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Investigating nests and eggs of modern and fossil amniotes

by

Ashley Lynn Ferguson

A dissertation

submitted in partial fulfillment

of the requirements for the degree of

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To the Graduate Faculty:

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## Investigating nests and eggs of modern and fossil amniotes Dissertation Abstract – Idaho State University (2022)

Nests and eggs represent the beginning of life for many vertebrates. Determining the nesting strategies of extant amniotes is crucial in elucidating the evolution and diversification of reproductive traits as nesting materials are poorly preserved in the fossil record. Avian and chelonian nests are particularly rare compared to non-avian dinosaurs.

The goal of this dissertation is to investigate and describe the sedimentology, taphonomy, and ecology of two fossil nesting localities and examine modern eggshell porosity via micro-CT images. I characterized two nesting localities, one from the Late Cretaceous Kaiparowits Formation in Grand Staircase-Escalante National Monument outside of Escalante, Utah, the second in the Eocene Bridger Formation east of Lyman, Wyoming, in terms of sedimentology and stratigraphy and taphonomy.

Eggshell from the Kaiparowits Formation is identified as testudine and unique characteristics of the shell unit height-to-width ratio, egg size, eggshell thickness, and ornamentation warrant the naming of a new ootaxa, *Testudoolithus tuberi*. The distribution of eggshell is interpreted as resulting from nest predation. The Bridger Formation eggshell material is similar since the distribution and preservation of half eggs is interpreted as being caused by predation. Both of the nesting localities are imbedded in greenish-gray mudstones overlying and under lithic sandstones, suggesting flood-plain deposition. The avian eggshell is named a new ootaxon as well, (*Doolithus bridgerensis*), from the number of observable ultrastructural layers.

Micro-CT images of modern rigid-shelled amniotes, including birds, crocodylians, a tortoise, and a gecko, reveals a complex network of internal pores that do not connect to the external surface of the eggshell. The functional pores tend to be cylindrical to trumpet-shaped in birds and pear-shaped in the tortoise, but are bowl-shaped in the gecko, conical in the

Х

crocodylian genera *Crocodylus* and *Osteolaemus*, and globular in *Melanosuchus niger*. Eggs in open and closed nesting strategies have generally similar functional eggshell porosity ranges of 0.1-0.8% sample volume. However, covered nesters generally have higher total porosities of 2.9-16.1%. The internal porosity arises from the interstitial spaces between mineralized egg units. The functional value of the internal porosity is yet unknown, but may improve properties of shell insulation and retention of water vapor.

Keywords: Kaiparowits, Bridger, eggs, taphonomy, porosity, nesting

# Chapter 1: Rare clutch of Cretaceous turtle eggs preserved in the Kaiparowits Formation of southern Utah

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### 1.1. Abstract

Although turtles are common Mesozoic fossils, their eggs and nests are rare. Here, we describe an in-situ clutch of turtle eggs from the Upper Cretaceous Kaiparowits Formation of southern Utah. The clutch is preserved in a green mudstone associated with aquatic terrestrial gastropods. Eggshell is broadly distributed across an area of  $3.75 \text{ m}^2$  with three nearly complete, spherical eggs ~2.9 cm in diameter, and another three partial eggs identified. The aragonitic eggshell is 0.7-1.2 mm thick and consists of closely packed, slightly domed shell units with a height to width ratio of 3.7:1 and nodular ornamentation  $53-71 \mu \text{m}$  in diameter. Eggshell orientation (concave up / concave down) of 54:46 is consistent with in-situ preservation. An eggshell porosity of 42.5 mm correlates to a humid nesting environment. Extrapolating from egg size, the producing adult carapace length is estimated at 24.45 cm with an average clutch size of 9.91 eggs. The unique attributes of the eggshell warrant naming of a new oospecies, *Testudoolithus tuberi*. This fossil occurrence is another example of exceptional preservation in the Western Interior Basin associated with the Campanian "taphozone."

Keywords: Ootaxonomy, Testudines, Taphonomy, Eggshell orientation, Predation, Volcanism

## **1.2. Introduction**

Nests, egg clutches, and gravid adults in the fossil record are relatively rare, yet provide a glimpse into the reproductive, developmental, and growth strategies of extinct taxa by preserving a narrow temporal window into an animal's reproductive cycle within a sedimentary sequence (Lawver and Jackson, 2017). Among amniotes, the most commonly preserved fossil nesting sites with egg material are from non-avian dinosaurs (Carpenter, 1999; Deeming, 2016; Varricchio and Jackson, 2016). By contrast, fossil turtle nests are rare, with only five autochthonous nests documented in the world (Lawver and Jackson, 2014, 2017; Jackson et al., 2018). Turtle eggs are distinctive among amniotes in that they are composed of aragonite, whereas all other amniote groups lay eggs made of calcite (Hirsch, 1983). Turtle eggs also have a distinct microstructure of



**Figure 1.1** — Comparison between the global number of genera of terrestrial taxa and the total amount of described turtle nests and eggs. Black dots represent a described ootaxon. Dashed line approximates the rate of new genera from 30 Ma to the Holocene. Modified from Cleary et al., 2020.

shell units with an organic core, where aragonite crystals radiate and an underlying shell membrane is visible in thin section (Hirsch, 1983). The unique composition of turtle eggs not only makes their identification easier, but the typically poorer preservation potential of aragonite versus calcite in continental settings might explain why turtle eggs are infrequently observed as fossils (Hirsch, 1996; Lawver and Jackson, 2014).

Considering the rise of turtle diversity through the Mesozoic-Cenozoic eras (Cleary et al., 2020), it is somewhat surprising that so few turtle eggs are recorded in the literature (Fig 1.1). In addition to four known Mesozoic turtle nests, four gravid turtles and an individual egg with an embryo are described from the Jurassic and Cretaceous strata. This includes an in situ clutch containing 23 spherical eggs from Albian (Lower Cretaceous) age rocks of the Tiantai Basin of Zhejiang, China (Jackson et al., 2008), a clutch of 26 eggs from the Campanian (Upper Cretaceous) Oldman Formation of Alberta, Canada (Zelenitsky et al., 2008), a clutch of 16 eggs with embryos from the Campanian (Upper Cretaceous) Judith River Formation of Montana (Lawver and Jackson, 2017), and a clutch of 15 eggs from the Upper Cretaceous Xiaguan Formation of Henan Province, China (Jackson et al., 2018). Gravid turtles are ideal when no embryonic material is preserved because they provide definitive identification of eggs to a particular taxon. Zelenitsky et al. (2008) described a gravid turtle from the Dinosaur Park Formation in southeastern Alberta and refer to both the turtle and the previously described clutch from the Oldman Formation as Adocus sp. Another gravid Adocus sp. specimen containing two eggs has been described from the Campanian (Upper Cretaceous) Kaiparowits Formation of southern Utah (Knell et al., 2011). A gravid Basilemys variolosa specimen from the Dinosaur Park Formation of Alberta has also been found, but not fully described (Lawver and Jackson, 2017). In addition, an individual egg from the Xiaguan Formation of China contained an embryo

from the Nanhsiungchelyidae family (Ke et al., 2021). Lastly, spherical objects within a eurysternid specimen from the Solnhofen in Germany (Upper Jurassic) have been interpreted as altered eggs (Joyce and Zelenitsky, 2002). Of all the clutches described, only one potentially showed signs of hatching (Jackson et al., 2018).

Here we describe a new occurrence of turtle eggs from the Late Cretaceous of Utah, which taphonomic evidence points to as an in-situ clutch. It was discovered within the middle unit of the Kaiparowits Formation, providing the latest example of exceptional preservation in the Campanian Western Interior Basin.

#### **1.3. Geologic setting**

The egg site is from the Kaiparowits Formation located in the Grand Staircase-Escalante National Monument in southern Utah (Fig 1.2). Situated in the Cordilleran Foreland Basin and the southern portion of the Western Interior Basin (WIB), the Kaiparowits Basin is along the western margin of the Western Interior Seaway, recording a complicated relationship between eustasy, tectonics, and sedimentation rates during the deposition of marine and terrestrial sediments (DeCelles and Giles, 1996). The extremely high abundance of terrestrial flora and fauna throughout the basin, well constrained radiometric dates, and its correlation with other stratigraphic units as far north as Alberta, make it an ideal location to investigate long-standing questions about continental-scale biogeography and diversity trends in the Upper Cretaceous (Roberts et al., 2005; Titus et al., 2005; Gates et al., 2010).

The Kaiparowits Formation is 1,005 m thick and was deposited in ~4 m.y. (Beveridge et al., 2020) and is composed of mudstones and sandstones deposited in a myriad of wet, subhumid channel, overbank, and lacustrine depositional environments. Because of this rapid

sedimentation, the preservation of the terrestrial vertebrates, invertebrates, plants, and microfossils is extremely high (Roberts et al., 2013). In addition, the presence of several bentonites throughout the formation, dating it to ~76.6-72.8 Ma, using maximum depositional age from detrital zircons and <sup>40</sup>Ar/<sup>39</sup>Ar on sanidine, suggests that the Kaiparowits Formation is coeval to other important fossiliferous Campanian formations throughout the WIB, such as the Dinosaur Park Formation, and sections of the Judith River, Two Medicine, Fruitland, and Aguja formations (Titus et al., 2005; Roberts et al., 2005, 2013; Beveridge et al., 2020).

The strata of the Kaiparowits Formation are dominantly mudstone and sandstone that weather into badland topography. The three lower informal members of the Kaiparowits



**Figure 1.2** — Extent of the Kaiparowits Formation within Grand Staircase-Escalante National Monument. The star represents the approximate location of the field area.

Formation are distinguished by sand:mud ratios. Both the lower and upper members have high channel/overbank ratios, while the middle member has a low channel/overbank ratio (Roberts, 2007). The lower member is sourced from the Mogollan Highlands with longitudinal flow to the northeast (Jinnah et al., 2009). The base of the middle member has tidally influenced sediments, and the upper portion of the middle member is the most fossiliferous section of terrestrial vertebrates in the formation (Roberts, 2007). These informal members accumulated extremely quickly, ~42 cm/ka, representing one of the fastest accumulations rates in the world (Roberts, 2007; Roberts et al., 2013). The Upper Valley Member consists of volcaniclastic, massive, muddy sandstones and sandy, bentonitic and organic-rich mudstones with a higher proportion of sandstone than mudstone at a 3:2 ratio (Beveridge et al., 2020). The deposits in the Kaiparowits Formation were deposited in a low-relief, inland alluvial plain setting, with paleosols, oxbow ponds, small lakes, and large channels suggesting a wet alluvial system.

#### 1.4. Methods

#### 1.4.1. Collection Methods

The specimen was initially discovered in 2009 and is housed at the Idaho Museum of Natural History (IMNH 2413/51224). Collection of the material included saturating the eggshell with Vinac and carefully jacketing sections of concentrated shell. The site was checked again in subsequent years to determine if any new material had become exposed via erosion. The specimens were then prepped from the bottom using standard hand tools at the IMNH.

A detailed decimeter-scale stratigraphic column of 42 m was constructed using a Jacobs Staff and Brunton compass. The section began at a cliff-forming sandstone unit and ended approximately parallel to the previously measured Kaiparowits Blues Ceratopsian (KBC)

measured section (Roberts, 2007) at a ridge-forming sandstone at a strike and dip of  $132^{\circ}/6.5^{\circ}$ . Detailed descriptions of each of the units were done in the field. Field mapping of the site consisted of a 1 m<sup>2</sup> grid separated into 10 cm intervals. The location of collected eggs was marked and surface and excavated eggshell fragments were tallied and their orientations recorded [i.e., concave up (CU), concave down (CD), or unknown (UK)]. Eggshell fragments designated as unknown indicate that the angle of the eggshell exceeded 45° to the horizontal plane or the fragments were disturbed during excavation. Tallies included only eggshell fragments measuring 1 cm<sup>2</sup> or greater in size. Eggshell counts were then compared to a null hypothesis of a 50:50 CU:CD ratio using a chi-square test with Yates's correction. Yates's correction is used here to accommodate the 2 × 2 contingency table and relatively small sample size (Ury and Fleiss, 1980; Yates, 1984).

#### 1.4.2. Sampling Methods and Calculations

A fragment of loose eggshell was removed from the site and prepared at Wagner Petrographic as a standard 30 µm thick petrographic radial thin section and viewed under a Leica MC 170 HD microscope. An additional fragment was mounted onto aluminum stubs, coated with 10 nm of gold, and imaged with a FEI Quanta 600 FEG scanning electron microscope (SEM) at the University of Utah Surface Analysis Lab. ImageJ software was used to measure structural features (Schneider et al., 2012).

Eggshell mineral composition was determined by X-ray powder diffractometry on a Bruker D2 PHASER X-ray diffractometer using Cu K $\alpha$  radiation. After cleaning eggshell fragments in an ultrasonic bath, the fragments were dried in an oven at 70°C overnight. The fragments were powdered to a uniform grain size using a quartz mortar and pestle. The powder was sprinkled on a 1" diameter glass base mount. The analysis yielded well defined ( $\geq$  2-sigma

above background) major diffraction lines between 20° to 50° 2θ. Data were analyzed using Bruker EVA search-match software and the Crystallography Open Database (COD) (Vaitkus et al., 2021).

The number of eggs laid and the carapace length of the female can be estimated based off the correlation of carapace length and egg mass in extant turtles (Elgar and Heaphy, 1989). The mass of the female turtle was calculated using Blueweiss et al., 1978 equation:

$$E = 0.41 W^{0.42}$$

where E is the individual egg mass and W is the maternal body weight. This regression includes turtles and other reptiles, which produces a conservative estimate of maternal mass. Carapace length of the gravid turtle that produced IMNH 2713/51224 was calculated using the positive correlation between egg mass and adult carapace length via the regression line from Elgar and Heaphy, 1989:

$$\ln(E) = 0.84 \ln(C_1) - 1.95$$

where E is the egg mass and  $C_1$  is the carapace length. In addition to the carapace length, Elgar and Heaphy, 1989 provide an equation to calculate the number of eggs in a clutch where  $C_s$  is the clutch size and  $C_1$  is the carapace length.

$$\ln(C_s) = 1.57\ln(C_l) - 6.34$$

Assuming a density of 1.13 g/cm<sup>3</sup> for rigid shelled turtle eggs (Lawver, 2017), the mass of an individual egg is calculated from the volume of a sphere. Eggshell porosity was determined by taking the total pore area of an egg ( $A_p$ , mm<sup>2</sup>) and dividing it by eggshell thickness ( $L_s$ , mm) (Tanaka et al. 2015). Total pore area is calculated by the following equation:

$$A_p = A \cdot A_s \cdot D$$

where A is the individual pore area,  $A_s$  is the surface area of the egg, and D is the pore density (Seymour, 1979). Average values for each variable were used to account for variation in the eggshell fragments.

