Brief Communication: Histology and Micro CT as Methods for Assessment of Facial Suture Patency

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ABSTRACT The extent of fusion in facial sutures has implications for topics ranging from biomechanics to phylogeny reconstruction. An unfortunate limitation of studying sutural fusion in skeletal specimens is that it is difficult to assess whether apparently patent sutures are in fact fused internally. Both histology and microcomputed tomography (CT) are potential tools for solving this, but relatively few studies have attempted to discern the limits of micro CT for visualization of microanatomical structures. We examined microanatomical aspects of facial sutures in adult cadaveric samples from captive bushbabies. Premaxillary and nasopremaxillary sutures were examined in serially sectioned snouts of four greater bushbabies (*Otolemur garnettii*) and four lesser bushbabies (*Galago moholi*). Sections containing sutures with osseous bridging were rated as "fused," and the presence or absence of grooves on the external side was recorded. One bushbaby was studied using micro CT prior to physical sectioning. *O. garnettii* and two of the *G. moholi* show multiple foci of fusion. Histological examination confirmed that sutural fusion is limited to the internal surface in numerous sections, resulting in an external notch. Such points of internal fusion could be clearly visualized in raw CT slices. The presence of such notches suggests that external examination can underestimate the degree of suture fusion. Thus, microanatomical evidence may be needed to fully assess biomechanical correlates and phylogenetic interpretations based on fusion of facial sutures. Our results also indicate micro CT may be a useful tool to obtain this evidence. Am J Phys Anthropol 138:499–506, 2009. ©2009 Wiley-Liss, Inc.

The extent of fusion of facial sutures has been used to interpret phylogenetic patterns (Wang et al., 2006a,b) and has important implications for the study of facial biomechanics (Rafferty et al., 2003; Herring, 2008). An unfortunate limitation of studying suture fusion in skeletal specimens is that it is difficult to assess whether apparently patent sutures are in fact fused internally (Wang et al., 2006b). Although this can be addressed by examining both endocranial and ectocranial surfaces of sutures (Key et al., 1994), it is feasible with facial sutures only when a cranium is bisected or damaged. This may explain the virtual lack of microanatomical analyses of facial sutures in primates, with the exception of humans (Pritchard et al., 1956).

Micro CT has emerged as a useful alternative to the destructive study of skeletal samples (Recinos et al., 2004; Stadler et al., 2006) and may prove a good tool for assessing suture fusion. Histological study of suture fusion yields superior results in terms of soft tissue identification and assessment of bone maturity (e.g., Bradley et al., 1996). However, this method requires destruction of skeletal specimens and is labor intensive. This study assesses the degree of fusion of two facial sutures in greater and lesser bushbabies. The extent of fusion of the premaxillary (PS) and nasopremaxillary (NPS) sutures is documented to assess the occurrence of externally undetectable fusion sites and to compare the effectiveness of histology versus micro CT for assessment of facial suture fusion.

MATERIALS AND METHODS

PS and NPS sutures were examined in four greater bushbabies (*Otolemur garnettii*) and four lesser bushbabies (*Galago moholi*). Fully sectioned midfaces of the *Otolemur* were available from a previous study (Smith et al., 2005). To study a smaller species that could be analyzed by both histology and micro CT, four specimens of *Galago* were prepared as described later. These animals were obtained as cadavers after being euthanized following unrelated studies (Schmechel et al., 1996) or after natural deaths in captivity at the Duke Lemur Center. Males and females were included for both species. Specimens of *Galago* ranged from 3.5 to 6.5 years of age and specimens of *Otolemur* ranged in age from 4 to 20 years of age. One additional *O. garnettii*, a neonate, was examined to view the relationship of the premaxilla to other bones with fully patent sutures. Adult snouts were decalcified in a formic acid–sodium citrate solution.

