



On the “cartilaginous rider” in the endocasts of turtle brain cavities

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Abstract

In recent years, paleoneurology became a very popular research field and hundreds of brain-endocasts were described. The interpretation of a dorsal protuberance of the brain-endocast puzzled researchers for a long time, the so-called (cartilaginous) rider. This is mainly because of technical limitations in the past and due to non-accessibility of comparative material. Using turtles as a case-study, we conducted a literature review and studied embryological data in addition to fossil and extant species' endocasts. We assessed three hypotheses on the origin of the rider as relating to 1) the pineal gland, to 2) the blood vessel system, and to 3) skull roof elements. Based on our integrated anatomical observations, we refute the pineal gland hypothesis (1) and an exclusive blood vessel explanation (2). However, we show that, in most cases, the cartilaginous origin applies (3). The related cartilages, mainly the anterior process of the chondrocranial tectum synoticum, can persist until adulthood. Its diversity is interpreted in regard to the mechanical support for the temporal skull region, the shape of which has been shown to be in turn related to neck retraction and jaw mechanics. Finally, we highlight the value of embryological data to provide profound hypotheses for evolutionary research despite its low quantitative evaluability. We argue that it should be studied in conjunction with modern computer-aided data acquisition whenever possible.

Keywords

brain, chondrocranium, pila antotica, primary braincase wall, tectum cranii, Testudinata

Introduction

Thanks to the development of computed tomography (CT) in the last 20 years and its increasing application to earth and life sciences, non-destructive analyses of

anatomical structures, otherwise not assessable for macroscopical research, became possible (Cunningham et al. 2014; Laaß et al. 2017; Rowe et al. 2011; Schillinger et

al. 2018; Witmer 1995, 1997). The braincase, that is covered by several dermal bones in the adult skull, can now be analyzed in great detail, particularly in regard to its complete shape as well as to its internal structures such as bones, blood-, and nerve canals (e.g., Ferreira et al. in press; Rollet et al. 2021). The braincase is mainly a derivative of the embryonic chondrocranium, which is initially related to the protection of sense organs – nose, eyes, ears –, cranial nerves, and the brain (Yaryhin and Werneburg 2018). The brain, however, does not attach closely to the braincase in most living reptiles (Edinger 1929; Hopson 1979), whereas they are closer in macrocephal birds and mammals (Early et al. 2020; Orliac et al. 2014; Knoll and Kawabe 2020). Instead, it is embedded in cerebrospinal fluid, which is produced via ultrafiltration by a highly vascular choroid plexus (tela choroidea) above the myelencephalon (Wyneken 2001). As such, it is difficult to correlate the internal shape of the braincase directly to the external anatomy of the brain.

Paleoneurology was established as a research program almost 100 years ago (Edinger 1929), and a number of original articles describing single endocasts of fossil vertebrates were published based on preservational natural casts (steinkerne) or on elastic caoutchouc/latex casts. After Edinger (1929), Hopson (1979) presented a unifying review on fossil brain endocasts, and a recent account provides a quantitative assessment to amniote paleoneurology and brain evolution using most modern methodology available (Dozo et al. in press). Given that most of the brain cavity is filled by cerebrospinal fluid in reptiles, there might be relatively little significance of endocast shape to inform detailed brain anatomy and evolution (Fig. 1A–B), besides the important examinations on proportional and general size changes (Hopson 1977; Koyabu et al. 2014; Lautenschlager et al. 2018; Weisbecker and Goswami 2014).

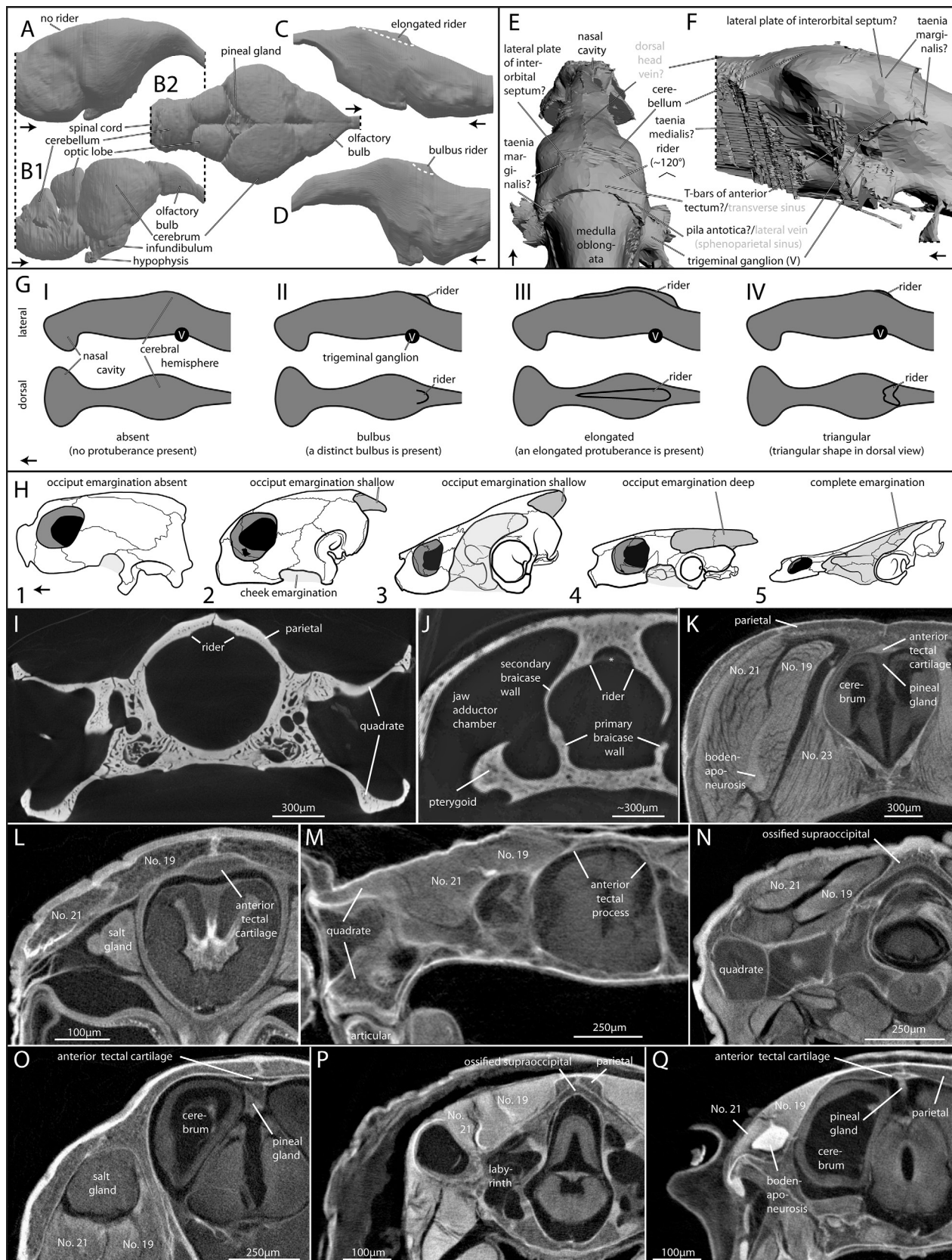
Only little work has been done to correlate endocast shape to actual brain anatomy in living vertebrates (e.g., Balanoff et al. 2016; Clement et al. 2021; Evers et al. 2019; Kim and Evans 2014), which was also problematic due to technical limitations for a long time. Contrast-enhanced computed tomography (Gignac et al. 2016; Lautenschlager et al. 2014) or magnetic resonance imaging

(MRI) (Evers et al. 2019) aid solving this issue nowadays; however, automated reconstructions are difficult for soft tissue, and manual reconstructions are time-consuming. Scientifically more difficult, however, is the fact that each taxon has an individual content of cerebrospinal fluid and individual brain and skull proportions (Jones 1979) making it difficult to generalize brain-endocast relationships (Paulina-Carabajal et al. 2013). That is, because the braincase not only serves as protective organ in the adult (Werneburg 2019/2020; Werneburg and Yaryhin 2019), but also as an anchor for dermatocranial bones (Pitirri et al. 2020) that underlie a variety of different morphofunctional constraints (Hanken and Hall 1993).

