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At Birth, Tarsiers Lack a Postorbital Bar or Septum

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ABSTRACT

Among primates, partial or complete posterior closure of the orbit has been widely accepted as a shared derived characteristic justifying an exclusive tarsier-anthropoid clade, while some regard the tarsier lateral orbit as an elaborated postorbital bar (POB). To test these competing hypotheses while minimizing the confounding effect of tarsier orbital hypertrophy, we compared tarsiers and other primates at early (fetal and newborn) ages using dissection, micro-CT scans and soft tissue histology. Our findings demonstrate unanticipated variation in the anatomy and development of the zygomaticofrontal (ZFA) articulation, which forms the orbit's lateral framework. Tarsiers uniquely exhibit a combination of two features: absence of a pre- and peri-natal frontal spur to join with the zygomatic to form the ZFA; and, the spur's substitution by an elaborate ligament, which envelops the eye laterally as an expansive postorbital membrane (POM) that merges with the anterolateral fontanelle of the lateral cranial vault. In lacking a frontal spur, tarsiers are distinct from strepsirhines, while the ligamentous structure of the POM distinguishes its ZFA from that of anthropoids, which is a typical facial suture at and prior to birth. The POM of tarsiers may be thought of as an accessory fontanelle, a structural compromise that provides flexible stability and spatial separation of bones while anticipating rapid postnatal growth of an enormously enlarged eye. We regard the tarsier POM as part of a neomorphic eyeball hypertrophy complex, and reject the hypothesis of derived homology of the postorbital septa of adult tarsiers and anthropoids on histological, developmental and functional grounds. Anat Rec, 00:000–000, 2013. © 2013 Wiley Periodicals, Inc.

Key words: Anthropoidea; Haplorhini; homology; postorbital septum; tarsiiforms; ocular hypertrophy; *Tarsius*

The orbit of adult primates is well supported laterally by the articulation of the frontal and zygomatic bones. In extant strepsirhines (lemurs, lorises, and allies) and Paleogene euprimates (adapiforms, fossil tarsiiforms, and primates of modern aspect), each of these bones projects toward one another with orbital processes,

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TABLE 1. Species, ages, and selected measurements of the specimens under study

Spec. no.	Species	Source	Age in days (if known)	Cranial length (mm)	CRL (mm)	Figure reference
P96	*Tarsius syrichta*	DLC	Fetal	24.85	48.73	3, 5E-G, S3
P94	*T. syrichta*	DLC	0	29.45	68	3A-C, 4B, 5A-D, H, 10, S3
P98	*T. syrichta*	DLC	6	29.15	?	5I, S3
013	*T. bancanus*	LM	1	31.7	?	1, 2, 4A
P696	*Cheirogaleus medius*	DLC	0	24.38	54.81	1
P888	*Microcebus murinus*	DLC	1	?	40.86	S2
P385	*Mirza coquereli*	DLC	0	25.52	60.32	7
P2502	*Lemur catta*	DLC	1	40.25	96.29	1
P6154	*Propithecus verrauxi*	DLC	Fetal	D	∼102.53	S2
OG101	*Otolemur garnettii*	DLC	0	36.02	82.09	6
3019	*Galago senegalensis*	LN	0	?	64.93	1
CJV177	*Callithrix jacchus*	WNPRC	Perinatal	?	?	1
CP11	*Cebuella pygmaea*	DWA	Perinatal	?	?	S2
SG10	*Saguinus geoffroyi*	CMZ	Fetal	21.25	55	8, 9, S5
SG11	*Saguinus geoffroyi*	CMZ	0	35.56	100	1
Ss5033	*Saimiri boliviensis*	SAPRC	Late fetal (Stillborn)	41.66	125	1, S4
Sq101	*Saimiri boliviensis*	SAPRC	0	53.18	132	S4
Alouatta3	*Alouatta seniculus*	DWA	Fetal	32.68	81.49	8, S5
Alouatta2	*Alouatta seniculus*	DWA	Fetal	45.54	118.47	1
12-17-01B	*Tupaia glis*	DMC	1?	23.06	?	1

Source: CMZ, Cleveland Metropark Zoo; DLC, Duke Lemur Center; DMC, Duke Medical Center; DWA, Dallas World Aquarium; LM, collection of Lawrence Martin; LN, collection of Leanne Nash; SAPRC, South Alabama Primate Research Center; WNPRC, Wisconsin National Primate Research Center.?, measurement unavailable.

forming a postorbital bar (POB). While all extant primates possess a functionally similar supportive structure (e.g., the lateral border of the human eye socket), only two groups exhibit a posterolateral extension of bone behind the eye: tarsiers and anthropoids (Pocock, 1918; Cartmill, 1980; Rosenberger et al., 2008). For nearly 100 years this character has been interpreted as a shared derived feature supporting the central phylogenetic dichotomy of crown euprimate evolution (Pocock, 1918; Szalay and Delson, 1979; Fleagle, 1999): the distinction between strepsirhines and haplorhines (tarsiers, anthropoids, and allies) and, more specifically, the sister-group relationship between tarsiers and anthropoids to the exclusion of many fossil tarsiiformes.

Among anthropoids, an effectively complete postorbital septum (POS) is produced by sheets of the zygomatic and alisphenoid that form extensive sutural contacts with one another, in addition to the zygomatic's contact with the frontal. In tarsiers, the extent of postorbital closure is far less and restricted to the upper part of the framework, leaving a relatively wide open confluence between the orbit and the temporal fossa behind it (see electronic Supporting Information Fig. S1). Here, too, the zygomatic and frontal meet to form a tripartite lateral orbital mosaic with the addition of a slip arising from the alisphenoid. While these particular similarities have been interpreted as homologies having the same overall functional explanation (Cartmill, 1980), to insulate the eye from mechanical interference generated by the adjacent temporalis muscle lying behind the orbit, they also present something of a paradox, for tarsiers have the largest eyes of all mammals relative to their body size (Polyak, 1957) while those of anthropoids are relatively small by comparison with other primates (Ross and Kirk, 2007). As a consequence of these proportional differences, the gross anatomy of their adult orbits differs and this has led to a fundamental disagreement. Some authors view the lateral orbital mosaic of tarsiers as a homologous version of the anthropoid POS, either preconfiguring the evolution of complete orbital closure or representing a vestige of closure that was lost in connection with eyeball hypertrophy (Cartmill, 1980). Others regard the tarsier's lateral mosaic as little more than an elaborated postorbital bar (Simons and Rasmussen, 1989; Rosenberger et al., 2008), modified to accommodate enormous eyeballs.

It is widely acknowledged that the derived orbital morphologies of tarsiers and anthropoids evolved directly or step-wise from a basal condition resembling a simple ring-like postorbital bar, like those found in Eocene fossil primates and modern strepsirhines (Hershkovitz, 1977; Cartmill, 1980; Rosenberger et al., 2008). However, absent paleontological evidence of the transformation, this model is inferred entirely from adult morphology. Here we present the first comparative assessment of perinatal development of this region in light of recent work documenting profound differences between tarsiers and other primates in the timing of eye growth (Jeffery et al., 2007; Cummings et al., 2012). The enormous tarsier eye grows disproportionately more in the postnatal period (∼66% in diameter) compared with anthropoids (∼38–50% in diameter) and quickly outpaces bony growth surrounding the eye (Cummings et al., 2012); in adults more than half the globe falls outside the margin of the orbit (Schultz, 1940). Moreover, a comparison of orbital diameter in fetal, 0-day-old and 6-day-old tarsiers suggests that the growth rate of the orbit accelerates rapidly in the perinatal timeframe (Cummings et al., 2012). To disentangle the morphological influence of hypertrophic eyeball expansion, we investigated late fetal and perinatal development of the zygomaticofrontal articulation (ZFA) at stages prior to the divergent postnatal orbital growth rates observed in

