, which first appears in the 16P specimen. Center 4 ossifies the internal lamina of the cochlear coil in a posterior to anterior direction. In the 20P specimen almost the entire cochlea is ossified, although a small portion above the foramen vestibuli remains in cartilage, and by day 25P ossification is complete.

The centers of ossification of the canalicular cartilage invade this structure from two areas, the postparietal dermal center and the cochlear centers. The first center appears in the 8P specimen in association with the postparietal bone. This dermal element contacts the postero-dorsal corner of the canalicular cartilage and once contact exists, endochondral ossification begins. Ossification from the cochlear region spreads dorsally and laterally into the canalicular region of the inner ear capsule beginning in the 20P specimen. This proceeds most rapidly along the canals and then spreads into the cartilages between and distal to the canals. The last regions of the canalicular cartilage to ossify are the lateral wall of the flocular cavity between the superior and lateral canals, and the ventro-lateral lip of cartilage below the lateral canal from which extends the styloid process. The distal tip of the styloid process remains in cartilage on postnatal day 30.

The basic pattern of ossification of the cochlea and canalicular regions of the skull in *M. eugenii* are the same, although the first appearance of ossification in the periotic of *M. eugenii* is in the 31P specimen. Cartilage remains in the canalicular region of the periotic in the oldest available specimen (52P).

DISCUSSION

A meaningful comparison of ontogenetic events between species requires that the timing of these events be standardized in some way. Wayne ('86a,b) and Creighton and Strauss ('86) have argued that birth in mammals may be one of the most variable developmental parameters. However, birth was taken as the standard in this study of marsupials because it has often been hypothesized that marsupial morphology is conservative at birth. The test of this hypothesis was one of the goals of the study. Further, comparison of the condition of the skull at birth in these marsupials with neonates of other mammalian and non-mammalian taxa may allow us to identify what adaptations are specific to the marsupial neonate. When we extend our discussion to comparisons with other taxa, we use birth as a stage for comparison, but focus primarily on the sequence and simultaneous occurrence of ontogenetic events, which are independent of specific timing.

Comparison of M. domestica *and* M. eugenii

Neonatal morphology

Although *Monodelphis domestica* and *Macropus eugenii* differ in gestation length by about 10 days (*M. domestica* = 14.5 days, *M. eugenii* = 25 days), in adult weight by a factor of approximately 70 (*M. domestica* adult

females weigh 60–100 gm, *M. eugenii* adult females weight 4,000–6,000 gm), and in the timing of postnatal events by a factor of more than 5 (*M. domestica* detaches from the teat at 14 days and is weaned at 50 days; *M. eugenii* detaches at 100 days and is weaned at 270 days) there is relatively little difference in neonatal size (*M. domestica* 75–100 gm; *M. eugenii,* 400–500 gm; Tyndale-Biscoe and Janssens, '88). Most significantly, there is little or no difference in neonatal morphology. The same suite of cranial bones is present at birth in both *M. domestica* and *M. eugenii.* All but one of the cranial bones present at birth surround the oral cavity; the sole exception is the exoccipital.

The premaxillae, maxillae, dentaries, palatines, and pterygoids all have begun ossification in the neonates of both species and rapidly ossify to become relatively robust bones. At birth the palatal shelves have elevated, and are reinforced by processes from the premaxillae, maxillae, and palatine bones, allowing the neonate to attach to the teat, suckle and breathe simultaneously. Ossification is best developed anteriorly (Fig. 9). The pterygoid bones in the neonate are just small bumps of ossification adjacent to the ventral surface of the cartilaginous ala temporalis. The dentary bones of the neonate are splints dorsal and lateral to Meckel's cartilage. The coronoid, condylar, and angular processes have not formed at birth, and the lower jaw is suspended by contact through the ear ossicles and the otic capsule (Filan, '91; Maier, '87a; Müller, '68a,b). At birth, the dentaries do not contact each other through a symphysis, but Meckel's cartilage is continuous across the midline.