## 1.5. Results

The mudstone unit containing the eggs and eggshell is over 10 m thick and varies in color from greenish-gray, tan, and darker grays. The unit overlies an ~ 2 m trough cross-bedded lithic to feldspatholithic arenite with indurated ~20-30 cm oblong to tube-like structures scattered throughout. These structures are often weathered a dark brown to dark red color compared to the surrounding light tan sandstone and act as paleodrainage indicators. A finer sandstone of similar composition and structure as the previous sandstone overlies the mudstone facies. Two horizons of reworked bentonitic mudstones were noted in the section as well (Fig 1.3) above the locality.

The eggshell material is embedded in a greenish-gray carbonaceous mudstone with evidence of poorly developed soil features such as slickensides and caliche nodules. Obscure rhizoliths occur on faint horizons with gypsum spar. Invertebrate fossils, mainly gastropods, are common with 299 mostly complete gastropods counted within the specimen jackets. Original shell material is preserved on the gastropods and at least five different taxa are represented (Tapanila and Roberts, 2013; Fig 1.4) such as *Viviparus* sp., *Lioplacodes subtortuosa, Lioplacodes* sp., *Planorbis chacoensis*, and a stylommatophoran. Besides the stylommatophoran, the gastropods are aquatic (Yen, 1947). While a majority of the gastropod material is fractured from gleying and pedogenic features, most are still identifiable. In addition to the invertebrate material, an ornithischian and tyrannosaur tooth, as well as a centrum (1.1 cm) were found



Figure 1.3 — Stratigraphic column through the eggshell locality compared to the lectostratotype of Roberts, 2007. Cl = claystone; Si = siltstone; Fs = fine grained sandstone;
Ms = medium grained sandstone; Cs = coarse grained sandstone; Cg = conglomerate.

associated with the eggshell material, though the centrum is directly associated with the main horizon of eggshell.

Three mostly complete eggs (Fig 1.5), the least compacted measuring 2.86 - 2.90 cm in diameter, occur underneath a distribution of eggshell spanning an area of  $3.75 \text{ m}^2$  (Fig 1.6A, B). The lithostatically compacted eggs are filled with the same greenish-gray mudstone in which



Figure 1.4 — Gastropods associated with the egg nesting site. A) *Viviparus* sp. B) *Lioplacodes* sp. C) *Lioplacodes subtortuosa*. D) Stylommatophora. E) *Planorbis chacoensis*.
Scale equals 2 mm (A-D) and 1 mm (E).

they occur and are in association with at least three other partial eggs and crushed eggshell representing an unknown number of additional eggs. The eggshell is light orange in color in situ and white when exposed to the surface. The similarity of preservation and the infilling sediment suggests that the eggshell material and eggs came from the same horizon and originally represented a clutch. The majority of eggshell fragments on the top of the clutch are predominantly parallel to the orientation of the three preserved eggs. Based on the most complete egg, the egg mass is calculated to be 14.43 g. This likely represents an overestimate since the eggs are fractured from lithostatic compaction and potentially wider than their original state. Assuming a mass of 14.43 g, the estimated carapace length of the female that laid IMNH 2713/51224 is 244.5 mm, laying 9.91 eggs per clutch. The number of eggs.

A total of 803 fragments of eggshell have been counted on the surface and in excavation within a  $3.75 \text{ m}^2$  area. The eggshell consists of mostly CU fragments (N=301), with 261 CD and



**Figure 1.5** — Close up of the three mostly complete eggs from the bottom of the main jacket (IMNH 2713/51224). Scale equals 1 cm.



**Figure 1.6** — View of eggshell clutch (IMNH 2713/51224) from the top (A) and bottom (B) of the jacket. A) Spread of eggshell from the top of the jacket. B) View of eggshell from the bottom of the jacket. This side of the jacket preserved three mostly intact eggs, indicated by the dotted outline. Eggshell fragments are an orange to tan color. Scale equals 5 cm.



**Figure 1.7** — Microstructure of IMNH 2713/51224. A) Radial thin-section of eggshell viewed through plane polarized light and (B) cross-polarized light. Outer shell surface is at the top of the image. Note the narrow and closely packed shell units, aragonitic radial ultrastructure, and cratering of the inner shell surface. C) SEM image of nodular surface ornamentation of the eggshell. D) SEM radial thin section showing view of shell units (su). Note how the shell unit widens toward the outer surface. Scale bars equal 200  $\mu$ m (A and B), 1 mm (C), and 500  $\mu$ m (D).

241 UK, respectively. Excluding the vertical fragments, this yields a 54:46 CU to CD ratio. Comparing the observed CU to CD ratio to an expected 50:50 ratio, it is not statistically significant ( $x^2(1) = 1.286$ , p < 0.257). The mostly complete and partial eggs were not counted and would cause the number of CU fragments to increase.

The surface of the eggshell is microscopically ornamented, with round nodes between pores 53-71  $\mu$ m in diameter (Fig 1.7C). This ornamentation is too small and numerous to be associated with the tops of shell units. Pores are numerous on the surface of the shell and range from spherical to oblong in shape and are straight cylinders in thin section. An average of 11.5 pores per mm, an average pore area of 1.26 x 10<sup>-3</sup> mm<sup>2</sup>, and a total egg area of 2642.10 mm<sup>2</sup> results in an eggshell porosity of 42.5 mm. Thickness of the eggshell ranges from 0.7 – 1.2 mm and consists of tall and relatively narrow shell units (307.7  $\mu$ m based on SEM; Fig 1.7D) with a width to height ratio of 3.7:1 at the widest point. Only the widest shell units were measured in



**Figure 1.8** — Representative eggshell X-ray diffraction profile of IMNH 2413/51224. Vertical axis is count rate; horizontal axis is degrees-2 $\theta$ . Vertical bars correspond to predicted 2 $\theta$  peaks and their intensities for an aragonite standard (COD No. 96-901-5718).

order to avoid possible oblong cuts in thin section. The shell units flare slightly towards the outer surface of the shell and are slightly domed at the top (Fig 1.7D). The aragonitic crystals are acicular and radiate nucleation sites from the inner shell surface. Some nucleation sites are visible in thin section, though most shell units are concave on the interior surface (Fig 1.7A). Under cross-polarized light, the eggshell displays a sweeping extinction pattern (Fig 1.7B). Viewing the eggshell samples with SEM and in thin-section, there appeared to be very little diagenetic alteration as microscopic and ultrastructural features are visible.

The results of the XRD analysis are represented by Figure 1.8. All of the samples showed a good match to  $CaCO_3$  in the aragonite phase (COD 96-901-5718; Putz and Brandenburg, n.d.) suggesting a lack of diagenetic alteration. The main peak appeared at 46.07 20.

#### **1.6.** Discussion

The shape of the shell units, needle-like crystals, and aragonitic composition confirm a testudine identification for IMNH 2713/51224 (Hirsch, 1983; Mikhailov, 1991). The XRD results suggest that very little diagenetic alteration has occurred to the eggshell, as well as the fact that ultrastructural features are observable in thin-section and SEM.

#### 1.6.1. Identity of the egg producer

Turtles are conspicuous members of the Kaiparowits Formation fauna, with a minimum of 14 taxa. Trionychid, baenid, and adocid specimens far exceed the other families represented in the formation (Hutchison et al., 2013). Examples of modern trionychid eggshell have thinner eggshell, smaller height-to-width ratios, and lack shell unit flaring and doming and therefore do not match the IMNH 2713/51224 eggs (Lawver and Jackson, 2017). Two gravid fossil *Adocus* sp. and a clutch have been described from the Kaiparowits (Knell et al., 2011) and Dinosaur Park

(Zelenitsky et al., 2008) formations and are similar to the eggs described here. The eggshell thickness from the *Adocus* sp. clutch fall within the thickness range of IMNH 2713/51224, domed shell units, and a similar, though lower, height-to-width ratio (2.5:1 - 3.5:1; Zelenitsky et al., 2008). However, the main difference between the *Adocus* sp. eggs and the eggs described here is the size of the eggs and the surface texture of the shell. The *Adocus* sp. eggs lack ornamentation and are larger by 17 - 33%.

Studies of freshwater and marine turtles suggest that egg size is less variable than clutch size within a population (Bjorndal and Carr, 1989; Wilkinson and Gibbons, 2005). This may indicate that the difference in egg size between the specimens described in this paper and those described for *Adocus* sp. from the Dinosaur Park (Zelenitsky et al., 2008) and Kaiparowits formations (Knell et al., 2011) is significant enough, in addition to the small ornamentation of the new material, to belong to a different turtle (Table 1.1). A significant degree of homoplasy occurs in extant turtle eggshell (Winkler, 2006; Lawver, 2017), therefore, this newly described eggshell material may belong to several different turtle taxa that could produce eggs of similar microstructure.

#### **1.6.2.** Depositional Environment and Taphonomy

The egg clutch was laid in a silty mudstone of overbank deposits. Weakly developed paleosols suggest waterlogged soils (Roberts et al., 2013) and the abundance of freshwater snails supports this interpretation. Mild pedogenic processes are indicated by the presence of caliche nodules and slickensides and suggest mild seasonal variation in precipitation and a high-water table (Roberts, 2007). The exquisite preservation of the gastropods, which includes nacreous carbonate, likely indicates that the assemblage is autochthonous. The types of gastropods represented at the site are consistent with pond or small lake environments with the terrestrial

Ootaxon	Specimen No.	Material	Age	Location	Shape	Length x Width (mm)	Thickness (mm)	<sup>5</sup> Ratio
Chelonoolithus braemi <sup>1</sup>	Guimarota 98-2	Eggshell fragments	Kimmeridgian, Upper Jurassic	Portugal	_	_	0.2	1:1
Emydoolithus laiyangensis²	IVPP V18544	Nearly complete egg	Upper Cretaceous	Shandong Province, China	Elongate	91 x 22	0.4-0.5	2:1 to 5:1
Haininchelys curiosa <sup>3</sup>	_	Eggshell fragments	Upper Paleocene	Belgium	_	_	0.25-0.3	1.2:1 to 2.3:1
Testudoflexoolithus agassizi <sup>4</sup>	MCZ 2810/HEC 49	Eggshell fragments	Pleistocene	Florida, USA	_	_	0.06-0.1	1:1 or 2:3
Testudoflexoolithus bathonicae <sup>4,5</sup>	MB(NH)37983/HEC 186	Egg in matrix	Bathonian, Middle Jurassic	England	Ellipsoidal	48 x 26	0.2-0.25	1:1
Testudoolithus? <sup>6</sup>	ZMNH M8856	Clutch of at least 15 eggs	Upper Cretaceous	Henan Province, China	Spheroidal	53 x 58	1.5	4.1 to 5.4:1
Testudoolithus? <sup>7</sup>	CUGW EH051	Egg with embryo	Upper Cretaceous	Henan Province, China	Spheroidal	54 x 59	1.8	5.1:1 to 7.5:1
Testudoolithus tuberi	IMNH 2713/51224	Clutch of at least 6 eggs	Campanian, Upper Cretaceous	Utah, USA	Spherical	28.6-29	0.7-1.2	3.7:1
Testudoolithus hirschi <sup>8</sup>	_	Eggshell fragments	Kimmeridgian, Upper Jurassic	Portugal	_	_	0.15	3:1
Testudoolithus jiangi <sup>9,10</sup>	ZMNH M8713	Clutch of 23 eggs	Albian, Early Cretaceous	Zhejiang Province, China	Spherical	35-52	0.7-1.0	2.5:1 to 3:1
Testudoolithus Iordhowensis <sup>11</sup>	AM F82183	Clutch of at least 10 eggs	Pleistocene	Lord Howe Island, Australia	Spherical	53.9	0.8	1.2:1
Testudoolithus rigidus⁴	UCM 55806/HEC 425	Half an egg	Lower Cretaceous - Pliocene	USA, Europe, Africa	Spheroidal	42 x 47	0.22-0.24	2:1
Testudoolithus zelenitskyae <sup>12</sup>	MOR 710	Clutch of at least 16 eggs	Campanian, Upper Cretaceous	USA and Canada	Spherical	34-39	0.66-0.76	3.15:1 to 5.5:1

Table 1.1. — Record of distinguishing characters of turtle ootaxa. Modified from Lawver and Jackson, 2014. References: 1, Kohring, 1998; 2, Q. Wang et al., 2013; 3, Schleich et al., 1988; 4, Hirsch, 1996; 5; Buckman, 1860; 6, Jackson et al., 2018; 7, Ke et al., 2021; 8, Kohring, 1999; 9, Fang et al., 2003; 10, Jackson et al., 2008; 11, Lawver and Jackson, 2016; 12, Lawver and Jackson, 2017.

stylommatophoran and non-avian dinosaur teeth potentially being washed into the pond from the floodplain (Tapanila and Roberts, 2013). A study of ten species of extant freshwater turtles from eight U.S. states and four countries were documented nesting an average of 19.7 m from a nearby shoreline, with some as close as 0.3 m to a stream and as far as 320 m (Bodie, 2001).

Eggshell porosity is influenced by a nests absolute humidity and has been used as a means of determining nest type (i.e., covered versus open) (Tanaka et al. 2015). Animals that lay eggs covered by soil or vegetation, such as crocodylians and most turtles, exhibit higher egg porosity and nest humidity. A value of 42.5 mm suggests a higher porosity relative to egg mass than species of open nesters. This in turn fits the interpretation of a more humid nesting environment.

Taphonomic studies of modern avian, crocodylian, and tortoise nesting sites reveal patterns in the abundance, orientation, condition, and spatial distribution of eggs, eggshell fragments, and bone. A number of these studies have used fragmented eggshell orientation (Hayward et al., 2000, 2011; Wang et al., 2013; Imai et al., 2015; Ferguson et al., 2017, 2018) and abrasion (Oser and Jackson, 2014) to distinguish transported assemblages from hatched, trampled, or predated nesting sites. For example, studies of hatched and predated tortoise, caiman, crocodile, gull, pelican, cormorant, heron, and common tern nests demonstrate that assemblages of fragmented eggshell favor an approximately 60:40 concave up to concave down ratio, despite differences in nesting strategies or predation (Hayward et al., 2000, 2011; Wang et al., 2013; Jackson et al., 2015; Ferguson et al., 2017, 2018; McGrath et al., 2020). However, distinguishing between hatched or predated nesting sites is difficult with eggshell orientation alone, but it appears effective in determining an in-situ collection of eggshell fragments with large enough sample sizes (Hayward et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2000, 2011; Jackson et al., 2015; Ferguson et al., 2005; Ferguson et al., 2005; Ferguson et al., 2005; Ferguson et al., 2005;

2017, 2018). An eggshell orientation of 54:46 CU to CD suggests the eggshell material is in situ and hasn't been influenced by fluvial transport in addition to autochthonous gastropods and fine-grained sediments.

