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An accumulation of turtle eggs with embryos from the Campanian (Upper Cretaceous) Judith River Formation of Montana

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ABSTRACT

A weathered accumulation of turtle eggs, interpreted as remnants of a single clutch composed of at least 16 turtle eggs (MOR 710) from the Campanian (Upper Cretaceous) Judith River Formation of north-central Montana, USA, represents a new oospecies *Testudoolithus zelenitskyae*. This ootaxon is diagnosed by the following unique combination of characters: spherical eggs 34–39 mm in diameter, 660–760 μm thick eggshell, shell unit height-to-width ratio of 3.15:1–5.5:1, and domed shell units. Estimated egg mass indicates that the egg-laying adult likely possessed a carapace 35.0–54.4 cm in length. Similarities between *T. zelenitskyae* oosp. nov. and *Adocus* sp. eggs, along with comparable body size, suggest that this taxon might have produced MOR 710. One egg exhibits abnormal multilayered eggshell, likely resulting from prolonged egg retention by the female turtle. At least five of these eggs, including the multilayered specimen, preserve embryonic remains that demonstrate a late stage of embryonic development. This suggests that death occurred just prior to hatching.

1. Introduction

Fossil turtle egg clutches, gravid adults, and turtle embryos are relatively rare in the rock record when compared to dinosaurian specimens (Lawver and Jackson, 2014). Jackson et al. (2008) report an in situ turtle egg clutch preserving 23 spherical eggs in three superimposed layers from the Albian (Lower Cretaceous) deposits of Tiantai basin of Zhejiang, China. Zelenitsky et al. (2008) describe a clutch of 26 eggs from the Campanian (Upper Cretaceous) Oldman Formation of Alberta, Canada and estimated that a female with carapace length of 49.5 cm produced the clutch. A possible tortoise clutch containing at least five eggs comes from the Pliocene Apolakkia Formation of Rhodes, Greece (Mueller-Töwe et al., 2011), and fossil egg clutches from the Pleistocene of Lord Howe Island, Australia are tentatively assigned to the stem turtle, *Meiolania platyceps* (Anderson, 1925; Gaffney, 1996; Lawver and Jackson, in press).

In contrast to these turtle clutches, fossil gravid turtles provide definitive assignment of eggs to a specific taxon. A gravid turtle and a turtle clutch from the Upper Cretaceous Dinosaur Park and Oldman formations, respectively, show similar eggshell microstructure. Zelenitsky et al. (2008) conclude that both are referable to *Adocus* sp. Likewise, Knell et al. (2011) report portions of two eggs within a gravid *Adocus* sp. from the Campanian (Upper Cretaceous) Kaiparowits Formation of Utah. Additionally, an undescribed gravid *Basilemys variolosa* from the Dinosaur Park Formation of Alberta, Canada preserves at least three eggs that were discovered when the specimen was inadvertently damaged (Braman and Brinkman, 2009). Finally, a eurysternid specimen from the Upper Jurassic of Solnhofen in Germany contains spherical objects that Joyce and Zelenitsky (2002) interpreted as highly altered eggs.

Fossil turtle embryos are known from North America (Zelenitsky, 1995; Clouse, 2001; Jackson and Schmitt, 2008; Zelenitsky et al., 2008; McGee, 2012), Asia (Mikhailov et al., 1994; Cohen et al., 1995; Fang et al., 2009), and Europe (Hemprich, 1932). Although, morphological analysis of these specimens could assist in determining their taxonomic affinity, only one specimen has been investigated. Using computerized tomography (micro-CT) McGee (2012) confirmed that *Adocus* sp. produced the clutch from the Oldman Formation, thereby agreeing with the previous identification of Zelenitsky et al. (2008).

Although rare, fossil turtle clutches, gravid females, and embryonic remains provide important information about the evolution of turtle reproduction and paleoecology. Here, we describe a weathered accumulation of turtle eggs (some containing embryos), which we interpret as remnants of a single clutch. The egg accumulation is from the Campanian strata of the Judith River Formation of north-central Montana, USA. Jackson and Schmitt (2008) briefly report the microstructure of the multilayered egg from this clutch when establishing criteria for recognition of egg abnormalities in the fossil record. However, a detailed description of the clutch was beyond the scope of their paper. Jackson and Schmitt (2008) simply referred to this egg as MOR 710, whereas here it is assigned as MOR 710B. We describe this weathered clutch, assign the eggs to parataxonomy, and discuss the implications for the evolution of turtle reproductive biology.

Institutional Abbreviations: AM, Australian Museum, Sydney, New South Wales, Australia; BMNH, The Natural History Museum, London, United Kingdom; HEC, Hirsch Eggshell Catalogue, University of Colorado, Boulder, Colorado, U.S.A; IVPP, Institute of Vertebrate Paleontology and Paleoanthropology, Beijing, China; LBA, L. Barry Albright field number; MCZ, Museum of Comparative Zoology, Cambridge, Massachusetts, U.S.A; MOR, Museum of the Rockies, Bozeman, Montana, U.S.A; NHMU, Natural History Museum of Utah, Salt Lake City, Utah, U.S.A; TMP, Royal Tyrrell Museum of Paleontology, Alberta, Canada; UCM, University of Colorado Museum, Boulder, Colorado, U.S.A; ZMNH, Zhejiang Museum of Natural History, Hangzhou, Zhejiang Province, China.