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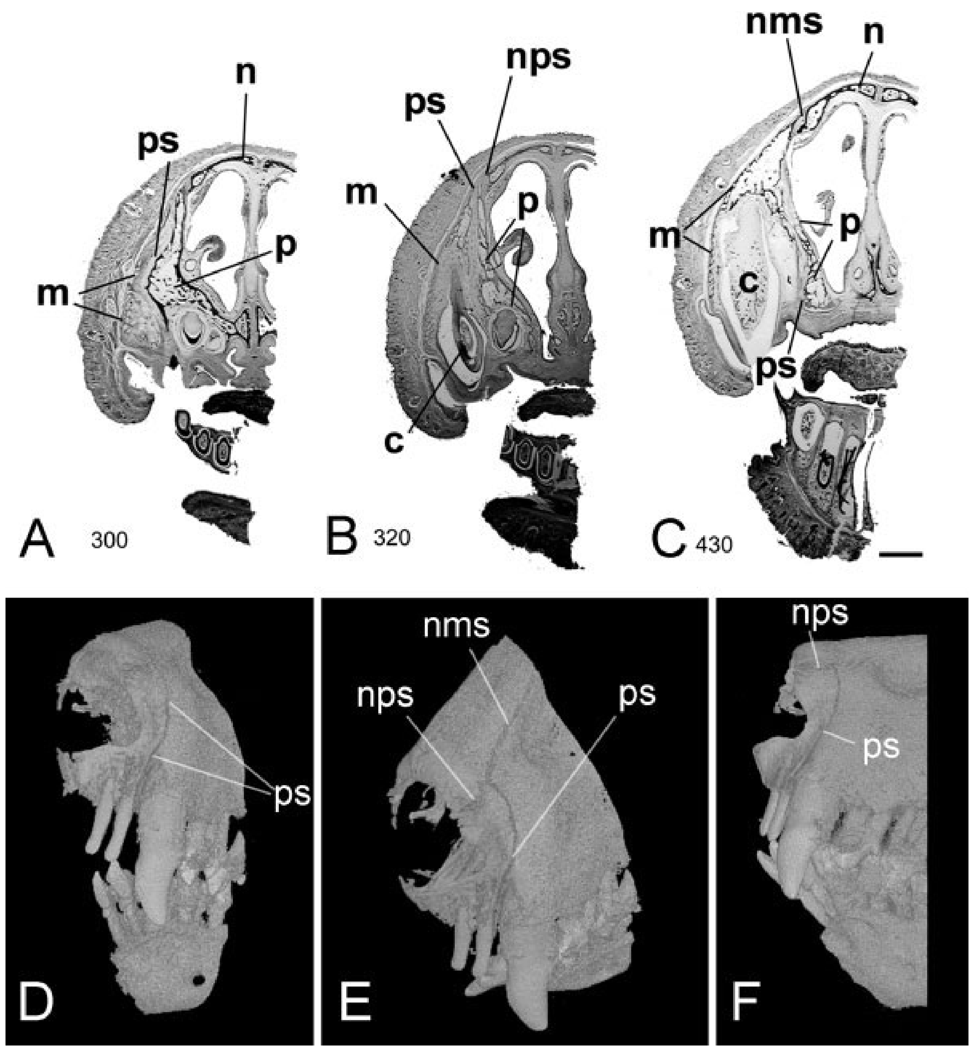

Fig. 1. (**A–C**) Three coronal sectional levels showing the relationships of midfacial bones in a neonatal greater bushbaby (*O. garnettii*). The premaxillary suture (ps) ends after the nasopremaxillary suture (nps) in its anteroposterior extent. However, the ps is hidden from external view posteriorly (C). (**D–F**) Gross external view of the sutures in an adult *G. moholi*, viewed via a three-dimensional reconstructions, in rotating views. c, canine; m, maxilla; n, nasal bone; nms, nasomaxillary suture; p, premaxilla. Scale bar = 1 mm.

Subsequently, the tissues were dehydrated, paraffin embedded, and serially sectioned in the coronal plane at 10–12 μm. Slides were stained alternately with hematoxylin–eosin and Gomori trichrome procedures. Prior to decalfication and embedding, one 4-year-old *G. moholi* was scanned using a Skyscan 1172 high-resolution micro CT scanner (40 kV, slice thickness at 8.85 μm) housed in the laboratory of J.W. Hagadorn, Department of Geology, Amherst College, Amherst, MA. Image processing and three-dimensional (3D) volume reconstruction were performed with VGStudio Max 1.2 (Volume Graphics GmbH, Heidelberg, Germany).

Figure 1A–C illustrates the location of the sutures in a neonatal bushbaby (*O. garnettii*). Figure 1D–F shows 3D reconstructions of the snout of a 4-year-old *G. moholi*, in which the PS and NPS appear to be patent externally.

Sutures were rated according to suture fusion or patency by microscopic examination. Sutures were viewed using a Leica DMLB photomicroscope (Leica Microsystems: Wetzlar, Germany). The existence or absence of a continuous bony bridge between the premaxilla and maxillary bones or between the PS and nasal bones was confirmed at high magnifications (200–630×) on every 10th section. Any osseous bridging constituted a rating of "fused" for a section. The presence or absence of a notch on the external side of the suture was also recorded. For each specimen, the number of sections with fused sutures was summed and divided by the total number of sections to calculate the percentage of the suture that was fused.

