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Searching for a Clue to Characterize a Crystalline Dinosaur's Eggshell of Baja California, Mexico

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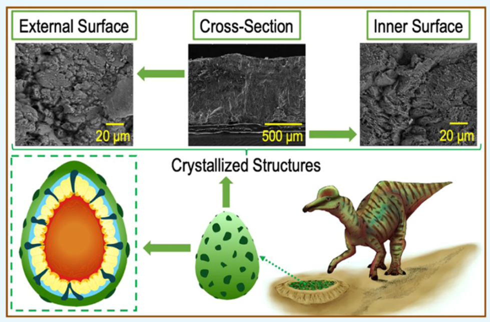
ABSTRACT: This work presents a detailed structural and morphological analysis of different dinosaur eggshells such as *Spheroolithus* (sample 1, 2), lambeosaurinae, *Prismatoolithus*, and one unidentified ootaxon performed by high-resolution scanning electron microscopy (HRSEM). These ancient eggshells of Late Cretaceous dinosaurs were collected in the coastal area of El Rosario, Baja California in Mexico. Additionally, a thorough study was performed on the elements present in the samples by different techniques such as energy-dispersive spectroscopy (EDS), X-ray fluorescence (XRF), and X-ray photoelectron spectroscopy (XPS). The XPS technique was performed to make an accurate identification of the compounds of two different types of eggshells (*Spheroolithus* sample 1 and *Prismatoolithus*). This contribution compares the surface of five different dinosaur eggshells of 74 Ma and their inner section to determine the morphology, distribution of the chemical elements present, as well as their relationship. The observed morphology of the ornithopod eggshells of the herbivorous species shows that the mammillary cones are in the form of columns with microaggregates and irregular pores. In contrast, in the theropod eggshells, the mammillary cones are observed in different forms with wider pores. Finally, the chemical components present in the structures of each of the samples were estimated using the information obtained from SEM-EDS, evidencing the presence of calcite, quartz, and albite in each of the samples. The composition reveals that eggshells contain Si, P, S, K, Ca, Mn, Fe, and Sr and trace elements such as Cr, Cu, and Zn. The presence of heavy metals may be an indication that the eggshells presented diagenetic alterations.



■ INTRODUCTION

Dinosaurs were one of the most enigmatic and interesting species of animals that have existed on earth. They lived in a variety of environments that ranged from forest to arid land, including the coast around the oceans.[1,2] Of these species, the first dinosaur was the Megalosaurus discovered and described by William Buckland at the end of the 18th century.[3,4] Later, Mary Ann Mantell identified the Iguanodon and the ankylosaurus *Hylaeosaurus* that presented great differences in the shape of bones, posture, and teeth compared to the first discovery.[4] In the second half of the 19th century, William Parker-Foulke found the first duckbill dinosaur fossil *Hadrosaur foulkii*.[5,6] The *Hadrosaurus* species belong to the Hadrosauridae family. They are a group of herbivores that lived approximately 80−66 million years ago during the Late Cretaceous period.[7] Subsequently, the theropod *Cryolophosaurus ellioti* belonging to the Tetanurae family was discovered in the Antarctica.[8] This family belongs to the carnivorous species called maniraptoran that lived between the Late Jurassic and Late Cretaceous period (160−66 million years back).[9]

All of the dinosaurs mentioned above faced a period of extinction along the earth, with the fossils found in different parts of the world being the only evidences of their existence. Information from the paleoenvironmental conditions, as well as diagenetic and thermal changes were investigated for fossils comprising bones, skeleton, tracks, skin impressions, gastroliths, coprolites,[10] nets,[11] and eggs or eggshells.[12,13] Since the sensational discovery of dinosaur eggs in Gobi desert by the Central Asiatic Expedition of the American Museum of Natural History in the early 1920s, the remains of fossil eggshells, eggs, and nest have been found in all of the continent and all Mesozoic deposits, most of them from the upper Cretaceous.[14,15] Just in North America alone, there exist at least 40 localities with fossils of eggs and eggshells distributed between Canada, the United States, and Mexico.[16]

Most of the works on the classification of fossil eggshells are based on their different characteristics such as the general morphology, pore shape, and thickness, but mainly the biocrystalline arrangement.[17−20] A few works focus on the element and the isotopic analysis that we carried out. These were done to find information about the possible body

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temperature of some of the dinosaur groups,[21] but above all, we wanted to infer their paleoenviromental conditions.[22,23]

Regardless of its size, morphology, and shape, the eggshell provides the embryo a stable and adequate medium called the homeostatic medium.[15] The egg is composed of two phases: an organic phase constituted of sugars, lipids, and proteins, and an inorganic phase mainly composed of calcium carbonate ($CaCO_3$) that corresponds to the mineral part of the eggshell (Figure 1). The function of the eggshell (based on its architecture) is to protect the embryo against external environmental agents that affect its development.[24,25]

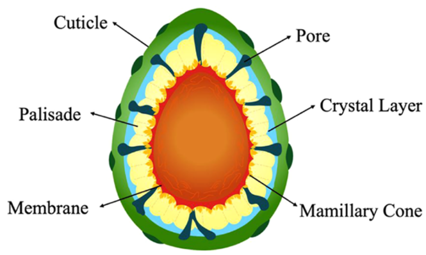

Figure 1. Schematic illustration of the parts of the avian eggshell.

A great diversity of Late Cretaceous dinosaurs has been discovered[26] in Mexico, where, at present, there only exist two localities with egg and eggshell fossil records: Cerro El Pueblo Formation at the south of Coahuila[27] and El Gallo Formation in Baja California.[28] Those discoveries lead to the studies of paleopathology,[29] ignites,[30] teeth,[31] and the paleoenvironment[32] of different species, with the Hadrosauridae family the most reported in the scientific literature.[33] These works, like most of them, are focused on the classification of eggshells; however, there is no information about the elemental composition of fossil eggshells found in this country. Therefore, we have studied the elemental composition and their distribution in different areas using different dinosaur eggshells. We have also studied the change they have undergone due to the paleoenvironmental and diagenetic conditions that caused the formation of exogenous inorganic sediments in these eggshells.

The majority of dinosaurs' eggshells found in old nests in different parts of the world are proved enough of their existence. The following questions arise: How were they originated? How did these species disappear? But most of all, how can we characterize the one unknown sample if it does not correspond to the others in the nest? None of these questions have a simple answer as we cannot travel back in time to be the direct observers of how that happened. Nor are there any living witnesses alive. However, some testimonies have been found recorded on the surface of Earth's rocks or fossilized samples in the best cases, but this information is, most of the time, hard to decode and rests there hidden. However, we have some techniques that can help us to unravel some of the mysteries that lie there hidden, such as spectroscopic and microscopic techniques.[34] These were performed to study the surface and cross-sectional morphology as well as the elemental composition of the external and internal surfaces of the eggshells of five samples: *Spheroolithus* (samples 1 and 2), Lambeosaurinae, *Prismatoolithus*, and one unidentified ootaxon. The aim of this research was to perform strategies to analyze the eggshell of the unidentified ootaxon to determine whether it belonged to either herbivorous (ornithopod) or carnivorous (theropod) dinosaurs.

The eggshell samples assigned to the oogenus *Spheroolithus* and *Prismatoolithus* were described and identified in a previous work.[28] However, based on the El Gallo Formation fossil record, it is believed that these eggshells were laid by ornithopod hadrosaur (Figure 2A) in the case of *Spheroolithus* eggshells, and by a theropod troodontid in the case of the *Prismatoolithus* eggshell (Figure 2B).[27] The Lambeosaurinae eggshell is assigned to this taxon thanks to association founded in the site. The unidentified sample is too damaged to identify and to classify into an oofamily.

All of these analyses aimed at giving information about the elemental composition of the dinosaur eggshells collected in Mexico contributing therefore to the paleontological research in the Americas, in the same way that has already been done in other continents.