In turtles, for example, neck retraction largely influences the shaping of the whole skull and already embryologically, the cartilaginous elements of the developing skull experience reorientations. Embryonic neck forces push the palatoquadrate against the braincase, closing the originally wide spaced cranioquadrate passage (Werneburg and Maier 2019). This and related architectural skull changes across turtle evolution influenced the function of the jaw musculature (Ferreira and Werneburg 2019; Jones et al. 2012; Werneburg 2011, 2012, 2013a, 2013b). To retain the ancestral jaw muscle power, the cranium became akinetic by stiffening the basicranial articulation and by forming a secondary braincase wall, among other changes (Rabi et al. 2013; Werneburg and Maier 2019). It would not be surprising if the braincase and endocranial cavity in turtles are also affected by those morphological changes.

In recent years, knowledge on endocast anatomy of fossil and extant turtle species has increased enormously, and all major taxa were analyzed by at least one specimen (Evers et al. 2019; Ferreira et al. 2020; Ferreira et al. in press; Hermanson et al. 2020; Lautenschlager et al. 2018; Paulina-Carabajal et al. 2013, 2017, 2019a,b). One curiosity, however, often appears in turtle endocast literature, namely, the presence and diversity of the so-called 'rider' or 'cartilaginous rider'. It represents, in most cases, a bulbous or elongated protuberance (Fig. 1), dorsal and anterior to the cerebellar part of the endocast, and it is also known in some other reptiles (Witmer et al. 2008). Alternative interpretations of the cartilaginous

Figure 1. Rider diversity and skull categories. A) Braincase endocast in *Emydura subglobosa* (IW92) with no rider on the endocast; B) Brain tissue of the same specimen as in A in lateral (B1) and dorsal view (B2); C) an elongated rider in *Chelydra serpentina* (UFR VP1); D) a bulbous-like rider in *Caretta caretta* (NHMUK1940.3.15.1); E–F) † *Plesiochelys etalloni* (modified after Paulina-Carabajal et al. 2013), in posterodorsal view (E) and in oblique lateral (F) view – gray labels based on Paulina-Carabajal et al. (2013), black labels with “?” based on the presented hypothesis; G) four rider categories discussed herein, outlines based on the endocast of † *P. etalloni*; H) morphotypes of posterior temporal emargination discussed herein, images modified after Werneburg (2012) and Ferreira and Werneburg (2019): from left to right: *Dermochelys coriacea*, *Chelonia mydas*, *Cuora trifasciata*, *Emydura macquarii*, *Chelodina expansa*. I) Image of a μ CT-scan of a macerated skull of *Malacochersus tornieri* (SMF-58702) in the cerebellum region. J) Image of a μ CT-scan of a macerated skull of *Podocnemis unifilis* (SMF-55470) in the cerebrum region; * = partly calcified cartilage of the anterior tectal process is visible. K–Q) Contrast-enhanced stainings using PTA-solution in K) *Platyστεrnon megacephalum* (R12559); L) a juvenile *Dermochelys coriacea* (IW1476), M) a juvenile *Chelus fimbriatus* (IW1148), N–O) a hatchling *Caretta caretta* (IW1681), P–Q) *Apalone spinifer aspera* (R12970). Abbreviations (following Werneburg 2011): No. 19 = musculus (m.) adductor mandibulae externus Pars profundus, No. 21 = m. adductor mandibulae externus Pars superficialis, No. 23 = m. adductor mandibulae internus Pars pseudotemporalis. For institutional abbreviations see caption to Table 1. Arrows in A–H indicate anterior.



rider were discussed in the literature: 1) as the cavity of the pineal gland (Paulina-Carabajal et al. 2013; cited after Paulina-Carabajal 2017; Deantoni et al. 2012), 2) as imprints of blood vessels (Paulina-Carabajal et al. 2017; Paulina-Carabajal et al. 2013; Paulina-Carabajal et al. 2019a,b; Witmer et al. 2008) and/or 3) as structures of

the skull roof (Evers et al. 2019; Gaffney and Zangerl 1968; Hopson 1979; Paulina-Carabajal et al. 2017; Paulina-Carabajal et al. 2019a,b). A detailed morphological survey on the diversity of this part of the endocranium and the morphological correlates to this area in the brain cavity is pending. Given the important pre-hatching develop-

mental processes of skull formation in turtles mentioned above, we examined embryonic head anatomy in turtles and observed anatomical relationships to the rider in the brain-cavity (Fig. 2). Moreover, the high resolution and coloration of histological sections allow clear distinction between tissues, which is sometimes difficult in gray-scaled enhanced contrast tomography. We compared our embryological findings to fossil and extant adult skull anatomy aided by 3D reconstructions based on μ CT images, discussing the potential functional significance of the rider.

Materials and Methods

We compared a series of μ CT-scans and brain-endocast reconstructions that we prepared for other studies (i.e., Evers et al. 2019; Ferreira et al. 2020; Ferreira et al. in press; Lautenschlager et al. 2018) as well as literature data on turtle brain endocasts to analyze the anatomy of the rider (Table 1; Werneburg et al. 2021). In fossils with natural cast (steinkern) preservation, as often published in old literature, the absence of a rider can be related to two alternative reasons: (a) sediment filled the cavity before the cartilaginous process of the supraoccipital (see results below) decayed or (b) the cartilaginous process was ossified and the specimen, which was fossilized, never had the space to correspond to the (cartilaginous) rider. In digital endocasts, the absence of the rider can only be attributed to the second option (b). Since it is a "digital filling" of the endocranial cavity, it does not matter when the cartilage decayed. In the fossilized specimens, the cartilage would never be there, so in the CT images, one would see it as an empty space, regardless of the stage at which the cartilage decayed. In this case, the absence of the rider would be direct evidence that it was not present in the respective taxon.

For our study, we had access to the embryonic histological collection of Prof. Dr. Wolfgang Maier, Zoologisches Institut der Universität Tübingen (ZIUT), Germany, which houses a number of turtle species that were collected by Stefan Eßwein around 1990. In addition, we studied sections of *Chelydra serpentina* (Inv.-Nr. Rept 1214; carapace length = 31 mm) from Phyletisches Museum Jena, Germany, and *Caretta caretta* from the lab of Prof. Dr. Shigeru Kuratani (SK-lab) in Kōbe, Japan. Sections are mainly stained with Azan after Haidenhain (Mulisch and Welsch 2015) and were photographed using a Canon EOS 650D camera under an Olympus BH-2 microscope. Specimens of interest were late term embryos.

