

# The origin of orbitotemporal diversity in lepidosaurs: insights from tuatara chondrocranial anatomy

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## Abstract

*Sphenodon punctatus*, the tuatara, is the last survivor of the formerly widely distributed group of Rhynchocephalia, which is the sister group of Squamata. The skull anatomy of *S. punctatus* and its fossil relatives is comparably well known; however, embryological data of skull development are rare, incomplete, and mostly represented by dated works. Knowing the anatomy of the chondrocranium of *S. punctatus* is crucial to an understanding of chondrocranial evolution in reptiles and particularly in lepidosaurs. Here, based on the historical histological collection of Hugo Schauinsland, we reexamined the anatomy of the fully formed chondrocranium in *S. punctatus* and describe a very early stage of its chondrocranium formation, which was not considered in any previous study. The architecture of the fully formed chondrocranium of *S. punctatus* represents one of the most complex ones among sauropsids. We observed a number of characters, that are absent in other reptiles and were never previously described in *S. punctatus*. We consider the robust lateral braincase wall in *S. punctatus* to represent an ancestral condition. In the lepidosaurian ancestor it likely had the potential for further diversification of the orbitotemporal region in squamates. Certainly, it provided extra mechanical strength to the chondrocranium as a whole. At the same time, the strong cartilaginous lateral wall of the chondrocranium in *S. punctatus* could also be a rudimentary form of the more distant ancestor of lepidosaurs, in which the chondrocranium played a more functional role.

## Key words

Chondrocranium; development; evolution; Lepidosauria; primary braincase, Rhynchocephalia; *Sphenodon punctatus*.

## Introduction

The lizard-like reptiles of the group Rhynchocephalia were successful and widely distributed during the Mesozoic, inhabited territories of Europe, Africa, and North America and included terrestrial and marine animals (CREE, 2014, FRASER, 1988, GILMORE, 1909, JONES & CREE, 2012, RASMUSSEN, 1981). Now, however, they are represented only by one species, *Sphenodon punctatus* (GRAY, 1842) (syn. “*Hatteria punctata*”), which is endemic to New Zealand (HAY *et al.*, 2010). Previously, based on allozyme variation, it has been divided into two species, *S. punctatus* and *S. guntheri* (DAUGHERTY *et al.*,

1990). However, subsequent nDNA analysis indicates that all populations are best regarded as one species: *S. punctatus* (HAY *et al.*, 2010).

Squamata and Rhynchocephalia are sister taxa and diverged about 250 million years ago (EVANS & JONES, 2010, JONES *et al.*, 2013, REST *et al.*, 2003). Therefore, as the only living representative of Rhynchocephalia, *S. punctatus* has been extensively studied to examine what it can tell about reptile evolution (BRENNAN, 2016, BROOM, 1906, CREE, 2014, EVANS, 2008, GISI, 1907, GORNAK *et al.*, 1982, GÜNTHER, 1867, HOPPE, 1934, JONES *et al.*,

2011, JONES & CREE, 2012, JONES *et al.*, 2012, REGNAULT *et al.*, 2017, SANGER *et al.*, 2015, SÄVE-SÖDERBERGH, 1946, SÄVE-SÖDERBERGH, 1947).

The skull anatomy of *S. punctatus* and its fossil relatives is relatively well-known (GÜNTHER, 1867, JONES *et al.*, 2011, JONES, 2008, JONES *et al.*, 2009, SIEBENROCK, 1894). However, embryological data of skull development in *S. punctatus* are rare and are mostly represented by very old studies (HOWES & SWINNERTON, 1901, SCHAUINSLAND, 1903, WYETH, 1924), and later studies mostly concern dermal ossification (RIEPEL, 1992, WERNER, 1962). Most data were summarized in the “Biology of Reptilia” book series (BELLAIRS & KAMAL, 1981), in which the authors concluded that the fully formed chondrocranium requires further, more detailed observations particularly in the nasal region.

Previous descriptions of the fully formed chondrocranium of *S. punctatus* differ from one another. Differences in overall shape are particularly surprising, this concerns especially the orbitotemporal region (HOWES & SWINNERTON, 1901, SCHAUINSLAND, 1901, WERNER, 1962). Most likely, the illustrated diversity is not the result of intraspecific variability, but an artifact of using different approaches to reconstruct the primordial skull (discussed by YARYHIN & WERNEBURG (2017)). In general, on a certain taxonomic (i.e., “family”) level, the chondrocranium represents a highly conserved organ (YARYHIN & WERNEBURG, 2018).

Here, we re-describe the fully formed chondrocranium of *S. punctatus* using three dimensional computer imaging. We evaluate how its structure compares to that of squamates and what this means for lepidosaurs as a whole. In particular, we concentrate on the orbitotemporal region, which experienced the greatest diversification among lepidosaurs (DAZA & BAUER, 2010, EVANS, 2008). We also describe a very early stage of chondrocranium formation, that was not considered in any study before.

## Materials and methods

**Specimens.** The original embryonic material used in our study was collected in New Zealand at the end of the 19<sup>th</sup> century by Prof. Dr. Hugo Schauinsland. He collected this material during his famous New Zealand expedition either on Stephens Island from April 1896 to May 1897 (SCHAUINSLAND, [1899] 1996) or on the Cook Islands from December 1896 to January 1897 (SCHAUINSLAND, 1898). The embryonic material was serially sectioned and, based on coloration of the sections, it was probably stained with hematoxylin. After long term storage, the sections became partially bleached and lost coloration, but they are still informative. The collection is housed at Übersee-Museum (Bremen, Germany), which was founded by Hugo Schauinsland in 1896 (SCHAUINSLAND, [1899] 1996).

In the current study, we describe two stages. The first is an early stage represented by 13 slides in total of three

different embryos sectioned in the sagittal, frontal, and transverse planes respectively. The second stage is older, has a fully formed chondrocranium, and it consists of 24 slides labeled as specimen “*Hatteria d*” (original labeling of Schauinsland). According to the information on the slides of “*Hatteria d*”, the thickness of sections is 15 µm. The thickness of the other sections is not recorded, but based on our own experience, we estimate that it is not more than 10 µm. On one series of the early embryo sections, the name “F. Zinsung” is written, possibly in reference to a technical assistant of Hugo Schauinsland. The other two series of the small embryos are labeled as “*Hatteria K*”, in which the letter “K” perhaps refers to the word “klein”, i.e. German for ‘small’. However, based on the progress of organ development, we conclude that the letters do not correspond to the letter-labeled stages of DENDY (1899).

Based on the developmental conditions, we infer that the earliest embryos of our study represent a stage that is slightly earlier than “stage P” described by Howes and Swinnerton (1901). The earliest developmental stage of the chondrocranium resembles that of other lepidosaurs and sauropsids in general (BELLAIRS & KAMAL, 1981, YARYHIN & WERNEBURG, 2017, YARYHIN & WERNEBURG, 2018), in which the first mesenchymal precursors of the chondrocranium are trabeculae, acrochordal, and parachordals. The specific shape of the chondrocranium anlagen closely resembles that of the crocodile *Mecistops cataphractus* (MÜLLER, 1967).