Turtle nests closer to a shoreline have an increased likelihood of predation (Marchand et al., 2002) and a modern taphonomic study of desert tortoise (Gopherus agassizii) and loggerhead sea turtles (Caretta caretta) demonstrated that only predation of nesting sites showed significant eggshell above the egg chamber (Jackson et al., 2015). In addition, hatched tortoise eggs only consist of ~25% of the original egg and the egg becomes infilled with sand and eggshell fragments. In the case of IMNH 2713/51224, the bottom of the egg chamber is indicated by the three most well-preserved eggs, whereas the top of the chamber has been destroyed from digging. This would explain the wide distribution of eggshell away from what would have been the original egg chamber. Many modern predators of turtle nests, such as gray foxes and racoons, scatter eggshell around the egg chamber and will often leave behind uneaten eggs. Depending on the predator, the eggs could be carefully removed with the chamber retaining its shape and fragmented eggshell deposited at a higher stratigraphic level, or almost completely destroyed leaving scattered eggshell among any eggs left behind in the remaining egg chamber (Jackson et al. 2015). The unusual distribution of eggshell, the presence of mostly complete eggs, and an autochthonous eggshell orientation of 54:46 CU to CD suggests the nest was predated rather than successfully hatched.

Turtle clutches are extremely rare when compared to the number of turtles that existed over geologic time (Fig 1.1). Turtle eggshell, unlike dinosaurian or crocodilian eggs, are composed of aragonite which often alters to low-Mg calcite (Al-Aasm and Veizer, 1986; Pederson et al., 2019) distorting micro- and ultrastructural features of the eggshell. Eggshell is

also prone to fracturing, abrasion, and dissolution. Modern experiments of calcitic eggshell corrosion increases with increased acidity and temperature, but even alkaline aqueous solutions (pH 7.4) result in some corrosion. This has led some researchers to conclude that preservation of eggshell is unlikely to occur in water saturated environments (Clayburn et al., 2004). However, in the eggshell example from the Kaiparowits Formation, the setting combines a perennially wet environment, deposition in overbank/pond sediments, and original aragonite preservation of intact aquatic gastropod shells (Tapanila and Roberts, 2013). The mismatch between limited experimental results on eggshell corrosion and fossil occurrences where aragonite is preserved warrants further study.

Occurrence of eggshell in the Kaiparowits Formation follows a trend, especially in the middle unit, of taxonomically broad, high-quality preservation of fossils. In the case of the eggshell, its depositional environment is favorable for burial. The clutch is located on a pond margin, in a system that shows evidence for perennial moisture (Roberts, 2007; Miller et al., 2013; Foreman et al., 2015), where ponds are long lasting and the margins might transgress over time, providing a mechanism for burial and preservation. There is some evidence that Cretaceous waters in the Kaiparowits were alkaline (e.g., abundance of carbonate nodules) which would elevate pH and slow corrosion.

Second, the foreland basin, especially during the time of middle unit deposition, accumulated sediments at an astonishing rate, owing to rapid subsidence and copious finegrained input from volcanic ashes (Roberts et al., 2005). Smectitic clays present within the mud rocks of the Kaiparowits Formation may have limited rock porosity, thereby limiting exchange of potentially caustic groundwaters with buried fossils. Primary aragonite is commonplace throughout the middle unit of the formation, though rare in other similar basins that lack volcanic

input. The preservation of eggshell in the Kaiparowits and Dinosaur Park formations is a further example of enhanced preservation along the greater WIB during Campanian time, a so-called taphozone (Roberts et al., 2013; Tapanila and Roberts, 2014) of regionally enhanced fossil preservation.

### **1.7. Systematic Paleontology**

Oofamily TESTUDOOLITHIDAE Hirsch, 1996

**Oogenus TESTUDOOLITHUS Hirsch**, 1996

Type Oospecies – *Testudoolithus tuberi* oosp. nov.

Holotype – IMNH 2713/51224, the remains of fragmented clutch composed of three mostly complete eggs and scattered fragmented eggshell.

Etymology – 'Tuber,' from the Latin word for bump or protuberance, after the round ornamentation on the surface of the eggshell.

Distribution – Kaiparowits Formation, Utah, U.S.A. Campanian (Late Cretaceous; ~76.0 - 75.5 Ma; Roberts et al. 2013) in age.

Diagnosis – *Testudoolithus tuberi* oosp. nov. differs from all other oospecies by the following unique characteristics: spherical eggs 29 mm in diameter; 0.7-1.2 mm thick eggshell; shell unit height-to-width ratio of 3.7:1; domed shell units that flare towards the external surface and 53-71 µm round nodes of dispersituberculate ornamentation on the shell surface.

Remarks – Hirsch (1996) designated two oofamilies of the testudoid basic type,

Tesdudoflexoolithidae and Testudoolithidae, based on the rigidity of the eggshell layer. Shell units that are generally wider than high and loosely adjoining constitute more pliable eggshell and fall within the Tesdudoflexoolithidae oofamily, whereas higher and tightly packed shell units that interlock consist of the rigid eggshell of the Testudoolithidae oofamily (Table 1.1). The interlocking and tall shell units suggests that IMNH 2713/51224 is within the Testudoolithidae family.

Testudoolithus tuberi is the most similar to two oospecies, Testudoolithus jiangi and Testudoolithus zelenitskyae (Fang et al., 2003; Jackson et al., 2008; Lawver and Jackson, 2017). Jackson and others (2008) describe T. jiangi as spherical eggs 35-52 mm in diameter with an eggshell thickness of ~ 0.7-1.0 mm and a shell unit to height-to-width ratio of 2.5:1 - 3:1. T. zelenitskyae eggs are smooth with a shell thickness of 660 - 760 µm and a minimum egg size of 34 - 39 mm (Lawver and Jackson, 2017) and a height-to-width ratio of 3.15:1 - 5.5:1. Though similar, the main difference between T. tuberi is the mosaic of characteristics such as egg size, doming and flaring of shell units, and the surficial ornamentation. The shell units of T. jiangi are not flared or domed. While flaring is observed in *T. zelenitskyae*, no ornamentation besides doming is mentioned for either species. Other ootaxa within Testudoolithus differ with thinner eggshell, smaller eggs, and differing height-to-width ratios (Hirsch, 1996; Lawver and Jackson, 2017), besides the eggs from the Xiaguan Formation (Jackson et al., 2018; Ke et al., 2021) which both have thicker shells and larger eggs. In addition, T. tuberi differs from Chelonoolithus braemi (Lawver and Jackson, 2017) and Haininchelys curiosa (Schleich, 1988) with significantly thicker eggshell and taller shell units. An elliptical egg shape distinguishes Emdyoolithus laiyangensis from T. tuberi (Wang et al., 2013).

#### **1.8.** Conclusions

IMNH 2713/51224 was collected from the Campanian (Upper Cretaceous) Kaiparowits Formation within Grand Staircase-Escalante National Monument and consists of a spread of
eggshell over an area of  $3.75 \text{ m}^2$  with six partially and mostly complete eggs. The site is interpreted as a turtle clutch originally consisting of ~10 spherical eggs. The eggshell is composed of aragonite with shell units interlocked with a height-to-width ratio of 3.7:1 and a thickness of 0.7-1.2 mm. These features, along with ornamentation  $53-71 \mu \text{m}$  in diameter makes *T. tuberi* oosp. nov. distinguishable from previously described eggs. The wide distribution of eggshell and presence of intact eggs are interpreted to be due to predation rather than hatching. The preservation of primary aragonitic eggshell and gastropods further hints at enhanced preservation along the WIB during Campanian time.

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#### Chapter 2: First avian eggs from the Bridger Formation, Wyoming

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#### 2.1. Abstract

Avian eggs and nests in the fossil record are rare, particularly when compared to their non-avian ancestors. Here, we describe an in-situ clutch of avian eggs from the Eocene Bridger Formation in southwestern Wyoming. Detailed stratigraphic correlation places the egg site 9-22 m below the Church Butte Tuff, with the range due to the uncertainty in dip. The eggs are preserved in an olive-gray mudstone within the Blacks Fork Member. Eggshell is distributed across an area of 2 m<sup>2</sup> with two concentrations of eggs and eggshell ~20 cm apart. The first concentration contains five partial eggs, three mammalian bones and an avian bone, whereas the second concentration contains three additional partial eggs, and five mammalian elements. The most complete egg measures 5.40 x 5.87 cm. The eggshell is 0.3-0.6 mm thick and consists of an indistinct and slightly undulating boundary between the mammillary and continuous layer with a mammillary layer to shell thickness ratio of 1:4. Eggshell orientation (concave up/concave down) of 59:41 is consistent with in-situ preservation. Extrapolating from egg size, the producing maternal bird's mass is estimated at 2.2 kg with an average clutch size of 3.79 eggs. The eggshell micro- and ultrastructural features are similar to those of modern Ciconiiformes and Gruiformes. The unique attributes of the eggshell warrant naming of a new oogenus and

oospecies, *Duoolithus bridgerensis*. The documentation of this ootaxa provides features similar to modern avian taxa and therefore may aid in understanding the evolution of avian reproductive biology.

Keywords: Ootaxonomy, Taphonomy, Eggshell orientation, Predation, Aves, Eocene

#### **2.2. Introduction**

Avian eggs from the Paleogene and Neogene are rare compared to their non-avian counterparts and generally lack embryonic material to determine the identity of the egg-laying taxa (Carpenter, 1999; Chandler and Wall, 2001; Deeming, 2016; Hirsch et al., 1997; Jackson et al., 2013; Kohring and Hirsch, 1996; Lawver and Boyd, 2018; Varricchio and Jackson, 2016). Without embryonic remains, other characteristics have been used to assign specific avian taxa to eggshell material, such as macroscopic features. Chandler and Wall (2001) assigned eggs from the Oligocene Brule Formation to a limpkin based on the similarity of porosity, size, asymmetry, and the association of *Badistorni aramus* to the locality. However, macroscopic features alone are unreliable in identifying avian taxa as egg macroscopic features can overlap among Aves (Stoddard et al., 2017).

Micro- and ultrastructural features of eggshell comparing modern and fossil material provides a means of identifying the egg-laying animal. For example, Hirsch and Bray, (1988) compared the macro- and microstructure of eggs from the Miocene and Oligocene of the Western Interior to modern owl eggs (*Strix aluco* and *Strix varia*). The authors describe the microfeatures of the fossil and modern eggs to be very similar with slender shell units and distinct mammillary knobs. Eggs from the Eocene Willwood Formation were designated as

belonging to a neognathe bird based on the presence of three structural layers and an indistinct squamatic texture (Jackson et al., 2013).

In addition to these eggshell characteristics, the taphonomy of a site can determine the amount of transport the eggshell has been subjected to (Hayward et al., 2011; Imai et al., 2015), as well as interpret the developmental mode of young associated with a nesting locality based on bone content (Ferguson et al., 2018, 2017; McGrath et al., 2020). Eggshell orientation has previously been used as a means of distinguishing transported assemblages from trampled, hatched, or predated nesting sites. For example, fragmented eggshell associated with successfully hatched gull (Ferguson et al., 2018; Hayward et al., 2011), common tern (Wang et al., 2013), tortoise (Jackson et al., 2015), caiman nests (Ferguson et al., 2017), and pelican and cormorant nests (Ferguson et al., 2018) favor a 60:40 concave up (CU) to concave down (CD) orientation, despite different nesting strategies. The amount of skeletal material associated with a semi-precocial gull nesting colony is about an order of magnitude less than material associated with altricial pelican and cormorant nests (Ferguson et al., 2018).

Isotopic studies of eggshell fragments provide knowledge of the paleoecological environment that fossil egg-laying animals lived in. Studies of modern reptile and bird eggshell carbonate suggest that the  $\delta^{13}$ C reflects the diet of the female during eggshell calcification, with  $\delta^{18}$ O reflecting the water consumed by the female (Erben et al., 1979; Folinsbee et al., 1970; Schaffner and Swart, 1991). An estimate of the fractionation of  $\delta^{13}$ C and  $\delta^{18}$ O allows the interpretation of existing vegetation and the composition of nearby water sources.

Here, we describe the stratigraphy, taphonomy, isotopic, and micro-and ultrastructural features of the first eggs collected from the Eocene Bridger Formation. We compare the eggs to previously described fossil eggshell and designate a new ootaxon. Lastly, we compare the eggs to

modern avian birds and discuss the identification of the egg-laying avian taxa and the interpretation of fossil egg preservation.

# 2.3. Geologic Setting

The Bridger Formation, located within the southern half of the Green River Basin, Wyoming (Fig 2.1) consists of up to 842 m of fluvial and lacustrine sediments (Evanoff et al., 1998; Murphey and Evanoff, 2007) that overlie and interfinger with the Laney Member of the Green



**Figure 2.1** — Location of the Bridger Formation within the Green River Basin. The star represents the approximate location of the field area.

River Formation (Sullivan, 1980). Structurally, the foreland basin is a large asymmetric northtrending syncline with mostly shallow dips  $(3^{\circ} - 5^{\circ})$  formed from Laramide-style uplift (Murphey et al., 2017; Roehler, 1992a). Sedimentation was continuous across most of the basin, with only a few erosive interruptions adjacent to syndepositional active thrust faults (Roehler, 1992a). Petrographic studies of the sands suggest that the sediments originated from the Challis volcanic field in central Idaho via the Idaho River (Chetel et al., 2011).

The strata of the Bridger Formation consist of interbedded gray and green mudstones and sandstones, brown claystones, gray to tan limestones, and gray to white tuffs with carbonaceous shales, red mudstones, and conglomerates being rarer (Roehler, 1992b; Sullivan, 1980). These sediments have been interpreted as fluvial flood-plain deposits, sediments from shallow freshwater lakes, and ashfall deposits (Brand, 2007; Buchheim et al., 2000; Roehler, 1993; Sullivan, 1980). Most of the sediment is volcanoclastic with channel form sandstone bodies trending to the southeast (Chetel et al., 2011; Surdam and Stanley, 1980).

The Bridger Formation is the stratotype of the Bridgerian North American Land Mammal Age (Wood et al., 1941). Except for the uppermost Turtle Bluff Member, which is earliest Uintan in age, the Bridger Formation spans the majority of the Bridgerian (Br-1b to Br-3; Gunnell, 1998) placing it in the Middle Eocene. Based on sanidine in various tuffs throughout the formation, the Bridger Formation is estimated to have been deposited between 49.09-45.57 Ma (Murphey et al., 2017). <sup>40</sup>Ar/<sup>39</sup>Ar ages of 49.05  $\pm$  0.16 Ma were derived from the lowest tuff, (i.e., Church Butte Tuff; Smith et al., 2008) and 46.14  $\pm$  0.44 Ma from the Basal E Tuff near the top of the formation (Evanoff et al., 1998).