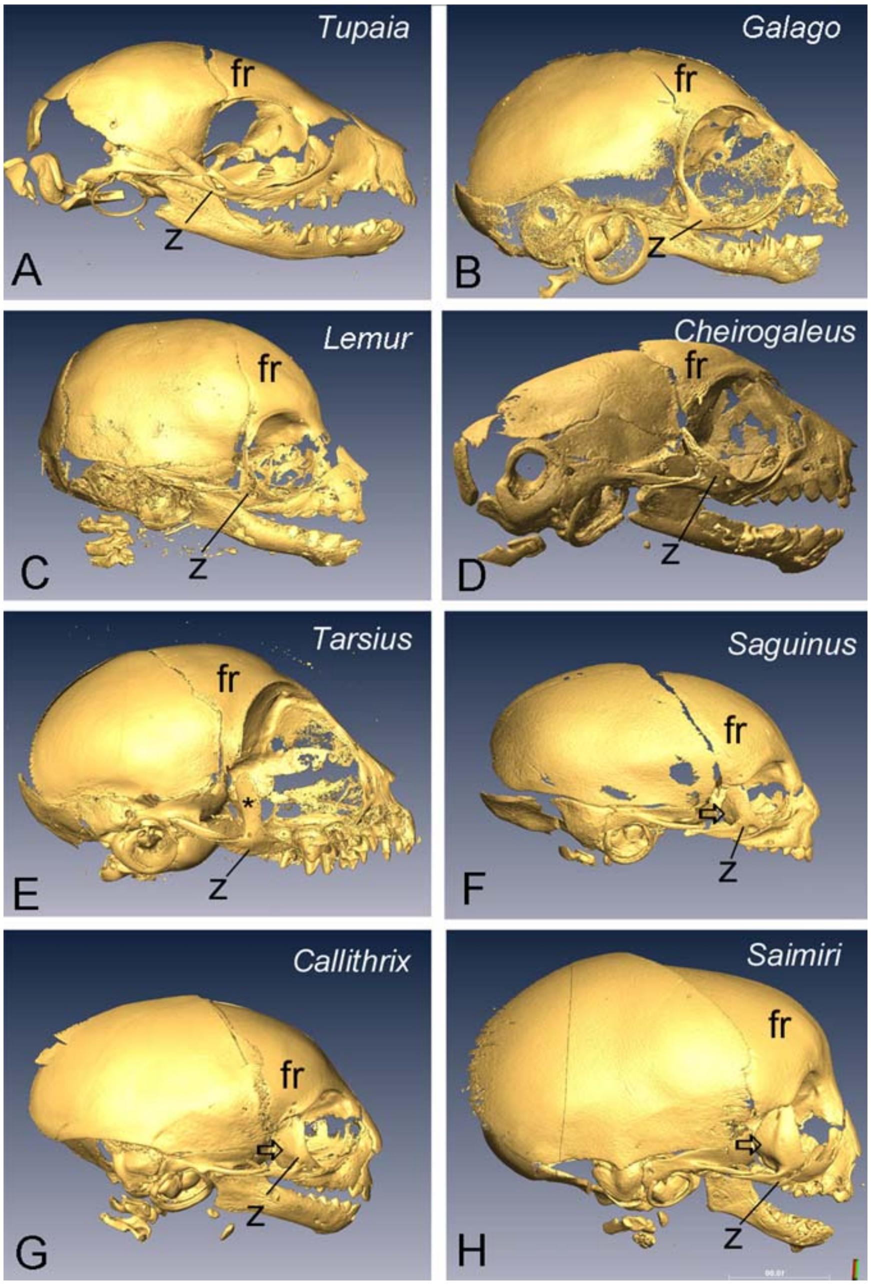

Fig. 1. Osteology of the zygomaticofrontal articulation (ZFA) in newborn primates. The ZFA of all newborn strepsirhines (**A–D**) is a complete or nearly complete postorbital bar (POB), formed by postorbital processes of the frontal (fr) and zygomatic (z) bones. The POB is incomplete in cheirogaleids at birth (D). In *Tarsius bancanus* (**E**), there is no direct articulation at the ZFA. The spur-like postorbital process of the frontal is lacking, leaving a gap between the frontal and the postorbital process of the zygomatic (*). The ZFA of anthropoids is fully established in an adult-like configuration at birth (**F–H**) in that a zygomaticofrontal suture is fully formed and a postorbital flange of the zygomatic (open arrows) extends towards the calvaria.

tarsiers and anthropoids, before the tarsier eye attains the extreme ectopic position found in adults.

MATERIALS AND METHODS

Cadaveric specimens of tarsiers (*Tarsius syrichta* and *T. bancanus*) at late fetal to infant ages were studied by gross dissection, microcomputed tomographic (microCT) imaging, and histology (Table 1). These specimens were compared to seven species of strepsirhines, five species of anthropoids, and the tree shrew (*Tupaia glis*). The comparative sample was selected to reveal morphological patterns in primates with varying eye sizes at birth (Cummings et al., 2012), with *Tupaia* selected to represent a model of an equally if not more primitive version of the primate POB.

The specimens were CT scanned prior to dissection or histological processing. Most were scanned using a Scanco

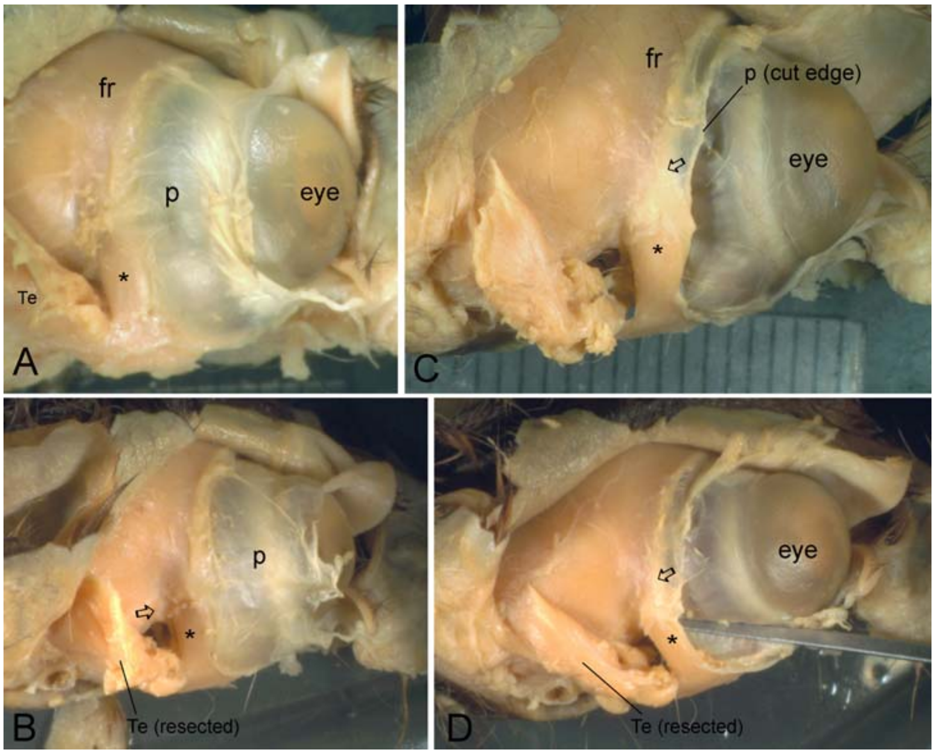

Fig. 2. Dissection of the lateral border of the orbit (right side) in *Tarsius bancanus* (1-day-old), showing (**A**,**B**) the relationship of the orbital process of the zygomatic bone (*) to the eye, with the temporalis muscle (Te) dissected away. In A and B, the periorbita (p) is intact. (**C**) Most of the periorbita is removed, except where it joins denser connective tissue (open arrows) between the orbital process of the zygomatic and the frontal bone (fr). (**D**) This forms a flexible syndesmosis.

vivaCT 75 scanner (55 kVp, 20.5 μm reconstructed voxel size) at Northeastern Ohio Medical University (NEOMED). Two specimens were microCT scanned at Johns Hopkins University using a dedicated small animal SPECT/CT imaging system (X-SPECT®, Gamma Medica-Ideas, Northridge, CA) (55 kVp, 142 μA, 50 μm spatial resolution; 33 μm minimum reconstructed voxel size). Surface reconstructions of skulls were produced for each image volume using Amira 5.3 software (Visage Imaging, GmbH).