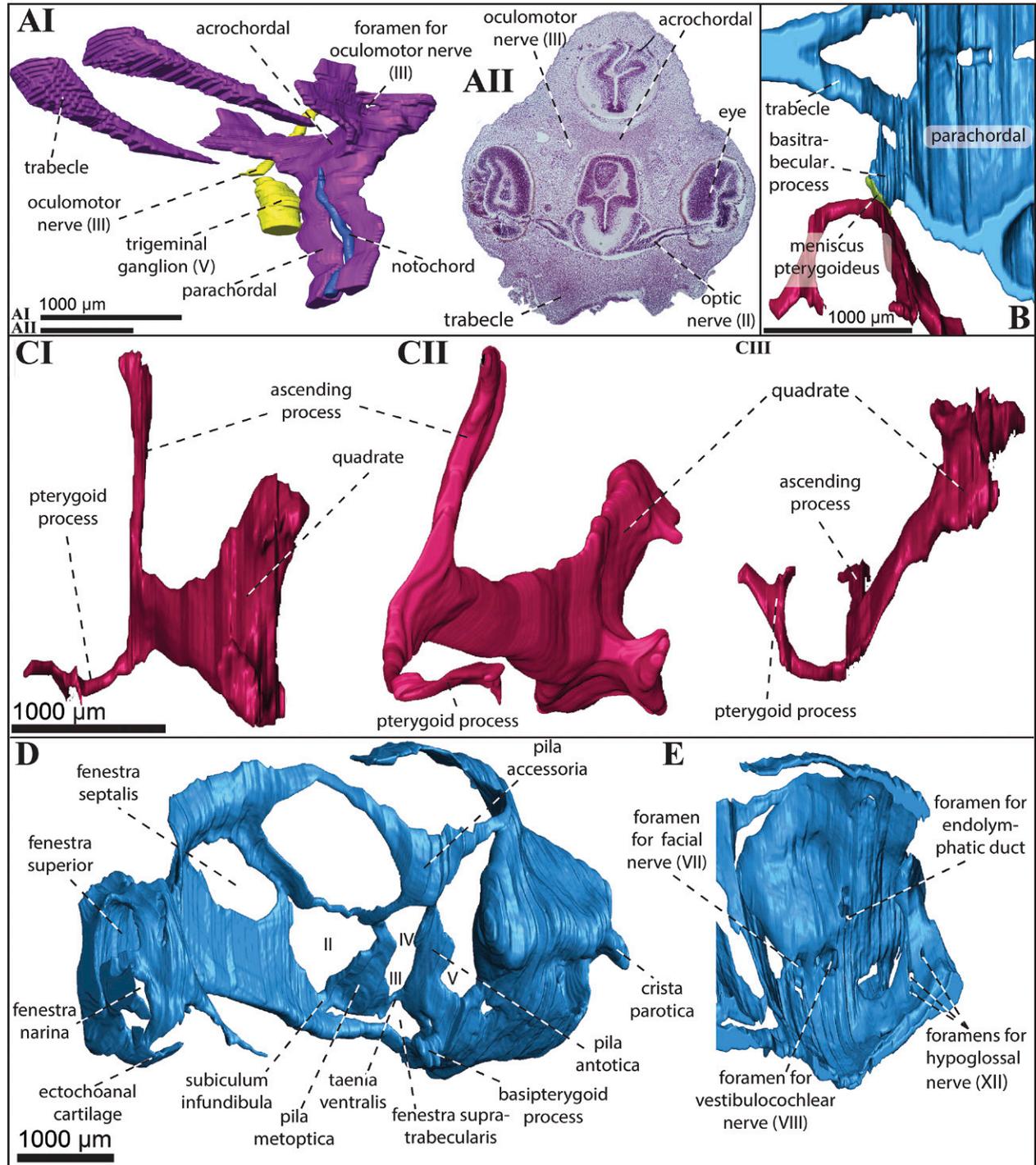
The collection of the Übersee-Museum contains several more slides. However, those sections provide no further information on development of the chondrocranium as they are of almost the same stage as “*Hatteria d*”, or even more advanced, and/or contain postcranial material.

**Image processing.** The sections were photographed with an Olympus BH2 microscope equipped with a Canon EOS 650D digital camera. Where necessary, the images were stitched together using the “Photomerge” option in Adobe PS CC software. Background cleaning and color adjustments were also performed using Adobe PS CC software.

**3D modeling.** Semiautomatic alignment of the image stack was done using Fiji software (SCHINDELIN *et al.*, 2012); the 3D-reconstruction was performed in Amira 5.0 software (Thermo Fisher Scientific) using manual segmentation tools.

## Results

**Early stage of chondrocranium differentiation** (based on the three small specimens). At this stage, the embryos show a weak differentiation of sensory organs. The olfactory organ is represented by the olfactory pits (with-



**Fig. 1.** 3D reconstructions of the chondrocranium of *Sphenodon punctatus* (A – specimen “F. Zinsung”, B–E – specimen “*Hatteria d*”): mesenchymal condensations of the chondrocranium anlage in an early embryo in an oblique ventral view with the nerves on the right hand side segmented. The notochord represents the midline (AI) cross section through the head of the same embryo (AII). Coronal section of a 3D reconstruction illustrating the basipterygoid articulation (B); pterigoquadrate in lateral (CI), frontal (CII), and ventral (CIII) view; chondrocranium in the oblique view, the left side is cropped for better illustration of the relationships of the elements in the orbitotemporal and the nasal regions (D); right otic capsule in medial view (E). Average positions of: the olfactory (I), optic (II), oculomotor (III), and trochlear (IV) nerves; V, trigeminal ganglion.

out lumen): the eyes are relatively small although lenses are already present; the optic chiasma is not developed; and the otic vesicles have just started to differentiate as labyrinth organ. However, these early embryos already have chondrocranium anlagen. Trabeculae, parachordals,

and the acrochordal already appear as mesenchymal condensations (Fig. 1AI, AII). Trabeculae are paired mesenchymal condensations that stretch along the forebrain. The density of the mesenchyme decreases in anterior direction and, thus, the trabeculae look more expanded

anteriorly. The acrochordal and the parachordals are aligned in the same plane along the posterior part of the brain. The mesencephalic flexure of the brain is prominent, thus the trabeculae are in an almost perpendicular plane to the acrochordal and the parachordals (Fig. 1AI). The acrochordal is represented by a very dense and robust mesenchymal condensation with clear margins (Fig. 1AII). From its dorsolateral edge, a mesenchymal plate arises, representing the primordial pila antotica, which is pierced by the oculomotor nerve (III). The anterior tip of the notochord reaches the acrochordal, but does not pierce it. Along the cranial part of the notochord, the paired mesenchymal and rod-like parachordals align. They closely attach to the notochord, but do not fuse to each other (Fig. 1AI). Anteriorly, the parachordals are mesenchymally connected to the acrochordal.