The second similarity between *Monodelphis domestica* and *Macropus eugenii* is the sequence of endochondral osteogenesis of the skull, which is identical in these two species, although the timing differs (Table 1). The first endochondral bone to begin ossification is the exoccipital; in both *M. domestica* and *M. eugenii* perichondral ossification is evident in this region at birth. The cranial base ossifies in a caudal to rostral direction: exoccipital, basioccipital, supraoccipital, basisphenoid, presphenoid, and orbitosphenoid. The elements of the visceral arches ossify in the reverse, rostral to caudal direction: ala temporalis, malleus, incus, and stapes. The ossification of the periotic is interpolated between the malleus and the presphenoid in both species. The stapes is the last endochondral bone to ossify in both species. In *M. domestica* this begins on postnatal day 25P, while in *M. eugenii* the stapes does not begin ossification until day 52P. Additionally, in both these taxa all dermal bones have begun ossification before any endochondral bone (with the exception of the exoccipital bone) begins ossification.

Finally, the relative rates of ossification of the bones of the facial skeleton relative to those of the auditory ossicles and the braincase, independent of their origin as either dermal or endochondral elements, are also similar in *Monodelphis domestica* and *Macropus eugenii.* In both taxa not only is the onset of ossification of bones surrounding the oral cavity early relative to other bones, but also the rate at which the bones of the face grow toward the adult configuration is accelerated. These differences set up a gradient of ossification in these two regions of the skull: The face contains multiple ossification centers at a time when the neurocranium is still housed in membrane and cartilage and the bones of the face have approached each other to form a solid structure when the bones of the braincase are isolated elements (Figs. 1–4). An excellent example of this pattern of growth is found in the ossification of the squamosal. The squamosal bone has two components, the zygomatic process which contributes to the posterior bar of the zygomatic arch, and the squamous portion which contributes to the sidewall of the braincase and also contacts the periotic. In *M. domestica* the zygomatic process is the first part of this bone to ossify and by day 3 it has approached the jugal to complete the zygomatic arch. This portion is functionally a part of the facial skeleton. The squamous portion grows very slowly over the side of the braincase and makes it first contact with other bones 20–25 days postnatally when it touches the alisphenoid and the parietal. The frontal and parietal bones in both species exhibit similar patterns of relatively slow growth over the braincase (Figs. 1–4). While the dermal bones of the face contact each other by day 8P in *M. domestica* and 14P in *M. eugenii,* the dermal bones of the neurocranium such as the frontal and parietal reach each other laterally between days 11P and 16P in *M. domestica* and day 30P in *M. eugenii.* The dermal bones do not roof the braincase until days 25–30P in *M. domestica* and after day 50 in *M. eugenii.*

Development of the alisphenoid

One difference between *Macropus eugenii* and *Monodelphis domestica* is the pattern of ossification of the alisphenoid. In *M. eugenii* perichondral ossification proceeds from a single cartilaginous process, lying between the second and third branches of the trigeminal nerve and considered to be homologous with the lamina ascendens of eutherian mammals (e.g., Goodrich, '30; Maier, '87a; Presley, '81, and references therein). *M. domestica* possesses two sites of perichondral ossification that grow from separate cartilaginous processes of the ala temporalis and which, on the basis of their relations to the branches of the trigeminal nerve, might be considered to be homologous to the lamina ascendens (between V_2 & V_3) and the processus ascendens (between V_1 & V_2), respectively (Figs. 11, 12). Maier ('87a) in a detailed study of the ossification of the sidewall of the braincase of *M. domestica* only reported a single center, which grows from the processus ascendens. Our report thus differs from Maier ('87a) and other previous studies of didelphids (e.g., Presley, '81; Toeplitz, '20). The discrepancy between our study and that of Maier is most likely due to the fact that at least a week separated Maier's earliest specimen (M0, "neonate") and the next oldest specimen (M7 "about 6 days old"). The two centers begin ossification on about day 3–4 and by the end of 7–8 days (in our dated specimens) the two ossification centers have fused. Maier's earliest specimen is before ossification has begun and his second specimen is old enough so that the two centers have already met; therefore he was not able to observe the fact that two centers grow independently. This difference provides an example of the usefulness of a finely graded series of embryos such as those available in our study.