First, surface reconstructions were examined to establish the extent of postorbital suture formation for the POB or POS. Next, all tarsiers, a selected strepsirrhine (*Otolemur*) and anthropoids (*Saguinus, Alouatta*) were dissected to examine non-osseous connective tissues in the postorbital region. An Olympus SZ40 dissection microscope was used to study most of the specimens; for *T. bancanus* a Leica MZ75 dissecting microscope was used. To establish the relationship between bone and soft tissue structures (e.g., the eye), CT reconstructions of bone (Fig. 1E) were compared to dissection photographs (Fig. 2). Illustrations were made after superimposing CT-based skull reconstructions with dissection photographs and CT slices windowed to show bone tissue and the position of the eyeball (the latter was seen as pixels in lower densities compared to bone).

The two infant (postnatal) tarsiers were paraffin embedded, and sectioned at 10 to 12 μm in the coronal plane. The other species had been partially sectioned for previous studies (Dennis et al., 2004; Smith et al., 2010). For the present study, the remainder of these paraffin blocks was sectioned through the postorbital region in the same manner as described for the infant tarsiers. Every 5th section was mounted and stained with either hematoxylin-eosin or Gomori trichrome. Selected sections were stained using Verhoeff's hematoxylin method for identifying elastic fibers (Humason, 1979). Using a Leica photomicroscope, the tissues were examined at 25 × −630× to describe histological structure of the ZFA. Suture morphology was assessed with respect to stages of development published by Pritchard et al. (1956).

RESULTS

Our reconstructions reveal four configurations of the ZFA at birth in our comparative sample. In the newborn *Tupaia glis,* a gracile, incomplete postorbital bar is present; the orbital process of the frontal is especially small and partially separated from the rest of the frontal bone (Fig. 1A). In most strepsirhines, the POB is fully established at birth, with sutural contact evident at the ZFA. The POB is established at some point prenatally, as is clear in the fetal sifaka (*Propithecus verrauxi,* see Supporting Information Fig. S2A). The tips of the zygomatic and frontal orbital processes that join at the ZFA may be slender (e.g., *Galago*, Fig. 1B) or wider, flat, overlapping elements (e.g., *Lemur*, Fig. 1C). One exception to this pattern is seen in perinatal dwarf and mouse

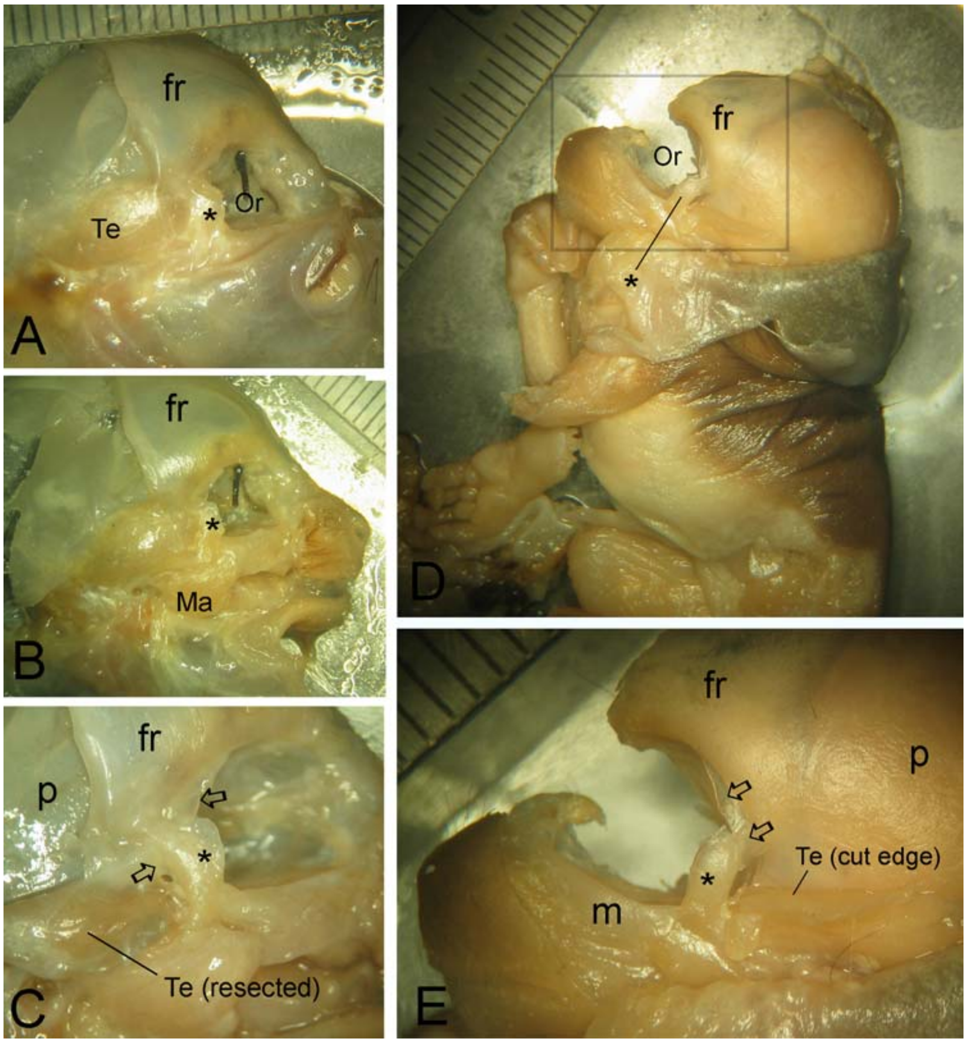

Fig. 3. (**A–C**) Dissection of the lateral border of the orbit (Or) in *Tarsius syricta* (0-day-old), emphasizing the relationship of the orbital process of the zygomatic bone (*) with the frontal bone (fr). (**D**,**E**) similar dissection in a late fetal *T. syrichta*. In (C and E) The temporalis muscle (Te) is dissected away, revealing that the orbital process of the zygomatic bone does not form a suture with the frontal bone. Instead, a thick membrane (open arrows) spans the distance between these two bones. p, parietal bone. Figs. A–C are the basis for the illustration in Fig. 4.

lemurs (cheirogaleids), where no sutural contact occurs between the orbital processes of the frontal and zygomatic bones (Fig. 1D; and see Supporting Information Fig. S2B). They resemble *Tupaia glis* in that zygomatic and frontal orbital processes are developed but the POB is incomplete. Similarly, in the tarsier there is also no direct articulation at the ZFA (Fig. 1E; and see Supporting Information Fig. S2C). Unlike the strepsirhines and tree shrews, however, the spur-like orbital process of the frontal is lacking, leaving a substantial gap between the frontal bone and the orbital process of the zygomatic. The frontal bone has a limited extension that reaches away from the orbital rim toward the orbital process of the zygomatic, either as a flat, short plate (Fig. 1E), or a slight prominence (see Supporting Information Fig. S2C). The most extensive zygomaticofrontal articulation is seen in newborn anthropoids. The anthropoid postorbital region is characterized by the apposition of frontal and zygomatic bones and a fully formed, linearly expansive suture at the ZFA (Fig. 1F–H; and see Supporting Information Fig. S2D). In addition, the orbit, even in fetal stages, is enclosed posterolaterally by a large lamina of bone extending from the orbital process of the zygomatic to meet the braincase at the alisphenoid, thus forming the complete POS (Figs. 1F–H; and see Supporting Information Fig. S1D). This posterior lamina of the zygomatic is not present in either strepsirhines or tarsiers (Fig. 1A–E; and see Supporting Information Figs. S2A–C).