#### **Fully formed chondrocranium** (specimen “*Hatteria K*”).

The chondrocranium of *Sphenodon punctatus* is very robust (Fig. 2) when compared to other lepidosaurs (BELLAIRS & KAMAL, 1981). The nasal region is represented by the nasal capsules, which are relatively short, and in lateral view, almost twice as high as wide (Fig. 2A). The orbitotemporal region contains all elements known for lepidosaurs (BELLAIRS & KAMAL, 1981, YARYHIN & WERNEBURG, 2018) and occupies almost the half of the whole chondrocranium length. The remainder of the chondrocranium is represented by the otic capsules fused together dorsally by the tectum synoticum and ventrally by the basal plate. The basicranial fenestra is not well developed, thus it is difficult to determine the boundary between the acrochordal and the basal plate.

#### The nasal region

Based on the suggestions of Bellairs and Kamal (1981), we studied the anatomy of the nasal region in greater detail. However, we did not detect any crucial difference in its anatomy when compared to previous descriptions of the nasal region (SCHAUINSLAND, 1901, SCHAUINSLAND, 1903, WERNER, 1962).

The paired nasal capsules of *S. punctatus* are separated by the nasal septum (Fig. 3B–E). The nasal capsule represents a single concha, which surrounds the olfactory organ. Its vestibule is anteriorly covered by a small cartilaginous cupola. This cupola is fused only ventrally and laterally with the lamina transversalis anterior and the lateral edge of the parietotectal cartilage, respectively (Fig. 1D). Thus, the dorsal and lateral margins of the cupola, together with the nasal septum and the dorsolateral margin of the parietotectal cartilage, encompass a large fenestra superior of complex shape (Fig. 1D). The complex shape of the fenestra superior is also due to the small incisura formed between the nasal septum and the medial margin of the cupola. The dorsal margin of the cupola, together with the nasal septum and the dorsolateral margin of the parietotectal cartilage, borders another fenestra, which is almost separated in two fenestrae itself. This

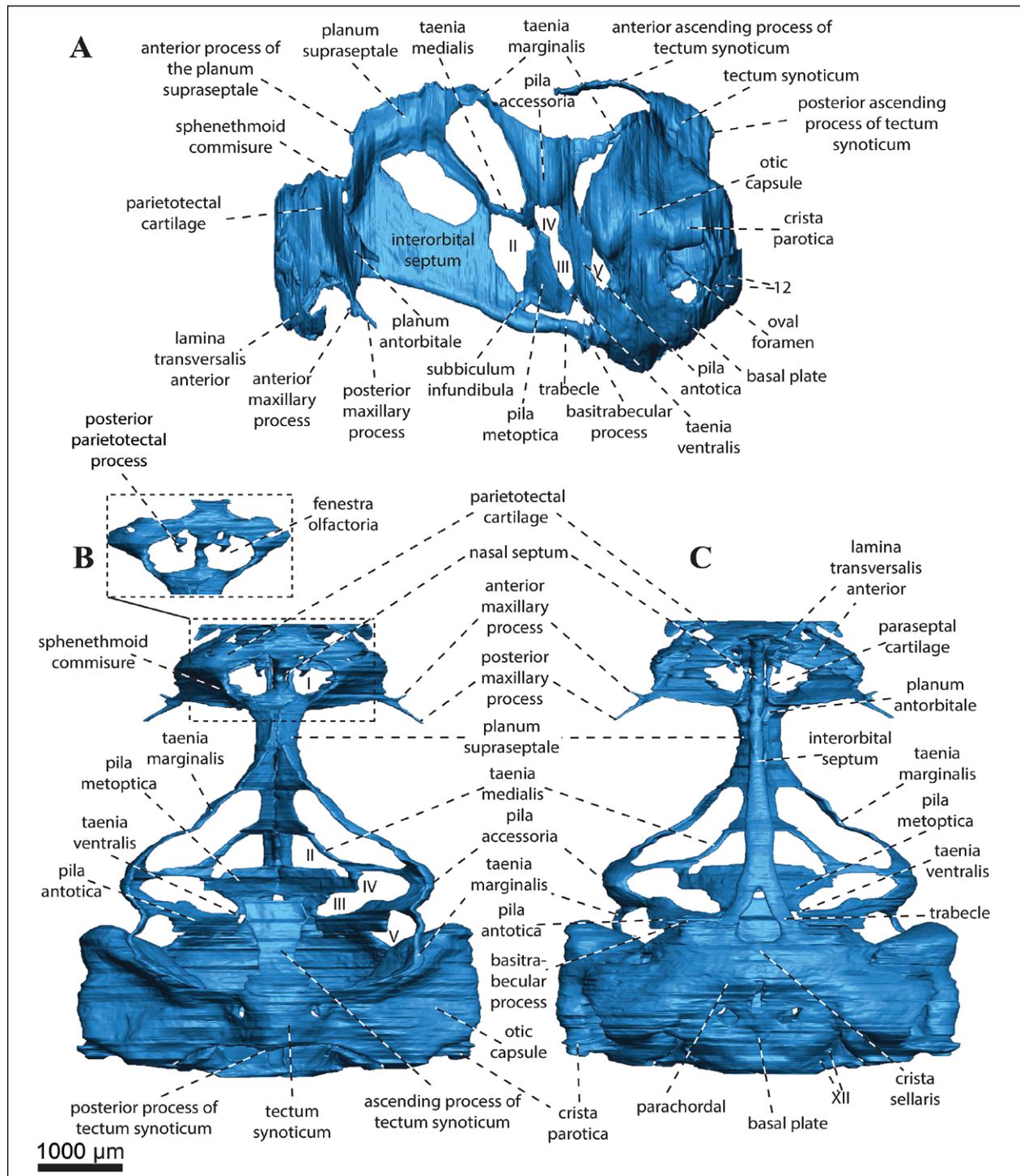
conditional separation is due to the presence of the lateral outgrowth of the cupola, which supports the most anterior part of the vestibule. Thus, the upper part of the fenestra represents the fenestra narina, which does not represent a true fenestra in *S. punctatus*, but only a part of the larger one.

The lower portion of the most anterior part of the nasal septum passes into the massive lamina transversalis anterior, which forms another small capsule that surrounds the anterior part of Jacobson’s organ (Figs. 2A, C; 3C, D). Behind the level at which the nasolacrimal duct and the duct of the Jacobson’s organ fuse, the lamina transversalis anterior divides into two further lamellae. The lateral one ends blindly and aligns with the ventrolateral part of the choanal groove thus representing the ectochoanal cartilage. The median lamella passes along the ventral edge of the nasal septum and turns to the rod shaped paraseptal cartilage (Fig. 2C), which is fused posteriorly with the planum antorbitale that covers the olfactory organ posteriorly (Fig. 2A, C).

