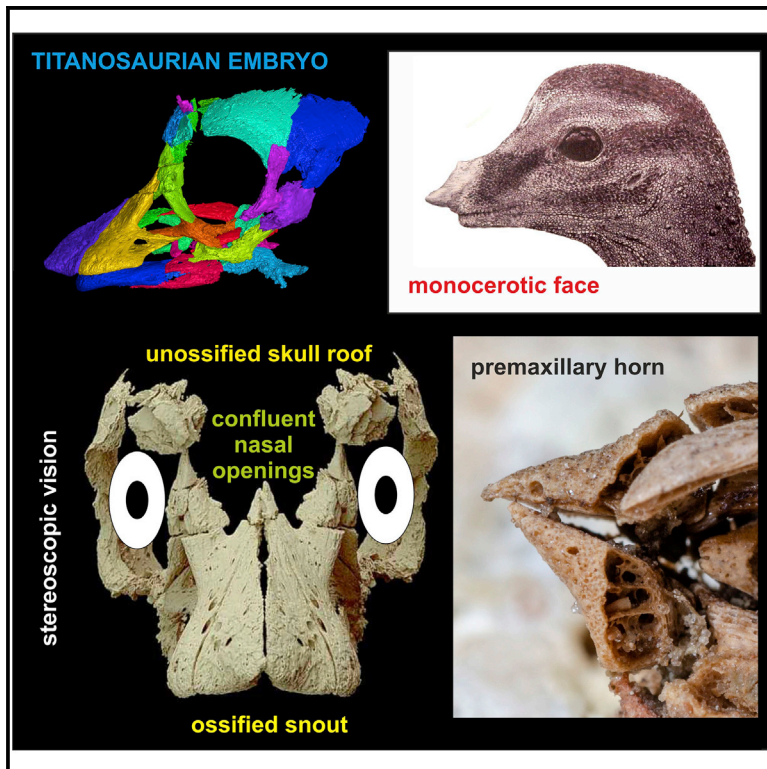# Current Biology

Specialized Craniofacial Anatomy of a Titanosaurian Embryo from Argentina

Graphical Abstract

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In Brief

Kundrát et al. report the first 3D preserved embryo representing a sauropod sauropodomorph. The analyses indicate an unusual monocerotic face and the early development of stereoscopic vision for a sauropod. It reveals significant heterochrony in prenatal cranial ossification when compared with non-sauropod sauropodomorphs.

Highlights

- The first 3D preserved embryonic skull of a sauropod sauropodomorph

- It differs in facial anatomy and size from the sauropod embryos of Auca Mahuevo

- It exhibits an unusual monocerotic and stereoscopic face for a sauropod

- Heterochronies in cranial ossifications occurred in the evolution of sauropods

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Specialized Craniofacial Anatomy of a Titanosaurian Embryo from Argentina

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SUMMARY

The first dinosaur embryos found inside megaloolithid eggs from Auca Mahuevo, Patagonia, were assigned to sauropod dinosaurs that lived approximately 80 million years ago. Discovered some 25 years ago, these considerably flattened specimens still remain the only unquestionable embryonic remains of a sauropod dinosaur providing an initial glimpse into titanosaurian *in ovo* ontogeny. Here we describe an almost intact embryonic skull, which indicates the early development of stereoscopic vision, and an unusual monocerotic face for a sauropod. The new fossil also reveals a neurovascular sensory system in the premaxilla and a partly calcified braincase, which potentially refines estimates of its prenatal stage. The embryo was found in an egg with thicker eggshell and a partly different geochemical signature than those from the egg-bearing layers described in Auca Mahuevo. The cranial bones are comparably ossified as in previously described specimens but differ in facial anatomy and size. The new specimen reveals significant heterochrony in cranial ossifications when compared with non-sauropod sauropodomorph embryos, and demonstrates that the specialized craniofacial morphology preceded the postnatal transformation of the skull anatomy in adults of related titanosaurians.

RESULTS AND DISCUSSION

First 3D Preserved Sauropod Sauropodomorph Embryo and Its Repatriation

Sauropodomorph embryology remains one of the least explored areas of the life history of dinosaurs. The first definitive discovery of sauropod embryos came with the finding of an enormous nesting ground of titanosaurian dinosaurs known as Auca Mahuevo, discovered in Upper Cretaceous deposits of northern Patagonia, Argentina [1, 2]. Recently, embryonic remains of the early sauropodomorphs *Massospondylus* and probably *Lufengosaurus* were reported from the Early Jurassic of South Africa [3] and China [4], respectively. Our study contributes yet another important specimen for understanding sauropodomorph ontogeny, in the form of a new specimen of titanosaurian embryo from Argentina, albeit of unknown provenance within Patagonia. The original egg was illegally exported from this country, and eventually brought to the attention of one of us (T.W.M.). After preparation exposed embryonic remains, the unique preservation and scientific importance of the specimen became evident and T.W.M. repatriated the fossil to the Museo Municipal "Carmen Funes" in Plaza Huincul, Neuquén Province, Argentina, where the specimen was catalogued under the number MCF-PVPH-874.

MCF-PVPH-874 represents a fragment of a fossil egg and the egg-filling sediments with the skull *in situ* (Figures 1A, 1B, and S1). No postcranial remains were recognized inside the egg as in other embryonic specimens from Auca Mahuevo (Figure 1C) [1]. The skull has been well exposed on its left side after chemical preparation, but we have also imaged the skull with propagation phase contrast synchrotron microtomography (Figures 1D, 1E, S2, and S3; Video S1) at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. The scan revealed many previously unknown anatomical details and the internal structure of the preserved bones and putative soft tissue (Figure S4).