Dissection of fetal and infant tarsiers reveals a thick, expansive postorbital membrane (POM) spanning the gap between the frontal and zygomatic bones (Figs. 2 and 3). This fan-shaped ligament is continuous with a thinner periorbital membrane at its anterior and posteroinferior limits. Anterosuperiorly, the tarsier POM extends along the orbital rim of the frontal bone (Figs. 2C, 3E, and 4). Deep to the temporalis muscle, the

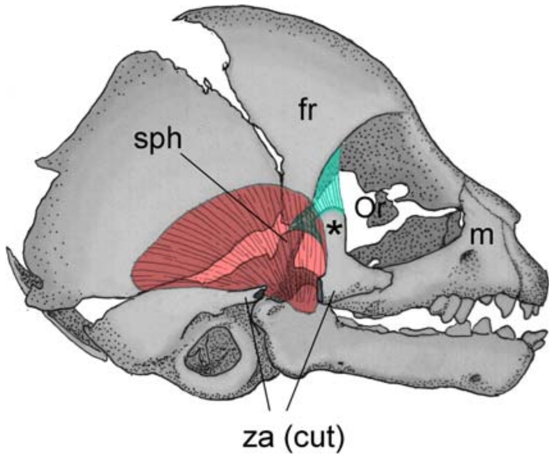

Fig. 4. The orbit (Or) in newborn *Tarsius syricta* (P0), emphasizing the relationship of the orbital process of the zygomatic bone (*) with the frontal bone (fr) (see Fig. 3). The zygomatic bone is not in sutural contact with the frontal bone. Instead, the postorbital membrane (blue) spans the distance between the bones. The temporalis muscle (emphasized in red) overlies the posterior extent of this membrane. m, maxilla; sph, sphenoid; za, zygomatic arch.

posterosuperior part of the POM sweeps backward from the orbital process of the zygomatic (Fig. 4). Histological serial sections (Fig. 5A) and dissections (Fig. 2) show the thick POM merging into the thinner periorbital fascia that extends anterior to the orbital margin. At the posterior limit, the POM unites with the calvaria by attaching to the tip of the alisphenoid (Fig. 5D) and then merging into the fontanelle situated between the frontal and sphenoid bones (Fig. 5E–G).

In trichrome procedures, the POM is a heavily collagenous structure, and in the 6-day-old *Tarsius* specimen, parallel bundles of collagen are visible (Fig. 5H,I, and Supporting Information S3A–D). A parallel arrangement of collagen is seen at all ages (Supporting Information Figs. S3A–C). In the 6-day-old specimen (Supporting Information Fig. S3C), collagen is organized into bundles and both fibroblast-like (Supporting Information Fig. S3C) and chondrocyte-like cells (Fig. 3D) are seen. Preparation with Verhoeff hematoxylin procedure reveals no discernable elastic fiber content (Supporting Information Fig. S3E), in contrast to elastic cartilage samples of the same specimens (Supporting Information Fig. S3F). Thus, rather than a sutural joint, the ZFA of fetal and infant tarsiers is a flexible syndesmosis, specifically a fan-shaped ligament (Figs. 2D, 3, and 4).

In perinatal strepsirhines, the POB is invested by and continuous with periosteal connective tissue (Fig. 6A–C). In the newborn cheirogaleid *Mirza coquereli,* there is an unossified cord that completes the POB (Fig. 7A,B). Made of collagenous connective tissue (Fig. 7C), the tissue is nonetheless highly cellular (Fig. 7D), and thus not a fully differentiated ligament. It joins the orbital processes of the ZFA and is continuous posteriorly with the periorbita, but it bears no attachment to other bones or calvarial membranes (Figs. 6E,F and 7C,D).

In anthropoids (Fig. 8), the ZFA emerges at even earlier developmental stages. In a fetal *Alouatta,* the bones of the ZFA are broadly apposed, although not yet forming an interlocking suture (Fig. 8A,B). In all newborn anthropoids studied, the ZFA exhibits closely approximated edges of the frontal and zygomatic bones with distinctly separated periostea; only vascular loose connective tissue separates them (Supporting Information Fig. S4A–E). In a fetal *Saguinus,* the zygomatic bone is not yet in contact with the frontal bone. Instead, a thin membrane spans the small separation between them (Fig. 9), a morphology distinct from both tarsiers and strepsirhines. Unlike the POM of tarsiers, this membrane is not appreciably more fibrous than the periorbital fascia anterior to it (Figs. 9C–H). It is densely cellular with some elastic fiber content (Supporting Information Figs. S5A,B). The two fetal *Alouatta* both have a fully established zygomaticofrontal suture present. In the smallest fetus (Supporting Information Figs. S5C–E), the suture has capsular (periosteal) layers for each front that border a densely cellular middle zone, in which fibers are sparse and variable in their orientation (Supporting Information Fig. S5E). Both anterior (Supporting Information Fig. S5F,H) and posterior (Supporting Information Fig. S5G,I) to this suture the frontal and zygomatic appear to be more widely separated, and are separated only by loose connective tissue.

DISCUSSION

Most mammals support their eyes posterolaterally with connective tissues other than bone (Jašarević et al., 2010; Herring et al., 2011), but mammals have evolved an osseous postorbital bar many times independently (Heesy, 2005). For those that form an osseous ring lateral to the eye, the frontal and zygomatic are always recruited, which may be inevitable given the location of the eye within the cranium. An enhanced framework in the form of a partition appears to have been selectively advantageous in rare cases, involving an extension of the wing-like alisphenoid that articulates with the zygomatic and frontal bones. Only *Tarsius* and anthropoids have such an arrangement. However, the limited osteological options to develop lateral orbital support means that similarities in the adult morphology of some taxa can mask underlying analogy, or homoplasy. With all Paleogene and extant euprimates having either an osseous ring-like POB or a sheet-like POS of some kind, a central question in primate evolution has revolved around the enhanced pattern of orbital support: Do the partial septum of tarsiers and the complete septum of anthropoids represent alternative states of a shared derived homology and, therefore, evidence of an exclusive sister-group relationship? The most compelling morphological argument put forth in support of this hypothesis is that in both cases the postorbital wall is formed not only by the original bar's zygomatic and frontal processes but by interposition of an alisphenoid element as well (Cartmill and Kay, 1978; Cartmill, 1980).