2. Materials and methods

The fossil turtle eggs studied are housed at the Museum of the Rockies (MOR) in Bozeman, Montana. Two multilayered eggshells were removed from an egg from MOR 710. One was etched with hydrochloric acid for about five seconds in order to better reveal the fine crystalline structure. Histological radial sections were made through eggshell fragments from both normal eggs (MOR 710A, C), the multilayered specimen (MOR 710B), and an embryonic costal element labeled MOR 710:EB 3 (the exact location of the section is indeterminate). Additional eggshell thin sections (MOR 710:ES 1–3) came from unspecified eggs within the clutch. Histological procedures follow Lamm (2013). Eggshell fragments were mounted on an aluminum stub, coated with 10 nm of gold, and imaged under a JEOL JSM-6100 scanning electron microscope (SEM) at 10 kV. Images included the inner surface and radial cross sections of the eggshell. Photomicrographs of histological sections of eggshell and bone were taken with a Nikon Digital Sight DS-5Mc camera and microstructural features were measured with the image analysis software ImageJ (Rasband, 1997; http://imagej.nih.gov/ij/). Assessment of potential diagenetic alteration of the eggshell included a Nikon eclipse 50i microscope equipped with cathodoluminescence (CL). Egg mass was calculated using Hoyt's (1979) equation:

$$\mathrm{Mass} = 0.000548 \times \mathrm{LB}^2$$

where L is egg length in mm and B is egg breadth in mm. Carapace length for the gravid female turtle that produced MOR 710 is estimated using the positive correlation between egg mass and adult carapace length (Elgar and Heaphy, 1989) and the regression line:

$$y = 0.0568x + 1.5811$$

The latter was derived from 63 species (Elgar and Heaphy, 1989: Appendix), where y is the egg mass and x is carapace length ($r^2 = 0.666$). Note that the regression equation provided in Elgar and Heaphy (1989: Fig. 1) contains a typographical error, which results in unrealistically small carapace lengths.

3. Geology

The Campanian (Upper Cretaceous) Judith River and Two Medicine formations in north-central Montana consist of eastward thinning, non-marine clastic deposits that record regressive-progradational phases of shoreline migration of the Western Interior Cretaceous Seaway (Fig. 1A, B) (Lorenz, 1981; Rogers, 1998). The temporally and lithostratigraphically correlative formations are now separated by the Sweetgrass arch, a north-south-trending anticline (Fig. 1A, B). The Judith River Formation in eastern Montana includes deposits of a broad lowland coastal alluvial plain (Rogers, 1998). Marine rocks of the Claggett and Bearpaw formations underlie and overlie the formation, respectively (Rogers, 1998). Eberth et al. (1992) and Goodwin and Deino (1989) dated rocks of the Judith River Formation in central Montana as 74.5 and 78.0 Ma, respectively, which corresponds to the middle to late Campanian (Fig. 1B).

MOR 710 comes from a sandy siltstone in a fining-upward stratigraphic sequence representing overbank deposits at the Egg White Site (MOR locality JR-122L) in Hill County, near Havre, Montana (Clouse, 2001; Fig. 1). This specimen occurred approximately 4 m laterally from a clutch of lambeosaurine eggs preserved beneath a bentonite layer within the so-called upper nesting horizon of the lower nesting ground (Clouse, 2001). According to Clouse (2001), this nesting ground lies in the upper half of the Judith River Formation, deposited during the latter half of the Bearpaw Seaway transgression. Invertebrate fossils within the nesting horizon consist of freshwater and brackish species, including large unionid bivalves, as well as unidentified small bivalves and gastropods (Clouse, 2001). Vertebrate fossils include shed theropod and ornithopod teeth and fragmentary turtle shells referable to *Basilemys* sp., *Adocus* sp., *Aspideretoides* sp., and an undescribed large-bodied terrestrial form.

4. Systematic paleontology

Oofamily Testudoolithidae Hirsch, 1996 sensu Jackson et al., 2008

Oogenus *Testudoolithus* Hirsch, 1996 sensu Jackson et al., 2008

Oospecies ***Testudoolithus zelenitskyae*** oosp. nov.
Figs. 2–3

Holotype. MOR 710, the weathered remains of a clutch composed of at least 16 turtle eggs.
Etymology. After Darla Zelenitsky in recognition of her initial description of *Adocus* eggs from Alberta, Canada and continuing contribution to oological research.
Type locality and age. MOR locality JR-122L, Egg White Site, north-central Montana, U.S.A, Judith River Formation, Upper Cretaceous (Campanian).
Diagnosis. *Testudoolithus zelenitskyae* oosp. nov. differs from all other oospecies in the following unique combination of characters: spherical turtle eggs 34–39 mm in diameter; 660–760 μm thick eggshell; shell unit height-to-width ratio of 3.15:1–5.5:1 and domed shell units.
Distribution. Judith River Formation, Montana, U.S.A. (Jackson and Schmitt, 2008, MOR 710), Oldman and Dinosaur Park formations, Alberta, Canada (Zelenitsky et al., 2008, TMP 1999.63.2 and TMP 2008.27.1, respectively), and Kaiparowits Formation, Utah, U.S.A. (Knell et al., 2011, NHMU 16868 [LBA-06-7]). All these formations are Campanian (Late Cretaceous) in age.
Description
Eggs. Eleven beige eggs (Fig. 2) measuring 34–39 mm in diameter occurred on a weathered surface eroded into a concretionary layer (Fig. 1C). The lithostatically compressed eggs are filled by sandy