Here we report on this exceptional specimen of sauropod embryo that exhibits cranial bones including braincase components keeping their original shape, mostly intact surfaces and articulation. These circumstances enabled us (1) to reconstruct the most plausible appearance of the skull in titanosaurian sauropods before hatching; (2) to describe anatomical characters based on intact cranial bones useful for taxonomic or ontogenetic comparisons; (3) to revise opinions on how babies of these giants may be hatched, in particular, if this process was facilitated by the rostral horn [5]; (4) to test some previous suggestions about sauropodomorph reproduction [6] and cranial ossification [7]; and (5) to reveal biological and geochemical characters that
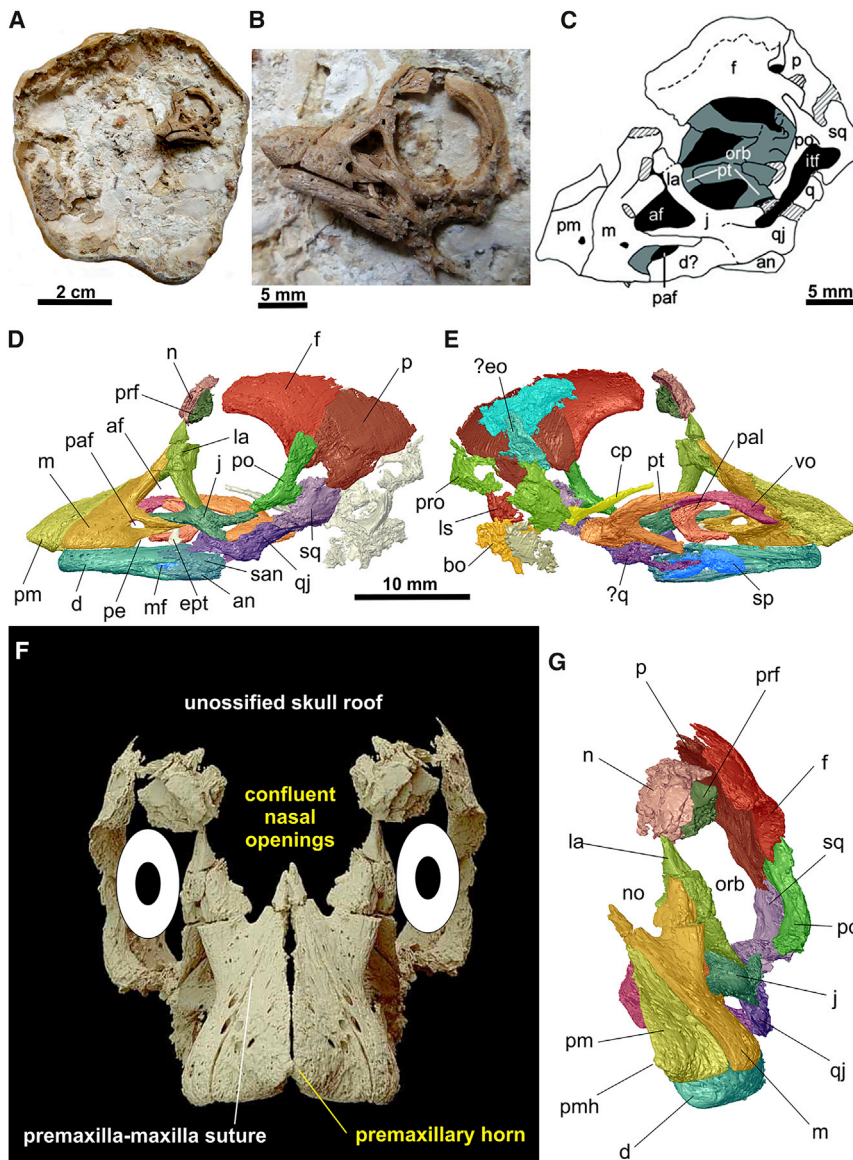
Figure 1. The New Specimen of Megaloolithid Egg Containing the 3D Preserved Skull of a Titanosaurian Embryo MCF-PVPH-874

(A) Sediment filling the egg with the embryonic skull *in situ*.

(B) Magnified perspective of the embryonic skull with the preorbital and orbital region in left lateral view.

(C) The previously described flattened embryonic skull MCF-PVPH-263 from Auca Mahuevo.

(D and E) Digital reconstruction of the cranial bones: D, in lateral view, and E, in medial view (see Video S1 and Figures S1–S3); notice mineralized portions of neurocranial bones (see Figure S4).

(F) Reconstruction of the skull in anterior view showing incomplete skull roof and antero-lateral orientation of orbits; notice the preserved premaxilla-maxilla suture, which suggests that the narial opening is anteriorly limited by the maxilla, with the premaxilla possibly restricted to the sagittal contact.

(G) The left part of the skull in anterior view; notice placement of the orbit and the posteriorly retracted narial opening (ellipse). af, antorbital fenestra; an, angular; bo, basioccipital; cp, cultriform process of the parabasisphenoid; d, dentary; eo, exoccipital; ept, ectopterygoid; f, frontal; itf, infratemporal fenestra; j, jugal; la, lacrimal; ls, laterosphenoid; m, maxilla; mf, mandibular fenestra; n, nasal; no, narial opening; orb, orbit; p, parietal; paf, preantorbital foramen; pal, palatine; pe, postdental emargination; pm, premaxilla; pmh, premaxillary horn; po, postorbital; prf, prefrontal; pro, prootic; pt, pterygoid; qj, quadratojugal; q, quadrate; san, surangular; sp, splenial; sq, squamosal; vo, vomer.