A 4-year-old male *G. moholi* was evaluated twice, once using micro CT slices and histological sections. Because thresholding is the most subjective aspect of image processing, we scored suture patency using raw, unthresholded CT slices. This assured consistent grayscale values among slices, thus guarding against the introduction of variation because of inconsistent image processing. We did use thresholding to render a 3D volume. However, that image is used for illustrative purposes; no data were drawn from the image. Micro CT slices were selected using Adobe Photoshop 8.0 at similar interslice intervals (approximately every 100 μm) to determine whether histology and micro CT yield similar results. The presence or absence of osseous bridges was rated for a comparison to the histological results and to assess interobserver reliability. After two investigators separately rated 65 sutures from 50 slices (every 5th slice across the extent of the NPS), interobserver reliability was assessed using Cohen's kappa test (using SPSS version 12). For all 65 sutures, this coefficient calculated at 0.88 (a value of 1) indicates complete agreement between paired values. However, the coefficient for the NPS alone (0.95) indicated greater reliability of suture rating compared with the PS alone (0.61).

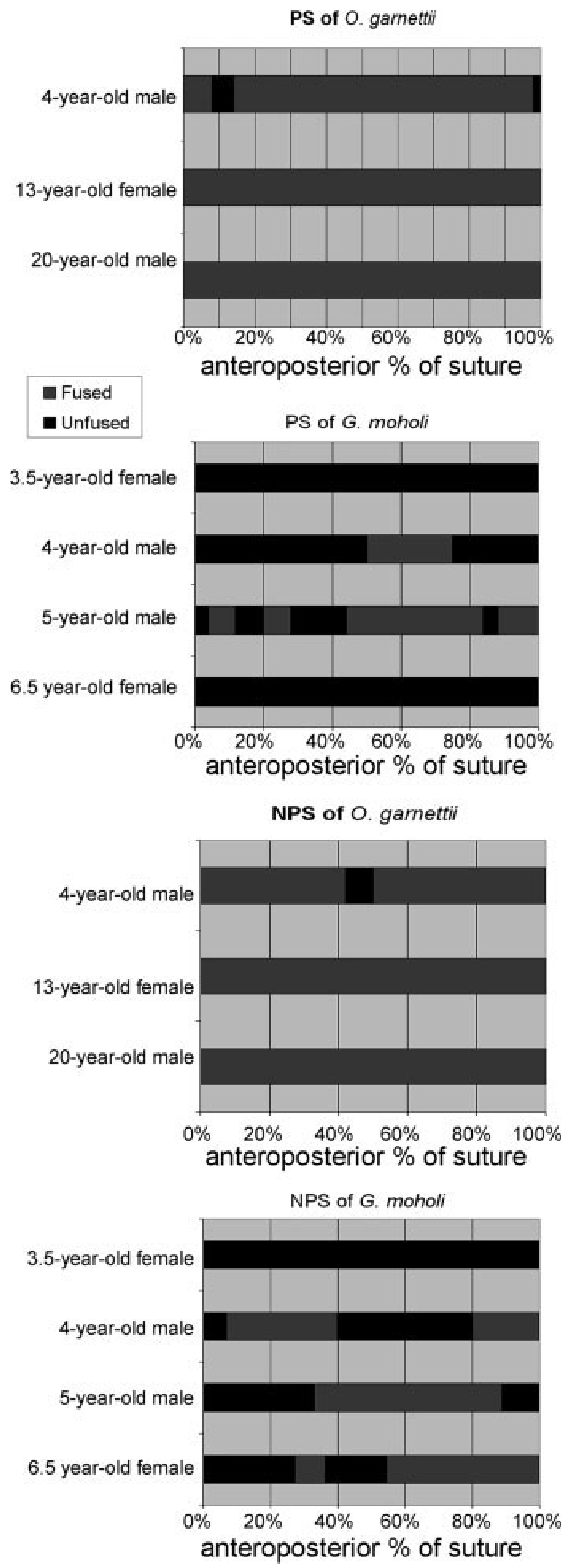

Fig. 2. Percentage of suture fusion in the premaxillary (PS) and nasopremaxillary (NPS) sutures in captive bushbabies. 0% is the anteriormost limit of the suture. In the NPS, more fusion was observed in older animals for both species. In the PS, female *G. moholi* had complete patency, whereas males had intermittent anteroposterior points of fusion. Only the youngest *Otolemur* had any patency in the NPS or PS.

TABLE 1. Percentage fusion of the nasopremaxillary and premaxillary sutures in greater and lesser bushbabies

Species	*Otolemur garnettii*			*Galago moholi*			
Age (years)	4	13	20	3.5	4	5	6.5
Premaxillary suture (percent fused)	92	100	100	0	23	68	0
Nasopremaxillary suture (percent fused)	92	100	100	0	53	56	54

RESULTS

In the larger species, *O. garnettii*, only the youngest specimen exhibits patency in the NPS or PS (Fig. 2; Table 1). One *Otolemur* (8-year-old male) had such complete fusion of sutures that the posterior end points were unclear (hence this specimen is excluded from Fig. 2). In the NPS, more fusion was observed in older animals for both species. The same was not true of the PS. Female *G. moholi* has complete patency, whereas males have intermittent anteroposterior points of fusion. Despite 100% fusion of both the PS and NPA in the oldest *O. garnettii*, an external notch was found in 6–23% of the sections. Figure 3 illustrates these notches in facial sutures of the 8-year-old male *O. garnettii*.