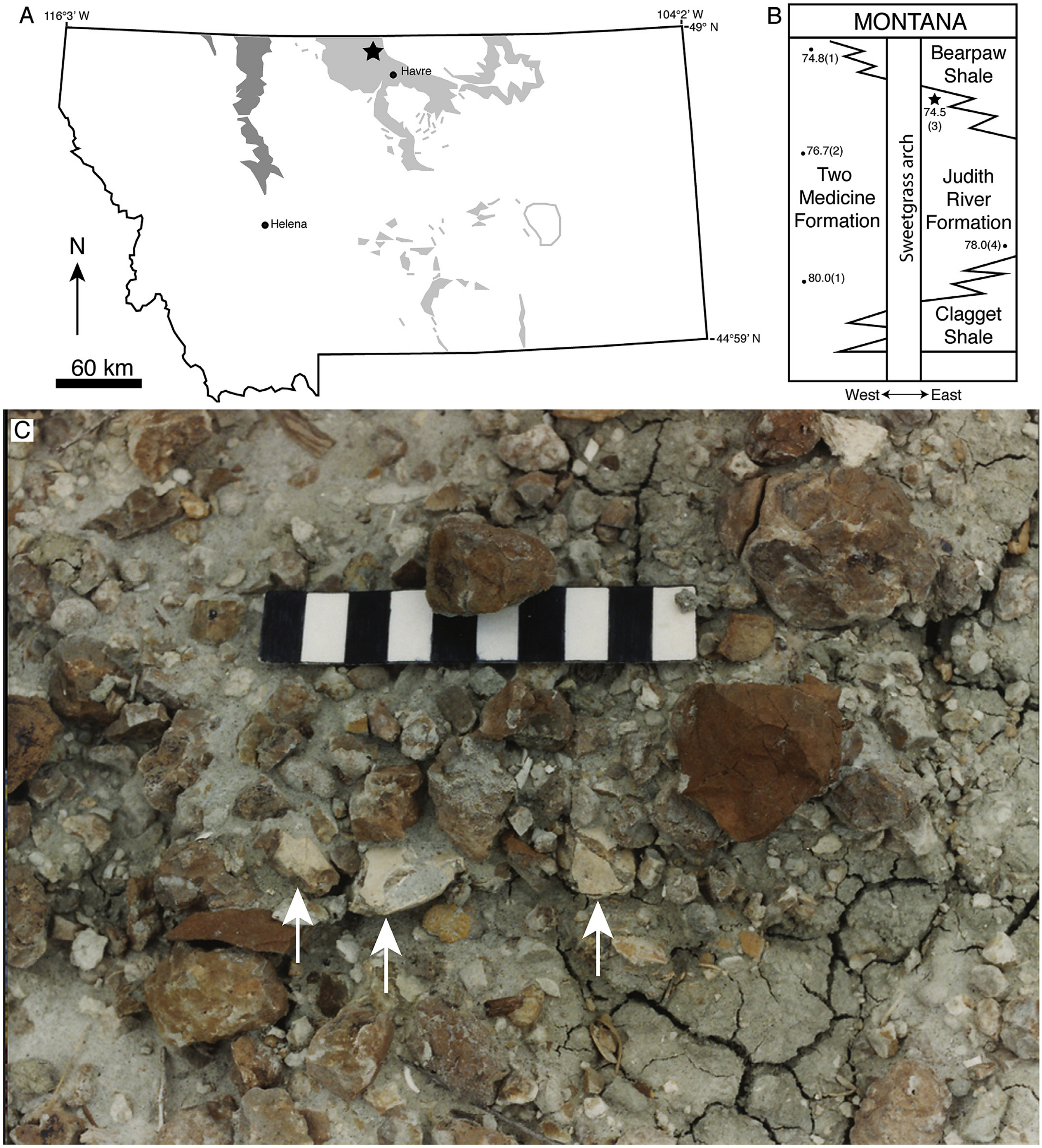

Fig. 1. The Campanian Judith River and Two Medicine formations and stratigraphically correlative units of Montana. **A**, Map of Montana showing exposures of the Judith River (light grey) and Two Medicine (dark grey) formations. Star represents the Egg White Site locality where MOR 710 was collected; **B**, Geologic cross section of north-central Montana showing the Two Medicine-Judith River clastic wedge with radiometric dates and references (modified from Jackson and Varricchio, 2010). Star represents the approximate location of the Egg White Site; **C**, Field photograph of the MOR 710 showing original site exposed in eroded concretionary layer (photo credit, V. Clouse). Arrows point to three of the eggs. Scale bar equals 10 cm. References: 1, Rogers et al. (1993); 2, Varricchio et al. (2010); 3, Eberth et al. (1992); 4, Goodwin and Deino (1989).

siltstone. These occurred in close association with one well-preserved egg of the same color and four highly crushed, orange eggs, which were found in situ within a sandy siltstone matrix. The similarity of preservation and in-filling sediment suggest all eggs came from the same horizon and originally represented a clutch. In addition, all eggs exhibit relatively intact eggshell on both sides and thus appear to be unhatched. We estimate egg mass as 21.5–32.5 g. The weathered condition of the material, however, prevents

Fig. 2. MOR 710A and C, two lithologically compressed eggs with normal eggshell structure. Scale bar equals 1 cm.

determination of the original number of eggs, their relative position to one another, and number of egg layers that once comprised the clutch.

Single-layered eggshell. The 660–760 μm thick eggshell is composed of a single layer of shell units; the latter average 135–240 μm wide, with a height-to-width ratio of 3.15:1–5.5:1 (Fig. 3A, B). Shell units consist of tightly interlocking acicular aragonite crystals that radiate from nucleation sites at the inner shell surface (Fig. 3C). These crystals feather out at their terminal ends and the shell units exhibit domed ornamentation on the external surface. Well-preserved craters, likely resulting from osteogenesis, occur at the base of some but not all shell units and measure 60 μm in diameter (Fig. 3B). A straight tube-like pore that measures 50 μm in diameter occurs between shell units and extends from the inner to the outer eggshell surfaces (Fig. 3A). Under cross-polarized light, the eggshell displays sweeping extinction. Remnants of 46–107 μm-thick shell membrane are preserved below the inner shell surface and consist of two layers, which also characterize extant turtle eggs (Fig. 3B; Hirsch, 1983).

Multilayered eggshell. MOR 710B includes two eggshell layers. Preservation within MOR 710B is highly variable. Some specimens display substantial diagenetic alteration, primarily dissolution of aragonite and reprecipitation of calcite (Fig. 3D). The inner and outer eggshells measure 500–540 μm and 340–380 μm, respectively, with a total thickness of 835–860 μm (Fig. 3E, F). The inner surface of the lower eggshell has a shattered appearance, with splinter-like fragments of eggshell randomly oriented within the secondary mineral matrix. The inner eggshell layer is typically thinner than the single layered eggshell described above, likely due to this alteration. Although sometimes slightly separated from the inner eggshell, the overlying eggshell conforms closely to the external surface of the underlying shell units. Two eggshell fragments from different areas of the egg show no evidence of nucleation sites between the inner and outer eggshell layers under SEM (Fig. 3E and unpublished image). With the exception of one or possibly two nuclei, radial thin section from different fragments also show an absence of nuclei (Fig. 3F). Shell units in the outer eggshell layer have a height-to-width ratio of 1.65:1, and their external surfaces are typically flatter and lack the domed shape of the underlying ones. No pores were observed in the outer eggshell, whereas this layer truncates a pore of the underlying eggshell in at least one area of the shell (Fig. 3E).