On the basis of ontogeny and histology, we present new observations on primate periorbital anatomy, particularly aspects that were previously described in comparisons of adult specimens. The mammalian eye may be said to develop and exist postnatally within a collagenous sac that continues from the foramina of the orbit at its deepest aspect, then following the contours of the orbit itself (being partially synonymous with the periosteum once bones form), and then extending superficially as the palpebral fascia (Warwick and Williams, 1973; Sisson et al., 1975). From a putative euprimate common ancestor possessing

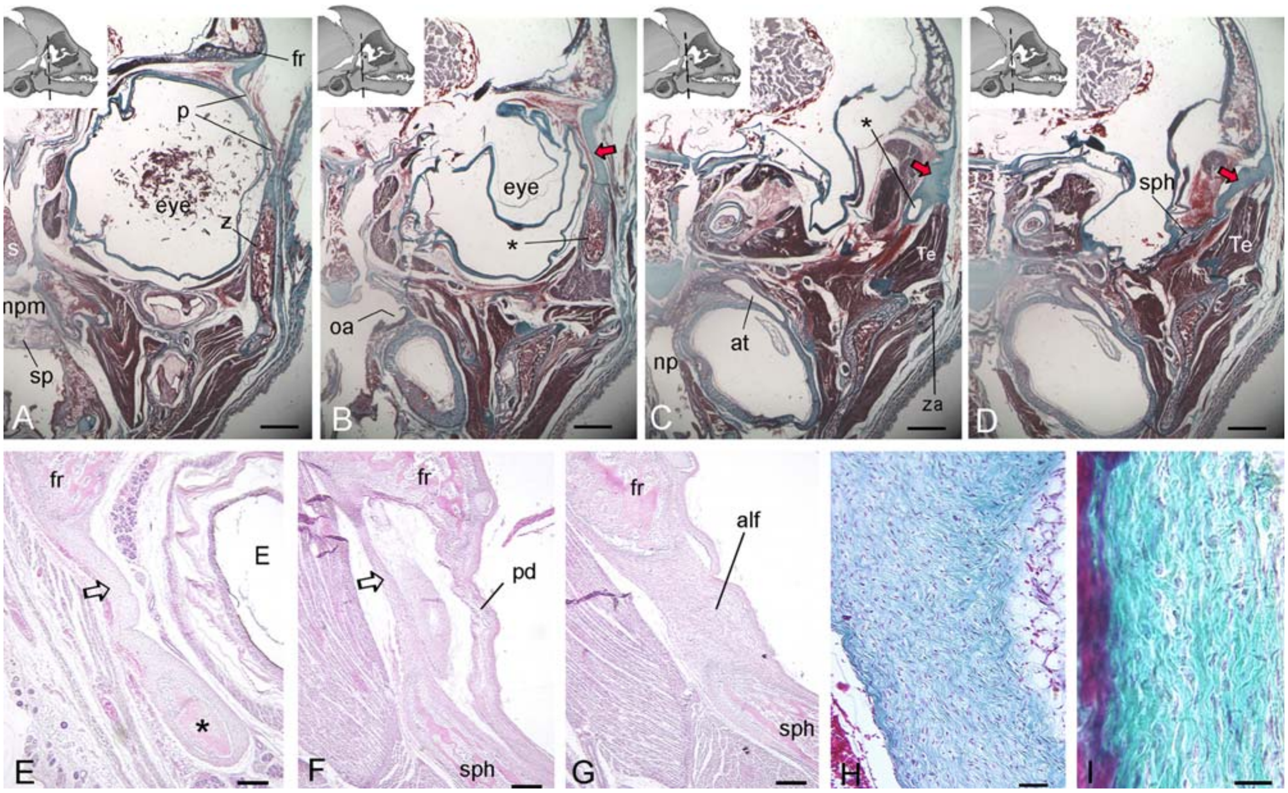

Fig. 5. (**A–D**): Histological sections of the same tarsier shown in Fig. 4 (contralateral side), from anterior to posterior, showing the soft tissue membranes spanning the distance from the frontal bone (fr) to the zygomatic bone (z) and sphenoid bone (sph). At all levels, the orbital process of the zygomatic bone is widely separated from the frontal bone. At its anterior limit, the periorbita (p, B) reflects outward from the bones deep to the skin over the eye. The periorbita is continuous with a thick ligament posteriorly (open arrows, B–D). This flat ligament, the postorbital membrane, connects the tip of the orbital process of the zygomatic (*) with the frontal bone (B,C). The posterior extent of this membrane merges into the anterolateral fontanelle. The anterior extent of this fontanelle intervenes between the sphenoid and frontal bones (open arrow, D). At these posterior levels, the temporalis muscle (Te) attaches (C,D). Insets: approximate level of each coronal section. (**E–G**) Histology of lateral orbit in late fetal *T. syrichta*, showing the gap between the orbital process of the zygomatic bone (*), and the frontal bone (fr) bridged by a thick postorbital membrane (open arrows). Posterior to the zygomatic, the membrane thins slightly, has an inferior attachment to the alisphenoid (sph) and converges toward the periosteal layer of the dura mater (pd). Together, the postorbital membrane and periosteal dura merge into the anterolateral fontanelle (alf). (**H**,**I**) High magnification view of the postorbital membrane in P0 (H: *T. syrichta*) and P6 (I: *T. syrichta*) tarsiers, showing thickness with numerous rows of parallel collagen fibers, organized into bundles in Fig. 5I. at, auditory tube lumen; E, eye; np, nasopharynx; npm, nasopharyngeal meatus; oa, opening of auditory tube; sp, soft palate. Stains: A–D, H,I, Gomori trichrome; E–G, hematoxylin and eosin. Scale bars: A (applies to A–D), 1 mm; E–G, 150 μm.; H, 50 μm; I, 20 μm.

a POB, one might envision expansion of the bar's posterolateral rim by ossification that follows a fascial plane, the periorbital membrane. It is precisely this collagenous sheet that is thought to create a "path" for expansion of the postorbital septum (Cartmill, 1980); the periorbita along that path is thought to become periosteum of the orbit. However, in mammals without a POS, the periorbita is partially a nonperiosteal membrane that separates soft tissues of the orbital and temporal fossae (Sisson et al., 1975; Cartmill, 1980).

In some mammals, a cord-shaped ligament exists in place of an osseous postorbital bar. It may be a fibrocartilaginous ligament in at least some cases (Jašarević et al., 2010). The connective tissue cord that unites the incomplete POB of cheirogaleids at the ZFA is a transient postorbital ligament. The cord is narrow, and it simply ties the frontal and zygomatic processes of the developing POB. One might interpret the POM of tarsiers as another example of a transient postorbital ligament that is sheet-like in its form, thus taking on the character of a broader membrane. Postnatal delay of zygomaticofrontal ossification in tarsiers could then be taken as an archaic euprimate phenomenon shared with some strepsirhines, either primitively or via parallelism. Indeed, while formation of the POB is generally a progressive prenatal process concurrent with eye growth (Cummings et al., 2012; Ramaswami, 1957), small strepsirhines like the cheirogaleids, with their allometrically enlarged eyes (Kay and Kirk, 2000), may also have a gap in the POB through late fetal and even early postnatal ontogeny, ostensibly allowing for rapid postnatal growth of the eye. However, their resemblance appears to be homoplasious for reasons that also bear on the adult morphology. The tarsier POM is anteroposteriorly far more expansive than the strepsirhine POB and its connective tissue precursor. It connects both facial and neurocranial bones, while the strepsirhine POB and its membranous ZFA bridge are restricted to the facial