Geographical Location of the Dinosaur Eggshells. The samples were collected in the El Gallo Formation (Late Campanian) in the town of El Rosario, Baja California. The Lambeosaurinae eggshells (II) were 3.4 km from El Rosario (30°3′25′N 115°45′37″W) (Figure 3); those of the *Prismatoolithus* eggshells (IV) and the unidentified ootaxon (V) were 1.2 km from point II (30°3′50″N 115°46′12″W), while the two *Spherooltihus* eggshells (I and III) were 2.1 km from point II (30°3′58″N 115°46′45″W).

■ RESULTS AND DISCUSSION

The samples studied were donated from the Institute of Geology from the National Autonomous University of Mexico (UNAM). These eggshells were dated and classified according to the classic protocols used in paleontology. However, there was not any approach or methodology to characterize these

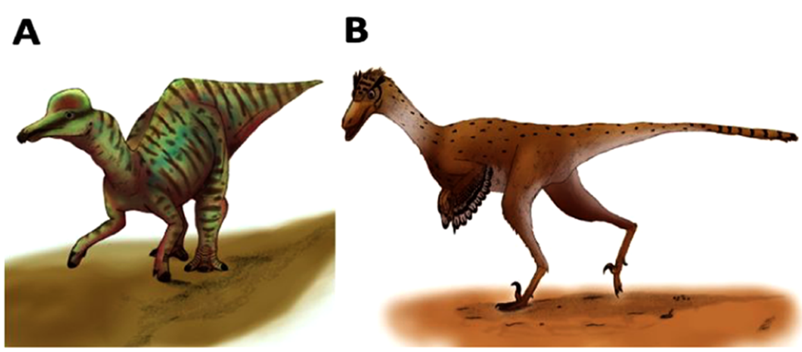

Figure 2. Illustrations of (A) ornithopod (herbivorous) and (B) theropod (carnivorous) dinosaurs belonging to the Hadrosauridae and Troodontidae families.

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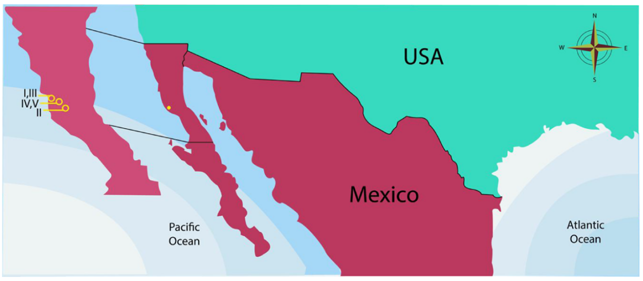
A



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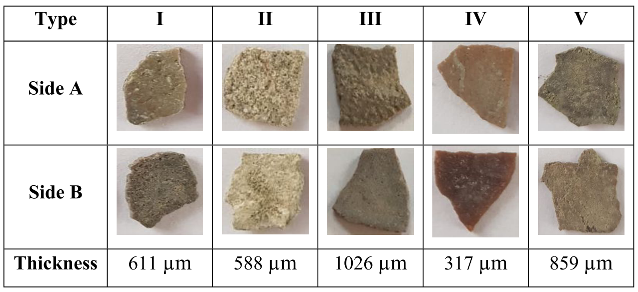

Type	I	II	III	IV	V
Side A					
Side B					
Thickness	611 µm	588 µm	1026 µm	317 µm	859 µm

Figure 3. (a) Map of the precise location where the eggshells were collected: (I) *Spheroolithus* sample 1; (II) Lambeosaurinae eggshell; (III) *Spheroolithus* sample 1; (IV) *Prismatoolithus*; and (V) unidentified ootaxon. The inset on the left-hand side is the amplified zone of the north part of the Baja California peninsula, where these samples were found. (b) Eggshells of the five types of dinosaurs: (I) *Spheroolithus* sample 1; (II) Lambeosaurinae eggshell; (III) *Spheroolithus* sample 2; (IV) *Prismatoolithus*; and (V) unidentified ootaxon. Side A. External surface; Side B. Inner surface.

samples by spectroscopic and structural methods. Figure 3 shows the optical photos of five dinosaur eggshells discussed in this work. They present dimensions of approximately 0.5 cm × 0.5 cm. We were able to appreciate that the theropod eggshell (IV) has a reddish coloration and smooth surface, while the ornithopod eggshells (I, II, and III) have opaque colors such as gray and light yellow with rough surfaces.

According to the images in Figure 3, the eggshells of the unidentified ootaxon (V) are similar to the eggshells corresponding to the ornithopod (I and III) in terms of roughness and thickness.

Morphological Characterization by Scanning Electron Microscopy (SEM). An intrinsic characteristic of the ornithopods' eggshells is that they present tubular calcite crystals. The phases are usually merged, making it difficult to discern them, and in some cases, present ornamentation in the outer layer.[14,15] The characteristic of *Prismatoolithus* eggshells from theropods, on the other hand, presents at least two structural layers in the eggshell, a mammillary layer in the lower part of the eggshell with a radial-tubular structure, followed by a prismatic layer in the upper part with a tubular or columnar structure. In addition, they have a smooth external surface as ornamentation.[35,36]

From the external and internal surfaces (Figure 4A,B), we can observe large deposition of granules forming larger aggregates, thus preventing the visibility of smaller particles due to the fusion of adjacent units. The inner surface of the ornithopod eggshells (Figure 4, types IB, IIB, and IIIB) presents units with fibrous structures similar to those observed in titanosaurid dinosaur eggshells,[37] which were probably

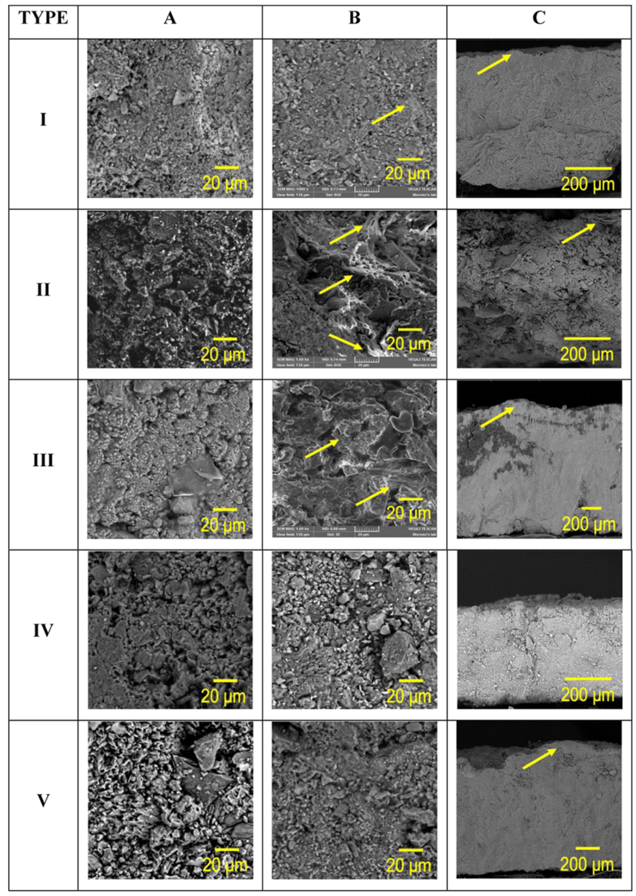

Figure 4. SEM images of the five dinosaur eggshells. (A) External surface; (B) inner surface; (C) cross section: (I) *Spheroolithus* sample 1, (II) Lambeosaurinae eggshell, (III) *Spheroolithus* sample 2, (IV) *Prismatoolithus*, and (V) unidentified ootaxon. The ornamentation (IC-VC) and fibrous structures (IB) are indicated by the yellow arrow.

formed due to the presence of the membrana testacea (indicated by the yellow arrow in Figure 4B). As for the therapod eggshells (Figure 4, type IVB), they present granular structures of different shapes and sizes distinct from those observed in the nonidentified ootaxon (Figure 4, type VB), which has microaggregates scattered over the entire internal surface, giving a soft and fibrous appearance in some areas of the eggshell.