Cathodoluminescence imaging of MOR 710B reveals dull blue luminescence throughout the specimen except for small areas of non-luminescence and/or bright orange luminescence that occur primarily between shell units at the inner shell surface, in pores between shell units and within dissolution cavities (Fig. 3D).

Embryonic remains. At least five eggs contain embryonic remains, including the multilayered specimen (MOR 710B) described above. Although the state of embryonic preservation in MOR 710D prohibits a detailed description, we provide a brief anatomic and histologic assessment based on the available information from this egg.

Partial preparation of MOR 710D reveals well ossified and partially articulated embryonic skeletal elements that include the skull, lower jaw, fore and hindlimbs, as well as numerous unidentifiable bones (Fig. 4A, B). The skull and lower jaw are preserved in ventral view. Although, the premaxillae and maxillae are articulated, the remaining exposed cranial elements are disarticulated and unidentifiable. The triturating surfaces of the upper and lower jaws, as well as the palatal bones are not exposed. The lower jaw includes both dentaries, which exhibit a weakly sutured symphysis. The left forelimb preserves the manus and a possible radius, whereas the hindlimb elements include the right tibia, right fibula, as well as both right and left ungual phalanges. Numerous other unidentifiable postcranial elements are exposed on the partially prepped surface of the egg.

A histological thin section of a costal element (MOR 710:EB 3) shows that the embryonic bone consists of primary woven bone with numerous osteocyte lacunae and bony trabeculae that radiate from the medullary cavity and surround large vascular canals (Fig. 4C, D). There is no evidence of compact bone or bone remodeling. Bone growth dominates in the anteroposterior direction.

5. Discussion

The needle-like aragonite crystals allow definitive assignment of MOR 710 to Testudines (Hirsch, 1983). The generally well-preserved aragonite structure shows some dissolution and calcite replacement, with small areas of bright orange luminescence and areas of non-luminescent. Bright orange luminescence is due to Mn^{2+} incorporation into the calcite crystal (Marshall, 1988), which is generally linked to elevated Mn^{2+} concentrations (Wendler et al., 2012). Areas of non-luminescence may result from increased Fe^{2+}, a main quencher element of CL in carbonates (Barbin, 2000; Boggs and Krinsley, 2006; Götte and Richter, 2009), or possibly because Mn^{2+} levels are below the detection limits (Barbin, 2000; Wendler et al., 2012). Some non-luminescent areas in the eggshell may represent open space in fractures that are unfilled by calcite.

In contrast to these small bright orange and non-luminescent areas, MOR 710B primarily exhibits a dull blue color, similar to CL imaging of extant and recent fossil turtle eggshell (Lawver and Jackson, in press; Lawver, unpublished data). Blue luminescence is thought to occur in the absence of activator elements (Wendler et al., 2012) and has been detected in the aragonite shells of *Nautilus* (Cephalopoda) that are only minimally affected by diagenetic overprint (Barbin et al., 1995). Blue luminescence in MOR 710B also suggests minimal diagenetic recrystallization of the aragonite eggshell.

5.1. Parataxonomy

Hirsch's (1996) parataxonomic classification for fossil turtle eggs and eggshells includes two oofamilies, Testudoflexoolithidae and Testudoolithidae. Flexible eggs with loosely abutting shell units comprise the former, whereas the latter includes rigid eggs with tightly packed shell units. The rigid structure of the eggshell excludes MOR 710 from Testudoflexoolithidae (Table 1).

Among oospecies assigned to Testudoolithidae, MOR 710 differs from *Chelonoolithus braemi* Kohring, 1998 and *Haininchelys curiosa* Schleich et al., 1988 in its thicker eggshell and a shell unit height-to-

