



FULL LENGTH ARTICLE

Fissures, folds, and scrolls: The ontogenetic basis for complexity of the nasal cavity in a fruit bat (*Rousettus leschenaultii*)

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Abstract

Mammalian nasal capsule development has been described in only a few cross-sectional age series, rendering it difficult to infer developmental mechanisms that influence adult morphology. Here we examined a sample of Leschenault's rousette fruit bats (*Rousettus leschenaultii*) ranging in age from embryonic to adult ($n = 13$). We examined serially sectioned coronal histological specimens and used micro-computed tomography scans to visualize morphology in two older specimens. We found that the development of the nasal capsule in *Rousettus* proceeds similarly to many previously described mammals, following a general theme in which the central (i.e., septal) region matures into capsular cartilage before peripheral regions, and rostral parts of the septum and paries nasi mature before caudal parts. The ossification of turbinates also generally follows a rostral to the caudal pattern. Our results suggest discrete mechanisms for increasing complexity of the nasal capsule, some of which are restricted to the late embryonic and early fetal timeframe, including fissuration and mesenchymal proliferation. During fetal and early postnatal ontogeny, appositional and interstitial chondral growth of cartilage modifies the capsular template. Postnatally, appositional bone growth and pneumatization render greater complexity to individual structures and spaces. Future studies that focus on the relative contribution of each mechanism during development may draw critical inferences how nasal morphology is reflective of, or deviates from the original fetal template. A comparison of other chiropterans to nasal development in *Rousettus* could reveal phylogenetic patterns (whether ancestral or derived) or the developmental basis for specializations relating to respiration, olfaction, or laryngeal echolocation.

KEYWORDS

Chiroptera, nasal capsule, nasal cavity, ontogeny, turbinates

1 | INTRODUCTION

The cartilaginous nasal capsule is the most rostral part of the chondrocranium and remains cartilaginous for most or

all of prenatal development in vertebrates. It provides the endochondral template for portions of the ethmoid bone, and source of certain cartilaginous cranial structures (e.g., septal cartilage), with some parts that are simply

transient and resorb later in development (Maier, 1993; Smith et al., 2008, 2012; Zeller, 1987). Our knowledge of the comparative morphology of the mammalian nasal capsule is quite extensive. The capsule is open rostrally and caudally (anterior and posterior nares), has opening(s) for olfactory nerves caudally and dorsally, and is mostly open ventrally (Fawcett, 1918, 1919, 1921). In most mammals, the superolateral boundaries of the nasal capsule form one of three developmental regions that arise from independent mesenchymal condensations and eventually fuse into a continuous tent-like cartilaginous covering that supports the developing nasal mucosa (de Beer, 1937; Zeller, 1987). From rostral to caudal, the three developmental regions are called the pars anterior, pars intermedia, and pars posterior (Maier, 1993; Smith & Rossie, 2008; and see Moore, 1981). As their mesenchymal condensations merge toward one another, these regions overlap with one another (as elegantly illustrated in Maier, 1993), and portions of the rostral and caudal regions jut inwardly, forming primordia of some of the larger turbinals (see Maier, 1993; Smith & Rossie, 2008 for more details).

The tripartite nasal capsule organization described above is known for a broad array of mammals, most of which were described using one or two late embryonic or early fetal specimens per species (e.g., Fawcett, 1918, 1919, 1921). These studies highlight that there are variations in nasal capsular form, some of which reflect the degree of nasal fossa complexity in the adult forms. For example, the relatively simple internal nasal cavity of anthropoid primates (monkeys, apes, and humans) develops from nasal capsules that are more quadrate or rounded in embryos, while in other primates the capsule is more elongate (Starck, 1975; and see De Beer, 1937). After the cartilaginous capsule initially forms in the primate embryo, further differences emerge between the nasal regions of anthropoids compared to the more complex anatomy observed in lemurs and lorises (Smith & Rossie, 2008). However, Smith, Eiting, and Bhatnagar (2015) contend that all primates develop reduced complexity in the paranasal region (specifically the frontal recess) compared to most other mammals, and that this is likely a consequence of orbital convergence typical of the Order Primates. Some of this reduction is hypothesized to relate to crowding of the interorbital space due to highly convergent (i.e., forward facing) orbits, which may affect postnatal bone modeling patterns (Cartmill, 1972; Smith, Craven, Engel, Bonar, & DeLeon, 2019). Thus, the ontogeny of nasal architecture is greatly prolonged across prenatal and postnatal timeframes in primates.

Whereas studies of embryonic and early fetal stages have been relatively common (see de Beer, 1937), there are relatively few studies that have examined later fetal and perinatal stages (as lamented by Maier & Ruf, 2014).

Broader age ranges of developing mammals have been studied for opossums (*Caluromys philander*—Macrini, 2014), tree shrews (*Tupaia belangeri*—Zeller, 1987), mouse lemurs (*Microcebus* spp.—Smith & Rossie, 2008), some afrosericida (e.g., tenrecids—Roux, 1947), a bat (*Myotis*—Frick, 1954, as cited and discussed in Zeller, 1987), the domestic cat (Terry, 1917), and of course humans (e.g., Slaby, 1960). Studies on these disparate taxa offer a glimpse into major variations among mammals, but we are a long way from understanding variation within taxonomic groups. Furthermore, the manner by which turbinals attain their complex form is understood in very few taxa, and this is but one aspect of nasal morphogenesis that remains obscured due to a lack of a broader comparative base of knowledge stemming from developmental series. Presley (1993) noted the hazards in drawing inferences about development using few or single prenatal samples, citing an instance of developmental reversal during embryogenesis of the sphenoid bone. The development itself evolves, and this may hinder the recognition of homology (Hall, 1995) or of neomorphic traits. Hence, a larger body of evidence on development is required to recognize precisely how specializations in nasal anatomy emerge.

In the present study, we expand our knowledge of age-related nasal capsule changes with a focus on Chiroptera (bats), an Order in which diversity of nasal form may be argued to exceed that in other groups of mammals, including primates (e.g., Bhatnagar & Meisami, 1998; Eiting, Smith, & Dumont, 2014; Pedersen, 1995; Santana & Lofgren, 2013). Among chiropterans, the pteropodids (fruit bats) have the least specialized, if at all, facial form compared to laryngeal echolocating bat species (Allen, 1882; Giannini, Macrini, Wible, Rowe, & Simmons, 2012). This also pertains to *Rousettus* spp. (Jurgens, 1963; Fehse, 1990, cited in Giannini et al., 2012), which have evolved a novel means of echolocation (oral clicks). Here, we examine a broad range of prenatal, early postnatal, and adult Leschenault's rousette fruit bats (Pteropodidae: *Rousettus leschenaultii*).

There are two overarching goals for this study. First, since pteropodids studied to date have a relatively unspecialized internal nasal morphology (Allen, 1882; Giannini et al., 2012; Starck, 1943), nasal capsule ontogeny in *Rousettus* may offer context to recognize developmental distinctions with more specialized bats, few of which have been studied ontogenetically to date (e.g., *Myotis*—Frick, 1954, as discussed in Zeller, 1987; *Rhinolophus*—Usui & Tikita, 2019). Second, in light of recent studies indicating postnatal plasticity of certain turbinals or parts of turbinals (Coppola, Craven, Seeger, & Weiler, 2014; Pang et al., 2016; Smith et al., 2007; Smith, Martell, Rossie, Bonar, & DeLeon, 2016; Van Valkenburgh et al., 2014), it may be possible to infer predominant mechanisms influencing

internal nasal morphology that are specific to a certain life stage or contiguous stages. We hypothesize that olfactory regions are shaped most strongly by prenatal chondral mechanisms (e.g., mesenchymal centers that chondrify), whereas structures that bear respiratory mucosa achieve their greatest complexity postnatally, via appositional bone outgrowths (Smith et al., 2016).

2 | METHODS

The sample studied (Table 1) is primarily from the collection of serially sectioned bat embryos, fetuses, and one adult assembled by KPB, and now maintained by TDS. Most of the specimens were previously prepared and described by Bhatnagar et al. (1996). Two additional specimens that were obtained at the same time, but not previously serially sectioned, were prepared recently for a different study (Smith et al., 2017) and were also appropriate for the present study. Since we did not have all external features of the specimens recorded, the prenatal stages (embryo or fetus in Table 1) are approximations only, based on the maturity of capsular tissues and the state of palate formation. The newly prepared specimens included one perinatal and one early postnatal specimen, according to collection notes (Bhatnagar et al., 1996). Two immature postnatal specimens (sectioned previously in Bhatnagar et al., 1996 and Smith et al., 2017) have numerous erupted deciduous teeth, well-mineralized unerupted replacement teeth, and M1 with one cusp nearly in alveolar eruption; these are referred to as an

“infants” throughout the manuscript. A histologically prepared adult includes sections through the rostral extent of ET I, allowing examination of its mucosa. Two adult skeletonized specimens from the Mammal Collection at the American Museum of Natural History (one female, AMNH 107418; one male, AMNH 107423) were also included to describe turbinal morphology throughout the nasal fossa.

Methods for histological preparation were previously reported in detail by Bhatnagar et al. (1996). Briefly, the samples were serially sectioned at 6–10 μm ; most were sectioned coronally, except for one perinatal specimen that was sectioned sagittally. Sections were stained with hematoxylin and eosin or by the Gomori trichrome procedure. We examined histological specimens using light microscopy, and observed and recorded regions of the nasal capsule regarding the maturation (mesenchymal condensation; chondrification) or degree of ossification. We photographed selected sections using an Axiocam MRc 5 Firewire camera attached to either a Zeiss stereomicroscope (X0.64 to X1.6 magnification) for low-magnification views or a Leica DMLB photomicroscope (X25 to X630) for high-magnification views. In order to gain a better understanding of the three-dimensional development of the bony turbinal skeleton and nasal fossa, we used the two specimens noted from an existing collection of preserved specimens (see above) for micro-Computed Tomography (micro-CT) imaging prior to histological sectioning. One was considered perinatal based on field notes, and the other was an older infant, with the partial eruption of deciduous teeth. These micro-CT

TABLE 1 *Rousettus leschenaultii* specimens used in the study, with stage and size measurements

Specimen no.	Age (stage)	CRL ¹ (mm)	HL ¹ /CL (mm)	Notes
R17	Prenatal (embryo)	7.0	3.2	SS
R22	Prenatal (embryo)	9.9	5.8	SS
Ro2	Prenatal (late embryo)	11.3	–	SS
Ro4	Prenatal (early fetal)	14.1	8.45	SS
Ro12	Prenatal (fetal)	18.5	13.5	SS
R24	? (Late fetal?)	34.1	–	SS
R26	Perinatal	–	–	SS; “near term” ¹
R34	Postnatal (infant)	–	–	SS; “young attached to nipple” ¹
Ro101	Perinatal?	–	21.36	CT; SS; 100 g fetus
Ro102	Infant	–	26.61	CT; SS; young of 139
Ro1	Adult	–	–	Serially sectioned
AMNH 107418	Adult	–	–	CT
AMNH 107423	Adult	–	–	CT

Note: ¹from Bhatnagar, Wible, & Karim, 1996.