The extent of fusion of the PS ranged from 0 to 68% in *G. moholi* and 92 to 100% in *O. garnettii* (Fig. 2, Table 1). The extent of fusion of the NPS ranged from 0 to 56% in *G. moholi* and 92 to 100% in *O. garnettii*. The youngest *O. garnettii* (4-year-old male) has a much greater extent of suture fusion (~90% in both sutures) compared with 3.5-year-old female *G. moholi* (no fusion in either suture) or a 4-year-old male *G. moholi* (23–50% fusion). It is notable that the 3D reconstructions of the 4-year-old male *G. moholi* (Fig. 1D–F) suggest that points of fusion of the PS may not be detectable externally. Histological observations reveal that limited, internal bridging is relatively common, with sometimes deep notches on the external side of the suture. In the 4-year-old *O. garnettii*, sutural fusion is limited to the internal side in as many as 31% of sections, resulting in a superficial notch. Even in animals with 100% fusion of the PS and NPS, deep external notches to these and other sutures are demonstrated by histology (Fig. 3A–D). Intermittent points of fusion are seen in the PS and NPS of most *G. moholi* and in the PS of the 4-year-old *O. garnettii*. This suture in the latter specimen (Figs. 2 and 4) is fused across 92% of its length, but has intermittent patency and some external notches (Fig. 4 - arrowheads). In both the PS and NPS, the degree of bridging across external and internal aspects of the suture varies (Figs. 4 and 5).

CT slices of *G. moholi* yield similar observations to histological sections despite differences in resolution. Observations of histological sections indicate 23% fusion of the PS and 50% for the NPS. Based on micro CT slices, 10 and 44% fusion is indicated, respectively, for these same sutures. However, using micro CT, some portions of the PS could not be assessed because of the thinness of the premaxilla (see Fig. 6) and the suture itself. Figure 5 illustrates a serial comparison of the NPS viewed by micro CT and subsequent histological sections. Despite an imperfect match between slice planes, analogous segments of the NPS are easily identifiable (Fig. 5, compare middle left and right images). On the far left column, the NPS is enlarged to show both patent sutures and apparent points of fusion. Histological observations

