Midline growth of the sphenoid bone in primates: A histological and micro-computed tomography study

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ABSTRACT

Objectives

The aim of the present study is to broaden our knowledge of the ontogeny of cranial base cartilaginous joints in primates.

Materials and Methods

A cross-sectional age sample of sixty-six specimens from four platyrrhine and three strepsirrhine genera were scanned using micro computed tomography, histology, and immunohistochemistry. Specimens were segmented, reconstructed, and measured using Amira software. Ontogenetic scaling of palatal, presphenoid, and basisphenoid length relative to cranial length was examined using standardized major axis (SMA) regression. After histological sectioning, selected specimens were examined using immunohistochemistry of antibodies to proliferating cell nuclear antigen.

Results

Our results support the hypothesis that the presphenoid in platyrrhines grows more rapidly compared to strepsirrhines, but this study establishes that most or all of this growth discrepancy occurs prenatally, and mostly at the presphenoseptal synchondrosis (PSept). All species have prolonged patency (here meaning absence of any bony bridging across the synchondrosis) of the intrasphenoidal and spheno-occipital synchondroses (ISS). However, immunohistochemical results suggest growth is only rapid throughout infancy, and mitotic activity is slowing during juvenile ages. The same is indicated for the PSept.

Discussion

These results demonstrate that platyrrhines and strepsirrhines do not follow the pattern of early fusion of ISS seen in humans. In addition, these primates have a more prolonged patency and growth at PSept compared to humans. Finally, results reveal that in bushbabies and tamarins, as in humans, synchondroses remain cartilaginous for a prolonged period after chondrocyte proliferation has slowed or ceased. In light of these results, it is time to reassess related processes, such as differences in timing of brain expansion.

Key Words: growth centers, synchondroses, proliferating chondrocytes

1 INTRODUCTION

Midline synchondroses (cartilaginous joints; Figs. 1a, b) of the cranial base are located between the occipital and sphenoid bones (spheno-occipital synchondrosis) and between the two midline elements that form the body of the sphenoid (intrasphenoidal synchondrosis). More anteriorly is the spheno-ethmoidal synchondrosis, between midline parts of the sphenoid and ethmoid bones (Lieberman et al., 2000). One additional synchondrosis exists between the sphenoid and the septal cartilage (prespheno-septal synchondrosis, Baume, 1968; Wealthall and Herring, 2006). The significance of these specialized joints is to elongate basicranial bones anteroposteriorly (Thilander and Ingervall, 1973; Wealthall and Herring, 2006; Hall and Precious, 2013), or in the case of the prespheno-septal synchondrosis, to promote directional growth of the midface (Wealthall and Herring, 2006; Smith et al., 2017).

Basicranial synchondroses have been implicated to play a role in angulation of the cranial base in anthropoid primates, including humans. It has been suggested that the timing or perhaps sequence of synchondroseal fusion may influence basicranial angulation (e.g., Scott, 1958; Lieberman et al., 2000). However, the study of synchondroseal patency versus fusion has been investigated across age in far too few primates, with a heavy bias toward catarrhines (e.g., Giles et al., 1981; Michedja, 1972), and including no strepsirrhines at all. Moreover, still fewer primates have been studied using histology, which is essential for documenting that growth is occurring in cartilage (e.g., see Wealthall and Herring, 2006).

There is very little understanding of the pace and duration in which cranial synchondroses grow during the postnatal time period. The few prior studies on human and nonhuman primates show clearly that they are highly active (e.g., in chondrocyte proliferation) during infancy (Michedja, 1972; Thilander and Ingervall, 1973). However, in humans and at least one Old World monkey, the spheno-occipital synchondrosis remains cartilaginous for some time *after* growth ceases, before eventually ossifying (Thilander and Ingervall, 1973; Heinkele et al., 1989). Thus, the duration of synchondroseal patency in dry skulls, which is well studied in primates (Michedja, 1972; Giles et al., 1981; Terhune et al., 2013; Balolia, 2015), does not correspond precisely to the active interstitial growth period. Further confounding the issue, there are mammals in which the spheno-occipital synchondrosis and intrasphenoidal synchondrosis remain cartilaginous throughout life, even after active growth ceases (e.g., mice, Dai et al., 2017). The uncertainty of the duration of growth in mammals broadly, along with the relatively deep and hidden position of intrasphenoidal synchondrosis and septal synchondroses (see Smith et al., 2017, 2021), requires more scrutiny using histological methods.

We use microcomputed tomography (μCT) to examine the degree of patency among midline basicranial bones across postnatal ontogeny in nonhuman primates and measure the basi- and presphenoid bones to infer the pace of growth. Further, we use histology and immunohistochemistry to identify growth characteristics (i.e., chondrocyte proliferation and hypertrophy - Wealthall and Herring, 2006) at selected postnatal ages.

1.1 Postnatal synchondroseal ontogeny in anthropoid primates

Numerous studies have examined ontogeny of primate cranial joints, most frequently sutures (e.g., Richtsmeier et al. 1993; Collard and O'Higgins, 2001; Cray et al. 2008, 2014, 2021). In some studies, the term “suture” is used to collectively refer to all joints between adjacent cranial bones (e.g., Dolan, 1971). But unlike sutures, which serve as passive growth “sites” and serve

biomechanical functions (Jasinoski et al., 2010; Curtis et al., 2013), synchondroses are considered growth *centers*; by virtue of growth occurring within the tissue itself (i.e., interstitial growth), synchondroses produce "tissue-separating force" that elongates or widens the cranial base (Baume, 1968; Hall and Precious, 2013; and see Hall, 2015). Thus, the pace of growth of synchondroses, and the timing of fusion are factors that actively affect basicranial morphology and indirectly affect midfacial form based on the continuity of the sphenoid and facial skeleton (Wealthall and Herring, 2006; Smith et al., 2017, 2021).

Among all primates, our knowledge of synchondroseal growth and closure in humans is by far most advanced, especially concerning prenatal development (e.g., Scott, 1958, Baume, 1968; Kodama, 1976; Kjaer, 1990; Jeffery and Spoor, 2002; Jeffery, 2005). Documentation of postnatal changes are equally detailed, at least in osteological terms. Among midline synchondroses, the spheno-occipital synchondrosis remains patent the longest, with most sources agreeing on a range of closure between 17 and 25 years (e.g., Krogman, 1930; Ford, 1958, Scott, 1958). Because of its prolonged patency, multiple developmental roles have been implicated for this joint, such as its great importance to basicranial elongation (Hoyte, 1975), a possible influence on basicranial angulation (Melsen, 1974), and having a role in integration of the basicranium and pharynx (Laitman and Crelin, 1976). More rostral synchondroses act as active growth centers for a far shorter timespan (Hoyte, 1975; Scott, 1958). The intrasphenoidal synchondrosis fuses perinatally (Scott, 1958; Baume, 1968; Shopfner et al., 1968). Similarly, the prespheno-septal synchondrosis undergoes fibrous degeneration near the time of birth (Baume, 1968; Melsen, 1974). There are certain discrepancies in the literature regarding the spheno-ethmoidal "synchondrosis" in humans. Lieberman et al. (2000) discuss this joint as one of three midline synchondroses that actively grow. However, other authors pointedly refer to it as a suture (Scott, 1958; Melsen, 1974), and a histological study demonstrated it is indeed a fibrous joint at birth in humans, in contrast to the rat (Baume, 1968); we recently confirmed this in other anthropoid primates (Smith et al., 2021).

Our prior knowledge of *comparative* synchondroseal ontogeny in primates is mostly based on skeletal samples, and preferentially focused on the spheno-occipital synchondrosis (e.g., Giles et al., 1981; Joganic, 2016), often to the exclusion of other midline synchondroses. In part, this limited focus is due to the difficulty in making external observations on the most anterior synchondroses, which are partially or completely hidden from view in undissected crania. The spheno-occipital synchondrosis remains patent postnatally for a prolonged period in apes (Krogman, 1930; Schultz, 1940, 1941; Balolia, 2015, Joganic, 2016), macaques (Michedja, 1972), baboon (Krogman, 1930), and green monkeys (Heinkele et al., 1989). Actual chronological ages are only known in the macaques and humans (discussed further below), but in all known catarrhines, the synchondrosis remains patent until adulthood (i.e., after full dental eruption). In the few studies assessing the timing of spheno-occipital synchondrosis fusion in platyrrhines, the joint similarly fuses at adult ages (Dolan, 1971, Schön, 1976). In studies that address multiple synchondroses of non-human primates, the intrasphenoidal synchondrosis fuses prior to the spheno-occipital synchondrosis (Dolan, 1971; Michedja, 1972). Studies of macaques using cephalometry (Sirianni and Van Ness, 1978) and radioactive labeling of proliferating chondrocytes (Giles et al., 1981) provide additional lines of evidence that the intrasphenoidal synchondrosis contributes less to basicranial length than the more posterior synchondrosis. Postnatal fate of more anterior midline synchondroses has rarely been discussed. Scott (1958) pointedly asserts that the intrasphenoidal synchondrosis of apes, like humans, fuses near the time

of birth. Smith et al. (2017) demonstrated a CT reconstruction of a newborn chimpanzee that supports this assertion.