Abbreviations: CL, cranial length (prosthion-inion); CT, micro-CT scanned; SS, serially sectioned.

scans were performed at Northeast Ohio Medical University (NEOMED) using a Scanco vivaCT micro-CT scanner. Scan parameters were 70 kVp and 114 μ A, and the resulting volumes were reconstructed using 20.5 μ m cubic voxels. Additionally, we micro-CT scanned the two adult skeletonized specimens using a GE V|tome|x micro-CT scanner with a 240 kV high-power directional X-ray tube. We scanned the complete crania at resolutions of 0.0460968–0.04948675 mm voxel size, and the rostra were scanned at 0.02768222–0.03103465 mm voxel size. We used Avizo v. 8.1.1 software (<https://www.fei.com/software/avizo3d/>) to manually segment voxels representing the bones surrounding the nasal fossa and individual turbinal bones from micro-CT data and generated three-dimensional surface models of turbinal skeletons for the infant and adult specimens. Micro-CT data and resulting three-dimensional surfaces are available on Morphosource (www.morphosource.org) by searching the bibliographic information for this article.

3 | RESULTS

3.1 | Early morphogenesis of the nasal capsule

In the 7.7 mm crown-rump length (CRL) embryo, the nasal sac has invaginated and the primordial olfactory and vomeronasal epithelia are visible (shown in Figure 1 of Bhatnagar et al., 1996). However, the mesenchyme of the midface is dispersed, with no evidence of the nasal capsule as yet. The 9.9 mm CRL embryo is the smallest specimen in our sample to possess visible turbinals (Figure 1a–c). The free rostral projection of ethmoturbinal (ET) I is formed, but internally this turbinal is supported only by dispersed mesenchyme throughout its length (Figure 1a,b). The inferior margin of the nasal capsule bends inward (medially) on both sides, and is emphasized by deep fissures dorsally and ventrally (Figure 1a,b). The paries nasi have progressed to a chondrified state anteriorly. However, while the paranasal cartilages are chondrified laterally, they transition to condensed mesenchyme superiorly and inferiorly; there is no mesenchymal condensation for the maxilloturbinal (MT) at this stage (Figure 1a,b). Caudally, the nasal fossa remains developmentally primitive, ending in a cul-de-sac that as yet lacks turbinals (cupular recess); the lateral wall of the nasal capsule (paranasal cartilages and orbitonasal lamina) is condensed mesenchyme (Figure 1c). The septal cartilage is the most advanced capsular element, well chondrified except for the superior margin in the caudal limits of the developing nasal fossa.

Chondrification of the nasal capsule is further progressed in the 14.1 mm CRL fetus. Rostrally and at

intermediate levels (i.e., pars anterior and pars intermedia), the paries nasi are bridged to the nasal septum, via the primitive roof of the capsule (the nasal tectum). This parietotectal complex is completely chondrified (Figure 1d). The turbinals are all visible in this specimen but are varied in their core tissue framework, as will be discussed below.

3.2 | Development of the MT

The MT is initially an inferior fold of nasal mucosa into which the inferior margin of the nasal capsule folds inward (medially). While our sample of embryos does not reveal the earliest phase of maturation of its connective tissue core, in the 14.1 mm CRL fetus the MT is rostrally supported by an oval chondrified process which laterally joins the inferior margin of the paranasal cartilage (Figure 1d,e). Passing caudally, the MT cartilage becomes more densely cellular (Figure 1f) and eventually transitions to condensed mesenchyme (Figure 1g).

In older prenatal specimens, the cartilage of the entire nasal capsule, including the MT, is less densely cellular (reflecting more matrix production), stains lightly bluish with trichrome, with crisp margins revealing the perichondrial interface (Figure 2a). The MT is notably more complex in fetuses than in embryos. While the largest embryo and early fetus (Figure 1e–g) possesses only the root lamella of the MT, the 18.5 mm CRL fetus has a dorsal and ventral bar projecting from the root (Figure 2a,d). The next-to-largest fetus is considerably larger in CRL (34.1 mm) and two major distinctions separate it from the 18.5 mm CRL fetus. First, the MT has commenced the ossification of its root (basal) lamella (Figure 2b). The secondary lamella and the tertiary plates remain cartilaginous. Second, the dorsal and ventral secondary lamellae each bear tertiary projections. In cross-section, these appear as bars projecting in either direction perpendicular to the secondary lamellae (Figure 2b); three-dimensionally, these would appear as a narrow plate anchored longitudinally to the secondary lamellae. In the 34.1 mm fetus and perinatal specimens, ossification of the root lamella is approximately within the middle third of the MT, while the caudal part (Figure 2e) and rostral part remain entirely cartilaginous. The caudally projecting part of the MT becomes unanchored by a root lamella (Figure 5e); most caudally this remains unossified in the infants (not shown).

In one of the perinatal specimens, the tertiary longitudinal plates bear evidence of appositional chondroblastic expansion at the margins (Figure 3). These sites of appositional growth correspond to peripheral margins of the tertiary lamellae that bend inwardly in the infants (Figure 2c). The infants have a further ossified MT; the secondary lamellae are fully ossified and the tertiary



FIGURE 1 Coronal sections of prenatal *Rousettus* revealing early morphogenesis of the nasal capsule; (a) to (c) and (e) to (g) are rostrocaudal sequences. In a 9.9 mm crown-rump length (CRL) embryo (a–c), the paranasal cartilages (PC) are most chondrified laterally, while the margins approaching the inferior and superior limits transition to a condensed mesenchyme (see *, a, b). At the inferior limit of the PC, the capsule folds inward (medially) in the direction of the nasal septum (NS). Fissures (arrows) help to define this region as a discrete fold, which will eventually house the maxilloturbinal. Caudally (c), the orbitonasal lamina (ONL) and the upper limits of the nasal septal cartilage (NS) are also condensed mesenchyme. At this stage, note the lower edge of the first ethmoturbinal (ET I) still has relatively dispersed mesenchyme (as evidenced by relatively lighter staining). In a 14.1 mm CRL fetus (d–g), the PC is well chondrified, with continuity to the nasal septum (d). The maxilloturbinal (MT) is well-chondrified rostrally, except for mesenchymal condensations (*) projecting dorsally and ventrally from the root lamella (e). Caudally the MT lacks dorsal and ventral lamellae (f) and then transitions to condensed mesenchyme (g). PT, parietotectal complex; RC, cupular recess. Hematoxylin–eosin stain. Scale bars: a, b, 100 μ m; c, d, 200 μ m; e–g, 50 μ m

plates are ossified except for their peripheral margins, which bear some cartilage remnants. It is noteworthy that the MT is not any more complex than the fetal specimens in terms of lamellar organization (Figure 2f). However, in adult *Rousettus*, examined by histology (Figure 2g) and by micro-CT reconstruction (Figure 4), the MT is more redundantly scrolled compared to all sub-adults. That is, in the adult the tertiary lamellae exhibit more elaborate edges that are expanded additional scrolls (Figures 2g and 4b,d,f); these are not observed when the MT initially ossifies (Figures 2f and 4a,c,e).

3.3 | Development of the ET complex

ETs commence development as mucosal folds, initially without any internal support aside from dispersed

mesenchyme (Figure 1b). In the 14.1 mm CRL fetus, only ET I is nearly fully chondrified (Figure 5a,d,g), aside from the rostral tip, which exists as condensed mesenchyme (e.g., Figure 1d). ET II is a mesenchymal condensation that adheres to the internal side of the orbitonasal lamina (Figures 6c and 7b). ET III has a chondrified base of the root lamella, which transitions to condensed mesenchyme near the tip (Figure 7b). In the 18.5 mm CRL fetus, all of these elements have fully chondrified (Figure 5b,e,h,k). ET I and II are initially distinct (Figure 5e). Their shared root lamella is secondarily developed (compare Figure 5g,h,i). Adjacent turbinals within the olfactory recess imply the turbinals form first as a simple mass with well-vascularized dispersed mesenchyme within its core, and subsequently, mesenchyme condenses within this projection (Figure 6b,d).