The lamina ascendens of *Monodelphis domestica,* reported here for the first time, meets all criteria for homology with the lamina ascendens of other mammals. It is a small, cartilaginous process of the ala temporalis, lying between V_2 and V_3 (de Beer, '37; Presley, '81; Presley and Steel, '76; Maier, '87a), with perichondral ossification that forms a portion of the alisphenoid. *M. domestica* thus possesses both a typical therian lamina ascendens as well as a typical "reptilian" processus ascendens. We conclude that the former process and its center of ossification in *M. domestica,* other marsupials such as *Macropus,* and eutherian mammals is a neomorphic ossification that is not homologous with the processus ascendens or the reptilian epipterygoid. Presley ('81, '89) and Presley and Steel ('76) propose, as do we, that the lamina and processus ascendens are not homologous. However, these authors observed an independent ossification center in the side wall of the braincase of *Didelphis.* We observed no such center in *M. domestica* and suggest that further study of didelphid marsupials, with a complete series of specimens (including cleared and stained specimens in which the ossification pattern of these two processes is best revealed) may resolve this discrepancy.

Functional adaptations of the marsupial neonate

As has been noted above the most characteristic features of marsupial cranial skeletal development are the accelerated development around the oral cavity, both in terms of the onset of ossification and more importantly the rate of bone growth, and the very late growth of the neural skeleton, again in both timing of onset and rate of ossification. The only exception to this latter statement is the exoccipital bone, which in both species is the only endochondral bone to begin ossification before all dermal bones have begun ossification, and is the only bone of the braincase to be ossified at birth. What are the functional consequences of this neonatal morphology?

The early ossification of bones around the oral cavity has been noted by previous authors, and this pattern along with the well-developed tongue and the robust chondrocranium have been considered by previous authors to be adaptations for suckling. We will consider these hypotheses below. The early ossification of the exoccipital bone is also likely to relate to neonatal behavior. As marsupial neonates migrate to the teat following birth, they move their heads in a side-to-side motion, presumably as part of their search for the teat (Tyndale-Biscoe, '73). The exoccipital may ossify early to support the atlanto-occipital joint and insertion of cervical muscles during this migration.

The slow and later ossification of the other elements of the neurocranium (both dermal and endochondral elements) is almost certainly related to a pattern of extended brain growth in marsupials. In both species most neural structures differentiate and grow during the early stages of postnatal development, whereas the equivalent period of neu-

rogenesis is intra-uterine in most eutherians. Renfree et al. ('82) demonstrated that the most rapid brain growth in *Macropus eugenii* occurs in the postnatal period up to 115 days, after which it slows down markedly. Similar qualification is not available for *Monodelphis domestica,* but at birth there is virtually no differentiation of forebrain structures (e.g., Fig. 7). In the first 2–3 days after birth, little brain growth occurs; most neural differentiation and growth occurs in the 2nd and 3rd weeks following birth (N.B. Cant, pers. comm.; Saunders et al., '89). The association between brain and neurocranial growth is not well understood; however, several studies indicate that skeletal growth is responsive to the primary growth of the brain (Bassett, '72; Hanken, '83; Koski, '75; Young, '59). Additionally, several studies have suggested that neural tissues are responsible for the induction of the bones forming the braincase (e.g., Schowing, '68; Hall, '87), suggesting a mechanistic relation between the relative rates of neural and neurocranial growth. We believe that the relatively slow ossification of the neurocranium is in response to the long period of brain growth observed in these marsupials.