However, the theropod eggshell (Figure 4, type IVC) presents a smooth appearance, without protuberance on the surface. Dinosaur eggshells have different ornamentation forms such as nodes, valleys, or ridges[38] distributed over the entire outer surface of the eggshell.[15] *Spheroolithus* eggshells (Figure 4, types IC and IIIC) present nodes distributed at different distances (this is indicated by the yellow arrow in Figure 4C), similar to those found in the unidentified ootaxon (Figure 4, type VC). In the case of the Lambeosaurinae eggshell (Figure 4, type IIC), we were unable to identify any ornamentations due to the wear that the eggshell presents. We could, however, observe a thin lamina in some areas of the surface, which probably corresponds to structures formed by the diagenetic processes. Apart from all of these characteristics, the ornithopods eggshells also present a calcite diagenetic layer of approximately ∼100 μm thickness, which makes the visibility of the ornamentation difficult.

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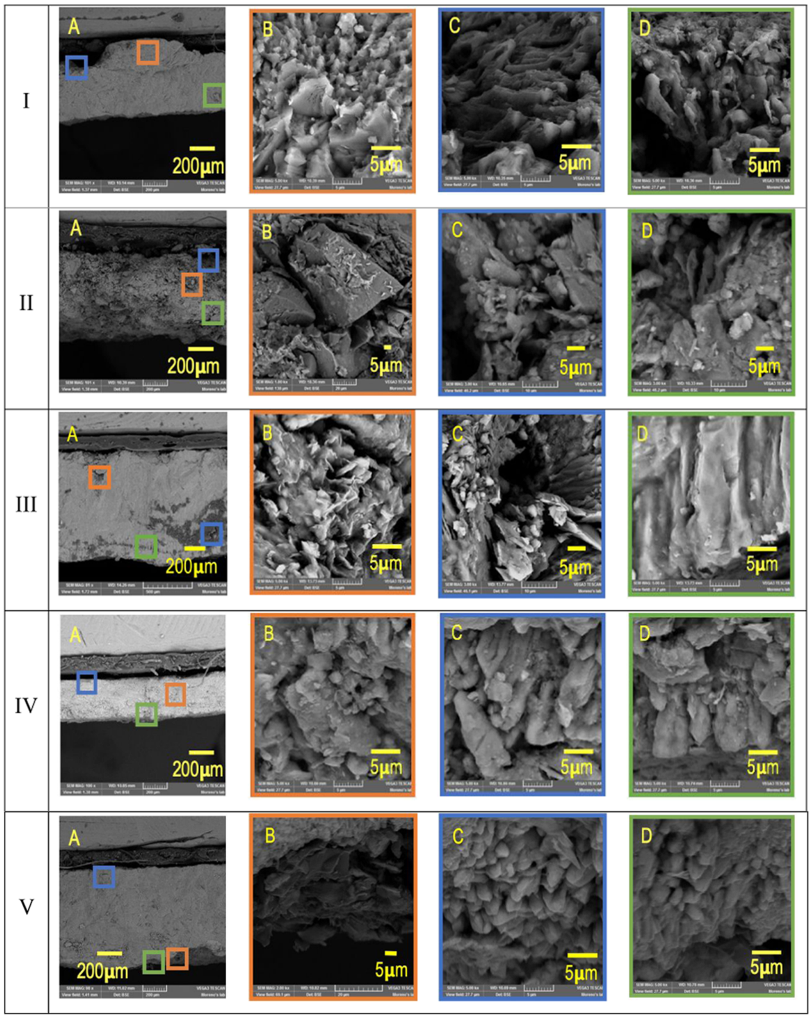

Figure 5. SEM images of the cross section of the five dinosaur eggshells. (A) Cross section; (B) structures observed in the cross section in different zones; (C) mammillary cones; (D) structures present in the cuticle: (I) *Spheroolithus* sample 1, (II) Lambeosaurinae eggshell, (III) *Spheroolithus* sample 2, (IV) *Prismatoolithus*, and (V) unidentified ootaxon. The outer surface is at the bottom of each image.

The structural morphology observed in the eggshells varies depending on the area of analysis. The *Spheroolithus* sample 1 (Figure 5, type I) is composed of layers of different morphologies not very well faceted (Figure 5, type IA) and of irregular structures with sizes ranging from 0.5 to 5 μm (Figure 5, type IB). The first layer of the eggshell fulfills the function of the base carrying out the process of crystallization and formation of the mammillary cones. This layer grows epitaxially, with a cone appearance (Figure 5, type IC), giving way to the formation of the mineral palisade composed mainly of calcium carbonate ($CaCO_3$), which is responsible for the hardness and shape of the eggshell.[39] We were able to observe the outer layer formed by a sheet of crystals arranged in parallel (Figure 5, type ID). A similar observation was also made in the eggshell of the *Spheroolithus*, sample 2 (type III), where the layers are presented in different morphologies (Figure 5, type IIIA). Here, we were able to appreciate more clearly a polycrystalline growth (Figure 5, type IIIB), mammillary cones growing out of the membrane (Figure 5, type IIIC), and a well-defined calcite diagenetic layer (Figure 5, type IIID).

The *Spheroolithus* eggshells belonging to ornithopod dinosaur, as does the Lambeosaurinae family eggshell (Figure 5, type IIA), present different structures with well-defined facets with sizes greater than 5 μm (some even ranging from 20 to 50 μm). This is what causes an increase in the roughness of the sample that can be easily spotted under an optical microscope. This roughness prevents the observation of the eggshell layers. However, the mammillary cones are shorter and separated from each other (Figure 5, type IIC); they present columnar structures in the outer layer of the eggshell (Figure 5, type IID), as those observed in the previous species (Figure 5, type ID).

The eggshells of the ornithopod dinosaurs present thin and elongated mammillary cones with a rounded termination arranged in parallel, very different from those observed in the *Prismatoolithus* eggshell (Figure 5, type IVC). The mammillary cones form clusters with broad juxtaposed bilayered structures that have an unrounded and columnar termination in the outer surface (Figure 5, type IVD). In addition, they have structures with sizes ranging from 0.5 to 10 μm (Figure 5, type IVB), while the nonidentified ootaxon presents a high similarity with the eggshells of ornithopods in terms of crystal size and appearance (Figure 5, type VB). We were able to distinguish a polycrystalline growth identical to the one observed in the *Spheroolithus* eggshell sample 2 (Figure 5, type IIIB), with thin mammillary cones and closely spherical structures (Figure 5, type VC), forming small columns located in the last layer of the eggshell (Figure 5, type VD) and thus adding more

