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Dinosaur eggs and nesting behaviors: A paleobiological investigation

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Abstract

Although dinosaur eggs were first discovered and identified in the late 1800s, limited attention was given to the scientific value of oological fossils in contrast to observations based on skeletal features. Here, we offer a review of Mesozoic saurischian egg materials, in comparison with extant crocodilians and avians, and their paleobiological interpretation based either on the presence of embryos in ovo or brooding adults on egg clutches. Our study focuses on the eggs of the oviraptorid *Citipati osmolka* (Mongolia), the troodontid *Troodon formosus* (North America), the theropod oospecies *Macroelongatoolithus xixiaensis* (China), the ornithothoracine bird (Argentina), an indeterminate theropod (Thailand), and titanosaurs (Argentina). Results show that (1) many oological characters and reproductive behaviors associated with modern birds are rooted among non-avian theropods, (2) there is a reproductive evolutionary cline from crocodilians to modern birds with (3) a noticeable pattern of coeval development between the accretion of eggshell layers, origination and size increased of larger air cells (inferred from egg polar asymmetry), and brooding/incubating behaviors. Most of these pre-adaptations are grouped in two main clades of the saurischian cladogram: one at the level of Oviraptorosauridae and the other at Troodontidae. Although undeniably these two theropod taxa seem to represent two important phases for the evolution of avian reproduction, the phylogenetic distance between these clades and Titanosauria cannot be ignored. As such, the reproductive features that appeared in concert in oviraptorids might have gradually evolved across more basal theropod clades. Although *Troodon formosus* by its egg shape and nesting behavior seems to be in this study the precursors of modern avian reproduction, the importance of small-bodied theropods such as those who laid the Phu Phok eggs cannot be dismissed and the eggs of such dinosaurs could suggest a closer phylogenetic ties to Aves than troodontids. At a higher level of inferences, there is a strong possibility that the evolution of these reproductive features is concurrent with profound physiological and metabolic changes that occurred in saurischian dinosaurs throughout their evolution.

Keywords: Dinosaur eggs and nests; Brooding; Incubation; Evolution of reproductive behaviors; Theropods

1. Introduction

Although the first dinosaur eggs were described as early as 1859 by Jean-Jacques Pouech (Buffetaut and Le Loeuff, 1994), only limited scientific attention has been given to dinosaur eggs since their first discovery. This situation partly resulted from the poor association between skeletal and oological material in the fossil record. Recently, oology (referring to the study of eggs, eggshells, and nests) took a new turn not only because of the discovery of fossilized eggs and nests on every continent (except Antarctica) but also due to the discovery of life assemblages preserving egg clutches with parent dinosaurs or embryos in ovo. Yet, only a limited amount of

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paleobiological information stemmed from the study of fossil eggs, nests, and eggshells (e.g., Kérourio, 1981; Williams et al., 1984; Sabath, 1991; Cousin, 1997; Clark et al., 1999; Kohring, 1999; Varrichio et al., 1999; Peitz, 2000; Zhao, 2000). One of the issues raised in this research is whether detailed oological observations can convey novel paleobiological information about the reproductive behaviors of dinosaurs, possibly their physiology including their metabolism, and shed light on which particular group of non-avian theropods is more closely related to birds. To achieve these objectives, a description of oological materials from well identified or associated saurischian dinosaur taxa including their taphonomic parameters and discussion of their paleobiological significance are provided through examination of the oviraptorid *Citipati osmolka* (Mongolia), the troodontid *Troodon formosus* (Montana), the theropod *Macroelongatoolithus xixiaensis* (China) oospecies, the theropod eggs from Phu Phok (Thailand), the ornithothoracine eggs from Neuquén (Patagonia), and the titanosaurid eggs from Auca Mahuevo (Patagonia). Although many dinosaur eggs have been discovered during the last century, the specimens studied in this investigation represent a set of well-documented fossils that are now curated in museums and offer a positive taxonomic identification through the above-mentioned associations in contrast to the material loosely classified in the egg parataxonomic literature.

2. Abbreviations

Institutional abbreviations: AMNH, American Museum of Natural History fossil reptiles, New York; BMNH, The Natural History Museum (Palaeontology Department), London; GMV, Geological Museum Vertebrate Beijing; IGM, Institute of Geology, Mongolia, Ulaan Baatar; IVPP, Institute of Vertebrate Paleontology and Paleoanthropology, Beijing; LACM, Natural History Museum of Los Angeles County; MCF-PVPH, Museo Carmen Funes, Plaza Huincul, Argentina; MCZ, Museum of Comparative Zoology, Harvard; MOR, Museum of the Rockies; MUCPV, Museo de Geología y Paleontología, Universidad Nacional del Comahue, Neuquén, Argentina; SK1-1, Sahat Sakhan Dinosaur Research Centre; YPM, Yale Peabody Museum of Natural History, New Haven; USC, University of Southern California.

Technical viations: CL, cathodoluminescence; BSEM, backscattered scanning electron microscopy; Fm, formation; MT, Membrana Testacea; OUT, operational taxonomic units; PLM, polarized light microscopy; SEM, scanning electron microscope; TLM, transmitted light microscopy; MT, membrana testacea.

3. Methods

Observations of the oological material rely on specimens acquired in the field by the first author, specimens in various museums, material kindly donated by institutions and private collectors now curated at the LACM. In an effort to incorporate the parataxonomic literature in this research, the descriptive terminology rests as much as possible on published nomenclature of eggs and eggshells (e.g., Mikhailov, 1991, 1997; Hirsch, 1996) but new terminology is introduced when needed (Grellet-Tinner and Norell, 2002) as explained in the text. For many years, dinosaur eggs regardless of their degree of association with skeletal remains were given oospecific names. Although we do not adhere to this terminology for phylogenetic reasons and because it has proven to be dubious and confusing, we feel that in order to facilitate communication the readers need to be informed that titanosaurid eggs have been named megaloolithid; troodontid eggs, prismatoolithid; and oviraptorid eggs, elongatoolithid in the parataxonomic classification.

Our specimens were tested and examined with several tools and methods that complement each other to observe and describe the eggshell morphology. Scanning electron microscopy (SEM) allows observation of the samples at various magnifications. This technique, in contrast to TLM or PLM, reveals tri-dimensional details of the microcrystalline arrangement within the shell units (Grellet-Tinner and Norell, 2002), the presence and thickness of structural layers, and the distribution of vesicles interpreted as the relics of former protein filaments interwoven in the crystalline matrix of the eggshell (Grellet-Tinner, 2005a). However, SEM observations are highly dependent on the quality and the orientation of the fracture of the eggshell specimen. TLM and PLM offer little in terms of three dimensional view but allow better observation of some structures that could be ambiguous in SEM and can be used to confirm the presence of various eggshell structural layers and the micro-crystalline arrangement within shell units. The “organic lines” that are visible in TLM and analogous to the vesicles and crystallographic cleavages in SEM observations are a good illustration of how SEM and TLM observations complement each others (Fig. 1A, B). In addition to SEM, TLM and PLM observations, CL and BSEM analyses were carried out in all our specimens to evaluate the alteration and/or replacement of carbonates in fossil eggshells (Amthor, 1993; Barbin, 2000). The main application of CL examinations is to reveal diagenetic textures that are otherwise invisible in TLM and SEM in order to attempt to discriminate those from biological structures.

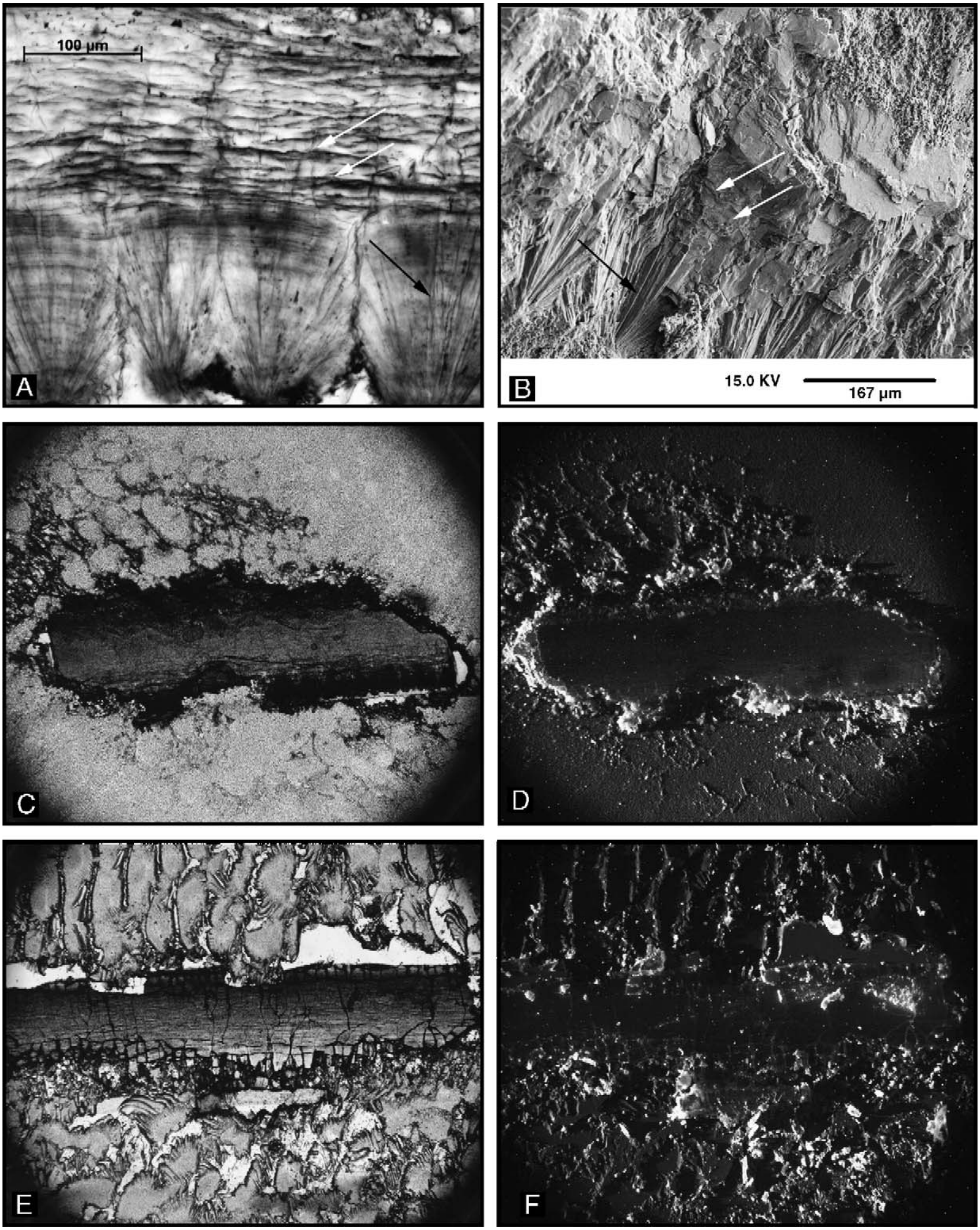


Fig. 1. A) TLM of *Deinonychus antirrhopus*. Black arrow points to the acicular calcitic crystals of layer 1, white arrows to the organic lines of layer 2. B) SEM of *Deinonychus antirrhopus*. Black and white arrows point to the same features as those in TLM. Vesicules (in SEM) are aligned in plans following the organic lines (in TLM). C and D) TLM and CL views of the eggshell of an extant emu. No luminescence is noted in this modern eggshell, thus validating the use of CL to distinguish diagenesis in fossil eggshells. E and F) TLM and CL views of an eggshell of an extant Rhea. Same observations as for the extant emu.

A fluorescent reaction indicating diagenetic alteration could be observed when samples are bombarded with high-energy electrons. This phenomenon is caused by the replacement of calcium or magnesium carbonates (Ca^{2+} or $Mg^{2+}CO_3$) mostly by manganese in the crystalline structure of the eggshell (Amthor, 1993). To test whether CL alone can discriminate diagenetic from biological structures in eggshell, several eggshells of modern birds were studied with CL. Although exposed to a broad range in intensity of the electron beam and time of exposure no luminescence was noted in modern specimens (Fig. 1C–F).

Eggs were photographed with a scale bar and perpendicular to the specimen to minimize parallax as they were measured. For every species, the digital image was imported into a graphic software package and the contour of the specimen was then drawn in order to discern the smallest variation in shape between poles, or between eggs that could be of the same oospecies or family. Variation in the eggshell surficial ornamentation according to its topological position (near the egg equator, poles, or in between) was also reported. When clutches are preserved, number of eggs, their spatial arrangement (i.e. number of egg rows and whether eggs fill up the entire clutch area), and the orientation of their long axis in reference to the sediment surface were noted.

Images were directly digitized at high resolution, and saved as Tagged Image Format Files (TIFF). Adobe Photoshop software (version 5.0) was used to enhance image contrast and brightness, and for measuring the samples. Egg length and diameter were measured using a Mitutoyo caliper, model # CD-8 CS. When multiple measurements were obtained, the average was calculated and recorded. Eggshell thickness was measured from the base of the layer 1 to the outermost inorganic layer. The eggshell structural layers were measured between their upper and lower lines of transition with adjacent layers and are numbered from the innermost to the outermost (closest to the external surface).

4. Material

MCF-PVPH 147 250, 264, 441, 442, 444, 445, 446, 262, 263, 264; titanosaurid egg material. Auca Mahuevo, Patagonia, Argentina; Campanian, Anacleto Formation.

IGM 100/979, 100/971, 100/1004, 100/1125; oviraptorid egg material. Ukhaa Tolgod, Gobi Desert, Mongolia; Campanian, Djadokhta Formation.

MOR 393, 363, 246, 748, 750, 393, 963, 676, 675; troodontid egg material. Egg Mountain and Island, Montana, USA; Campanian, Two Medicine Formation.

LACM 7477/151450; macroelongatoolithid egg material. Sanlimiao, Henan, China; Maastrichtian, Zoumagang Formation.

An uncatalogued clutch housed at the Nanyang Museum containing 13 pairs of eggs (featured in the 1996 National Geographic Magazine); macroelongatoolithid eggs. Sanlimiao, Henan, China; Maastrichtian, Zoumagang Formation.

MUCPv: ornithothoracine egg material. Neuquén, Patagonia, Argentina; Campanian, Bajo de la Carpa Formation.

SK1-1 to SK1-4: theropod egg material. Phu Phok, Sakhon Nakhon, Thailand; Early Cretaceous, Sao Khua Formation.