From literature, late term embryos of following species were compared: *Apalone spinifera* (Fig. 2C) (Sheil 2003), *Caretta caretta* (Fig. 2A) (Kuratani 1999), *Chelonia mydas* (Fig. 2M–R') (Parker 1880), *Chelydra serpentina* (Fig. 2D) (Sheil and Greenbaum 2005), *Chrysemys picta* (Fig. 2E) (Shaner 1926), *Emys orbicularis* (Fig. 2G) (Kunkel 1912), *Eretmochelys imbricata* (Fig. 2H–I) (Fuchs 1915; Sheil 2013), *Macrochelys temminckii*

(Fig. 2J) (Sheil 2005), *Pelodiscus sinensis* (Fig. 2K) (Sánchez-Villagra et al. 2009), *Phrynosoma hylarum* (Fig. 2L) (Bona and Alcalde 2009), *Podocnemis unifilis* (Fig. 3B) (Sheil and Zaharewicz 2014), and *Trachemys scripta* (Fig. 2M) (Tulenko and Sheil 2007).

Results

Observations of μ CT slices of enhanced contrast stained specimens (with phosphotungstic acid, i.e. PTA, see Ferreira et al. 2020) do not show any brain tissue filling the rider cavity (Fig. 1K–Q). It instead shows the supraoccipital bone remaining cartilaginous more anteriorly, which is clearly seen due to different gray-scales in the images (Fig. 1K–O). As a consequence, the endocasts reconstructed based on enhanced contrast-stained specimens do not show the rider (not used for comparison in our study as outlined in Table 1), in contrast to those based on μ CT images of unstained macerated or fossil skull specimens (Fig. 1I–J).

A survey on a variety of extinct and extant turtle adult brain-endocasts (Table 1; Werneburg et al. 2021) allowed us to categorize the diversity of the rider-region in the cerebellar area in four morphotypes (Fig. 1G): I) absent (no protuberance is present), II) bulbus (a distinct bulbus is present), III) elongated (an elongated protuberance is present), IV) triangular (the rider has a short triangular shape in dorsal view). These categorizations only describe general shapes, and there are fluent transitions between the states and the prominence of riders varies among taxa. Rider categories also show inter- and – as known for *Chelonia mydas* for example (Table 1) – even intraspecific variation in the endocast. Intraspecific variation might be related to ontogenetic age and size of the adult specimens. Many stem- and many crown turtles show a bulbus-like rider, cryptodires often have an elongated rider, but a number of notable exceptions exist in all groups – hampering any robust phylogenetic conclusion. A triangular rider shape was only rarely observed (Table 1). There is, however, a clear tendency that a bulbus-like rider corresponds with no (Fig. 1H1) or a shallow (2–3 in Fig. 1H) occiput emargination (i.e., posterodorsal emargination *sensu* Werneburg 2012), whereas an elongated rider (Fig. G–III) corresponds with a deep occiput (4 in Fig. 1H) or a complete (5 in Fig. 1H) emargination (see Table 1).

Histological analyses of late term embryos revealed detailed anatomy of the structures surrounding the cerebellum and cerebrum (Fig. 2). We had a particular focus on the space for the cerebrospinal fluid, on blood vessels, and on bones and cartilages. Details will be described and will be compared to temporal coverage categories (Fig. 1H, Table 1) in the discussion section.

A literature survey on the diversity and the development of the tectum synoticum in late term embryos revealed that a clearly defined anterior tectal process is present in all turtles. Length of the late embryonic pro-

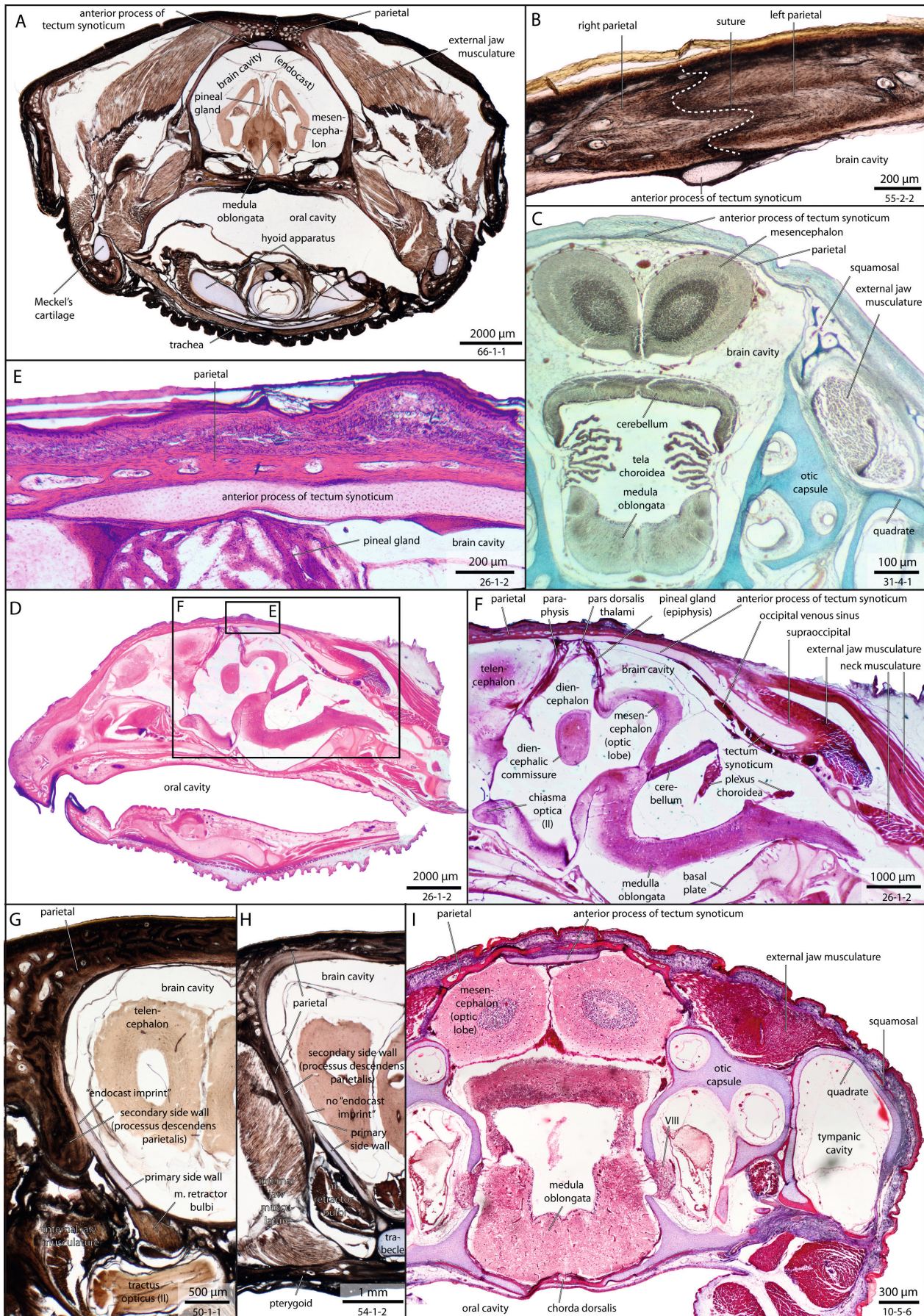


Figure 2. Histological sections of late term embryos and a juvenile turtle. A–B, G–H) *Pelomedusa subrufa* (ZIUT, carapace length, CL = 96 mm); C) *Chelonia mydas* (ZIUT, CL = 24 mm); D–F) *Chelydra serpentina* (CL = 31 mm); I) *Chelodina longicollis* (ZIUT, CL = 18 mm; head length, HL = 11.7 mm). Section numbers in the right lower corner. A–C, G–I) cross, D–F) sagittal sections.