The calcified neurocranial elements provide some morphological clues about their identity and potentially refine estimates of a prenatal stage of the titanosaurian skull. The elements identified as basioccipital, prootic, and exoccipital are comparably as incomplete as those that occur around days 47 to 50 of *in ovo* development of the alligator neurocranium (Figure S4E). At hatching, the neurocranium is usually well ossified in the alligator, whereas small cartilaginous streaks remain between basicranial bones in precocial species of modern birds [9].

Adopting the bone mineralization scale of the modern precocial archosaurs provides a simplified, but so far the only, framework for reasonable assessment of the developmental age of dinosaur embryos [10]. Assuming the presence of developmental precociality in titanosaurians (due to hypothetical lack of any parental care), embryo MCF-PVPH-874 has already undergone four-fifths of its *in ovo* development. The absolute length of the incubation period, however, remains unknown for any sauropod dinosaur.

Recently, embryos of the non-sauropod sauropodomorph *Massospondylus carinatus* (BP/1/5347a) were found to be only 60% through their incubation period (IP) using cranial

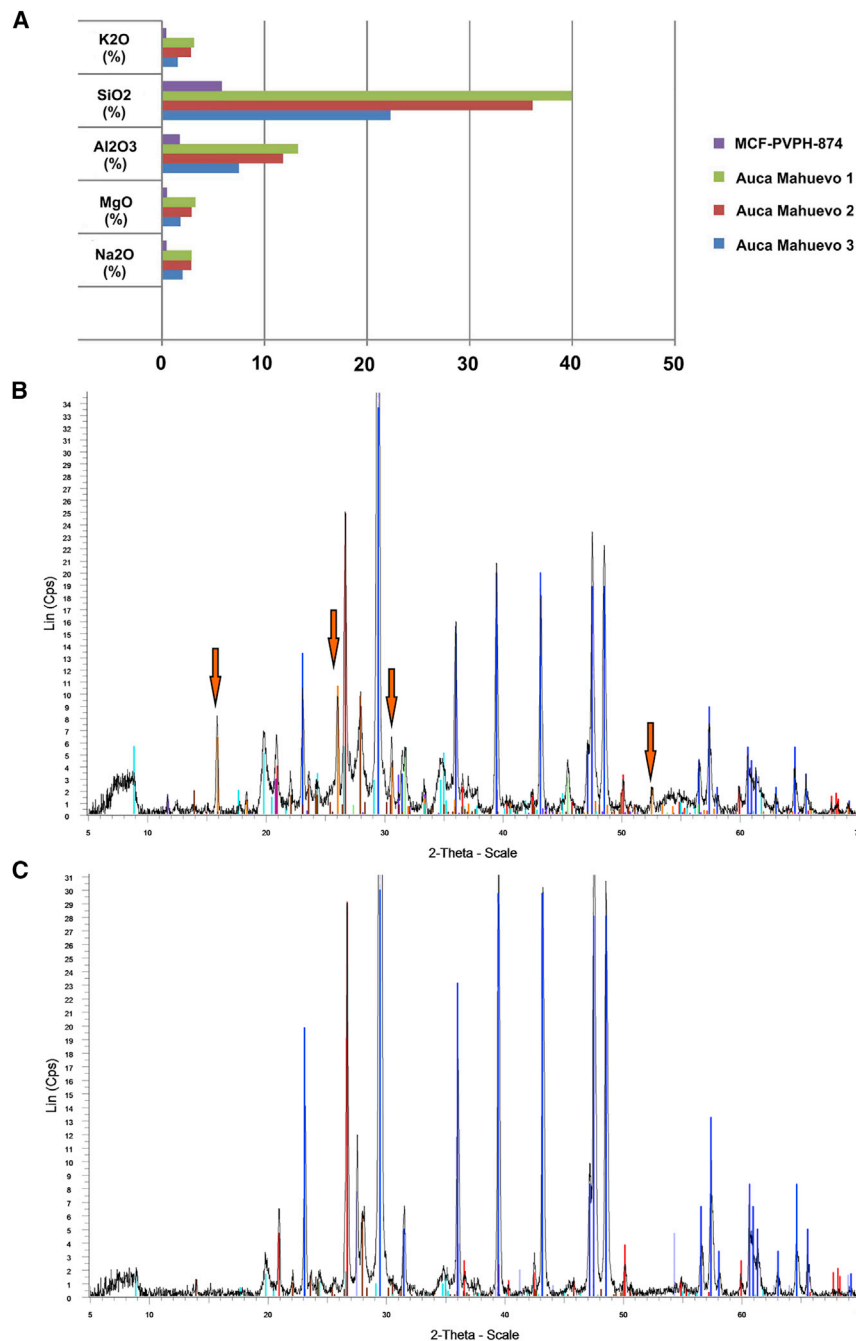distinguish the new specimen from previously described titanosaurian embryos from Auca Mahuevo [1, 2].

Cranial Ossifications and Stage Assessment

Most of the MCF-PVPH-874 skull is well ossified except for the roof (Figures 1F and 1G). The skull roof fenestra remains largely open and the bone ossifications surrounding it are slightly in advance of the condition seen in a 50-day embryo of the American alligator [8]. Notably, the retroarticular process (posterior end of the Meckel's cartilage) starts to mineralize around day 44 in the alligator [8], whereas no fossilized remains of the articular have been observed in MCF-PVPH-874 (SOM). Furthermore, we found that most of the embryonic braincase elements of MCF-PVPH-874 were lightly calcified and preserved at the stage of acquiring their surface contours at the time of death. The only elements with definable outlines include the cultriform process of the parabasisphenoid and the laterosphenoid (Figure S4D).