This hypothesis and the data from the present study are in contrast to a recent hypothesis made by Hall and Hughes ('87). In this paper Hall and Hughes hypothesize that the bones of the neurocranium in marsupials may form sutures early in postnatal ontogeny, preventing further expansion of the brain. They then claim that this hypothesis would help to explain the generally small brains of marsupials relative to placentals of the same body size (Jerison, '73), as the early ossification and late neural growth would place a constraint on neural capacity. However, our data show that growth of the neurocranium is extended, rather than truncated, in these two marsupial species. Our hypothesis on the relation between brain growth and the growth of the neural skeleton awaits further study for confirmation, and we consider marsupial cranial development to be an excellent system with which to study the mechanistic relations between neural and cranial growth.

In contrast to the neurocranium, the facial skeleton exhibits an accelerated rate of ossification. As noted above, this is most often attributed to the functional demands of suckling (e.g., Hall and Hughes, '87; Hill and Hill, '55; Hughes and Hall, '88; Maier, '87a; Tyndale-Biscoe and Renfree, '87). Although no detailed functional analysis of the oral apparatus has ever been attempted, there have been several hypotheses concerning suckling in marsupial neonates. It has been postulated that the striated muscular extension from the abdominal muscles into the teat of the female express milk into the neonates mouth without active participation by the neonate (Barbour, '63; Bolliger and Gross, '60). This model of marsupial "suckling" was refuted by failure to elicit milk letdown with electrical stimulation of these muscles (Enders, '66). In contrast to this hypothesis, McCrady ('38) describes hearing suckling sounds from newborn *Didelphis* recently attached to the teat, and Griffiths and Slater ('88) describe the sucking of liquid from an inflexible pipette by marsupial and monotreme neonates. This phenomenon has also been reported by Jurgelski ('71). The neonate is clearly capable of some active form of suckling.

The hypothesized method of suckling in marsupials has been called a "pump-sucking" mechanism. By this mechanism the neonate is thought to first push the tongue against the teat toward the roof of the mouth, and then pull the tongue away from the teat, creating negative pressure in the posterior portions of the oral cavity and drawing milk into the mouth (Filan, '91; Griffiths and Slater, '88). Such suckling requires well-developed tongue musculature as well as skeletal support for the palate in order to be effective and most likely forces are developed in the posterior half of the oral cavity, where the bulk of the teat lies (e.g., Fig. 8). In addition, as discussed by Filan ('91) the robust ear ossicles are probably important in buttressing the mandible against the skull. She points out that it appears there is little movement of the jaw, and that in the neonate, there is probably little movement at either the dentary-squamosal joint or the joint between the auditory ossicles and the braincase. Maier ('87a, '89) believes that the robust chondrocranium and the large cartilaginous ala temporalis (which contacts the lamina parieto-orbitalis in many marsupial neonates and actually fuses with it in some, e.g., *Dasyurus*) is a structural adaptation to resist deformation of the skull during contraction of the jaw muscles while suckling.

Until experimental work on the actual mechanism of suckling and documentation of the distribution of forces during suckling

have been completed, it is impossible to corroborate or refute the hypotheses that the precocious ossification or robust chondrocranium are related specifically to any particular suckling force. And although the rapid growth of the bones surrounding the oral cavity is generally considered to give rigidity to the palate for the mechanical demands of suckling, the reticulated configuration of the bones forming the palate in the earliest neonates may not provide a great deal of structural rigidity. The greatest concentration of bone in the neonate is anterior where a virtually complete ring of bone surrounds the oral cavity; more posteriorly, where the bulk of the teat lies, the bones are smaller splints and do not form as solid a structure (Figs. 8, 9). At least two additional functions are likely to be performed by the precociously ossified oral cavity. First, because the oral bones are best developed anteriorly rather than near the bulk of the teat, it is possible that in the earliest stages the bones surrounding the oral cavity provide a ring of bone that helps hold the enlarged teat in the oral cavity. The configuration of the bones is likely to be effective in resisting the tensile forces generated by the suspension of the neonate. Additionally, the precocious ossification of the palatal region may function to maintain the patency of the nasal passage. When the neonate is attached to the teat, breathing is necessarily nasal, and while the cartilages of the nasal septa are well developed anteriorly, posteriorly the swollen teat and any oral movements involved in suckling might compress the airway. The palatine bone wraps the nasal passage in the region of the teat and may be important in protecting the airway (Figs. 8, 9).