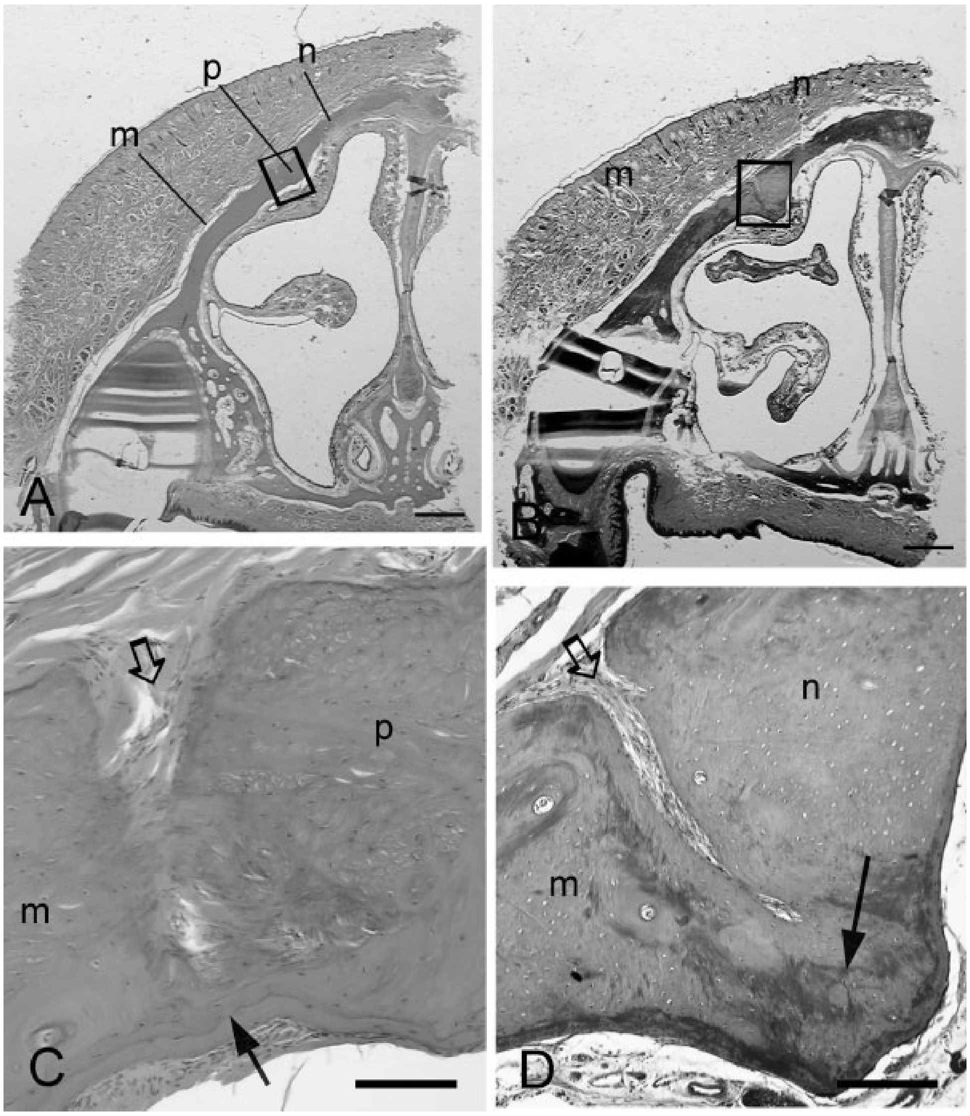

Fig. 3. The PS of an 8-year-old *O. garnettii* was completely fused (**A**, **B**; boxes are enlarged in **C** and **D**). Although fusion was clearly visible (C, D: arrows), an external groove (open arrow) was present in some sections. In Figure D, the nasal process of the premaxilla is completely fused to the nasal and maxillary bones at the point of fusion. m, maxilla; p, premaxilla; n, nasal bone. Scale bars. A, B = 1 mm; C, D = 100 μm.

clearly confirm a limited presence of internal osseous bridging (far right column). Lamellar bone is apparent in some portions of the fused suture (Fig. 5, bottom right).

DISCUSSION

The rostral facial skeleton and its sutures have been studied with respect to biomechanics, aging, dietary correlates, and phylogeny (Ravosa et al., 2000; Wang et al., 2006a,b). Although they differ in microanatomy (Pritchard et al., 1956), calvarial and facial sutures respond similarly to mechanical loads (Herring, 2008). Epigenetic influences on sutural complexity, fusion, and patency have been related to dietary transitions (Byron, 2006) and feeding biomechanics (Wang et al., 2006a,b). Unfortunately, the influence of these factors on the results of this study remains unclear, and functional considerations associated with diet are not within the scope of this study.

Nevertheless, our observations of PS and NPS fusion have implications for studies of the biomechanical properties of apparently patent sutures. Patent sutures ameliorate strain on bone during mastication (Behrents et al., 1978; Hylander, 1979; Herring et al., 2001). Because of their role in redistributing forces, factoring in sutures may be critical to finite element modeling (Ross, 2005).

Aside from epigenetic influences, few studies have considered factors affecting facial suture fusion. Detailed observations of sutural fusion based on the external view are available for macaques of known sex and age. On the basis of external rating, Wang et al. (2006a,b) found that facial sutures generally remain patent longer than calvarial sutures, but reported substantial variation in timing of fusion in all sutures. The NPS and PS remain externally patent longer in female than in male macaques. The limited bushbaby sample studied here suggests a similar degree of variability may exist for strepsirrhines, and the dichotomy between the two genera suggests a broader analysis may reveal influences of diet, sex, and body size on the extent of facial suture fusion.

This microanatomical study of bushbabies represents a more detailed level of scrutiny than previous studies of primate facial sutures. Both micro CT and histological observations confirm partial fusion of the PS and NPS that may not be externally detectable. The impact of partial fusion on the biomechanics of facial sutures has not