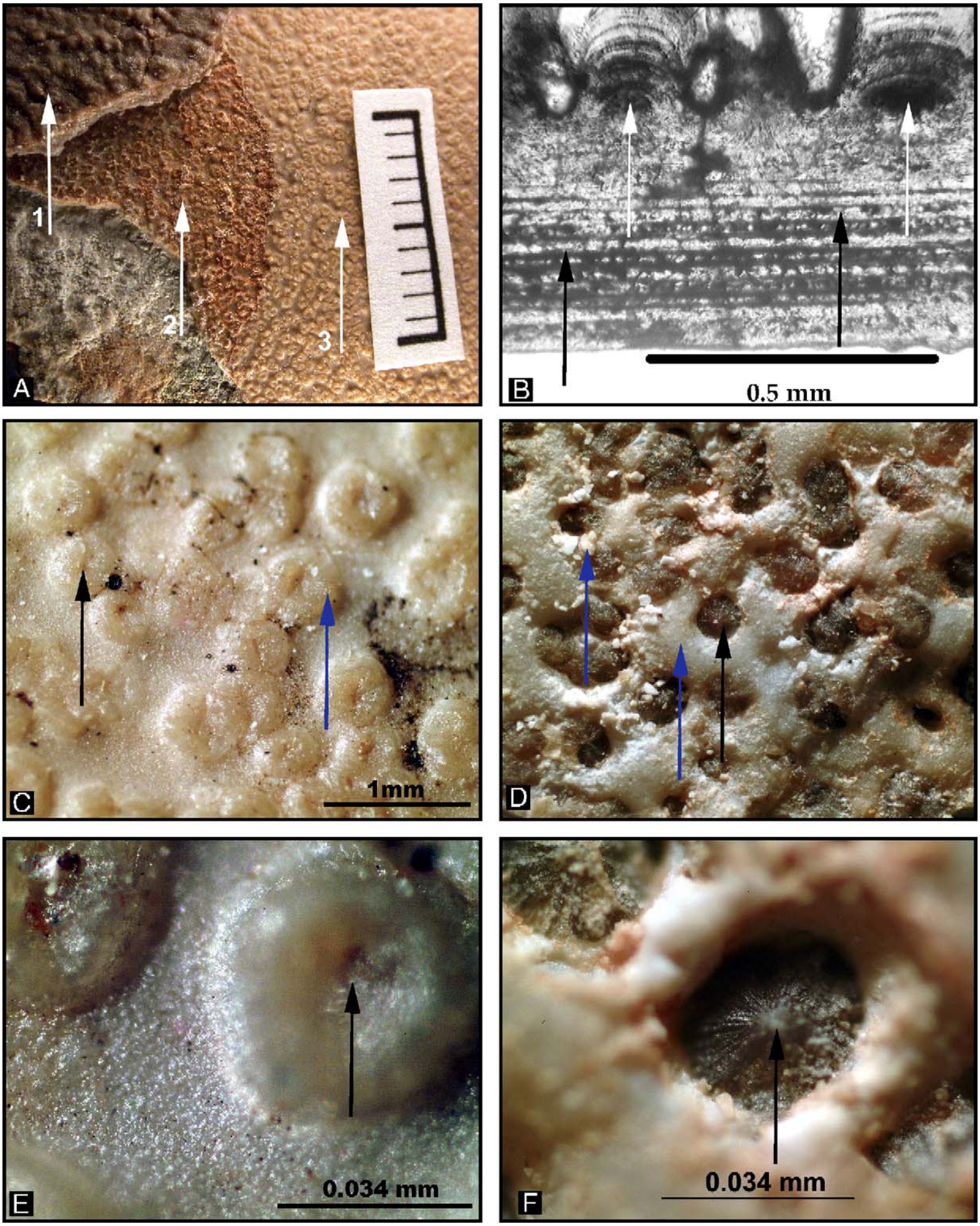
5. Observation and interpretation

5.1. Titanosaurid eggs

Occurrences where embryonic dinosaur remains are preserved in ovo are extremely rare. However, the Early Campanian site of Auca Mahuevo, in Argentina (Neuquén Province, northwestern Patagonia), has produced large number of eggs with embryonic skeletal remains allowing a titanosaurid identification of this material (Chiappe et al., 1998, 1999, 2000, 2001), eggs with membrana testacea cast found still adhering to the inner eggshell surface (Fig. 1A), and embryonic skin casts found in ovo (Dingus et al., 2000). At this locality, the MT interpreted as the result of biomediation processes (Grellet-Tinner, 2005a) is recovered in two morphs: a fibrous state that appears as parallel strands of the protein precursors (Fig. 2B) and a solid calcite layer with dome-like protuberances that are the molds of the mammillary tips (Fig. 2C–F). Soft tissues are rarely fossilized and their recovery is generally limited to only a few Lagerstätten (Bottjer et al., 2002), thus by their quality and abundance at the locality of Auca Mahuevo the preserved MT can raise the status of this site to that of a Lagerstätte (Grellet-Tinner, 2005a).

At this locality, eggs and clutches are found in huge quantities over an area exceeding 10 km^2 (Chiappe et al., 1998, 1999, 2000, 2001), and in few occasions these eggs were recovered in excavated and rimmed nests preserved in sandstone (Chiappe et al., 2004). The egg spatial arrangement in these nests and random clutches resembles that of modern crocodilians and chelonians, suggesting that titanosaurs had two functioning ovaries and laid their eggs en masse (a massive amount of eggs laid at once). Hence, these oological and nesting observations suggest that the titanosaur reproductive mode was closer to basal reptilians than modern avians.

Titanosaurid eggs (MCF-PVPH 147, 250, 262, 263, 264) display an ubiquitous spherical to subspherical general shape (Fig. 3A). Therefore, two diameters were measured. The greatest diameter ranges between 125 and 140 mm, the smallest diameter is 10% to 20% shorter. However, these two measurements might be



slightly biased by the taphonomic processes expressed as fractures and various degrees of compression, due to sedimentary compaction. Experimentation by Hayward et al. (2000) using *Gallus gallus* eggs documented that when fresh and hollow (blown) eggs are buried in sand under controlled environments and submitted to a compressive force of 700 kg, they were compressed up to 85% of their original shape. In addition, it is likely that egg resistance to compressive pressures could be correlated to the number of structural layers in a given eggshell (Grellet-Tinner, 2005b). Mollusc shells are biomineralized calcium carbonate tissues and are known to demonstrate an increased resistance to external pressure as the shell accumulates more structural layers, particularly when the internal crystallization varies in direction from layer to layer (Kamat et al., 2000). Similarly, the biomineralized calcium carbonate chicken egg with its tri-laminated and thin eggshell structure (Grellet-Tinner, 2000) and differing *C*-axis of calcium carbonate crystals in each layer offers more resistance to external stress than the single layered and thicker titanosaurid eggshells (Grellet-Tinner, 2005b). Therefore, considering the mono-layered structural composition of titanosaurid eggshell and all the above considerations there is a strong likelihood that the sub-spherical shape of these eggs results from taphonomic processes.

The outer eggshell surface of the titanosaurid eggs displays nodes, some coalescing into longer structures (Fig. 3B), with pore apertures located in the depressions in between them (Fig. 3 C). In well-preserved specimens, the average diameter and height from the base to the apex of the nodes is 0.58 and 0.28 mm, respectively, with inter-nodular values that range from 0.52 to 1.60 mm. Cousin and Breton (2000) speculated that the nodular appearance of megaloolithid eggshells from the Late Cretaceous of France is an indicator of the substrate, or nesting material surrounding the eggs. The nodular surface of the titanosaurid eggs from Auca Mahuevo could therefore be interpreted as a specialization to facilitate gas and water vapor conductance through the pores located at the base of each node, by preventing nesting debris from obstructing their apertures (e.g., Sabath, 1991; Grellet-Tinner et al., 2004). Following this assumption and considering that the grain size of the siliciclastic sediments at the Auca Mahuevo site is overall smaller than the minimum internodal distance necessary for the pores to be functional, it is possible to safely infer that the Auca Mahuevo nests, in order to be efficient in respect to the eggshell ornamentation, might have contained some vegetation (Grellet-Tinner et al., 2004). This interpretation is independently supported by the recent discovery of carbonized remains, presumably of plants, inside some of these nests (Chiappe et al., 2004) and interestingly, the concept of nests including vegetation has already been suggested for Late Cretaceous megaloolithid French sites such as those of Rennes-Le-Chateau (Cousin, 1997; Cousin and Breton, 2000; Erben, 1970), and at l′Arc (Kérourio, 1981).

In the radial section, the eggshell is composed of a single structural layer consisting of acicular calcitic crystals radiating from nucleation centers located above the MT (Fig. 3D) crisscrossed by a network of horizontal and vertical pore canals (Grellet-Tinner et al., 2004). Pore canals enable the diffusion of gases and water vapor through the eggshell (Paganelli, 1980). Their size, geometry, and number reflect a specialization to the habitat where nesting occurs (Williams et al., 1984; Cousin and Breton, 2000). According and based on the description of Williams et al. (1984) of the "Type 31" megaloolithid eggs from the Upper Maastrichtian of France, which have a pore system that matches in size, shape, and geometry that of the Auca Mahuevo eggs, Grellet-Tinner et al. (2004) suggested that the Patagonian eggs must have been incubated in high moisture

Fig. 2. A) The original calcium carbonate section of MCF-PVPH 441 adheres to the upper surface of the calcified MT. Arrow 1 point to the eggshell, arrow 2 to the space occupied by a freshly pried eggshell exposing the upper surface of the MT, and arrow 3 points to the rest of the exposed MT surface. B) Thin section of MCF-PVPH 445 clearly showing the preserved filamentous MT (black arrows) and the nucleation centers of eggshell units (white arrows). C) Upper surface of the MT from MCF-PVPH 441. Black arrow points to the center of one of the dome-like structures which is the cast of an eggshell nucleation center also called organic core by other authors. White arrows points to the wall of this dome-like structure. The exquisite preservation of the MT has only been documented in rare occurrences, but never as detailed as this one. Such a preservation implies that the MT was not exposed at all or only for a short time to the atmosphere as it would have otherwise desiccated and detached from the eggshell surface. D) Inner surface of the original calcium carbonate eggshell of MCF-PVPH 441. Black arrow points to the tip of an eggshell nucleation center, and blue arrows to calcified MT still adhering to the inner surface of MCF-PVPH 441. E) The black arrow points to the central depression where the tip of the core would have been located. F) The black arrow 1 points to the apex of a core. Note the blue and pink discolorations of the MT reminiscent to what is observed in stratified microbial communities. This observation coupled with the over all fine preservation of the MT advocate a bio-mediation during the fossilization process, likely induced by bacterial activities. The presence of eggshell nucleation centers with the matching negative counter slabs of calcified MT demonstrate that eggshell formation of these titanosaurid eggs is similar to that of modern birds where the eggshell units grows out of the MT from nucleation centers.

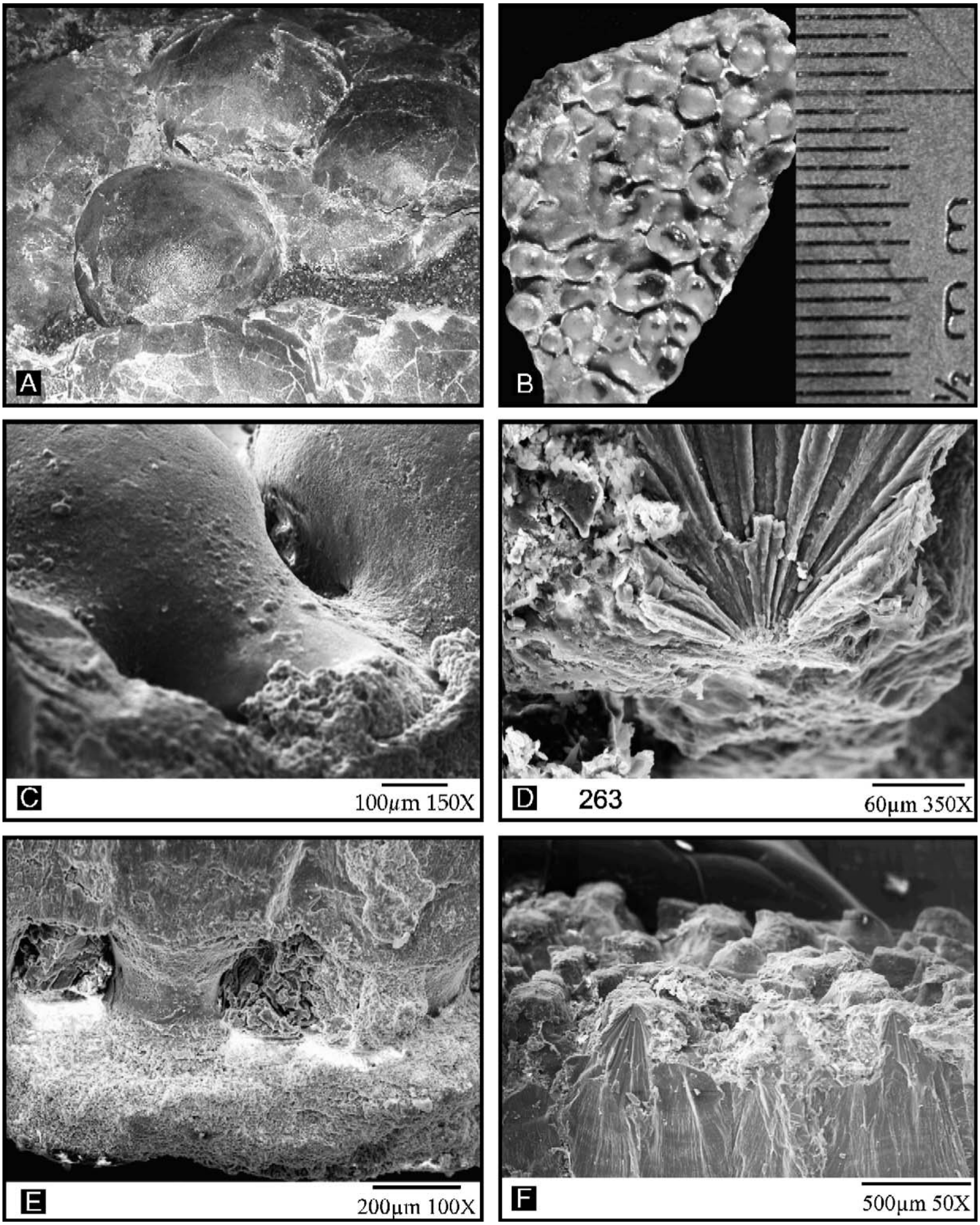


Fig. 3. A) Egg clutch recovered from the quarry site in egg-bed 3 (LACM 149648). B) Eggshell surface with single and coalescent nodes (MCF-PVPH 442). Scale on the side. C) Detail of two pore apertures at the base of a node on an outer-eggshell surface (MCF-PVPH 444). D) Calcite crystals radiate from a nucleation center, also called organic core, at the base of each eggshell unit (MCF-PVPH 263). E) View of the junction of the base of three eggshell units with a poorly preserved MT (arrow) showing the horizontal network of pore canals infilled with secondary calcite (MCF-PVPH 444). F) View of the inner eggshell surface. Note the base of many eggshell units (MCF-PVPH 263).

conditions. This interpretation is therefore consistent with the presence of vegetation in the nests.