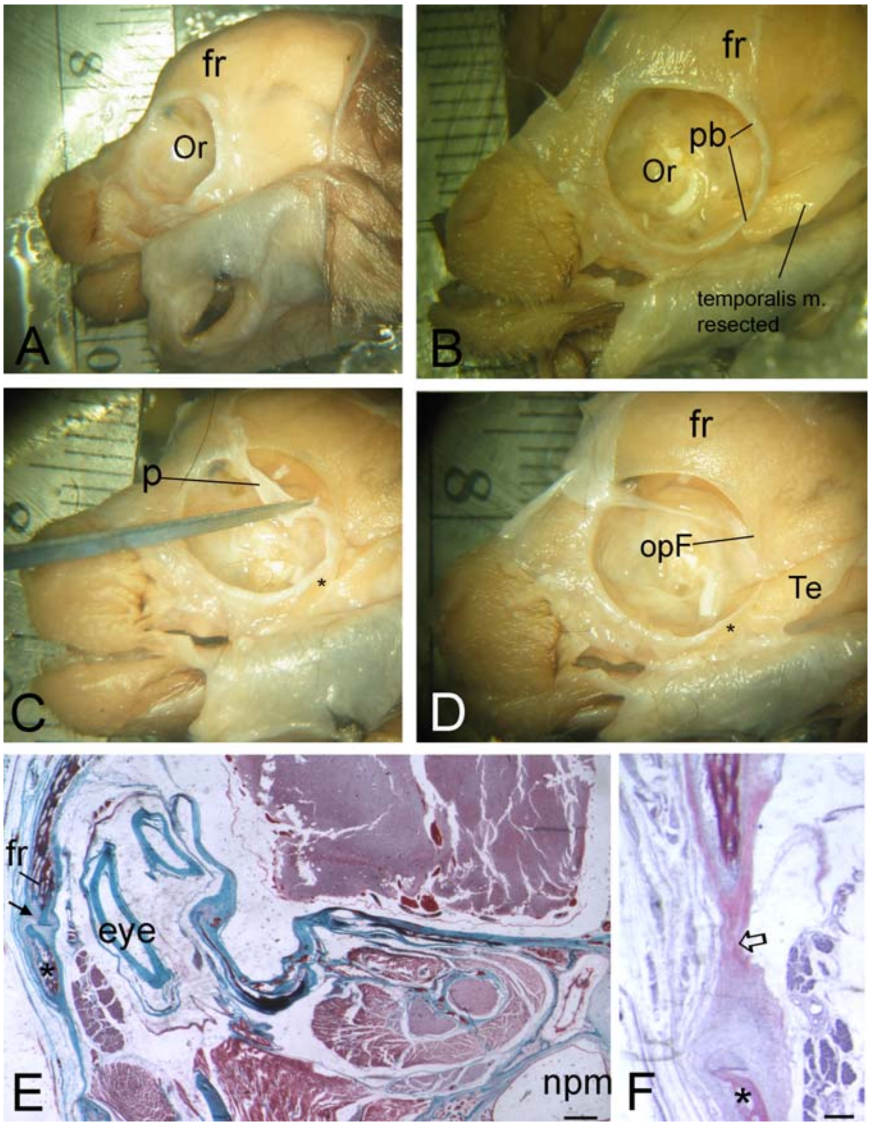

Fig. 6. Dissection of the orbital region in *Otolemur garnettii*. (**A**) Skin and eye removed, temporalis muscle undisturbed. (**B**) The thin postorbital bar (pb) is emphasized after resection of the temporalis m. (**C**) The periorbita (p) is partially stripped aside. (**D**) The periorbita is intimately connected with sutural margins of the zygomaticofrontal suture. After the connective tissue is fully stripped from the post orbital bar, gracile tips of the orbital process of the frontal bone (opF) and orbital process of the zygmomatic bone (*) remain. A small portion of the zygomatic bone broke away upon removal of the periorbita. In histological sections of the same specimen (contralateral size), the frontal (fr) and zygomatic bones form a suture (small arrow, **E**). Posterior to the suture, the connective tissue between these bones thins (open arrow, Fig. **F**) and disappears, forming no association with the calvaria. Or, orbit; Te, temporalis muscle. Stains: E,F, Gomori trichrome. Scale bars: E, 500 μm; F, 200 μm.

skeleton. In other words, in tarsiers the POM matches the spatial arrangement of the lateral orbit of adult tarsiers, including its connection to the calvaria. This last point is critical, because it corresponds with a prominent osseous feature that also argues for nonhomology of the lateral orbit in tarsiers and strepsirhines: the spur-like zygomatic process of the frontal bone typical of postorbital bars, precociously present in strepsirhines (Ramaswami, 1957) and common among other mammals with a POB (Heesy, 2005), is absent in tarsiers (Fig. 1E). It has either been lost or suppressed and modified developmentally.

Could the tarsier POM represent an unusually thickened portion of the periorbita that presages the haplorhine POS? Our findings suggest that it does not. The zygomaticofrontal suture is not formed until some time after the first postnatal week in *Tarsius syrichta*; it is delayed in *T. bancanus* as well. Instead, the posterolateral border is completed by the POM. Small extensions of the frontal bone also indicate ossification occurs within the POM (e.g., Fig. 1E, and see Supporting Information Fig. S2C). Anthropoids, in contrast, have a fully formed osseous postorbital septum well established at birth (Schwartz, 2003), even in small species with