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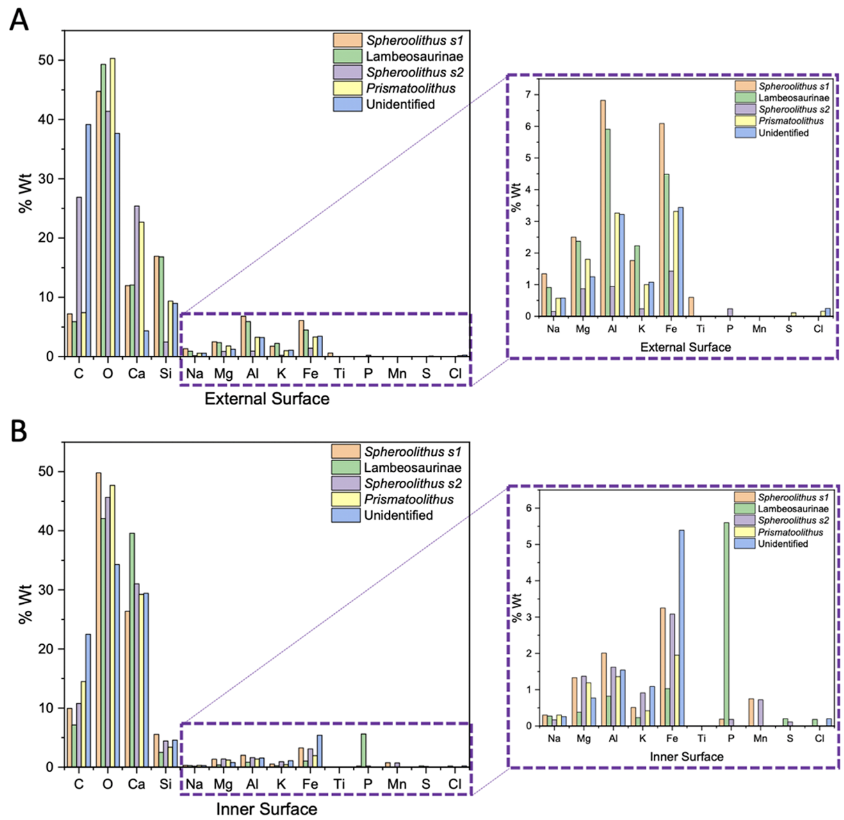

Figure 6. Elemental composition in the percentage of molecular weight of the eggshells of the five dinosaurs. (A) External surface; (B) inner surface; orange: *Spheroolithus* sample 1, green: Lambeosaurinae eggshell, yellow: *Spheroolithus* sample 2, purple: *Prismatoolithus*, blue: unidentified ootaxon. The inset shows a zoom over the area where the percentage value of the elements present in the tables is very low.

evidence to the idea of the eggshell belonging to ornithopod dinosaurs corresponding probably to the Hadrosauridae family.

Elemental Analysis by Energy-Dispersive Spectroscopy (EDS). EDS analysis was performed on both sides of the eggshells. Here, the presence of calcium (Ca), oxygen (O), silicon (Si), and carbon (C) was mainly observed (Figure 6A,B). These elements are the main components of the eggshell and of the wollastonite.[40] This is one of the standards of the compounds that can be found in the sample (see Supporting Information Table S1). Wollastonite is a common mineral that is obtained in the reaction of calcium carbonate ($CaCO_3$) with silicon dioxide (SiO_2, due to the silicification) in the eggshell. This synthesis happens in the soil at high temperatures and pressures during progressive metamorphism.[41] In addition, elements such as magnesium (Mg), iron (Fe), potassium (K), sodium (Na), and aluminum (Al) are also found in smaller quantities, as observed in the inset of Figure 6A,B, respectively. These elements are mixed with silicon oxide,[42] forming albite, a common mineral that is also composed of aluminum, potassium, and sodium.[43]

Of the elemental composition obtained, the calcium content is inversely proportional to the content of silicon on both sides, being higher on the outer side than on the inner side (Figure 6A,B). The decrease in the calcium content is probably an indication that other elements have infiltrated through diffusion, replacement, or recrystallization processes carried out during fossilization.[44]

The presence of elements such as magnesium (Mg), phosphorus (P), and manganese (Mn) is an indication that sedimentary materials such clay minerals were filtered inside the eggshells. Thanks to this, the elements and/or minerals that are not part of the composition of the eggshell could also be observed. The evidence that the eggshell has undergone diagenetic changes during fossilization implied a change in the elemental composition of the eggshell.

In addition, the EDS mapping of the studied area, framed with a white line (Figure 7 and Supporting Information Figures S6−S9), allowed us to observe that carbon, potassium, iron, manganese, phosphorous, sulfur, and chlorine, in the case of Supporting Information Figures S6−S9, are distributed more evenly throughout the sample compared to the other elements. Oxygen, sodium, aluminum, silicon, and magnesium are found mainly in areas where calcium is present in a lower concentration due to the spatial location of the atoms within the structures formed in the eggshells. Furthermore, Figure 7 shows a larger structure framed in yellow, in which oxygen, sodium, aluminum, and silicon are the most characteristic elements, as is the structure shown in Supporting Information Figure S9.

X-ray Fluorescence (XRF) Elemental Analysis. X-ray fluorescence analysis is a technique that helps determining the elemental composition of a sample with high precision compared to the analysis obtained by EDS. Therefore, the samples were analyzed to corroborate the data previously obtained. Supporting Information Table S2 shows the mass concentration (mg kg^{-1}) of each of the elements found in the samples. Being calcium the main component of the eggshells, its high concentration is adequate for the type of sample. Furthermore, silicon and phosphorous are slightly in excess compared to aluminum, potassium, or iron, elements that are naturally found in the soil.[45] Moreover, the presence of sodium, magnesium, and chlorine was observed with values

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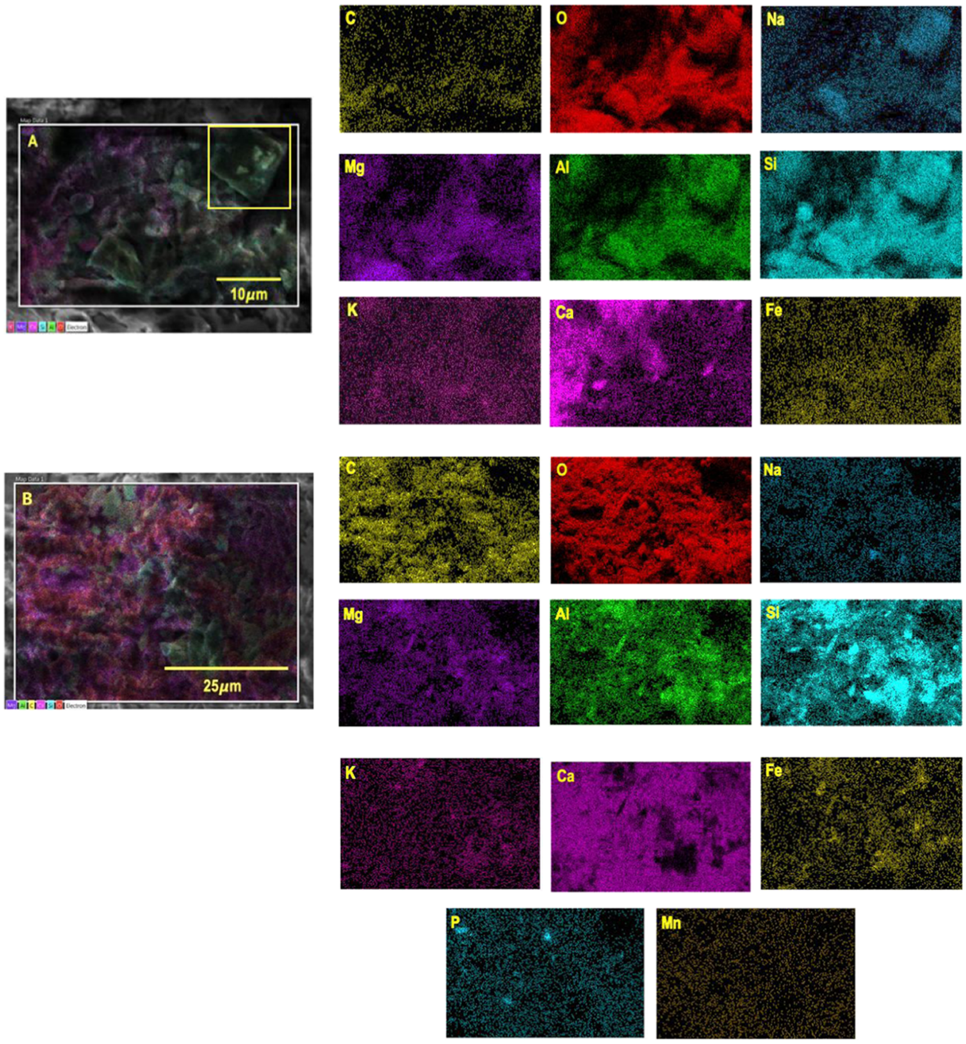

Figure 7. Images of the elemental distribution EDS (in color) of the surface eggshell of the *Spheroolithus* sample 1. (A) External surface, (B) inner surface.