1.2 Histology of the midline synchondroses in non-human primates

Whether intrinsically active as growth centers, or active in response to external stimuli (see further discussion in Dean, 1982), basicranial synchondroses are crucial to the formation of species-specific morphology for the cranial base as well as orientation of the midface. Their critical importance in growth is seen when the growth centers are congenitally absent, as in achondroplasia and some syndromic craniosynostoses. In these cases, the anteroposterior (rostro-caudal) basicranial length is reduced (Cohen et al, 1985; McGrath et al., 2012), which is further correlated with midfacial growth deficits (Richtsmeier, 1987; Perlyn et al., 2006; Goldstein et al., 2014; Marulanda et al., 2017). The organization of chondrocytes within the synchondrosis indicates interstitial growth in progress. Similar in organization to epiphyseal growth plates, a zone of resting or reserve chondrocytes serves as a pool of cells that become mitotic and organize into longitudinal proliferating columns. As proliferating chondrocytes approach the ossification centers for the bodies of midline basicranial bones, they undergo hypertrophy (Fig. 1c). Proliferation, concomitant matrix production, and hypertrophy all propel basicranial bones apart. The cartilaginous synchondrosis does not lengthen, however, since there is a balanced resorption of cartilage closest to bone with replacement by bone (Moore, 1981). In most synchondroses, the resting zone of cartilage is flanked by proliferating chondrocytes directed anteriorly and posteriorly, with mirrored growth in both directions (Fig. 1a). However, some synchondroses are unidirectional, such as the presphenoseptal synchondrosis of most mammals, including strepsirrhines (Fig. 1a). In platyrrhines and cercopithecoids that have been studied, a more radial growth occurs at this synchondrosis at birth (Smith et al., 2017, 2021; Fig. 1b); in addition, Mano et al. (2021) found the presphenoid is proportionally larger in platyrrhines at birth compared to perinatal strepsirrhines. Based on this difference, growth patterns of the sphenoid may vary among primates.

The synchondroses of only few non-human primate species (and no strepsirrhines or platyrrhines) have been studied histologically across age. In the spheno-occipital synchondrosis of humans, zones of proliferating and hypertrophic chondrocytes increase in dimensions from birth until ages 3 or 4, after which they diminish in size (Thilander and Ingervall, 1973). From later childhood through about age 15, the matrix is described by Thilander and Ingervall to become more fibrous, and the zone of hypertrophy loses its columnar organization. Osseous union commences at 16 to 17 years in females and two years later in males, although cartilaginous remnants remain for a variable amount of time. A study by Heinkele et al. (1989) on green monkeys (*Cercopithecus aethiops*) examined a far shorter age range in terms of growth (mixed dentition to late adulthood) but similarly found a transitional period in young adults in which the spheno-occipital synchondrosis remains partially cartilaginous, and yet interstitial growth had ceased subsequent to some osseous bridging across the joint. Only one study has examined the intrasphenoidal synchondrosis in an age range of non-human primates, specifically *Macaca mulatta* (Michedja, 1972). In specimens of known age, this study demonstrated shifting growth characteristics in the intrasphenoidal synchondrosis relative to the spheno-occipital synchondrosis. At birth, the spheno-occipital synchondrosis had more diminutive zones of interstitial growth (e.g., proliferating chondrocytes) compared to intrasphenoidal synchondrosis, but these zones expanded considerably at each age studied, and was still exhibiting growth characteristics at the oldest age examined, 24 months. In contrast, intrasphenoidal synchondrosis

decreased steadily at six and twelve months. At 24 months, Michedja observed “narrower bands” (presumably referring to the zones of proliferating and/or hypertrophic chondrocytes), and close approximation of the presphenoid and basisphenoid ectocranially. Based on this, the author suggested cessation of growth was imminent. The intrasphenoidal synchondrosis is thus active as a growth center in *Macaca mulatta* for a more protracted timeframe than in humans. And yet, we know little of this synchondrosis in other primates aside from its persistence at birth in all known primates (Smith et al., in 2021), with the exception of humans (Baume, 1968).

1.3 Goals and hypotheses

Because sampling in prior studies of basicranial synchondroses have been biased toward anthropoids, and especially apes, macaques, and humans (Baume, 1968; Michedja, 1972; Adams and Harkess, 1972; Heinkele et al.,1989), there is no basis for making assertions on functional roles of synchondroses in primates generally. Strepsirrhine and platyrrhine primates are examined here to place prior results on catarrhines in a comparative context. Specifically, we test the hypothesis that the two midline segments of the sphenoid grow at different rates in platyrrhines (Mano et al., 2021). Another consideration of our study is exploratory. Primates may be exceptional in fusion of cranial synchondroses, since in some mammals, synchondroses remain cartilaginous perpetually (e.g., Dai et al., 2017). However, since no studies have examined synchondroseal ontogeny in strepsirrhines, and only rarely have platyrrhines been studied, it is as yet unclear whether eventual fusion of cranial synchondroses is typical of all primates.

MATERIALS AND METHODS

2.1 Sample

Sixty-six subadult cadaveric primates were used in this study (Table 1). Specimens were derived from the Duke Lemur Center (*Galago moholi, Lemur catta, Otolemur crassicaudatus, Varecia spp.*), Lemur Conservation Foundation (*Lemur catta, Otolemur crassicaudatus, Varecia spp.*), Dumond Conservancy (*Aotus nancymaae*), Dallas World Aquarium (*Cebuella pygmaea*), and the New England Primate Research Center (*Saguinus oedipus*). All specimens died of natural causes and were fixed in formalin by immersion, or were frozen and later fixed by immersion in formalin.

2.2 Micro computed tomography

All specimens were scanned using μCT at Northeast Ohio Medical University (NEOMED) using a Scanco vivaCT 75 scanner (scan parameters: 70 kVp; 114 mA). The

volumes were reconstructed using 20.5-30 μm cubic voxels (depending on head size) and exported as 8-bit TIFF stacks for three-dimensional reconstructions (DeLeon and Smith, 2014; Smith et al., 2014). All three-dimensional reconstructions were carried out using Amira ® 2019.1 software (Thermofisher). Juvenile specimens in which at least M1 was fully erupted were selected for histology. The species selected have markedly different rates of dental development. For example, the 57-day-old *Galago* had fully erupted M1 and M2, and partially erupted M3. The oldest *Saguinus* studied, at 117 days postnatal age, had fully erupted deciduous teeth, and a partially erupted M_1; this specimen was older than typical weaning age for this species (50 days, Kappeller and Periera, 2003). In addition to the subadult sample, we also examined selected adult specimens available to us as μCT datasets in order to determine whether all synchondroses eventually fuse. This sample included two adult *Galago moholi,* one adult *Saguinus oedipus,* and one adult *Aotus nancymaae*. These specimens were used to establish whether synchondroses fuse in the species under study.

2.3 Histological methods and microscopic study of synchondroses

Ten newborn specimens were previously sectioned (e.g., Smith et al., 2015; 2021), and four older specimens were newly prepared for the present study. Serial sectioning was conducted at Slippery Rock University (SRU) using routine paraffin embedding after decalcification in a formic acid-sodium citrate solution. Sections are 10 μm thick, and every fourth to tenth section was mounted on glass slides. Slides were alternately stained using Gomori trichrome or hematoxylin-eosin procedures (for more details see DeLeon and Smith, 2014). Selected specimens of *Galago* (two newborns, one infant, one juvenile) and *Saguinus oedipus* (three newborns, one infant, one juvenile) were immunohistochemically studied to establish the mitotic characteristics of chondrocytes within sphenoidal synchondroses. The juvenile *Galago* was among the specimens frozen prior to fixation in formalin. Because of this, it was expected to allow identification of cartilage but in the end had some limited value for characterization of cellular characteristics (see below).

Synchondroses are microscopically recognized as a specialized type of hyaline cartilage. Here we studied synchondroses for characteristics indicating growth, namely, proliferating and hypertrophic chondrocytes (Fig. 1a). We examined three midline synchondroses: the spheno-occipital synchondrosis, the intrasphenoidal synchondrosis, and the prespheno-septal synchondrosis (Fig. 1b). The spheno-ethmoidal synchondrosis is a fibrous joint at birth in anthropoid primates and does not exhibit growth characteristics in newborn strepsirrhines studied to date (Smith et al., 2021).

2.4 Immunohistochemistry

Based on availability of cadavers at similar stages, selected specimens of *Saguinus* and *Galago* (one newborn *Galago,* three newborn *Saguinus*, one one-month-old from each species, and one older subadult from each species) were prepared using immunohistochemistry to detect proliferating cell nuclear antigen (PCNA), a marker of mitotic cells. Briefly, sections were deparaffinized and rehydrated to water. A short antigen retrieval step was accomplished in boiling Sodium Citrate Buffer for 2 minutes followed by cooling. Endogenous peroxidase was

blocked with 3% hydrogen peroxide in methanol, and then 1% Goat Serum was used to block non-specific binding. Sections were then incubated with PCNA primary antibody (AbCam, Cambridge, MA, USA, ab18197) diluted 1 to 3000 in Goat Serum for 2 hours at room temperature. After three washes with phosphate-buffered saline (PBS), sections were incubated with Goat Anti-Rabbit Secondary Antibody conjugated for HRP (AbCam, ab6721) for 1 hour at room temperature and were subsequently washed again with PBS three times. Finally, sections were exposed to 3,3'-diaminobenzidine (DAB) (Vector Laboratories, Burlingame, CA, USA) for 3 minutes, and the reaction was stopped with water. Sections were counterstained with Fast Green 0.1% solution diluted 1:10 in water, and the sections were dehydrated, cleared, and mounted with Permount (Fisher Scientific, Waltham, MA, USA).

Since duration of preservation and condition prior to initial preservation varied or are unknown, and initial treatment (frozen first, or straight to formalin) varied, we assumed that our results would vary in intensity of immunoreactions. Selected sections of each specimen were also prepared as negative controls, in which the primary antibody was omitted. These were carefully assessed in cases of weak reactivity of test slides from the same specimen. In the negative control slides, DAB staining was absent or barely detectable.

2.5 Morphometric Analysis

Morphometric measurements of the entire subadult sample (Table 1) were made using Amira 2020.1 software. In each subadult specimen, the basicranial bones were segmented using the magic wand tool. Next, the 3D measurement tool was used to measure the basisphenoid, presphenoid, palatal, and cranial length. Basisphenoid and presphenoid lengths were collected from the midline ventral margins of the bones. Palatal length was the distance from prosthion to the midline of the palate on its posterior margin. Cranial length was the distance from prosthion to inion.