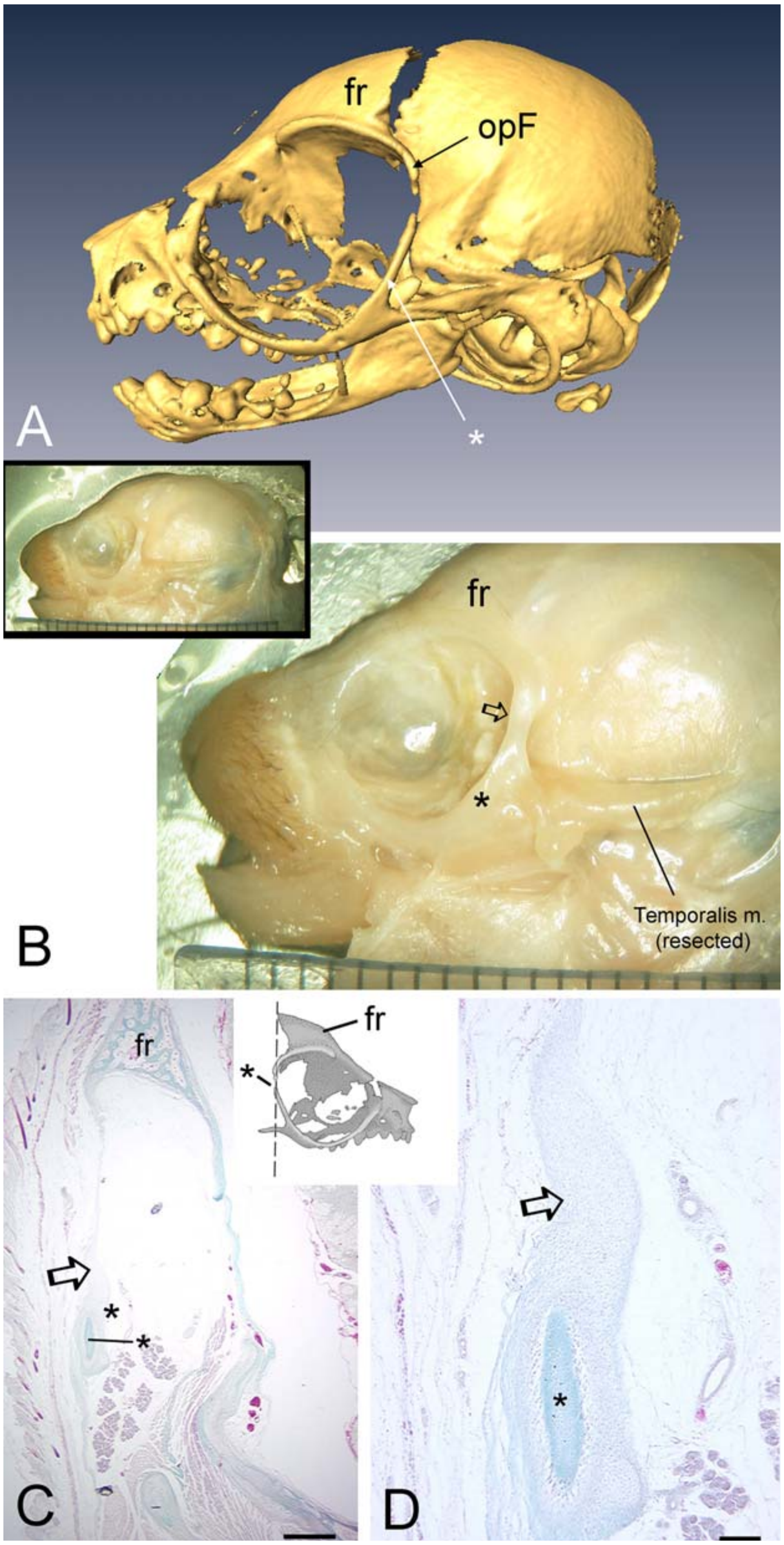

Fig. 7. In *Mirza coquereli* (**A**, CT reconstruction) the postorbital bar is incomplete at birth, with a small gap between the orbital process of the zygomatic (*) and the orbital process of the frontal bone (opF). Dissection of the same specimen reveals a cord-like ligament completes the postorbital bar (open arrow, **B**). Histological examination reveals that the postorbital bar of *Mirza* is preceded by a transient, thick ligament (**C**,**D**). fr, frontal bone. Stains: C, D, Gomori trichrome. Scale bars: C, 100 μm; D, 50 μm.

relatively large eyes (Fig. 1H). Furthermore, the ZFA of anthropoids is histologically distinct from that of the tarsier at birth. All anthropoids studied thus far exhibit a typical facial suture at the ZFA at the time of birth. This suture develops between two facial bones with distinctly separated periostea (Sperber, 2001). At the perinatal age, only loose connective tissue separates them (Supporting Information Fig. S3), as previously described for immature facial sutures (Pritchard et al., 1956; Sperber, 2001). In contrast, the tarsier POM is a ligamentous joint. As a fan-shaped sheet, its role is to support the large eye posterolaterally before bony support is fully formed. The possibility that there is some fibrocartilage in the POM would be in keeping with previous descriptions of the postorbital ligament of the rabbit (Jašarević et al., 2010).

To propose that the tarsier configuration evolved into an anthropoid-like facial suture would require a transition between distinct joint types with different mechanical functions and biological roles in the two groups. The POM of tarsiers is unique among extant primates, representing a morphology that is distinct from any stage of development in anthropoids. Its structure relates to the function of enclosing protruding orbital contents, similar to the purpose of the more typical postorbital ligament of some other mammals that lack an osseous septum (Jašarević et al., 2010). In the case of infant tarsiers, it is most likely that the adaptive evolution of this tissue arrangement was driven by mechanical requirements associated with a uniquely large pulse of eyeball growth, which occurs soon after birth (Cummings et al., 2012). The POM of tarsiers has more in common with a neurocranial fontanelle, an expansive collagenous intersection of more than two calvarial bones (Enlow and Hans, 1996; Opperman, 2000), than a facial suture. In this sense, it can be thought of as an “accessory fontanelle,” a spatial separator and structural compromise that provides flexible mechanical stability while anticipating rapid postnatal eye growth.

Thus, *Tarsius* possesses neither a POB nor a POS at birth. It is neither a suture as seen in late fetal and newborn anthropoids; nor is it like the thin membranous precursor sheet (periorbita) that precedes suture formation in prenatal anthropoids; nor is it the same as the connective tissue cord that transiently attaches the zygomatic and frontal orbital processes in perinatal cheirogaleids (e.g., Fig. 7D). By extension, our observations indicate that the entire postnatal trajectory of POS development differs between anthropoids and tarsiers. Closure of the lateral orbital mosaic in anthropoids is completed by inward and posterior growth of a lamina off the orbital process of the zygomatic, whereas in tarsiers it is primarily an outward extension derived from the frontal bone. In tarsier specimens spanning fetal and

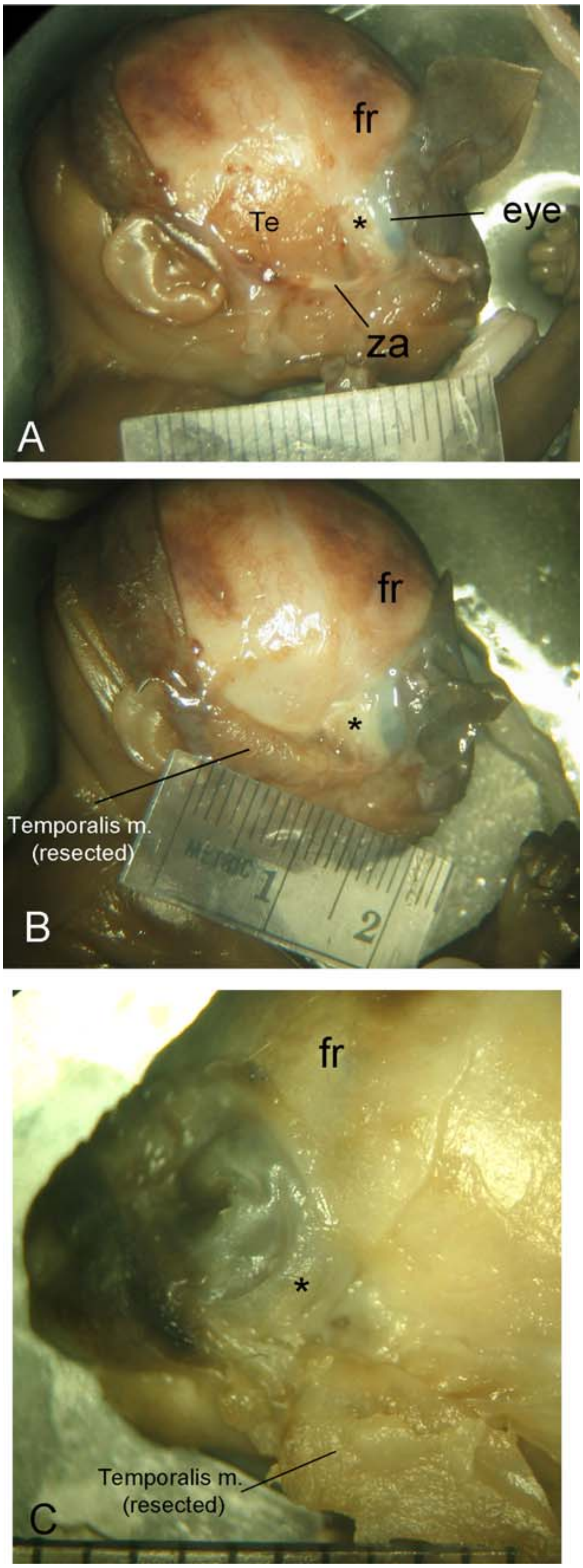

Fig. .8

Fig. 8. (**A**) Dissection of lateral orbit in a late fetal howler monkey (*Alouatta seniculus*). Here, the orbital process of the zygomatic bone (*) is in direct contact with the frontal bone (fr). This bone is also expanded posteriorly with broad proximity to the frontal, as shown after resection of the temporalis muscle (**B**). Note the broad contact between the frontal and zygomatic bones. (**C**) A fetal *Saguinus* has a zygomatic process that is not fully in contact with the frontal bone, although the postorbital lamina does extend posteriorly. za, zygomatic arch. Rulers = mm.