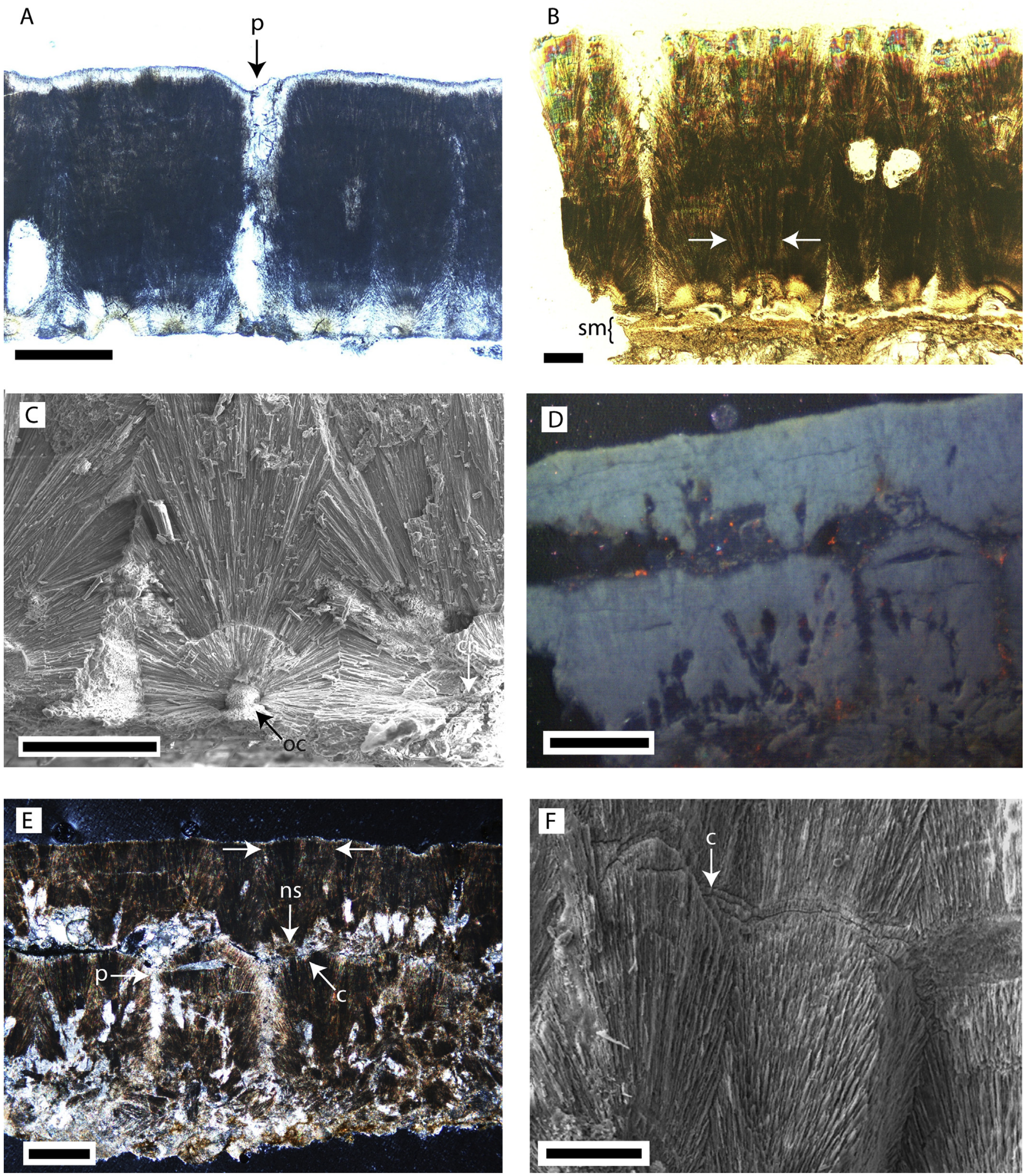

Fig. 3. Radial thin sections and SEM images of MOR 710 eggshell. **A**, MOR 710A showing straight tube-like pore. Inner and outer shell surfaces are at the top and bottom of image, respectively. Scale bar equals 250 μm; **B**, MOR 710:ES 1 under cross-polarized light. Note the closely packed, narrow shell units (between horizontal arrows), acicular aragonite crystals, and craters at the inner shell surface. Scale bar equals 100 μm; **C**, Radial SEM from MOR 710B with preservation of the former organic core. Scale bar equals 90 μm; **D,** Eggshell from MOR 710B under cathodoluminescence showing dull blue luminescence, with bright orange and non-luminescent areas representing alteration associated with secondary calcite and open fractures, respectively. Scale bar equals 250 μm; **E**, MOR 710B under cross-polarized light showing multilayers comprising the eggshell. Note the close contact of the two eggshells and a rare nucleation site (ns) between layers. Shattered structure and secondary replacement on the left decreases apparent shell thickness. Scale bar equals 250 μm; **F**, SEM micrograph of multilayered eggshell from MOR 710B etched with hydrochloric acid. Note absence of nuclei and close confirmation of inner and outer eggshell layers. Panel F is reprinted from Cretaceous Research, Volume 29, Jackson and Schmitt, 2008; and Scanning, Volume 24, Jackson et al., 2002. Scale bar equals 10 μm. **Abbreviations**; **c**, contact; **cn**, cratered nuclei; **ns**, nucleation site; **oc**, organic core; **p**, pore; **sm**, shell membrane. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