For each species, we examined ontogenetic scaling of presphenoid, basisphenoid and palate lengths versus cranial length (CL) using standardized major axis (SMA) regression (Warton et al., 2006). We considered the scaling of palate length relative to cranial length to assess relative growth differences in midfacial and overall cranial lengths across the different primate clades represented. We compared regression slopes separately for presphenoid, basisphenoid and palatal relative to CL. All measurements were natural log (*ln*) transformed. We used the *sma()* function of the *smatr* package in R to test the null hypothesis of isometric scaling and equivalent elevations with an alpha level of 0.05. In the first set of analyses, we tested the effect of taxon on scaling relationships. The *sma()* function uses a likelihood ratio statistic to test the null hypothesis that the slopes were similar across taxa. It uses a Wald statistic to test the null hypothesis that the elevation (or y-intercepts) were similar across taxa, but this is only meaningful where there is no significant difference in slopes. In the second set of analyses, we tested scaling relationships of these three metrics relative to cranial length within taxa. Again, the *sma()* function uses a likelihood ratio statistic to test slope and a Wald statistic to test elevation.

2.6 Predictions

Previously, we established that at birth, strepsirrhine and platyrrhine primates all exhibit at least three midline synchondroses with growth characteristics: the spheno-occipital synchondrosis, the intrasphenoidal synchondrosis and the prespheno-septal synchondrosis. We predict all three exhibit prolonged postnatal patency. However, we predict growth characteristics will persist the longest in the spheno-occipital synchondrosis of all species, supporting the hypothesis of uniquely early cessation of growth in the intrasphenoidal synchondrosis of humans, and perhaps all hominoids (Scott, 1958). Finally, we predict contrasting growth patterns in the sphenoid of platyrrhines compared to strepsirrhines; based on prior observations on prenatal and newborn primates (Mano et al., 2021), we specifically predict the presphenoid grows more rapidly in platyrrhines compared to strepsirrhines.

3 RESULTS

3.1 Postnatal osteology based on µCT reconstructions

The midline spheno-occipital and intrasphenoidal synchondroses remain unfused for all primates across the subadult age ranges studied, from birth to juvenile ages (Figs. 2, 2). In strepsirrhines, the spheno-occipital synchondrosis appears to become reduced more than intrasphenoidal synchondrosis with age (Fig. 2), whereas the reverse is true in anthropoids (Fig. 3; Fig. S1).

The prespheno-septal synchondrosis is difficult to assess osteologically, being hidden by the palate and vomer from a ventral perspective. In strepsirrhines, the anterior margin of the presphenoid is slightly rounded or blunt ended, with an extended gap rostrally where it articulates with the midline septal cartilage. In subadult lemurids, there is no trace of a mesethmoid ossification center (the future perpendicular plate of the ethmoid) within the septum. In contrast, both galagid species possess this ossification center at birth, and it becomes a large downwardly oriented crescent by juvenility (Fig. S2). The anthropoids studied here possess a taller presphenoid body compared to strepsirrhines, with a more rounded anterior margin (Fig. S3). In subadult anthropoids, there is a large gap anterior to the presphenoid, along its cartilaginous interface (Figs. S2, S3). The perpendicular plate is large and relatively tall in the adult *Saguinus* (Fig. S2). A view of the piriform aperture also suggests an overall increase in height of adults compared to late infant or juvenile monkeys, with the ossified parts of the septum also becoming taller in adults (Fig. S3). The greater extent of unossified cartilage is also indicated by reconstructions of late infant and juvenile monkeys compared to adults; in the former specimens, the cribriform plate is lacking, presumably still cartilaginous (Fig. S4).

3.2 Morphometric analysis based on µCT measurements

Measurements from micro-CT reconstructions demonstrate the relative growth of the sphenoid bodies and palate compared to cranial length (Table 2). Scaling of basisphenoid length relative to CL showed no significant differences in slope among taxa (likelihood ratio = 3.082, $p = 0.798$), and the common slope of basisphenoid scales with positive allometry relative to CL (likelihood ratio = 32.665, $p < 0.001$). In post hoc tests, most taxa show positive allometry of the basisphenoid relative to CL (Table 2, Figure 4a). One exception iss *Cebuella*, where this sample

shows a slight but nonsignificant negative allometry (slope = 0.898). Visual inspection of the scaling relationship of basisphenoid relative to CL suggests that Cebuella has a longer basisphenoid relative to CL compared to the other taxa. Statistical tests of elevation are not reported, because although the slopes are not significantly different, the fit lines do intersect and the y-intercepts are not consistent with the order observed here. Early prenatal elongation of the basisphenoid associated with expansion of the petrous temporal region may explain the unique features of *Cebuella.*

The common slope of PS scales with positive allometry relative to CL (likelihood ratio = 77.32, $p < 0.001$). However, scaling of the PS relative to CL shows a significant difference in slope among taxa (likelihood ratio = 16.988, $p = 0.009$). Post hoc tests shows that the PS scaling coefficients of *Aotus nancymaae* and *Cebuella pygmaea* are significantly greater than the other taxa (Table 2, Figure 4b). In other words, the presphenoid is growing at a greater rate in *Aotus* and *Cebuella* compared to the other taxa. The relationship of PS and CL is not significantly different from isometry in *Saguinus* (Table 2). Based on visual inspection, the PS is relatively longer in the platyrrhines than in the strepsirrhines (Figure 4b). Statistical tests of elevation are not reported, because the slopes are significantly different

Scaling of PL relative to CL shows a significant difference in slope among taxa (likelihood ratio = 21.506, $p = 0.001$). The common slope of PL scales with positive allometry relative to CL (likelihood ratio = 50.002, $p < 0.001$). Post hoc tests show that for most taxa, PL scales with positive allometry relative to CL (see Table 2). Exceptions included *Saguinus* and *Varecia*, neither of which are significantly different from isometry (Table 2). Based on visual inspection, the PL is relatively shorter in the platyrrhines than in the strepsirrhines (Figure 4c). Again, statistical tests of elevation are not reported, because the slopes are significantly different.

A second set of analyses comparing scaling relationships within each taxon clearly shows the elongation of PS relative to basisphenoid, scaled by CL (Figure 5). Most taxa have no significant differences among slopes of basisphenoid, PS, or PL relative to CL (see Table 2). However, the rate of elongation of the PS is greater than that of basisphenoid or PL in *Aotus*, *Lemur*, and *Varecia*.

3.3 Histological and immunohistochemical Findings

Histology confirms cartilage presence across all ages in all three midline synchondroses studied in both species (Figs. 6, 7). In both species, rows of proliferating and hypertrophic chondrocytes can be seen in all synchondroses and birth and one-month of age (Figs. 6a-f; 7a-f). However, the columnar organization is less apparent or absent in juveniles (Figs. 6g-i; 7g-i). The juveniles of both species also exhibit deeply basophilic matrix in the hypertrophic zone (Figs. 6g-i; 7g-i).

At every age, PCNA-reactive chondrocytes are present at least within the proliferating zone and at least some cells in the resting zone. Results were not uniform across specimens in terms of intensity of reactivity, which may be an artefactual phenomena related to the degree of preservation. However, in all but one specimen, it was possible to detect signals above

background level, as confirmed by comparison to negative controls. We also make comparisons between synchondroses in each specimen.

In a newborn *Galago,* the two bipolar synchondroses (spheno-occipital and intrasphenoidal synchondroses) had a similar organization with hypertrophic zones at both sides appearing slightly wider than the adjacent proliferating zones (Figs. 6a, b). In the presphenoseptal synchondrosis, the hypertrophic zone is notably larger than the more posterior synchondroses (Fig. 6c). In general, PCNA reactivity is weak or moderate, but no background staining is seen, and control slides show no signal at all by comparison (Figs. S5a, b). The proliferating chondrocytes are in columns of five to six cells, and these are the most highly reactive for PCNA (Figs. 6a,b, see insets). Fewer chondrocytes in the hypertrophic zone are PCNA+. In the unipolar prespheno-septal synchondrosis, the hypertrophic zone is much wider than the proliferating zone, and the latter has columns of three to four PCNA+ chondrocytes (Figs. 6c, and inset).

In the one-month-old *Galago,* both the spheno-occipital synchondrosis and intrasphenoidal synchondrosis are proportionally reduced in patency, but still show an organization reflecting bipolar growth (Figs. 6a, b). PCNA reactivity in each of these synchondroses is strong in most chondrocytes, including many in the resting zone (Figs. 6d, e). There is some background staining of mineralized matrix apparent in some sections (e.g., Fig. 6e, see inset). The prespheno-septal synchondrosis is similar in organization to the newborn, and PCNA+ cells are in both hypertrophic and proliferating zones (Figs. 6f, and inset).

In the juvenile *Galago*, the spheno-occipital and intrasphenoidal synchondroses remain cartilaginous and are more diminished in degree of patency compared to the one-month-old, although some distortion related to freezing that preceded fixation may have occurred (Figs. 6g, h). Despite freezing, PCNA reactivity could be detected in the proliferating chondrocyte zone of the spheno-occipital and intrasphenoidal synchondroses (Figs. 6g, h, see inset). The intrasphenoidal synchondrosis has a region of degraded matrix in the center of the resting zone, although it is impossible to say whether this may be artefactual (e.g., freezing damage). The prespheno-septal synchondrosis exhibits a hypertrophic and small proliferating zone, although no PCNA reactivity could be detected (Figs. 6i, see inset). There is some limited background staining of mineralized matrix apparent in some sections (e.g., Fig. 6i, see inset). All three synchondroses exhibit a highly basophilic matrix in the zone of hypertrophic cartilage.