The planum antorbitale has an asymmetric cone shape (Fig. 2A, C). It is fused with the parietotectal cartilage along its dorsolateral aspect and with the paraseptal cartilage ventrally (Fig. 3E). The medial edge of planum antorbitale closely approaches the nasal septum, but does not fuse with it (Fig. 2C). The most dorsolateral aspect of the planum antorbitale is stretched out as a process that bifurcates in two parts (Fig. 2A–C) – the short one is oriented anteriorly, representing the anterior maxillary process, and the relatively long one is oriented posteriorly (Fig. 2B, C). The latter represents the posterior maxillary process and travels along the lateral and median surfaces of the palatine and maxillary respectively. The most posterior part of the posterior maxillary process is encapsulated by these two bones.

The parietotectal cartilage forms the roof of the nasal septum (Figs. 2A–C, 3C). It is medially fused with the dorsal aspect of the highest portion of the nasal septum, which sharply decreases in height just behind the level of confluence with the parietotectal cartilage (Fig. 3B–E). Slightly posteriorly to the level of fusion with the nasal septum, the parietotectal cartilage gives rise to a small process that travels posteroventrally down along the ventral edge of the nasal septum. This process is swollen in its middle part forming a cartilaginous bulb (Fig. 2B). Between this process and the nasal septum, the olfactory nerve enters the nasal capsule. In our studied specimen of *S. punctatus*, this process is asymmetric. On the right side, the bulb becomes fused with the nasal septum, forming a foramen for the olfactory nerve. This is different from the incisura of the other side, where the bulb does not fuse with the nasal septum (Figs. 2B, 3D). Based on the topology of this process, we propose to name it ‘the posterior parietotectal process’.

More laterally to the process, the parietotectal cartilage is fused with the sphenethmoid commissure, which is also fused posteriorly with planum suprasedptale. Together with the nasal septum, all these structures encompass a relatively large fenestra olfactoria (Fig. 2B).



**Fig. 2.** 3D reconstruction of the fully formed chondrocranium of *Sphenodon punctatus* (based on specimen “*Hatteria d*”) in lateral (A), dorsal (B) and ventral (C) view. A few of the most anterior sections of the nasal region are missing and were probably lost during the sectioning process. The pterygoquadrate is not shown here but the position of the pterygoquadrate in relation to the chondrocranium is shown in red in Fig. 3A. Average positions of: the olfactory (I), optic (II), oculomotor (III), and trochlear (IV) nerves; V, trigeminal ganglion; XII, foramen for the hypoglossal nerve.

### The orbitotemporal region

The orbitotemporal region of *S. punctatus* is inseparably fused with the nasal region. The nasal septum gradually becomes the interorbital septum (Fig. 2A, C); its anterior portion is as deep as the nasal septum in its highest part.

Thus, between the two deep regions of both septa, there is a depression on the dorsal edge indicating a border between these regions. The interorbital septum reaches its maximum height in its middle portion, then it gradually reduces in height, and at the posterior level of the planum suprasedale, the interorbital septum abruptly decreases

in depth and passes into a short trabecula communis (Figs. 2A, 3F, G). In front of the pituitary, the trabecula communis bifurcates in paired trabeculae. They fuse with the crista sellaris posteriorly, limiting the pituitary fenestra from the lateral sides (Fig. 2C).

The dorsal edge of the interorbital septum continues with a paired planum suprasedale (Figs. 2A, B, 3F). It starts behind the midpoint of the depression mentioned above and is inextricably linked with the interorbital septum up to its highest point, where both planum suprasedale become interrupted by the big fenestra septalis, filled with a thin membrane. The fenestra septalis is positioned along the remaining part of the dorsal edge of the interorbital septum. Posterior to the fenestra septalis, the interorbital septum again becomes tightly connected with planum suprasedale (Fig. 1D).

The planum suprasedale supports the olfactory tract and the bulb from below and represents a thin cartilaginous plate. In the anterior portion of the planum suprasedale and along the expansion of the interorbital septum, in the area of the olfactory bulb, the planum suprasedale forms an open channel. However, in its middle portion, up to the middle plane of the fenestra septalis, the lateral edges of the planum suprasedale surround the olfactory tract and approach one another, almost forming a tube (Fig. 3F). Posteriorly, the planum suprasedale bifurcates into: 1) a ventral part, i.e. the remaining part of planum suprasedale that is open and relatively narrow and becomes taenia medialis; and 2) a dorsal part that becomes taenia marginalis (Fig. 2A).

The middle part of the frontal edge of planum suprasedale is fused with the sphenethmoid commissure, and the most anterior and ventral area of the planum suprasedale bears a very short anterior process. Shortly before, the interorbital septum becomes the trabecula communis, and just posterior to the optic chiasma, there is a very short process (subiculum infundibula). This is fused with a paired polygonal plate, the pila metoptica (Figs. 1D, 2A), that is dorsolaterally oriented and supports the brain. Both pilae metoptica are fused to each other ventrally at the level of trabecula communis and are posteriorly separated along the level of the trabeculae. In lateral view, the frontal edge of the pila metoptica has an almost orthogonal position relative to the trabecula communis. The dorsal part of the pila metoptica is connected with taenia medialis. Thus, together with the posterior ridge of the interorbital septum, all these structures surround the triangular optic nerve foramen. From the point of fusion of the pila metoptica and taenia medialis, another rod-shaped cartilage branches out. It is slightly shorter than taenia medialis and travels laterally in an almost horizontal plane to fuse with the anteroventral edge of a broad concave cartilaginous plate representing “pila accessoria”, or lamina accessoria (see discussion).

The posterior edge of the pila metoptica reaches the anterior third of the trabeculae, where it fuses with the lower anterior edge of pila antotica, forming a short cartilaginous bridge – that we name taenia ventralis (Fig. 1D). This arrangement also forms a fenestra supratretecularis,

which is restricted ventrally by the trabeculum and dorsally by the taenia ventralis, pilae metoptica et antotica (Fig. 1D). The pila antotica represents a relatively broad rod of cartilage (Figs. 1D, 3H). Ventrally, it is connected to the crista sellaris, then travels slightly anterior to meet the pila metoptica via the taenia ventralis, after which it passes almost directly dorsally. Above the level of the trigeminal ganglion (V), the pila antotica becomes narrower and, at the level of the most anterior prominence of the otic capsule, it fuses with the posteroventral edge of the lamina accessoria.

The lamina accessoria is a concave, trapezoid cartilaginous plate, which divides taenia marginalis into an anterior and a posterior part that connects respectively to the planum suprasedale anteriorly and to the otic capsule posteriorly. The most posterior end of the taenia marginalis attaches to the dorsal aspect of the otic capsule, near the tectum synoticum (Fig. 1D).

The basitrabecular process represents a lateral outgrowth of the posterior end of the trabeculum. It has a thin connection with the trabeculae and is strongly fused posteriorly with the acrochordal cartilage. Laterally, it articulates with a small flattened cartilage – the basiptyergoid meniscus (Figs. 1B, 3H).