Conservative morphology in marsupial neonates

The state of ossification in the neonates of *Monodelphis domestica* and *Macropus eugenii* supports the hypothesis that morphology is consistent in marsupial neonates (Lillegraven, '75; Lee and Cockburn, '85) and, as we will show, presents a pattern of cranial ossification not documented in any other mammal. Two factors contribute to our belief that this pattern of cranial ossification can be generalized across marsupials. First, as already described, the two species chosen for this study represent marsupials that are distinct phylogenetically, in adult morphology, and in their natural and life history (see above). They present two virtual extremes within marsupials. Second, previous studies include brief descriptions of neonates in other marsupial taxa that are in accordance with our findings (e.g., Esdaile, '16; Hill and Hill, '55; Nesslinger, '56; Tyndale-Biscoe, '73). Nesslinger ('56) reported that the premaxilla, maxilla, palatine, pterygoid, exoccipital and also the tympanic, nasal, and lacrimal are bones present at birth in *Didelphis*. However, the true ages of Nesslinger's specimens were known in fewer than 35% of the cases, so that these bones may not be present at birth. Of particular interest are reports of animals such as *Dasyurus* (Hill and Hill, '55) that suggest similar craniofacial patterns even in taxa considered to be especially altricial (Hughes and Hall, '88). Obviously, corroboration of the hypothesis of conservative neonatal morphology awaits further data from many more marsupial taxa. The single report in discordance with this hypothesis of conservation is that of Gemmell et al. ('88). These authors, using the alizarin clearing and staining technique, report that no bone is present at birth in either *Isoodon macrourus* or *Trichosurus vulpecula*. However, when the first bones do appear by this technique (2–3 days postnatal) they are quite robust and resemble those seen in our cleared and stained specimens 2–3 days postnatal. It is likely that histological techniques would reveal earlier presence of bone (as mentioned by these authors). The order of ossification is not detailed for all cranial bones, but appears to follow the same order as observed here.

The similarities in neonatal cranial ossification patterns do not, however, lead to obvious similarities in adult morphology (e.g., adaptations for dietary specialization or tooth morphology and replacement patterns, both of which are quite different in the Didelphidae and Macropodidae). The results of this study indicate that even in two species with apparently similar functional constraints on morphology at a particular stage of development, and subsequent similarity in developmental patterns, there is still the capacity for generation of very different mature morphologies. This specific finding is in contrast to the more general hypothesis of Lillegraven ('75) and Lee and Cockburn ('85) that the developmental strategy of marsupials may limit potential body morphs (see Kirsch, '77c for another specific contrasting example.

Comparisons with monotremes

Although a number of authors (e.g., Kuhn, '71; Watson, '16) have discussed the develop-

ment of the skull in monotremes, only the studies by Gaupp ('08) and to a lesser extent, de Beer and Fell ('36), possess sufficient stages, covering the appropriate time period, to allow comparison of the sequence of onset of ossification in marsupials and monotremes. Gaupp ('08) provides one of the most detailed studies of a complete series of bones in his study of the development of the cranium in the echidna (*Echidna aculeata*). Cranial ossification first appears in "stage" 44 of Gaupp (who does not specify how his individuals were staged; however, they were numbered in chronological order). At this stage, the only bone to exhibit ossification is the premaxilla. At stage 45 the maxilla and squamosal appear, and at stage 46 most dermal bones are present: the parietal, frontal, squamosal, nasal, septomaxilla, parasphenoid, vomer, palatine, premaxilla, tympanic, gonial, and dentary. The last bone to appear is the pterygoid, which is not present until stage 49.