In newborn *Saguinus oedipus*, the spheno-occipital synchondrosis is more widely patent than the intrasphenoidal synchondrosis, with a bipolar organization of hypertrophic and proliferating chondrocytes zones (Figs. 7a, b). PCNA reactivity is most pronounced and widespread in the proliferating chondrocyte zone in each of these synchondroses (Fig. 7a, b, see insets). The prespheno-septal synchondrosis has a wide hypertrophic zone and a narrower proliferating zone, in which rows of proliferating chondrocytes are not as well-organized into

columns compared to the other synchondroses. The cell nuclei in this most anterior synchondrosis are only moderately PCNA reactive (Figs. 7c, and inset).

In the one-month-old *Saguinus oedipus,* the spheno-occipital synchondrosis remains more widely patent than the intrasphenoidal synchondrosis (Figs. 7d, e). The zone of proliferating chondrocytes has better organized columns in spheno-occipital synchondrosis than in the intrasphenoidal synchondrosis. In addition, there are more numerous chondrocytes in columns in the spheno-occipital synchondrosis than in the intrasphenoidal synchondrosis; in both synchondroses, all proliferating and some hypertrophic chondrocytes are highly or moderately reactive (Figs. 7d, e, see insets). As in the newborns, the prespheno-septal synchondrosis has a wide hypertrophic zone and a narrower proliferating zone. Chondrocytes are organized into short columns of two to five each with strongly PCNA reactive nuclei (Figs. 7f, and inset).

In the juvenile *Saguinus oedipus*, all three midline synchondroses remain cartilaginous, with mostly lightly basophilic matrix (Fig. 7g-i). No areas of fibrous matrix were observed. The spheno-occipital and intrasphenoidal synchondroses appear nearly equal in degree of patency (Figs. 7g, h). On the ectocranial side, the spheno-occipital synchondrosis exhibits some bony bridging, connecting the basi- and presphenoid bodies. The zone of growth appears relatively diminished compared to the one-month-old; the zone of hypertrophic chondrocytes is relatively reduced, and the matrix appears mineralized (Figs. 7g, h). However, PCNA-reactive proliferating chondrocytes are still detectable (Figs. 7g, h, see insets). Aside from the dark-stained matrix of the hypertrophic zone at the prespheno-septal synchondrosis, there is no clear organization of proliferating chondrocytes (e.g., columns); the cartilage has the same appearance as septal cartilage more rostrally. Furthermore, PCNA reactivity is widespread and evenly dispersed among chondrocytes throughout the septum (Figs. 7i, see inset). PCNA reactivity is weaker than in other specimens, but well above very limited background staining; no signal at all was apparent in the synchondroses prepared as a control slide (Figs. S5c, d).

Overall, the *Saguinus* specimens were more homogenous in the reactivity levels among specimens compared to the *Galago* specimens. This suggests preservation was more consistent, although other factors will be discussed below.

Osteological views from three-dimensional reconstructions, as well as histological sections of older subadult and adult specimens reveal additional detail on the fusion of sphenoidal synchondroses. In *Galago*, subadults and adults of known age establish that the spheno-occipital synchondrosis begins to fuse at approximately three months of age (Fig. 8a), whereas the intrasphenoidal synchondrosis remains patent for an extended time period; it is completely patent in a four-year-old adult (Fig. 8b). An adult of unknown age demonstrates partial fusion of the intrasphenoidal synchondrosis (Fig. 8c(. The sequence of fusion is unclear in our anthropoid samples, but our oldest subadult *Saguinus* appears to exhibit incipient bony bridging on the ectocranial aspect of the joint (Figs. 8d, e). An adult *Aotus* demonstrates both synchondroses do eventually fuse in this species (Fig. 8f).

4 DISCUSSION

Even though the limited histological literature on primates clearly shows patency alone does not indicate active growth within a synchondrosis (Thilander and Ingervall, 1973; Heinkele et al., 1989), fusion is sometimes discussed as synonymous with growth cessation. For instance, Alhazmi et al., 2017 asserts that whereas the spheno-occipital synchondrosis is partially fused in humans, "cranial base and facial growth are still active" (p 13). More explicitly, Marulanda et al. (2017) states that in humans, "synchondroses act as growth centers, providing sites for rapid bone growth until fusion" (p 11408). At one level, these statements are imprecise. But they also reflect our lack of a broad understanding of synchondroseal growth based on a comparative perspective. Until recently, no studies have assessed synchondroseal histology in strepsirrhine or platyrrhine primates. Our knowledge of these growth centers was limited to osteological determination of patency/fusion, mainly focusing on catarrhine primates (Krogman, 1930; Giles et al., 1981; Joganic, 2016). Here, we have broadened our perspective on the nature of growth in midline sphenoidal synchondroses and establish that most are patent and actively growing during infancy but are likely slowing and perhaps ceasing growth during juvenility.

4.1 The presphenoid grows faster in the platyrrhines than in the strepsirrhines

Metric data indicate longitudinal growth at the intrasphenoidal and spheno-occipital synchondroses in all species, occurring throughout all subadult ages studied, extending our observations of prenatal stages (Mano et al., 2021). Qualitative results on patency of the synchondroses across age suggest the intrasphenoidal synchondrosis of anthropoids may have a less significant postnatal role in basicranial elongation compared to the same synchondrosis in strepsirrhines. However, histology of *Galago* and *Saguinus* suggests both the intrasphenoidal and spheno-occipital synchondroses are active growth centers for a similar duration (see below).

Morphometric results revealed that, relative to cranial length, palatal length is isometric in anthropoids but positively allometric in strepsirrhines. Although presphenoid length is also notably positively allometric in *Aotus,* this is not a consistent trend among anthropoids. However, across postnatal age, all three anthropoids exhibit the same broad separation of y intercepts of presphenoid and basisphenoid length, in contrast to all strepsirrhines (Fig. 5). This strongly indicates a faster *prenatal* rate of growth of presphenoid compared to basisphenoid occurs in anthropoids. Previously, we established that at an early fetal stage, *Saguinus* has far more proliferating and hypertrophic chondrocytes at the anterior end (prespheno-septal synchondrosis) than at the posterior end (intrasphenoidal synchondrosis) of the presphenoid (Mano et al., 2021). This, along with the tall and convex shape of the anterior end of presphenoid in newborn and older platyrrhines, indicates that most presphenoid growth occurs anteriorly, at the interface with the midfacial skeleton.

Aside from the confirmation of accelerated presphenoid growth in platyrrhines, our morphometric results provide no strong picture of suborder differences in growth of the prechordal and postchordal sphenoid elements. Yet, with the small samples sizes, a clear pattern may be elusive at this time. Fortunately, growing availability of high-resolution scans can resolve this in the future.

The platyrrhines studied have prolonged postnatal patency of the intrasphenoidal synchondrosis, and it does not fuse at birth in *Macaca mulatta* (Michejda, 1972). Thus,

anthropoid traits such as more angular CBA and relatively reduced midfacial projection, cannot be explained by the timing of the intrasphenoidal synchondrosis fusion alone. As far as we know at this time, humans might be distinct in early cessation of growth at the intrasphenoidal synchondrosis. However, the intrasphenoidal synchondrosis may be fused early in the chimpanzee (Smith et al., 2017) and other apes, but we currently lack high resolution CT data to assess this in detail.

A further implication of the suborder differences in presphenoid and basisphenoid lengths is that presphenoid growth may have a direct role influencing facial growth and orientation. We suggest the prespheno-septal synchondrosis has the most important role in this influence, based on the invasive position of this synchondrosis, protruding into the nasal cavity (which has long been known in platyrrhines - Ashley Montagu, 1943). It may prove to be the case that its most important direct influence on midfacial growth is realized late prenatally and during early infancy, when this synchondrosis is most actively engaged in chondrocyte proliferation and hypertrophy (Mano et al., 2021; see below).

4.2. All midline synchondroses exhibit interstitial growth postnatally, with a faster pace during early infancy

Here, we reveal the first histological evidence on midline synchondroses in cross-sectional age samples of one strepsirrhine and one platyrrhine. Microanatomical characteristics, including preliminary evidence derived from PCNA labeling, reveal some similarities to data on humans. Compared to juveniles, in both species PCNA reactivity is more prevalent at birth and during infancy, when proliferating chondrocytes appear more numerous in the spheno-occipital synchondrosis. However, the same is true of the intrasphenoidal synchondrosis in both *Galago* and *Saguinus*. The prespheno-septal synchondrosis, in both species, also shows better evidence for growth activity at the two earlier ages.

It is important to acknowledge the preservation of tissues for immunohistochemistry was far from ideal, and this is especially so for the bushbabies, which except for one frozen specimen were all stored in formalin for decades prior to histology. Both the freezing and prolonged fluid storage can explain relatively weak signaling (Figs. 6a-c; g-i). An additional unknown variable is the likely variable amount of time it took to discover the remains. The inevitable and related variation in preservation can explain between-specimen differences in reactivity levels, and perhaps some aberrant findings. Despite these shortcomings, the PCNA immunohistochemical results provide insight into whether mitosis is occurring postnatally in synchondroses of *Galago* and *Saguinus*. We conclude prolonged growth via chondrocyte proliferation is occurring in all synchondroses of both species. PCNA reactivity also reinforces microanatomical observations in the form of chondrocytes columns. By itself, the finding that the intrasphenoidal synchondrosis is mitotic during infancy provides a strong contrast to humans, which fuse this joint at birth. Intriguingly, Giles et al. (1981) noted less chondrocyte proliferation in the intrasphenoidal synchondrosis compared to the spheno-occipital synchondrosis in newborn macaques. This, this with evidence of perinatal fusion of this growth center in humans and perhaps great apes (Baume, 1986; Smith et al., 2017), suggests that the intrasphenoidal synchondrosis might be of reduced importance in all catarrhines compared to other primates.