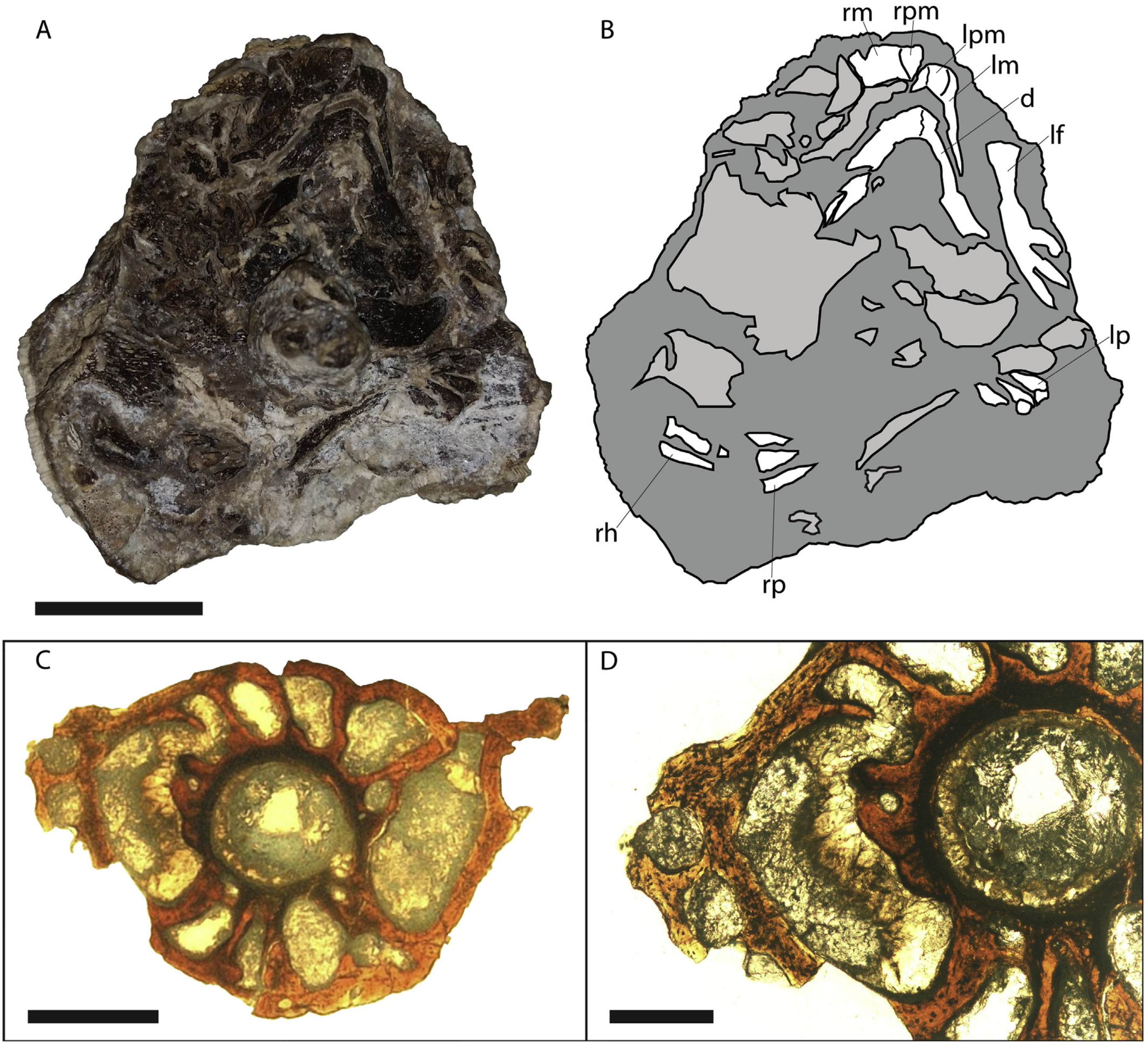

Fig. 4. MOR 710D embryo. **A**, Partially prepared egg showing exposed embryonic skeletal elements. Scale bar equals 1 cm; **B**, Line drawing of A. White represents identifiable skeletal elements, whereas light grey areas are unidentifiable elements; **C**, Histological thin section (MOR 710:EB 3) of a costal element showing the medullary cavity and vascular canals infilled with secondary calcite crystals, as well as trabeculae growing in the posteroanterior direction. Scale bar equals 0.5 mm; **D**, Enlargement of C showing numerous randomly arranged osteocyte lacunae. Scale bar equals 0.25 mm. **Abbreviations**: **d**, dentary; **lf**, left forelimb; **lm**, left maxilla; **lp**, left pes; **lpm**, left premaxilla; **rh**, right hindlimb; **rm**, right maxilla; **rp**, right pes; **rpm**, right premaxilla.

width ratio of 3.15:1–5.5:1. MOR 710 can be distinguished from *Emydoolithus laiyangensis* Wang et al., 2013 in overall eggs shape, thicker eggshell, and a greater height-to-width ratio. Thicker eggshell also distinguishes MOR 710 from *Testudoolithus hirschi* Kohring, 1999, and *T. rigidus* Hirsch, 1996. MOR 710 is similar to *Testudoolithus jiangi* Jackson et al., 2008 (Fang et al., 2003) and the eggs fall within the ranges of egg size and eggshell thickness of this ootaxon; however, MOR 710 has a greater shell unit height-to-width ratio.

5.2. Taxonomy

MOR 710 could potentially represent the eggs of one of several turtle taxa known from the Judith River Formation: *Axestemys splendida*, *Aspideretoides* sp., *Plastomenus* sp., *Basilemys* sp., *Neurankylus eximius*, *Plesiobaena antiqua*, and *Adocus* sp. (Sahni, 1972; Vitek, 2012; Joyce and Bourque, 2016). The trionychids *Axestemys splendida* and *Plastomenus* sp. seem unlikely candidates because extant trionychid eggs differ from MOR 710 in their thinner eggshell (0.1–0.2 mm), smaller height-to-width ratio, and lack of domed external surfaces (Fig. 5). A gravid *Basilemys variolosa* from the stratigraphically correlative Dinosaur Park Formation in Alberta, Canada could potentially provide identification; however, Braman and Brinkman (2009) failed to describe the eggshell microstructure, thereby precluding comparison to MOR 710. The eggs of *N. eximius* and *P. antiqua* are currently unknown, although *N. eximius* may have been large enough to produce a similar sized clutch as MOR 710. Taxa from the Judith River Group of Alberta include *Judithemys sukhanovi* and *Boremys pulchra*; however, eggs of these taxa are also currently unknown. Additionally, *J. sukhanovi* may have been capable of producing a clutch as large as MOR 710.

The eggshell microstructure of most eggs within MOR 710 is most similar to eggs preserved within a gravid *Adocus* sp. and a fossil egg clutch from Alberta, Canada (Zelenitsky et al., 2008). Similarities include thick eggshell, with shell units taller than wide, and a domed external surface (see Table 2). In her unpublished master's thesis Zelenitsky (1995) named these *Adocus* sp. eggs *Testudoolithus magnirigidus*; however, this name does not meet the criteria of the International Committee on Zoological Nomenclature (Article 8.1.3) that requires simultaneously obtainable copies and wide distribution. Consequently, this oospecies is a nomen nudum (Lawver and Jackson, 2014) and we name the new ootaxon *Testudoolithus zelenitskyae*. A significant amount of homoplasy occurs in extant turtle eggshells (Winkler, 2006; Lawver, 2012) and, therefore, *Testudoolithus zelenitskyae* oosp. nov. could potentially belong to another, as yet undescribed extinct taxa of close phylogenetic affinity to *Adocus*. Therefore, we assign the eggs to parataxonomy for better comparison with other egg types as well as allowing for the possibility of homoplastic morphology shared among multiple taxa.

5.3. *Embryonic remains*

The embryonic remains provide only limited information. Nevertheless, some morphological and histological comparisons are possible with comparison to the extant trionychid *Apalone spinifera*. For example, preservation of anteroposteriorly projecting bony trabeculae of the costal elements (Fig. 4C, D) coincides with the initial expansion of this element in order to form the costal plates of the carapace. In *A. spinifera* this expansion begins no later than Stage 23 (Sheil, 2003), thus suggesting that the fossil embryos obtained 23 of 25 embryonic developmental stages prior to their demise. Additionally, embryos of the more distantly related taxa, *Chelydra serpentina*, *Macrochelys temminckii*, and *Phrynops hilarii* also possess well-developed phalanges at stage 23 (Sheil, 2005; Sheil and Greenbaum, 2005; Bona and Alcalde, 2009). Finally, the well-ossified embryonic bones that characterize all eggs within