The tips of the eggshell units (mammillae) are particularly visible and exhibit a perfect conic shape in specimen MCF-PVPH 263 (Fig. 3F), which contains an embryo. Contrary to expectations based on our knowledge of avian ontogeny, the mammillae do not display a pronounced concave appearance that results from the absorption of calcium by the embryo during its late stage of development. This pattern normally results from the dissolution of calcium carbonate at the inner tips of the mammillae by the carbonic acid that originates from the CO_2 exhaled by the breathing embryo (Packard and DeMarco, 1995). The lack of calcium resorption associated with the presence of a well-ossified skull in MCF-PVPH-263 suggests that calcium utilization from the eggshell may have happened at a later ontogenetic stage than in modern birds.

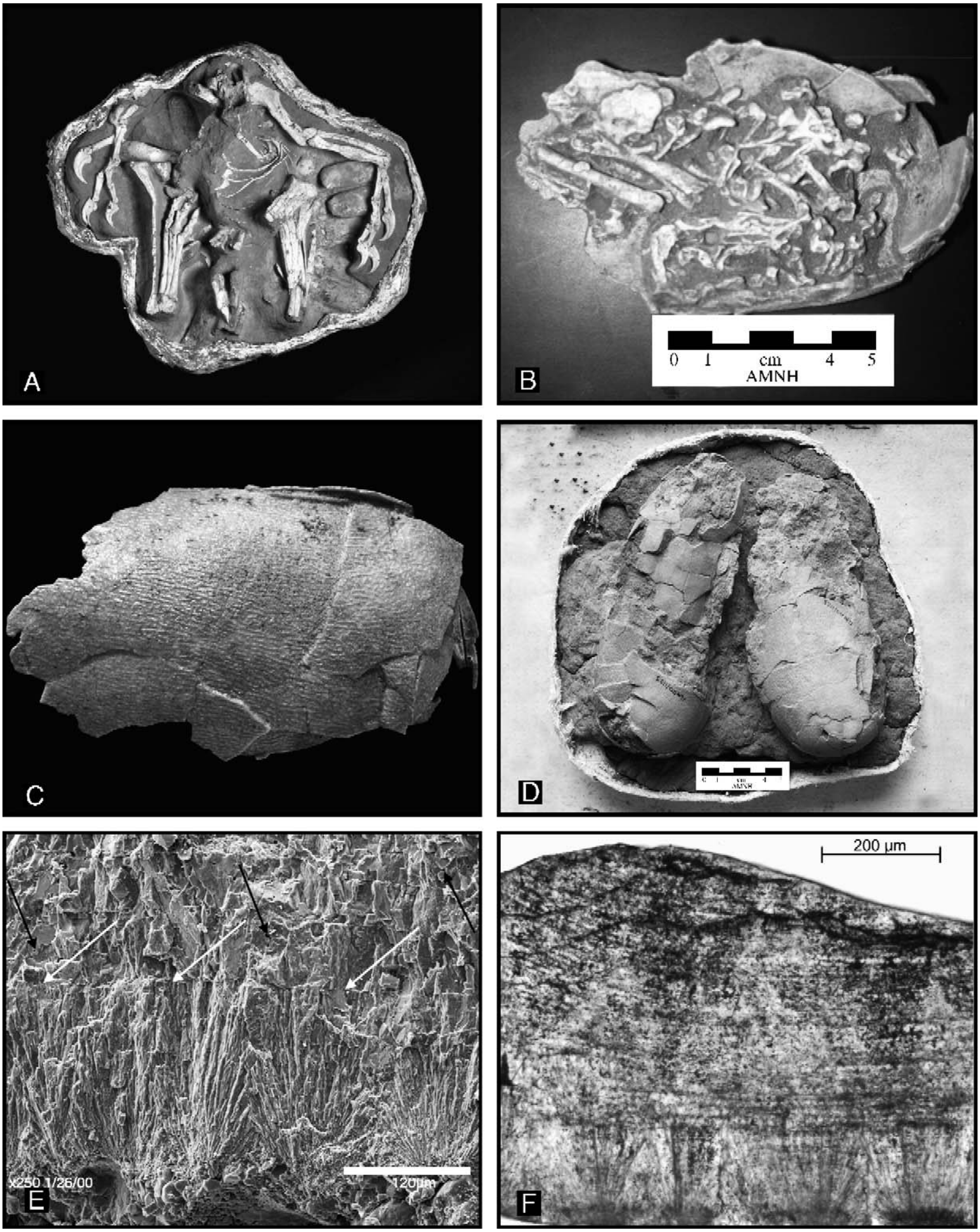
5.2. Oviraptorid (*Citipati osmolskae*) eggs

Assemblages of oviraptorids (IGM 100/979, 1004) brooding their nests (Fig. 4A) and an oviraptorid embryo (Fig. 4B) in ovo (Norell et al., 1994, 2001) have been collected from the Campanian Djadokhta Formation of Mongolia and from equivalent stratigraphic units in Inner Mongolia, China (Dong and Currie, 1996). The IGM 100/979 clutch consists of fifteen eggs arranged in pairs that are partially exposed below the skeleton of an adult oviraptor. The paired eggs are orientated sub-horizontally, slightly tilted toward the center (Fig. 4A) and are radially aligned in two superposed layers (Clark et al., 1999). According to Clark et al. (1999), the best-exposed egg is 18 to 19 cm in length and width variation ranges from 6.5 to 7.2 cm in the exposed eggs. IGM 100/979 and similar other life assemblages (Dong and Currie, 1996) provide clear evidence that the center of the nest is devoid of eggs. The egg type of IGM 100/979, and IGM 100/1004 have been assigned to the elongaloolithid oofamily (e.g., Zhao, 1975; Mikhailov, 1992; Clark et al., 2001), a parataxonomic classification mostly based only on few microstructural features, egg shape, eggshell surficial ornamentation that correspond with the fine longitudinally oriented ridges (linearituberculate ornamentation) that grades into dimples or smooth areas near or at the poles of IGM 100/971 and IGM 1001125 (Fig. 4C). Oviraptorid eggs and all elongatoolithids are commonly recovered in clutches where they are paired and in a horizontal to sub-horizontal position in one or more superposed rows with an empty space in the center (Dong and Currie, 1996; Clark et al., 1999). Two paleobiological interpretations could be inferred from these observations. First, oviraptorids had two functioning ovaries and second they produced paired eggs in a monoautochronous sequence (Clark et al., 1999).

Although in IGM 100/979 none of the eggs are completely exposed, their shape appears to be elongate with parallel sides and with two rounded poles. The original study of IGM 100/979 reported that one of the poles was less rounded than the other (Norell et al., 1995) but a subsequent description stated that they were similar in shape and size (Clark et al., 1999). However, the study of IGM 100/1125 (an isolated pair of oviraptorid eggs) clearly shows that the two poles are not identical (Fig. 4D) as the pole tilted inside the clutch is slightly more tapered than the other pole. This feature is interpreted as resulting from the presence of a small air cell, a feature departing from the absence of air cell in extant crocodilians but not yet fully comparable by its size to that of modern avians (thus a proto-avian air cell). The sub-horizontal orientation of the eggs with the blunt pole up and having a higher pore concentration coupled with the linearituberculate ornamentation between poles, is thought to have acted as an air conveyor (Sabath, 1991). This suggests that these eggshell structures facilitated the air circulation along the eggs to their blunt poles and indirectly supports the presence of an air cell at the blunt pole.

Preservation of IGM 100/979 shows that there was little disturbance of the clutch and skeleton resting on it, thus suggesting that the adult specimen is preserved in the position in which it died, here interpreted as a brooding posture. Loope et al. (1998) interpreted the depositional setting of the Djadokhta Formation sands as low energy debris flows from semistable eolian dunes, a geological setting consistent with the latitudinal paleobearing of the Djadokhta Formation estimated at 30° north, a latitude known for desert environments. The burying sediments would have come from destabilized sand dunes during large rainstorm events. The fact that when brooding modern birds would tend to abandon their nest at the approach of a deadly threat (Winkler, 2004) and that the oviraptors were recovered on their clutches, suggest that the debris flow must have been low energy and the brooding oviraptorid should have been dead or lethargic at the time of burial for it not to flee. As documented (Davies and Curry, 1978) modern paleognath birds, namely *Dromaius novahollandiae* (emu) are known to incubate their brood for 40 days (personal observation) with very limited food or water intake, initiating an energy depletion that can reach lethargic levels. If oviraptorids brooded their clutches, even as a simple act of protection, for similarly long periods of time, it could explain their burial atop their egg clutches. At the same time this would advocate that the behavior of a long brooding period predates the most recent common ancestor of modern birds.

The eggshell of these oviraptorid eggs is relatively thin, averaging 570 μm but ranging from 500 to 641 μm, according to whether the measurement was taken at the apex of a ridge or in at the bottom of a trough. The



eggshell radial sections viewed in SEM display a bilayered structure (Fig. 4E), where layers 1 and 2 average 169 and 401.59 μm, respectively, and where the contact between the two structural layers is aprismatic (Fig. 4E) (sensu Grellet-Tinner and Norell, 2002). The base of the shell units in layer 1 consists of bundles of acicular crystals of rhombohedric form that create fans of equal width and height radiating from the cores (Fig. 4E, F) to the boundary between layers 1 and 2. The distinct layer 2 (Fig. 4E, F) consists of calcitic crystals with a *C*-axis that are orientated 90° with respect to those of layer 1. The outermost section of this layer appears undulatory (Fig. 4F) due to the linearituberculate surficial ornamentation aligned with the long axis of the egg. Black organic lines, a terminology used in the parataxonomic literature, are visible in TLM throughout layer 2 (Fig. 4F). These lines are likely the voids left by a proteinous mesh originally intertwined with the inorganic calcium carbonate of the eggshell (Fig. 1A, B) as evidenced by SEM where vesicles are oriented in continuous horizontal lines (Fig. 1A, B). This observation further supported by the micron size of these vesicles, confirms that they are the molds of the original protein fibers embedded in the eggshell (Grellet-Tinner, 2005b). In contrast to those of the dromaeosaurid *Deinonychus antirrhopus* (Grellet-Tinner and Makovicky, submitted for publication), these black lines are equally distributed throughout layer 2 (Fig. 4F) and their wavy or sinusoidal appearance does not always parallel the undulation of the eggshell outer surface. This pattern that was previously interpreted as the result of erosion (Norell et al., 2001), is viewed here as a character of biological origin possibly adding a strengthening component to the eggshell structure. As previously mentioned, studies on shells of mollusks have shown that resistance to pressure increases with the number of structural layers, particularly when the internal crystallization varies in direction from layer to layer (Kamat et al., 2000). Shells of mollusks and eggshells share a common denominator, as they both are the product of biomineralization, thus sharing the same properties. Although thin, the eggshell of *C. osmolskae* is composed of two structural aprismatic layers. The relative thinness of this eggshell was compensated by a novel bi-layered structure interpreted here to cope with the external stress originating from superposed layers of eggs and possibly the weight of the brooding parent who partially covered the eggs with its appendages. Moreover, these observations and interpretations suggest a coeval evolution between brooding behavior and accretion of a second layer in the eggshell structure.

5.3. Troodontid (*Troodon formosus*) eggs

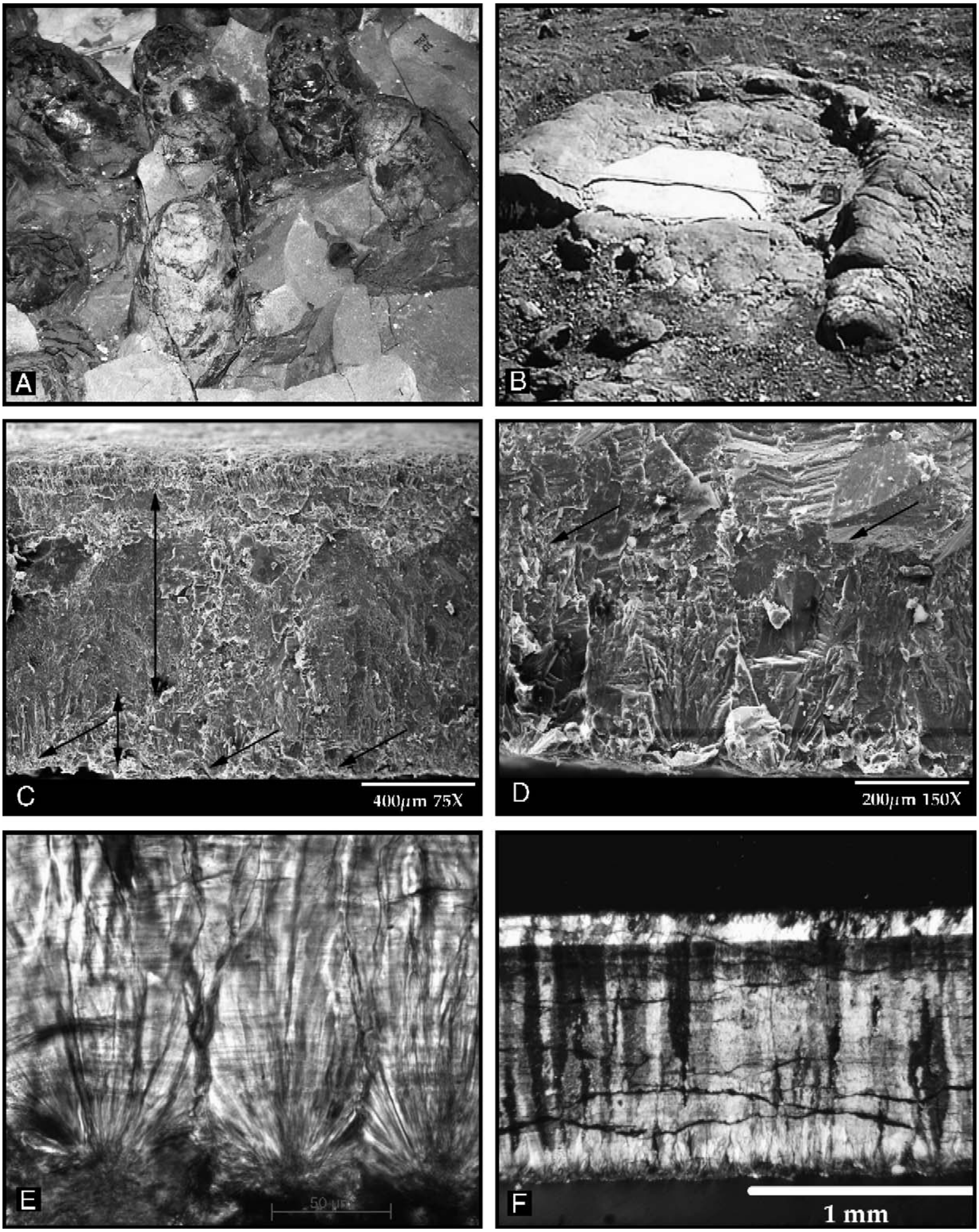
Troodon formosus was the first discovered species of the troodontid family, a medium-sized coelurosaur from the Late Cretaceous of North America. Although numerous specimens of *T. formosus* were found associated with eggs, eggshell fragments, and nests (Varricchio et al., 1997, 2002), it is not clear that this oological material has been fully interpreted. Therefore, a review and new interpretation of this oological material and the reproductive strategy of *T. formosus* are offered here.