# 2.4. Methods

#### 2.4.1. Collection methods

The specimens were discovered in 2017 and are housed at the University of Washington Burke Museum (UWBM 102922-102925, 118216-118217). Collection of the material included saturating the eggshell with Butvar-72 and carefully jacketing sections of concentrated shell. The site was surveyed in subsequent years to determine if any new material had become exposed via erosion. The specimens were then prepped from the bottom using standard hand tools at the Idaho Museum of Natural History (IMNH).

A detailed decimeter-scale stratigraphic column was constructed using a Jacobs Staff and Brunton compass. The section began at a sandstone unit on the northwestern edge of "Freya's Field" and ended to the southeast of the field along exposed buttes at a ~1-2 m thick, laterally continuous sandstone unit at a strike and dip of 038%/3° to the southeast. Detailed descriptions of each of the units were done in the field. Marker beds in the field area were mapped with GPS points. The GPS data points were recorded with a Geo XT 7 Trimble device receiving real-time differential corrections in 2015. Field mapping of the site consisted of a  $1 \text{ m}^2$  grid separated into  $10 \times 10$  cm intervals. The location of the collected partial eggs was marked and surface and excavated eggshell fragments were tallied, and their orientations recorded [i.e., concave up (CU), concave down (CD), or unknown (UK)]. Eggshell fragments designated as unknown indicate that the angle of the eggshell exceeded  $45^{\circ}$  to the horizontal plane or the fragments were disturbed during excavation. Tallies included only eggshell fragments measuring 1 cm<sup>2</sup> or greater in size. Eggshell counts were then compared to a null hypothesis of a 50:50 CU:CD ratio using a chi-square test with Yates's correction. Yates's correction is used here to accommodate the  $2 \times 2$  contingency table and relatively small sample size (Ury and Fleiss, 1980; Yates, 1984).

# 2.4.2. Sampling methods and calculations

Two fragments of loose eggshell were removed from the site and prepared at Spectrum Petrographic as a standard 30 µm thick petrographic radial thin section and viewed under a Leica MC 170 HD microscope. An additional fragment was mounted onto aluminum stubs, coated with 10 nm of gold, and imaged with a FEI Quanta 600 FEG scanning electron microscope (SEM) at the University of Utah Surface Analysis Lab. ImageJ software was used to measure structural features (Schneider et al., 2012).

Isotopic samples consist of 10 eggshell fragments, five fragments each, from the two jackets that produced partial eggs. The samples provided ~0.3 mg of powder collected via crushing by a sterilized mortar and pestle in the Geochemistry Lab in the Department of Geosciences at Idaho State University. Before powdering the samples, the material was cleaned with acetone and isopropyl alcohol to remove any adhesives and then washed with distilled water in an ultrasonic bath and dried in an oven overnight. After powdering, the material reacted with anhydrous oversaturated phosphoric acid at 90°C for 12 minutes. Carbon and oxygen isotope compositions were then compared to V-PDB (Vienna Pee Dee Belemnite). Eggshell mineral composition was determined by X-ray powder diffractometry on a Bruker D2 PHASER X-ray diffractometer using Cu Ka radiation. After cleaning eggshell fragments in an ultrasonic bath, the fragments were dried in an oven at 70°C overnight. The fragments were powdered to a uniform grain size (10's mg) using a quartz mortar and pestle. The powdered samples were sprinkled on 1" diameter glass base mount. The analysis yielded well defined ( $\geq 2$ -sigma above background) major diffraction lines between  $20^{\circ}$  to  $50^{\circ}2\theta$ . Data were analyzed using Bruker EVA search-match software and the Crystallography Open Database (COD) (Vaitkus et al., 2021).

In addition to the microstructure of the eggshell, egg size can be used to narrow down the mass of the female bird and the number of possible eggs in a clutch (Blueweiss et al., 1978). The mass of the female bird and individual egg weight was calculated using the following regression from Blueweiss et al. 1978:

$$E_b = 0.26 W^{0.77}$$

where  $E_b$  is the individual egg weight, and W is the maternal body weight. Clutch size of the gravid female bird that produced the eggs was calculated using the positive correlation between clutch weight and maternal body weight from Blueweiss et al. 1978:

$$C = 1.24 W^{0.74}$$

where C is the clutch weight and W is the maternal body weight. Assuming a density of 1.09  $g/cm^3$  (Williams et al., 1984) for avian eggs, the mass of an individual egg is calculated from the volume of an ellipse.

#### 2.5. Results

# 2.5.1. Regional stratigraphic position of Freya's Field

Stratigraphic context of Freya's Field fossils was assessed with two approaches, a highresolution stratigraphic section that inferred thickness by correcting for a 3-degree dip measured from various bedding plane surfaces of locally exposed sandstone units as well as through tracing and GPS recording of marker bed units.

Freya's Field is an extensively exposed flat area that consists of an expanse of exposed bedding planes of silt and sandstone. Most of the extensively distributed in situ fossil finds occur between 1,983.5 m and 1,984.5 m Height Above Ellipsoid or HAE. The lowest fossil site was recorded at 1,982 m while the highest site was at 1,989 m (Fig 2.2). If dips measured from



Figure 2.2 — View of field area from Google Earth Satellite Imagery.

Inverted triangles mark bases of high-resolution stratigraphic sections. X's mark top of highresolution sections. Dashed lines show paths of high-resolution section. Crosses indicate GPS data points representing marker bed outcrops. The numbers inside color-rimmed ovals connected to GPS points are the elevation recorded as Height Above Ellipsoid in units of meters. Small white-filled, black-rimmed circles are locations of vertebrate fossils. The white-filled, black-rimmed star is the egg site. The distribution of fossils demarcates "Freya's Field". The marker beds are those classically recognized and mapped by Evanoff et al., 1998 and Murphey and Evanoff, 2011 as well as others (e.g., Alexander and Burger, 2001) except for the "Lower mudstone". This lower mudstone is significant only because of its utility in marking the upper boundary of the fossiliferous horizon of Freya's Field.

sandstones are more broadly reflective of bedding attitude in the area, then taking dip into account suggests these fossil localities could actually be spread out over as much as 15-20 m of stratigraphy (Fig 2.3).

In any case, the fossiliferous section is capped by a dark grey mudstone that is well exposed along the East side of the locality and to south of it (Fig 2.2: Lower mudstone). The base of this bed is at ~1,990 m. The highest fossil recovered at 1,989 m is about 4 m geographically from an outcrop of this lower mudstone. Thus, stratigraphically speaking, the fossiliferous part of the sequence likely comes within a meter or less of this lower mudstone (Fig 2.3). The lower mudstone is recorded in the base of the middle of the three high-resolution stratigraphic sections.



**Figure 2.3** — Stratigraphy of the Freya's Field area.

A) Generic stratigraphic section of Blacks Fork Member of Bridger Formation, modified from Murphey and Evanoff, (2011). Boundary between Br1b and Br2 is the Lyman Limestone. Colored layers highlight marker beds that were observed and mapped in Freya's Field. The color scheme matches that used in Figure 2.2. B) Marker bed local elevations. This section magnifies the elevational position of marker beds exposed around Freya's Field. Meter levels are Height Above Ellipsoid elevations. These elevations are not necessarily reflective of stratigraphic thickness but are consistent with stratigraphic intervals published by other authors (see text). Position of each marker bed is an average of readings taken on multiple outcrops for each bed (see Figure 2.2). Abbreviations: LMs, Lower mudstone (not a regionally recognized marker bed); CBT, Church Butte Tuff (broadly recognized marker bed dividing Lower and middle parts of Bridger B); CWL, Cottonwood Limestone (also known as Cottonwood White Layer, which is very visible in Grizzly Buttes area but locally absent near Church Butte); JRL, Jackson Ridge Limestone; BMTL, Black Mountain Turtle Layer (broadly recognized marker bed denoting boundary between middle and upper parts of Bridger B); SCL, Sage Creek Limestone (extensive bench-forming limestone that forms the boundary between Bridger B and Bridger C). C) High resolution stratigraphic section. Three high resolution lithostratigraphic sections were mapped using a Jacob staff (see Figure 2.2 for geographic location of these sections). They are separated by alluvium covered areas. The lower section is in the fossiliferous zone of Freya's field. The middle section includes the lower mudstone, and the upper section crosses the Church Butte Tuff.

No further mapping could be accomplished to the North or West of the locality due to extensive cover by quaternary fill and vegetation. However, the Paleogene beds become

increasingly well exposed to the southeast as elevation rises towards the terrace capped by the Sage Creek Limestone (Fig 2.2). The next bed identifiable to the southeast is the Church Butte Tuff. It was identified by its similarity in outcrop appearance to verified exposures of it at Grizzly Buttes (Alexander and Burger, 2001). The highest of the three high-resolution sections crosses an exposure of the Church Butte Tuff (Fig 2.2; Fig 2.3). Although no obvious tuff-like characteristics were observed locally (e.g., euhedral biotite crystals), the low relief exposures were deeply weathered preventing us from sampling and examining fresh rock given the time constraints we had while in the field. The Church Butte Tuff occurs at 1,999-2,002 m. Thus, it is likely at least 9-13 meters above the highest fossil exposures. Based on dips used in the high resolution-section it is possible that the distance is even greater: possibly 17-22 m (Fig 2.3).

The next marker bed observed in the sequence is the Cottonwood Limestone. It has a more uniquely diagnostic and extensive appearance than the Church Butte Tuff in the area. Outcrops were recorded at elevations between 2,007-2,009 m. Thus, it appears to be between 5-10 m above the Church Butte Tuff. This is probably greater than the 5-meter interval between these beds noted by Murphey and Evanoff, (2011) as "typical" of other areas, but probably less than the 10–12-meter interval observed between these beds to the southwest at Grizzly Buttes (Murphey and Evanoff, 2011; DMB unpublished data). Thus, since Freya's Field is to the southwest of Jackson Ridge and Church Butte in the direction of Grizzly Buttes, this intermediate thickness makes sense.

Above the Cottonwood Limestone is the Jackson Ridge Limestone. We identified it based on its similarity in outcrop appearance to exposures at Grizzly Buttes and by the fact that it is a limestone. Outcrops appear at 2,012-2,014 m. Thus, it appears to be 3–7 m above the Cottonwood Limestone. A 3–7-meter interval is close to what Murphey and Evanoff (2011)

report for these beds around Grizzly Buttes (6 m). However, they do not indicate a thickening of this interval to the southwest as they do for the interval between the Church Butte Tuff and Cottonwood Limestone, so finding more similarity in the thickness of this interval between Freya's Field and Grizzly Buttes may be consistent with previous observations.

Just above the Jackson Ridge Limestone, the slope of the terrain steepens and remains steep until the terrace is reached. We noted a possible exposure of the Black Mountain Turtle Layer at 2,070 m. However, this assessment is rather uncertain due to physical difficulty of evaluating lithologic characteristics and tracing beds here. Thus, the boundary between the middle and upper parts of Bridger B remains uncertain at Freya's Field. However, the base of the Sage Creek Limestone is unmistakably prominent and occurs at 2,111 m (about 9 m below the top of the terrace). If nothing else, we can say that the interval between the Church Butte Tuff (marking the beginning of the middle of Bridger B) and Sage Creek Limestone (marking the top of Bridger B) is around 103 m. This can be compared to the generalized section published by Murphey and Evanoff (2011, their figure 3) that puts the base of Sage Creek 125 m above the Church Butte Tuff. If it is correct that some dip to the southeast must be factored in here, it is possible that the actual stratigraphic thickness is closer to 125 m here as well. Elevational intervals between the Church Butte Tuff and Sage Creek Limestone in the Grizzly Buttes area also seem to be around ~100 m (DMB unpublished data). The Sage Creek exposures used to judge the interval around Grizzly Butte are to east-southeast of the Church Butte Exposures. Given the proximity of these exposures to the Moxa arch (Murphey and Evanoff, 2011) it seems unlikely that the dip would generally be to the southeast and causing elevational differences to under-estimate the stratigraphic thickness of this section. If anything, we might expect elevational differences to over-estimate slightly.

# 2.5.2. Eggshell locality and eggshell description

The mudstone unit containing the eggs and eggshell overlies a structureless dark brown to burgundy sandstone coarsening upward from a fine to medium sand (lithic arenite, Fig 2.3C) with casts of unionids, about 8–9 cm in maximum length, near the lower contact of the mudstone. The contact between the sandstone and mudstone is sharp. Within the mudstone are three 10 cm scale lenses of sandstone with similar composition as the previous sandstone, occurring above the eggshell horizon. A fractured carapace of a turtle was found several meters from the egg site in the same mudstone unit. The smaller sandstone lenses pinched out before reaching the site of the turtle. Several meters of sediment above the egg bearing unit are obscured by vegetation.

The eggshell material is embedded in a friable greenish-gray mudstone with occasional centimeter scale pockets of higher sand content. Besides the eggshell material, the rock is devoid of other sources of calcium carbonate. The eggshell is concentrated on the same horizon and consists of eight partial eggs, all with signs of lithostatic compaction. Two main jackets of material were collected and prepped and referred to as J1 (UWBM 118216) and J2 (UWBM 118217), respectively (Fig 2.4). The two jackets were collected ~20 cm apart. The first jacket and nearby smaller jackets consist of approximately five partial eggs, four CU and one CD, and four bones.

Within J1, the two partial eggs in the main jacket are separated by 12.17 cm measured from the center of each. This jacket contains the largest egg portion out of all the material collected, which appears to be half an egg transected lengthwise, measuring 5.40 cm by 5.87 cm. This measurement is likely an overestimate due to compaction. The other egg within the jacket is about a quarter of a complete egg and is CD. The bone material is associated with an

approximate half egg (UWBM 102923) with an element directly underneath the egg. The ends of the element are missing, but the thickness of the cortical bone suggests it is mammalian. Other associated elements include the proximal epithesial plate and shaft of two mammalian tibiae, and one additional unknown long bone, likely belonging to a bird based on the thickness of the



Figure 2.4 — View from bottom of UWBM 118216, i.e., Jacket 1 (A) and UWBM 118217,

i.e., Jacket 2 (B). Red dashed line indicates location of in situ bones. Scale is 2 cm.

cortical bone in cross section. The other two eggs are approximately a quarter to a half egg, respectively (UWBM 102924 and 102922). UWBM 102922 measures 3.60 cm by 4.70 cm and represents the lower range of egg size, taking into account that this specimen has both lithologic compactions vertically and laterally, as indicated by shell fragment overlap.