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Figure 2. Whole Rock Geochemistry of the Eggshell and Sediment Samples from Northern Patagonia

(A) Comparisons of X-ray fluorescence-based analyses of MCF-PVPH-874 and three samples from Layer 3 of Auca Mahuevo; notice significantly lower amount of several specific oxides in MCF-PVPH-874.

(B) High-resolution X-ray diffractometry spectrum of the Auca Mahuevo specimen showing the presence of the tectosilicate mineral analcime (orange colored peaks and arrows).

(C) High-resolution X-ray diffractometry spectrum of MCF-PVPH-874; notice the absence of the analcime.

Geochemistry Signatures

To investigate the provenance of MCF-PVPH-874 further, we analyzed the whole-rock geochemistry of three samples of fossil eggshell and sediment from Layer 3 of Auca Mahuevo and one sample derived from MCF-PVPH-874. The X-ray fluorescence (XRF) analysis revealed that all samples from Auca Mahuevo contain higher amounts of several specific oxides than sample MCF-PVPH-874 (Figure 2A). The samples were also analyzed by X-ray diffractometry (XRD) to identify the principal minerals present. The XRD spectra revealed the presence in all Auca Mahuevo samples of the tectosilicate mineral analcime (Figure 2B).
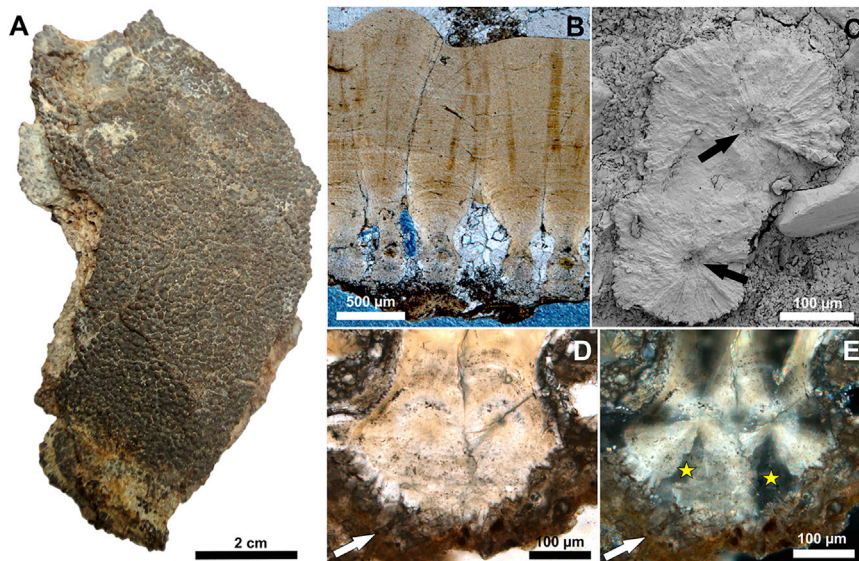
Analcime is indicative of the lowest (zeolite) grade of metamorphism or diagenesis of a sedimentary facies. Such diagenesis would happen on a regional scale. The XRD spectra thus suggest that there has been pervasive zeolitic alteration at Auca Mahuevo. It is inconceivable that the analcime is detrital, for example washed into the basin from elsewhere, as this mineral is exceedingly rare in source rocks and is soft and easily broken down. It is most probably a widespread diagenetic product as a result of decomposition/alteration of the less stable silicates [12]. Analcime, however, is totally absent from the sample of MCF-PVPH-874 (Figure 2C), indicating that this specimen did not undergo such regional diagenesis/metamorphism and derives from a different locality than the egg layer of Auca Mahuevo 3 [2].

ossification sequences of living saurians [7]. Having compared MCF-PVPH-874 (~75% of IP) with BP/1/5347a (~60% of IP), we suggest that significant developmental heterochronies between snout and calva had occurred in the evolution of titanosaurian sauropods. In fact, MCF-PVPH-874 exhibits rather a reversed ossification pattern of advanced ossifications of the snout (*contra* incomplete sutures in BP/1/5347a). Moreover, MCF-PVPH-874 also shows much delayed ossification of posterior roofing bones as in crocodilians [8] and birds [11], *contra* complete interfrontal and interparietal sutures in BP/1/5347a.

Eggshell Microstructure and Classification

Embryo MCF-PVPH-874 was found inside an egg referable to Megaloolothidae [13] (Figures 3A and 3B). The monolayered eggshell consists of fan-shaped units, whose tops constitute tubercular surface ornamentation (Figure 3B). Having compared the eggshell parameters among megaloolithid eggs from several

Figure 3. Eggshell Microstructure of MCF-PVPH-874

(A) The fragment of the original egg.

(B) Radial thin section through the monolayered eggshell of the megaloolithid type showing fan-shaped units.

(C) Inner surface of the eggshell exposing the coalescent mammillary tips with central concavities (arrow).

(D) The coalescent inner (mammillary) tips of the eggshell units in radial view; notice a fibrous mat underlying the innertips.

(E) The same perspective in polarized light revealing deep resorption pits (asterisk); notice a close association between the innertips and underlying fibrous structure that likely represents the shell membrane (arrow).

sites in northern Patagonia, we found that MCF-PVPH-874 has a significantly thicker eggshell (1.6–2 mm) than the specimens from Auca Mahuevo (0.6–1.3 mm) [6]. The eggshell thickness is more similar to specimens from the Mansilla I and II localities (1.8–2 mm) in the Río Negro province [14]. It is also comparable to the partial egg specimen (1.7–2.1 mm) found near Neuquén City (the same stratigraphic unit that contains Auca Mahuevo) [15].