The overall egg length of *T. formosus* ranges from 12 to 16 cm. These eggs are elongated and asymmetrical (Fig. 5A) with their narrow poles averaging respectively 3 cm in diameter and their blunt end 7 cm (Varricchio et al., 2002). This significant difference in shape and size between the two poles argues for the presence of a fully developed air cell like those of modern avians in contrast to the proto-air cell of *C. osmolskae* (Grellet-Tinner, 2005b).

The largest clutch assigned *T. formosus* (MOR 963) contains up to 24 eggs that stand vertically or at a slight angle with their narrow poles embedded in the sediment (Fig. 5A) (Varricchio et al., 1997, 1999, 2002). These 24 eggs (Fig. 5B) occupy an oval space of 45 by 56 cm within a rimmed nest 1 by 1 m (Varrichio et al., 1999). Another clutch of this species, MOR 246, has eggs that stand sub-vertically leaning towards the center (Varricchio et al., 2002). In both cases, egg pairing was mentioned (Varrichio et al., 1999, 2002) and supported by statistical analysis (Varricchio et al., 1997). Yet, this claim relies solely on the spacing of the narrow end of the eggs, their trend and plunge angles, and also on an a priori assumption that the eggs were laid in pairs (Varricchio et al., 1997). However, a different statistical analysis conducted by Varricchio et al. (1997) based

Fig. 4. A) IGM 100/979. Life assemblage with at least 2 superposed rows of eggs and a brooding (oviraptor) *Citipati osmolka*. B) IGM 100/971. *C. osmolka* embryo in ovo. C) IGM 100/971 external surface. Note the overlapping eggshell fragments indicative of compressive forces after burial. D) IGM 100/1125. Paired eggs are indicative of monoautochronic ovideposition. Note the slight angles between these two eggs also observed in Macroelongatoolothid eggs, and the minor but visible difference in size between the two poles indicative of the presence of proto-air cells. E) SEM Presence of two aprismatic layers (with arrows show the demarcation) with layer 1 consisting of shell units made of acicular and radiating calcitic crystals. F) TLM view of the organic lines (black arrows) in layer 2. In contrast to those of *D. antirrhopus* these lines are equally compacted and either follow the aspect or are in opposition of the eggshell surficial ornamentation (as here shown).

on the blunt poles (the poles not originally buried in sediments) came to the conclusion that pairing was not present and that the eggs were randomly positioned in a bowl-like depression. The interpretation of the eggs' arrangement within these clutches is important for inferring the reproduction aspect of this theropod. Should the spatial distribution of these eggs be paired it would imply that *T. formosus* retained two functional ovaries

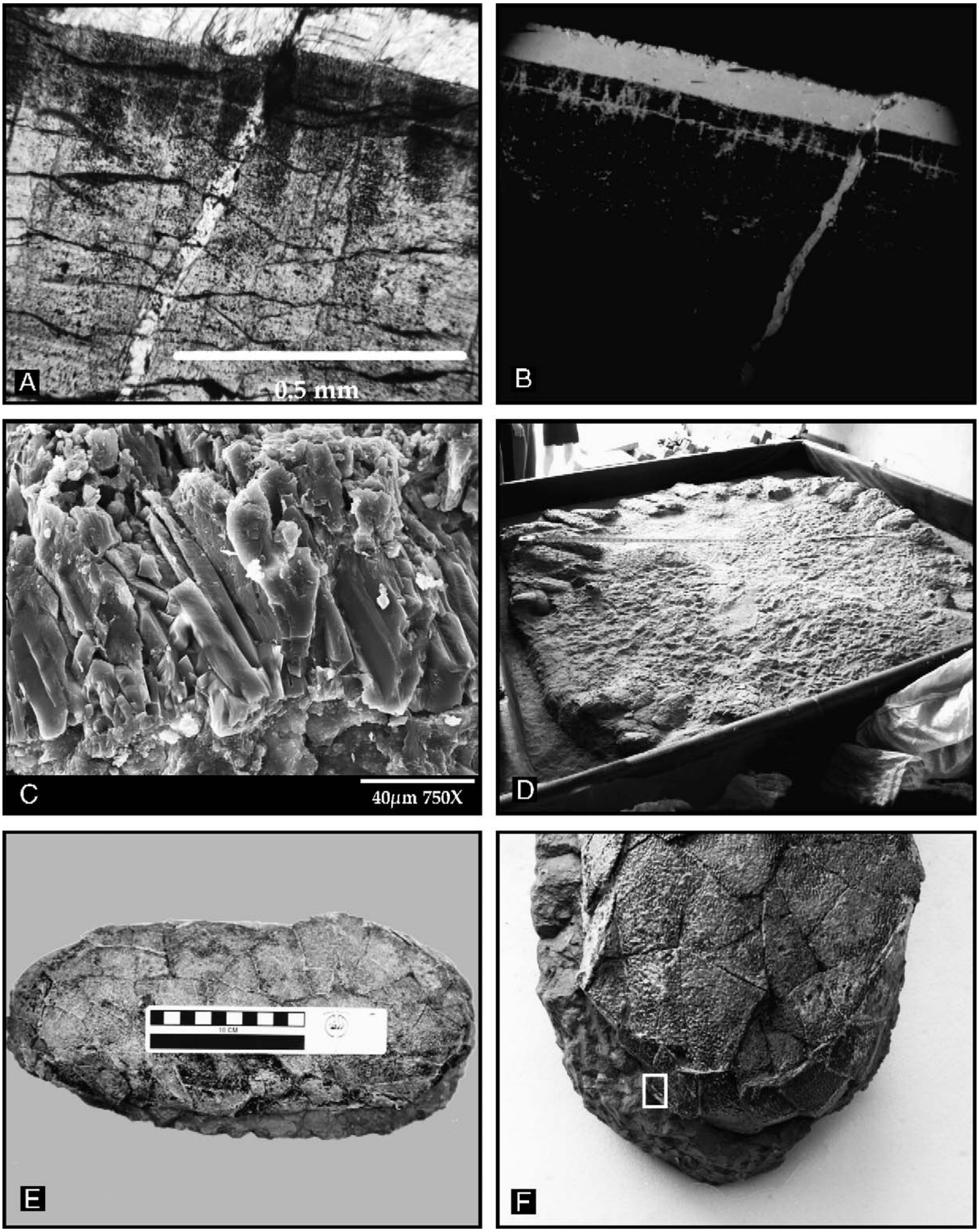


and a monoautochronous ovideposition like *C. osmolskae*. Alternatively, unpaired eggs distribution could be interpreted as the result of having only one functional ovary at any given time, a condition known presently only in extant birds. Another argument that could refute the egg pairing stems from the geometry of the nest and the eggs. According to Varricchio et al., (1997, 1999, 2002), the nest is a bowl-shaped depression where the conical eggs have their narrow ends down and their blunt ends in contact with each other, filling the top area of the clutch. Having the widest section of these eggs in close contact and filling up the upper space of a bowl-shaped depression implies that the position of their lower and narrower sections is random being primarily influenced by the position of their blunt poles. Therefore, any analysis relying on their narrow pole spatial position as conducted by Varricchio et al. (1997) should be regarded with caution. Furthermore, post-deposition manipulation for this theropod cannot be ruled out as suggested by Horner (1987). In view of these observations, the presence of paired eggs in *T. formosus* clutches, hence whether *T. formosus* had one or two functional ovaries at any given time, cannot be resolved by the spatial distribution of the eggs. However, the egg geometry of this extinct theropod could provide an unexpected clue to whether *T. formosus* had one or two functional ovary at any given time. *Troodon formosus* eggs markedly resembles that of modern avian eggs by their asymmetrical shape and modern birds are known to rotate their eggs in the oviduct prior to ovideposition (Smart, 1995), a peristaltic movement that could be facilitated by having one single egg at the time in the oviduct. Thus, the bird-like asymmetrical shape of *T. formosus* eggs could be better as a proxy to infer whether this theropod had only one functional ovary, than their relative position in clutches.

The eggshell MOR 246 is relatively thin averaging only 965 μm and its radial section viewed in SEM displays a bi-layered structure (Fig. 5C) as substantiated by TLM, PLM observations and CL analysis. However, Varricchio et al. (2002) speculate that *T. formosus* eggshell might have three structural layers, although they did not corroborate the presence of this structural feature by independent analyses. Unlike the eggs of *C. osmolskae*, the contact between the two structural layers is gradual, giving this eggshell a prismatic character (sensu Grellet-Tinner and Norell, 2002) and making it difficult to distinguish layer 1 from layer 2. Nevertheless, the boundary between these two layers can be approximated by the increased amount of vesicles typical of layer 2 over those in layer 1 (Fig. 5D), as seen in SEM. The 191 μm-thick layer 1 consists of long blade-shaped calcite crystals that extend from the basal spherulites (Fig. 5E). Layer 2 averages 721 μm in thickness and consists of calcitic crystals with their *C*-axis that are moderately differently oriented in respect to those of the bladed-shaped crystals in layer 1. Yet, this difference in orientation does not completely mask the prismatic columns of the eggshell units originating in layer 1 (Fig. 5F), as it would for the aprismatic eggshells of *C. osmolskae* and *D. antirrhopus* (Makovicky and Grellet-Tinner, 2000). SEM observations reveal an outermost layer with a thickness that varies from 129 to 78 μm (Figs. 5F, 6A, B) that consists of calcite that exhibits a needle like crystal habit (Fig. 6C) similar to aragonitic needles growing upon micritic grains in marine environments (Tucker and Wright, 1990). This observation coupled with the presence of a funnel-like pore opening below this layer and a strong red fluorescence falsifies the biological origin of this structure. Interestingly, there is a line visible in SEM and that fluoresces under CL. It separates the upper section of layer 2 from the rest of this layer (Fig. 6B), a probable cause for the interpretation of three structural layers in *T. formosus* (Varricchio et al., 2002). However, this line fluoresces in a similar fashion to the diagenetic calcite layer that blankets the eggshell outer surface, the micrite that invaded a pore canal, and some former organic lines adjacent to the external eggshell surface, thus suggesting that this line is a diagenetic artifact. Furthermore, neither SEM, TLM or PLM observations denote any structural difference within layer 2 or change of crystallographic or extinction patterns at or around this demarcation line, corroborating CL observations and refuting the presence of three layers in this eggshell. Nevertheless, the bi-layered structure of *T. formosus* eggs should not be sufficient by itself to support the weight of a brooding adult as recovered at least in one MOR specimen, unless this troodontid would possess a novel mechanical adapta-

Fig. 5. A) Egg clutch of *Troodon formosus* (MOR 675) prepared from below, as such the exposed sections of the eggs are their lower sections normally buried in the substrate. Note the conic and asymmetrical shape of these eggs, and the tremendous difference in their pole sizes. In contrats to oviraptorid eggs, *T. formosus* eggs are “planted” with their long axis at an angle at, or around 90° to the substrate. B. (MOR 963), egg clutch in a rimed nest. C) SEM view of *T. formosus* eggshell. Note the presence of two prismatic eggshell structural layers which contact is unclear. Double black arrows highlight these two layers, and single black arrows point to the concave base on the eggshell units. Note the ubiquitous presence of a surficial diagenetic layer of calcite that could be confused with a structural eggshell layer. D) SEM. Long blade-shaped crystals at the base of each eggshell unit. E) TLM. Same as 5.1 D. F) TLM. Note the diagenetic calcite layer that blankets that outer eggshell surface.

tion. The vertical arrangement of *T. formosus* eggs coupled with their partial burial in the substrate offers an innovative compromise where the weight of the brooding parent is transferred onto one pole of the egg that is partially buried eliminating the risk of damaging the clutch (Zhao and Ma, 1997). Hence, although the eggshell is only bi-laminated, the mechanical properties inferred from egg spatial position and



burial would support at least an avian brooding (so far inferred as a protective) behavior for *T. formosus.*

Reptilian and avian nests are viewed as species-specific microenvironments that optimize egg incubation and hatching (Proctor and Lynch, 1993). As an understanding of the behaviors of extinct animals can only be extrapolated from observations of their living relatives, observations of pluvianid nesting and incubating behaviors provide interesting clues about the possible nesting behavior of *T. formosus*. Pluvianids display a suite of interesting nesting and incubating behaviors which result in patterns of egg spatial distribution and clutch geometry similar to those observed in troodontids, but they also live in climatic environments that compare favorably with those inferred for *T. formosus* where the paleoclimate is best described as arid with summer convective showers (Varricchio, 1993). The pluvianid *Pluvianus aegyptius* partially buries its eggs by digging a depression in sandbars (Howell, 1979). Egg Mountain and Egg Island, the two localities where *T. formosus* have been recovered were islands in a local lacustrine system (Varricchio, 1993). *Pluvianus aegyptius* eggs fill a bowl-like depression without leaving any empty space and they are positioned in a sub-vertical position, pointed pole down with only the blunt pole of these eggs exposed periodically. Eggs partially buried or buried only a few centimeters below the surface in hot and arid environments could easily reach lethal levels of 40 °C if not attended by the parents whose primary thermoregulation strategy is to cool down their clutch with water from nearby water sources (Grant, 1982). *Pluvianus aegyptius* follows this incubating model by transferring heat at night and by cooling down the eggs during the day by wetting them with water transported in the plumage (Howell, 1979). This uncommon incubating strategy consists of utilization of thermo-inertia from the sand as a heat source and active involvement of the parent to insure that its eggs stay below lethal levels during extremely hot conditions (Howell, 1979). The similarity of the paleoenvironments of *T. formosus* with that of extant pluvianids, coupled with nesting structure and clutch arrangement that resemble each other, suggest that *T. formosus* could have displayed similar nesting and incubating behaviors. This interpretation and the lack of empty space in the center of the clutch (in contrast to *C. osmolskae*), coupled with the presence a skeletal remains of adults on the eggs could indicate that *T. formosus* would have therefore already developed a form of incubation (thermoregulation) like modern pluvianids rather than brooding behavior limited to clutch protection like oviraptorids.