The second jacket (UWBM 118217) contains three partial eggs, all CU, and five bones. The farthest partial eggs are separated by 8.25 cm, with the center egg butted up against the largest of the eggs and 6.70 cm from the smaller egg. The larger of the eggs measures 5.69 cm by 5.22 cm, similar to the size of the half egg in J1. The bones include a centrum, phalanx, astragalus, and two unknown elements. All appear to be mammalian based on cortical bone thickness and overall morphology. The most complete eggs from each of the jackets are relatively spherical and have smooth outer surfaces. Each partial egg appears to have been truncated by weathering with the outer surface of the eggshell weathered as well.

Based on the most complete egg, the egg mass is calculated to be 97.7 g. This likely overestimates mass, as the eggs have been fractured by lithostatic compaction and are potentially wider than their original state. Assuming a mass of 97.7g for a single egg, the maternal bird's mass is estimated as 2.2 kg (about the size of an average chicken hen) with a clutch weight of 369.9 g, or three to four eggs (3.79 eggs) in a clutch.

A total of 138 fragments of eggshell have been counted on the surface and in excavation within a 2 m<sup>2</sup> area. The surface eggshell consists of mostly vertical fragments (N=32), with 14 CU and 11 CD in total (56:44 CU to CD ratio). The 111 fragments and eggs collected during excavation yielded 45.9% CU (N = 51), 32.4% CD (N = 36), and 21.6% UK (N = 24). Excluding the eggshell material of UK orientation, the normalized total percentages from the excavated

material yielded 58.6% CU and 41.4% CD orientations. The in-situ observed CU to CD ratio is not significantly different from 50:50 ( $x^2(1) = 0.979$ , p < 0.322).

The surface of the eggshell is smooth and slightly weathered, with round and infrequent pores, likely angusticanaliculate. However, the thin-section did not cut through a pore to confirm



**Figure 2.5** — Microstructure of UWBM 102923. A) View of the smooth external surface of the eggshell. The black arrow points to a texture within the upper portion of the continuous layer (CL). Surface angusticanaliculate pore. B) SEM radial section of mammillary layer (ML) showing tabular structure of the calcite. Surface of the eggshell at the top of the image. C) Close up of squamatic of the eggshell at the top of image. D) Radial thin section under plane polarized light. Line indicates the boundary between the CL and ML. E) Radial thin section under crossed polarized light. Extinction patterns indicate the presence of crystalline prisms. Black line distinguishes the CL and ML boundary. Scale bars equal 500  $\mu$ m (A), 10  $\mu$ m (B), 40  $\mu$ m (C), and 200  $\mu$ m (D and E).

narrow pore canals. The mammillary layer is relatively thin, averaging 0.15 mm. Some nucleation sites are visible in thin section (Fig 2.5D) and the mammillary cones are spaced an average of 0.12 mm

apart measured from center-to-center from the radial thin section. A radial-tabular ultrastructure is visible in both SEM and thin-section in the mammillary layer, the latter where radial ultrastructure is more easily observed (Fig 2.5B, D).

The boundary between the mammillary and continuous layers is indistinct and slightly undulating and is marked by the absence of vertical lines indicating shell units in thin-section.

The mammillae are well formed. In SEM, the transition between the layers is designated by the change from tabular to squamatic ultrastructure. The squamatic texture continues from the top of the mammillary layer to the top of the eggshell, with no signs of an external layer, usually visible as densely packed vertical calcite crystals in SEM and a color change (more translucent) in thin-sections of avian eggs (Jackson et al., 2013; Jackson and Varricchio, 2010; Lawver and Boyd, 2018; Mikhailov, 1991; Varricchio and



Figure 2.6 — Plot of  $\delta^{13}$ C and  $\delta^{18}$ O in Bridger Formation eggshell. J1 and J2 refer to the two main jackets collected.  $\delta^{13}$ C are converted values.

Barta, 2015). Wedge-shaped prismatic units are visible in the continuous layer under polarized light, but are hidden under plain light by the squamatic texture.

Data for the ten eggshell samples for isotopic analysis is summarized in Table 2.1 and Figure 3.6. Viewing the eggshell samples with SEM, there appeared to be very little diagenetic alteration as microscopic and ultrastructural features were visible. Both  $\delta^{18}$ O and  $\delta^{13}$ C ranges are relatively narrow, with a standard deviation of less than 1‰. The values of  $\delta^{18}$ O vary from -3.3‰ to -5.8‰ (Table 2.1) across the two jackets with an average of -4.7 ± 0.5‰. When split into the two jackets, the oxygen data averages to -4.9 ± 0.5‰ for J1 and -4.4 ± 0.7‰ for J2. After applying a fractionation of -16‰ calculated from the diet of carbohydrate feeders

(Sarkar and Bhattacharya, 1991), the  $\delta^{13}$ C signatures vary by a similar amount to the  $\delta^{18}$ O values, from -23.0‰ to -25.2‰ with an average of -24.2 ± 0.6‰. Separating J1 and J2, the average  $\delta^{13}$ C values are -24.5 ± 0.46‰ and -23.8 ± 0.5‰, respectively.

The results of the XRD analysis are represented by Figure 2.7. Sample N2.2 was used to represent the signatures of all ten isotopic powdered samples. All of the samples showed a good match to CaCO<sub>3</sub> in the magnesium-rich calcite phase (Entry No. 96-900-1298; Putz and Brandenburg). The main peak appeared at 29.6 20. Based on the XRD results and the lack of a significant amount

Sample ID	$\delta^{13}C$	δ <sup>13</sup> C	δ <sup>18</sup> Ο	
Sample ID	VPDB	VPDB Food	VPDB	
N1.1	-8.5	-24.5	-5.0	
N1.2	-9.2	-25.2	-4.3	
N1.3	-8.7	-24.7	-5.0	
N1.4	-7.7	-23.7	-5.8	
N1.5	-8.3	-24.3	-4.8	
N2.1	-8.1	-24.1	-4.7	
N2.2	-7.5	-23.5	-3.8	
N2.3	-8.2	-24.2	-5.5	
N2.4	-7.0	-23.0	-3.3	
N2.5	-8.4	-24.4	-4.6	
Ave for Nest 1	8.5	-24.5	-5.0	
Ave for Nest 2	7.8	-23.8	-4.4	
σ for N1	0.5	0.5	0.5	
σ for N2	0.5	0.5	0.8	
Ave for all	-8.2	-24.2	-4.7	
$\sigma$ for all	0.6	0.6	0.7	

Table 2.1 — Oxygen and carbon isotope				
values from Bridger Formation eggshell. The				
food column is calculated from a -16‰				
fractionation (Sarkar and Bhattacharya, 1991).				

of diagenetic calcite on the eggshell samples in SEM and thin-section, diagenesis has not significantly altered the isotopic composition of the material.

# 2.6. Systematic paleontology

Oofamily MEDIOOLITHIDAE Kohring and Hirsch, 1996

Oogenus Duoolithus oogen. nov.

Type Oospecies – Duoolithus bridgerensis

Etymology – *Duoolithus* comes from Latin 'duo', meaning two, referring to the two structural layers, and the Greek term 'oolithus', meaning 'egg stone.'

Diagnosis – Within Medioolithidae, *Duoolithus* oogen. nov. is diagnosed as follows: 1) two structural layers consisting of the continuous and mamillary layers; 2) smooth surface; 3) eggs slightly asymmetric in shape; 4) shell thickness of 0.3-0.6 mm.



**Figure 2.7** — Representative eggshell X-ray diffraction profile (sample N2.2). Vertical axis is count rate; horizontal axis is degrees- $2\theta$ . Vertical bars correspond to predicted  $2\theta$  peaks and their intensities for a high-Mg calcite standard (COD No. 96-900-1298).

Oospecies – Duoolithus bridgerensis oosp. nov.

Holotype – UWBM 118216, two partial eggs, one being about half of a complete egg.

Additional Material – Six additional partial eggs (UWBM 102922, 102924, 102925, and 118217) in various conditions of completeness from the same locality.

Etymology – After the Bridger Formation in which the material was found.

Distribution - "Freya's Field" east of Lyman in Uinta County, Wyoming. Eocene (Br2

NALMA), Bridger Formation.

Diagnosis – An indistinct and slightly undulating continuous and mammillary layer boundary; a smooth outer surface with angusticanaliculate pores; mammillary layer to total shell thickness ratio of 1:4; shell thickness of 0.3-0.6 mm; egg size of 5.40 x 5.87 cm.

Differential Diagnosis – Medioolithid eggshell material that differs from all other oospecies in the following unique combination of characters: most complete egg size of 54.0 x 58.7 mm; smooth outer shell surface; eggshell thickness between 0.3 - 0.6 mm (N = 11) composed of two structural layers; indistinct and slightly undulating contact between continuous and mammillary layers; and a 1:4 mammillary to total eggshell thickness ratio.

Description – *D. bridgerensis* is the most similar to two oospecies, *Medioolithus geisltalensis* and *Microolithus wilsoni* (Table 2.2) within the Medioolithidae oofamily (Jackson et al., 2013; Kohring and Hirsch, 1996). Kohring and Hirsch (1996) describe *Medioolithus geisltalensis* as a smooth, spherical egg (axis 90 mm) with an eggshell thickness of ~ 0.8-1.0 mm. *Microolithus wilsoni* eggs are also smooth with a shell thickness of 0.6 mm and a minimum egg size of 30 mm x 37 mm (Jackson et al., 2013). The thickness of the mammillary layer to the continuous layer is 1:3 in the three oogenera (Jackson et al., 2013; Kohring and Hirsch, 1996). Though similar, the main difference between *D. bridgerensis* and the two other taxa is the number of ultrastructural

layers, with the former having two and the later having three (Table 2.2). In addition, *M. geisltalensis* occasionally has paired pore openings, which were not observed in *D. bridgerensis*.*M. wilsoni* also has a more abrupt transition from the mammillary to continuous layer.

The last Medioolithid taxa, *Incognitoolithus ramotubulus* (Hirsch et al., 1997), differs from *D. bridgerensis* by distinct grooves and pits on the surface and an "aepyornithid" pore pattern. *I. ramotubulus* is also thicker, from 1.27 – 1.43 mm, and is estimated to be an ellipsoidal egg 75 x 100 to 90 x 120 mm in size. The various taxa of *Ornitholithus* (Dughi and Sirugue, 1962) are distinct from *D. bridgerensis* by being much larger eggs (150 x 200 mm and 200 x 400 mm), thicker eggshell, a significantly different ML:TST (mammillary layer to total shell thickness) ratio, and most being ornamented (Donaire and López-Martínez, 2009; Dughi and Sirugue, 1962; Kohring and Hirsch, 1996; Table 2.2). Both *Metoolithus jacksonae* and *nebraskensis* differ from *D. bridgerensis* by the nodular surface ornamentation and three structural layers (Jackson et al., 2013; Lawver and Boyd, 2018).

There are several specimens that have not been designated as ootaxa that have been described (Table 2.2). A specimen designated AMNH 7499; HEC 98 from the Miocene Sheep Creek Formation of Nebraska (Hirsch and Bray, 1988) is similar to *Duoolithus bridgerensis*, however, the egg is smaller and the shell thickness is thinner overall. The number of structural layers of AMNH 7499 is difficult to determine from the published images. Hirsch and Bray, (1988) tentatively assigned the specimen, along with SDSM 2558; HEC 25 and SDSM 248; HEC 15 from the Oligocene White River Group, as belonging to a strigiform based on the spherical egg shape, similarity in ultrastructure, and the presence of skeletal material nearby in the Batesland Formation of South Dakota (Miller, 1944). The two SDSM specimens differ from modern owl eggs in that they have surface ornamentation.

					Thickness			
Ootaxon/Specimen	Location	Layers	Pores	Egg size (mm)	(µm)	Surface	ML:TST	Reference
Miocene-Sheep Creek Formation								
AMNH 7499; HEC 98 - Strigiformes	Nebraska	_	_	44 x 47	340-400	S	_	1
Oligocene-Brule Formation								
Metoolithus jacksonae	North Dakota	3	?	10.7 x 8.5**	650-900	N, U	1:2.70-1:2.75	2
SDSM 2558; HEC 25 - Strigiformes	South Dakota	_	_	34.2 x 34.3**	630	Ν	_	1
SDSM 248; HEC 15 - Strigiformes	South Dakota	_	_	43.7 x 45.3	710-760	Ν	_	1
GCVP 3610, 3682, 3958 - Limpkin	South Dakota	_	_	58.11 x 44.13	_	S	_	3
Eocene-Bridger Formation								
Duoolithus bridgerensis	Wyoming	2	NB?	54 x 58.7	300-600	S	1:04	this paper
Eocene-Geiseltal								
Medioolithus geiseltalensis - Crane?	Germany	3	NB	90 x 90	700-1000	S	1.3-1.4	4, 5
Eocene-Chadron Formation								
Metoolithus nebraskensis	Nebraska	3	?	45 x 60	750-900	N, U	1:04	6
Eocene-DeBeque Formation								
				75 x 100; 90 x				
Incognitoolithus ramotubulus	Colorado	2	В	120	1140-1430	S	1:3.5-1:3.8	4, 5
UCM 47531; HEC 302	Colorado	2	NB	35 x 53	450-500	S	?	4
Eocene-London Clay								
	United	2	2		206	c		7
Feeene Willwood Fermation	Kinguoin	5	ŗ	_	290	3	_	7
Adiana alithus wilsoni	Muchaing	2		20 x 272	600	c	1.4	C
	Wyoming	с с		50 X 57 !	700 800	s c	1.4	0
	Wyoming	5 2		04 X 89	700-800	s c	1.4	4
USNM 336564 - Paracathartes	wyoming	3	ŗ	65 X 45"	-	5	ſ	8
howardae	Wyoming			60 x 85		S		8
Paleocene-Fort Union Formation	, 0	_	-		_		—	
PU 16961 - Lithornis celetius	Montana	3	?		415	S		8
Paleocene-Tremp, Other				_			_	
Ornitholithus	France, Spain	2	В	200 x 400	1600-3000	S, R, N	1:12 - 1:6	5, 9, 10

**Table 2.2** — Record of avian eggs from the Paleogene of North America and Europe.

Modified from Jackson et al. (2013). Abbreviations and symbols: B, branching; ML, mammillary layer; N, nodes; NB, nonbranching; R, ridges; S, smooth; TST, total shell thickness; U, undulating; \* unprepared egg, \*\* fragment. References: 1) Hirsch and Bray (1988); 2) Lawver and Boyd (2018); 3) Chandler and Wall (2001); 4) Hirsch et al. (1997); 5) Kohring and Hirsch (1996); 6) Jackson et al. (2013); 7) Grellet-Tinner and Dyke (2005); 8) Houde, (1988); 9) Dughi and Sirugue (1962); 10) Donaire and López-Martínez (2009).