Table 1. Endocast specimens compared in this study. Rider category (Fig. 1G) and emargination types (Fig. 1H) are listed. Note that these classifications are only tentative, rough, and partly subjective given the great diversity in shape and prominence of riders and emarginations among turtles. Institutional abbreviations: AMNH, American Museum of Natural History, USA; CAMSMB, Sedgwick Museum of Earth Sciences, UK; DMNH, Denver Museum of Nature and Science, USA; FMNH, Field Museum of Natural History, USA; GPIT, Paleontological Collection Tübingen; IVPP, Institute of Vertebrate Paleontology and Paleoanthropology, China; IW, Ingmar Werneburg Private Collection; MB, Museum für Naturkunde Berlin, Germany; MIWG, Museum of Isle of Wight Geology, UK; NHMUK, Natural History Museum, UK; PIMUZ, Laboratory collection of Paläontologisches Institut und Museum der Universität Zürich, Switzerland; R, Reptile collection of SMNS; SMF, Senckenberg Museum Frankfurt, Germany; SMNS, Staatliches Museum für Naturkunde Stuttgart, Germany; USNM, United States National Museum, USA; WGJ, Walter G. Joyce Private Collection; YPM, Yale Peabody Museum, USA; ZMB, Zoologisches Museum Berlin, Germany; UFRVP, Université de Fribourg, Switzerland. For 3d-models reconstructed by us (*), please see Werneburg et al. (2021).

| Major taxon | Species | Collection number | reference | Rider category | Posterior emargination |
|-------------------------------|---|--|---|-------------------|------------------------|
| Proganochelyidae | † <i>Proganochelys quenstedtii</i> * | MB 1910.45.2 (Berlin specimen) | Lautenschlager et al. (2018) | Bulbus | Shallow |
| | † <i>Proganochelys quenstedtii</i> * | SMNS 16980 (Stuttgart specimen) | Lautenschlager et al. (2018) | Absent/bulbus | Shallow |
| Meiolaniidae | † <i>Gaffneyania auricularis</i> | MPEF-PV 10556 | Paulina-Carabajal et al. (2017) | Absent | Shallow |
| | † <i>Meiolania platycephala</i> | MMF 13825a | Paulina-Carabajal et al. (2017) | Bulbus | Shallow |
| | † <i>Naomichelys speciosa</i> * | FMNH-PR-273 | Paulina-Carabajal et al. (2019); Werneburg and Joyce (2021) | Bulbus | Shallow |
| | † <i>Niolamia argentina</i> | MLP 26–40 | Paulina-Carabajal et al. (2017) | Bulbus | Shallow |
| Baenidae | † <i>Eubaena cephalica</i> * | DMNH 96004 | Ferreira et al. (in press) | Elongated | Deep |
| | † <i>Plesiobaena antiqua</i> | UCMP 49759 | Gaffney (1982) | Triangular | Shallow |
| Plesiochelyidae | † <i>Plesiochelys etalloni</i> | MH 435 | Paulina-Carabajal et al. (2013) | Triangular | Deep |
| Testudines, Sandownidae | † <i>Sandownia harrisi</i> * | MIWG3480 | Ferreira et al. (in press) | Bulbus/elongated | Deep |
| Pleurodira, Bothremydidae | † <i>Bothremys cooki</i> | AMNH 2521 (Type skull) | Gaffney and Zangerl (1968) | Bulbus/ Elongated | Deep |
| | † <i>Bothremys barberi</i> | FMNH PR 247 | Gaffney and Zangerl (1968) | Bulbus/ Elongated | Deep |
| | † <i>Chedighaii hutchisoni</i> | KUVP 14765 | Deantoni (2015) | Absent/bulbus | Deep |
| Pleurodira, Chelidae | <i>Chelodina reimanni</i> * | ZMB 49659 | Ferreira et al. (in press); Lautenschlager et al. 2018) | Bulbus/ Elongated | Deep |
| | <i>Emydura subglobosa</i> * | IW92 | Ferreira et al. (in press) | Absent | Shallow |
| Pleurodira, Podocnemidae | † <i>Yuraramirim montealtensis</i> | MPMA 04-0008/89 | Ferreira (2018) | Elongated | Shallow |
| | <i>Podocnemis unifilis</i> * | SMF-55470 | Ferreira et al. (in press) | Elongated | Deep |
| | <i>Erymnochelys madagascariensis</i> | AMNH living Reptiles 63579 | Gaffney and Zangerl (1968) | Absent | Deep |
| Cryptodira, Protostegidae | † <i>Rhinocelys pulchriceps</i> * | CAMSMB_B55775 | Evers et al. (2018), Ferreira et al. (in press) | Bulbus | Shallow |
| Cryptodira, Xinjiangchelyidae | † <i>Annemys sp.</i> * | IVPP-V-18106 | Ferreira et al. (in press) | Elongated | Deep |
| | † <i>Xinjiangchelys radiplicatoides</i> * | IVPP V9539 | Ferreira et al. (in press) | Bulbus/ Elongated | Deep |
| Cryptodira, Trionychidae | <i>Apalone spinifera</i> * | FMNH 22178 | Ferreira et al. (in press) | Elongated | Deep |
| | <i>Pelodiscus sinensis</i> * | IW576-2 | Ferreira et al. (in press); Lautenschlager et al. (2018) | Elongated | Deep |
| Cryptodira, Emyderna | <i>Platysternon megacephalum</i> * | SMF-69684 | Ferreira et al. (in press) | Absent | Shallow |
| | <i>Trachemys scripta</i> * | See Evers et al. 2019 for specimen information | Ferreira et al. (in press) | Bulbus | Deep |

| Major taxon | Species | Collection number | reference | Rider category | Posterior emargination |
|--------------------------|---------------------------------|------------------------------------|---|-------------------|------------------------|
| Cryptodira, Geocemydidae | <i>Cuora amboinensis</i> * | NHMMUK69.42.145_4 | Ferreira et al. (in press) | Bulbus/elongated | Deep |
| | <i>Rhinoclemmys funereal</i> * | YPM12174 | Paulina-Carabajal et al. (2017), Ferreira et al. (in press) | Bulbus | Deep |
| Cryptodira, Testudinidae | <i>Chelonoidis chilensis</i> | MPEF-AC 25 | Paulina-Carabajal et al. (2017) | Bulbus | Deep |
| | <i>Gopherus berlandieri</i> * | AMNH-73816 | Paulina-Carabajal et al. (2017) | Elongated | Deep |
| | <i>Kinixys belliana</i> * | AMNH-10028 | Paulina-Carabajal et al. (2017) | Absent | Deep |
| | <i>Malacochersus tornieri</i> * | SMF-58702 | Ferreira et al. (in press); Lautenschlager et al. (2018) | Bulbus/elongated | Deep |
| | <i>Testudo graeca</i> * | YPM14342 | Paulina-Carabajal et al. (2017) | Bulbus | Deep |
| | <i>Testudo hermanni</i> * | AMNH134518 | Paulina-Carabajal et al. (2017) | Bulbus/elongated | Deep |
| Cryptodira, Cheloniodea | † <i>Corsochelys haliniches</i> | CNHM PR 249 | Zangerl (1960) | Elongated | Shallow |
| | <i>Caretta caretta</i> | CNHM 31022 | Zangerl (1962) | Bulbus/ Elongated | Shallow |
| | <i>Caretta caretta</i> * | NHMMUK1940.3.15.1 | Ferreira et al. (in press) | Bulbus | Shallow |
| | <i>Chelonia mydas</i> | CNHM 22066 | Zangerl (1962) | Bulbus/ Elongated | Shallow |
| | <i>Chelonia mydas</i> * | ZMB-37416MS | Ferreira et al. (in press) | Absent | Shallow |
| Cryptodira, Chelydridae | <i>Chelydra serpentina</i> * | UFR VP1 | Ferreira et al. (in press) | Elongated | Deep |
| | <i>Macrochelys temminckii</i> * | GPIT-PV-79430 (syn. GPIT/RE/10801) | Ferreira et al. (in press); Lautenschlager et al. (2018) | Bulbus/elongated | Shallow/Deep |

cess differs among species and to individual degree of development. *Caretta caretta* and *Podocnemis unifilis* late term embryos show aberrant, T-shaped appearances of this process (Fig. 3A–B).