## The otic and occipital region

The base of the chondrocranium is represented by an almost equilateral tub-shaped cartilaginous plate (Fig. 2C). At the midline, it is divided by the notochord in two parts (left and right). The cranial portion of the notochord is thick and highly vacuolated posteriorly (Fig. 3I, J), but becomes significantly thinner anteriorly. It is never completely embedded into the cartilaginous mass. The notochord lies on the ventral surface of the posterior half and in the most anterior part of the cartilaginous plate. In the remaining part of this plate, the notochord lays just between the parachordals. Thus, the most anterior portion of the plate represents crista sellaris (~ acrochordal; see YARYHIN & WERNEBURG (2017)), and the remaining part is derived from the parachordals (Fig. 2C). The anterior portion, which is divided by the notochord, represents the unfused parachordals. The posterior portion with the notochord laying on the dorsal the surface represents the fused parachordals, i.e. the basal plate. It is noteworthy that there is no space between the notochord and the medial edge of the parachordal, therefore, a true basicranial fenestra is absent and, instead, there is only a slit between the parachordals containing the notochord (Fig. 2C). In our specimen this slit is interrupted in its midsection by two thin closely positioned cartilaginous connections between the parachordals one above and one below the notochord (Fig. 2C).

Posteriorly, the basal plate bears a pair of occipital arches. These arches are oriented dorsolaterally and positioned nearly perpendicularly to each other (Fig. 3J). The occipital arches are delimited from the basal plate by the foramina for the hypoglossal nerves (XII). There

are three foramina on the left and two on the right side. The occipital arches do not meet dorsally, instead they are closely attached laterally (but not fused) to the posteromedial surfaces of the otic capsules (Fig. 3J).

The otic capsule is short; it is more than one and a half times higher than long (Fig. 2). Ventrally, it has a broad and strong fusion with the basal plate and the parachordal, which is called basicapsular commissure. This connection is interrupted anteriorly by the foramen for the facial nerve (VII). The internal surface of the capsule has five openings (Fig. 1E): 1) the foramen for the facial nerve (VII); 2) the foramen for the vestibulocochlear nerve (VIII); 3) a foramen for the endolymphatic duct; 4) above the endolymphatic foramen a small foramen for a blood vessel entering the intracapsular space; and 5) a relatively large opening in the area of the posterior semi-circular canal.

The posterolateral surface of the otic capsule bears a crest, which becomes higher in posterior direction and represents crista parotica. Immediately below it, there is the big roundish fenestra ovalis.

Posterodorsally, both otic capsules are connected by a broad cartilaginous bridge – the tectum synoticum (Fig. 2A, B), that covers the endolymphatic sacs. Anteriorly, the tectum synoticum bears a long robust ascending process, which has very short paired processes (Figs. 2A, B, 3I). The tectum synoticum also bears very short posterior processes (Fig. 3J).

### Pterygoquadrate (palatoquadrate)

The pterygoquadrate cartilage in *S. punctatus*, consisting of the quadrate, the ascending process, and the pterygoid process, articulates with the basipterygoid-meniscus near the base of the ascending process (Fig. 1C). The quadrate cartilage is C-shaped in frontal view and is aligned in an almost vertical position in relation to the chondrocranium. Anteriorly, it extends along the parachordal and in front of the level of the trigeminal ganglion (V), where it is fused with the ascending process. The ascending process is a relatively long almost vertical cartilaginous rod. Dorsally, it reaches the level of the taenia marginalis, which curves around its mesial surface (Fig. 3H). The pterygoid process stretches from the base of the ascending process along the acrochordal cartilage, then it extends to the anterior level of the pituitary fenestra, before it abruptly bends laterally and bifurcates in two short processes.

## Discussion

### Early stage and tempus optimum in *S. punctatus*

The architecture of the fully formed chondrocranium of *S. punctatus*, is perhaps one of the most complex among sauropsids (BELLAIRES & KAMAL, 1981). Nevertheless,

we observed a number of characters that are absent in other reptiles and that were never previously described in *S. punctatus* or – to our knowledge – in any other sauropsid. These include (1) an unusual cartilage beam in the nose that we call posterior parietotectal process, (2) a posterior fusion of the basipterygoid process to the parachordal, and (3) a ventral connection of pila antotica and pila metoptica that we call taenia ventralis (shown but not described by Schauinsland 1903).

Among the existing descriptions and illustrations of the fully developed chondrocranium, the general shape of our specimen could be compared only with the descriptions of the embryonic skull provided by Schauinsland (1901, 1903) and Werner (1962). The reconstruction of Howes and Swinerton (1901) is more schematic and disproportionate when compared to the above-mentioned descriptions. In the Schauinsland-descriptions, the chondrocranium, especially the nasal region, is somewhat schematically reconstructed, hence only the general shape matches our observation. Analyzing the structures described and illustrated by Werner (1962), we estimate that his specimen is slightly more advanced in development than the one described here. The main differences are the advanced ossification of the basicranium and a break in the anterior taenia marginalis as well as the absence of a few structures, like taenia ventralis, the posterior fusion of the basipterygoid process, or the posterior parietotectal process in the fenestra olfactoria: they were perhaps already transformed in this later stage.

