1	Preservation of aragonite in Late Cretaceous (Campanian) turtle eggshell
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15	
16	Abstract
17	Among amniotes, turtles are the only clade that lay aragonitic eggs. Because aragonite is a
18	metastable mineral, unequivocal preservation of aragonite in fossil turtle eggs has only been
19	reported from Pliocene deposits. Here, we report in situ preservation of aragonite in a turtle

20 egg from the Judith River Formation (Campanian) of Montana, USA. We utilized electron

21 backscatter diffraction (EBSD) and Raman spectroscopy to explore the carbonate mineral

22 polymorphs in the eggshell. The EBSD maps show the presence of both aragonite and calcite

23	with the former preserved as needle-like crystal, a feature of all turtle eggshells. The presence
24	of aragonite is independently validated by Raman maps. This dual approach provides direct,
25	unambiguous evidence that the record of aragonitic turtle eggshell dates back at least to the
26	Campanian (ca. 76 Ma) and supports the hypothesis that aragonitic eggshell is a
27	synapomorphy of all turtles. The presence of pristine aragonite also indicates minimal
28	taphonomic alteration at the fossil locality. Consequently, diverse invertebrate or vertebrate
29	fossils from localities with aragonitic turtle egg may provide high quality, unaltered
30	palaeoenvironmental information.
31	Keywords: aragonite; EBSD; eggshell; Judith River Formation; Raman spectroscopy; turtle
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33	1. Introduction
34	Although the main biomineral of vertebrates is calcium phosphate, eggshells of amniotes
35	are composed of calcium carbonate (i.e. calcite, aragonite, and vaterite). Most amniotic
36	eggshells are composed of calcite (Mikhailov, 1997; Dauphin et al., 2021) with a few rare
37	avian eggshells coated with vaterite (Hauber et al., 2018; Portugal et al., 2018). Unlike
38	eggshell from all other amniotes, turtle eggshell mostly consists of aragonite (Hirsch, 1983;
39	Kusuda et al., 2013; see also Baird and Solomon, 1979; Al-Bahry et al., 2009 for the presence
40	of calcite and vaterite). Previous studies on fossil turtle eggs have shown that the
41	characteristic acicular (needle-like) crystals of eggshells are morphologically similar to those
42	of modern turtles (Hirsch, 1983; Kusuda et al., 2013; Lawver and Jackson, 2014). However,
43	few studies have shown the unaltered preservation of aragonite. Hirsch (1983, 1996), Hirsch
44	and Lopez-Jurado (1987), Zelenitsky et al. (2008), and Mueller-Töwe et al. (2011) used X-
45	ray diffraction (XRD) and Kohring (1990) used staining to test for the presence of aragonite
46	and argue for the preservation of aragonite in Late Jurassic to Pliocene turtle eggs. Among

47 these studies, only Mueller-Töwe et al. (2011) presented a supporting XRD diffractogram 48 of aragonite from Pliocene eggs but other studies did not present XRD diffractograms, which 49 are essential for unequivocal confirmation of aragonite. Therefore, Pliocene has been the 50 oldest age for confirmed aragonite in turtle eggs. Considering that turtles may have originated 51 as early as 265–242 Ma (Lyson and Bever, 2020), there is a very large temporal gap between 52 the earliest turtles and the oldest confirmed aragonitic egg.

53 Detecting aragonite in the fossil record has been an important issue in invertebrate palaeontology and marine palaeoenvironmental studies because it provides a direct evidence 54 of the aragonite- and calcite-sea secular variation in the Phanerozoic (Balthasar et al., 2011; 55 56 Janiszewska et al., 2017). Accordingly, invertebrate palaeontologists/zoologists have actively 57 applied imaging techniques to detect *in situ* presence of aragonite (e.g. Cusack et al., 2008; Taylor et al., 2008; Balthasar et al., 2011; Janiszewska et al., 2017, 2018; Stolarski et al., 58 59 2021) by using crystallographic approaches (e.g. EBSD or XRD) and/or a vibrational spectroscopic approach (e.g. Raman spectroscopy); the combination of both approaches 60 provides manifest evidence for phase identification (Balthasar et al., 2011; Stolarski et al., 61 2021). Because turtle eggs are the only aragonitic eggs among amniotes, fossil turtle eggs 62 provide the best opportunity to introduce these technical advancements to vertebrate 63 64 palaeontology.