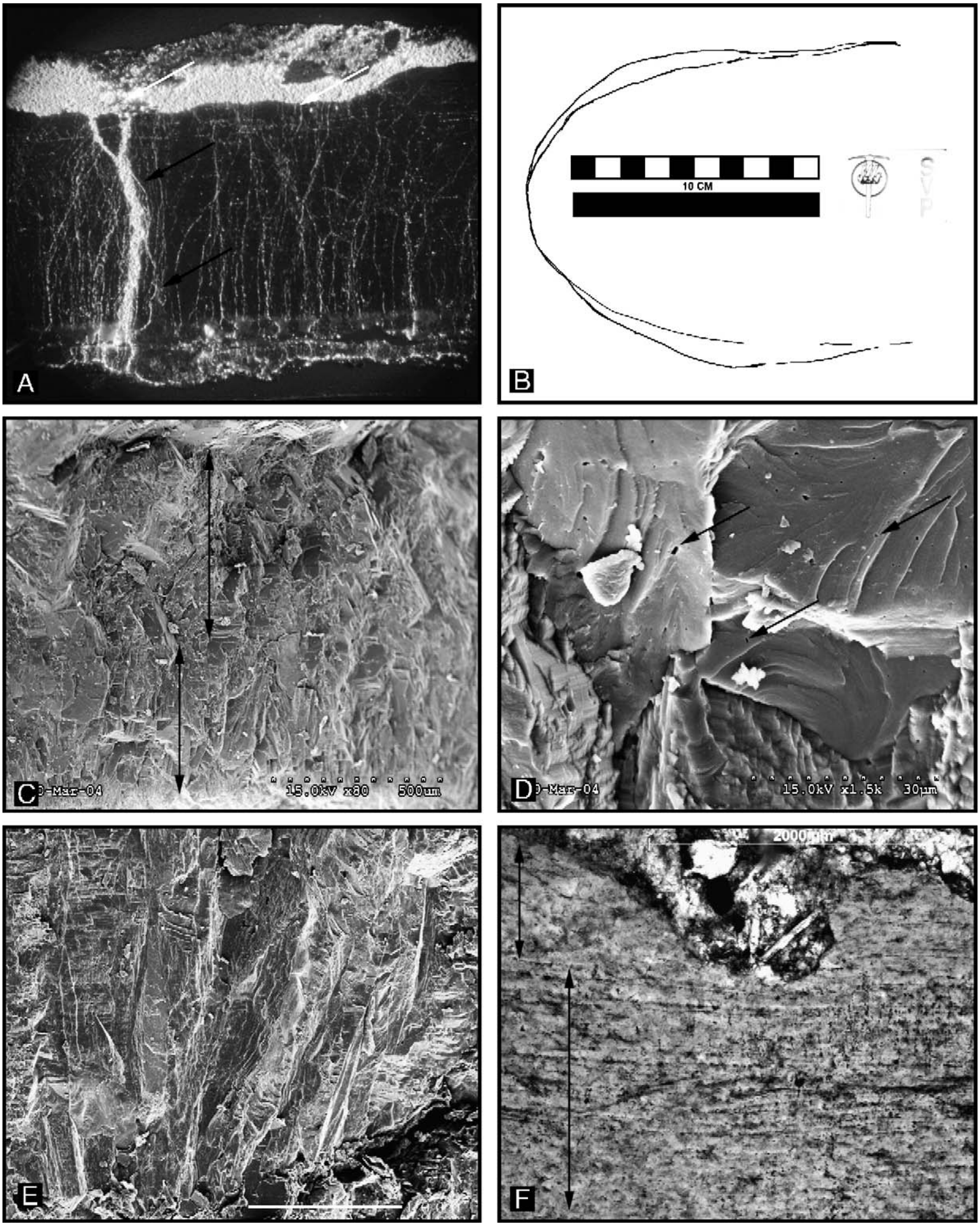
5.4. Theropod macroelongaloolithid eggs

While conducting a geological exploration in the Xixia County, China in 1974, the 12th Geological Team of the Henan Geological Bureau discovered several spherical objects with an ornamented surface that were soon after identified as dinosaur eggs (Fang et al., 1994). Since then, numerous fossil dinosaur eggs and eggshells have been discovered in the Chinese Upper Cretaceous red beds (Fang et al., 1994). These eggs come in different shapes and preservation states, but none of them until now were originally reported to be associated with embryonic remains. Consequently, Chinese researchers (Zhao, 1975) first adopted a binominal nomenclature to classify this egg and eggshell cornucopia (parataxonomic classification). One group of interest, the elongaloolithid eggs includes the *Macroelongatoolithus* oogenus (from Xixia County), which eggs measure approximately 45 by 16 cm and a sizeable egg clutch from Sanlimiao (Xixia County) (Currie, 1996) of approximatively 2 m in diameter comprising at least 13 pairs of macroelongaloolithid eggs (Fig. 6 D) revisited by the first author at the Nanyang Museum (Fig. 6D).

The macroelongatoolithid egg LACM 7477/151450 was recovered from the Zoumagang Fm. (Maastrichtian), 10 km North East of Sanlimiao, Xixia County, Henan Province, China. LACM 7477/151450 is oval

Fig. 6. A) TLM view. Note the pore canal that obliquely crosses the eggshell thickness and widens in its lower section. B) CL. Note the line that separates layer 2 in two sub-sections, possibly indicative a third structural layer. However, close examination of these two images refutes the presence of such a third layer by the lack of change of crystallographic or extinction patterns below and above the line and by the presence of the same diagenetic material that has invaded this line, the pore canal, and covered the eggshell surface. C) SEM of the diagenetic calcite layer that blankets that outer eggshell surface. Note the needle-like crystal habit similar to aragonitic needles growing on micritic grain in marine environments. D) *Macroelongatoolithus xixiaensis,* a clutch from the Zoumagang FM (Maastrichtian), of at least 13 pairs of eggs that were laid in a large ring of 75 inches in diameter (as portayed by the National Geographic 1996 Issue). E) F.A.3818.2001-1, an oblong egg from the Zoumagang FM (Maastrichtian), Xixia County, Henan Province. Although middly altered as substanciated by the presence of overlapping eggshell fragments, this egg is well enough preserved to obtain its 14 by 35 cm dimensions. F) Detail of the overlapping eggshell fragments indicating that F.A.3818.2001-1 was buried whole and subsequently submitted to taphonomic forces that compressed and cracked its surface. The white squarre shows the place where an eggshell fragment was taken for microstructural examination. Although dispersituberculate on the rest of the specimen, the surficial ornamentation is smooth at the poles.

and occurs with its long axis half embedded in the original reddish matrix (Fig. 6E). The eggshell is fractured into several medium and small size fragments that have been dislocated from their original position with a minor amount of overlap (Fig. 6F), and is covered by a thin layer of white calcite. The pattern of fractures suggests that the egg was exposed to compression after it has been buried and after the matrix had already solidified



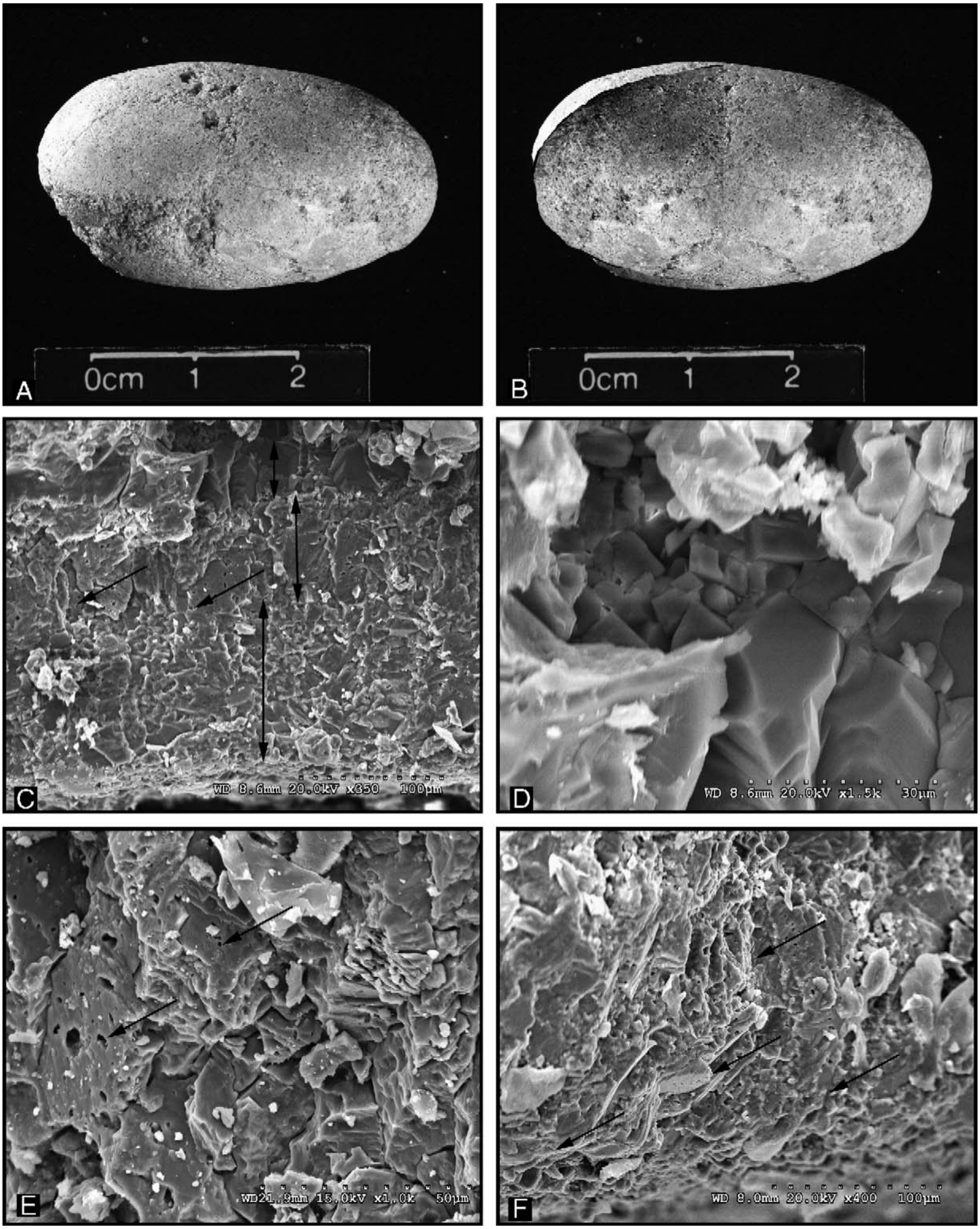
thus limiting any possible lateral extension. It also indicates that LACM 7477/151450 was complete and possibly not fractured at burial (Mueller-Töwe et al., 2002). The white calcite blanket shows that a second diagenetic phase took place not only after the compression phase but also after the egg was buried and the silty matrix was transformed into siltstone. This second phase was likely associated with hypersaturated pore fluids in respect to $CaCO^3$ and its precipitation. The direct consequences of these processes are that the eggshell ornamentation has been eroded and/or is partially masked by diagenetic $CaCO^3$, as observed in CL (Fig. 7A), and secondly, that the egg dimension could be slightly biased, as eggshell fragments overlap at the egg periphery. The egg measures 14 by 35 cm, and its length to width ratio is 2.5. None of these dimensions match the oospecies included in the elongatoolithid family as described by Li et al. (1995), Fang et al. (1994) and Carpenter (1999). According to Fang et al. (1994), *M. xixiaensis* is known to reach 45 cm in length and 16.5 cm in width. A range of 38 to 60 cm in length for this oospecies is also mentioned and concurs with dimension given by other authors (Li et al., 1995; Carpenter, 1999). Among the other large oospecies generally included in the elongatoolithid family (e.g. *Elongatoolithus, Macroolithus*), none are as long as LACM 7477/151450 and all are within the lower 20 cm range. Without further information, observations of LACM 7477/151450 would suggest that the size range of *M. xixiaensis* needs to be extended. This egg displays a slight morphological difference between its two poles, a condition already observed for the eggs of *C. osmolskae* (see previous description). In order to quantify this difference in shape, the perimeter of LACM 7477/151450 was digitally outlined, cut into two halves along the equator, and one half was rotated 180° and then overlain over the other. The wider outline shows that one end was more blunt than the other (Fig. 7B). This observation concurs with the observation of Fang et al. (1994) description of *M. xixiaensis* with ''one end obtuse and the other taper'' and also supports Carpenter's (1999) elongatoolithid description where ''One end is usually rounded, while the other is somewhat pointed''. Also consistent with the obsevation of Li et al. (1995) of *M. xixiaensis*, the 13 pairs of eggs of the macroelongatoolithid clutch (Currie, 1996) now housed at the Nanyang Museum (Fig. 6D) are distinctively paired and almost parallel to each other or forming a 13° angle among themselves, a feature that Li et al. (1995) attributed to the muscular contraction of the dinosaur fallopian tubes during (monoautochronic) ovideposition. The eggs of the Nanyang clutch average 39–40 cm in length, a measure also congruent with what has already been reported and observed in other *M. xixiaensis* specimens.