Our ability to infer later development, in late infancy and juvenility, is more limited based on sample size. Although PCNA results might be weighed more cautiously, microanatomical features allow two general observations. First, growth in the three synchondroses continues during later infancy. Second, the proliferation in the prespheno-septal synchondrosis is slowing earlier than in the two more posterior synchondroses. However, the PCNA reactivity in the septum itself (Fig. 7i) illustrates that more rostrally situated septal cartilage can continue to influence midfacial growth (and see Wealthall and Herring, 2006). In the available juveniles, the cartilage of the spheno-occipital and intrasphenoidal synchondroses in *Galago* and *Saguinus* lack clear evidence of cartilage matrix breakdown. Given the age of the animals studied here, this is consistent with the prior findings on *Cercopithecus.* Although the sample examined by Heinkele et al. (1989) was entirely of unknown chronological age, all dentally immature monkeys were described to have a continuously cartilaginous SOS with a clear "cellular zonation," including columns of proliferating chondrocytes. One of the subadult *Cercopithecus* had more pronounced mineralization of cartilage matrix. In adult *Cercopithecus*, the amount of mineralized matrix increased, and bridging began to occur, until only islands of remnant cartilage remained. Further work would be needed to determine if the synchondroses fuse in a similar manner in strepsirrhines and platyrrhines, but in the species examined here, eventual fusion is certain (Fig. 8).

The fusion of the spheno-occipital synchondrosis in humans is similar to that described for *Cercopithecus* above. Thilander and Ingervall provided histological evidence that bridging of bone across SOS begins at 12-13 years of age in girls and two years later in boys (revising closure to two years earlier compared to the prior radiological assessment by these authors cited above). Prior to this fusion, the upper (endocranial) part of the spheno-occipital synchondrosis becomes increasingly fibrous in humans by about 6-7 years of age, and closer to the age of fusion, chondrocytes in the hypertrophic zone lose their columnar organization (Thilander and Ingervall, 1973). After fusion, the spheno-occipital synchondrosis the spheno-occipital synchondrosis may contain islands of cartilage even at 20 years of age, similar to descriptions of young adult *Cercopithecus* (Heinkele et al., 1989). Our sample includes no young adults so we cannot assess whether fusion proceeds similarly to descriptions of SOS in catarrhines. However, a broad similarity among *Galago, Saguinus*, *Macaca mulatta* (Michedja, 1972) and humans (Thilander and Ingervall, 1973) is indicative of relatively fast growth during early infancy (evidenced by organization of hypertrophic and proliferating chondrocyte zone), and indications of slowed growth near the time of M1 eruption in all primates. The emphasis on growth during early postnatal ages may be a similarity of primates to mammals generally. In mice, proliferating chondrocytes become fewer while hypertrophic chondrocytes more numerous (Dai et al., 2017). In an adult mouse, there are no proliferating or resting chondrocytes. Expression of mitotic markers gradually decrease; very little is observed in the adult (Dai et al., 2017).

Histological changes in the intrasphenoidal and prespheno-septal synchondroses of hominoids is only known well in humans. Baume (1968) describes both as relatively unimportant for active growth perinatally. He asserts the cessation of growth is "imminent" at birth in the intrasphenoidal synchondrosis (calling it the presphenoid synchondrosis), based on "chondrofibrosis" of the joint and some bony bridging at the periphery. He further states that at the junction of the septal cartilage and the presphenoid (i.e., the prespheno-septal synchondrosis), the cartilage is in a state of degeneration. An understanding of these joints in perinatal apes as sorely needed.

4.3 Fate of synchondroses

Due to the rarity of histological studies and the few species examined previously, the duration of active growth in synchondroses is unknown in all but a few primate species. It is not even clear that synchondroses fuse completely in all primates, although our observations suggest fusion may be common in primates. The spheno-occipital synchondrosis has been most studied in mammals. Dai et al. (2017) found as mice age, the spheno-occipital synchondrosis becomes narrower, but remains in adults. Indeed, many mammals possess sphenoid bones that are separable in adults (Esteve-Altava, 2022).

Although we may lack sufficient samples to establish the duration of active synchondroseal growth to the number of days or weeks, the available juvenile specimens used for histology suggest certain late-stage characteristics reminiscent of epiphyseal plates at similar stages, such as highly mineralized cartilage matrix adjacent to the zone of ossification, and poorly organized columns or lack of columns in zones of proliferation (Roach et al., 2003). The results thus suggest active growth is ceasing at about two months in *Galago* and at about four months in *Saguinus*. The spheno-occipital synchondrosis remains unfused in humans for many years after cessation of growth, and this seems virtually certain to be the case in *Galago* as well. An interesting finding is the longer patency of the intrasphenoidal synchondrosis than the spheno-occipital synchondrosis in *Galago*. This is the reverse of what is known in the anthropoids studied to date (Michedja, 1972; Thilander and Ingerval, 1973). The eventual fusion of both the spheno-occipital and intrasphenoidal synchondroses is demonstrated in *Galago* and *Aotus*, consistent with the pattern seen in apes (Joganic, 1981), and consistent with the observation that primates may exhibit greater consolidation of cranial bones through fusion compared to many other mammals (Esteve-Altava, 2022).

4.5 Implications for future studies

With this increased knowledge of the timing of synchondroseal growth activity, it is time to reassess other lines of evidence. For example, our knowledge of the timing of brain growth in primates is rather limited. We know within Anthropoidea there are great disparities in the timing of brain growth (Leigh, 2004). Assessment of these disparities in tandem with observations on timing of synchondroseal growth and cessation of growth should clarify whether synchondroses are indeed pacemakers of basicranial growth (Hall, 2015) or may simply keep pace with brain expansion (Thilander and Ingervall, 1973).

A second line of inquiry going forward is which synchondrosis and what aspects of its ontogeny could best explain basicranial angulation. There has been disagreement concerning the primary site of basicranial flexion, for example, with some authors asserting macaques and/or humans primarily flex due to growth characteristics of the spheno-occipital synchondrosis (Melsen, 1974; Sirianni and Van Ness, 1972), whereas as other authors attribute this to the intrasphenoidal synchondrosis (Scott, 1958; Michedja, 1972). Furthermore, future studies must determine whether the timing of cessation of synchondroseal growth to the degree of basicranial angulation (Lieberman et al., 2000) or if intrinsic growth characteristics are more explanatory. Of note among intrinsic variables is the possibility of differential growth of chondrocytes through

the thickness of the joint (Hoyte, 1973; Sirianni and Van Ness, 1978; Giles et al., 1981) or anteroposterior sides of the joint (Michedja, 1972).

Lastly, further inquiry into the timing of synchondroseal contributions to midfacial growth is needed. It emerges here that the prespheno-septal synchondrosis is active in growth at least through infancy in at least some strepsirrhines and platyrrhines. Humans (and perhaps other hominoids) differ, as this synchondrosis is in a state of degeneration perinatally in humans (Baume, 1968), which likely contributes to midfacial reduction. Presumably, this synchondrosis degenerates prenatally in all hominoids, but this requires histological study.

Conclusions

Here, we have broadened our perspective on the nature of growth in midline sphenoidal synchondroses and establish that most are patent and actively growing during infancy, but likely cease growth during juvenility in strepsirrhine and platyrrhine primates. Three midline sphenoidal synchondroses (spheno-occipital synchondrosis, intrasphenoidal synchondrosis, prespheno-septal synchondrosis) are most active in growth during infancy, in common with prior findings on catarrhines (e.g., Michejda, 1972; Thilander and Ingerval, 1973). However, a clear contrast is apparent in ontogeny of the intrasphenoidal synchondrosis, in that all monkeys and all strepsirrhines exhibit a more prolonged patency at this site compared to humans (and possibly apes), and our histological results demonstrate proliferating chondrocytes are present through infancy in at least some species. Moreover, our findings challenge the idea that the spheno-occipital synchondrosis is the main contributor to basicranial elongation in all primates.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Data Availability Statement

Any scans used in the present study are available on request from the corresponding author (TDS), or from Valerie DeLeon or Chris Vinyard. Interested researchers are encouraged to first check a MorphoSource project page at:

https://www.morphosource.org/dashboard/collections/000368935/edit?locale=en&

On this project page, μCT scan volumes of whole bodies or heads of specimens used in the present study are freely available for download as TIFF stacks.

In addition, another MorphSource project page includes freely downloadable three-dimensional surfaces of sphenoid bones from selected specimens studied here. These are accessible at:

https://www.morphosource.org/dashboard/collections/000367594/edit?locale=en&

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Figure legends:

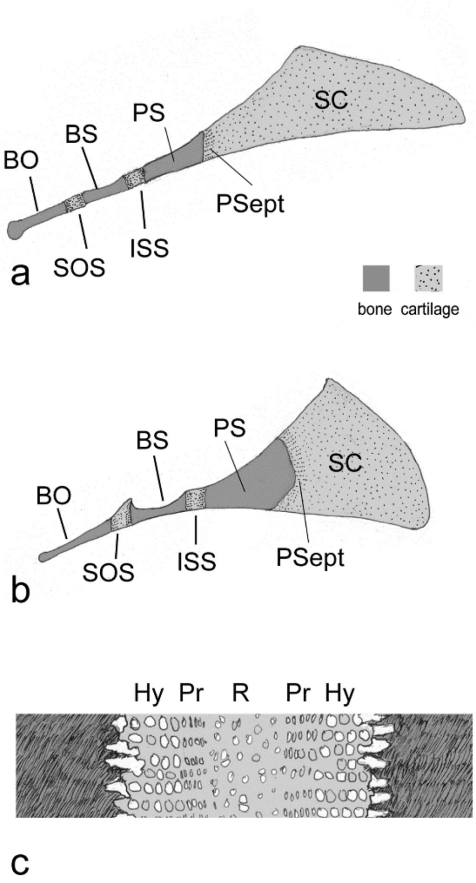

Figure 1: Bone *as a tissue* lacks the capacity for interstitial growth, or internal growth as opposed to additive growth on the perimeter (Hall, 2015). Hence, cranial synchondroses and epiphyseal plates are crucial islands within bones *as individual organs*, allowing them to retain the capacity for localized interstitial growth. In primates, as in many other mammals, at least three synchondroses exist in the midline. Shown in a, from posterior to anterior, are the spheno-occipital synchondrosis (SOS), intrasphenoidal synchondrosis (ISS), and the prespheno-septal synchondrosis (PSept). These are growth centers intervening between the basioccipital (BO), basisphenoid (BS), presphenoid (PS), and septal cartilage (SC). In some primates these all are oriented parallel to the long axis of the cranial base, but in some primates, PSept has a radial orientation (b). c) The "mirror-image" organization of a typical synchondrosis, which intervenes between two bone (dark grey). There is a central resting zone of cartilage (R) that serves as a pool for new chondrocytes. On both anterior and posterior sides of the resting zone, chondrocytes flatten and arranges themselves into columns as they divide, the zone of proliferation (Pr). Proliferating chondrocytes closest to bone enlarge and form the zone of hypertrophy (Hy), cartilage closest to bone in continually replaced by bone.