Other non-ootaxa specimens differ from *D. bridgerensis* by having three ultrastructural layers (USNM 336570, USNM 336564, PU 16961, MNING 2005-1; Grellet-Tinner and Dyke, 2005; Houde, 1988), larger eggs, or more elliptical in shape (USNM 336564, GCVP 3610, 3682, 3958; Chandler and Wall, 2001; Houde, 1988). Beyond overall size estimates and overall porosity, GCVP 3610, 3682, and 3958 do not have microstructural information to assist with identification, though the authors suggest the macrofeatures look most similar to modern limpkins and associated the eggs to the fossil taxon *Badistornis aramus*. Several other taxa have also been assigned to various *Lithornis* species (USNM 336570; PU 1696; MNING 2005-1) and to *Paracathartes howardae* (USNM 336564) based on their proximity to skeletal material (Grellet-Tinner and Dyke, 2005; Houde, 1988; Table 2.2). Without embryonic material these designations to avian taxa are tentative. Two additional eggs (UCM 47602; HEC60-62, UCM 47531; HEC 302) described by Hirsch et al., 1997 have two structural layers, but are ovoid in shape and have a prismatic basic type rather than ornithoid.

# 2.7. Discussion

#### 2.7.1. Identity of the egg layer

Several microstructural and ultrastructural features designate *Duoolithus bridgerensis* as theropod, such as squamatic ultrastructure and at least two zones with a clear boundary. Squamatic texture is the main diagnostic characteristic of the ornithoid basic type. Other features include: layers composed of calcite, confirmed by XRD analysis; and the mammillary cones are built up by radial or radial and tabular ultrastructure and have blocky calcite cleavage (Grellet-Tinner and Chiappe, 2004; Grellet-Tinner and Dyke, 2005; Jackson et al., 2013; Jin et al., 2010; Lawver et al., 2016; Lawver and Boyd, 2018; Mikhailov, 1997a; Zelenitsky et al., 2002). In addition, the eggshell material was recovered from Eocene sediments, ruling out a non-avian dinosaurian source for *D. bridgerensis*.

The calculated clutch weight based on the most complete egg of 5.40 cm by 5.87 cm is 369.9 g, or three to four eggs in a clutch. This suggests that there are potentially two clutches of eggs at the locality, and each jacket represents the predated remains of an individual nest. However, it is difficult to assess the true number of eggs due to the fracturing. Based on half eggs there appears to be at least eight eggs between the two nests.

The Green River Basin is host to several different orders of avian groups (Table 2.3). Mikhailov (1997b) created an atlas of SEM images of modern avian groups, revealing that a few orders are similar to *Duoolithus bridgerensis*. Ciconiiformes (stork-like birds) eggshell is characterized by a ML:CL (mammillary layer to continuous layer) of 1:2.5 to 1:3 and an external zone that only differs from the squamatic zone in the fine characteristics of the squamatic texture (weakly scaled in the external zone and looser and larger scales in the squamatic zone). The eggshell thickness of this group ranges from 0.1 - 1.5 mm with the fossil eggshell falling within

Order	Taxon	Formation	Reference
Anseriformes	Presbyornis pervetus	GR, W	Wetmore, 1926
Apodiformes	Eocypselus rowei	GR	Ksepka et al., 2013
Caprimulgiformes	Prefica nivea	GR	Olson, 1987
Caprimulgiformes	Fluvioviridavis platyrhamphus	GR	Mayr and Daniels, 2001
Charadriiformes?	Nahmavis grandei	GR	Musser and Clarke, 2020
Ciconiiformes	Juncitarsus gracillimus	B <i>,</i> GR	Olson and Feduccia, 1980
Ciconiiformes	Uintornis lucaris	B <i>,</i> GR	Marsh, 1872
Ciconiiformes	Uintornis marionae	B <i>,</i> GR	Feduccia and Martin, 1976
Ciconiiformes	Eobucco brodkorbi	B <i>,</i> GR	Feduccia and Martin, 1976
Ciconiiformes	Vadaravis brownae	GR	Smith et al., 2013
Coliiformes	Celericolius acriala	GR	Ksepka and Clarke, 2010
Coliiformes	Anneavis anneae	GR	Houde and Olson, 1992
Coraciiformes	Botauroides parvus	B <i>,</i> GR	Shufeldt, 1915
Coraciiformes	Primobucco mcgrewi	GR	Brodkorb, 1970
Coraciiformes	Paracoracias occidentalis	GR	Clarke et al., 2009
Calliformos	Amitabba urbeinterdictoncie		Gulas-Wroblewski and Wroblewski,
Gainformes	Annitabna arbsinteraictensis	D, GK	2003
Galliformes	Gallinuloides wyomingensis	GR	Mayr and Weidig, 2004
Gastornithiformes	Gastornis sp.	GR	Grande, 2013
Gruiformes	Eocrex primus	W	Wetmore, 1931
Gruiformes	Geranodornis aenigma	В	Cracraft, 1969
Gruiformes	Palaeophasianus meleagroides	В	Shufeldt, 1913
Gruiformes	Messelornis nearctica	GR	Hesse, 1992
Gruiformes	Aletornis bellus	В	Wetmore, 1940
Gruiformes	Aletornis gracilis	В	Wetmore, 1940
Gruiformes	Aletornis nobilis	В	Wetmore, 1940
Gruiformes	Aletornis pernix	В	Wetmore, 1940
Gruiformes	Aletornis venustus	В	Wetmore, 1940
Leptosomiformes	Plesiocathartes wyomingensis	GR	Weidig, 2006
Leptosomiformes	Plesiocathartes major	GR	Weidig, 2006
Lithornithiformes	Calciavus grandei	GR	Nesbitt and Clarke, 2016
Lithornithiformes	Pseudocrypturus cercanaxius	GR	Houde, 1988
Musophagiformes	Foro panarium	GR	Olson, 1992
Passeriformes	Zygodactylus grandei	GR	Smith et al., 2018
Passeriformes	Eozagodactylus americanus	GR	Weidig, 2010
Passeriformes	Eofringillirostrum boudreauxi	GR	Ksepka et al., 2019
Pelecaniformes	Limnofregata azygosternon	GR	Olson, 1977
Pelecaniformes	Limnofregata hasegawai	GR	Olson and Matsuoka, 2005
Pelecaniformes	Limnofregata hutchisoni	GR <i>,</i> W	Stidham, 2015
Piciformes	Neanis kistneri	GR	Feduccia, 1973
Psittaciformes	Cyrilavis olsoni	GR	Ksepka et al., 2011
Psittaciformes	Cyrilavis colburnorum	GR	Ksepka et al., 2011
Psittaciformes	Avolatavis tenens	GR	Ksepka and Clarke, 2012
Psittaciformes	Tynskya eocaena	GR	Mayr, 2000
Strigiformes	Protostrix saurodosis	В	Wetmore, 1937
Strigiformes	Protostrix leptosteus	В	Wetmore, 1937

Table 2.3 — Eocene avian fauna of the Green River Basin. B – Bridger; GR – Green River;

W-Wasatch.
this range. The order Gruiformes (rails, coots, cranes, bustards, etc.) also have similar eggshell characteristics, such as a ML:CL of 1:2 to 1:3.5 and an obscure external zone, though more distinct than in Ciconiiformes. The thickness of the eggshell in Gruiformes is also thinner (0.08 – 0.4 mm) and there is more variation in the appearance of prisms and the thickness of the external layer. Though Ciconiiformes appears to be the most similar to *D. bridgerensis*, without embryonic material associated with the eggs our interpretation of the nester remains tentative.

#### 2.7.2. Stratigraphic placement, sedimentology, and paleoenvironment of egg site

High-resolution stratigraphy and marker bed tracing suggest Freya's Field is likely in the upper part of the Lower Bridger B. Depending on actual dips in the area, the highest fossil occurrences of the site are between 10 and 27 meters below the Church Butte Tuff. The fossil site is indubitably well above the "Lower Red Bed" which is also a tuff and occurs ~33 meters above the Lyman Limestone, the base of Bridger B (Alexander and Burger, 2001; Murphey and Evanoff, 2011). The fossiliferous sequence of Freya's Field is between 8-20 m thick depending again on whether the beds are more horizontal or actually dipping to the southeast in the area.

The eggs are located within a fluvial overbank deposit based on the fine sediments and terrestrial taxa located near the site. A large channel sandstone underlies a thick sequence of mudstone in which the eggs are located. Thin ribbons of sandstone above the locality are indicative of crevasse-splay deposits. The sandstones show relatively few sedimentary structures such as crossbedding. The mudstone is greenish-gray in color, suggesting an anoxic and more consistently wet environment (Nadon, 1993; Potter et al., 2005). Deposition of floodplain mudstones allowed for the eggshell material to remain in-situ by slow accretion of sediment.

In addition to the sedimentological evidence of little transport of the eggs, the orientations of the eggshell agree with this interpretation. Studies of modern nesting localities of

birds (Ferguson et al., 2018; Hayward et al., 2011, 2000; McGrath et al., 2020; Wang et al., 2013), crocodilians (Ferguson et al., 2017), and tortoise nests (Jackson et al., 2015) suggest that eggshell orientation correlates to the manner in which the fragments accumulated. In particular, a predominance of CU eggshell is characteristic of hatched or predated nesting sites, whereas more CD eggshell suggests trampling by young, wind, or water transport. Chick trampling produces a ratio of 40:60, whereas fluid transport generates skewed CD ratios of at least 80% (Hayward et al., 2011; Imai et al., 2015). A ratio of 59:41 CU to CD eggshell fragments and partial eggs correlate with previous studies of hatched or predated nesting sites which are typically between 60-70% CU.

Very few skeletal elements are associated with the egg material. The bones themselves also show no signs of weathering or abrasion. Skeletal elements, when exposed to the surface for long periods of time, become cracked and spall sections of the outer cortical bone (Behrensmeyer, 1978; Behrensmeyer et al., 2003; Tappen, 1994). The amount of time it takes for bones to degrade depends on the size and robustness of the elements, and the climate. Here, the elements belong to small mammals and birds and therefore suggests the elements were not exposed for extended periods of time on the surface. The avian elements do have longitudinal fractures and fractures in the cortical bone of birds does occur more rapidly than in mammals, however, smaller elements generally stay intact longer (Behrensmeyer et al., 2003) and the other elements showed no signs of weathering. We interpret the fragmented avian elements to have occurred pre-deposition. The lack of skeletal elements is typical of nesting animals with semiprecocial or precocial young (Ferguson et al., 2018, 2017; Jackson et al., 2015; Wang et al., 2013). A lack of skeletal elements at the Bridger site, as well as most of the elements belonging

to mammals, rather than the nesting animal, leads us to believe that the site is reflective of a semiprecocial reproductive mode.

The presence of longitudinal (from pole to pole) half eggs suggests those particular eggs did not successfully hatch. Hatched eggs are typically distinguishable by the removal of the larger, more rounded end by the hatching chick, whereas depredated eggs are generally broken on the side of the egg (Hayward et al., 2000; Marks and Hall, 1992; Rearden, 1951; Sooter, 1946). The spread within each jacket of the eggshell material also suggests predation, as one would expect the eggs to be closer together if still being tended to.

Isotopic analyses of  $\delta^{18}$ O and  $\delta^{13}$ C is often used to determine information regarding the water consumed and the diet of animals. Isotopic values of eggshell material are linearly related to the water consumed by the female (Erben et al., 1979; Folinsbee et al., 1970; Schaffner and Swart, 1991; Sarkar and Bhattacharya, 1991). Studies of captive Mallards, quail, and falcons suggest that shell carbonate values reflect the integrated diet of the span of 3-5 days prior to shell formation (Hobson, 1995). Therefore, the average  $\delta^{18}$ O value of -4.65 ± 0.508‰ reflects the fluids the females were consuming near the egg locality. Morrill and Koch, 2002 determined that the  $\delta^{18}$ O of aragonitic unionid shell from the Laney Member of the Green River Formation, which interfingers with the lower Bridger Formation, is reflective of low elevation continental precipitation at -7.42  $\pm$  1.9‰. The unionid sample was collected from limestone and likely represents a lacustrine environment. This value is slightly lighter relative to the egg samples and likely reflects a mixture of water sources such as lakes and rivers. Values of  $\delta^{13}$ C from fossil amber samples during the middle Eocene are  $-21.7 \pm 0.4\%$  (Tiger Mountain Formation, Washington; Tappert et al., 2013). The variation in  $\delta^{13}$ C signatures in amber and resin samples seen throughout geologic time generally correlates with marine  $\delta^{18}$ O and indicates that resins that formed during higher global temperatures are more enriched in <sup>13</sup>C. The variation from the Tiger Mountain sample and the Bridger Formation eggshell sample  $\delta^{13}$ C values (-24.15 ± 0.6‰) may be due to a difference of geologic age and location on the continent. The depleted signature of the eggshell, relative to potential marine values is more consistent with a diet composed of C<sub>3</sub> plants. The ratio between <sup>13</sup>C/<sup>12</sup>C of terrestrial C<sub>3</sub> plants is ~-26.5‰ (Craig, 1953), whereas C<sub>4</sub> plants average ~-12.6‰ (Vogel, 1978). A heavier  $\delta^{13}$ C signature from our samples may represent a higher trophic level or natural variation in C<sub>3</sub> plants. Plants that are categorized as C<sub>4</sub> do not evolve until the Oligocene (Sage, 2004) and therefore are not expected to contribute to  $\delta^{13}$ C values.

### 2.8. Conclusion

UWBM 102922-102925, 118216 and 118217 was collected from an olive-gray mudstone in the Blacks Fork Member of the Eocene Bridger Formation in southwestern Wyoming. Highresolution stratigraphy and GPS data places the fossiliferous horizon ~9-22 m below the Church Butte Tuff, with the range due to uncertainty in the dip of the stratigraphic units. The site consists of two main concentrations of eggshell over an area of 2 m<sup>2</sup> with eight partial eggs. The site is interpreted as two clutches originally consisting of approximately five and three eggs. The eggshell is composed of high-Mg calcite with a continuous and mammillary layer with an indistinct and slightly undulating boundary between the two. The mammillary layer and total shell thickness ratio is 1:4 with a shell thickness of 0.3-0.6 mm. These features make *Duoolithus bridgerensis* oogen and oosp. nov. distinguishable from previously described eggs. The distribution of eggshell and presence of partial eggs, some of which are cut from pole to pole, are interpreted to be predated rather than hatched. The lack of fossil avian bones and presence of

mammalian bones is suggestive of a semiprecocial reproductive mode. The micro- and ultrastructural features of the eggshell is most similar to the avian orders Ciconiiformes and Gruiformes, both of which are represented by several species from the Wasatch, Green River, and Bridger formations. The documentation of additional ootaxa, particularly from the Paleogene, leads to new features similar to modern avian taxa and therefore may aid in understanding the evolution of avian reproductive biology.