Discussion

The origin of the rider in the dorsal part of the endocast was alternatively explained by three hypotheses in the past. Based on our comparisons, we reject the pineal-gland-hypothesis (1), we argue that the vessel-hypothesis applies, if at all, only rarely (2), and consider the skull roof hypothesis as applying in most cases (3). In particular, we show that an anterior process of the cranial tectum and other elements of the primordial (chondrocranial) skull can persist and differentiate until adulthood and leave traces in the brain cavity.

Pineal gland

The pineal gland is a dorsal median projection of the brain, laying between the telencephalic hemispheres anteriorly and the mesencephalic optic lobes posteriorly and can be associated with other outgrowths of the dorsal diencephalon (Fig. 1B', 2F). The leatherback marine turtle *Dermochelys coriacea* has as adult a dorsal expansion

of the endocranial cavity (* in Fig. 4C: note, the section is not complete median and the cavity is here filled with cartilage), which houses a very elongated pineal gland (Wyneken 2001: fig. 192). Paulina-Carabajal et al. (2013) interpreted this pineal cavity (partly) as the endocast rider that was first described for two extinct marine († *Bothremys cooki* et *barberi*) and one extant [*Erymnochelys* (“*Podocnemis*”) *madagascariensis*] pleurodire turtle (Gaffney and Zangerl 1968).

Like *D. coriacea*, other extant marine turtles (Cheloniodea) also have a relatively long pineal gland, which is, however, not surrounded by bone in a separate dorsal cavity (Fig. 1Q; (Wyneken 2001: fig. 193–194, 196–200; discussed by Paulina-Carabajal et al. 2017), making the pineal-gland-hypothesis very unlikely. Compared to other extant marine turtles, however, the adult leatherback has a particularly shortened and highly domed skull (Nick 1912; van Bemmelen 1896; Wegner 1959) with several related characteristics (Fig. 4C–D). The jaw musculature, for example, extends in a straight line between the skull roof and the lower jaw in adults (Schumacher 1972). Consequently, the trochlear mechanism at the otic capsule, usually present in cryptodires (Ferreira et al. 2020; Ferreira and Werneburg 2019; Werneburg 2012), was lost in this species (Burne 1905; Poglayen-Neuwall 1953; Schumacher 1972; Werneburg 2013b). The proportional skull changes in *D. coriacea*, in our opinion, are also mirrored in the orientation of internal skull structures, the anatomy of which can only be understood from an ontogenetic perspective and can, hence, help interpreting the

origin of the pineal cavity in this species. That is, in a hatchling studied by us the pineal gland is clearly placed below the anterior tectal cartilage, and no pineal cavity is yet formed (Fig. 1K).

Vascular system

In the studied late term embryos, there is – with the notable exception of *Chelodina* with its flattened skull (Fig. 1H–5, 2I) – always a conspicuous distance between brain tissue and the surrounding skull elements, which is filled with cerebrospinal liquid. Blood vessels have enough space within this liquid (Fig. 2F) and do not leave any imprint to the internal surface of the skull in turtles that could represent a rider. In the stained CT-scans, we never identified any imprinting vasculature on the dorsal and lateral braincase wall. However, there is clear evidence that there is, in most cases, much space between brain tissue and the borders of the brain-cavity in adult turtles also (Edinger 1929; Evers et al. 2019; Ferreira et al. in press; Wyneken 2001; Zangerl 1960). Only in few specimens, such as in the hatchling of *Podocnemis erythrocephala* studied herein, brain tissue comes in close contact to the skull in the cerebellar region (Fig. 1B; see also Fig. 1). *Podocnemis*, however, shows a typically elongated (cartilaginous) rider in the endocast as is also known for specimens with a lot of space between brain tissue and the border of the brain-cavity. A hypothesis that the “rider” on the endocast surface is *exclusively* caused by blood vessels in all turtles must, hence, be refuted.

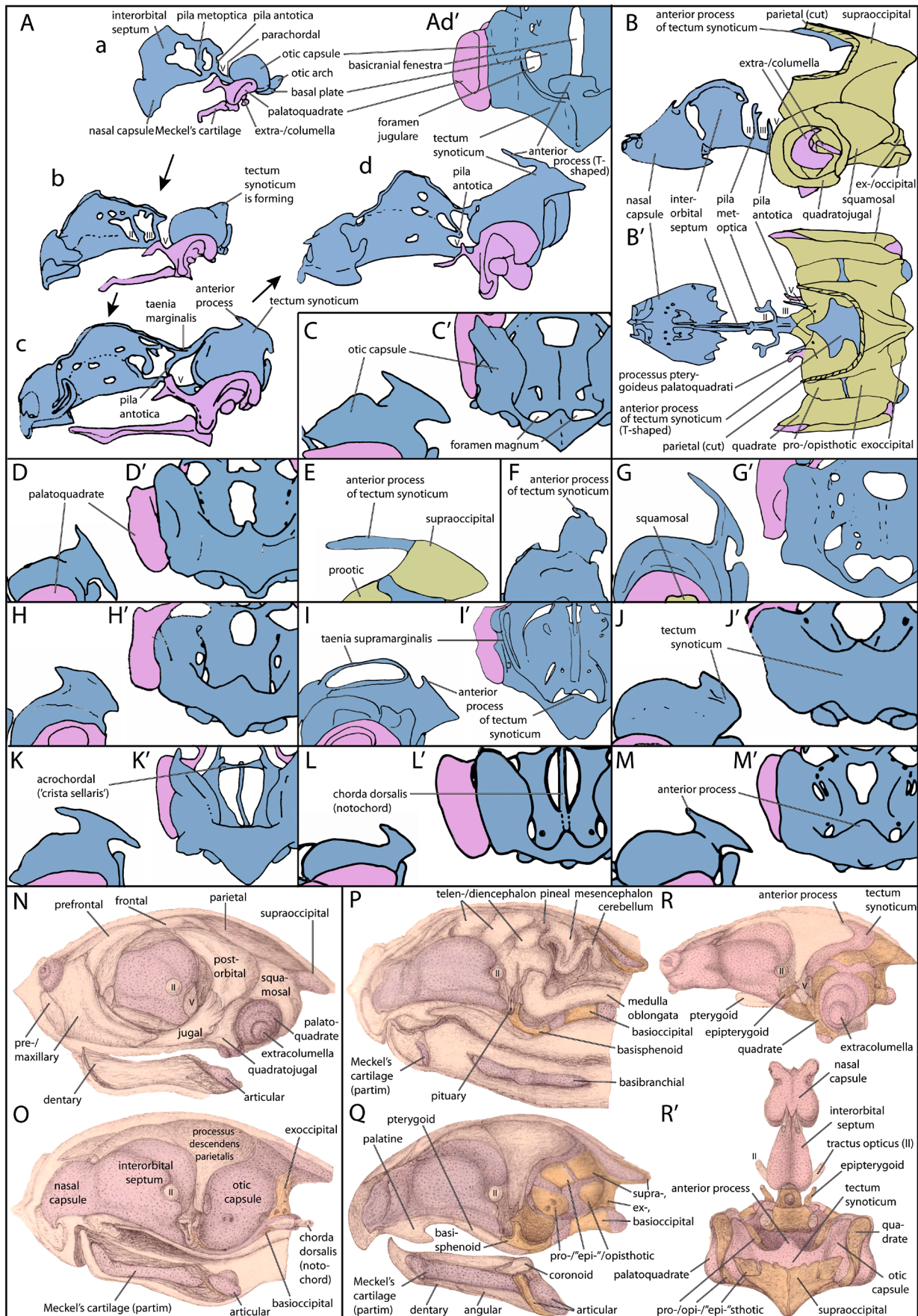
Although we never found any indication of the following development (maybe partly also because of limited data), one could argue that later in ontogeny the brain could – completely or partly – expand such way that it pushes the blood vessels against the braincase internally. However, the skull elements surrounding the brain, the cartilaginous chondrocranium and the dermal skull roof bones – parietal and frontal mainly –, are already well-developed around hatching (Fig. 2D–F, I) and a vascular imprint to the braincase would require enormous forces or reorganizations of the developing skull, which is not conceivable, particularly when considering the soft nature of the vessels. Also, the brain cavity apparently grows faster