Finally, we observed significant central concavities on inner tips of single and coalescing shell units in MCF-PVPH-874 (Figure 1C) contrasting with an absence of any sign of calcium resorption in specimen MCF-PVPH-263 from Auca Mahuevo. The resorption pits are deeply excavated and merged in a fibrous mat that probably represents the fossilized remains of the shell membrane (Figures 3D and 3E). Thus, we provide for the first time evidence that titanosaurian embryos utilized eggshell-derived calcium relatively long before they approached the hatching stage.

Developmental Disparity in the Evolution of Titanosaurian Face

Embryo MCF-PVPH-874 shows one of the most complete and articulated skulls known from a titanosaurian dinosaur from Patagonia. In fact, only a few titanosaurian skulls are known globally, including that of *Tapuiasaurus macedoi* [16] from Brazil; *Bonitasaurus salgadoi* [17], *Antarctosaurus wichmannianus* [18], and *Sarmientosaurus musacchioi* [19] from Patagonia; and *Rapetosaurus krausei* from Madagascar [20]. MCF-PVPH-874 is cranially most similar to the previously described embryos from Auca Mahuevo, MCF-PVPH-272 and MCF-PVPH-263 [1, 2]. It is assigned to titanosaurians based on the presence of several characters such as a distinct notch ventral to the antorbital fenestra (postdental emargination), a rostrally extended quadratojugal, and the presence of a mandibular fenestra [1, 2, 21].

Noteworthy are the number of anatomical details that differ in MCF-PVPH-874 from the two previously described specimens (SOM). The maxilla is rostrally longer (character 1) and the premaxilla-maxilla suture inclines postero-dorsally, contrasting with a short snout and vertically oriented suture in MCF-PVPH-272 and 263. A preantorbital fenestra of MCF-PVPH-874 opens ventral rather than posterior to the rostral rim of the antorbital fenestra (character 2); this condition is also seen in *Tapuiasaurus* [16]. The lacrimal of MCF-PVPH-874 projects more vertically (character 3) as in skeletally mature specimens (see below), and contrasts markedly with the rostrocaudally inclined lacrimal in MCF-PVPH-272 and 263. Furthermore, a rostrodorsal rim on the postdental emargination is slightly angled (character 4) in MCF-PVPH-874, as in *Tapuiasaurus* [16], *Antarctosaurus* [17], *Sarmientosaurus* [19], and *Rapetosaurus* [20], whereas it is notched in the embryos MCF-PVPH-272 and MCF-PVPH-263 and in *Bonitasaura* [17]. The jugal of MCF-PVPH-874 has a deeply incised caudal margin (character 5) and recalls the condition in *Tapuiasaurus* [16]. Unlike MCF-PVPH-272 and 263 [2, 21], the quadratojugal and squamosal contact directly (character 6) as in titanosaurians from South America and Africa [16–20]. These characters most probably had undergone post-hatching remodeling to some extent, and should therefore be used with caution when searching for relatives of MCF-PVPH-874 among known titanosaurian taxa. It appears that embryo MCF-PVPH-874 is most similar in morphology to *Tapuiasaurus* (characters 1, 2, and 4–6) from the Early Cretaceous of Brazil [16], suggesting a hypothetical affinity of MCF-PVPH-874 to nemegtosaurid titanosaurians.

Differences between the new titanosaurian embryo and previously described specimens [1, 2, 17] are also expressed in quantitative terms. In terms of size proportions, MCF-PVPH-874 is significantly smaller (25%–35%) than MCF-PVPH-263. In comparison to the latter, the new specimen has a shorter dorsoventral diameter of the orbit (8.9 mm versus 11.9 mm), antero-posterior length of the antorbital fenestra (4.1 mm versus 6.4 mm), and the maximum length of the postorbital (8.4 mm versus 10.9 mm; measured from the anterior contact with the jugal to the most postero-dorsal extension of the postorbital). Despite the metric differences, the cranial ossification patterns are almost identical. A reasonable explanation of this developmental