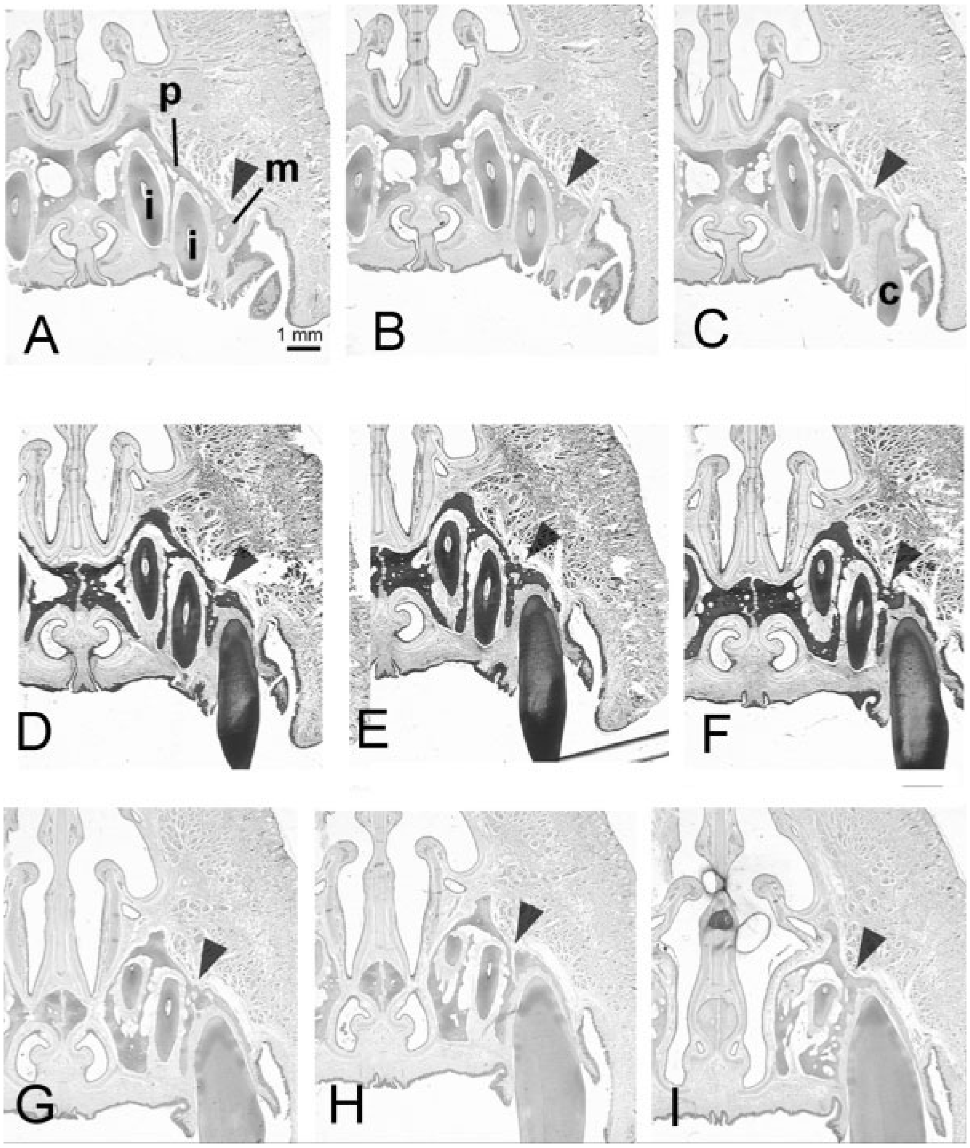

Fig. 4. Anteroposterior sequence of serial sections (through the premaxillary suture arrowheads) of a 4-year-old *O. garnettii*. Note the intermittent points of fusion. Serial sections are shown at every 100 μm across the suture. c, canine; i, incisor; m, maxilla; p, premaxilla.

been considered. In contrast, much scrutiny has been given to partial fusion of the mandibular symphysis (e.g., Beecher, 2005). The degree of fusion of facial sutures must be given similar weight in future experimental and modeling studies of facial biomechanics.

Methodological considerations

External rating of suture fusion and patency has been used to reveal patterns associated with age, function, and phylogeny (Rafferty et al., 2003; Wang et al., 2006a,b; Cray et al., 2008). Although histology remains the gold standard for assessing sutural morphology (Bradley et al., 1996), the necessarily destructive process is unsuitable to the study of relatively rare samples, such as skeletal remains of endangered primates. Micro CT has emerged as a potentially useful alternative (Recinos et al., 2004; Stadler et al., 2006).

This study is the first micro CT analysis of facial suture patency and fusion, with the exception of the midpalatal suture (Korbmacher et al., 2007). In this study, observations using both histology and micro CT clearly demonstrate the existence of fusion in the facial sutures of bushbabies. The lower percentage of fusion detected by micro CT may be the result of poorer resolution compared with histological sections. Despite this limitation, matched comparisons show that micro CT can detect osseous bridges at analogous points in the same individual (see Fig. 5).

It is beyond the scope of this study to compare microanatomical and gross methods for assessing suture fusion. However, both histology and micro CT show deep notches overlying points of internal fusion. This suggests that such sutures may be misinterpreted as patent. Indeed, reconstructions of the external bony surfaces imaged using micro CT show no clear visible indication of fusion. The PS in particular appears as a continuous groove in the specimen (Fig. 1E), suggesting these external notches may mislead raters viewing the suture from the external view.