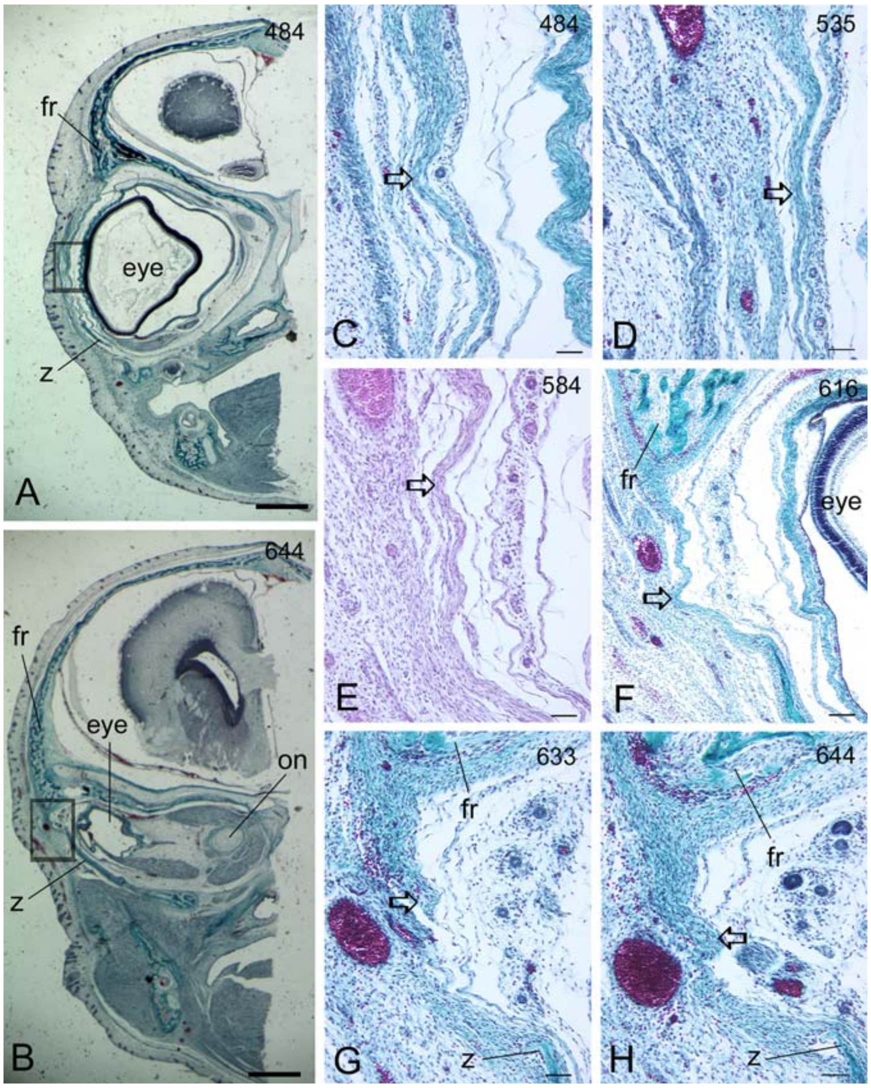

Fig. 9. (**A**,**B**) A fetal tamarin (*Saguinus geoffroyi*) showing the posterior orbit just anterior (A) and at the very posterior limit (B) to the zygomaticofrontal joint. The boxes show the location of higher magnification views that are shown in **C** and **H**, respectively. Plates **D** through **G** show serial sections between C and H (serial section levels show on top right of each plate). Note there is little difference in the periorbital fascia (open arrows) throughout the series. Stains: Gomori trichrome except in E (hematoxylin and eosin). fr, frontal; on, optic nerve; z, zygomatic. Scales: A,B, 1 mm; C–H, 50 μm.

early postnatal ages, there is no apparent ossification in the portion of the POM that reaches between zygomatic and alisphenoid. In older subadult tarsiers, the alisphenoid develops a thin band of bone (Schwartz, 2003) that extends beyond the braincase and contacts the zygomatic. Eventually, ossification occurring within the POM at its attachments to the frontal, zygomatic, and sphenoid forms a plate-like, partial POS (Fig. 10). This plate is the partial POS of tarsiers, and its ossification within a broad membrane explains, in part, why this region differs anatomically from anthropoids in the adult state. The osteology of previous studies has thus missed a critical nuance by not considering ontogeny (but see Schwartz, 2003).

We suggest the presence of the POM in the lateral orbit of perinatal tarsiers represents a unique phenotype rather than a transitional attribute that reflects heterochronic differences between tarsiers and anthropoids. There is currently no paleontological basis from which to infer that the last common ancestor of tarsiers and anthropoids had hypertrophic eyes along with a POS. Given the tarsier's extreme specialization, it seems more likely that the POM is part of an integrated system of perinatal support and subsequent ossification pathway, evolved to support the extraordinary adult eye size (which by adult age is larger in volume than the tarsier's own brain—Castenholtz, 1984).

CONCLUSIONS

Recognizing homology in adult organisms with greatly divergent morphologies can be immensely challenging. Basic similarities (or differences) in body plans can

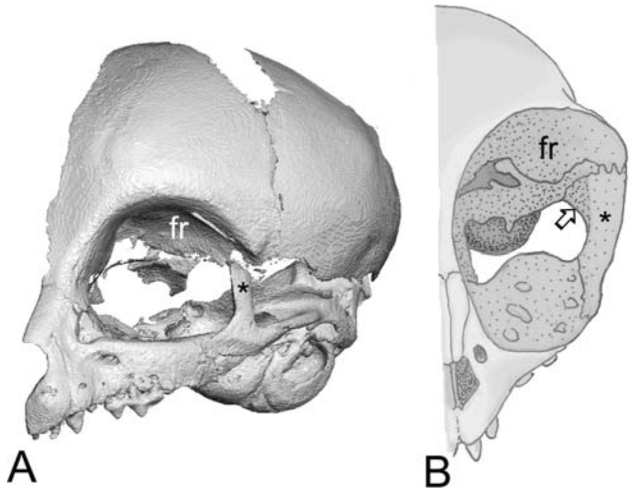

Fig. 10. Comparison of a newborn *Tarsius syrichta* (**A**) to an adult *Tarsius bancanus* (**B**, based on AMNH # 106649). Note that the orbital process of the zygomatic (*) remains spatially separated from the calvaria in the adult and, unlike anthropoids, only meets the braincase through a bridge formed by processes that grow outward from the orbit postnatally. The only nonsize related or proportional change in this specimen involves the development of a small medial flange that grows inward to meet the alisphenoid (open arrow, B).

become obscured under a host of adaptations in the adult (Gould, 1977). It is precisely this dilemma that has created a challenge when comparing the orbits of tarsiers and anthropoids. In such comparisons, the most informative evidence to distinguish between homology and analogy may be developmental.

In considering developmental evidence, we are mindful that ontogeny itself is subject to selection, and phenotypes that are structurally homologous may emerge by different mechanisms (Hall, 1999). However, the late prenatal/early postnatal morphology of tarsiers appears to be adapted to a unique ontogenetic trajectory. In other words, the structure at birth is functionally well designed for tarsiers but not for any known anthropoids (living or fossil). These results, detailing profound gross and histological differences between tarsiers and anthropoids at birth, are consistent with the notion that tarsiers evolved a novel postorbital morphology that corresponds with their specialized, enormously enlarged eyes. The evolution of enormously large eyes in tarsiers presents an instance where selection for an intensely growth-dependent adult morphology profoundly influences the ontogenetic program and resultant morphology of functionally related structures; here, the lateral orbital mosaic. The tarsier postorbital region is morphologically, histologically and ontogenetically distinguished from that in tree shrews, strepsirhines and anthropoids as follows: (i) prenatal loss or suppression of the orbital process of the frontal bone (which itself could be a haplorhine synapomorphy); (ii) its replacement by a unique POM that makes the ZFA functionally analogous to a fontanelle; (iii) postnatal development and ossification of what may be a secondary orbital process of the frontal bone within the POM; and, (iv) a different mechanism and timing for fusion of the zygomatic with the alisphenoid. In sum, this suggests the entirety of the lateral orbital mosaic of *Tarsius* is a neomorphic structure.

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