than the brain itself, resulting in larger endocranial spaces in older individuals (Ferreira et al. in press). Moreover, in case the soft-tissued vessels are attached to the inner wall of the skull, the probability of vessels to fossilize and then to leave an imprint on the endocast must be considered a highly exceptional event and such fossilized vessels on the skull bones were never recorded or described in any digital reconstruction in turtles.

When considering the blood-vessel hypothesis, Paulina-Carabajal et al. (2013) referred to Witmer et al. (2008), who described blood vessels on the surface of the endocast in *Crocodylus johnstoni*. However, the related CT-based reconstruction was done on a postmortem crocodile in which air “fortuitously had entered the encephalic venous system” (p. 72), which cannot be taken as a hard evidence to discuss the origin of the “rider”. Usually, the blood vessels collapse after death making fossilization even more unlikely. Nevertheless, the correspondence particularly of the sphenoparietal sinus of the crocodile to the v-shaped “rider” and its ventrolateral extension towards the trigeminal ganglion, as interpreted as endocast structures for † *Plesiochelys etalloni* (Fig. 1F–F’) by Paulina-Carabajal et al. (2013), is stunning, but appears to be a reconstruction artifact based on a scan available to us. It might be possible that a very elongated rider as seen in † *Pl. etalloni* might correspond to the dorsal longitudinal sinus along the skull roof (sensu Witmer et al. 2008). Nevertheless, we found no such correspondence in the sampled histological sections or μ CT data, and, hence, consider this hypothesis unlikely (i.e., it rather represents the suture between the frontals). In most cases, the elongated rider might be too massive to correspond to a vessel rather than to the anteriorly extended anterior process of tectum cranii like in *Chelydra serpentina* (Fig. 2D–F).

Late term *Ca. caretta* embryos (Kuratani 1999) and hatchling *Podocnemis unifilis* specimens (Sheil and Zaharewicz 2014) have a very curious shape of the anterior process of the tectum synoticum with anterolateral extensions giving the process a T-shaped appearance in dorsal view (Fig. 3Ad’–B). These observations suggest that among turtles, the adult tectum cranii might develop curious shapes, including anteriorly pointing and converging branches depending on the specific skull architecture and morphofunctional requirements in the adult. It

Figure 3. Late embryonic diversity of the anterior (tectal) process in different turtle species in lateral (normal letter) and dorsal (letter with ‘) views (redrawn from cited references). In Ad’ and C–L’, only parts of the chondrocrania are shown with the left otic capsule for orientation. Earlier stages are shown in C, D, J, M), post-hatching specimens are shown in P–R’. A) four developmental stages of *Caretta caretta* (Kuratani 1999), embryos with a carapace length (CL) of a) 9.2–9.7 mm, b) 11.6–12.6 mm, c) 13.1–14.1 mm, d) > 16.6 mm; B) hatchling of *Podocnemis unifilis* (Sheil and Zaharewicz 2014) with different ossification in the braincase (dermal bones not shown, only parietal cut); C) *Apalone spinifera* (Sheil 2003); D) *Chelydra serpentina* (Sheil & Greenbaum 2005); E) *Chrysemys marginata* (Shaner 1926); F) *Emydura subglobosa* (Werneburg & Yaryhin 2019), G) *Emys orbicularis* (Kunkel 1912); H) *Eretmochelys imbricata* (Sheil 2013); I) *Eretmochelys imbricata* (Fuchs 1915); J) *Macrochelys temminckii* (Sheil 2005); K) *Pelodiscus sinensis* (Sánchez-Villagra et al. 2009); L) *Phrynops hillarii* (Bona & Alcalde 2009). M) *Trachemys scripta* (Tulenko & Sheil 2007); N–R’) *Chelonia mydas* (Parker 1880); N–O) embryo two-thirds ripe (head length: ~ 14.8 mm) with N) all bones in lateral view and O) a median sagittal section; P–R’) ripe young (head length: ~ 23.3 mm) in P) median section, Q) more lateral sagittal section, R) a similar section with less dermal bones shown, and R’) a dorsal view on the chondro-/neurocranium. Coloration in A–L follows Werneburg & Maier (2019: fig. 1): Blue, chondrocranium (cartilage); purple, viscerocranium (cartilage); green, bone (endochondral ossification). Images not to scale.



might even make connections to elements of the primary braincase wall (Fig. 2G–H), including taenia marginalis or pila antotica (Paluh and Sheil 2013), leaving traces on

the lateral aspect of the rider (see black labels Fig. 1E–F as possible alternative to interpret the structures found in † *Pl. etalloni*).