Data from both external and microanatomical observations can reveal important information about sutural fusion. Micro CT-scanning provides a new method for investigating the phenomenon and can accomplished

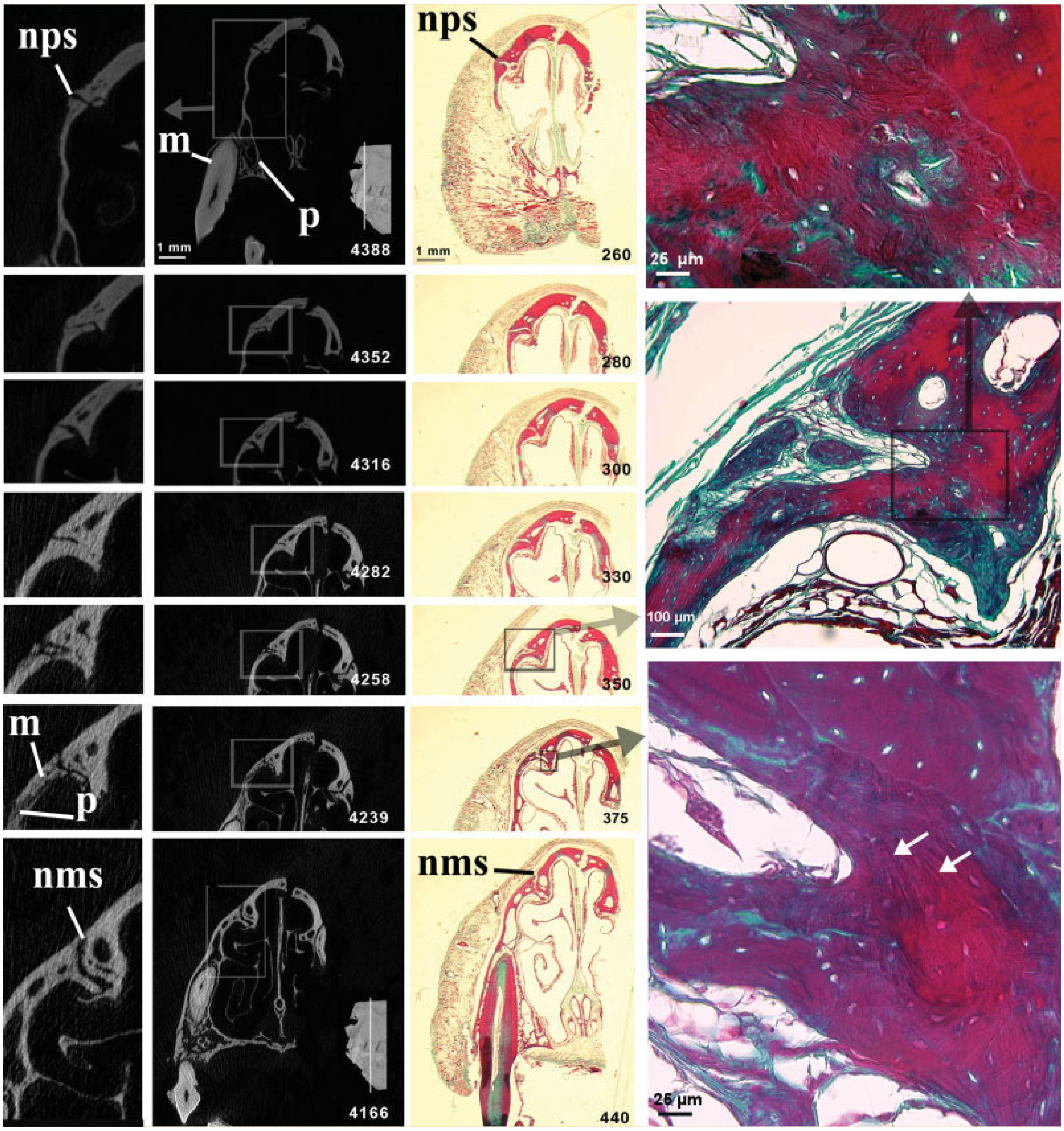

Fig. 5. Four-year-old *G. moholi* showing micro CT slices (left) next to analogous serial histological sections (right), in which facial sutures (such as the nasopremaxillary suture, NPS) are seen at an analogous level. Sections range from anterior (top) to posterior (bottom). Left and right columns illustrate enlarged views of central columns (see box for location of enlarged view). Scout images (top and bottom left) show approximate levels of the first and last CT slices. Numbers on the central CT and histological images indicate serial slice or section level (slice thickness = 8.5 µm; section thickness = 10 µm). The NPS had both patent and fused segments (sections 260–300 and 330–374, respectively). Note that fusion can be inferred from CT slices (CT slice 4258) and that all fusion sites are overlain by an external notch. Osseous bridging of the NPS as inferred from some CT slices is confirmed by histology (far right; see double arrows). m, maxilla; nms, nasomaxillary suture; p, premaxilla.