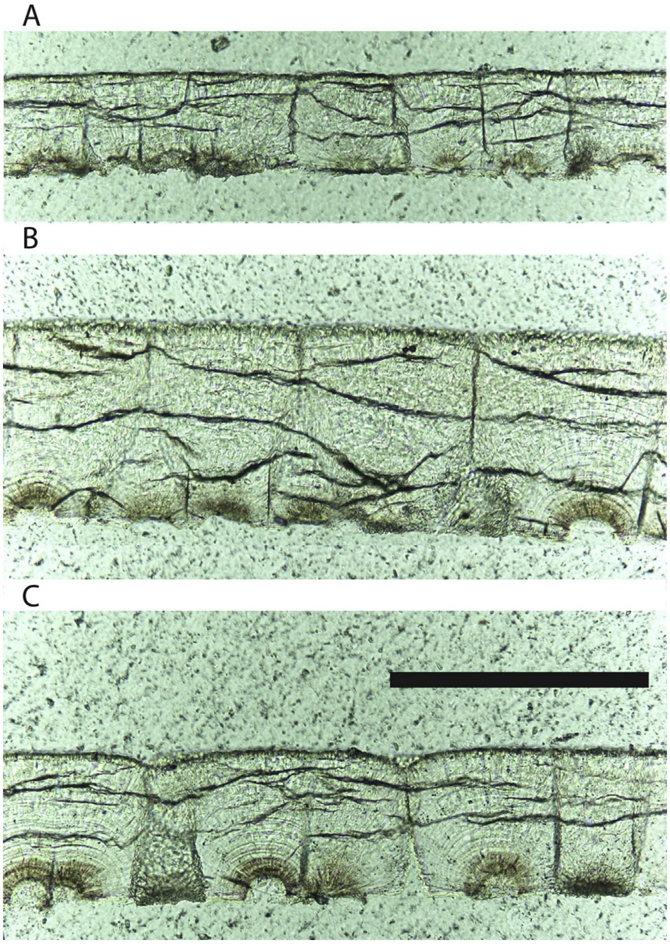

Fig. 5. Radial thin sections of extant trionychid eggshell. **A**, *Apalone mutica* eggshell (ES 203); **B**, *Apalone ferox* eggshell (ES 204); **C**, *Apalone spinifera* eggshell (ES 205). Scale bar equals 250 μm.

Table 1
List of turtle ootaxa and their distinguishing characteristics.

Ootaxon	Holotype: Material	Geographic and temporal distribution	Egg shape	Length × width (mm)	Eggshell thickness (mm)	Shell unit (height:width)
Testudoflexoolithus agassizi[1]	MCZ 2810/HEC 49: Eggshell fragments	Florida, USA; Pleistocene	–	–	0.06–0.1	1:1 or 2:3
Testudoflexoolithus bathonicae[1,2]	MB(NH)37983/HEC 186: An egg imbedded in matrix	England; Bathonian, Middle Jurassic	Ellipsoidal	48 × 26	0.2–0.25	1:1
Chelonoolithus braemi[3]	Guimarota 98-2: Eggshell fragments	Portugal; Kimmeridgian, Upper Jurassic	–	–	0.2	1:1
Emydoolithus laiyangensis[4]	IVPP V18544: A nearly complete egg	Shandong Province, China; Upper Cretaceous	Elongate	91 × 22	0.4–0.5	2:1 to 5:1
Haininchelys curiosa[5]	-: Eggshell fragments	Belgium; Upper Paleocene	–	–	0.25–0.3	1.2:1 to 2.3:1
Testudoolithus hirschi[6]	-: Eggshell fragments	Portugal; Kimmeridgian, Upper Jurassic	–	–	0.15	3:1
Testudoolithus jiangi[7,8]	ZMNH M8713: A clutch of 23 eggs	Zhejiang Province, China; Albian, Early Cretaceous	Spherical	35–52	0.7–1.0	2.5:1 to 3:1
Testudoolithus rigidus[1]	UCM 55806/HEC 425: Half of an egg	U.S.A., Europe, Africa; Lower Cretaceous - Pliocene	Spheroidal	42 × 47	0.22–0.24	2:1
Meiolania platyceps eggs[9]	AM F82183: A clutch of at least 10 eggs	Lord Howe Island, Australia; Pleistocene	Spherical	53.9	0.8	1.2:1
Testudoolithus zelenitskyae	MOR 710: A clutch of at least 16 eggs	U.S.A. and Canada; Campanian, Upper Cretaceous	Spherical	34–39	660–760	3.15:1 to 5.5:1

Modified from Lawver and Jackson (2014).
References: 1, Hirsch (1996); 2, Buckman (1859); 3, Kohring (1998); 4, Wang et al. (2013); 5, Schleich et al. (1988); 6, Kohring (1999); 7, Fang et al. (2003); 8, Jackson et al. (2008); 9, Lawver and Jackson (in press).

MOR 710 also suggest that termination of embryonic development occurred close to hatching.

5.4. *Multilayered eggshell*

The multiple eggshell layers of MOR 710B closely resembles a pathological condition reported in extant turtle eggs, whereby one or more additional eggshell layer is deposited over the original egg (Erben et al., 1979; Ewert et al., 1984; Jackson and Varricchio, 2003). However, the presence of an embryo in an advanced stage of development in a pathological egg is surprising. Here, we discuss alternative explanations that may account for this unusual occurrence.

First, the additional layer is biological in origin. This multilayered condition is relatively common in extant turtles, currently described for at least nine taxa (Jackson et al., 2004 and reference therein). The additional shell layers result from prolonged retention of one or more eggs in the uterus due to physiological or environmental stresses (Erben et al., 1979; Ewert et al., 1984). The abnormal layer(s) often block the pore canals of the underlying eggshell, and termination of embryonic development often results from asphyxiation. However, in at least one case an extant turtle embryo from a multilayered egg survived to hatching and died shortly thereafter (personal communication, M. Ewert, May, 2001, Bloomington, IN). If the outer eggshell in MOR 710B resulted from egg retention, the pores in the inner and outer eggshells may have aligned and extended through both layers in some areas of the egg, permitting limited but potentially adequate embryonic gas exchange during embryonic development. For example, Ewert et al. (1984) described a pore in an abnormal *Rhinoclemmys areolata* eggshell that extended straight through three superimposed layers.