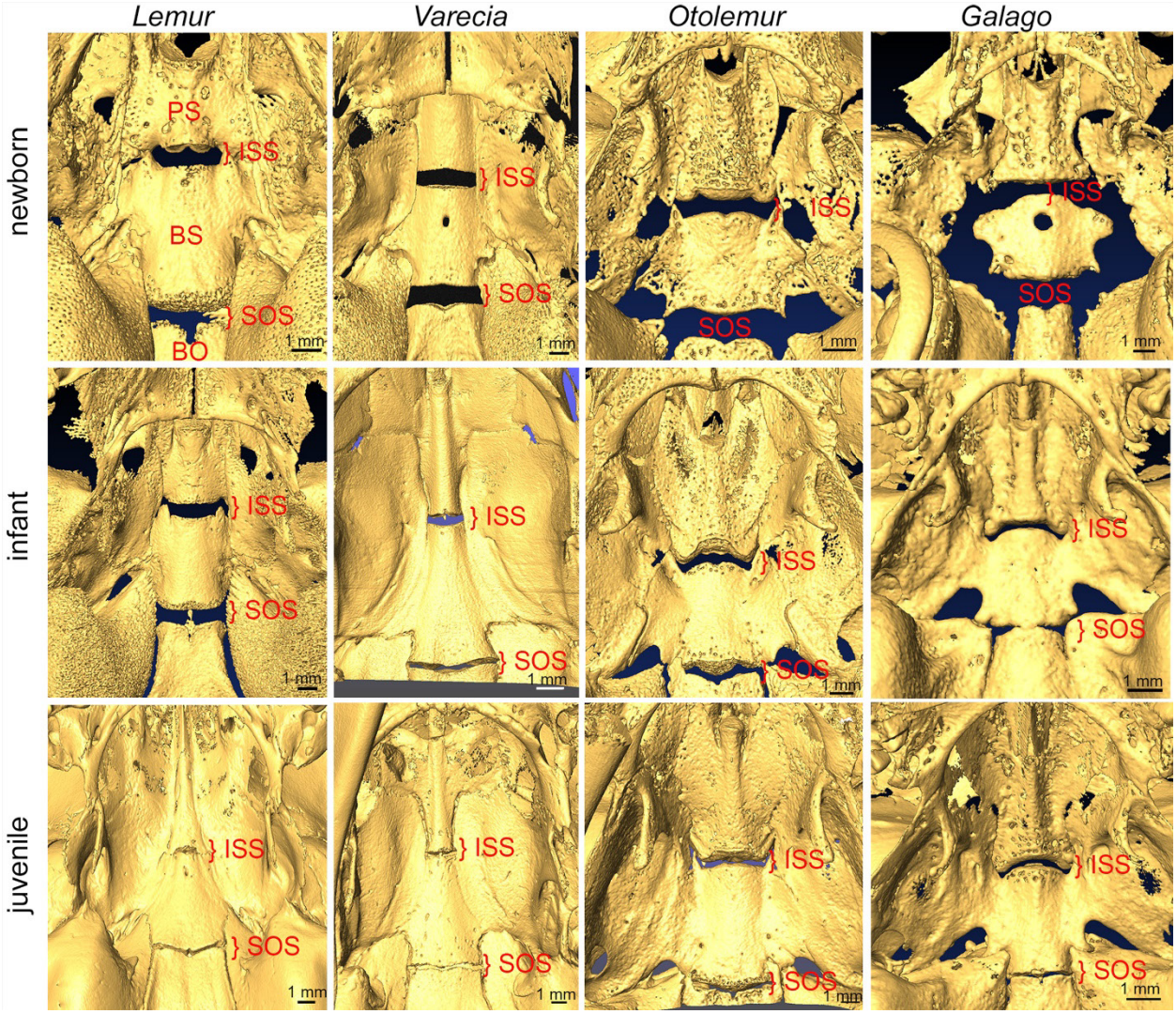

Figure 2: Age series of sphenoidal synchondroses among four species of strepsirrhines from newborn to juvenile. Micro-CT reconstructions in magnified view in the ventral perspective. Note that the spheno-occipital synchondrosis (SOS) appears to close more than the intrasphenoidal synchondrosis (ISS) in most of these species at older ages.

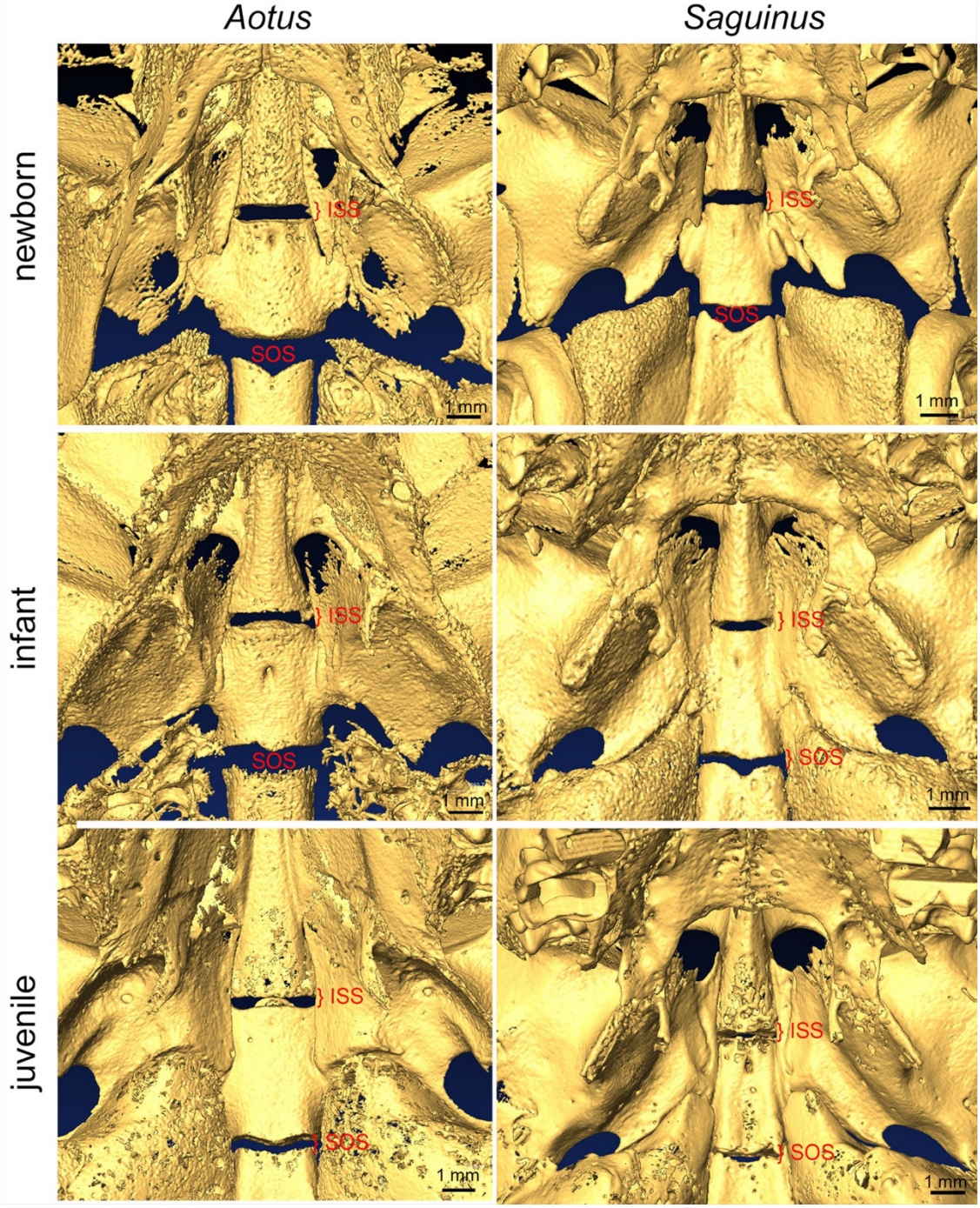

Figure 3: Age series of sphenoid synchondrosis among two species of anthropoids from newborn to juvenile. Micro CT reconstructions in magnified view in the ventral perspective. The intrasphenoidal synchondrosis (ISS) and spheno-occipital synchondrosis (SOS) remain relatively patent through juvenility. Although the oldest tamarin exceeded weaning age, if aged dentally it could be categorized as an advanced infant stage, since M1 is not fully erupted.