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#### Chapter 3: Investigating fossil eggshell porosity using micro-CT scans

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# 3.1. Abstract

Vertebrates that lay rigid-shelled eggs construct a variety of nests, generally characterized as open, cavity, or mound nests. Functional porosity of amniotic egg shell is correlated with nest type in extant archosaurs and has been used to infer the nesting behaviors of extinct taxa. Eggs that are less porous are characterized as belonging to open nests, and more porous egg belong to cavity or mound nests. Traditional methods of pore visualization such as thin-sections or scanning electron microscopy, only view an object in 2D and require destructive practices. Here, we image the porosity of 13 eggshell fragments via models created from micro-CT imagery. Fragments of shell were collected from a gecko, tortoise, five bird, and six crocodylian species. Three-dimensional data were obtained at a resolution of 8.15-25.18 µm and rendered and processed using PerGeos and ZBrush software. Eggs in open nests have functional eggshell porosity ranges of 0.1-0.8% total volume. Only Gekko gecko and Anas platyrhynchos have total porosities higher than 2% overall volume. Mound and cavity nesting crocodylian eggs have functional eggshell porosity ranges of 0.1-3.3% and total porosities of 2.9-16.1% of the total volume, however, despite being a cavity nester, Malacochersus tornieri has lower total and functional pore percentages. The internal porosity comprises most of the eggshell porosity, and only a small percentage is connected from outside to inside the shell. The internal porosity arises from the spaces between mineralized

egg units, with the exception of *Gekko gecko*. The functional value of the internal porosity is yet unknown, but may improve properties of shell insulation and retention of water vapor.

### **3.2. Introduction**

Amniotes are a diverse clade that use a variety of nesting strategies in continental settings that are classified as open nests and closed nests (these include cavity and mound nests) (Brazaitis and Watanabe, 2011; Hansell and Overhill, 2000; Murray et al., 2020). Open nests are characterized by eggs being exposed to the environment (Hansell and Overhill, 2000) and are often incubated by the parent, as in birds. Cavity nests are made by digging a hole in sediment where eggs are deposited and buried (Jackson et al., 2015); and mound nests are formed by placing vegetation and sediment over a clutch of eggs (Brazaitis and Watanabe, 2011; Hansell and Overhill, 2000). By observing the behaviors of extant relatives, inferences have been made for nesting behaviors in extinct groups (Ferguson et al., 2018, 2017; Tanaka et al., 2015; Varricchio et al., 2015; Wiemann et al., 2017). However, application to extinct taxa is limited due to a lack of preserved nest structures, pliable eggs, and nesting materials (Carpenter, 1999; Deeming, 2016). Eggshell micro- and ultrastructure represents a method of investigating nesting ecology in both fossil and extant taxa.

The study of eggs and eggshell micro- and ultrastructure, including porosity and surface ornamentation, has a deep literature (e.g., Mikhailov et al., 1996; Hirsch, 1996). We use the classification scheme of Mikhailov et al. (1996) in this study. Several authors have related eggshell features to open and closed nest strategies in modern (Hirsch, 1996; Mikhailov et al., 1996) and extinct amniotes (Deeming, 2006; Jackson et al., 2008; Sabath, 1991; Seymour, 1979; Tanaka et al., 2015; Varricchio et al., 2013). For example, animals that construct open nests lay eggs having fewer pores, whereas eggs in cavity or mound nests tend

to contain more pores (Packard et al., 1979), or thinner eggshell relative to mass (Seymour and Rahn, 1978).

Traditionally, the pores of eggs are described using a variety of methods such as radial and tangential thin-sections, scanning electron microscopy (SEM) (Mikhailov, 1997; Packard et al., 1989), and casting (Tyler, 1965, 1964, 1957; Tyler and Simkiss, 1959). Thinsections and SEM imagery are rapid and reliable, but provide a limited view of the eggshell and pores, and requires sample destruction or gold plating. Though casting reproduces some of the eggshell porosity in 3D, it is limited to porosity that connects through the shell, and excludes "internal porosity," defined here as the pores that do not connect through both surfaces of the eggshell.

Here, we use micro-CT scans of modern taxa to directly observe the total 3D porosity of eggshells, including representatives of open and closed nesting strategies. We apply this method to a number of animals that lay rigid-shelled eggs, including birds, crocodylians, a tortoise, and a gecko to examine patterns between nesting type and habitat among these taxa.

Taxa		Nest Type	Institution	
Geckos	Gekko gecko	open	Montana State University	
Turtles	Malacochersus tornieri	cavity	Montana State University	
Birds	Anas platyrhynchos domesticus	open	Montana State University	
	Anser anser domesticus	open	Montana State University	
	Eudromia elegans	open	Montana State University	
	Meleagris gallopavo	open	Private Donation	
	Struthio camelus	open	Montana State University	
Crocodylians	Crocodylus intermedius	cavity	St. Augustine Alligator Farm	
	Crocodylus niloticus	cavity	St. Augustine Alligator Farm	
	Crocodylus porosus	mound	Billabong Sanctuary - Australia	
	Crocodylus siamensis	mound	St. Augustine Alligator Farm	
	Melanosuchus niger	mound	St. Augustine Alligator Farm	
	Osteolaemus tetraspis	mound	St. Augustine Alligator Farm	

Table 3.1 — List of taxa modeled in this study, their nesting habit, and providing

institution.

### 3.3. Methods

### 3.3.1. Sample selection

Eggshell material selected for this study include taxa across the spectrum of the clade of Amniota that lay rigid shelled eggs. This includes a gecko, a tortoise, crocodylians, and birds (Table 4.1). Pliable eggs are preserved less often in the fossil record and are therefore excluded in this study. The gecko and avian samples are from successfully hatched eggs, whereas the tortoise and crocodylian samples are from unsuccessful nests.

The species were selected to represent various clades of taxa that lay rigid shelled eggs and various nesting habitats such as tropical, desert, and montane. For example, the pancake tortoise *Malacochersus tornieri* is a cavity nester like some crocodylians, however; the species is endemic to Kenya and Tanzania in semi-arid deserts and savannas with the exception of a population in Zambia (Mwaya et al., 2018). The avian species selected are representative of orders near the base of the avian clade and are more closely related to nonavian theropods. The mass of the eggs was calculated by the formula:

$$M = 5.48 \times 10^{-4} \times L \times B^2$$

where L is the length and B is the breadth of the egg (Hoyt, 1979). For eggs where a length and breadth could not be measured, an average mass was used from the literature. Table 4.2 provides a list of variables and equations.

# 3.3.2. Micro-CT scanning and image segmentation

Variable	Definition and unit		
В	Widest breadth of egg (mm)		
L	Egg length (mm)		
Ls	Eggshell thickness (mm)		
М	Egg mass (g)		
Fp	Functional pores		
T <sub>p</sub>	Total pores		
Equation	Reference		
$M = 5.48 \times 10^{-4} \times L \times B^2$	Hoyt, 1979		

**Table 3.2**—List of variables used in this study and the corresponding unit.

Eggshell samples (~1 cm<sup>2</sup> in size) are imaged by micro-CT with a voxel resolution of  $8.15 - 10.28 \ \mu\text{m}$ . As an exception, half of the egg of *Crocodylus porosus* (~14.2 cm<sup>2</sup>) was scanned at 25.18  $\mu$ m resolution. Shell samples were scanned by a SkyScan1173 Micro-CT system running at 40-60 kV and 120-200  $\mu$ A. The specimens were mounted to a rod vertically by Play-Doh to prevent movement during their 180° rotation with projections collected at approximately every 0.5°. Image stacks were rendered using PerGeos version 4.1. We segmented porosity using built-in thresholding functions following unsharp masking and preserve smoothing image filters to reduce noise (Fig 3.1.1). Two files were segmented for



**Figure 3.1** — Methodology sequence used in this study, e.g., ostrich egg. Step 1, render the eggshell and pores in PerGeos; Step 2, verify model alignment; Step 3, remove floating and improper data; Step 4, separate functional from non-functional pores.

each sample and exported as STL files; shell and total pores ( $T_p$ ). Using ZBrush 2022.05, the 'shell' and the 'pores' models were aligned (Fig 3.1.2), the data were cleaning included removing noise from thresholds, ~50 voxels in size (Fig 3.1.3). This process provides a clean pores model and shell model to identify functional ( $F_p$ ), or through-going pores, and internal pores ( $T_p$ - $F_p$ ). A separate sub-tool of the functional pores is created after removal of the internal pores (Fig 3.1.4).

#### **3.4. Description of Eggshell and Porosity**

# 3.4.1. Gekko gecko

The eggshell of *Gekko gecko* is 0.277 mm thick with a domed to lenticular shaped dispersituberculate ornamentation ranging in size from 0.054 - 0.193 mm in length and 0.014 - 0.064 mm in height (Fig 3.2A). The pores are bowl-shaped structures and are large relative to the size of the sample piece, taking up 2.2% of the overall volume (Table 3.3). Out of the 12 pores observed in the sample, only two connect to the outside of the eggshell via a short node at the apex of the bowl-shape (Fig 3.2A, Fig 3.4). The functional pores account for 0.6% of the overall volume of the sample.

### 3.4.2. Malacochersus tornieri

*Malacochersus tornieri* has the thinnest eggshell out of the species sampled at 0.206 mm thick. The surface of the eggshell is marked by small, unevenly dispersed circular divots. Occasionally the divots merge and create a track of circles (Fig 3.2B). The functional pores are generally pear-shaped with a round to irregular base (Fig 3.3). Additional pores are spherical to lenticular in shape. The internal pores (0.37% overall volume) originate from the egg interior and do not extend beyond the outer half of the eggshell. Most internal pores are gaps in the rugged interior texture of the eggshell such as between shell units. The functional pores only account for 0.03% of the volume of the eggshell sample (Table 3.3)



Figure 3.2 — 3D representation of *Gekko gecko* (A) and *Malacochersus tornieri* (B) eggshell and pores. From top to bottom; eggshell in slightly oblique plan view, pores in slightly oblique plan view, and a cross section of the pores. Connected pores represented by an orange color. Scale bar equals 1 mm.

# 3.4.3. Aves

The surface of *Anas platyrhyncos* eggshell has a microscopic rough texture (Fig 3.4A) and a shell thickness of 0.325 mm (Table 3.3). The functional pores of *A. platyrhyncos* are relatively straight and occasionally are wider near the eggshell surface or near the shell membrane (Fig 3.3) and account for 0.4% of the overall volume. Internal porosity (2.7%) occurs between mammillary bodies and often creates a lateral network of pore space that occasionally connect to functional pores. These internal pores do not extend beyond the ouer half of the eggshell in radial view, and are spherical to anastomosing in shape. The laterally connected internal pores favor a direction from bottom-left to top-right in Figure 3.4A.

The *Anser anser* eggshell sample is 0.581 mm thick (Table 3.3) and the surface of the shell is smooth (Fig 3.4B). The functional pores are 0.4% of the overall volume and are



Figure 3.3 — Comparison of the functional pores of the gecko, tortoise, avian, and

crocodylian species. Each group is represented by a silhouette and color.

slightly wider near the eggshell surface forming a narrow trumpet shape (Fig 3.3). Internal pores connect laterally between mammillary bodies and often connect functional pores together (Fig 3.4B). The internal porosity does not extend beyond the outer half of the shell. Laterally connected pores tend toward bottom-left to top-right in Fig 3.4B.

The surface of *Eudromia elegans* eggshell is smooth (Fig 3.5A) and has a shell thickness of 0.225 mm (Table 3.3). The functional pores are tubular to trumpet shaped (Fig 3.3) and account for 0.4% of the overall sample volume. The internal pores are less common in the *E. elegans* sample than the previously described eggshell specimens with less lateral connectivity (Fig 3.5A). The laterally connected pores are oriented in a direction similar to *A. platyrhyncos*, and *A. anser*. Similarly, the internal pores do not pass through the outer half of the eggshell.

*Meleagris gallopavo* eggshell is also smooth and 0.407 mm thick (Table 3.3). The *M. gallopavo* pores are more globular near the external surface and are less narrow in the center of the eggshell (Fig 3.3). Six of the observed *M. gallopavo* pores are linked together at the tops of the pores. The functional pores are 0.1% of the sample volume, with additional

Specimen	Nest Type	T <sub>p</sub> %	F <sub>p</sub> %	M (g)	L <sub>s</sub> (mm)
Gekko gecko	Open	2.2	0.6	-	0.277
Malacochersus tornieri	Cavity	0.4	0.03	19.7	0.206
Anas platyrhynchos	Open	3.1	0.4	83.7	0.325
Anser anser	Open	1.5	0.4	165.5	0.581
Eudromia elegans	Open	1.6	0.4	35.8	0.225
Meleagris gallopavo	Open	1.6	0.1	111.9	0.407
Struthio camelus	Open	1.1	0.8	1465.0	2.11
Crocodylus intermedius	Cavity	2.9	0.1	67.5	0.347
Crocodylus niloticus	Cavity	11.4	0.3	90.5	0.536
Crocodulus porosus	Mound	16.1	3.3	111.6	0.343
Crocodylus siamensis	Mound	5.8	0.3	112.8	0.345
Melanosuchus niger	Mound	3.3	1.5	121.9	0.455
Osteolamus tetraspis	Mound	5.4	0.9	76.8	0.295

Table 3.3 — List of porosity characteristics per sample. Percentages represent a

percentage of total volume of the eggshell sample.





**Figure 3.5** — 3D representation of *Eudromia elegans* (A), *Meleagris gallopavo* (B), and *Struthio camelus* (C) eggshell and pores. From top to bottom; eggshell in slightly oblique plan view, pores in slightly oblique plan view, and a cross section of the pores. Connected pores represented by an orange color. Scale bar equals 1 mm.

porosity increasing the value to 1.6%. Lateral connectivity of functional and internal pores is also observed and is aligned in a similar direction to *A platyrhyncos, A. anser,* and *E. elegans* (Fig 3.5B). Internal pores do not extend beyond the inner half of the eggshell.