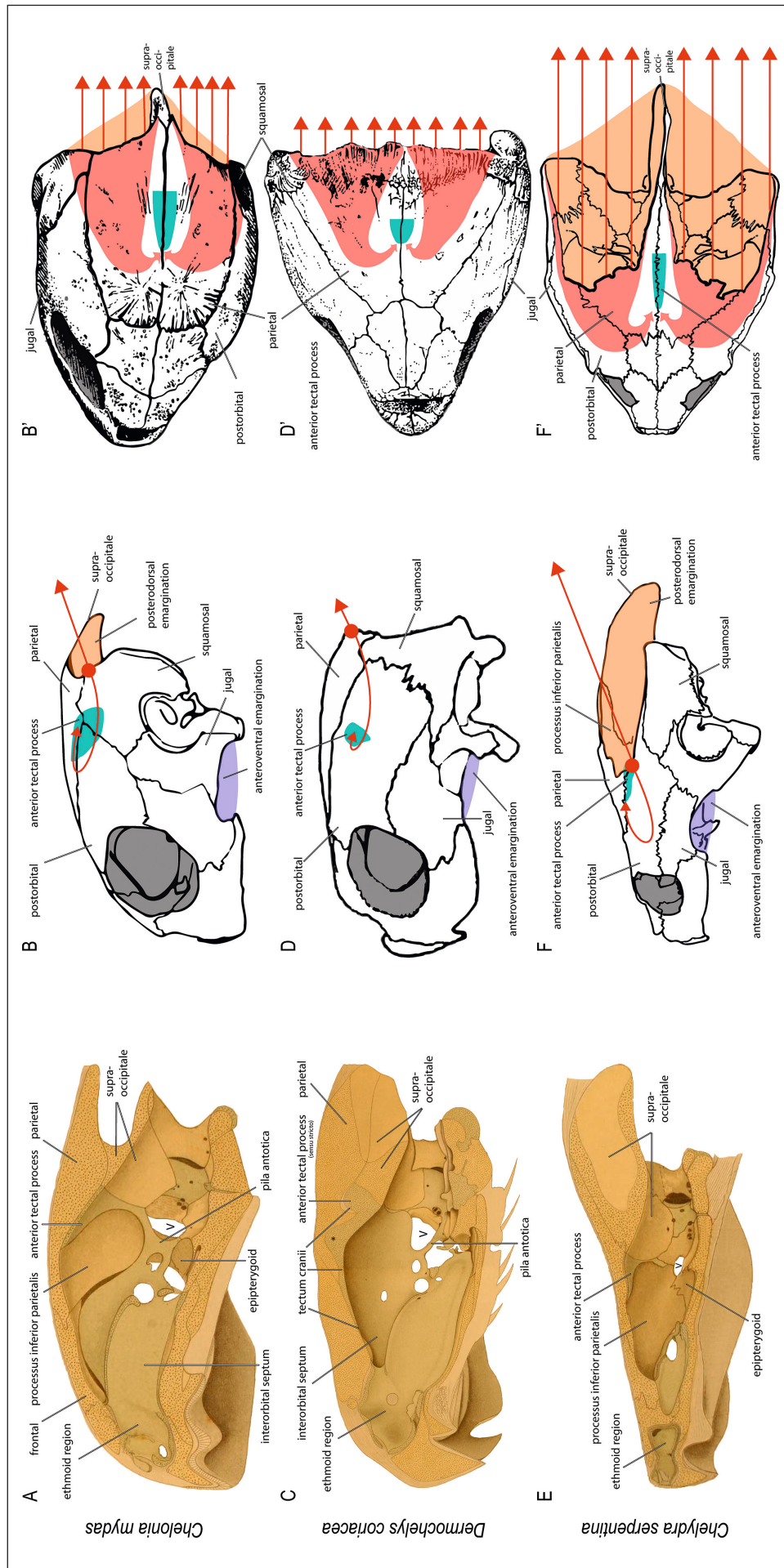


Figure 4. Illustration of the cartilaginous anterior process of the supraoccipital and hypothesis on the force transmissions in the skull. Note the different degrees of ossification in the skull in A, C, and E (note: these images are not exactly on the midline). In *D. coriacea* (C), tectum cranii is fully cartilaginous and continuous with the interorbital septum and – like in *Chelo. mydas* (A) – with the orbitotemporal region. In *Chely. serpentina* (E), only the cartilaginous process and few remainders of the cartilaginous cranium are present. The schematic illustrations show the approximate position of the anterior tectal process (blue). Bended arrows indicate suggested force transmissions in the skull: in lateral skull view (B, D, F), they are indicated by a thin bended arrow due to visualization reasons; in dorsal views (B', D', F'), those arrows correspond to the broad red bended arrows in the skull. The straight arrows indicate pulling neck forces, the relative degree of which are indicated by the depth of the posterodorsal emarginations. It is hypothesized that these forces in the skull are absorbed by the anterior tectal process, which has an altering relative length and position in each species. * in C indicates the spatial shift of the skull roof vs. the braincase resulting in a dorsal excavation, which is here filled with cartilage of tectum cranii – more medial, there is a space for the dorsally erected pineal gland (figs 192, 201 and 202 in Wyneken, 2001, and discussed by Paulina-Carabajal et al. 2013, 2017). Images modified after Nick (1912) (A, C, E), after Gaffney (1979) (B, F, F'), after Zangerl (1948) (D), and after Wyneken (2001) (B', D').

The anterior process of the supraoccipital

In the late embryonic skull of turtles, the chondocranium is well differentiated and already possesses some endochondral ossifications (visible in Fig. 2C–I, 3B, N–R'). In the primordial skull, the otic capsules are dorsally connected via a tectum synoticum (Fig. 3Ad'), which dorsally borders the foramen magnum (Fig. 3C'). In its anterior part, it is dorsolaterally covered by the parietals and usually ossifies endochondrally as the supraoccipital (e.g., Fig. 3B/B') (Sheil 2002). This cartilaginous bridge forms an anterior median process in all turtle embryos, which can be partly embedded in the ventral surface of the parietals and it projects into the brain-cavity (Fig. 2–3). The imprint of this anterior process of tectum synoticum to the skull roof bones clearly represents the anatomical correlate to the “rider” of the brain-endocast in late embryonic turtles. We never found the process to imprint the actual brain in the embryos, although it can closely align to its surface in late term specimens (Fig. 2F).

There is a striking diversity in the relative length and orientation of this late embryonic process in different species. This is, first of all, related to the developmental age of the embryos as visible in *Eretmochelys imbricata* (Fig. 3H vs. 3I) (Fuchs 1915; Sheil 2013) and *Chelydra serpentina* (Fig. 2D–F vs. 3D) (Sheil and Greenbaum 2005; see also Rieppel 1976, 1993). The older the embryo, the longer the process appears to be. Second, there are taxonomic differences among species at late embryonic stage (e.g., Fig. 3F vs. 3G). Whether, to which degree, and at which time of development the process will be replaced by endochondral ossification, and whether it will continue to grow through post-hatching development or remain as small cartilaginous process cannot be evaluated herein and is certainly different among species. It is clear, though, that at least in some taxa the most anterior part of this process remains cartilaginous after hatching, as confirmed by the μ CT images of PTA-stained specimens (Fig. 1K–L, O, Q). The tectum usually ossifies from posterior to anterior (Werneburg and Yaryhin 2019) suggesting that the extension of the rider might be influenced by the completeness of ossification of the supraoccipital.

In this context, little research has been done on cartilaginous structures in post-hatching, juvenile, and adult turtle skulls. It is known, however, that at least in the marine turtles *Dermochelys coriacea* (Fig. 4C) and *Chelonia mydas* (Chelonioidae) (Fig. 4A, 3P–R') and in the snapping turtle *Chelydra serpentina* (Chelydridae) (Fig. 4E) the embryonic tectum synoticum partly persists as the cartilaginous tectum cranii in the adult (Nick, 1912; Parker 1880; Wyneken, 2001: labeled as “cartilaginous part of brain case” in fig. 201–202), whereas the posterior part of the tectum is ossified as supraoccipital. This is also confirmed by our observations of the μ CT images of PTA-stained specimens (Ferreira et al. in press), which clearly show that, at least in some taxa, the most anterior region of the supraoccipital is cartilaginous in juvenile and small adults (Fig. 1K–L, O, Q). The anterior process of the cranial tectum, ventrally embedded between the

parietals, spatially corresponds to the rider protuberance on the endocast. In *D. coriacea*, *Chelo. mydas*, and *Chely. serpentina*, the proportions of the late term embryonic and the juvenile/adult cartilaginous process are – in relation to the rest of the skull – relatively similar. To which degree in the adult the cartilaginous process is calcified cannot be evaluated herein. In a juvenile specimen of *Pelomedusa subrufa* with a carapace length of 9.6 cm, one of the largest turtles ever treated with histological methodology (pers. comm. Wolfgang Maier), we still found a long cartilaginous process in place, whereas the rest of the skull is well ossified (Fig. 2A–B, G–H).

Functional considerations on the anterior tectal process

Considering the actual presence of a dorsally covered bony or cartilaginous anterior part of the supraoccipital, the anterior process might have some relation to the architecture of the temporal region (sensu Joyce 2007), which could be tested in future biomechanical analyses. The anterior process of the tectum synoticum is an ancestral feature also present in other reptiles (Howes and Swinerton 1901; Werneburg and Yaryhin 2019; Yaryhin and Werneburg 2018) and serves as a general anchor (Pitirri et al. 2020) and potential force buffer for dermal roof bones.