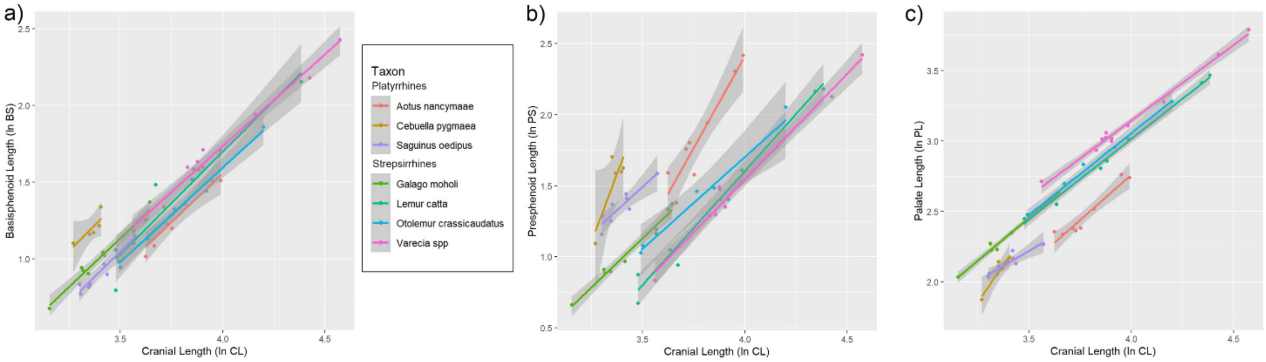

Figure 4. Scaling relationships for a) basisphenoid (BS), b) presphenoid (PS), and c) palate length (PL) relative to cranial length (CL). All values are ln transformed. Separate SMA regressions were performed for each taxon (see color-coded legend), and 95% confidence intervals for slope estimates are indicated by shadowed regions.

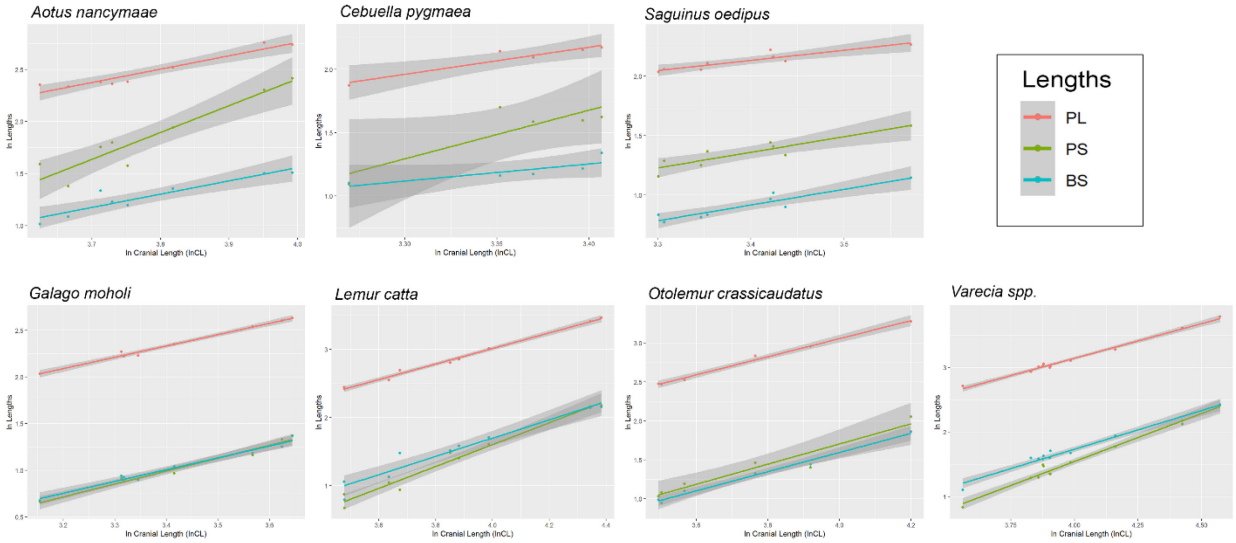

Figure 5. Scaling relationships of basisphenoid (BS), presphenoid (PS), and palate length (PL) relative to cranial length (CL) within each taxon. Note the extreme difference in elevation of the PS regression line in platyrrhines (top row) relative to strepsirrhines (bottom row) and the similarity in slopes for most taxa. These results indicate that platyrrhines have pronounced prenatal growth of the presphenoid relative to the basisphenoid.

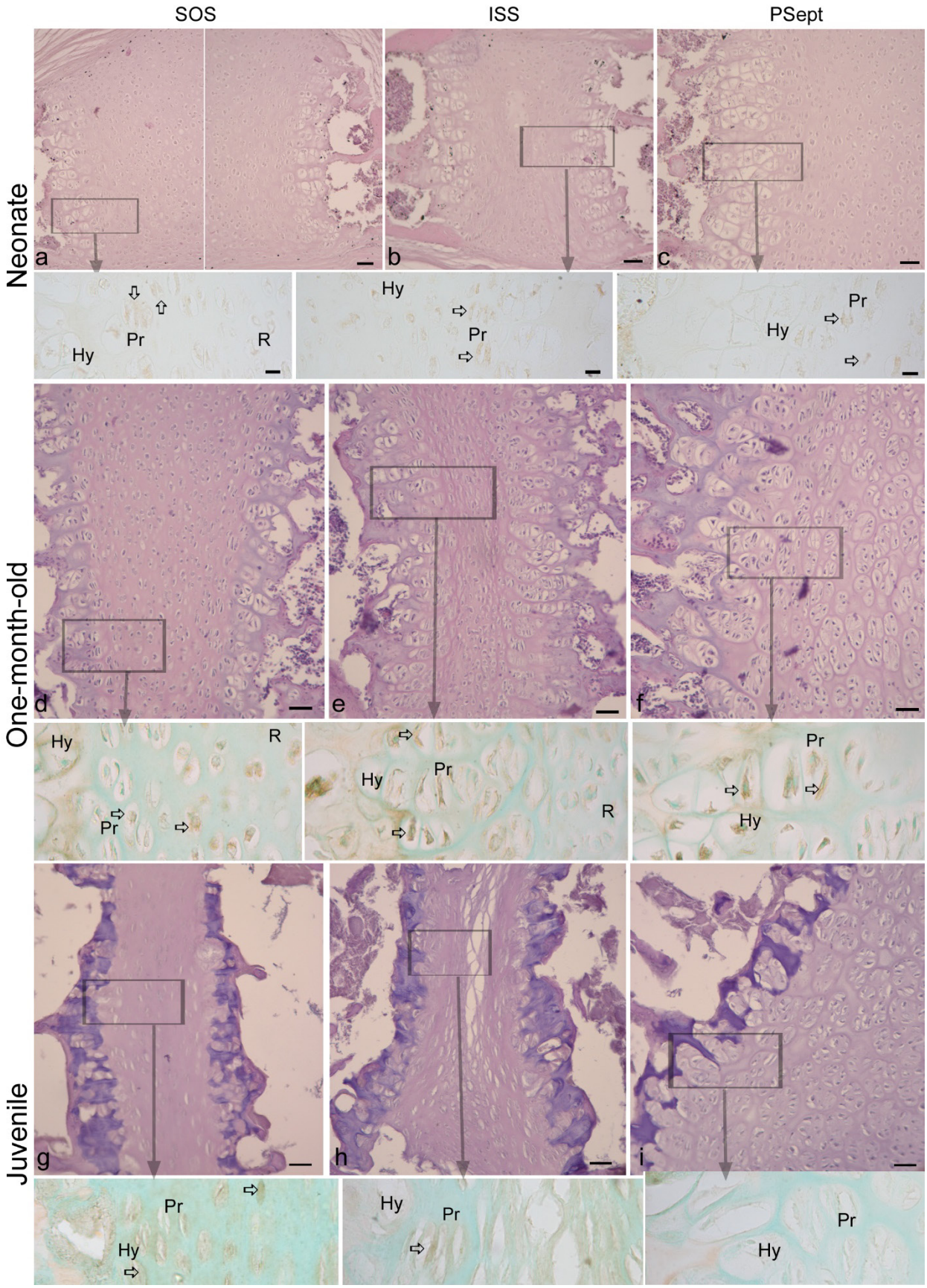
SOS
ISS
PSept
Neonate
One-month-old
Juvenile
a
b
c
d
e
f
g
h
i
Hy
Pr
R

Figure 6: *Galago moholi* showing the three midline sphenoidal synchondroses in H & E staining (a-i) and PCNA immunohistochemistry, with fast green counter stain (insets below each image). At birth (a-c) and one-month (d-f), low magnification reveals multiple hypertrophic cells forming columns in all synchondroses and immunohistochemical results reveals columns of multiple PCNA+ chondrocytes in the proliferating zone and sparse PCNA+ hypertrophic chondrocytes (see insets below each image). Immunohistochemical preparations are from sections near to those at the top row, and the approximate position is indicated by semitransparent boxes in a-c. Resting (R), proliferating (P), and hypertrophic (H) chondrocyte zones are indicated. PCNA + chondrocytes are indicated by open arrows. At the newborn and one-month ages, immunohistochemical results reveals ubiquitous PCNA reactivity to chondrocytes, although signaling is weak in the newborn. In the juvenile, low magnification reveals a more restricted hypertrophic zone in each synchondrosis, with deeply basophilic matrix (g-i) and immunohistochemical results reveals fewer PCNA+ chondrocytes organized into columns, especially in the two more anterior synchondroses (h, I, see insets). ISS, intrasphenoidal synchondrosis (ISS), PSept, prespheno-septal synchondrosis, SOS, spheno-occipital synchondrosis (SOS). Scale bars: a-i, 40 μm (insets,10 μm).