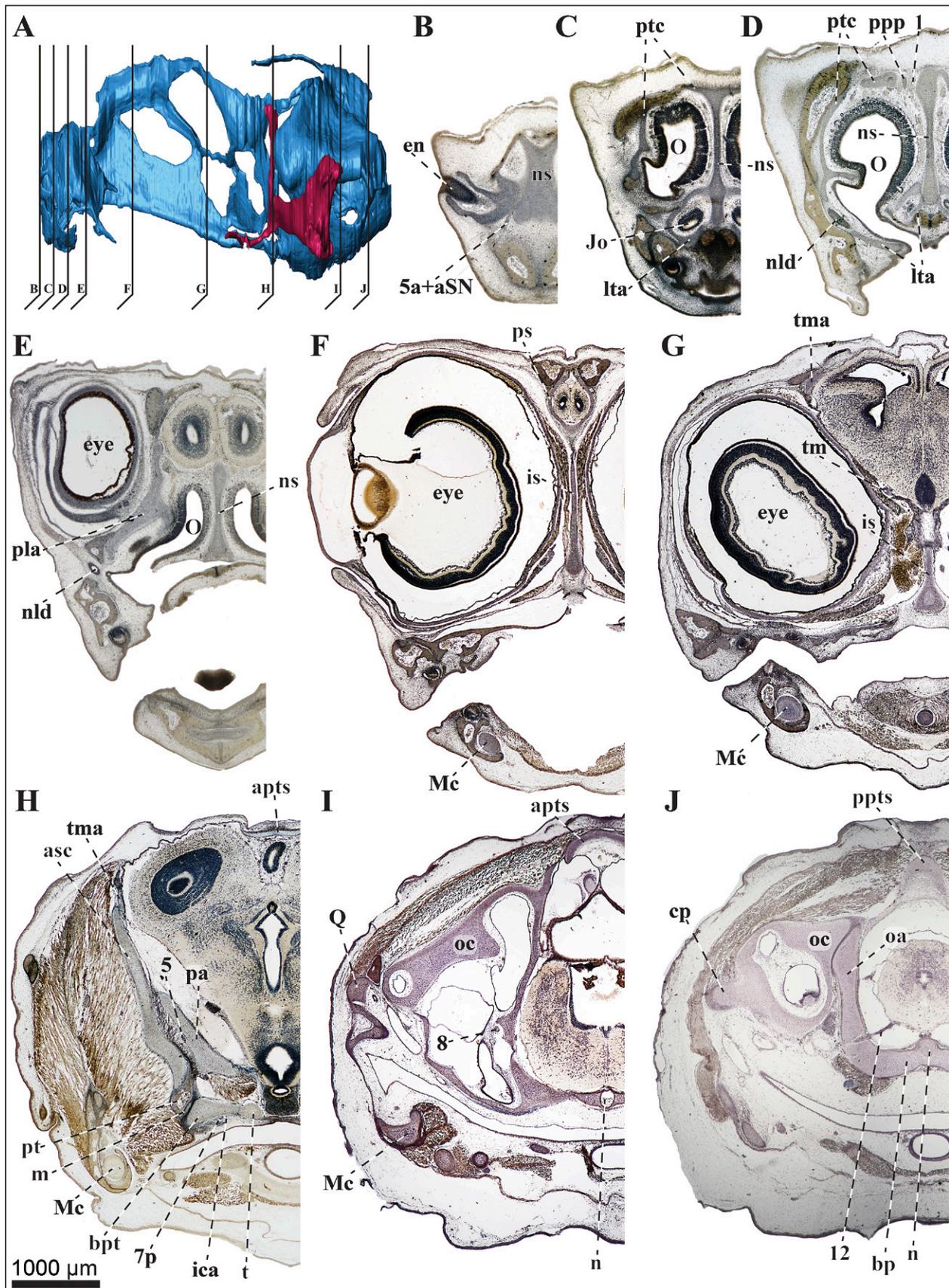
Our findings correspond with those of Schauinsland (1901, 1903) and fulfill the tempus optimum criteria (WERNEBURG & YARYHIN, in press). The tempus optimum is defined as the condition, in which:

(1) the ethmoid region is already chondrified anteriorly, and thus the posterior is also chondrified because chondrification of the cranium always develops from posterior to anterior.

(2) the basicranium has not undergone modifications by ossification which would have obscured its original form. The tempus optimum is considered the ideal state for consistent comparisons among different species in an evolutionary context (HÜPPI *et al.*, 2018, WERNEBURG & YARYHIN, in press). Given the important phylogenetic position of *S. punctatus* as the sister taxon to all other living lepidosaurs, a well-defined tempus optimum stage, described here, will be helpful for comparative and phylogenetic analyses in the future.

### Side wall evolution in lepidosaurs

Through evolution, the vertebrate skull has undergone modifications that may be related to the functional load of the chondrocranium (JONES *et al.*, 2017, WERNEBURG & MAIER, 2019), in which the orbitotemporal region experienced the most comprehensive changes (DE BEER, 1937). In non-amniotes, the chondrocranium is a particularly important structure during the larval period as it serves as



attachment site for jaw musculature (WERNEBURG, 2019, ZIERMANN *et al.*, 2018) and has a protective role for the sense organs and the brain. In those animals, the orbito-

temporal region of the chondrocranium is mostly represented by a robust and broad cartilaginous plate supporting the brain (KEMP, 1999).

In amniotes, the larval stage was reduced and direct development was enabled by the emergence of the amniotic egg, which provides an aquatic milieu inside the amniotic membrane and releases the animals from reproduction in water (LAURIN, 2010). This shift reduced the importance of a free-living larval stage as well as a fully functionally loaded chondrocranium. The disappearance of a free-living larval stage in amniotes, new living environments, and new types of locomotion and feeding behaviors triggered the further evolution of sense organs and the nervous system (SUMIDA & MARTIN, 1997). All these factors led to significant changes in the orbitotemporal region of the chondrocranium, which remained as a scaffold for the future bony skull (DE BEER, 1937, WERNEBURG, 2019).

In reptiles, the orbitotemporal region of the chondrocranium was transformed into a gracile network of cartilaginous bridges (taeniae) and columns (pilae), instead of the wide cartilaginous plate of early vertebrates (DE BEER, 1937). One possible explanation for these transformations could be to release space for the relatively large eyes in most non-mammalian amniotes. This pattern is particularly evident in squamate and avian embryos (DUFAYRE & HUBERT, 1961, HAMBURGER & HAMILTON, 1951). Mammalian embryos possess relatively small eyes, but the relative size of the brain has increased significantly compared to the ancestral amniote condition (KOYABU *et al.*, 2014). These secondary increases required an additional structural support from below and the side. Thus, the lateral wall of the chondrocranium had to reestablish broad lateral plates in the orbitotemporal region of sauropsids and mammals independently. These walls, however, are achieved differently when compared to non-amniotic vertebrates (DE BEER, 1937) and establishing homology of detailed parts among amniote groups remains outstanding.

In reptiles, the chondrocranial lateral wall, if present [it is completely absent in snakes and highly reduced in geckos (BELLAIRES & KAMAL, 1981)], consists of the pila antotica and pila accessoria (Fig. 4). The latter also participates in the formation of the taenia marginalis in most reptiles. The pila metoptica, as a separate chondrification, gives rise to the taenia medialis (YARYHIN & WERNEBURG, 2018). Among squamates, one can distinguish four different morphotypes of the chondrocranium lateral wall (Fig. 4A–D).

*Squamate type I:* In this most complete type, the pila metoptica, taenia medialis, pila antotica, and pila acces-

soria are present and are fused to each other (Fig. 4A). It is visible in lacertids (GAUPP, 1900, KAMAL & ABDEEN, 1972, RIEPPEL, 1977, YARYHIN & WERNEBURG, 2018) and in iguanids (OELRICH, 1956).

*Squamate type II:* This type is similar to type I, but the pila antotica is largely reduced during development. There can be a remainder at the basicranium and/or at the base of the pila accessoria (Fig. 4B). This condition is found in varanids (SHRIVASTAVA, 1964), agamids (ZADA, 1981), chamaeleons (VISSER, 1972), and skinks (EL-TOUBI & KAMAL, 1959, JEREZ *et al.*, 2015).