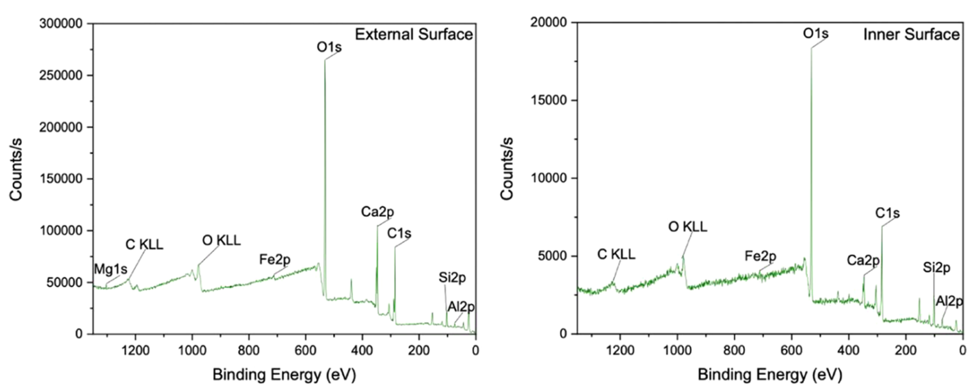

Figure 8. X-ray photoelectron spectra (XPS) of the eggshell of the *Spheroolithus* sample 1 (external and inner surfaces). The electronic state of some elements presented in the eggshell surface corresponds to the observed by EDS and XRF.

that are considered unreliable. However, the presence of metals like chromium (Cr), copper (Cu), zinc (Zn), and strontium (Sr), which were not observed by EDS, was detected by XRF. This provides information about the strength of the energy of the electronic levels, as the depth of the signal produced by XRF is greater than the one produced by EDS, allowing determination of these elements, which are in very low concentrations.

Determination of the Electronic State of the Elements by X-ray Photoelectron Spectroscopy (XPS). X-ray photoelectron spectroscopy is used to determine the elemental composition and chemical and electronic states of the elements present on the surface of a sample at a depth of

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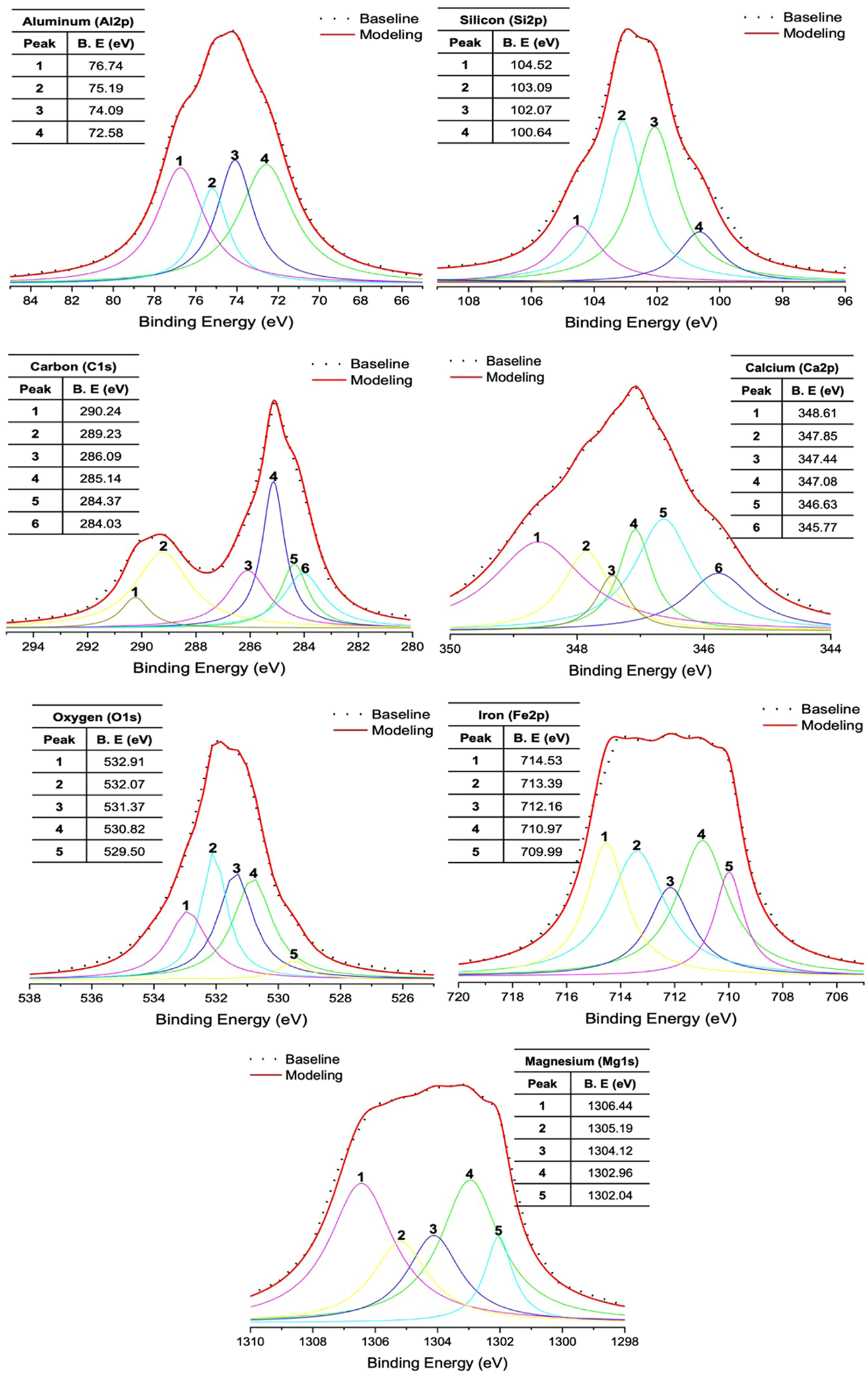

Figure 9. Deconvoluted X-ray photoelectron spectra of the external surface of the eggshell *Spheroolithus* sample 1. The tables show the binding energies of each compound; binding energy (B.E.).

1–10 nm.[46] Due to the presence of metals such as chromium, copper, zinc, and strontium detected in the XRF analysis but undetected in EDS, we decided to corroborate whether these elements could only be found inside or whether they could also be found on the surface of the samples. Based on these data, we found that these atoms were surrounded by the main elements of the eggshell (Ca, C, O). Compared to that of the bulk, the metals had a much higher binding potential on the surface, causing an increase in reactivity.

According to the spectra obtained from the ornithopod and theropod eggshells (Figures 8 and S11), the elements that are evident are calcium (Ca 2p, ≈347 eV), oxygen (O 1s, ≈532 eV), carbon (C 1s, ≈285 eV), silicon (Si 2p, ≈102 eV), aluminum (Al 2p, ≈74 eV), iron (Fe 2p, ≈712 eV), sodium

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(Na 1s, ≈1072 eV), and magnesium (Mg 1s, ≈1303 eV). The intensities corresponding to oxygen and carbon are the most notable (with the exception of calcium on the external surface), probably due to the presence of organic molecules formed on the surface of the eggshells and to the calcite diagenetic layer formed by the fossilization process. The decrease in some of the signals is caused by electrons that are elastically dispersed before leaving the surface of the sample, causing a reduction in the kinetic energy of the electrons and, therefore, a decrease in the intensity of the peaks.