Originally, in early Testudinata, such as † *Proganocheilus quenstedtii* (Gaffney 1990), the temporal skull region was almost completely covered by temporal bones (Werneburg 2012; Abel and Werneburg in press). With the emergence and increase of neck retraction through turtle evolution (Werneburg et al. 2015a; Werneburg et al. 2015b), marginal reductions of the dermatocranial skull armor evolved to buffer neck muscle forces during neck retraction (Werneburg 2015).

As there is variation in rider shape among turtles, and the tectum might serve as anchor and/or buffer for biomechanical forces, some aspects of rider diversity roughly seem to be consistent with variation in skull roof emargination with very short, bulbus-like riders being associated with no or only a shallow occiput emargination and elongated riders associated with deep occiput or complete emargination. This relation is tentative and needs rigorous quantitative examination beyond the scope of this study. If rider shape and length were ultimately found to be correlated with emargination, this would reinforce current hypotheses on how important neck functionality and associated characters are for skull disparity and development in turtles.

We speculate that forces transmitted along the reduced temporal coverage during neck retraction are buffered along the postorbital area in a posterodorsal and medial direction into the anterior process of the tectum synoticum (red arrows in Fig. 4B/B', D/D', F/F'). A shorter process in marine turtles with shallow emargination such as *Chelonia mydas* may compensate forces over the skull more posteriorly (Fig. 4B, B'), whereas a longer process in taxa with deep posterior emargination such as *Chelydra serpentina* may compensate neck forces more anteriorly

over the skull (Fig. 4 F/F'). This tentative association, however, may not be very distinct in future biomechanical analyses given that other factors certainly affect the temporal region and the skull roof, such as different skull proportions, jaw muscle action, and growth patterns.

The shape of the adult endocast rider cannot be easily associated to the length of the anterior tectal process (see Table 1; Werneburg et al. 2021), because of different modes of fossilization in extinct taxa and/or different degrees of ossification in the anterior tectal process. Nevertheless, it is noticeable that some stem turtles with their full temporal coverage, such as † *Pr. quenstedtii* (Lautenschlager et al. 2018) or † *Meiolania platyceps* (Paulina-Carabajal et al. 2017), only show a bulbus-like appearance of the rider, which might correspond to a short tectal process. Other stem turtles with deep occiput emarginations, such as the baenid paracryptodire † *Eubaena cephalica* (Evers and Benson 2019; Joyce et al. 2016; Rollot et al. 2018) can show elongated riders indicating to an elongated anterior tectal process.

Embryonic evidence for functional morphology

The collected images on the late embryonic stages from the literature (Fig. 3) are not fully comparable among each other because first, they are just random snapshots on ontogenetic development and the length of the anterior process might grow quickly through ontogeny (Fig. 3A). Second, some of the images are based on reliable histology-based 3D-reconstructions (e.g., Fuchs 1915), but most of the images are based on the more critical clearing and staining methodology, which has fundamental impact on the exposure of small and thin cartilage and relative positioning of structures inside the partly enzymatically digested embryo (discussed by Yaryhin and Werneburg 2017). Nevertheless, despite those limitations, the relative lengths of the process in most cases fit relatively well to the respective emargination types (compare to Fig. 1H): A short embryonic process is generally associated to an absent or a shallow posterior emargination (1–3 in Fig. 1H) (*Eretmochelys imbricata*: Fig. 3H–I, *Emydura subglobosa*: Fig. 3F, *Chelonia mydas*: Fig. 3N–R'; *Caretta caretta*: Fig. 3Ad). A longer process is generally associated to a deep posterior emargination (4 in Fig. 1H) (*Chelydra serpentina*: Fig. 2D–F – note in Fig. 3D only an early stage of this species is shown, same for *Macrochelys temminckii* in Fig. 3J and *Apalone spinifera* in Fig. 3C; *Podocnemis unifilis*: Fig. 3B; *Chrysemys picta*: Fig. 3E; *Emys orbicularis*: Fig. 3G). *Pelodiscus sinensis* (Fig. 3K) and *Trachemys scripta* (Fig. 3M) do not fit perfectly to this categorization, which might be due to the mentioned methodological issues.

Within some extant turtle taxa, e.g. *Terrapene* and chelid pleurodires, a dermal coverage of the temporal region is completely lost by evolutionary expansion of the anteroventral emargination (5 in Fig. 1H) (Werneburg 2012). Compared to all other species studied herein, the histological sections of *Chelodina longicollis* (Fig. 2I)

and the enhanced contrast stained μ CT of *Chelus fimbriatus* (Fig. 1M) reveal an extremely broad anterior process. This suggests different functional constraints. How neck retraction forces are transmitted in species without temporal skull coverage is not understood (Werneburg 2015). Neck muscles insert to a dense temporal fascia (Werneburg 2013a), the forces within also need to be buffered. The primary function of the temporal armor was the stabilization of the mobile quadrate. Through turtle evolution, the quadrate was fixed to the braincase (Werneburg and Maier 2019) and the temporal armor was freed and could get reduced in a way to react to increased neck retraction forces but still to keep lateral bracing between quadrate and upper jaw. Without any temporal coverage, the stability of the lateral bracing was lost and the temporal fascia and the quadratojugal ligament (Jones et al. 2012; Werneburg 2013a) might not be enough to withstand forces on the skull. In this context, the very broad anterior tectal process in type 5 (5 in Fig. 1H) species (Fig. 3I) appears to stabilize the contralateral parietals to keep integrity of the skull roof (sensu Pitirri et al. 2020). We see short tectal processes and shallow emarginations in chelids and chelonoids, and long processes with deep emarginations in emydids and chelydrids. This might be a pattern explained by functional, but also phylogenetic associations. *Chelodina* and *Chelus*, with their broad process and complete (*Chelodina*) or peculiar (*Chelus*) emargination, seem to support the functional scenario, but these are only two data points. However, we present only a tentative causal mechanism for the functional association, which can be explicitly tested by biomechanical analyses in the future.

Conclusions

Evaluating the origin of a rider on the top of brain endocasts requires consideration of the tissues surrounding the brain cavity, including their ontogeny. Alternative explanations for the rider in turtle endocasts, such as the cavity of the pituitary gland or blood vessel imprints on the endocranial cavity, do not hold after our analyses of histological sections and μ CT scans of PTA-stained specimens. Instead, we present clear evidence for the persistence of a cartilaginous tip of the supraoccipital bone, a remnant of the embryonic tectum synoticum, which causes the occurrence of a 'rider' in endosseous endocasts of turtle braincases. As such, the anatomy of the chondrocranium and its persistence until adulthood needs to be studied in detail for a profound interpretation of unique endocast structures, and we consider it as the major source for morphological variation of the endocast rider herein. Our considerations on these structures can only count as a preliminary examination of this topic. Cartilaginous structures were rarely studied before. This is, because comparative anatomy is mainly a subject of paleontologists today, who – in the past – rarely integrated embryonic or non-bone data to their analyses, but the field is changing.

Our paper highlights the need to integrate paleontology, zoology, and embryology to enable a holistic view on skull evolution (sensu Maier 1999). Turtles are a particularly suitable taxon to conduct such a research agenda, because of their exhaustive fossil record, a considerable extant diversity, and the relatively easy access to embryos. Nevertheless, comprehensive (and high quality) histological series are rare and, in many cases, they only represent random data points in ontogeny, data difficult to analyze via modern quantitative research programs. Despite this limitation, we show that embryology enables drawing fundamental hypotheses on organismal evolution and needs to be considered as valuable data source also in the future. An association of modern computer based μ CT-analyses – mainly applicable to older ontogenetic stages (juveniles, adults) – and traditional histology – mainly applicable to early ontogenetic stages (embryos) – enables two different viewpoints for an ultimately more comprehensive understanding on organismal morphological evolution (Maier 2020; Maier and Werneburg 2014).

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