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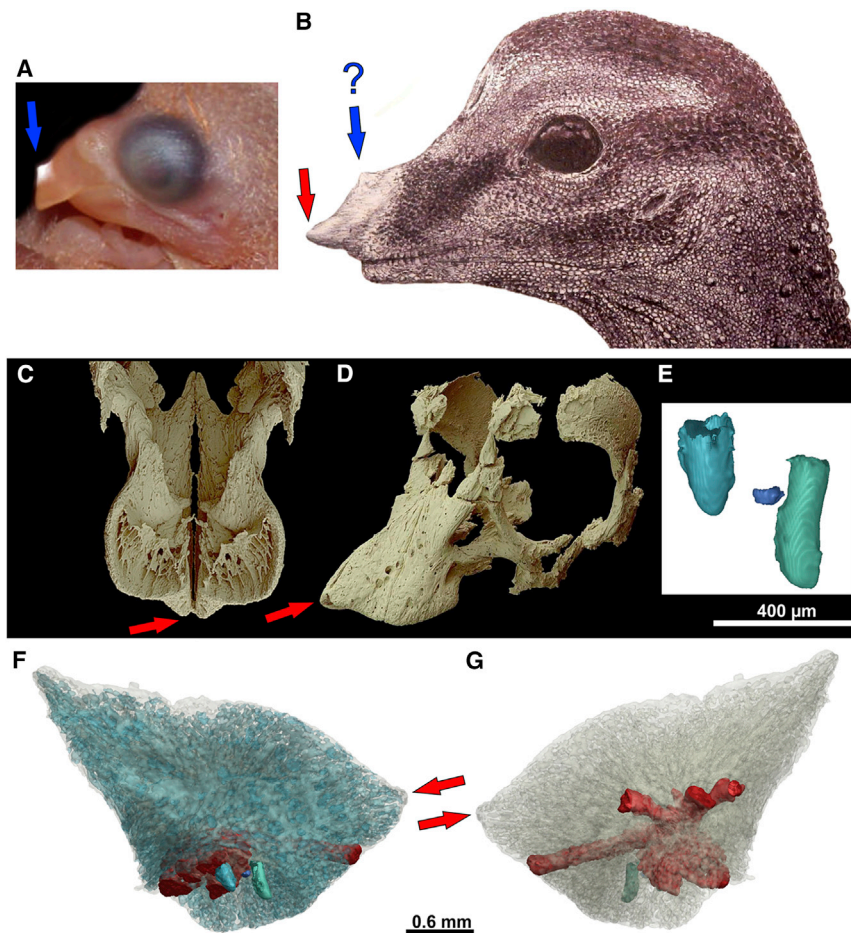
Figure 4. Reconstruction of the Craniofacial Anatomy of the MCF-PVPH-874 Titanosaurian Embryo

(A) Placement of the egg tooth in modern bird embryo.

(B) Reconstruction of the head appearance by Vladimír Rimbala; the blue arrow points to the probable placement of the keratinous egg-tooth and the red arrow to the premaxillary horn including both keratinous and bone components.

(C) The craniofacial region in ventral view showing the premaxillary and maxillary alveoli and the rostral premaxillary projection forming a basis of the horn-like process (red arrow).

(D) The skull in antero-ventral view; notice rostral pointed projection (red arrow).

(E) 3D rendered first mesial premaxillary teeth.

(F and G) 3D rendering of the opaque and semi-transparent premaxilla in medial and lateral views; notice distribution of the neurovascular canals, premaxillary teeth *in situ*, and the premaxillary horn (red arrow). See also Figure S1.

limit of the dentary recalling growth disparity of the upper and lower jaw in modern birds (Figures 4A and 4B). Unlike modern birds, the rostral process projects dorsal to the rostral rim of the premaxilla including alveoli and preserved teeth in MCF-PVPH-874 (Figures 4C–4E and S1D). Therefore, in contrast to previous reconstructions of MCF-PVPH-272 and 263 [5], we suggest that the rostral process lacks occlusion with dentary teeth and is actually free of alveoli. It is likely that a thicker corneous sheet developed over the rostral process and gave a horn-like appearance to the embryonic face of MCF-PVPH-874 (see red arrow in Figure 4B).

disparity could be that the ossification rates of MCF-PVPH-874 increase developmental variability in the same species that could correspond to different incubation and environmental conditions. Alternatively, MCF-PVPH-874 could represent a titanosaurian taxon different to that of MCF-PVPH-263 and 272.

In Ovo Cranial Development

MCF-PVPH-874 further provides the first 3D perspective of a sauropod sauropodomorph embryo. The preserved configuration of cranial bones reveals several breakthroughs in our understanding of the early development of the titanosaurian skull.

First, it shows that some of the characters discussed above (1 and 3) may be less different among the titanosaurian embryos if structures are compared and viewed at corresponding angles. Thus, we refine the appearance of these characters based on the 3D preservation of MCF-PVPH-874 and interpret the differences with the Auca Mahuevo specimens as artifacts due to postmortem deformation.

Second, it reveals that several transforming morphologies such as an elongated snout and retracted external narial openings, which have been hypothesized to appear during a juvenile period of titanosaurian ontogeny [5], are in fact already present in the MCF-PVPH-874 embryo prior to hatching.

Third, the premaxilla is slightly vaulted dorsally and forms a massive broad-based spike protruding far beyond the rostral

Fourth, unlike adults, the orbits face anterolaterally in the embryo MCF-PVPH-874 (Figures 1F and 1G). Thus, we assume that early juveniles of titanosaurian sauropods might benefit from a temporary ability of at least a partial binocular vision that would provide a much better visual perception. Putative stereoscopy was suggested for *Bajadasaurus*, a sauropod from Patagonia [22]; however, it was assumed based on a rather dorsal than anterior placement of orbits. The stereoscopy with "dorsally" projecting orbits became functional (e.g., in predator detection) either when the head was held with the snout facing ventrally on an erected neck or when the animal was grazing and the eyes faced anteriorly.

The Titanosaurian Premature Monocerotic Face

The interior of the premaxilla is canalized by a set of neurovascular canals radiating from the original ossification centrum with the two longest canals projecting toward the rostral process (Figures 4F and 4G). Three neurovascular branches project toward the external surface; such configuration most probably supported cell proliferation and sensory functions of the overlying skin. Another set of neurovascular canals projects lingually and terminates in the alveolar bone. Beside those, there is a single, the

longest, neurovascular canal that extends to the base of the premaxillary horn on the ventral side (Figure 4G).

The bony rostral process of the premaxilla has been interpreted as a functional analog [5] of the keratinous egg-tooth seen in modern crocodiles and birds [23–25]. In contrast to this, a functional egg tooth (consisting of dentin and enamel) develops from rudiments laid down in the premaxillary bone and grows at significantly higher rates exceeding other teeth in size in Squamata [26]. Furthermore, two functional egg teeth were reported in Gekkota [26], increasing the developmental diversity of the overall concept of egg teeth.