To determine the chemical bonding and compounds on the surfaces of each of the samples, the deconvolution of each of the electronic states was carried out, determining thus different functional groups from spheroolithus eggshell (Figures 9 and S10) (to see the compounds of each spectrum, see the Supporting Information). The aluminum (Al 2p) spectrum was resolved into four curves. The peaks at 76.74 eV and 74.09 eV are related to oxide (Al_2O_3) and hydroxide ($Al(OH)_3$) compounds, while the peak at 75.19 eV is associated with aluminosilicates (Al_2SiO_3) and metallic Al is founded at 72.58 eV. Silicon (Si 2p) spectrum had the same behavior as aluminum, with four curves at 104.52, 103.09, 102.07, and 100.64 eV, associated with elemental silicon, Si $2p_{3/2}$, Si $2p_{1/2}$, and silicon oxide (SiO_2). The nonmetallic element such as carbon (C 1s) presents a peak at 286.09 eV, which is associated with acetyl compounds (O−C−O). At 284.37 eV, compounds with nonoxidized alkane-type carbon groups (C−C) were observed, as well as carboxylic groups (C═O) at 289.23 eV. In addition, metal carbonates were observed at 290.21 eV, which are probably associated with $CaCO_3$ and Na_2CO_3. Besides, the C sp^2 and C sp^3 peaks were detected at 285.14 and 284.03 eV, respectively. Oxygen (O 1s), another nonmetallic element, corroborated the information obtained from the C 1s, the signals corresponding to the C−O group belong to alcohol and ether groups at 532.91 eV, and metal oxides at 530.82 and 529.50 eV are observed.[47,48]

The binding energies of the characteristic signals of alkali and alkaline-earth metals such as sodium (Na 1s) for the case of *Prismatoolithus* eggshell (Figure S11−S13), calcium (Ca 2p), and magnesium (Mg 1s) indicate that compounds linked as oxides (CaO at 346.63 eV, MgO at 1304.12 eV), hydroxides ($Mg(OH)_2$ at 1302.04 eV, NaOH at 1073.26 eV), aluminosilicates ($Na[AlSi_3O_8]$ at 1072.18 eV, chemical composition of albite), phosphates ($Mg(H_2PO_4)_2$ at 1306.44 eV, Na_3PO_4 at 1069.79 eV), bicarbonates ($NaHCO_3$ at 1071.06 eV), carbonates ($CaCO_3$ at 347.08 eV and 348.61 eV, $MgCO_3$ at 1305.19 eV), chlorides ($CaCl_2$ at 347.85 eV), sulfates ($CaSO_4$ at 347.44 eV), and metallic magnesium at 1302.96 eV are present, and finally, iron (Fe 2p), the only transition metal, confirming the presence of Fe_2O_3, Fe_3O_4, FeO, and FeOOH, at 710.97, 713.39, 709.99, and 712.16 eV, respectively.[48,49]

Taking into account these results, a methodology to obtain information about the intramineral proteins, present in the dinosaur eggshells, could be developed since a study of the eggshell of a dinosaur carried out by μ-XANES determines the presence of a cluster formed by the S−S/S−H groups of L-cysteine. This could be an indication of the plausible presence of the proteins or of the remaining peptides that were isolated by the mineral phase.[50]

■ CONCLUSIONS

The samples were optically observed determining that the eggshells of the unidentified ootaxon presented high similarities to the eggshells of the *Spheroolithus* (samples 1 and 2) and to the Lambeosaurinae (herbivorous dinosaurs), in terms of texture, roughness, and thickness. As for the coloration, the unidentified ootaxon looks more like *Spheroolithus* eggshells. However, colors are not entirely relevant when characterizing, as they can fluctuate during diagenesis. Using techniques such as SEM, we observed that the eggshells presented structures with different morphologies of sizes ranging from 0.5 to 50 μm, making it possible to identify the ornamentation, whereas the nonidentified ootaxon has nodes in the cuticle, characteristics that correspond to ornithopod eggshells. Furthermore, the mamillary cones observed in this eggshell are approximately cylindrical as those observed in *Spheroolithus* eggshells.

On the other hand, the eggshells are composed mainly of calcium, carbon, silicon, and oxygen. Other elements such as sodium, potassium, aluminum, and iron are also present in moderate concentration, but only on the surface level; however, traces of chromium, copper, zinc, and strontium, were determined internally. This variation in the composition of the eggshell, which is composed mainly of calcium carbonate (corroborated by XPS), is an indication that the eggshells underwent diagenetic changes, replacement, and recrystallization process during fossilization.

All of these analyses aimed at giving information about the elemental composition of the dinosaur eggshells collected in Mexico, contributing therefore to the paleontological research in the Americas, in the same way that has already been done in other continents. Furthermore, being able to determine whether an egg of an unidentified ootaxon of dinosaur corresponds either to a theropod or to an ornithopod dinosaur will be, without a doubt, a great contribution to the world of structural analysis of paleontological samples.

■ EXPERIMENTAL SECTION

Cleaning and Identification of Eggshell Surfaces. The eggshells were first washed with 5% ethylenediaminetetracetic acid (EDTA) solution for 30 min to remove the organic contamination present in the samples; then, they were washed with Milli-Q water and air-dried. After that, the corresponding sides of every eggshell were identified with an optical microscope SZH10 OLYMPUS and were placed on carbon tape to identify the external and internal sides of the eggshells. Once the sides were identified, we submitted the samples to the four different methodologies mentioned below.

Morphological Characterization by Scattering Electron Microscopy (SEM). For these analyses, we used an SEM-TESCAN VEGA 3 SB scanning electron microscope with a voltage of 15 keV in backscattering electrons (BSE). All samples were gold-coated to improve the conductivity. These samples were observed on both sides of the eggshell and radial section. The allocation of the side was carried out by observing the radial section of eggshells where the curvature was correctly appreciated. The thickness of each of the samples was obtained from the radial section SEM images using ImageJ software.

Energy-Dispersive Spectroscopy (EDS) Mapping Using High-Resolution SEM (HRSEM). The analyses by HRSEM and EDS mapping were performed using a JEOL

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JSM-7800F microscope with a 2 kX magnification to 15 keV. In the case of EDS, the process was finalized when the number of counts was in 2000, and for the mapping, the processing time was 4 min. The samples were used without gold coating.

Elemental Characterization by X-ray Fluorescence (XRF). For this technique, the samples were placed on a slide suitable for equipment use (after treatment) as it was previously described. We used an X-ray tube with Rh anode (potential difference, 30 keV; amperage, 250 μA), located at an angle of 0° with respect to the normal of the sample. The irradiation time of each sample was 900 s. X-ray detection was mediated using an SDD detector (Amptek), located at an angle of 45° with respect to the normal of the sample. The detector resolution was 140 eV to 5.9 keV. The data obtained were analyzed by the QXAS software of the International Atomic Energy Agency. Accuracy was determined using analytical methods for geogenic materials published by Espinosa et al.[34]

X-ray Photoelectron Spectroscopy (XPS). K-Alpha Surface Analysis from Thermo was used, with a monochromatic AL Kα line to 12 kV and 40 W of power at a relative angle of 30°. The general recognition was made using a neutralizer that generates a cloud of argon ions of 3 keV energy and 30 W power over the analyzed area. The spectra are obtained under two conditions: in a broad general scan (0–1350 V) with 1 eV/step and 100 eV of step energy, and in mode of small windows with 0.1 eV/step and 50 eV of step energy.