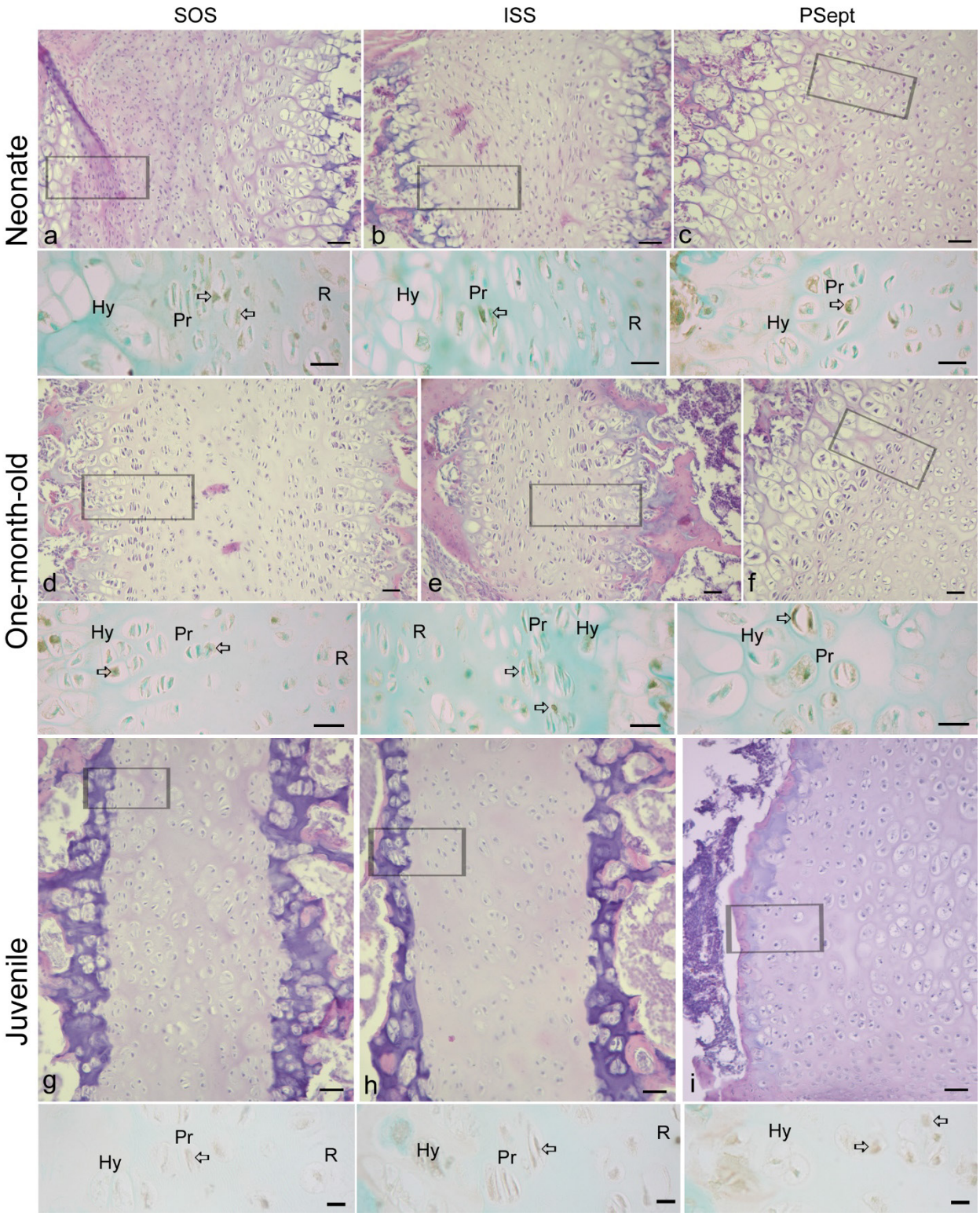

Figure 7: Newborn *Saguinus oedipus* showing the three midline sphenoidal synchondroses in H & E staining (top row) and PCNA immunohistochemistry (bottom row, with fast green counterstain). Immunohistochemical preparations are from sections near to those at the top row, and the approximate position is indicated by semitransparent boxes in a-c. Resting (R),

proliferating (P), and hypertrophic (H) chondrocyte zones are indicated. PCNA + chondrocytes are indicated by open arrows. In the newborn and one-month specimens, low magnification reveals multiple hypertrophic cells forming columns in all synchondroses (a-f) and immunohistochemical results reveals columns of PCNA+ chondrocytes in the proliferating zone (a-f, see insets). Relatively few, sparse hypertrophic chondrocytes are PCNA+. In the juvenile, low magnification reveals a more restricted hypertrophic zone in each synchondrosis, with deeply basophilic matrix (a-c) and immunohistochemical results reveals PCNA+ chondrocytes are still detectable at this age (d-f). Reactive tissue to the left in f is marrow. ISS, intrasphenoidal synchondrosis (ISS), PSept, prespheno-septal synchondrosis, SOS, spheno-occipital synchondrosis (SOS). Scale bars, a-c, 50 μm (insets, 20 μm); d-f, 40 μm (insets, 20 μm); g-i, 40 μm (insets, 10 μm).

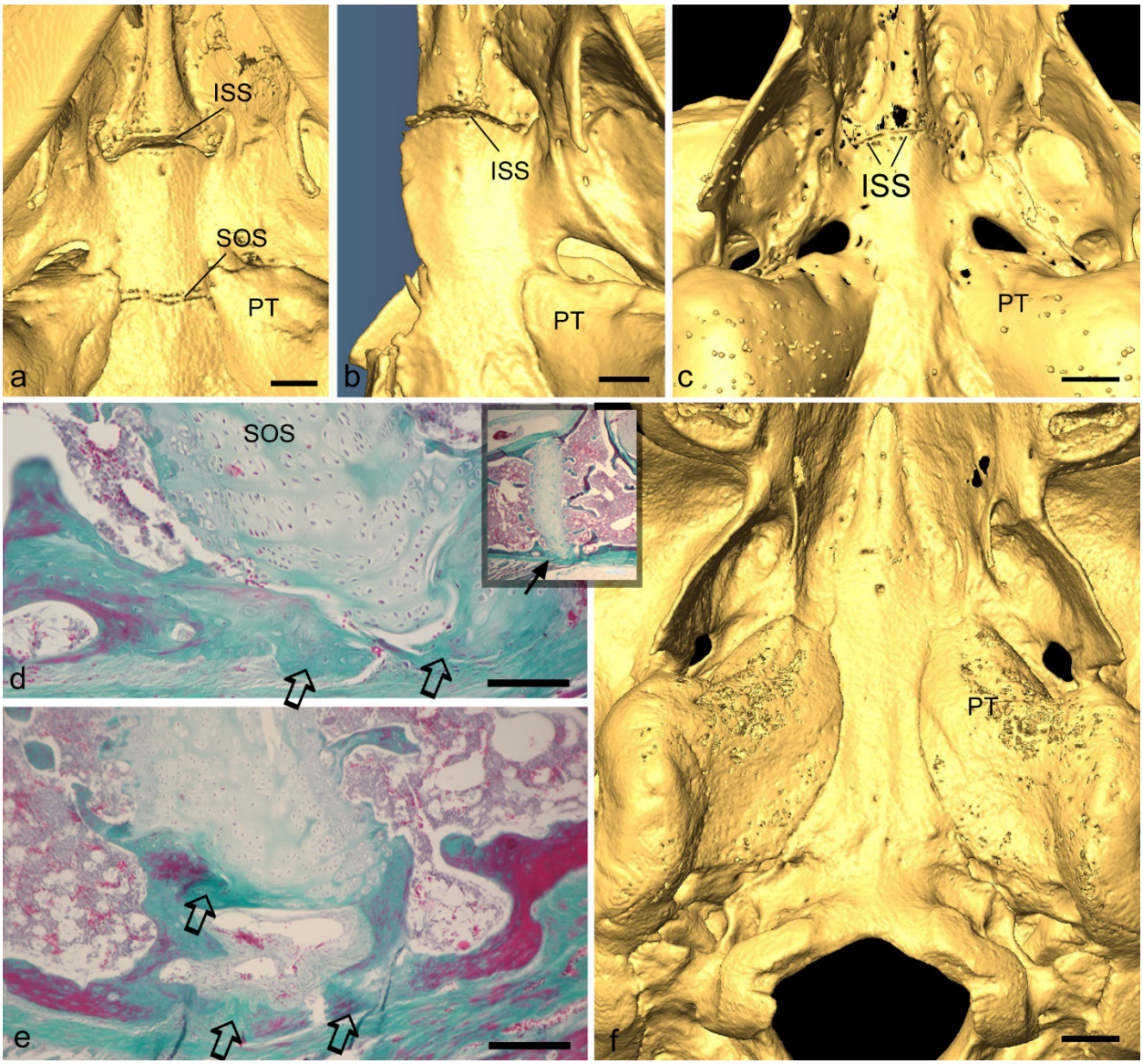

Figure 8: Closure of midline, sphenoidal synchondroses. a-c, basicranial view of juvenile and adult *Galago moholi.* In the juvenile with mixed dentition (a), incipient closure of the spheno-occipital synchondrosis (SOS) is apparent, whereas the intrasphenoidal synchondrosis (ISS) remains patent. In a four-year-old adult, ISS remains patent, though narrower, and SOS is obliterated. In an older adult of unknown age (c), ISS is only faintly visible. d, e: 117-day-old *Saguinus oedipus,* showing histology of SOS. Two sagittal cross-sectional levels are shown, one centrally through the SOS (c; inset with arrow shows site of magnification), and another near the lateral limit of SOS (e). These are two locations of several cross-sections where bony bridging appears imminent (open arrows; bone stained dark green/red). f, Basal view of sphenoid and occipital bone in and adult *Aotus nancymaae,* of unknown age. Note there is no trace of either synchondrosis, e.g., see the space between the apices of the petrous temporal (PT), which brackets the prior SOS location. Scale bars, a-c, 1 mm; d, e, 200 µm; inset 300 µm; f, 2.5 mm.