The eggshell and pores of *S. camelus* are unique among the avian eggs in this study. The shell is comparatively thick at 2.1 mm (Table 3.3), and the shell surface is smooth. The functional pores create elaborate, outwardly branching pores originating at the interior of the shell and splitting into multiple smaller pores at the surface (Fig 3.3). The functional pores account for 0.8% of the overall volume of the sample (Table 3.3). Laterally branching of the internal pores is not as interconnected as in the other avian species. Unlike the other species, internal pores extend beyond from inner to outer half of the eggshell, with pockets of pore space occurring in the center of the eggshell with no branches (Fig 3.5C). The network of pores favors a specific direction of top-left to bottom-right in *S. camelus* when looking at plan view of the fragment.

#### 3.4.4. Crocodylians

Eggshell of *C. intermedius* is irregular and pitted on the surface with no distinct pattern (Fig 3.6A). The sample is 0.347 mm thick. Functional pores are pyramidal to conical in shape, with the narrow portion at the outer surface of the eggshell (Fig 3.3). These pores only account for 0.1% of the overall volume. Internal pores are similar in shape to the functional pores, but can also be irregular and branch laterally in between shell units (Fig 3.6A). The internal pores extend outward to just below the outer surface of the eggshell.

The *C. niloticus* sample is 0.536 mm thick (Table 3.3) and has a sagenotuberculate ornamentation (Fig 3.6B) consisting of low reticulate ridges  $0.041 \pm 0.007$  mm in height. The anastomosing texture of the ornamentation appears to follow a specific direction, from top-left to bottom-right in plan view. The functional pores are pyramidal to conical in shape and expand irregularly in the direction of the shell interior (Fig 3.6B). These pores account for

0.3% of the overall sample volume. Internal porosity is conical to irregular-shaped and often branches laterally in two main directions, bottom-left to top-right and top-left to bottom-right in plan view. Internal pores often extend outward into the outer half of the eggshell.

The sample for *C. porosus* is the only one where half an egg was imaged. Shell thickness averaged 0.343 mm. The egg texture is smooth with the variation in functional pore density visible in the model (Fig 3.7A), with higher densities near the equator. The functional pores are pyramidal to conical in shape (Fig 3.3) and consist of 3.3% of the imaged volume. Lateral branching of the pores often connects multiple pores together. However, most of the pore volume consists of the lateral branching of internal pores (16.1% total porosity; Table 3.3). Most of the connections are oriented from the top-left to bottom-right of the sample image in plan view. Most pores extend outward in to the outer half of the eggshell.

*C. siamensis* eggshell is smooth and 0.345 mm thick. As with other species in the genus, the functional pores are conical in shape (Fig 3.3) and account for 0.3% of the total sample volume (Table 3.3). Lateral branching is not as extensive as in the other species of *Crocodylus* (Fig 3.7B) and a preferred direction is not visible. The pores are situated between shell units, with most pores terminated just below the eggshell outer surface.

*Melanosuchus niger* eggshell has ornamentation similar in structure to *Caiman latirostris* (Fernández et al., 2013), comprising of lacunae and tower-like structures with thin bridges connecting the towers (Fig 3.8A). The towers generally have two layers that bridge adjoining towers and are rounded on the outermost surface. The ornamentation adds  $0.384 \pm$ 0.046 mm to the eggshell thickness with a total thickness of 0.455 mm (Table 3.3). The pores occur between the ornamentation. *M. niger* pores range from conical to globular in shape and have irregular branches. Lateral branching of the pores generally does not reach adjacent pores. Pores that make it through the shell are about twice the diameter of internal pores accounting for 1.5% of the total volume.







Figure 3.7 — 3D representation of *Crocodylus porosus* (A) and *Crocodylus siamensis* (B) eggshell and pores. Section A is a sample from a quarter egg with the pole at the top of the image. From top to bottom; eggshell in slightly oblique plan view, pores in slightly oblique plan view, and a cross section of the pores. Connected pores represented by an orange color. Scale bar equals 1 mm.



representation of Melanosuchus niger (A) and Osteolameus tetraspis (B) eggshell and pores. From top to bottom; eggshell in slightly oblique plan view, pores in slightly oblique plan view, and a cross section of the pores. Connected pores represented by an orange color. Scale bar equals 1 mm.

**Figure 3.8** — 3D

The eggshell of *Osteolameus tetraspis* is smooth on the surface (Fig 3.8B) and averages 0.295 mm thick (Table 3.3), the thinnest of the six species sampled. The functional pores are conical in shape (Fig 3.3) and consist of 0.9% of the total sample volume. Lateral branching of the pores is extensive with patches of functional and internal pores with lateral extensions. The branching of the pores is oriented in bottom-left to top-right and top-right to bottom-left directions in plan view. Internal pores commonly can extend to just below the eggshell external surface.

# **3.5. Discussion**

### 3.5.1. Eggshell morphology of open nesters

The open nesters in this research are represented by five avian species (Table 3.1) and *Gekko gecko*. The avian eggs have smooth external surfaces, though *A. anser* has a microscopically rough texture (Fig 3.4A). *Gekko gecko* eggshell is the only sample of open nesters with ornamentation. Despite the small size of *G. gecko* eggs, the eggshell is thicker than *E. elegans* (Table 3.3).

The shape of the functional pores as well as mass and eggshell thickness match those previously described for *S. camelus* (Ar et al., 1974; Tyler and Simkiss, 1959), *A. platyrhynchos, A. anser* (Tyler, 1964; Ar et al., 1974; Rokitka and Rahn, 1987), *M. gallopavo* (Ar et al., 1974; Rokitka and Rahn, 1987), and *E. elegans* (Ar et al., 1974; Board and Perrott, 1979; Tyler and Simkiss, 1959). The pores of *G. gecko* were described as retecanaliculate, or a series of funnel shaped channels that occur on the edges of calcitic concretions that are easily detached (Packard and Hirsch, 1989). Our sample was collected from a successfully hatched egg, therefore the pits may have been created from the dissolution of the concretions during incubation and would cause the pore space to be larger. The percentage of functional porosity ranges from 0.1-0.8%.

Our data show that most of the porosity of each fragment are pores that never connect to the outside of the shell (internal porosity range of 0.3 to 2.7%; Table 3.3). The pores occur between mammillary bodies in the avian taxa. The *G. gecko* sample differs from the avian species in that the pores are distributed haphazardly and are not associated with shell units. In the avian taxa, the lateral branching of the functional and internal pores is often parallel and follows a specific orientation, suggesting that the mammillary bodies are arranged in a lenticular pattern (Fig 3.4 and 3.5). The *E. elegans* and *S. camelus* functional pores do not connect to each other via lateral porosity.

# 3.5.2 Eggshell morphology of cavity and mound nesters

Our study includes three cavity-nesting species (*Malacochersus tornieri, C. intermedius*, and *C. niloticus*) and four mound nesters (*C. porosus, C. siamensis, Melanosuchus niger*, and *Osteolameus torneri*). The two crocodylian cavity nesters (Table 3.3) have thicker eggshell relative to bird eggs of similar mass, whereas the mound nesting crocodylians have thinner eggshell compared to avian eggs of similar mass. *M. tornieri* has the thinnest eggshell of all of the sampled species (0.206 mm; Table 3.3) which falls within the range of avian eggs of similar mass (Ar et al., 1974).

Two crocodylian species, *C. niloticus* and *M. niger*, have ornamented eggshell and are a cavity and mound nester, respectively. All of the other species have smooth eggshell, suggesting that the ornamentation is not related to the nesting strategy. *M. niger* eggshell micro- and ultrastructure has not been described previously in the literature. However, the tower or spire-like ornamentation is similar to *Caiman latirostris* (Cedillo-Leal et al., 2017; Fernández et al., 2013). Personal observations by the lead author suggest that *Caiman yacare* eggshell also has similar ornamentation. Work by Cedillo-Leal et al. (2017) interpreted the ornamentation of *C. latirostris* as a means of increasing embryo survival when an egg is inundated with water. The authors found that the roughness of the ornamentation encouraged
bubble formation, potentially facilitating gas exchange despite being under water. This type of ornamentation may be a trait of all caiman eggshell.

The pores of cavity and mound nesters appear as gaps between shell units. Crocodylian pores have been described as angusti- or tubocanaliculate in structure, or straight pores that can be relatively thin (0.01-0.1 mm) or thick (0.05-0.2 mm) (Carpenter, 1999; Marzola et al., 2015; Mikhailov, 1997). However, our models show that the pores of the genera *Crocodylus* and *Osteolameus* are more pyramidal to conical in shape with the widest portion of the pore near the egg membrane and therefore may be better suited to a lagenocanaliculate classification (Mikhailov, 1997). *M. niger* pores are irregularly shaped, and previous descriptions of *Caiman latirostris* eggshell structure suggests a similar pore structure (Cedillo-Leal et al., 2017; Fernández et al., 2013). The pores of *M. tornieri* are pearshaped and the sample is the least porous of all of the imaged species (T<sub>P</sub> of 0.4%). The lower porosity of *M. tornieri* might be due to the desert environment that it typically lives in (Mwaya et al., 2018) as a way to prevent embryo desiccation.

All of the crocodylian species have some degree of internal lateral branching. The functional porosity is comparable to open nesters, with the exception of *C. porosus* and *M. niger*. However, the internal porosity, mostly composed of lateral branches, is higher than the open nesters (2.8-12.8%; Table 3.3). The internal pores have two preferred directions that create an X pattern in plan view (Fig 3.6, 3.7, 3.8).

## 3.5.3. Why have internal porosity?

Is the internal porosity of eggshell functional? Or is it merely a haphazard feature resulting from shell units that don't fully mineralize the internal volume of the eggshell? CT scans reveal regular patterns of internal porosity within most of the studied eggs, that is, the porosity is not a haphazard trait, but rather it is a feature of the shell. What value these internal pores may have is yet unknown. At the least, these additional pores may simply be

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'evolutionary spandrels' (Gould and Lewontin, 1979), where the pores are a byproduct of the shape of the shell units.

In this study we find that open nesters have eggshell with less internal porosity than closed nesters, with the exception of the pancake tortoise. The percentage of functional pores between open (0.1-0.8%) and closed nesters (0.03-3.3%) overlaps, however the most porous eggshells belong to closed nesters (Table 3.3). This suggests that the presence of additional pores may increase the surface area of the functional pores and therefore increases the rate of gas exchange through the membrane to the embryo. Internal pores could also act as insulation to minimize fluctuations in temperature or humidity as the embryo is developing. Most reptiles and birds cannot develop properly at incubation temperatures below 20°C and above 35°C (Du and Shine, 2015). An investigation on the insulator properties of avian nests found that quail eggs set on a table to cool from 55°C to room temperature took from 40-65 minutes (Lamprecht and Schmolz, 2004). The influence of these pores is poorly understood and requires further investigation.

# **3.6.** Conclusions

- 1. CT scans reveal the 3D network of porosity in eggshell is regular, and primarily comprises internal pores concentrated at the inner part of the shell.
- 2. Eggs associated with open nesting strategies have functional eggshell porosity ranges of 0.1-0.8% total volume, however, only *Gekko gecko* and *Anas platyrhynchos* have total porosities higher than 2% overall volume.
- 3. Mound and cavity nesting crocodylian eggs have functional eggshell porosity ranges of 0.1-3.3% and total porosities of 2.9-16.1% of the total volume, however, despite being a cavity nester, *Malacochersus tornieri* has lower total and functional pore percentages.

- 4. Open nesters have less internal porosity than covered nesters, with the exception of *Malacochersus tornieri*.
- 5. Only a small percentage of porosity is through-going from outside to inside the shell. Internal porosity arises from the interstitial spaces between mineralized egg units, with the exception of *Gekko gecko*. The functional value of the internal porosity is yet unknown, but may improve properties of shell insulation and retention of water vapor.

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## **Chapter 4: Conclusions**

In this dissertation I describe two new eggshell localites, a turtle site from the Campanian (Upper Cretaceous) Kaiparowits Formation within Grand Staircase-Escalante National Monument and a bird site from the Blacks Fork Member of the Eocene Bridger Formation in southwestern Wyoming. The turtle site consists of a spread of eggshell over an area of  $3.75 \text{ m}^2$  with six partially and mostly complete eggs. The site is interpreted as a turtle clutch originally consisting of ~10 spherical eggs. The eggshell is composed of aragonite with shell units interlocked with a height-to-width ratio of 3.7:1 and a thickness of 0.7-1.2mm. These features, along with ornamentation  $53-71 \mu m$  in diameter makes *T. tuberi* oosp. nov. distinguishable from previously described eggs. The wide distribution of eggshell and presence of intact eggs are interpreted to be due to predation rather than hatching. The preservation of primary aragonitic eggshell and gastropods further hints at enhanced preservation along the WIB during Campanian time.

The avian eggs were collected from an olive-gray mudstone. High-resolution stratigraphy and GPS data places the fossiliferous horizon ~9-22 m below the Church Butte Tuff, with the range due to uncertainty in the dip of the stratigraphic units. The site consists of two main concentrations of eggshell over an area of 2 m<sup>2</sup> with eight partial eggs. The site is interpreted as two clutches originally consisting of approximately five and three eggs. The eggshell is composed of high-Mg calcite with a continuous and mammillary layer with an indistinct and slightly undulating boundary between the two. The mammillary layer and total shell thickness ratio is 1:4 with a shell thickness of 0.3-0.6 mm. These features make *Duoolithus bridgerensis* oogen and oosp. nov. distinguishable from previously described eggs. The distribution of eggshell and presence of partial eggs, some of which are cut from pole to pole, are interpreted to be predated rather than hatched. The lack of fossil avian bones and presence of mammalian bones is suggestive of a semiprecocial reproductive mode. The

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micro- and ultrastructural features of the eggshell is most similar to the avian orders Ciconiiformes and Gruiformes, both of which are represented by several species from the Wasatch, Green River, and Bridger formations. The documentation of additional ootaxa, particularly from the Paleogene, leads to new features similar to modern avian taxa and therefore may aid in understanding the evolution of avian reproductive biology.

Micro-CT images of modern rigid-shelled amniote taxa reveal the 3D network of porosity, and for the first time a comparison of the internal versus functional porosity in eggshell. The eggs associated with open nesting strategies have functional eggshell porosity ranges of 0.1-0.8% total volume, however, only *Gekko gecko* and *Anas platyrhynchos* have total porosities higher than 2% overall volume. The mound and cavity nesting crocodylian eggs have functional eggshell porosity ranges of 0.1-3.3% and total porosities of 2.9-16.1% of the total volume, however, despite being a cavity nester, *Malacochersus tornieri* has lower total and functional pore percentages. Open nesters have less internal porosity comprises most of eggshell porosity, and only a small percentage is through-going from outside to inside the shell. The value of internal porosity arises from the interstitial spaces between mineralized egg units, with the exception of *Gekko gecko*. The functional value of the internal porosity is yet unknown, but may improve properties of shell insulation and retention of water vapor.

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