■ ASSOCIATED CONTENT

Supporting Information

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> Images, graphics, and maps of the elemental composition of the eggshells by the energy-dispersive spectroscopy (EDS) as well as the possible standards. Also, the mass concentration of elements recorded in the dinosaur samples by X-ray fluorescence spectroscopy (XRF), their electron state, and binding energy by X-ray photoelectron spectroscopy (XPS); analysis of the elemental composition of eggshell by scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) (Figures S1–S5); standards obtained by EDS according to the elemental composition present in the eggshells of the five dinosaurs (Table S1); analysis of energy-dispersive spectroscopy (EDS) mapping (Figures S6–S9); elemental analysis by X-ray fluorescence spectroscopy (XRF); mass concentration (mg kg^{-1}) of elements recorded in the dinosaur samples (Table S2); electronic state of the elements by X-ray photoelectron spectroscopy (XPS); binding energy of electronic states of the elements presents in the *Spheroolithus* sample 1 eggshell obtained by XPS (Table S3); deconvoluted X-ray photoelectron spectra of the inner surface of the eggshell *Spheroolithus* sample 1, and tables showing the binding energies of each compound (Figure S10); compounds determined by deconvolution of XPS spectrum of the *Spheroolithus* eggshell (Table S4); binding energy of electronic states of the elements presents in the *Prismatoolithus* eggshell obtained by XPS (Table S5); X-ray photoelectron spectroscopy (XPS) spectra of the eggshell of the *Prismatoolithus* (external and inner surface); electronic state of some elements presented in the eggshell surface corresponding to the observed by EDS and XRF (Figure S11); deconvoluted X-ray photoelectron spectra of the external surface of the eggshell *Prismatoolithus*; tables showing the binding energies of each compound (Figure S12); deconvoluted X-ray photoelectron spectra of the inner surface of the eggshell *Prismatoolithus*; tables showing the binding energies of each compound (Figure S13); and compounds determined by deconvolution of XPS spectrum of the *Prismatoolithus* eggshell (Table S6) (PDF)

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The manuscript was designed and written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Cullen, T. M.; Longstaffe, F. J.; Wortmann, U. G.; Huang, L.; Fanti, F.; Goodwin, M. B.; Ryan, M. J.; Evans, D. C. Large-scale stable isotope characterization of a Late Cretaceous dinosaur-dominated ecosystem. *Geology* **2020**, *48*, 546−551.

(2) Kuang, H.; Liu, Y.; Ning, Z.; Peng, N.; Xu, K. Cretaceous stratigraphy, paleoenvironment and terrestrial biota in Shandong Province. *Open J. Geol.* **2019**, *9*, 650−653.

(3) Buckland, W. Notice on the Megalosaurus or great fossil lizard of stones field. *Trans. Geol. Soc. London* **1824**, *1*, 390−396.

(4) Farlow, J. O.; Brett-Surman, M. K. *The Complete Dinosaurs*; Farlow, J. O.; Brett-Surman, M. K., Eds.; Indiana University Press: USA, 1997; Chapter 1 and 2.

(5) Foulke, W. P. Remarks on fossil bones, shells, and wood, particularly historical remarks on the collection of Hadrosaurus foulkii Leidy. *Proc. Acad. Nat. Sci. Philadelphia* **1859**, *10*, 213−215.

(6) Prieto-Márquez, A.; Weishampel, D. B.; Horner, J. R. The dinosaur Hadrosaurus foulkii, from the Campanian of the East Coast of North America, with a reevaluation of the genus. *Acta Palaeontol. Pol.* **2006**, *51*, 77−98.

(7) Case, J. A.; Martin, J. E.; Chaney, D. S.; Reguero, M.; Marenssi, S. A.; Santillana, S. M.; Woodburne, M. O. The Firtst duck-billed dinosaur (Family *Hadrosauridae*) from Antarctica. *J. Vertebr. Paleontol.* **2000**, *20*, 612−614.

(8) Hammer, W. R.; Hickerson, W. J. A crested theropod dinosaur from Antarctica. *Science* **1994**, *264*, 828−830.

(9) Lü, J.; Xu, L.; Liu, Y.; Zhang, X.; Jia, S.; Ji, Q. A new troodontid theropod from the Late Cretaceous of central China, and the radiation of Asian troodontids. *Acta Palaeontol. Pol.* **2010**, *55*, 381−388.

(10) Lucas, S. G. *Dinosaurs: The Textbook*; 6th ed.; Lucas, S. G., Ed.; Columbia University Press: USA, 2016; Chapter 12.

(11) Andrews, R. C. *The New Conquest of Asia: A Narrative of the Explorations of the Central Asiatic Expeditions in Mongolia and China 1921-30*; American Museum of Natural History: West Hartford, USA and New York, 1932.

(12) Grellet-Tinner, G.; Chiappe, L.; Norell, M.; Bottjer, D. Dinosaur eggs and nesting behaviors: a paleobiological investigation. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **2006**, *232*, 294−321.

(13) Grellet-Tinner, G.; Sim, C. M.; Kim, D. H.; Trimby, P.; Higa, A.; An, S. L.; Oh, H. S.; Kim, T. J.; Kardjilovm, N. Description of the first lithostrotian titanosaur embryo in ovo with Neutron characterization and implication for lithostrotian Aptian migration and dispersion. *Gondwana Res.* **2011**, *20*, 621−629.

(14) Mikhailov, K. E. *Fossil and Recent Eggshell in Amniotic Vertebrates: Fine Structure, Comparative*; Palaeontological Association, 1997; pp 1−80.

(15) Carpenter, K. *Eggs, Nests, and Baby Dinosaurs: A Look at Dinosaur Reproduction*; Carpenter, K., Ed.; Indiana University Press: USA, 1999; Chapter 5.

(16) Tanaka, K.; Zelenistky, D. K.; Williamson, T.; Well, A.; Therrien, F. Fossil eggshells from the Upper Cretaceous (Campanian) Fruitland Formation, New Mexico. *Hist. Biol.* **2011**, *23*, 41−45.

(17) Jackson, F. D.; Varrcchio, D. J. Fossil eggs and eggshell from the lowermost two medicine formation of western Montana, Sevenmile Hill Locality. *J. Vertebr. Paleontol.* **2010**, *30*, 1142−1156.

(18) Barta, D. E.; Brundridge, K. M.; Croghan, J. A.; Jackson, F. D.; Varricchio, D. J.; Jin, X.; Poust, A. W. Eggs and clutches of the Spheroolithidae from the Cretaceous Tiantai Basin, Zhejiang province, China. *Hist. Biol.* **2014**, *26*, 183−194.

(19) Xie, J. F.; Zhang, S. K.; Jin, X. S.; Li, D. Q.; Zhou, L. Q. A new type of dinosaur eggs from Early Cretaceous of Gansu Province, China. *Vertebr. Palasiat.* **2016**, *54*, 1−10.

(20) Fernández, M. S.; Wang, X.; Vremir, M.; Laurent, N. D.; Kaiser, G.; Dyke, G. A mixed vertebrate eggshell assemblage from the Transylvanian Late Cretaceous. *Sci. Rep.* **2019**, *9*, No. 1944.

(21) Eagle, R. A.; Enriquez, M.; Grellet-Tinner, G.; Pérez-Huerta, A.; Hu, D.; Tütken, T.; Montanari, S.; Loyd, S. J.; Ramirez, P.; Tripati, A. K.; Kohn, W. J.; Cerling, T. E.; Chiappe, L. M.; Eiler, J. M. Isotopic ordering in eggshells reflects body temperatures and suggest differing thermophysiology in two Cretaceous dinosaur. *Nat. Commun.* **2015**, *6*, No. 8296.

(22) Cojan, I.; Renard, M.; Emmanuel, L. Palaeoenviromental reconstruction of dinosaur nesting sites base on a geochemical approach to eggshells and associated palaeosols (Maastrichtian, Provence Basin, France). *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **2003**, *191*, 111−138.

(23) Montanari, S.; Higgins, P.; Norell, M. A. Dinosaur eggshell and tooth enamel geochemistry as an indicator of Mongolian Late Cretaceous paleoenvironments. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **2013**, *370*, 158−166.