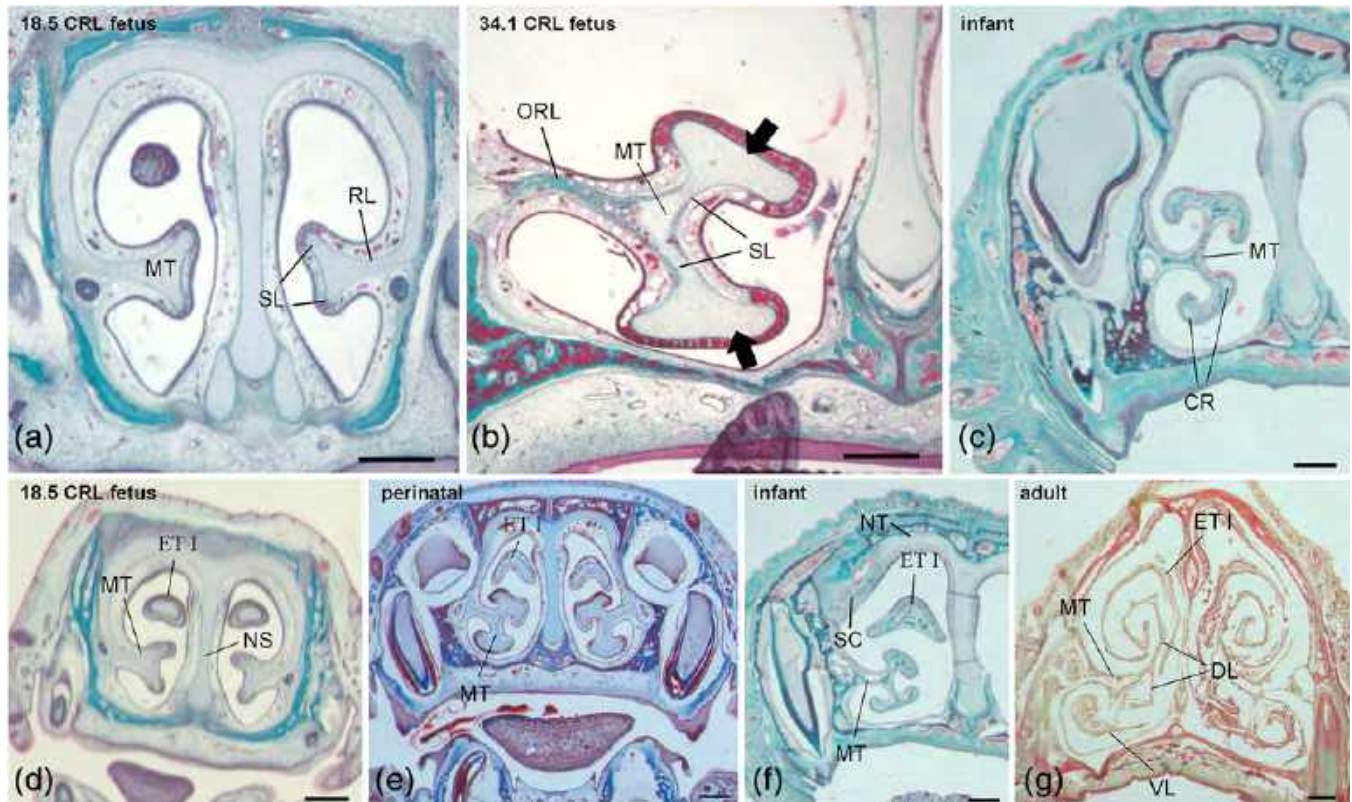


FIGURE 2 Micrographs of coronal sections showing the development of the maxilloturbinal (MT) in *Rousettus* across prenatal and postnatal age, showing the level of greatest complexity (top row) and association with the first ethmoturbinal (ET I; bottom row). After chondrification, the early fetal MT (a) bears simple dorsal and ventral secondary lamellae (SL) from its cartilaginous root lamella (RL). In an older fetus (b), the MT is now ossifying at its root lamella (ORL) and the secondary lamellae bear tertiary plates (bold arrows). The MT completes ossification postnatally; the infant *Rousettus* (c) reveals the newly ossified appearance. The dorsal and ventral lamellae end in outwardly convex crescents. Some cartilaginous remnants (CR) are seen in the secondary lamellae. ET has extensive overlap with MT, with the closest spatial association between MT and the free (unattached), rostral projection of ET I (d–g). Initially, the rostral projection is a horizontal bar (d). Perinatally, it remains cartilaginous, but has assumed a dorsally convex appearance (e). In the infant, it has ossified in this shape (f). In adults, the medial and lateral margins of the convex ET I become extended medial and lateral, ventrally projecting vertical lamellae; these have extensive overlap with the dorsal scroll of MT (g). The nasoturbinal is rudimentary, but a small semicircular crest (SC) is visible and partially ossified where it projects away from the nasal tectum (NT). NS, nasal septal cartilage. Scale bars, 0.5 mm

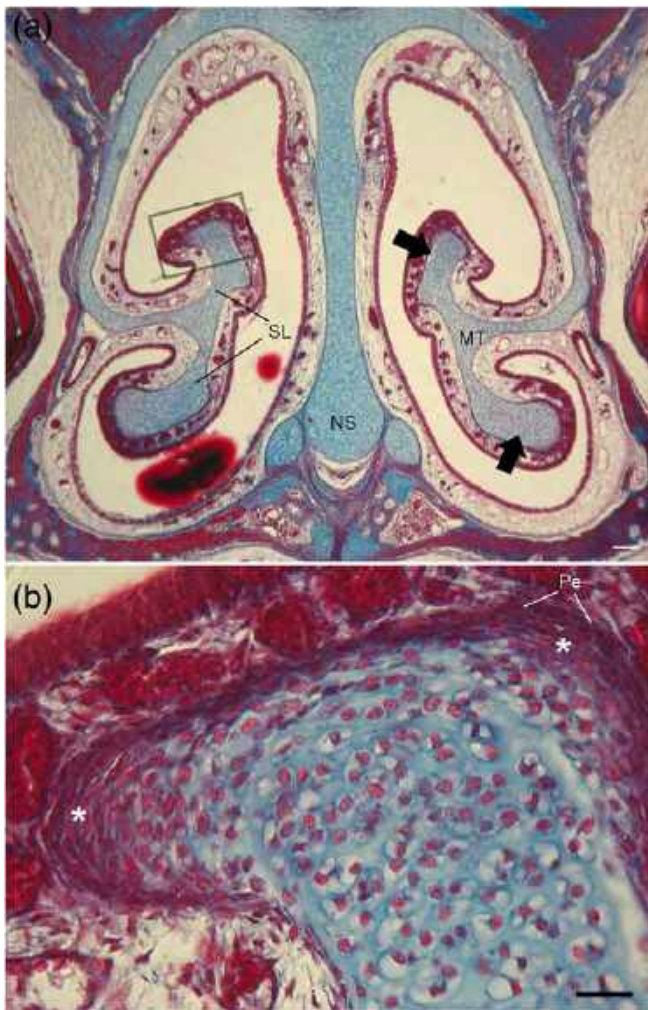


FIGURE 3 Coronal section of a perinatal *Rousettus* (a) showing the maxilloturbinal (MT). Rostrally, the secondary lamellae (SL) bear tertiary plates (arrows). These plates bear evidence of appositional cartilaginous growth (*) at their peripheral edges (b, enlarged from the superimposed box in a). The MT is ossified at the root more caudally. Note the appearance of a dense perichondrial (Pe) capsule surrounding the cartilage (b). Gomori trichrome stain. Scale bars: a, 100 μ m; b, 20 μ m

A cross-age comparison also suggests a slight change in the extent to which turbinals are sequestered within a dorsocaudal cul-de-sac of the nasal chamber, the olfactory recess. This recess is formed where a plate of cartilage (bone, postnatally) meets the alar processes of the vomer bone, and thereby creates a dorsal, caudally enclosed space that is separate from the inferior respiratory tract (Figure 5g–l). In the 14.1 mm fetus, only a very small portion of ET I–III is within this recess (Figure 5g) and ET IV is the primary turbinal in the enclosed space (Figure 5j). In fetal and older specimens, extensive parts of all turbinals are nested within the olfactory recess (Figure 5h,i,k,l).

The single frontoturbinal (FT) is observable in the 14.1 mm fetus (Figure 5a,d); it is supported by condensed mesenchyme rostrally, but only dispersed mesenchyme caudally. It is fully chondrified in the smallest (18.5 mm) fetus (Figure 5b,e), and is more complex in the next larger (34.1 mm) fetus by virtue of secondary lamellae (compare Figure 7c,d).

The nasoturbinal is rather rudimentary throughout development. The semicircular crest portion is clearly visible at all stages and is partly ossified in the infants (Figure 2f). Caudally, the nasoturbinal merges with the nasal tectum.

The ETs remain cartilaginous until the perinatal stage, when initial signs of endochondral ossification are seen in the rostral projection of ET I, ventrally and near to the rostral-most leading edge (not shown). In the infants, ossification has spread to the rostral tip (Figure 2f). However, the caudal extent of ET I remains cartilaginous (Figure 5i,l). When comparing the infant to adult specimens, the rostral extension of ET I becomes elaborated with plates that project ventrally toward the base of the nasal fossa on both medial and lateral sides (Figures 2d–g and 4). These descending plates are uniformly covered with respiratory mucosal surfaces in the histologically sectioned adult.

An outgrowth of the turbinals, concomitant with chondrification, might be inferred from a comparison of the 14.1 fetus compared to the 18.5 fetus, resulting in an increased complexity of the olfactory recess (Figure 7b,c). In both perinates, ET II to IV and the FT remain unossified (not shown). But in the infants, all of the ETs and the FT have ossified portions (Figure 5c,f,i,l). The caudal limits of all ETs and the FT (Figure 7e) remain cartilaginous.

A comparison of the infant to adult specimens in the depth of the ET complex (Figure 7e,f; just rostral to the olfactory recess) suggests the nasal fossa expands in the dorsal and lateral directions postnatally. The differences between the infants and two adult specimens suggest a slight increase in the relative height of the frontal recess occurs postnatally (Figure 7e,f). Correspondingly, some turbinals are more scrolled (e.g., ET II, nasoturbinal) or have more elongated basal lamella (ETs and FT) in the adults (Figure 7f) compared to the infants or newborns. However, the basic cross-sectional configuration of the ETs at the level shown in Figure 7, and deeper into the olfactory recess, remains remarkably similar from late fetal stages to adult ages (see Figure 7d–f). As noted above, this presents a contrast to the rostral projection of ET I (Figure 4).

We currently lack intervening juvenile stages to establish the mechanism(s) for turbinal growth during this transition, but it is notable that more posterior turbinals (including ETs and FT) are partially cartilaginous at least