de Beer and Fell ('36) provide a good description of 5 stages of cranial ontogeny in the platypus *Ornithorhynchus*, including details of ossification. Particularly relevant in a comparison with marsupials are their stage 4 ("recently hatched young") and stage 5 ("nestling"). The younger specimens (prehatching) in their study show no signs of ossification. Specimen 4 of de Beer and Fell is the first to exhibit any ossification and, although there is no way of knowing the order of appearance of these bones prior to this stage, in this specimen the premaxilla, septomaxilla, vomer, maxilla, palatine, "mammalian pterygoid," squamosal, nasal, frontal, parietal, tympanic, prearticular, and dentary are ossified. The "echidna pterygoid," prevomer, and jugal show no sign of ossification. The plates in this paper (Plates III, IV, V; Figs. 12–19) do provide some information on the relative amounts of ossification at hatching. Of particular interest are the bones around the oral cavity, which are not larger or better developed than the other cranial bones. The one exception is the premaxilla which is expanded into massive supports for the "egg tooth." Griffiths ('78) states that the bones present in the "monotreme" hatchling are the premaxillae, maxillae, palatines, squamosals, pterygoids, dentaries, as well as the nasals, frontals, septo-maxillae, and parietals. Thus there are no data to suggest a precocial ossification of bones surrounding the oral cavity.

Endochondral ossification in monotremes appears similar in sequence and timing to that seen in marsupials. In de Beer and Fell's ('36) specimen 5 the basioccipital, exoccipitals, supraoccipital, basisphenoid, and the alisphenoids have begun ossification. The ossification of these five bones prior to the ossification of other endochondral bones is also seen in *Monodelphis domestica* and *Macropus eugenii* (Table 1), but without younger specimens it is not possible to determine the sequence of appearance of these bones in monotremes. Gaupp ('08) observes no endochondral ossification in any specimen except his oldest specimen (stage 51). In this specimen ossification has begun in the supraoccipital and the pleurooccipitals. Watson ('16) observes ossification of the periotic, orbitosphenoid, and presphenoid in his 250 mm stage.

To summarize, Gaupp ('08), de Beer and Fell ('36), and Griffiths ('78) concur in the description of osteogenesis of monotremes (the specimens examined by Kuhn, '71; and Watson, '16, are late, after most dermal ossification has been initiated, but do not contradict these results). Monotremes appear to share with marsupials the extended period of endochondral ossification and the general pattern of dermal bones preceding endochondral bones in ossification. The pattern of brain growth of monotremes would be of interest in order to test our hypothesis on the relation between extended neurogenesis and osteogenesis. However, unlike marsupials, monotremes do not appear to exhibit precocious ossification and growth of bones around the oral cavity. This lack of precocious ossification around the oral cavity in monotremes, which do not attach to a teat, serves to corroborate the hypothesis that the marsupial configuration relates to attachment and suckling. However, it does not distinguish between the relative significance of passive attachment and active suckling, because monotreme neonates are capable of suction (Griffiths, '88). The lack of attachment and suckling in monotremes may, however, contradict the hypothesis that the robust chondrocranium of marsupials is a functional adaptation to active suckling (Maier, '87a), as the chondrocranium of monotremes is more massive than that of marsupials (de Beer and Fell, '36).