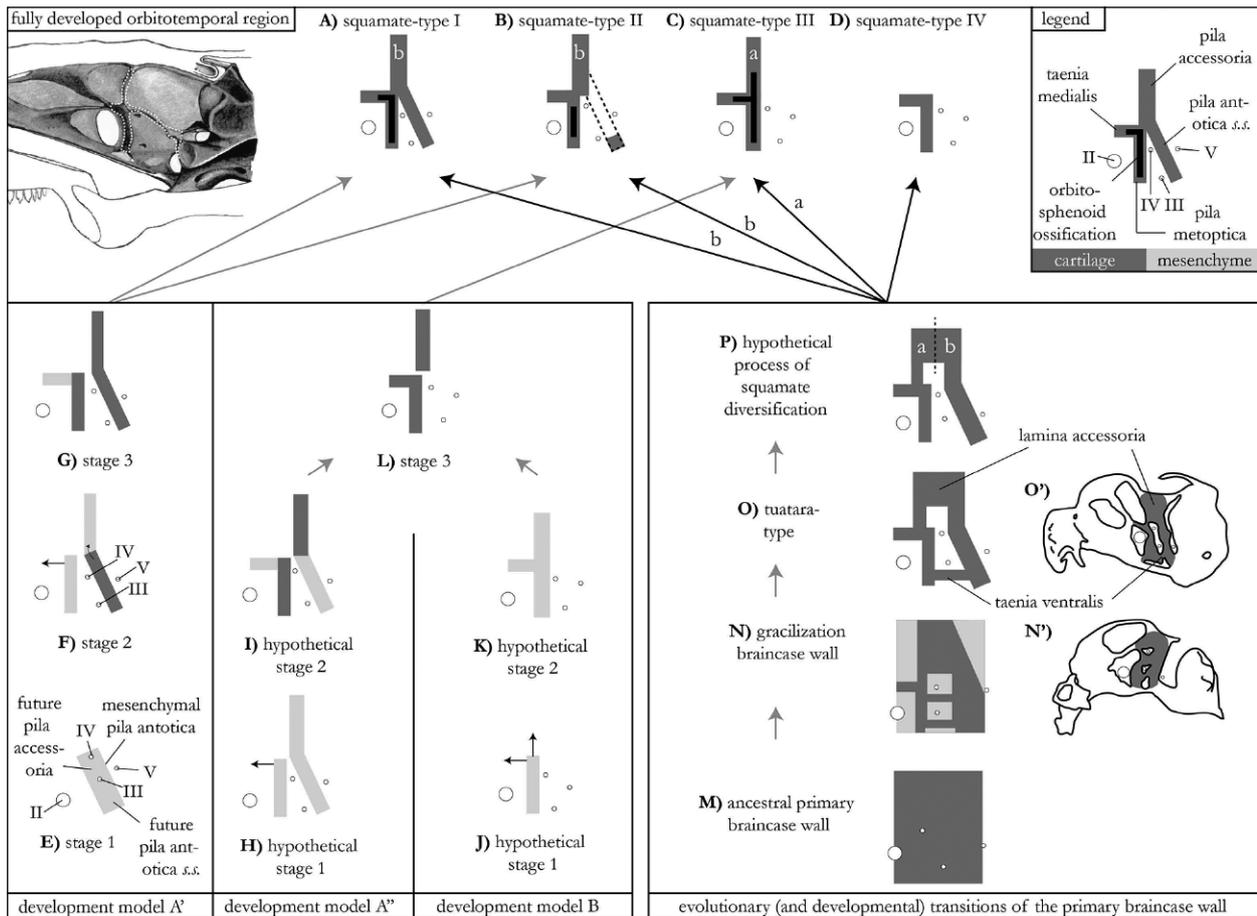
*Squamate type III:* In gymnophthalmids, the pila antotica is completely absent (Fig. 4C) (HERNANDEZ-JAIMES *et al.*, 2012). Also note that the pila accessoria does not always follow on dorsally from the pila metoptica suggesting that the homology of the pila accessoria may differ slightly from type I and II.

*Squamate type IV:* In this type, visible in geckos only, the pila metoptica and taenia medialis are present (BELLAIRES & KAMAL, 1981). Pila antotica and pila accessoria are not formed at all (Fig. 4D). However, observations of the earliest developmental state of the chondrocranium in gecko *Ascalobotes (Tarentola?) fascicularis* suggests the presence of the broad outgrowths of the mesenchymal acrochordal plate pierced by the oculomotor nerve (SEWERTZOFF, 1900) that might be homologous to the pila antotica in other reptiles. The development of this pattern needs to be clarified in future studies.

How these four morphotypes relate to developmental sequences and evolutionary polarity remains uncertain.

Recently, it has been shown that in lacertid lizards (type I) the pila accessoria develops as the distal part of the mesenchymal pila antotica and can be distinguished as an individual chondrocranial structure only after the fusion of pila metoptica to the mesenchymal pila antotica (KOVTURN & YARYGIN, 2010, YARYHIN & WERNEBURG, 2018), hence separating the latter into a pila accessoria dorsally and pila antotica *sensu stricto* ventrally (Fig. 4E–G): developmental model A'. This might also be true for other lizards with a type I configuration such as iguanids (OELRICH, 1956) as well as type II lizards such as skinks (YARYHIN & WERNEBURG, 2017). In development, pila accessoria first appears as a mesenchymal condensation only separated by the oculomotor nerve from the mesenchymal precursor of pila antotica (Fig. 4E). Chondrification develops from ventral to dorsal, hence,

← **Fig. 3.** Cross sections through the embryonic head of *Sphenodon punctatus* (specimen – “*Hatteria d*” section thickness: 15 µm) arranged in anterior (B) to posterior (J) order; level of each section is shown on the 3D reconstruction in the lateral view (A). aSN, subnarial artery; asc, ascending process; apts, ascending process of the tectum synoticum; bp, basal plate; bpt, basitrabecular process; cp, crista parotica; en, external nares; ica, internal carotid artery; is, interorbital septum; Jo, Jacobson’s organ; Ita, lamina transversalis anterior; m, basipterygoid meniscus; Mc, Meckel’s cartilage; n, notochord; ndl, nasolacrimal duct; ns, nasal septum; O, olfactory organ; oa, occipital arch; oc, otic capsule; pa, pila antotica; pla, planum antorbitale; ppp, posterior parietotectal process; ppts, posterior process of the tectum synoticum; ps, planum suprasedale; pt, pterygoid; ptc, parietotectal cartilage; Q, quadrate; t, trabeculae; tm, taenia medialis; tma, taenia marginalis; 1, olfactory nerve; 5, trigeminal nerve; 5a, ophthalmic nerve; 7p, palatine branch of the facial nerve; 8, acoustic nerve; 12, hypoglossal nerve.



**Fig. 4.** Evolution and development of the orbitotemporal region in lepidosaurs; schematic illustrations (in lateral view) of the diversity (A–D) and developmental models (E–L) in squamates and development (M–P) in *Sphenodon punctatus* (Rhynchocephalia). The illustration in the left upper corner shows the fully developed orbitotemporal region in an iguanid lizard (modified after OELRICH (1956)), the white dashed line indicates the original position of the elements shown on the legend in the right upper corner. For further details, see text.

first pila antotica *s.s.* (Fig. 4F) and then pila accessoria (Fig. 4G) chondrify (Yaryhin & Werneburg, 2018).