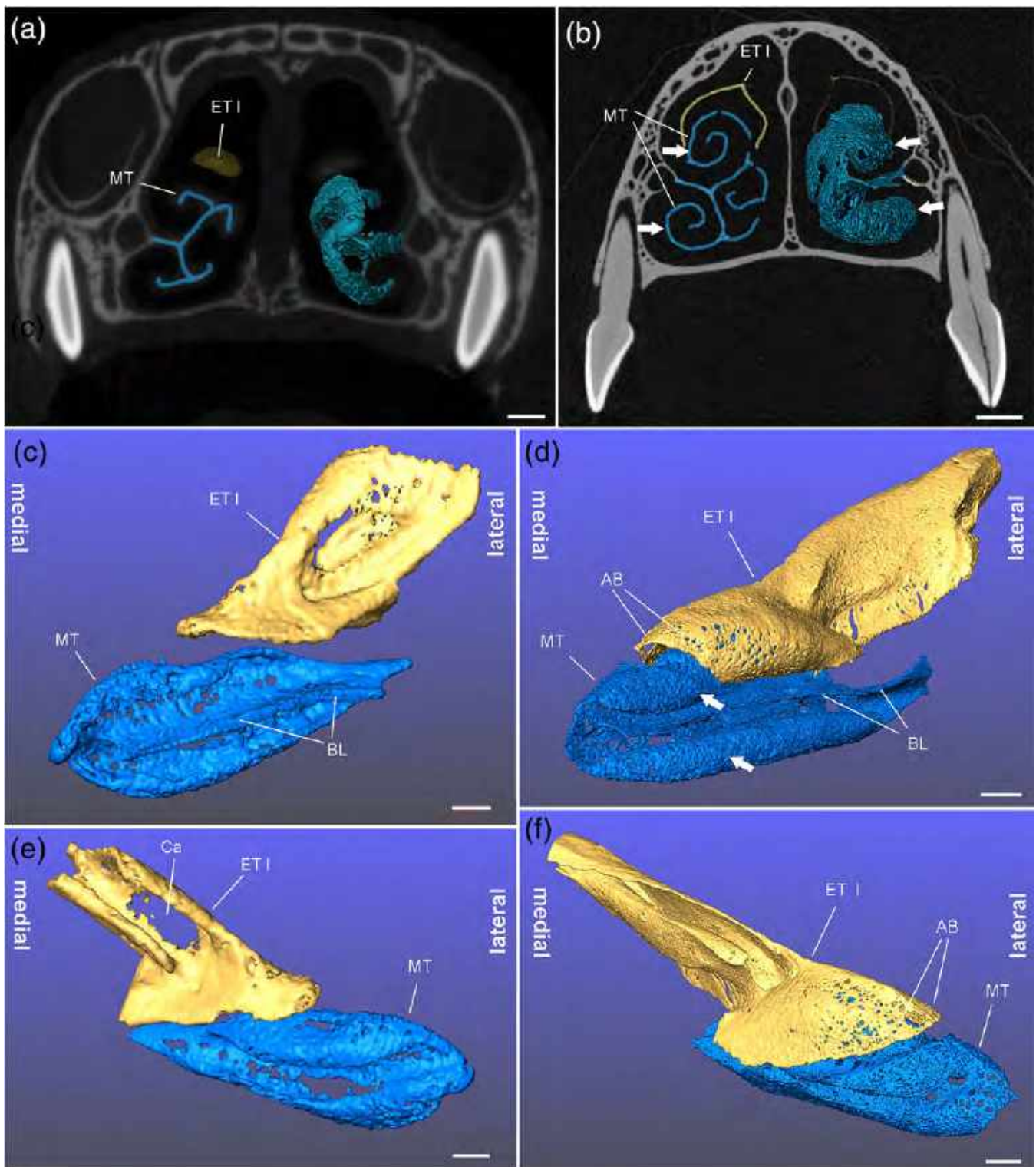


FIGURE 4 A comparison of the maxilloturbinal (MT) rostral limit of the first ethmoturbinal (ET I) in an infant (a, c, e) and adult (b, d, f) *Roussettus*. In the top row (a, b) are coronal slices through the MT where it interfaces with ET I. The bottom two rows (c–f) are anterolateral and anteromedial views, respectively, of three-dimensional reconstructions of the two bones. Note the MT has more elaborate scrolls (arrows in b and d) emanating away from the margins of the tertiary bars of the MT. Also note that in the adult ET I is expanded medially and laterally, closely interfacing with the dorsum of the MT. We infer this expansion is “appositional bone” (AB, see d, f) which emanates out from fully ossified rostral margins of ET I seen in the infant (c, e). Note that in the histologically sectioned adult (not shown), the medial and lateral flanges of the rostral projection of ET I are covered solely with respiratory mucosa. BL, basal (root) lamella; Ca, cartilaginous region of ET I. Scale bars: a, c, e, 0.5 mm; b, d, f, 1 mm

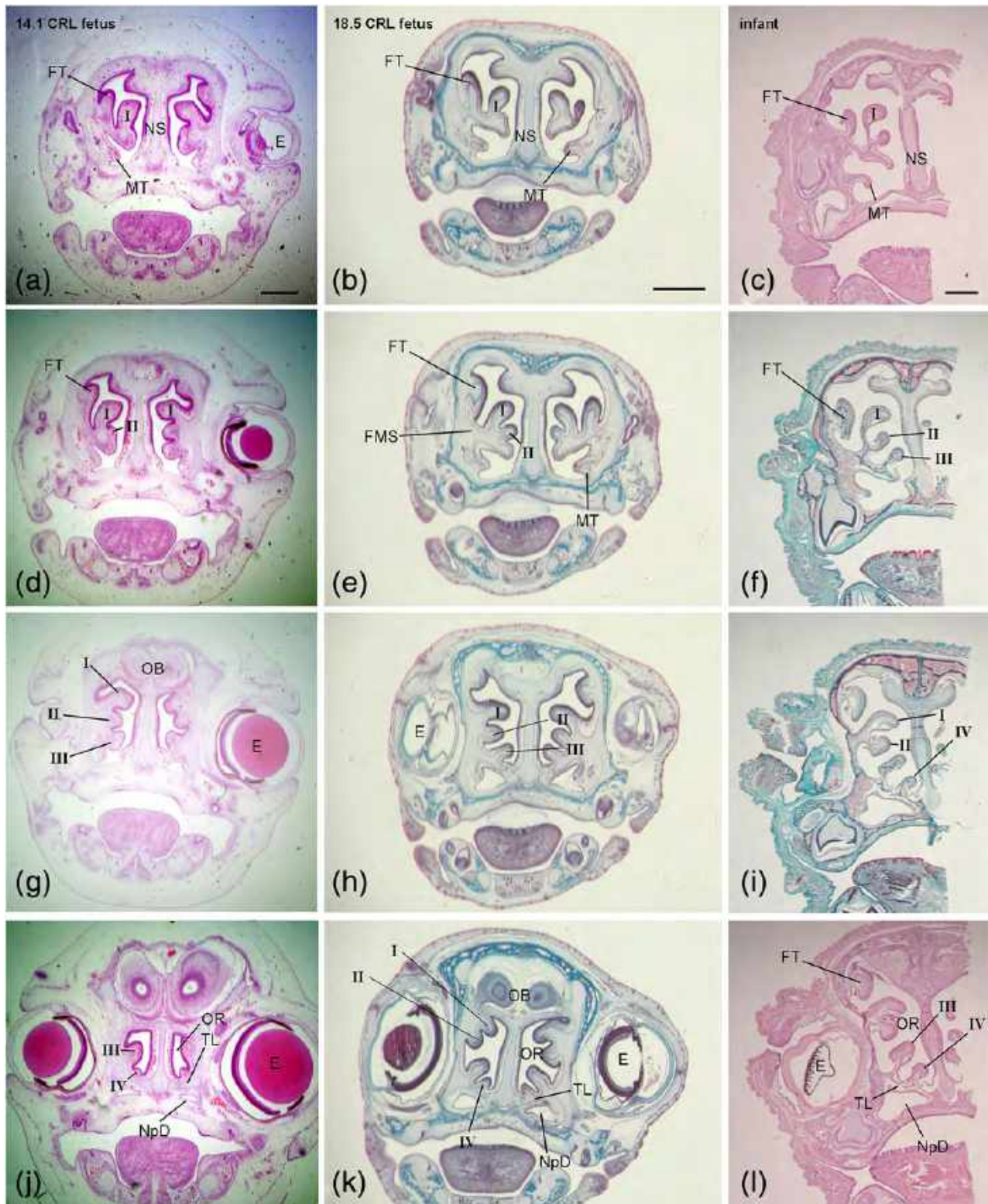


FIGURE 5 Coronal sections revealing development of the ethmoturbinal (ET) complex, represented at early fetal (left column), fetal (middle column), and early postnatal stages (right column). Top to bottom represents a rostrocaudal sequence in all columns. In the early fetus, ET I is fully chondrified (a, d). ET III is prominent (g), but ET II and IV are small, with no cartilage formed (g, j). All ETs are chondrified in the early fetus (b,e,h), including ET IV (k). In the infant, most ETs have ossified (c,f,i,l), although ET I remains cartilaginous posteriorly (i, l). The frontoturbinal (FT) remains cartilaginous throughout prenatal and early postnatal ages. It is also positionally stable, emanating from the frontomaxillary septum (FMS, a.k.a., lateral root of ET I) rostrally (e). The transverse lamina (TL) is complete in the early fetus (h) and the infant (i); it is ossified in the latter. In the early fetus, the TL is a mucosal bridge that is not fully chondrified within (j). The TL divides the more dorsal olfactory recess (OR) from the paired nasopharyngeal ducts (NpD; see j, k, l). E, eye; NS, nasal septum; OB, olfactory bulb. Scale bars: a, 0.5 mm (the same scale bar applies to d, g, and j); b, c, 1 mm (the same scale bars apply to e, f, h, i, k, and l)

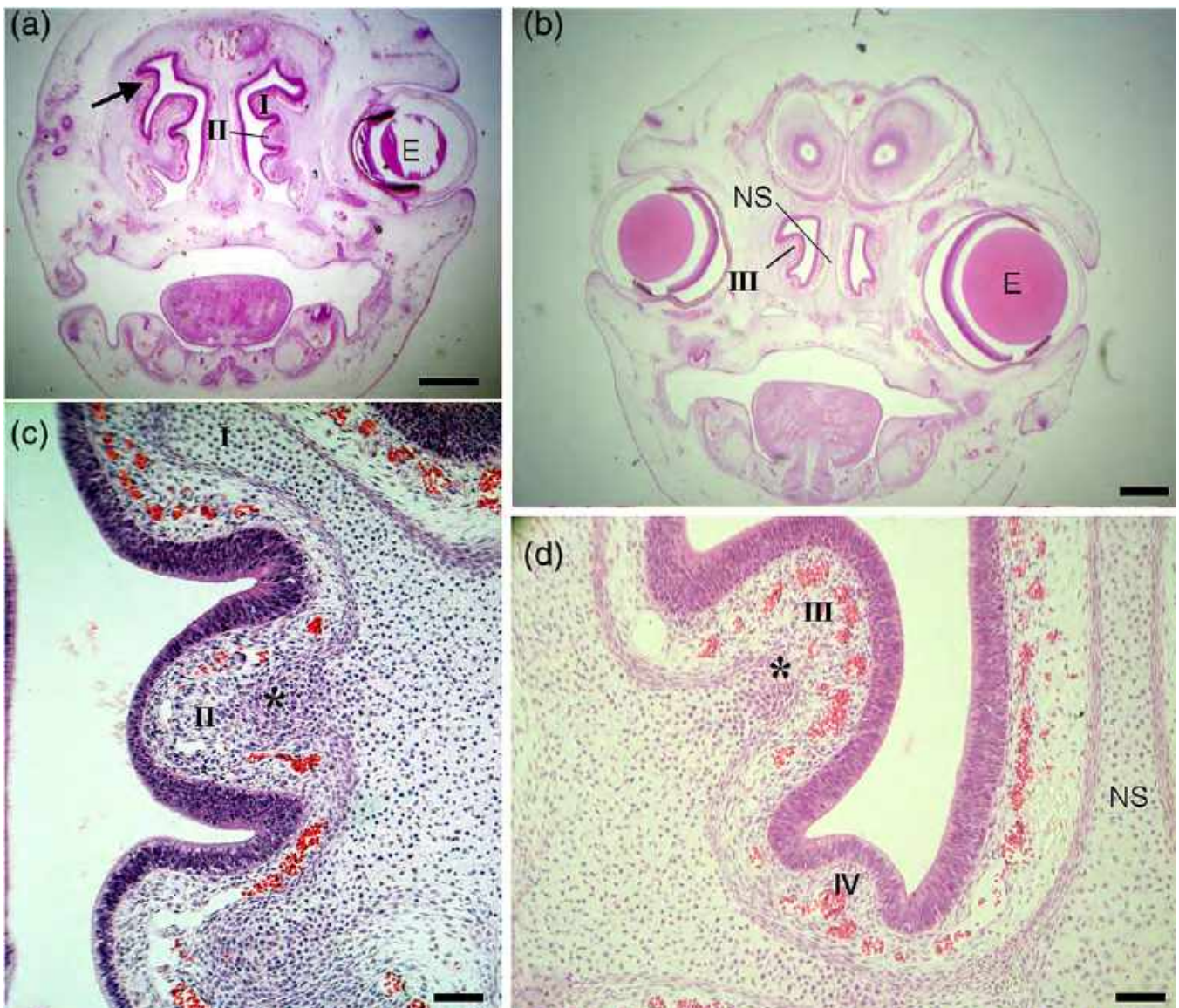


FIGURE 6 Coronal sections revealing early morphogenesis of the ethmoturbinal complex in a 14.1 CRL early fetal *Rousettus*. (a) At the level where ET I connects to the lateral nasal wall, this turbinal is fully chondrified (enlarged in c, see I). ET II (II) is in a state of partial chondrification (c). In (a), the incipient frontoturbinal is indicated by an arrow. (b) ET III is similarly in a state of partial chondrification (enlarged in d). Note condensed mesenchyme (*) at the tips of ET II and III. ET IV is a mucosal fold with only vascularized dispersed mesenchyme within it (IV, d). E, eye; NS, nasal septum. Scale bars, a, b, 0.5 mm; c, d, 50 μ m