with relative ease. Our results demonstrate that data derived from micro CT-scanning can be highly reliable, as evidenced by the small interobserver error in rating sutural fusion for the NPS. However, the thin posterior portion of the premaxilla (and the PS) was difficult to detect in CT slices (see Fig. 6), and this may account for the low interobserver reliability of ratings for this suture. Thus, our preliminary assessment of the use of micro CT for assessment of suture fusion indicates that high resolution is essential. Because a thin layer of soft tissue intervenes between the ossified fronts of facial sutures, the 8.85 μm pixel resolution used here should

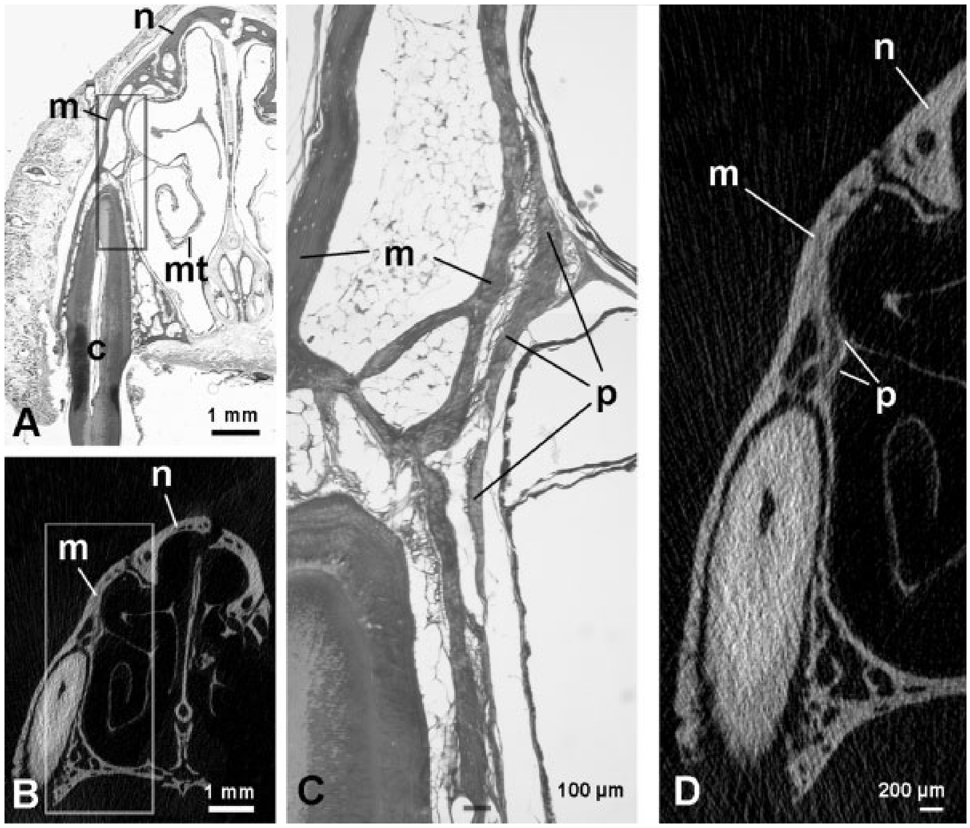

Fig. 6. Four-year-old *G. moholi* showing histology (**A**, **C**) next to analogous micro CT slices (**B**, **D**). Although the differences in precise plane of sections do not allow analogy of all structures, the posterior limit of the premaxilla (p) is at the same level in histology and CT slices. The insets of A and B are enlarged in C and **D**, respectively. Note the suture separating the premaxilla from the maxilla (m) is clearly visible histologically (C), but micro CT at this pixel resolution did not allow identification of the suture in this or adjacent slices (D). c, canine; mt, maxilloturbinal; n, nasal bone.

be sufficient for most sutures. Sutures separated by extremely thin layers of soft tissue, such as the PS, may need to be assessed with finer pixel resolution. The availability of representative histological specimens of a species is optimal for comparison to CT slices to infer whether a suture can be rated at a given resolution.

CONCLUSIONS

Studies of sutural complexity based on external evidence suggest that partial fusion is of ontogenetic and functional importance (Bradley et al., 1996; Burrows et al., 1999), and some studies exclude partially fused sutures from analysis (Yu et al., 2003). In light of intermittent and sometimes undetectable points of fusion, microanatomical evidence may be needed to fully assess fusion of facial sutures. In addition, the degree of internal bridging may provide another dataset for investigating the phylogenetic implications or sex-related patterns of suture fusion. The findings of this study indicate that micro CT is a potentially useful tool to inform such studies while avoiding the destructive consequences of histology.

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