The absence of the pila antotica in some clades such as gymnophthalmids (type III) and geckos (type IV) (Fig. 4C–D) might be due to insufficient embryonic sampling and might simply represent reduction of a preexisting pila antotica following the pattern of orbitotemporal region development in lacertids (Fig. 4: development model A' → squamate type II). Conversely, their pila accessoria (type III and IV) could also represent a distal chondrification of the mesenchymal precursor of pila antotica, as could be the case in gymnophthalmid lizards (HERNANDEZ-JAIMES *et al.*, 2012) (Fig. 4: developmental model A''). This second assumption derives from observations in lacertids as described above, in which the pila accessoria is derived distally from the mesenchymal precursor of the original pila antotica. However, in lacertids (Fig. 4: developmental model A'), chondrification always expands from the base of pila antotica towards its distal end, and there are no separate centers of chondrification (Fig. 4F) (YARYHIN & WERNEBURG, 2018). A third scenario could instead involve the pila accessoria being developed from an outgrowth of the taenia medialis and/or the pila metoptica (Fig. 4: developmental model B).

The specific shape of the orbitosphenoid, which is supposed to stem from within the pila metoptica (BENOIT *et al.*, 2017, BEVER *et al.*, 2005, DE BEER, 1937), could possibly hint to an origin of pila accessoria different to pila antotica. The extent of ossification of the orbitosphenoid in squamates appears to be associated with the diversity in the orbitotemporal region of the chondrocranium and may reflect the extension and derivatives of the pila metoptica (Fig. 4A–C). This inference is only tentative as only very few studies (LEDESMA & SCARPETTA, 2018, OELRICH, 1956, SÄVE-SÖDERBERGH, 1946, TARAZONA & RAMIREZ-PINILLA, 2008) provide detailed observations on orbitosphenoid appearance, which, in addition, are often just a developmental snapshot of a potentially more complex expansion of the ossification. In squamate type I, in which the pila antotica is present in the later stages of development (Fig. 4A), the orbitosphenoid ossification includes both the pila metoptica and taenia medialis, thus separating it from pila accessoria as recorded for the iguanid lizard *Ctenosaura* (OELRICH, 1956). In squamate type II, in which the pila antotica is lost during development (Fig. 4B), the orbitosphenoid ossifies only within pila metoptica, as observed in varanid lizards (SÄVE-SÖDERBERGH, 1947), and the taenia medialis

remains cartilaginous and is fused with pila accessoria without interruption. As the pila metoptica is the only ventral connection for the pila accessoria in this case (no second, ventral pila antotica *s.s.* connection is possible), the fusion of taenia medialis and the original pila antotica might form, for stabilization purposes, a complex structural pattern at the fusion point of both elements preventing the expansion of the orbitosphenoid ossification beyond pila metoptica. In squamate type III, in which that pila antotica is completely absent during development (Fig. 4C), the orbitosphenoid ossification is not confined to the pila metoptica, but spreads into the pila accessoria and the taenia medialis (HERNANDEZ-JAIMES *et al.*, 2012). This contiguous state perhaps suggests an early ontogenetic connection forming a smooth fusion between elements (Fig. 4: development model A'') or potentially an outgrowth of the pila accessoria from the pila metoptica and/or taenia medialis (Fig. 4: development model B) naturally reflected in the threefold ossification pattern. The anatomical condition in *S. punctatus* could help clarifying the nature and origin of these patterns as it may resemble an ancestral condition. In *S. punctatus*, pila antotica exists for a long period of chondrocranium developments which is likely the plesiomorphic state given the condition in outgroup representatives such as crocodiles, birds, and turtles (PALUH & SHEIL, 2013). The overall robust cartilaginous structure (Fig. 4N') (HOWES & SWINNERTON, 1901) also more closely resembles that of those animals (PALUH & SHEIL, 2013). Based on these considerations and since *S. punctatus* is the last survivor of a previously extremely diverse clade (EVANS & JONES, 2010), it is a good outgroup taxon to understand the diversification of squamate chondrocrania.

The 'pila accessoria' in *S. punctatus* is a conspicuously wide plate (Fig. 2A) that extends dorsally from the node of fusion of pila metoptica and taenia medialis. This arrangement perhaps supports the notion that the narrow pila accessoria of squamates represents either the posterior (type I and II) or anterior (type III) part of this structure: a lamina accessoria (Fig. 4A, B, C, P). Therefore, we hypothesize, that this wide lamina accessoria of *S. punctatus* represents the ancestral continuum of two types of pilae accessoria, i.e. of (a) squamate type I/II and (b) squamate type III (a and b in Fig. 4A–C, P). In a previous study of chondrocranium formation, *S. punctatus* was shown to develop both the pila antotica and metoptica from a single cartilaginous plate early in development (Fig. 4 M–N') (HOWES & SWINNERTON, 1901). A similar condition was also reported for turtles (KURATANI, 1987, TULENKO & SHEIL, 2007), but the mechanism of separation and transformation of this region remains unclear. The *S. punctatus*-type of pila accessoria (Fig. 4O–O') appears to present the ancestral condition for lepidosaurs. It persists as broad primary wall in *S. punctatus* but in squamate evolution either (a) the anterior part of the accessorial plate remains associated with the pila metoptica/taenia marginalis complex (squamate type III) or (b) the posterior part remains associated with the original pila antotica (squamate type I/II) (Fig. 4P).

The spatially distinct development of the pila metoptica and pila antotica in squamates evidently reflects a derived state, e.g. based on outgroup comparisons: in turtles and crocodiles, it develops from one cartilaginous plate (MÜLLER, 1967, TULENKO & SHEIL, 2007). This type of chondrocranial lateral wall development is apparently present also in mammals, where the hypochiasmatic cartilage, topographically homologous to the pila metoptica in reptiles, has a mesodermal origin but develops spatially separately from the acrochordal derivatives, which are also of mesodermal origin (MCBRATNEY-OWEN *et al.*, 2008). The ventral connection between the pila metoptica and pila antotica (taenia ventralis) appears to represent a remainder of the ancestral primary braincase wall inherited from a non-amniotes ancestor (Fig. 4M–O).

The ontogeny of the primary lateral braincase wall in *Sphenodon punctatus* (Fig. 4N–O) (HOWES & SWINNERTON, 1901) suggests that in squamate evolution, the originally broad braincase wall opened up in its middle around the small foramina of oculomotor (III) and trochlear (IV) nerve. Those enlarged foramina eventually coalesced with the loss of the taenia ventralis and resulted in an anterior (pila metoptica/taenia medialis) and a posterior (pila antotica *s.s.*) part. In different squamate taxa, either the anterior (a in Fig. 4) or the posterior (b in Fig. 4) part of the accessorial plate remained resulting in type I/II or type III. The functional consequences of different shapes of the orbitotemporal region in squamates is not well understood. The largely akinetic adult skull of *S. punctatus* (JONES *et al.*, 2011) however, might have some association to its robust orbitotemporal region, whereas the more gracile pattern in squamates might have some connection to higher degrees of cranial kinesis.

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