near the apices of lamellae. Some portions of basal (root) lamellae are ossified. We may, therefore, infer that post-natally, both chondral growth and bone modeling create the extensions of the scrolls and basal lamellae.

3.4 | Development of the paranasal region

Rousettus lacks an “anterolateral” recess (i.e., the portion of the lateral recess anterior to the frontomaxillary septum—see Smith & Rossie, 2008) or a maxillary

recess at any stage. However, a frontal recess is present. This space is located where the lateral root of ET I connects to the lateral wall, thus forming the floor of the recess (e.g., see Figure 5e). The recess is made more complex by the expansion of the FT in larger fetuses (e.g., Figure 7d). At birth, the recess remains surrounded by cartilage, but in the infant, the cartilage has receded to expose a large dorsomedial extent of the recess wall, immediately dorsal to the nasal tectum. Here, there are numerous osteoclasts, suggesting limited secondary pneumatization is occurring to expand the nasal cavity (Figure 8).



FIGURE 7 Coronal sections of *Roussettus* revealing the increasing complexity of the olfactory recess across age. In early fetal *Roussettus* (a, b), the cartilaginous part of the transverse lamina is a mucosal bridge with condensed mesenchyme at the core. Posteriorly the cartilage of the transverse lamina (TL) is differentiated (see Figure 3d). In the early stages, the frontoturbin is a small bulge (see Figure 5a). In the 18.5 mm CRL fetus (c), the TL and ETs are entirely cartilaginous. ET I and II share a common root lamella rostrally (see Figure 2e), but for most of their length, they are distinct projections. The frontoturbin (FT) is a small projection that lacks its eventual crescent shape and the frontal recess (FR) that it occupies is shallow. In a 34.1 mm CRL fetus (d), ET I and II share a root, the frontomaxillary septum (FMS), which borders the FR ventrally. No maxillary recess is observed at any level. The FT is now larger and crescent shaped. Ossification of all ethmoturbinas as well as the TL is underway at birth, and becomes complete early postnatally, as in the infant (e), in which only the apical parts of ET I and ET II, as well as the caudal parts of the FT, are still cartilaginous. (f) CT slice showing a similar level in an adult. Note the ethmoturbinas appear somewhat longer compared to the infant, and ET II is scrolled inward. *, turbinas that are mesenchymal condensations; E, eye; NS, nasal septum; NpD, nasopharyngeal duct; ONL, orbitonasal lamina; PN, paries nasi. Scale bars, a, c, e, 0.5 mm; b, d, f, 1 mm

4 | DISCUSSION

Previous work on age series of the nasal capsule and other chondrocranial elements has revealed some generalized ontogenetic trends among a diverse array of mammal species. At the tissue level, the nasal capsule undergoes the same route of differentiation of cartilages and endochondral bones,

first forming mesenchymal condensations; these often form at specific sites that presage major structural parts of the nasal capsule, such as the orbitonasal lamina of the pars posterior (Smith & Rossie, 2008; Zeller, 1987). Whereas this stage is broadly similar across mammals, in one bat (*Myotis*) the mesenchymal condensation was described to be an initially continuous mass, rather than exhibiting the more typical

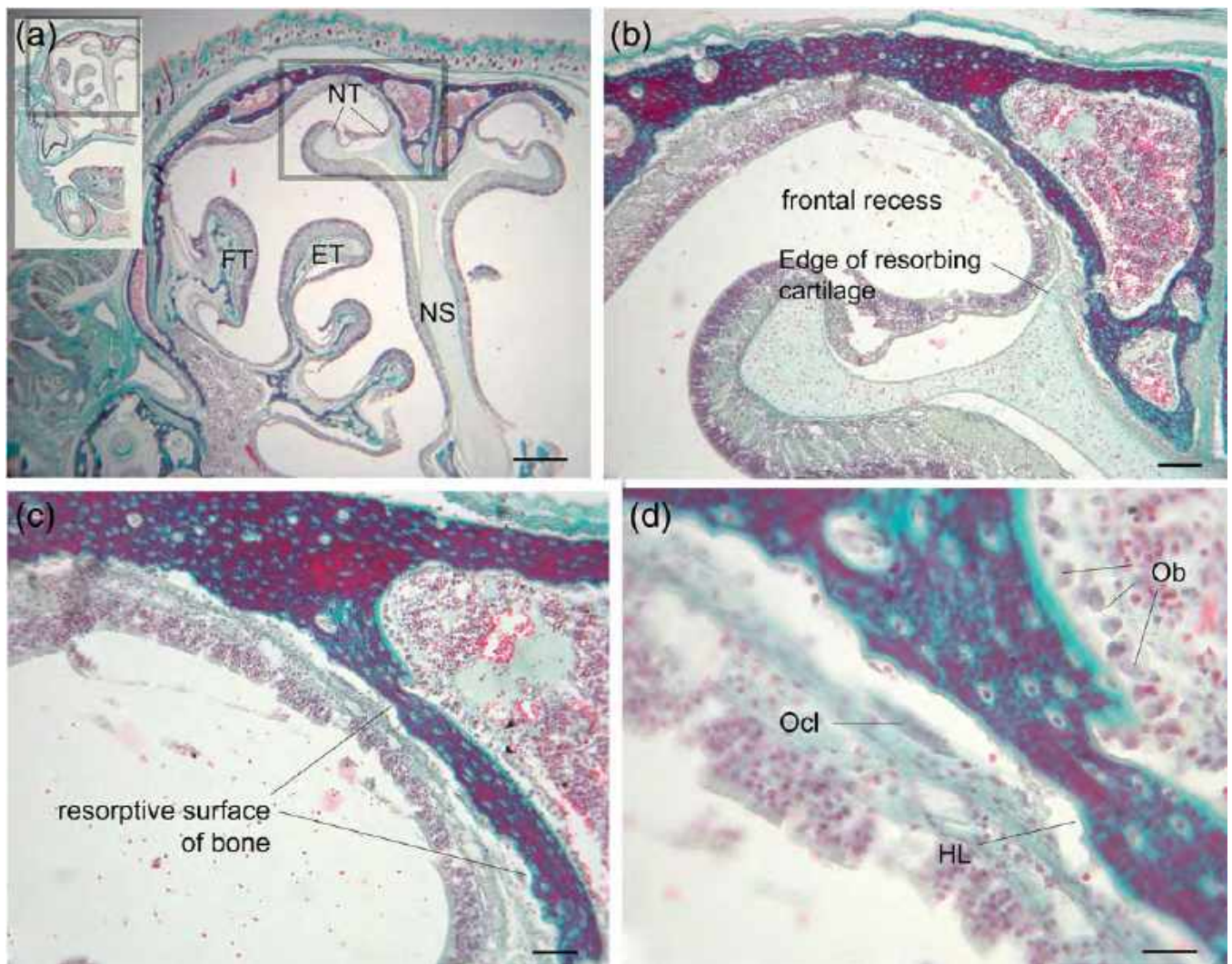


FIGURE 8 (a) The frontal recess of an infant *Rousettus* is pictured (inset shows the location of (a)), with the frontal recess framed within a box. (b) Enlarged from the box in (a), the margin of resorbing cartilage is indicated. Compare this to perinatal specimens (e.g., 7c) in which the capsule is still complete within the frontal recess. (c, d) Resorptive surface of the bone, showing the scalloped appearance, and osteoclasts (Ocl), osteoblasts (Ob), and Howship's lacunae (HL). NS, nasal septal cartilage; ET, ethmoturbinal, FT, frontoturbinal. Scale bars, a, 0.5; b, 100 μm ; c, 50 μm ; d, 20 μm

sequestered condensations (denoting centers for the three “pars”) which later merge (described by Zeller (1987), citing Frick, 1954). This suggests very early phases of nasal capsule development are rather malleable, and we should cast a look at more taxa to draw inferences on how this contributes to specializations in nasal form.

Chondrification within mesenchymal condensations is rapid, based on comparisons of staged embryos (mouse lemurs; Smith & Rossie, 2008). But a unique aspect of nasal capsule cartilage among chondrocranial regions is the varied fate of its parts: some persist as cartilage, some ossify, and some are resorbed (Maier, 2000; Schaeffer, 1920; Smith et al., 2008). In addition, some nasal structures are elaborated postnatally by appositional bone growth (Zeller, 1987; Smith et al., 2006). These diverse trajectories of development

for capsular components are known to yield variation in form (e.g., Smith et al., 2006) and may contribute to functional specialization. For this reason, our new observations on *Rousettus leschenaultii* are discussed in the context of previous work using developmental series across mammals.

4.1 | Comparative development

4.1.1 | Early morphogenesis: Embryogenesis and early ossification of the nasal capsule

The earliest *Rousettus* embryos in our sample do not depart in any obvious way from previous descriptions of

mammalian embryos. The nasal septal cartilage, a direct extension from the trabecular plate (within which midline parts of the sphenoid ossify), is the first element to appear as a mesenchymal condensation (Smith & Rossie, 2008; Terry, 1917; Zeller, 1987). Turbinals in *Rousettus*, *Microcebus* (Smith & Rossie, 2008) and all other mammals described to date (see Dieulafe, 1906) first appear as bulges or folds and initially lack mesenchymal condensations within. According to Dieulafe (1906), the bulging of turbinals in early stages is not due to evagination, but rather “fissuration,” or the formation of clefts into the nasal wall that result in projecting contours. Dieulafé asserted that after fissuration, the mesenchymal condensation and chondrification contribute to turbinal outgrowth.