Comparisons with eutherians

The most obvious difference in osteogenesis between the marsupials examined here

and placentals relates to the appearance of ossification relative to birth. In *Monodelphis domestica* and *Macropus eugenii* newborns, the only bones that have begun to ossify at birth are the dermal dentary, premaxilla, maxilla, palatine, pterygoid, and the endochondral exoccipital. Ossification of the remaining cranial bones begins after birth and in the case of endochondral bones can extend far into postnatal life. In contrast, placental newborns exhibit ossification in almost all of their cranial bones. The only consistent exceptions are the ossification centers of the nasal septum and the middle ear cartilages, which sometimes ossify postnatally in placentals with unusually short gestation periods and/or altricial neonates (e.g., hamsters or small insectivores such as *Sorex;* (van Arsdel and Hillemann, '51; Vogel, '73). However, more important than the state at birth (because as noted above, birth is a relatively labile point in mammalian ontogeny) is the fact that eutherians and metatherians differ in the sequence of cranial ossification and growth rate of cranial bones. A review of the literature on cranial osteogenesis in a wide variety of eutherians reveals no species with a pattern of ossification resulting in a stage similar in morphology to the marsupial neonates (Beatty and Hillemann, '56; Danielson and Kihlstrom, '86; Drews, '33; Johnson, '37; Mall, '06; Schrenk, '89; Starck, '67; Strong, '25; Zeller, '87).

This literature on cranial ossification in eutherians suggests that three features distinguish the ossification pattern of most eutherians from that observed in marsupials. First, although in eutherians, like in marsupials, the premaxillary, dentary and maxillary bones are amongst the first bones to ossify, eutherians generally exhibit ossification in a number of other bones at the same time, most often the frontal, squamosal, and parietal. Bones such as the palatine, pterygoid and nasal, which are early in marsupials, often begin ossification in eutherians after most other dermal bones of the skull have initiated ossification. Second, there is no tendency for a separation of the period of ossification of dermal and endochondral bones. Several endochondral bones, most often the exoccipital, basioccipital, and alisphenoid, begin ossification before many dermal bones have begun. And third, and probably most significant functionally, is that the rate of ossification and bone growth shows no marked distinction between most bones of the face and the neurocranium. For example, illustrations in Schrenk ('89, stage 1 and 2 *Ctenodactylus*) and Zeller ('87, age 34E *Tupaia*) demonstrate that when the palatal region shows a level of ossification comparable to the neonatal marsupial (Figs. 8, 9), most other bones of the cranium are also well ossified and similarly robust. In this third character, eutherians appear to resemble monotremes (e.g., illustrations in Kuhn, '71).

At this point it is difficult to make explicit comparisons, because it appears from the literature that there is much more variation in the sequence of ossification in eutherians than in marsupials. This difference is most likely due to the fact that virtually all eutherian cranial ossification occurs in utero, when little or no requirement for response to functional demands is imposed, in contrast to marsupials. Marsupials most certainly experience a greater functional constraint on the process of ossification than do eutherians. Of the taxa for which detailed information exists, *Tupaia* (Zeller, '87) most closely resembles the pattern observed in the marsupials examined here. Whether a marsupial-like sequence is primitive in eutherians, as suggested by Zeller, can only be addressed by further comparative work.

Comparisons with non-mammalian tetrapods

The literature expressly concerned with ossification sequences in non-mammalian tetrapods is sparse, with most papers on cranial development describing single or a limited number of developmental stages. However, a few papers on ossification sequence provide a reasonably broad sample of other tetrapod taxa. These include data on turtles (Shaner, '26), squamate reptiles (Franklin, '45; Haluska and Alberch, '83; Kamal and Hammouda, '65), and a variety of birds and amphibians (de Beer, '37; Hanken, '83; Wake and Hanken, '82; Wake et al., '83). Although incomplete, a picture of the cranial ossification patterns, but not ossification rate, can be obtained from these papers and compared with the pattern found in the marsupials discussed in this paper. The evolution of the vertebrate skull is a study of bone loss and fusion, so that many of the bones found in lower vertebrates have no specific homologues in mammals.