Egg diameter is related to the age of the dinosaur and its pelvic opening (Carpenter, 1999). In addition, immature birds would have a tendency to lay smaller eggs than mature females of the same species (personal observation) but overall, egg size is related to the size of the parent (Rahn et al., 1975). The huge size of *M. xixiaensis* eggs is therefore proportional to the body size of a large dinosaur species as further attested by the sizeable empty space in the clutch center. In addition, the extreme elongation of these macroelongatoolithid eggs suggests that there was a need to include more nutrients. As observed in modern birds, precocial chicks require more energy to develop than altricial dinosaurs, thus require the presence of more yolk in the egg (Noble, 1995; Carpenter, 1999) which in turns requires an egg with a larger volume to support precociality. When considering the size limitation exerted on the diameter of an egg by the diameter of the pelvic opening (Rahn et al., 1975; Carpenter, 1999), it would seem plausible that one way to increase the volume of an egg for these non-avian theropods was by elongating its shape, a common feature to all elongatoolithid eggs, to support precocial chicks as suggested by Clark et al. (1999) for *C. osmolskae*.

Where the calcitic coating is thin, it is possible to notice a variation of the surficial ornamentation from reticulated and randomly oriented, low vermiculate ridges at the blunt pole, to ridges that are oriented parallel to the long axis toward the equatorial regions, finishing as low and somewhat connected nodes at the pointed pole. Eggshell fragments were sampled from the blunt pole area as per agreed with the curator of the LACM. The eggshell is relatively thick averaging 1373 μm without accounting for the external ornamentation. The surficial ornamentation is dispersituberculate and displays mushroom-like nodes with slight

Fig. 7. A) CL reveals that, as LACM 7477/149736b, the outer surface of F.A.3818.2001-1 is blanketed by a layer of diagenetic calcite. Note the "Y"-shaped pore canal that bends midway between the eggshell surfaces. B) As *C. osmolka*, F.A.3818.2001-1 poles display a faint asymmetry. The F.A.3818.2001-1 contour was drawn, then bisected and the two halves were overlapped to evaluate their degree of asymmetry. C) The eggshell structure of F.A.3818.2001-1 is bi-laminated with an aprismatic contact between the two layers as observed on this SEM image. D) This SEM image shows a greater amount of vesicules (black arrows) in layer 2 of F.A.3818.2001-1. E. In SEM, the shape of the spherulite crystals that forms the base of the eggshell units of F.A.3818.2001-1 approach an acicular aspect due to their length by width high ratio and prolong into long blade-shaped crystals. E) TLM. The organic lines of F.A.3818.2001-1 divide layer 2, in two sub-layers with the upper section of this layer thinner than the lower sub-section.

indentations at their bases, a feature quite noticeable in TLM (Fig. 7F) and CL. The outer and inner eggshell surfaces are connected by straight pore canals that sometimes branch in a shallow ‘‘Y’’ near the outer eggshell surface. LACM 7477/151450 radial section viewed in SEM displays a bi-layered structure (Fig. 7C), where the contact between the two structural layers is aprismatic, but this condition is not as abrupt as the



aprismatic contacts observed in eggshells with radiating acicular crystals in layer 1 of *C. osmolskae*, *D. antirrhopus*, or those of modern paleognaths (Grellet-Tinner, 2000). The change of crystallographic orientation between layers 1 and 2 is further supported by a significant higher number of vesicles in layer 2 (Fig. 7D) helping to differentiate each layer (Fig. 7C, D). Layers 1 and 2, respectively, average 307 and 1066 μm. The bases of the shell units in layer 1 consist of long and thin spherulitic crystals that have nearly an acicular aspect (Fig. 7E). The microstructure of this eggshell supports its interpretation as being co-specific to *M. xixiaensis* (Li et al., 1995).

Similarities between the eggs of the oviraptorid *C. osmolskae* (Clark et al., 1999) and those of *M. xixiaensis* and *C. osmolskae* suggest similar reproductive physiology and behaviors. These similarities include the presence of: (1) two aprismatic structural eggshell layers, (2) surficial eggshell ornamentation, (3) egg pairing, (4) egg shape, (5) a small air cell (proto avian air cell) not as developed as those of modern birds denoted by a slight asymmetry between the two poles, (6) absence of eggs in the center of the clutch, and (7) narrow poles of the eggs pointing down toward the center of the clutch. However, some features differentiate the clutches of these two species: (1) the absence of multiple superimposed egg rows in *M. xixiaensis* as observed in *C. osmolskae* and other oviraptorids (Clark et al., 1999; Dong and Currie, 1996), (2) the size difference in the eggs, and (3) the size of the empty space in the center of the clutch. All the shared characters between these two lineages, suggest that the dinosaurs which laid *M. xixiaensis* eggs were theropods closely related to oviraptorids that likely similarly brooded their eggs sitting in the center of their clutch, with the understanding that brooding is here interpreted as a protective behavior instead of one that implies a thermal exchange. However, the few autapomorphies of *M. xixiaensis* indicate that the parent species was considerably larger than any known oviraptorids.

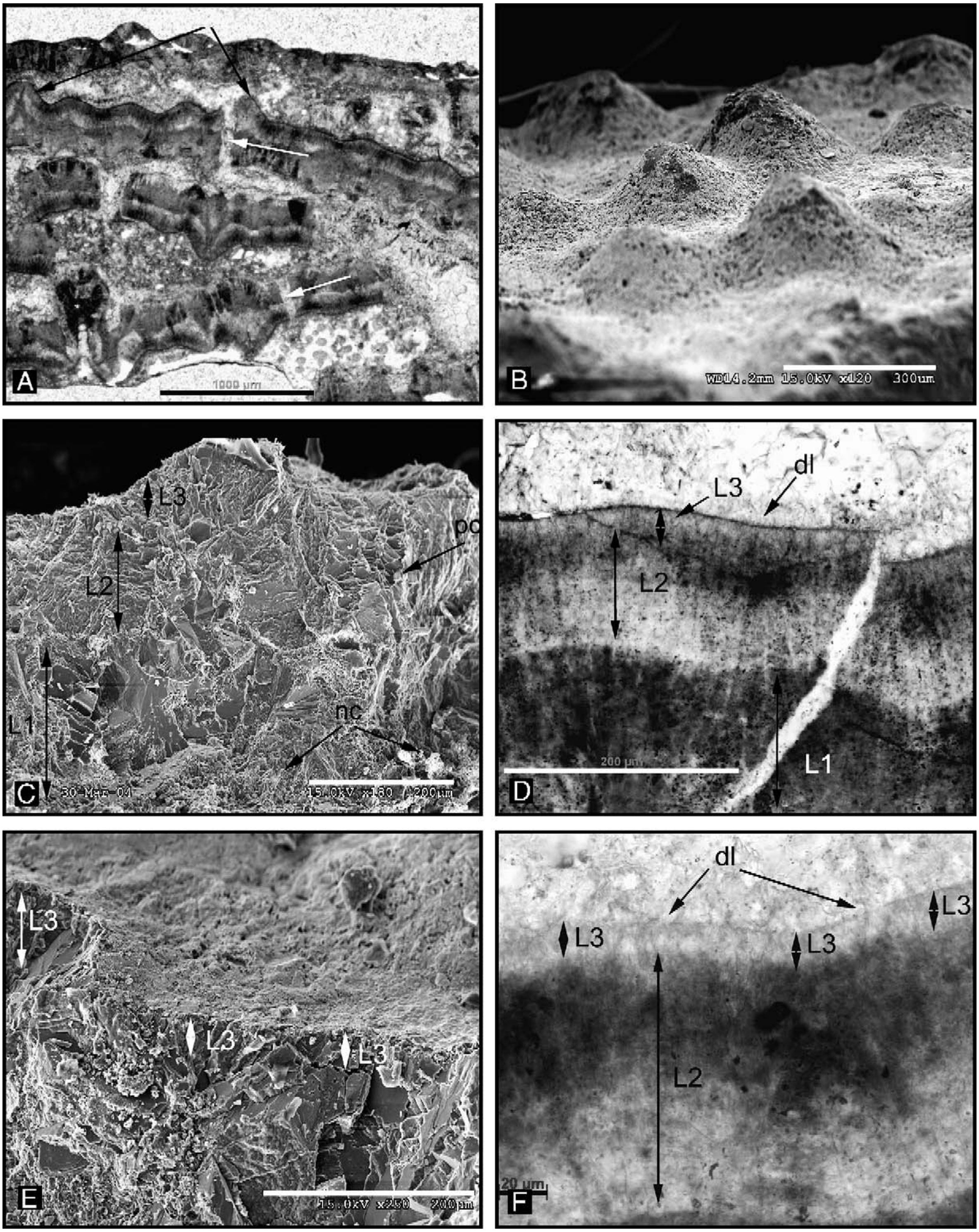
5.5. Ornithothoracine eggs

Dozens of small asymmetrical eggs have been found in exposures of the Cretaceous Bajo de la Carpa Formation of Neuquén City, Patagonia, Argentina. A recent publication describes several of these eggs and their embryonic remains in ovo and phylogenetically brackets them between the two avian Ornithothoraces and Ornithuromorpha nodes (Schweitzer et al., 2002). This discovery is important because it allows for the first time the association between the morphology of avian Mesozoic eggs and a particular clade of basal avialans.

The eggs we studied came from the same collection as those previously published (Schweitzer et al., 2002). They have undergone only slight taphonomic deformation as attested by their well-preserved shape. Their asymmetric, oval shape (Fig. 8A) favorably compares with that of the modern chicken (*Gallus gallus*) but their dimensions of 45 mm (length) by 27 mm (equatorial diameter), as reported by Schweitzer et al. (2002) implies a lesser volume (about 150% smaller) than *G. gallus*. Our measurements of a “complete” egg from the same locality are 20% smaller than those previously reported (Schweitzer et al., 2002), a discrepancy easily justified by the absence of preserved eggshell on the egg surface and amplified by a possible surficial erosion of the sandstone endocast of this egg during diagenesis. To test this hypothesis, another egg with a fully shelled section was digitally ‘‘lassoed’’ and pasted onto the whole egg (Fig. 8B). Two conclusions came forth from this test: 1) the shape is proportionally identical between the corresponding sections of these two specimens, thus suggesting that the overall shape of the “complete” egg is real, and 2) eggs that are only partially preserved consist of the halves that have a tapered pole (without air cell). This taphonomic feature coupled with the presence of sandstone infilling and the absence of visible crushed and cracked eggshell on the egg surface not only indicated that the preserved partial eggs were hatched and then filled up with the surround-

Fig. 8. A) MUCPv eggs from Bajo de la Carpa Fm. (Campanian), in Neuquen, Patagonia. Although most of the specimens consist only of preserved halves a few complete eggs display a shape like those of extant chicken. They are oval, with one pole more inflated than the other to accommodate an air cell. They average 45 by 27 mm. B) In order to estimate the asymmetry between the two poles, the pictures of the few eggs that were intact were inverted and pasted over the commonly found broken halves. This confirmed the presence of asymmetrical poles but also that the commonly preserved halves were the pointed poles, which is not surprising considering that most modern birds destroy the air cell and the surrounding section during hatching. C) As those from Phu Phok, the MUCPv eggshell structure is tri-laminated (black double arrows in this SEM) with prismatic contact between layers 1 and 2 and aprismatic contact between layers 2 and 3. The latter could be a diagenetic artifact. Equally important, the thickness of layer 1 far exceeds that of layer 2, as already observed in the Phu Phock eggs. D) SEM of the eggshell surface with a cover of euhedral crystals supporting a diagenetic phase that could have potentially modified the contact between layers 2 and 3. E) SEM shows a greater amount of vesicles (black arrows) present in layer 2 that helps to distinguish this layer from layer 1. F) SEM shows that the bases of the eggshell units display a substantial amount of acidification, a pattern congruent with the previously mentioned diagenetic hypothesis. This results in eggshell units truncated at their base and flaring at a 45° angle.

ing sandy matrix prior to fossilization, but more importantly, demonstrate a strong similarity with the hatching process in modern seagulls where the same egg sections are left intact resting on the ground surface (Hayward et al., 2000). The polar region that contains the air cell where the chick pips at the time of hatching, as well as the region immediately adjacent to the air cell is generally destroyed during hatching in seagull colonies



(Hayward et al., 2000). To further test the hypothesis that the missing pole of MUCPv eggs is as obtuse (the one containing the air cell) as that of modern avians, a complete fossil egg was digitally bisected in the center and the two poles were superimposed. This independent test supports the previous conclusion that the missing poles in the five eggs are indeed those that contained the air cell and suggests that this extinct bird already displayed a modern avian hatching strategy.

Eggshell thickness excluding a surficial diagenetic layer material that exhibits euhedral crystal habits (Fig. 8D) averages 285 μm. The eggshell structure is composed of three distinct structural layers (Fig. 8C). Layer 3, the outermost layer, averages 49.26 μm in thickness, a value exceeding by 12 μm that which is reported by Schweitzer et al. (2002). In the studied specimens, layer 3 is also characterized by having only a few vesicles and by having a very distinct boundary with layer 2 (aprismatic condition, see Grellet-Tinner and Norell, 2002). This feature is particularly interesting because presently there is no record of eggshell displaying simultaneously a prismatic boundary between layers 1 and 2 and an aprismatic boundary between layers 2 and 3. This condition could be linked to the degree of diagenetic re-crystallization and a preferential calcium carbonate deposition in layer 3, but nevertheless it does not exclude a biological origin. Both features were not mentioned in Schweitzer et al. (2002). In contrast to the layers 2 and 3, the contact between layers 1 and 2 is undefined (prismatic condition, sensu Grellet-Tinner and Norell, 2002). Nevertheless, layer 2 is easily differentiated from layer 1 by having a higher number of vesicles that are also larger than those in layer 1 (Fig. 8C, E). The 95.56 μm thickness of layer 2 is nearly twice that of layer 3 and markedly differs from the 132 μm value mentioned in Schweitzer et al. (2002). The thickness of layer 1 averages 137.2 μm, a value that also departs from the previously reported 91 μm (Schweitzer et al., 2002) and far exceeds that of layer 2, a feature unknown in modern birds or non-avian theropods. The base of layer 1 lacks the cores of the mammillae as it suffers from acidification, a feature denoted by the interval in between each unit. As a result, the cores themselves have weathered out and consequently each eggshell unit seems to originate from a flat, horizontal line that flares out on either side at a 45 degrees angled-V-shape. Another consequence of the dissolution is that the eggshell units originating at the base of the layer 1 are well defined (Fig. 8F) and extend without major crystallographic interruption into layer 2 but abruptly stop at the boundary of layers 2 and 3 (Fig. 8C). Considering the siliciclastic nature of the egg endocasts, it is probable that some acidification of the eggshell (Hayward et al., 1991) occurred during the fossilization process, a pattern already observed in similar sedimentary contexts for the Late Cretaceous megaloolithid eggs from Uruguay (Faccio, 1994) and for extant colonies of seagulls (Hayward et al., 1997). No pore canals or openings are readily visible in the observed specimens.