Smith and Rossie (2008) identified spatial trends in the formation and tissue maturation of the nasal capsule in *Microcebus* spp. First, the nasal tectum develops in an anteroposterior and mediolateral direction. The tectum joins the paries nasi rostrally first, via a bridging of condensed mesenchyme which chondrifies into the “roof” of the capsule, thus creating a parietotectal complex. This is described for amniotes generally (de Beer, 1937), and *Rousettus* may be inferred to follow suit based on characteristics of the embryos examined here (e.g., Figure 1a,b). More caudally, Smith and Rossie observed that the mesenchymal condensations of the orbitonasal lamina form after those for the paranasal cartilages. This is most certainly the case in *Rousettus* (compare Figure 1b and c). Finally, Smith and Rossie observed that the ETs undergo the earliest phases of maturation in a rostrocaudal sequence in *Microcebus*. This too characterizes *Rousettus*. However, it is possible ET III is slightly more advanced in development than ET II or ET IV at the time of their initial chondrification. This appears to be the case in the 14.1 mm *Rousettus* fetus (Figure 6), and also in the late embryo of *Microcebus* (see Figure 6a in Smith & Rossie, 2008). In all other respects, rostral turbinals appear to form condensations, chondrify, and ossify before more caudal turbinals. Moreover, maturation of individual turbinals matches the same pattern, occurring first at the rostral end, and progressing caudally (with the exception of the MT, which begins ossification near mid-length).

The rostral to the caudal sequence of capsular maturation was also observed in *Tupaia* (tree shrews) by Zeller (1987), who noted that the hind portion of the paries nasi (orbitonasal lamina) remains separated at first after the parietotectal complex is fused. In addition, the ETs chondrified after most of the more rostral elements, except that the “rostrum” itself—including the anterior cupula—also lags behind the parietotectal complex in chondrification.

Other features in our *Rousettus* series also match previous descriptions of other mammals, such as the initially

ventral chondrification of the septal cartilage, which proceeds dorsally across embryonic stages (Terry, 1917). Like *Rousettus*, the paries nasi are more advanced in development at their lateral limits in the cat (Terry, 1917) and in *Microcebus* (Smith & Rossie, 2008).

The role of mesenchyme in extending chondrified parts of the nasal capsule was emphasized by Smith and Rossie (2008) who noted that the core tissues of more posterior ETs mature well after the pars posterior has ossified. In *Rousettus* too, mesenchymal condensations appear as masses directly opposed to the inner surface of existing cartilage (see Figures 1g and 6c). We suspect mesenchymal condensations play their role prior to the development of a fully developed perichondrium.

The above sequence of nasal capsule maturation might be inferred to be ancestral to chiropterans, in that it bears a basic resemblance to other not closely related mammalian orders such as Primates (Smith & Rossie, 2008). The extent to which deviations from this pattern may lead to midfacial diversity is currently unclear. Hypothetical perturbations in tissue maturation could easily be imagined to reduce or increase turbinal complexity. Once chondrified, the interstitial growth of cartilage can directly expand nasal dimensions (Smith et al., 2017; Wealthall & Herring, 2006). The capacity of capsule maturation and growth might explain why the capsule grows differentially, such that in some mammals rostral outgrowth is more uniform whereas in others the caudal capsule expands more (Zeller, 1987). Further comparative study may clarify how early events influence adult midfacial morphological diversity.

4.1.2 | Late fetal and postnatal development: Endochondral ossification, appositional expansion, and pneumatization

Once the nasal capsule is fully chondrified and possesses a perichondrial capsule (e.g., Figure 3), the avenues for increased complexity are profoundly altered. Mesenchymal outgrowths are no longer observed. However, there is clear evidence that differential growth of cartilage by appositional expansion occurs (Figure 3), and the site corresponds precisely with the location of flanges that project from tertiary plates of the MT in older staged animals. Endochondral ossification is another potential means of tissue expansion, reflecting interstitial expansion via chondrocyte hypertrophy. This may cause an increase in the length of the root lamella until ossification completes. To our knowledge, chondral mechanisms for generating turbinal complexity have not been discussed and deserve more scrutiny.

Our sample of adults is not large enough to establish the degree of intraspecific variation in turbinal morphology.

However, the greatest age differences were observed in the respiratory region (MT; in which turbinal bearing respiratory mucosa are more greatly scrolled in adults (MT) or greatly expanded anteriorly (anterior projection of ET I). The differences in the olfactory region (e.g., within the olfactory recess) are more subtle, with some increased scrolling of ET II and the nasoturbinal. This is consistent with age-changes in other mammals. It is well known that turbinals continue to become more complex even after ossification completes. This expansion, via appositional bone growth, has been observed in pigs, dogs, *Tupaia*, and several primate species, and its contribution to MT complexity is well documented (e.g., Martineau-Doizé & Martineau, 1986; Smith et al., 2016; Trépanier, Martineau, & Martineau-Doizé, 1988; Wagner & Ruf, in press; Zeller, 1987). Thus, in *Rousettus*, we infer that the age-differences in the MT and ET I are likewise the result of appositional growth and not the intraspecific variation.

Two paranasal spaces that can expand by the folding of the nasal capsule (i.e., primary pneumatization) and later osteoclastic resorption of bone bordering the recess (i.e., secondary pneumatization) include the maxillary and frontal recesses (Witmer, 1999; Rossie, 2006; Smith, Rossie, Cooper, Mooney, & Siegel, 2005). Overall, pneumatization is very limited in *Rousettus*. The lack of a maxillary recess shows primary pneumatization (i.e., due to cartilage folding) is limited, notable since most mammals possess a maxillary recess (Moore, 1981). The secondary pneumatization observed in the frontal recess (Figure 8) commences postnatally, which is in keeping with the timing observed in many primates (Smith et al., 2012). The location of the osteoclastic activity is consistent with the hypothesis that secondary pneumatization is dependent on local loss of capsular cartilage (Witmer, 1999; Smith et al., 2005, Smith et al., 2012). This pneumatic expansion appears to increase vertical dimensions of the nasal fossa but does not create a cavity within the frontal bone, as found in many primates (Rossie, 2006).

Cartilage resorption is a relatively unexplored mechanism with respect to its influence on nasal morphology. In primates, chondroclasts are observed near parts of the pars anterior and pars intermedia in perinatal specimens, and there is also evidence that cartilage degenerates by other means (Smith et al., 2012). To some extent, this may be related to a loss of complexity of the internal nose in primates generally, and haplorrhine primates especially (Smith et al., 2017). In this respect, *Rousettus* differs notably. The posterior cupula remains cartilaginous through at least early postnatal age in *Rousettus*, while it breaks down before birth in most primates (Figure 9; Smith et al., 2017). Still, the possibility that cartilage resorption affects nasal capsular ontogeny should be considered on a broader array of bats, especially in light of

the notable degree of nasal diversity across the order (Göbbel, 2000; Wible & Bhatnagar, 1996).

A final mechanism implicated in nasal diversity is late fetal and postnatal bone modeling under the influence of dental eruption and mastication, as well as the growth of the dentition and soft tissue structures (e.g., brain, eyes). Because these structures share skeletal boundaries, they may indirectly affect nasal architecture by influencing bone modeling. In primates, the convergent eyes are known to be associated with medial orbital resorption in small-bodied species, which can inhibit the expansion of different regions of the nasal fossa (Smith et al., 2014). Although pteropodid bats do have relatively large, forward-facing eyes, they are also ectopic (extrude out of the skeletal boundaries of the orbit—Harvey et al., 2016), so the eyes do not likely constrain interorbital expansion. Still, such “trade-offs” are unaddressed by our current sample due to the lack of a full postnatal cross-sectional age series. Future morphometric studies should examine how the different turbinals expand postnatally in tandem with other growing structures. The present study suggests nonolfactory regions may expand more than olfactory regions (Figure 4), as is known based on quantitative data on other mammals (e.g., Smith et al., 2007). Whether these structural changes reflect optimizing airflow for odorant delivery, adding additional surface area for air-conditioning, or both, remains another question for further exploration.

4.2 | Synopsis of developmental mechanisms for complexity in the nasal region in mammals

The observations provided here along with prior results on developmental series emphasize several distinct mechanisms for increased complexity of nasal capsule elements, most described previously. These mechanisms are placed in context by virtue of the timing at which they act and influence morphology (Figure 9).

Prenatally, there are two mechanisms that are restricted mostly or entirely to early development (embryogenesis): fissuration and mesenchymal growth. Fissuration produces the earliest appearance of the turbinals. The bulging appearance of early turbinals is not the result of outgrowth, but rather they are “sculpted” by a sinking in of tissue at their margins, or fissuration (Dieulafe, 1906). Our smallest embryo has a simple, sac-like nasal fossa while the 9.9 mm CRL specimen has already undergone fissuration; the process presumably accounts for the distinct margins of larger turbinals such as ET I and MT in the smallest embryo (e.g., Figure 1a,b). However, this mode of formation does not apply equally well to all turbinals. In contrast, more caudal ETs initially bulge via mesenchymal expansion,