Generally, with only a few exceptions, dermal ossification begins before endochondral

ossification in non-mammalian tetrapods. In *Chrysemys* there is no overlap in onset of dermal and endochondral ossification. There is considerable overlap in the periods of dermal and endochondral ossification in snakes, birds, and amphibians, including the Anura and Gymnophiona. However, the great majority of dermal bones have begun ossification before the bulk of endochondral ossification in these groups (*Elaphe,* Haluska and Alberch, '83; *Natrix,* Franklin, '45; *Psammophis,* Kamal and Hammouda, '65; *Dermophis,* Wake and Hanken, '82; *Rana,* de Beer, '37).

The data on non-mammalian tetrapods may eventually provide an interesting phylogenetic perspective on cranial ossification, but thus far, no consistent pattern emerges. Although dermal ossification generally precedes endochondral ossification, the exceptions are numerous and variable. However, with the exception of *Dermophis mexicanus* (Amphibia: Gymnophiona), the exoccipital is always in the first group of endochondral ossifications, and the palatine and pterygoid are in the first group of dermal ossifications to appear. This is the same pattern observed in both *Monodelphis domestica* and *Macropus eugenii.* The pattern of cranial ossification in *Dermophis* is notable in another context because it appears to be derived and adaptive to its peculiar mode of development (Wake and Hanken, '82). *Dermophis* is a viviparous species with an intraoviducal feeding period late in its development. Secretions from the oviducal epithelium are stimulated by the teeth on the lower jaw of fetal *Dermophis.* The secretions are then actively ingested by the fetus. Wake and Hanken suggest that the early ossification of the palatoquadrate and articulare in *Dermophis* is a derived pattern associated with the inception of jaw movement during intraoviducal feeding. This is an example of a novel ossification sequence probably associated with the feeding requirements of early development. The parallels between *Dermophis* and the two marsupials in this study are potentially informative as they emphasize the role that functional requirements may have in shaping the patterns of early cranial ossification in vertebrates.

CONCLUSIONS

Several significant points emerge from our study of *Monodelphis domestica* and *Macropus eugenii.* The same cranial bones are ossified at birth—premaxillae, maxillae, palatines, pterygoids, mandibles, and exoccipitals—and they have similar morphologies, supporting the hypothesis that marsupial neonates are conservative in morphology at birth. The relatively slow ossification of the neurocranial bones appears to follow the long period of rapid brain growth (as reported by Renfree et al., '82) rather than dictate early cessation of neural growth (contra Hughes and Hall, '88). The early ossification and rapid growth of the bones surrounding the oral cavity in marsupial neonates appears to be a derived pattern associated with the functional requirements of attachment, maintenance of the airway, and suckling. Thus cranial ossification in marsupials is influenced by at least two independent gradients or processes: the acceleration of the facial region due to a number of functional demands, and the deceleration of the neurocranium following the relatively slow neurogenesis.

The literature on tetrapod cranial ossification sequences reveals no other groups exhibiting this pattern during their ontogeny, although the literature suggests that the tendency for dermal bones to begin ossification before endochondral bones may be primitive. The published information on monotreme cranial ossification (de Beer and Fell, '36; Gaupp, '08; Kuhn, '71; Watson, '16) reveals some similarities with *Monodelphis domestica* and *Macropus eugenii* in overall ossification sequence, particularly regarding the extended period of ossification onset of the endochondral bones of the skull. However, monotremes do not share with marsupials the precocious ossification of the oral region. The published data on eutherians reveals no underlying pattern in the sequence of osteogenesis, although it appears that, like marsupials, the dentary, maxillary, and premaxillary are among the first bones to begin ossification. Where eutherians apparently differ from marsupials is that these bones do not grow exceptionally rapidly and in most cases ossify at the same rate as most other cranial bones. In this, eutherians resemble monotremes. In placentals there is no discernible difference in the timing of dermal and endochondral, or viscerocranial and neurocranial ossification and in this, placentals appear to be derived. The pattern of marsupial cranial osteogenesis represented by these two marsupial species shows greater overall similarities to the Monotremata than to the

Eutheria, but possesses particular patterns that differ significantly from both.

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