Although the specimens studied here are not the same as those studied by Schweitzer et al. (2002), the lack of congruence of the thicknesses of layers 1 and 2 is difficult to explain unless the values were inverted by mistake in the 2002 publication. The erosion of layer 1 could possibly reflect the disparity between its values but layer 2 in either case is sandwiched between layers 1 and 3, and thus cannot be thinned out by an erosion process. Together, the aprismatic character at the boundary of layers 2 and 3 that establishes an unquestionable upper limit for layer 2 and the marked increase of the size and number of vesicles, coupled with the change of crystal orientation between layers 1 and 2 that provides a lower limit for layer 2 support the accuracy of the present measurements.

The hatching pattern observed in these ornithothoracine eggs, which preserves the pointed pole and its adjacent region, the gross aspect of the few intact eggs, and the tri-laminated and prismatic eggshell structure, advocate that the extinct bird species that laid these eggs had a similar nesting behavior to extant birds. Furthermore, the similarities of egg shape and eggshell structure between this species and extant birds indicate that this extinct ornithothorace had a modern avian

Fig. 9. Abbreviations: L1, eggshell structural layer 1; L2, eggshell structural layer 2; L3, eggshell structural layer 3; pc, pore canal; nc, eggshell units nucleation centers; dl, diagenetic line. A) Thin section of an entire egg. Note the multilayered and multidirectional arrangement of the eggshell fragments indicative of a compression of the egg, as it was still whole. Black arrows point to some of the rarer and taller surficial nodes. White arrows point to fractures in the eggshell that likely occurred during a third diagenetic stage. B) SEM of the nodular surficial ornamentation. Note the bimodal distribution of the height of the nodes. This type of ornamentation has been previously reported with elongatoolithid eggs, a parataxonomic family that has been traditionally associated with non-avian theropods. C) SEM of eggshell radial section. Note the distinct presence of three eggshell structural layers, a pore canal and eggshell units broken at their base, leaving only a conic stub at their point of origination. D) Thin section of eggshell radial section. As for the SEM, note the presence of the three structural eggshell layers and also of a black diagenetic line (dl) here interpreted as bacterially mediated micrite. E. SEM showing eggshell structural layer 3 in more detail. F) Thin section showing the difference of crystallographic orientation between L2 and L3. Note also the presence of the micrite diagenetic line on the outer eggshell surface.

reproductive system. However, the present observations demonstrate that unlike other presently described theropod oological material, this basal ornithothoracine species laid eggs with a thicker structural layer 1 than layer 2.

5.6. Theropod eggs from Phu Phok (Thailand)

To date, no positively identified Mesozoic theropod eggs with dimensions as small as 18 by 11 mm have been ever reported until four very small eggs about the size of a goldfinch's egg were discovered in the Lower Cretaceous Sao Khua Formation of Thailand (Buffetaut et al., 2005). One of them (SK1-1) still contains an embryo in ovo encased in calcite. Three small bones can be seen in cross-section in the hard calcitic matrix that fills the crushed shell. One of the bones just shows the outline of a hollow sub circular shaft. The other two appear to be located in what may be roughly their original anatomical position. The larger bone shows a hollow oval shaft continued on one side by a long curved tapering process. The co-occurrence of a hollow and relatively thin-walled bones indicates that these embryonic bones are those of a small theropod, however their preservation prevents determination of whether they were avian or not.

The four fossil eggs are crushed, but one of them is sufficiently preserved to assess its original size and shape. The asymmetric oval shape best compares with that of *Gallus gallus* but its dimensions of 18 mm (length) by 11 mm (equatorial diameter), with an estimated volume (Sabath, 1991) of 1.15 cm^3, match that of the extant *Carduelis carduelis* (Goldfinch). The presence of multiple stacked layers of eggshell (Fig. 9A) indicates that the eggs were not infilled, and thus neither cracked, nor opened, prior to and during burial (Mueller-Töwe et al., 2002). The overlap of the eggshell fragments near the equatorial portion suggests the lateral expansion of the shell was limited as the egg was crushed after early lithification of the encasing sediment (Mueller-Töwe et al., 2002). Crushing was followed by weathering (pore fluid) and calcite re-deposition, as determined in TLM by a faint black line outlining the eggshell outer surface (Fig. 9A, D, F) and CL. Closer examination reveals this line consists of calcitic epitaxial growth possibly of microbial origin (Tucker and Wright, 1990). CL observations show the multiple diagenetic phases to which this material has been submitted by the variation of the intensity of luminescence throughout in the eggshell structure.

The shell thickness, excluding surficial ornamentation, averages 353.9 μm. This value approaches that of *Gallus gallus* and is 30% thicker than the ornithothoracine eggs from the Late Cretaceous of Argentina (Schweitzer et al., 2002). The eggshell outer surface is deeply ornamented with nodes that display a bimodal size distribution (Fig. 9B). The taller nodes average 183 μm in height, while the more numerous and smaller ones are half that size (92.3 μm). Funnel-like structures breaking the taller nodes may be interpreted as pore canals, but they are more likely fissures resulting from compression during a late diagenetic phase (Fig. 9A). Despite intense recrystallization, three prismatic structural layers are still visible in radial section (Fig. 9C–F), a group of characters presently known to be synapomorphic for ornithothoraces (Schweitzer et al., 2002). Layer 1 is poorly preserved and only a few truncated mammillary crystals are visible at its base (Fig. 9C). The aspect of some of these crystals shows that they are not acicular but blade-like. Layer 1 averages 207 μm and is 1.7 times bigger than layer 2 (Fig. 9C, D, F), a character shared with the basal ornithothoracine eggs from Neuquén (personal observation) but not yet observed in other well identified non-avian theropods or Neornithines. Layer 3 is conspicuous and is 32 μm thick (Fig. 9C–F). The transition between layers 1 and 2 is gradual rather than sharp representing a prismatic condition (sensu Grellet-Tinner and Norell, 2002), as observed in troodontids (Varricchio et al., 2002) and neognath birds (Grellet-Tinner, 2000; Grellet-Tinner and Chiappe, 2004).

When compared to the eggs of other theropods for which eggs have been positively identified, the eggshell of the Phu Phok eggs is similar in its prismatic structure to eggshell of *T. formosus*. However, *T. formosus* differs in its thick bi-layered eggshell (700 to 1000 μm) and lack of surficial ornamentation. In addition, *T. formosus* eggs have a much larger volume of 450 cm^3 (Varricchio et al., 2002) and are elongated (12 to 16 cm) and possess one pole (7 cm) that is at least twice as big as the other (3 cm). *C. osmolskae* eggs are long and wide (18 by 7 cm) and possess a 500 to 640 μm thick bi-layered eggshell that displays an aprismatic structure with acicular crystals in layer 1, and its eggshell surface is characterized by a linearituberculate ornamentation. Although admittedly very little is known about positively identified eggs of basal avians, the Phu Phok eggs share a similar shape with the previously reported ornithothoracine eggs from Neuquén (Argentina) and a tri-laminated, prismatic eggshell structure where layer 1 is at least 1.4 times bigger than layer 2. However, they differ from these Argentinean eggs by their pronounced surficial ornamentation that is presently typical although not exclusive of non-avian theropods. This would indicate that the parent species could have been a primitive avian still retaining an eggshell ornamentation typical of non-avian dinosaurs (Grellet-Tinner, 2005b) or was a non-avian theropod that already displayed a mosaic of avian characters.

6. Discussion

As a model of archosaurian reproduction, the two extant groups phylogenetically bracketing non-avian dinosaurs (crocodilians and birds) differ in a number of features. Crocodilians produce large clutches of symmetrical eggs that are laid en masse from two functioning ovaries in contrast to birds that produce a limited amount of asymmetrical eggs that are laid in a monoautochronic mode from a single functional ovary (Proctor and Lynch, 1993). The typical descent of an egg from the left ovary (in most extant birds) to the shell gland follows a complex path very similar in every bird species, though differing in its duration (Parker and Haswell, 1910; Taylor, 1970). Calcitic covering of the ovum takes place in the shell gland (Makita, 1981) and other diagnostic features of eggs are partly controlled by peristaltic movements in the shell gland (Taylor, 1970; Deeming and Ferguson, 1995). Crocodilian eggshell is composed of one structural, calcitic layer (Schleich and Kästle, 1988) while modern birds display at least three layers (Mikhailov, 1997; Grellet-Tinner and Chiappe, 2004). Parental involvement for crocodilians is generally limited in assisting the hatchlings during and after hatching in contrast most of the birds invest a considerable amount of time and energy to incubate their eggs and rear their brood (Proctor and Lynch, 1993). Nests are simple for the former consisting of holes dug in the sand or vegetal mounds, but generally turn into elaborated structures for the latter as avian reproductive physiology (Romanoff and Romanoff, 1949) is crucial to controlling clutch size, chemical composition (Dauphin, 1990), eggshell morphology, and nest morphology (Proctor and Lynch, 1993).

Although the taxa studied in this research represent only snapshots of the entire picture of saurischian evolution, they reveal reproductive and oological trends. Such trends include a reproductive evolutionary cline from basal archosaurs (as inferred from crocodilians) to modern birds that is manifested by a successive acretion of eggshell layers, an autochronous ovideposition, the atrophy of one ovary or the evolutionary development of a single functioning ovary, and the change from limited parental care to a brooding or protective phase of the eggs that finally reaches a fully avian incubating behavior. All these transformations are indicative of a physiological and behavioral evolution of the reproductive system and likely reflects a profound physiologic change that occurred in saurischian dinosaurs.

Like modern crocodilians, the titanosaurs from Auca Mahuevo laid numerous, symmetrical but mostly subspherical eggs "en masse", in rimmed nests (Chiappe et al., 2004). However, titanosaurs developed innovative adaptations observed in their eggshell structure that are not shared with non-dinosaurian archosaurs adapted to their nesting environments, namely an (nodular) eggshell ornamentation – a saurischian dinosaurs synapomorphy – allowed for a greater air flow for eggs in the nests and an eggshell thickness is perforated by numerous straight and "Y" shaped pore canals originating between surficial nodes, a condition interpreted as a response to a high nesting moisture content.

Whether the acicular nature of the calcitic eggshell is an environmental adaptive strategy or reflects a different chemical equilibrium in the reproductive tracts of the titanosaurs remains untested, but it markedly differs from the eggshell microstructure of crocodilians. Interestingly, this acicular calcitic crystallization structure is also present in layer 1 of *D. antirrhopus* (Makovicky and Grellet-Tinner, 2000) and *C. osmolskae* eggs (Grellet-Tinner, 2005b) but absent in troodontid and fully formed eggs of most extant bird eggshell. However, developmental studies have revealed that during oogenesis the bladed-calcite crystals in modern birds have an acicular state that transforms into the bladed morph (Board and Sparks, 1995). Observations of fossil specimens combined with oogenesis in modern birds advocate that acicular calcite crystals seen in the eggshell microstructure of titanosaurid eggs are synapomorphic for saurischian dinosaurs.

Although rare, few life assemblages consisting of a brooding parent on egg clutch have been reported in the fossil record (Norell et al., 1994; Clark et al., 2001). Therefore, it would seem plausible that considering the vast expanse of the sedimentary egg-bearing layers of Auca Mahuevo and the commonly found titanosaurid skeletal remains in this stratigraphic layer in other localities devoid of eggs that, if present, such life-assemblages would have been recorded. Consequently, the lack of adult skeletons in the sedimentary egg-bearing layers of Auca Mahuevo coupled with the observed clutch structure and oological features would advocate that titanosaurs like modern crocodilians, did not spend time brooding their egg clutches. When considering all the characters at hand, the titanosaurids from Auca Mahuevo still share a number of reproductive characters with crocodilians, namely symmetric eggs, one eggshell structural layer that is calcitic, lack of a defined air cell and "en masse" ovideposition. These reproductive features and other outlined above suggest these dinosaurs retained two functional ovaries and lack of brooding behavior. However, the titanosaurs from Auca Mahuevo and possibly others from France already differ from primitive archosaurs by having a pronounced surficial eggshell ornamentation that supported high moisture content in their nests (Grellet-Tinner et al., 2004), numerous pores to facilitate gas exchanges between the growing embryos in rimmed nests (Chiappe et al., 2004), and acicular calcitic crystals (Grellet-Tinner et al., 2004).

The oviraptor *C. osmolskae* IGM 100/979 and other oviraptorid fossils have been found sitting on their nests in an avian posture (Dong and Currie, 1996). In such life assemblages, oviraptors sit atop an egg clutch with their axial skeleton in lodged in the space in the center of the clutch devoid of eggs, and the eggs are paired and arranged in a sub-horizontal position forming up to three superposed layers. While this type of life assemblage suggests brooding activity, it falsifies at the same time the possibility of any incubation by the presence of multiple rows of eggs lying in a sub-horizontal position on top of each other and by the absence of evidence of egg manipulation from one to another. This brooding behavior is interpreted as an intermediary phylogenetic precursor to avian incubation (Norell et al., 1995). In addition, *Citipati osmolskae* laid long oval eggs that averaged 7 by 18 cm with a polar asymmetry not yet as pronounced as that of extant birds. The elongate aspect of these eggs is interpreted as a response to a physiological requirement where there was a need to incorporate more nutrients into the eggs, suggesting that the chicks could have been precocial. Precociality implies a longer incubation time than altriciality and if as inferred oviraptorids brooded their clutches for long periods of time, it could explain their burial atop their egg clutches by low energy "mass wasting" of sediments from destabilized dunes (Loope et al., 1998). As such, *C. osmolskae* burial atop their egg clutch advocates that the behavior of long brooding periods predates the most recent common ancestor of modern birds. Interestingly, brooding first appears phylogenetically in the same taxon known to lay slightly asymmetrical eggs with a faint polar asymmetry, here correlated to the presence of a small air cell (proto avian air cell), a feature that is absent in reptilian eggs (Iverson and Evert, 1995), as their eggs are symmetrical.