DEVELOPMENTAL MECHANISMS THAT PROMOTE TURBINAL COMPLEXITY

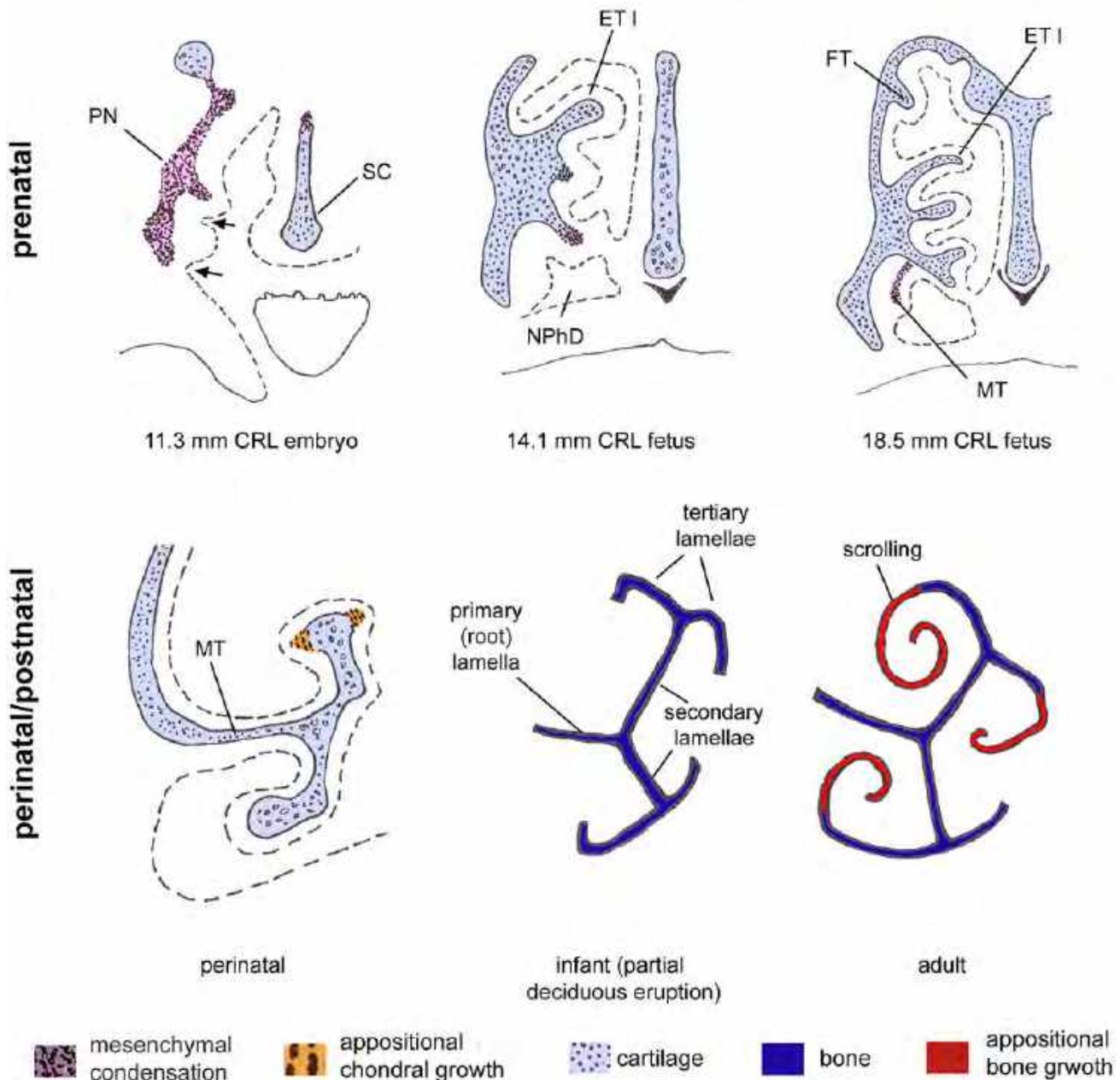


FIGURE 9 Summary of mechanisms that enhance the complexity of turbinal bones in *Roussettus*. Prenatally, a sinking in of nasal epithelium at specific sites (i.e., “fissuration”, see arrows, top left) creates initial folds that presage turbinals. Soon after, mesenchymal condensations (top left) establish the primitive morphology of the chondral template; later prenatally, secondary mesenchymal condensations form and chondrify. Perinatally and postnatally (bottom row), chondral growth shapes tertiary lamellae. Finally, differences between subadults (neonates, infant) and adults in our sample suggest lamellae attain a scrolled morphology via appositional bone growth after the chondral template is fully ossified. Interstitial growth of cartilage (not shown) and the process of endochondral ossification (e.g., see Figure 2b, may cause further modifications)

forming after chondrification of the pars posterior (Smith & Rossie, 2008). This is true in *Roussettus* as well, where there is initially well-vascularized dispersed mesenchyme within its core (Figure 6b,d). Fissuration and perhaps some mesenchymal proliferation influence the primordial form of the

turbinals. One might speculate that mesenchymal tissue volume bears a relationship to the eventual size of certain turbinals. In this regard, the unusual early development of the nasal capsule in *Myotis* is intriguing. The nasal capsule is described to be a continuous, rather tripartite

mesenchymal mass when it first forms (Frick, 1954, as discussed in Zeller, 1987). This could imply reduced mesenchymal tissue volume resulting in reduced complexity. Importantly, the nasal fossa of adult *Myotis* is itself described as having reduced complexity compared to some other bats (Bhatnagar & Kallen, 1974). Based on their extreme variations in nasal morphology (e.g., Curtis, Smith, Bhatnagar, Brown, & Simmons, 2020), bats provide ideal models to test whether mass and complexity of the capsule blastema bear a relationship to adult nasal diversity.

After initial chondrification, mesenchymal condensations appear on the inner surfaces of fully chondrified parts of the nasal capsule in mammals. A direct role of condensing mesenchyme in extending the MT in a caudal direction, and in the initial formation of secondary lamellae is also implied by our observations (Figure 1). The MT is usually described somewhat simply, as an inwardly folded portion of the paries nasi (e.g., Sperber, 2000). While descriptively true of a fully chondrified capsule, it belies that proliferating mesenchyme provides a clear means for augmenting existing chondrified parts of turbinals. Secondary lamellae of the MT are also shown to form this way in *Rousettus*.

Once a turbinal is fully chondrified, two additional mechanisms for increasing complexity are provided owing to the growth capacities of the cartilage itself: appositional and interstitial growth. These are active throughout fetal development and continue postnatally. Our study supports the hypothesis that these mechanisms are the primary means by which olfactory regions are shaped (Smith et al., 2016). Indeed, the posterior part of ET I is remarkably similar in adults compared to infants in which ossification is not quite complete (Figure 4), while the rostral part becomes much more elaborate across age. The same is true of other mammals in which ET I develops a rostral elongation that overlaps the MT and shares in its respiratory function (Smith et al., 2007; Smith & Rossie, 2008). However, the appositional growth of the chondral templates may influence subtle ornamentation (e.g., flanges) on any turbinals. Based on *Rousettus*, this mechanism may produce limited variation, and mostly in the late fetal/perinatal period.

Postnatally, the ossified turbinals may become elaborated with additional scrolls or flanges. This appositional growth, or *Zuwachsknochen*, is a notable mechanism by which neomorphic structures may form (Maier, 1987; Zeller, 1987) and is really the only avenue of chondral bone for rapid growth following its fully ossification. The observations on ET I and MT in *Rousettus* bear great similarity to age changes observed in strepsirrhine primates (e.g., Smith et al., 2007). The expanded bone surfaces are confirmed to bear respiratory mucosa in *Rousettus*, and this finding supports the hypothesis that “respiratory”

structures achieve their greatest complexity postnatally, via appositional bone outgrowths (Smith et al., 2016). Pneumatization within the frontal recess is a final mechanism observed in infant *Rousettus*, which expands this paranasal space. Fluid dynamics airflow modeling (e.g., Eiting et al., 2014; Eiting, Perot, & Dumont, 2015) may provide the optimal means for assessing the importance of pneumatization to adult internal nasal form, as is the case for appositional turbinal growth.

5 | CONCLUSIONS

Nasal capsule development in *Rousettus* proceeds similarly to many previously described mammals. In the future, this bat may provide a useful system for comparison to other species with more derived nasal morphology. Our findings also support the hypothesis that different developmental mechanisms influence turbinal complexity at different life stages (Smith et al., 2016). Specifically, discrete mechanisms for increasing complexity of the nasal capsule include those that are restricted to the early embryonic timeframe (fissuration and mesenchymal proliferation) and those that occur later and rely on the capacity of cartilage (appositional and interstitial chondral growth) and bone (appositional bone growth) to grow.

Future studies that focus on the relative contribution of each mechanism during development across mammal species may help draw critical inferences on the extent to which nasal morphology is reflective of the original cartilaginous template, or bauplan. Basic tissue-level comparisons among bats could be phylogenetically informative (e.g., see Sears, 2009, on primordial limb tissues). In addition, future ontogenetic comparisons to *Rousettus* could reveal the developmental basis for derived functional specializations associated with laryngeal echolocation in other groups of bats.

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AUTHOR CONTRIBUTIONS

Abigail Curtis: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; writing-original draft; writing-review and editing. **Kunwar Bhatnagar:** Conceptualization; data curation; formal analysis; investigation; methodology; resources; writing-original draft; writing-review and

editing. **Sharlene Santana:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; writing-original draft; writing-review and editing.