We do not know the exact *in ovo* position of the head of MCF-PVPH-874; however, given the curled position of extant reptiles [27, 28] of comparable ossification stage and dorsally projected egg tooth, it might be difficult to explain how the rostral process facilitated breaking the eggshell during hatching in titanosaurians. Consequently, if an egg tooth was retained in titanosaurian embryos, it seems more likely that it developed on the dorsal surface of the snout, regardless of the presence of a premaxillary horn-like structure. The most plausible topographic placement of a functional egg-tooth would be over the vaulted dorsal surface of the premaxilla (see blue arrow in Figure 4B). This egg tooth would likely have been shed shortly after hatching, whereas the bony premaxillary horn probably persisted for some time in juvenile titanosaurians from Patagonia.

In summary, embryo MCF-PVPH-874 contains the best 3D *in ovo* preserved embryonic skull of a sauropod sauropodomorph dinosaur or dinosaur in general. It is noteworthy that MCF-PVPH-874 shows significant heterochronic changes in ossification of cranial bones when compared with the non-sauropod sauropodomorph embryos [7]. It appears that the embryos of the massospondylid sauropods develop the skull roof before the snout while the embryos of titanosaurian sauropods exhibit the reversed pattern. Given the fact that we do not know how long these sauropod embryos developed inside their eggs, it is difficult to assess how much these prenatal heterochronies remained expressed in the cranial morphology at hatching. We assume, however, that the delayed closure of the skull roof in the titanosaurian sauropods might be linked with development of the dorsal dural sinus that is a prominent endoneurocranial structure in sauropods [29].

The specimen MCF-PVPH-874 implies that titanosaurian hatchlings hatched with a temporary monocerotid (single-horned) face, posterodorsally retracted narial openings, and early binocular vision. Although we do not disregard the hatching scenario of titanosaurian embryos using the boney ''egg-tooth'' *sensu* García [5], our observations of MCF-PVPH-874 make it equally reasonable to consider other alternatives such as using the epidermal prominence (thickening located on the median line between the narial and jaw edge) as in modern crocodilians and birds or by taking advantage of temporary hypertrophied musculature. Furthermore, the new specimen shows the first real cranial geometry of a titanosaurian sauropod from South America and reveals a putative affinity to nemegtosaurids. MCF-PVPH-874 differs from previously described embryonic skulls of titanosaurian dinosaurs discovered in Auca Mahuevo in morphological, developmental, and geochemical parameters.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Fossil Samples		
3D *in ovo* preserved titanosaurian embryo	This paper	MCF-PVPH-874
Flattened *in ovo* titanosaurian embryo	[1, 2, 16]	MCF-PVPH-263
Flattened *in ovo* titanosaurian embryo	[1, 2, 16]	MCF-PVPH-272
Biological Samples		
Embryos of *Alligator mississippiensis*	This paper + [7]	CIB-Am1-4
Deposited Data		
Scanned Data	http://paleo.esrf.eu	N/A
Software and Algorithms		
Mimics v.12.3 and v.13.1	Materialise, Belgium	N/A
VGStudio Max 2.2	Volume Graphics, Germany	N/A
Blender v.2.78	https://www.blender.org/	N/A

RESOURCE AVAILABILITY

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Martin Kundrát (martin.kundrat@upjs.sk).

Materials Availability
The MCF-PVPH-874 specimen is available for study at Museo Carmen Funes at Plaza Huincul in Argentina.

Data and Code Availability
The scanned data of the MCF-PVPH-874 specimen will be made publicly available on the ESRF paleontology online database at http://paleo.esrf.eu. The published article includes all codes generated or analyzed during this study.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The described specimen, MCF-PVPH-874, was analyzed from first-hand observations including 3D virtual high-resolution models. The fossil has been repatriated back to Argentina and has become an integral part of the collection of Museo Municipal "Carmen Funes" in Plaza Huincul where it will be available for future investigation.

The experimental subjects include the new 3D *in ovo* preserved specimen of titanosaurian embryo (MCF-PVPH-874) described in this study and two previously described [1, 2] flattened *in ovo* preserved titanosaurian specimens (MCF-PVPH-263 and 272) for comparative reasons. These specimens are housed at the Museo Municipal "Carmen Funes", Avenida Córdoba, Plaza Huincul 8318, Neuquén, Argentina.

The modern archosaur experimental subjects used in this study include four different developmental stages of *Alligator mississippiensis* from Collections of the Center for Interdisciplinary Biosciences, Technology and Innovation Park, Pavol Jozef Šafárik University in Košice, Slovak Republic. These specimens were donated to Martin Kundrát by Dr. James Perran Ross from the Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida, USA.

METHOD DETAILS

Provenance of the analyzed specimen
The specimen, MCF-PVPH-847, originally represented by a complete (unprepared) egg was bought by Terry W. Manning from an Argentinian dealer in Tucson in 2001, who is known to have collected only in Patagonia. John Nudds has the name and details of this company (which now deals solely in European minerals) and met the dealer in Tucson in 2015 who told him that the specimen had come from the Allen Formation of Bajo de Santa Rosa in Río Negro Province. The prepared specimen was repatriated to the Museo Municipal "Carmen Funes" in Plaza Huincul, Neuquén Province, Argentina, where the specimen was catalogued under the number MCF-PVPH-874.