Another feature that is characteristic of *C. osmolskae* is the pairing of its eggs. Such a spatial arrangement is indicative that this oviraptorid still had two functioning ovaries, as did sauropods and likely all primitive saurischians, but advocates for a monoautochronic ovideposition (Clark et al., 1999; Norell et al., 2001; Sato et al., 2005), and no post-partum manipulation of the eggs by the parent. In addition to this mosaic of characters indicating an avian trend, the eggshell of *C. osmolskae* is structurally divided into two aprismatic layers, an intermediary step between the mono-layered crocodilian and tri-layered avian eggshell structures. Kamat et al. (2000) argued that molluscs achieve a great mechanical equilibrium by increasing the number of structural layers of their shell and emphasized that differences in crystallographic orientation among layers enhances this trend. It is worth noting in that respect that, although the volume of *C. osmolskae* eggs is at least equal to that of the Auca Mahuevo titanosaurs, their eggshell is twice as thin although the eggs have to support the weight of overlying eggs and the partial weight of a brooding adult (e.g. appendicular skeleton). As such, this eggshell structure fulfills two mechanical constraints: An eggshell that is thin enough to be broken by the hatching embryo and a need for resistance to greater external forces (load from the other eggs and the brooding adult). To augment the mechanical stress resistance, we speculate that the linearituberculate surficial ornamentation of the eggshell, which was previously interpreted as a feature that solely favors air circulation among the eggs in a clutch and that keeps the pore apertures free of any obstruction (Sabath, 1991), could also act as a feature that increases the mechanical resistance to external stress by the presence of longitudinal grooves and ridges.

Comparison of *M. xixiaensis* eggs with oviraptorid eggs is necessary to better understand the reproductive characters of the theropod lineage that laid the *M. xixiaensis* eggs. As in *C. osmolskae*, *M. xixiaensis* eggs are elongate with a faint air cell, are paired, form a circle with an empty space in the center of the nest, and possess bi-laminated eggshell (Fig. 1). However they differ from *C. osmolskae* by their gigantic size, by not being in superposed rows in the clutch, and by the presence of elongate blade-shaped crystals in layer 1 of the eggshell. The synapomorphies characterizing the eggs of these two theropod groups indicate that the animals had the same general reproductive behaviors. However, the autapomorphies of *M. xixiaensis* suggest a few differences. The gigantic size of *M. xixiaensis* eggs is indicative of a larger theropod than *C. osmolskae*, as supported by the meter and a half wide empty space in the clutch center. However, the difference of the eggshell crystallography (elongated, blade-shaped/acicular for oviraptor) cannot yet be attributed to a difference in egg size. Although, elongated blade-shaped crystals have no known biological relevance yet, they are closer to acicular by their aspect ratio than the other types of blade-shaped morphs (in the calcite crystallographic spectrum). To summarize, the theropod lineage that laid *M. xixiaensis* eggs shared with oviraptorids similar proto-avian characters, namely: brooding, monoautochronic ovideposition, empty space in the center of the clutch, paired eggs laid on the perimeter of a circle, ornamentation composed of modified ridges, and a bi-laminated and aprismatic eggshell structure and, although, two characters depart from oviraptorids: the presence of a single row of eggs in the nest that could easily be correlated with weight bearing constraints of the brooding parent, this dinosaur species likely possessed similar reproductive behavior and system to oviraptorids.

In many respects, the troodontid *T. formosus* offers intriguing perspectives on the evolution of avian reproductive parameters in non-avian theropods not observed in *C. osmolskae,* and *M. xixiaensis*. First, *T. formosus* eggs are asymmetrical and conical. This asymmetry is far more pronounced than that of *C. osmolskae* and *M. xixiaensis* and closely resembles the condition commonly observed in modern birds. This feature indicates the presence of a large air cell and suggests that troodontids already possessed an avian-like air cell instead of a proto-avian air cell as inferred for oviraptorids and elongatoolithid eggs. Second, in contrast to other known theropods but similar to extant birds, troodontid eggshells lack surficial ornamentation. Third, the short and blade-shaped calcite crystals of eggshell layer 1 are similar to the condition observed in modern neornithines. Fourth, in *T. formosus* eggshell structure the prismatic condition of the contact between layers 1 and 2 is similar to the neornithine condition. Lastly, the novel spatial arrangement of *T. formosus* eggs mimics that of modern pluvianids. Several interpretations could be made about the parental care behavior and reproductive physiology of this troodontid. We hypothesize that, in addition to a monoautochronic ovideposition, *T. formosus* had only one functional ovary and already had developed an avian incubation in contrast to the brooding behavior observed in more basal theropods lineages. The incubation is best described as sporadic parental involvement to avoid a drastic temperature change of the eggs that are vertically embedded in the sediment with their large poles (with air cell) upward and barely exposed above the sediment, an incubation behavior compatible with the hot environments where this troodontid species lived (Varricchio, 1993) and possibly similar to that of extant pluvianids (e.g. Grant, 1982; Howell, 1979). To summarize, the presence of unquestionable egg asymmetry implying a fully developed air cell, eggs vertically embedded "heads up", a prismatic eggshell layer with bladed-shape crystals in layer 1, and an adult sitting atop the semi-buried egg clutch (Varricchio et al., 1997, 1999), advocate that troodontids adopted an avian reproduction mode and incubation behavior. Furthermore, all these avian characters seem to originate concomitantly within the same species, possibly indicating a coeval development between egg shape, eggshell structure, and nesting behaviors (Zhao, 2000) with novel reproductive physiology. The major difference between neornithines and *T. formosus* is its bi-laminated eggshell structure implying a difference in mechanical strength of the eggshell that has been compensated by the position of the eggs in the stratum.

As predicted by their phylogenetic proximity to extant birds, the Neuquén ornithothoracine asymmetric eggs shaped like those of modern galliform birds are indicative of the presence of a fully developed air cell, Also, their eggshell bears no surficial ornamentation, which contrasts with the condition of most of the non-avian dinosaurs. Most importantly, the eggshell structure is tri-laminated and prismatic and has short blade-shaped calcite crystals in layer 1. However, one ubiquitous feature differentiates the eggshell structure of these enantiornithines from that of modern birds. The thickness of layer 1 exceeds that of layer 2, so much that the ratio of the two layers is close to 1.4. Interestingly, this character state has not been observed yet in non-avian theropods except the Phu Phok eggs. Although no live assemblages associating egg clutches with adults were recovered (Schweitzer et al., 2002), all the oological characters and hatching patterns advocate that basal ornithothoraces would have had a modern avian reproductive system and nesting behaviors.

The features of the egg and eggshell from Phu Phok are more diagnostic than the poorly preserved skeletal remains of the embryo in ovo, and by comparison with other identified oologic material offer a novel perspective of the transition from non-avian to avian theropods. The best-preserved egg displays the oval and asymmetric shape characteristic of modern birds and basal ornithothoraces from Neuquén. The eggshell structure is tri-laminated with prismatic contacts between layers. Moreover, these eggs share with the basal ornithothoraces the unique character state of eggshell layer 1 being thicker than layer 2 by a ratio of 1.4. This feature alone suggests a closer phylogenetic proximity between the Thai and the Patagonian eggs than with either non-avian theropods or modern birds. In contrast to the Neuquén eggs, the Thai eggs still display a pronounced nodular ornamentation. Eggshell ornamentation is presently known in non-avian saurischians (Grellet-Tinner, 2005b) and its presence could associate these eggs with non-avian theropod lineages. However, all the well-identified eggs of non-avian theropods had a bi-laminated eggshell, had an eggshell structural layer 2 thicker than layer 1, and were at least 400 times bigger than the Phu Phok eggs. To summarize, the Phu Phok eggs by their mosaic of characters coupled with their extremely small size argue that they are likely the product of either a small non-avian theropod phylogenetically close to primitive avians, or have been laid by a bird that is more primitive than enantiornithines and phylogenetically bracketed between the Avialae and Ornithothoraces nodes.

The studied suite of oological characters and the coeval evolution of the reproductive and nesting behaviors are optimized on a cladogram (Fig. 10) that disregards skeletal-based character optimization. A few patterns are ubiquitous. Regardless, of the position of the Phu Phok eggs with respect to Aves, most of the oological characters and reproductive behaviors associated with modern birds are rooted among non-avian theropods. Although possibly attributed to a sample bias, most of these pre-adaptations are grouped at two main clades of the saurischian cladogram: one at the level of Oviraptorosauridae, and the other at Troodontidae. Although it is undeniable that these two theropod taxa represent important steps in the evolution of avian reproduction, the phylogenetic distance separating Oviraptorosauridae and Titanosauria, for instance, cannot be ignored as a bias. It is likely that the reproductive features that seem to have appeared in toto in oviraptorids might have evolved sequentially in more basal theropod clades.

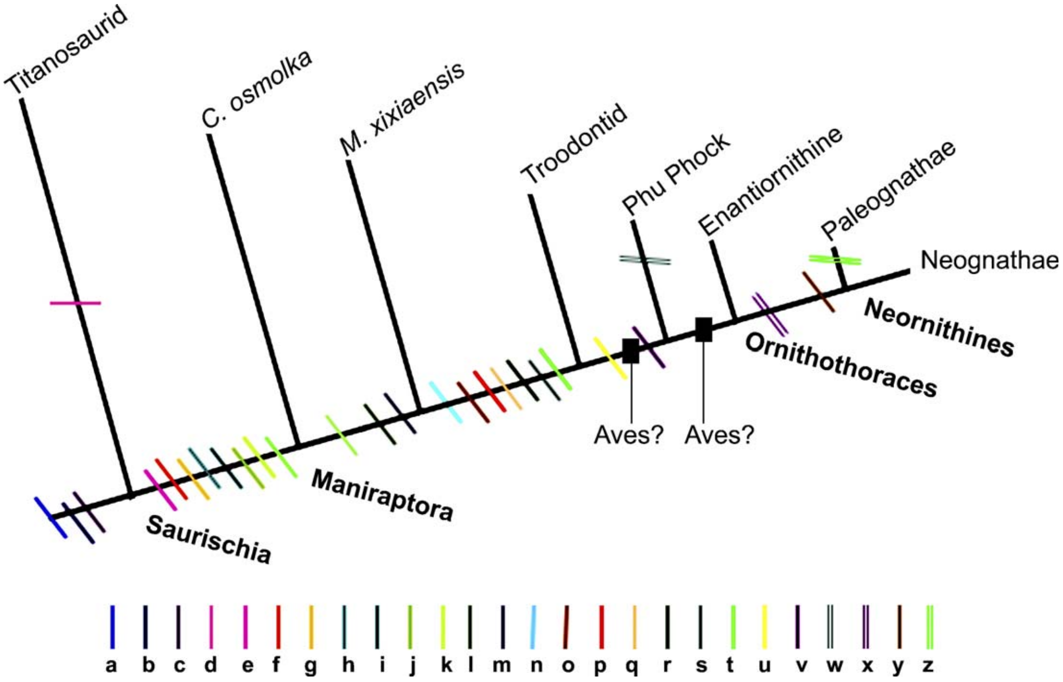


Fig. 10. Cladogram solely based on the optimization of oological and reproductive characters of the studied taxa. Note the coeval appearance of brooding behavior with a change in the architecture of the nest, egg, and eggshell in oviraptorids and a second similar advent at the level of troodontids when incubation developed a) presence of surficial ornamentation; b) acicular crystals as building blocks of the eggshell structure; c) eggs contained within a rimed nest; d) nodular ornamentation in titanosaurids; e) presence of two aprismatic layers; f) presence of acicular crystals limited in layer 1 and crystal orientation of layer at 90 from that of layer 1; g) linearituberculate ornamentation; h) elongated eggs; i) presence of a proto-air cell; j) appearance of a monoautochronic ovideposition as indicated by the eggs arranged in pairs; k) eggs are laid on the perimeters of circles that superposed in 2–3 layers and with an empty space in the center of the clutch; l) presence of brooding behavior; m) differentiation of organic lines within layer 2, creating 2 sub-divisions; n) presence of blade-shaped crystals in layer 1; o) presence of a single circle of eggs; p) presence of a fully developed air cell; q) no space devoid of eggs in the center of the clutch; r) reduction from two to one functioning ovary; s) presence of two prismatic eggshell structural layers; t) eggs are vertically oriented in the substrate with air cell up; u) absence of eggshell surficial ornamentation; v) presence of proto-avian incubation; w) presence of bi-modal nodular ornamentation; x) presence of three prismatic eggshell structural layers; y) layer 1 wider than layer 2; z) presence of aerial nests; s-1) character reversal for the Paleognath clade: prismatic eggshell boundaries reverse to an aprismatic character state.

Although physiological evolution in organisms, here underlining reproductive systems, cannot be directly preserved in the fossil record, it could be inferred by tiered levels of interpretation. Admittedly, such interpretations are speculative but sometimes are the only available means to infer evolutionary changes that are not directly observed in the fossil record. As such, the oological and nesting observations we made with their first level of inferences indicate that profound metabolic changes should have occurred in saurischian dinosaurs during the Mesozoic to parallel the reproductive cline observed in the species studied in this investigation.

7. Conclusion

Undoubtedly, eggs and eggshells by their shape, structure, and microstructure are excellent paleobiological indicators presenting innovative aspects of saurischian evolution. This research has demonstrated that, like the feathers of modern birds, most of the oological characters and reproductive behaviors associated with modern birds are rooted among non-avian theropods. Although the studied taxa in this work represent only a fraction of saurischian dinosaurs, they clearly reveal a reproductive evolutionary cline from archosaurian crocodiles to modern birds, and a noticeable pattern of coeval development between the acretion of eggshell layers, acquisition and size increase of air cells, and brooding/incubating behaviors. These oological and reproductive characters demonstrate a change in the reproductive organs from two functioning ovaries that deliver huge quantities of eggs in one ovideposition to a single functioning ovary with a daily (or more) monoautochronic ovideposition, through a stage where two ovaries were still functioning with a monoautochronic ovideposition. It is likely that physiological transformations occurred concomitantly with the above-mentioned evolution of oological characters and nesting behaviors, reflecting perhaps a profound change of metabolism prior to the rise of birds.

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