(24) Tullett, S. G. The Porosity of Avian Eggshells. *Comp. Biochem. Physiol., Part A: Physiol.* **1984**, *78*, 5−13.

(25) Hincke, M. T.; Nys, Y.; Gautron, J.; Mann, K.; Rodriguez-Navarro, A. B.; McKee, M. D. The Eggshell: Structure, Composition and Mineralization. *Front. Biosci.* **2012**, *17*, 1266−1280.

(26) Ramírez-Velasco, A. A.; Hernández-Rivera, R. Diversity of Late Cretaceous dinosaurs from Mexico. *Bol. Geol. Min.* **2015**, *126*, 63−108.

(27) Aguillón-Martínez, M.; Zelenitsky, D. K.; Brikman, D.; Eberth, D. Eggshell fragments from the uppermost Cerro del Pueblo Formation (Upper Cretaceous; Mexico). *J. Vertebr. Paleontol.* **2004**, *24*, 33A.

(28) Cabrera-Hernández, J. S. Descripción e identifiación de cáscaras de huevo de dinosaurios fósiles y de dos dinosaurios perinatales de la formación El Gallo (Cretácico tardío) El Rosario, Baja California, México. Bachelor Dissertation, Universidad de Guadalajara: Jalisco, México, 2018.

(29) Ramírez-Velasco, A. A.; Morales-Salinas, E.; Hernández-Rivera, R.; Tanke, D. R. Spinal and rib osteopathy in *Huehuecanauhtlus tiquichensis* (Ornithopoda: Hadrosauroidea) from the Late Cretaceous in Mexico. *Hist. Biol.* **2016**, *29*, 208−222.

(30) Gudiño-Maussán, J. L.; Aguilar, F. J.; Hernández-Rivera, R. Aplicación de fotogrametria digital para el registro de las huellas de dinosaurio de la Formación Cerro del Pueblo (Campaniano Tardio), Coahuila, México. *Bol. Soc. Geol. Mex.* **2018**, *70*, 307−324.

(31) Torres-Rodríguez, E.; Montellano-Ballesteros, M.; Hernández-Rivera, R.; Benammi, M. Dientes de terópodos del Cretácico Superior del Estado de Coahuila, México. *Rev. Mex. Cienc. Geol.* **2010**, *27*, 72−83.

(32) Fastovsky, D. E.; Clark, J. M.; Strater, N. H.; Montellano, M.; Hernández-Rivera, R.; Hopson, J. A. Depositional enviroments of a Middle Jurassic terrestrial vertebrate assemblage Huizachal Canyon, Mexico. *J. Vertebr. Paleontol.* **1995**, *15*, 561−575.

(33) Ramírez-Velasco, A. A.; Hernández-Rívera, R.; Servín-Pichardo, R. *Hadrosaurs: The Hadrosaurian Record from Mexico*; Eberth, D. A.; Evans, D. C., Eds.; Indiana University Press: USA, 2014; Chapter 19.

(34) Espinosa, A. A.; Miranda, J. Elemental Analysis of soils as possible resuspended dust sources in Mexico City. *J. Environ. Res.* **2013**, *7*, 1015−1020.

(35) Moreno-Aranza, M.; Canudo, J. I.; Gasca, H. M. Unusual theropod eggshells from the Erarly Cretaceous Blesa formation of the Iberian Range, Spain. *Acta Palaeontol. Pol.* **2014**, *59*, 843−854.

(36) Sellés, A. G.; Galobart, À. Reassessing the endemic European Upper Cretaceous dinosaur egg *Cairanoolithus*. *Hist. Biol.* **2016**, *28*, 583−596.

(37) Grellet-Tinner, G. Membrana Testacea of titanosaurid dinosaur eggs from Auca Mahuevo (Argentina): Implications for exceptional preservation of soft tissue in Lagerstätten. *J. Vertebr. Paleontol.* **2005**, *25*, 99−106.

(38) Kurzanov, S. M.; Mikhailov, K. E. *The Age of Dinosaur in Russia and Mongolia: Dinosaur Eggshells from the Lower Cretaceous of Mongolia*; Benton, M. J.; Shishkin, M. A.; Unwin, D. M.; Kurochkin, E. N., Eds.; Cambridge University Press: U.K., 1989; Chapter 28.

(39) Dauphin, Y.; Cuif, J. P.; Salomé, M.; Susini, J.; Williams, C. T. Microstructure and chemical composition of giant avian eggshells. *Anal. Bioanal. Chem.* **2006**, *386*, 1761−1771.

(40) Trubnikov, I. L.; Solov'ev, L. A.; Lupeiko, T. G. Synthesis of wollastonite in salt melts. *Glass. Ceram.* **1989**, *46*, 488−489.
(41) Vichaphund, S.; Kitiwan, M.; Atong, D.; Thavorniti, P. Microwave synthesis of wollastonite powder from eggshells. *J. Eur. Ceram. Soc.* **2011**, *31*, 2435−2440.
(42) Flicstein, J.; Schieber, M. Micro segregation of impurities in hydrothermally grown quartz crystals. *J. Cryst. Growth.* **1974**, *24−25*, 603−609.
(43) Marshall, D. B.; Wilson, C. J. L. Recrystallization and peristerite formation in albite. *Contrib. Mineral. Petrol.* **1976**, *57*, 55−69.
(44) Alexander, G. L.; McMahon, S.; Matthews, J. J.; Still, J. W.; Brasier, A. T. Petrological evidence supports the death mask model for the preservation of Ediacaran soft-bodied organism in South Australia. *Geology* **2019**, *47*, 215−218.
(45) Hoefler, H.; Streli, C.; Wobrasuchek, P.; Ovari, M.; Zaray, G. Analysis of low Z elements in various environmental samples with total reflection X-ray fluorescence (TXRF) spectrometry. *Spectrochim. Acta, Part B* **2006**, *61*, 1135−1140.
(46) Biesinger, M. C.; Payne, B. P.; Grosvenor, A. P.; Lau, L. W. M.; Gerson, A. R.; Smart, R. St.C. Resolving surface chemical states in XPS analysis of first row transitions metals, oxides and hydroxides: Cr, Mn, Fe, Co and Ni. *Appl. Surf. Sci.* **2011**, *257*, 2717−2730.
(47) Thermo Scientific. XPS Simplified/Elements Table. https://xpssimplified.com/periodictable.php (accessed May 21, 2020).
(48) Naumkin, A. V.; Kraut-Vass, A.; Gaarenstroom, S. W.; Powell, C. J.. *NIST Standard Reference Database 20. NIST Standard Reference Database*, 2012.
(49) Ferreira, F. V.; Souza, L. P.; Martins, T. M. M.; Lopes, J. H.; Mattos, B. D.; Mariano, M.; Pinheiro, I. F.; Valverde, T. M.; Livi, S.; Camilli, J. A.; Goes, A. M.; Gouveia, R. F.; Lona, L. M. F.; Rojas, O. J. Nanocellulose/bioactive glass cryogels as scaffolds for bone renegeration. *Nanoscale* **2019**, *11*, 19842−19849.
(50) Legorreta-Flores, A.; Davila-Tejeda, A.; Velásquez-Gonzáles, O.; Ortega, E.; Ponce, A.; Castillo-Michel, H.; Reyes-Grajeda, J. P.; Hernández-Rivera, R.; Cuéllar-Cruz, M.; Moreno, A. Calcium carbonate crystals shapes mediated by intramineral proteins from eggshell of ratite birds and crocodiles. Implications to the eggshell's formation of a dinosaur of 70 million years old. *Cryst. Growth. Des.* **2018**, *18*, 5663−5673.

https://dx.doi.org/10.1021/acsomega.0c03334
ACS Omega 2020, 5, 25936−25946