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Chemical Preparation

The analyzed specimen, MCF-PVPH-874, was *in ovo* prepared by Terry W. Manning using a chemical method that etches away only 10 μm of the rock a day. He found that the usual concentration of 5% acetic acid was too strong, being prone to erode the very delicate bones of the preserved embryo. At less aggressive concentrations of 0.5%–2.0%, however, the acid solution becomes locally stratified, with saturated acetate solution next to the fossils inhibiting progressive action of the acid. This problem was overcome by agitating the solution, which allowed the active acid to remain in direct contact with the exposed bone. A small quantity of wetting agent and an algal inhibitor were added to the etching solution to maintain the rate of reaction with the specimen and prevent subsequent appearance of microorganisms. At intervals during the acid treatment, digested matrix was carefully washed away, using weak, precisely controlled jets of distilled water; even fine needles were too coarse for this stage of the process. Then the specimen was washed in running tap water, air-dried and finally the exposed bone was protected with dilute Paraloid B72 in acetone.

Geochemistry

The whole-rock geochemistry of the samples was determined by X-ray fluorescence (XRF) techniques using a PANalytical Axios wavelength-dispersive spectrometer (WDS) fitted with a Rhodium X-ray tube. Major element analysis was performed using the *Omnion* package optimized for geological samples. Trace element data were obtained using the *Protrace* trace element package using standard conditions. Loss on ignition (LOI) was determined at 110°C and 1100°C. Samples were analyzed as pressed powder pellets (12 g sample, 3 g Hoechst wax, C micro powder). The samples were also analyzed by X-ray (powder) diffractometry (XRD) to identify the principle minerals present. The XRD data were obtained using a Bruker D8 Advance diffractometer equipped with a Göbel mirror and a Lynxeye detector. Soller slits on the X-ray tube, the antiscatter slits, were kept wide open (3°C). The diffraction patterns were acquired using Cu *Kalpha1* radiation with scans over a range of 5°-70° 2 θ, using a step size of 0.02° 2 θ and a count time of 0.2 s/step. Patterns were matched to standards from the ICDD (International Centre for Diffraction Data) database using Eva version 14. Major elements data is provided as weight percentages (wt %) expressed as their oxides), while trace-element concentrations are expressed in parts per million (ppm). These data reveal a number of geochemical differences.

Physical thin-sectioning

The petrographic thin sections were prepared according to the following methodology: 1) eggshell samples were embedded in bicomponent epoxy resin (Lamit 109; Kittfort); 2) the embedded samples were ground on a Montasupal grinder (Germany) using SiC (grain size: 400–600 nm); 3) warm reimpregnation of the ground surface with EpoFix (Struers); 4) fixation of the samples to slides using epoxy resin (type 109); 5) fixed samples were sectioned using a diamond knife (diameter 150 mm, Struers); 6) fixed samples were thinned on the Montasupal grinder using the abrasives of 240, 400 and 600 grits combined with ultrasound cleaning to reach a thickness of 0.2 mm; 7) final manual abrasion using 1000 grit SiC to reach a thickness of 30 μm; and finally 8) the sections were cover-slipped using a synthetic resin or polished on the Planopol TS (Struers).

Scanning electron microscopy

Regarding eggshell microstructure analysis, the selected samples of the specimen MCF-PVPH-874 were broken into fragments, some of which were prepared as standard petrographic thin sections (w 30 mm) to be studied using both transmitted and polarized light microscopy (see above). The other fragments were mounted on aluminum stubs, coated in gold (30 nm), and imaged under the Zeiss Supra 35-VP (Carl Zeiss SMT, Oberköchen, Germany) field emission scanning electron microscope (SEM) equipped with a VPSE detector for low vacuum conditions, a Robinson BSD for backscattered electron imaging, and coupled with an EDAX Apex 4 (Ametekh, Mahwah, USA) EDS-detector for dispersive X-ray microanalysis. Structural parameters such as shell/ layer/crystalline unit dimensions and pore width were measured with software of the Zeiss Supra 35-VP SEM facility.

Wholemount skeletal staining

We used *in toto* combined Alcian Blue-Alizarin Red staining in this study following the procedure described by Wassersug [30]. Wilde-type embryos of *Alligator mississippiensis* were collected under the supervision of James Perran Ross in Florida in 1999, 2001 and 2003, and are donations of the Florida Museum of Natural History and the Fish and Wildlife Conservation Commission, USA. Photographs were taken using a Spot RT camera (Diagnostic Instruments) set on a Nikon SMZ-U dissecting microscope, and were assembled using Adobe Photoshop and CorelDRAW X5.

3D Imaging

The *in ovo* preserved embryonic skull, MCF-PVPH-874, was scanned at beamline ID 19 of the European Synchrotron Radiation Facility. The scans were collected with propagation phase contrast synchrotron microtomography using a pink beam with two different energies 71.2 KeV and 115 keV. The scanned data of the complete and a partial specimen has an isotropic voxel size of 5.06 μm, 7.46 μm and 30 μm, respectively. The reconstructed slices were converted into 16-bit .tif image stacks that were concatenated to obtain a single stack covering the area of interest. To reduce the data size for morphological and histological observations, a second version of the reconstructed scan was calculated with a 2x2x2 binning and an 8-bit conversion. Mimics v.12.3 and v.13.1 (Materialise HQ, Leuven, Belgium) were used for the segmentation and 3D rendering of the skull. The internal structure and vasculature of the premaxilla was reconstructed using VGStudio Max 2.2 (Volume Graphics, Heidelberg, Germany). The mirrored reconstruction of the skull was rendered in Blender v.2.78 (GPL software, Blender Foundation, https://www.blender.org/).