A phenomenon known as egg capping offers an alternative explanation for the second layer in MOR 710B. Although rare in avian species (≤1%), a portion of a hatched egg occasionally “caps” an adjacent, unhatched egg (Derrickson and Warkentin, 1991; Verbeek, 1996). If this shell fragment is not removed by the adult, the embryo may suffocate. Derrickson and Warkentin (1991) speculated that egg capping might occur more often in clutches with greater hatching asynchrony and egg size variability. Hauber (2003), however, felt that this was not feasible to address because of the lack of published comparative data. Further, Hauber (2003) reports that no eggs in his study (0%) were covered by eggshell from conspecific eggs, whereas egg capping occurred in 33% of eggs from parasitized nests. The parasitic eggs were larger and hatched earlier than the host eggs, thereby increasing the potential for egg capping.

We interpret the multiple layers in MOR 710B as an abnormality for the following reasons. 1) Egg capping is unreported in extant turtles and other reptile eggs, likely because of their incubation in a substrate or vegetation mound, rather than in open nests characteristic of birds. 2) The inner surface of the outer layer of MOR 710B follows the contour of the underlying shell units (Fig. 3E, F) and its outer surface lacks the domed shape of normal eggshell. 3) None of the eggs in the turtle clutch appear hatched; to the contrary, the eggs contain well-formed embryos that suggest an advanced stage and synchronous embryonic development; missing eggshell most likely resulted from recent weathering. 4) Most importantly, the base of the shell units in the outer eggshell layer lack nucleation sites (Fig. 3E, F). We identified only one or possibly two nuclei in samples viewed in thin section (Fig 3E) and none under SEM. If the second layer in MOR 710B represented a fragment from a previously hatched egg one would expect nucleation sites at the base of each shell unit. These are clearly absent in most areas of the eggshell (Fig. 3E).

As a final note, in extant multilayered eggs, shell membrane may or may not occur between the inner and outer eggshell layers (Ewert et al., 1984). This variation also occurs in Mesozoic multilayered eggshells (Jackson et al., 2008). If present in MOR 710B, decay of the membrane may have provided a conduit for ground water, thus contributing to more extensive recrystallization and precipitation of calcite between the layers in some areas of the egg (Fig. 3E).

5.5. *Ecological inferences*

The size, shape, and estimated mass (21.5–32.5 g) of eggs preserved in MOR 710 allow anatomical and ecological interpretations. Extant turtles that lay spherical eggs are generally of large body size (Elgar and Heaphy, 1989) and have large clutches because this shape permits simultaneously shelling of more eggs in the uterus prior to oviposition (Ewert, 1979; Iverson and Ewert, 1991). We estimate carapace length for the gravid female turtle that produced MOR 710 as 35.0–54.4 cm using the positive correlation between egg mass and adult carapace length (Elgar and Heaphy, 1989). However, it is worth noting that rigid-shelled turtle eggs have a density of 1.126, whereas birds have a density of 1.03–1.09 (Iverson and Ewert, 1991). This indicates that the egg mass and carapace lengths calculated here represent minimum estimates. These egg characteristics (i.e., size, shape, mass) suggest that MOR 710 was produced by a large bodied taxon. This inference is further supported by carapace length estimates of the gravid *Adocus* sp. specimens from Alberta and Utah (Zelenitsky et al., 2008; Knell et al., 2011). The 40.5 cm long Alberta adult preserves at least five eggs, with a diameter of approximately 35–40 mm (Zelenitsky et al., 2008). Likewise the 50–55 cm-long gravid adult from Utah preserves at least two eggs with diameter of 35 mm. Both gravid specimens (e.g., Zelenitsky et al., 2008; Knell et al., 2011) are estimated to have originally contained more eggs than observed.

Table 2
Egg/eggshell characteristics of *Testudoolithus zelenitskyae* oosp. nov. and *Adocus* sp. specimens discussed in the text.

Specimen	Material	Formation	Egg shape	Length × width (mm)	Eggshell thickness (mm)	Shell unit (height:width)
MOR 710	Weather clutch of at least 16 eggs	Judith River Formation	Spherical	34–39	0.66–0.76	3.15:1–5.5:1
NHMU 16868 (LBA-06-7)[1]	Gravid *Adocus* sp. preserving at least two eggs	Kaiparowits Formation	Spherical	35	0.25–0.28	2.5:1
TMP 1999.63.2[2]	Gravid *Adocus* sp. preserving at least five eggs	Oldman Formation	Spherical	35–40	0.73–0.81	2.5:1
TMP 2008.27.1[2]	Clutch of at least 26 eggs	Dinosaur Park Formation	Spherical	40 × 42 40 × 43	0.5–0.65	3.5:1

Note: All specimens are Late Cretaceous, Campanian in age.
References: 1, Knell et al. (2011); 2, Zelenitsky et al. (2008).

6. Conclusions

MOR 710 comes from the Campanian (Upper Cretaceous) Judith River Formation of north-central Montana and consists of a weathered accumulation of eggs interpreted as remnants of a clutch originally composed of at least 16 spherical turtle eggs, at least one of which displays an additional, abnormal eggshell layer. Normal eggs exhibit a 660–760 μm thick eggshell comprised of a single layer of tightly interlocking aragonite shell units with a height-to-width ratio of 3.15:1–5.5:1. In contrast, the abnormal eggshell includes 500–540 μm and 340–380 μm-thick inner and pathological shell layers, respectively. The pathological layer likely represents a retained egg from the same or different nesting attempt due to physiological or environmental stress experienced by the female turtle. When compared to the extant trionychid, *Apalone spinifera*, the embryonic bones in at least five eggs (including the multilayered specimen) demonstrate a late stage of development. This indicates that these embryos were close to hatching when death occurred. The similarity of the eggshell microstructure between the Judith River eggs and that of *Adocus* sp. (Zelenitsky et al., 2008; Knell et al., 2011) suggests that this taxon might have produced MOR 710. Further descriptions of taxonomically identifiable fossil turtle eggs, such as those from the Canadian gravid *Basilemys variolosa* specimen, will help support or refute this identification.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cretres.2016.08.012.