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REFERENCES

- Allen, H. (1882). On a revision of the ethmoid bone in the Mammalia, with special reference to the description of this bone and of the sense of smelling in the Chiroptera. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 10, 135–171.
- Bhatnagar, K. P., & Kallen, F. C. (1974). Morphology of the nasal cavities and associated structures in *Artibeus jamaicensis* and *Myotis lucifugus*. *The American Journal of Anatomy*, 139, 167–189.
- Bhatnagar, K. P., & Meisami, E. (1998). Vomeronasal organ in bats and primates: Extremes of structural variability and its phylogenetic implications. *Microscopy Research and Technique*, 43, 465–475.
- Bhatnagar, K. P., Wible, J. R., & Karim, K. B. (1996). Development of the vomeronasal organ in *Rousettus leschenaulti* (Megachiroptera, Pteropodidae). *Journal of Anatomy*, 188, 129–135.
- Cartmill, M. (1972). Arboreal adaptations and the origin of the order Primates. In R. Tuttle (Ed.), *The functional and evolutionary biology of primates* (pp. 97–122). Chicago: Aldine.
- Coppola, D. M., Craven, B. A., Seeger, J., & Weiler, E. (2014). The effects of naris occlusion on mouse nasal turbinate development. *The Journal of Experimental Biology*, 217, 2044–2052.
- Curtis, A. A., Smith, T. D., Bhatnagar, K. P., Brown, A. M., & Simmons, N. B. (2020). Maxilloturbinal aids in nasophonation in horseshoe bats (Chiroptera: Rhinolophidae). *The Anatomical Record*, 303, 110–118.
- de Beer, G. R. (1937). *The development of the vertebrate skull*. Chicago: Chicago University Press.
- Dieulafe, L. (1906). Morphology and embryology of the nasal fossae of vertebrates. *The Annals of Otology, Rhinology, and Laryngology*, 15, 1–584.
- Eiting, T. P., Smith, T. D., & Dumont, E. R. (2014). Olfactory epithelium in the olfactory recess: A case study in New World leaf-nosed bats. *The Anatomical Record*, 297, 2105–2112.
- Eiting, T. P., Perot, J. B., & Dumont, E. R. (2015). How much does nasal cavity morphology matter? Patterns and rates of olfactory airflow in phyllostomid bats. *Proceedings of the Royal Society B*, 282, 20142161.
- Fawcett, E. (1918). The primordial cranium of *Erinaceus europaeus*. *Journal of Anatomy*, 52, 211–250.
- Fawcett, E. (1919). The Primordial Cranium of *Miniopterus schreibersi* at the 17 millimetre total length stage. *Journal of Anatomy*, 53, 315–350.37.
- Fawcett, E. (1921). The primordial cranium of *Tatusia novemcincta* as determined by sections and models of the embryos of 12 millimetre and 17 millimetre C.R. Length. *Journal of Anatomy*, 55, 187–218.
- Fehse, O. (1990). Ein Beitrag zur Morphologie des Primordialcraniums von *Rousettus aegyptiacus* (Geoffroy 1810), (Megachiroptera, Mammalia). Diplomarbeit, Eberhard-Karls-Universität, Tübingen.
- Frick, H. (1954). *Die Entwicklung and Morphologie des Chondrocraniums von Myotis Kaup*. Stuttgart: Thieme.
- Giannini, N. P., Macrini, T. E., Wible, J. R., Rowe, T. B., & Simmons, N. B. (2012). The internal nasal skeleton of the bat *Pteropus lylei* K. Anderson, 1908 (Chiroptera: Pteropodidae). *Annals of Carnegie Museum*, 81, 1–17.
- Göbbel, L. (2000). The external nasal cartilages in Chiroptera: Significance for intraordinal relationships. *Journal of Mammalian Evolution*, 7, 167–201.
- Hall, B. K. (1995). Homology and embryonic development. *Evolutionary Biology*, 28, 1–37.
- Harvey, B. M., Bhatnagar, K. P., Schenck, R. H., Rosenberger, A. L., Rehorek, S. J., AM Burrows, V. B., ... Smith, T. D. (2016). Membranous support for eyes of strepsirrhine primates and fruit bats. *Anatomical Record*, 299, 1690–1703.
- Jurgens, J. D. (1963). Contributions to the descriptive and comparative anatomy of the cranium of the Cape fruit-bat *Rousettus aegyptiacus Leachi* Smith. *Annale Van Die Universiteit Van Stellenbosch, Series A*, 38, 3–37.
- Macrini, T. E. (2014). Development of the ethmoid in *Caluromys philander* (Didelphidae, Marsupialia) with a discussion on the homology of turbinal elements in marsupials. *The Anatomical Record*, 297, 2007–2017.
- Maier, W. (1987). The ontogenetic development of the orbitotemporal region in the skull of *Monodelphis domestica* (Didelphidae, Marsupialia), and the problem of the mammalian alisphenoid. In H.-J. Kuhn & U. Zeller (Eds.), *Morphogenesis of the mammalian skull* (pp. 71–90). Verlag: Hamburg.
- Maier, W. (1993). Cranial morphology of the therian common ancestor, as suggested by adaptations of neonate marsupials. In F. Szalay, M. J. Novacek, & M. C. McKenna (Eds.), *Mammal phylogeny* (pp. 165–181). New York: Springer-Verlag.
- Maier, W. (2000). Ontogeny of the nasal capsule in cercopithecoids: A contribution to the comparative and evolutionary morphology of catarrhines. In P. F. Whitehead & C. J. Jolly (Eds.), *Old World monkeys* (pp. 99–132). Cambridge: Cambridge University Press.
- Maier, W., & Ruf, I. (2014). Morphology of the nasal capsule of primates—with special reference to Daubentonia and Homo. *The Anatomical Record*, 297, 2018–2030.
- Martineau-Doizé, B., & Martineau, G.-P. (1986). Topography and differential growth of the nasal ventral concha (Os conchae nasalis ventralis) of the piglets from birth to six weeks of age. *American Journal of Veterinary Research*, 47, 416–421.
- Moore, W. J. (1981). *The mammalian skull*. London: Cambridge University Press.
- Pang, B., Yee, K. K., Lischka, F. W., Rawson, N. E., Haskins, M. E., Wysocki, C. J., ... Van Valkenburgh, B. (2016). The influence of nasal airflow on respiratory and olfactory epithelial distribution in felids. *The Journal of Experimental Biology*, 219, 1866–1874.

- Pedersen, S. C. (1995). Cephalometric correlates of echolocation in the Chiroptera: II fetal development. *Journal of Morphology*, 225, 107–123.
- Presley, R. (1993). Preconception of adult structural pattern in the analysis of the developing skull. In J. Hanken & B. K. Hall (Eds.), *The skull* (Vol. 1, pp. 347–377). Development. Chicago: University of Chicago Press.
- Rossie, J. B. (2006). Ontogeny and homology of the paranasal sinuses in Platyrrhini (Mammalia: Primates). *Journal of Morphology*, 267, 1–40.
- Roux, G. (1947). The cranial development of certain Ethiopian "insectivores" and its bearing on the mutual affinities of the group. *Acta Zoologica*, 28, 165–397.
- Santana, S. E., & Lofgren, S. E. (2013). Does nasal echolocation influence the modularity of the mammal skull? *Journal of Evolutionary Biology*, 26, 2520–2526.
- Schaeffer, J. P. (1920). *The nose, paranasal sinuses, nasolacrimal passageways, and olfactory organ in man*. Philadelphia: P. Blakiston's Son & Co.
- Sears, K. E. (2009). Differences in the timing of prechondrogenic limb development in mammals: The marsupial-placental dichotomy resolved. *Evolution*, 63(8), 2193–2200.
- Slaby, O. (1960). Die frühe Morphogenese der Nasenkapsel beim Menschen. *Acta Anatomica*, 42, 105–175.
- Smith, T. D., Rossie, J. B., Cooper, G. M., Mooney, M. P., & Siegel, M. I. (2005). Secondary pneumatization in the maxillary sinus of callitrichid primates: insights from immunohistochemistry and bone cell distribution. *Anatomical Record*, 285, 677–689.
- Smith, T. D., & Rossie, J. B. (2008). Nasal fossa of mouse and dwarf lemurs (primates, cheirogaleidae). *The Anatomical Record*, 291, 895–915.
- Smith, T. D., Bhatnagar, K. P., Rossie, J. B., Docherty, B. A., Burrows, A. M., Mooney, M. P., & Siegel, M. I. (2007). Scaling of the first ethmoturbinal in nocturnal strepsirrhines: Olfactory and respiratory surfaces. *The Anatomical Record*, 290, 215–237.
- Smith, T. D., Rossie, J. B., Docherty, B. A., Cooper, G. M., Bonar, C. J., Silverio, A. L., & Burrows, A. M. (2008). Fate of the nasal capsular cartilages in prenatal and perinatal tamarins (*Saguinus geoffroyi*) and extent of secondary pneumatization of maxillary and frontal sinuses. *The Anatomical Record*, 291, 1397–1413.
- Smith, T. D., Rossie, J. B., Cooper, G. M., Durham, E. L., Schmeig, R. M., Docherty, B. A., ... Burrows, A. M. (2012). Microanatomical variation of the nasal capsular cartilage in newborn primates. *The Anatomical Record*, 295, 950–960.
- Smith, T. D., Kentzel, E. S., Cunningham, J. M., Bruening, A. E., Jankord, K. D., Trupp, S. J., ... DeLeon, V. B. (2014). Mapping bone cell distributions to assess ontogenetic origin of primate midfacial form. *American Journal of Physical Anthropology*, 154(3), 424–435.
- Smith, T. D., Eiting, T. P., & Bhatnagar, K. P. (2015). Anatomy of the nasal passages in mammals. In R. L. Doty (Ed.), *Handbook of olfaction and gustation* (3rd ed., pp. 37–62). New York: Wiley.
- Smith, T. D., Martell, M. C., Rossie, J. B., Bonar, C. J., & DeLeon, V. B. (2016). Ontogeny and microanatomy of the nasal turbinals in lemuriformes. *The Anatomical Record*, 299, 1492–1510.
- Smith, T. D., McMahon, M. J., Millen, M. E., Llera, C., Engel, S. M., Li, L., ... DeLeon, V. B. (2017). Growth and development at the sphenoethmoidal junction in perinatal primates. *The Anatomical Record*, 300, 2115–2137.
- Smith, T. D., Craven, B. A., Engel, S. M., Bonar, C. J., & DeLeon, V. B. (2019). Nasal airflow in the pygmy slow loris (*Nycticebus pygmaeus*) based on a combined histological, computed tomographic and computational fluid dynamics methodology. *The Journal of Experimental Biology*, 222(Pt 23), jeb207605. <https://doi.org/10.1242/jeb.207605>
- Sperber, G. (2000). *Craniofacial development*. Hamilton: B.C. Decker.
- Starck, D. (1943). Beitrag zur Kenntnis der Morphologie und Entwicklungsgeschichte des Chiropterenocraniums. Das Chondrocranium von *Pteropus semindus*. *Zeitschrift für Anatomie und Entwicklungsgeschichte*, 112, 588–635.
- Starck, D. (1975). The development of the chondrocranium in primates. In W. P. Luckett & F. S. Szalay (Eds.), *Phylogeny of the Primates* (pp. 127–155). New York: Plenum.
- Terry, R. J. (1917). The primordial cranium of the cat. *Journal of Morphology*, 29, 281–434.
- Trépanier, H., Martineau, G.-P., & Martineau-Doizé, B. (1988). Distribution of the mineralized tissues in the nasal ventral conchae (*os conchae nasalis ventralis*) of piglets after birth: Relationship with atrophic rhinitis. *The Anatomical Record*, 222, 237–244.
- Usui, K., & Tikita, M. (2019). Normal embryonic development of the greater horseshoe bat *Rhinolophus ferrumequinum*, with special reference to nose leaf formation. *Journal of Morphology*, 280, 1309–1322.
- Van Valkenburgh, B., Pang, B., Bird, D., Curtis, C., Yee, K., Wysocki, C., & Craven, B. (2014). Respiratory and olfactory turbinals in feliform and caniform carnivorans: The influence of snout length. *The Anatomical Record*, 297, 2065–2079.
- Wagner F, Ruf I. 'Forever young' – Postnatal growth inhibition of the turbinal skeleton in brachycephalic dog breeds (*Canis lupus familiaris*, Canidae, Carnivora). *The Anatomical Record*. (in press). <https://doi.org/10.1002/ar.24422>.
- Wealthall, R. J., & Herring, S. W. (2006). Endochondral ossification of the mouse nasal septum. *The Anatomical Record*, 288, 1163–1172.
- Wible, J. R., & Bhatnagar, K. P. (1996). Chiropteran vomeronasal complex and the interfamilial relationships of bats. *Journal of Mammalian Evolution*, 3, 285–314.
- Witmer, L. M. (1999). The phylogenetic history of the paranasal air sinuses. In T. Koppe, H. Nagai, & K. W. Alt (Eds.), *The paranasal sinuses of higher primates* (pp. 21–34). Berlin: Quintessence.
- Zeller, U. (1987). Morphogenesis of the mammalian skull with special reference to *Tupaia*. In H. J. Kuhn & U. Zeller (Eds.), *Morphogenesis of the mammalian skull* (pp. 17–50). New York: Springer.

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