Detecting aragonite preservation in fossil eggs is important for both palaeontology and taphonomy of fossil bearing deposits. First, it provides the most direct evidence for the unique history of turtle reproduction. Considering the fact that (recently extinct) nontestudines turtle *Meiolania* laid aragonitic eggs (Lawver and Jackson, 2016), it is likely that aragonitic eggs have been synapomorphic to all turtle, but palaeontological evidence is very scarce. Indeed, although there are numerous reports of fossil turtle eggs, many of these

71	studies reported that fossil turtle eggs are composed of calcite rather than aragonite (e.g.
72	Hirsch, 1983; Fukuda and Obata, 1991; Isaji et al., 2006; Cadena et al., 2019; Moreno-
73	Azanza et al., 2021). Only through fossil turtle eggs with preserved aragonite, we can gather
74	palaeontological evidence that aragonite has been a main carbonate phase for all turtle eggs
75	since the cladogenesis of the Testudinata (shelled-turtles). Secondly, because aragonite is
76	metastable and can be easily converted into calcite in many sedimentary settings (Hall and
77	Kennedy, 1967; Balthasar et al., 2011), preserved aragonite carries significant taphonomic
78	implications (e.g. Hall and Kennedy, 1967; Fricke et al., 2008) for the fossil locality. In this
79	study, we report the preservation of aragonite in turtle eggshells from the nonmarine Judith
80	River Formation (Campanian, Late Cretaceous) of Montana.

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## 2. Geological Setting

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The holotype of the ootaxon (egg-type) Testudoolithus zelenitskyae (catalogue number: 84 Museum of the Rockies (MOR) 710) used in this study was recovered from the Egg White 85 Site (Fig. 1; MOR locality JR-122L; north-central Montana) of the Judith River Formation 86 87 (Lawver and Jackson, 2017). Vertebrate fossils (including dinosaur egg clutches) from the Judith River Formation near Havre were mostly found in the Coal Ridge Member above the 88 89 mid-Judith discontinuity (Rogers et al., 2016). Thus, MOR 710 very likely came from the 90 fossiliferous Coal Ridge Member (Lawver and Jackson, 2017). The age of the Coal Ridge Member is well constrained by absolute age dating as between 76.2 and 75.2 Ma, middle to 91 92 late Campanian (Rogers et al., 2016).

#### 3. Material and method

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96	Lawver and Jackson (2017) provided a detailed morphological description of MOR 710.
97	Briefly summarizing the description, the eggs are beige in colour, 34–39 mm in diameter,
98	with eggshell 660–760 $\mu$ m thick. The eggshell consists of a single layer with acicular
99	crystals, a diagnostic feature of turtle eggshells (Hirsch, 1983; Schleich and Kastle, 1988;
100	Kusuda et al., 2013), but some parts are of the eggshells are composed of two overlapping
101	layers (Lawver and Jackson, 2017).
102	EBSD and Raman spectroscopy were used to identify the carbonate phases of MOR 710.
103	We further investigated chemical composition and morphology by using scanning electron
104	microscope (SEM) and energy dispersive X-ray spectroscopy (EDS). In addition, the
105	presence of thermally altered organic matter in the eggshell was tested by using Raman
106	spectroscopy. For EBSD and Raman spectroscopy analyses, we followed established
107	protocols (Moreno-Azanza et al., 2013; Choi et al., 2019, 2021). Detailed methodologies are
108	presented in the Text S1.
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110	4. Results
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112	EBSD phase analysis shows that aragonite is dominant in MOR 710 (Fig. 2A), which still

preserves acicular crystals (Fig. 2B, C; Fig. S1). The aragonitic eggshell is mainly composed

of a single layer but some parts have an additional layer (Figs. S1, S2). Calcite is usually 114

present in the inner part (Fig. 2A) and it is likely that this calcite represents alteration of 115

original aragonite (see below). In backscattered diffraction (BSE) images, the calcite is 116

typically brighter than associated aragonites (Fig. S1), indicating the presence of elements
heavier than calcium. EDS analysis (Fig. S1) shows that, compared to aragonite, calcite is
enriched with O, Mg (very weakly), Mn, and Fe, while depleted Ca. The depletion of Mg and
enrichment of Ca in aragonite is similar to that of relic aragonite reported by Balthasar et al.
(2011). Because Mn, Fe, and Mg can be enriched in diagenetic calcite (converted from
biogenic aragonite; Brand, 1989), the three elements in the calcite of MOR 710 are most
probably exogeneous.

Raman spectroscopy maps show that aragonite and calcite phases detected by EBSD yield 124 dominant aragonite and calcite Raman peaks, respectively (Fig. 2D-F, H). The consistency 125 between the EBSD and RS maps is explicitly represented by calcite/aragonite ratio mapping 126 127 (Fig. 2F). Notably, whereas EBSD maps yield binary mineral identification, Raman spectroscopy maps show the presence of aragonite and calcite more continuously so that 128 129 subtle transformations from aragonite to calcite (e.g. point iii in Fig. 2F, H) are more effectively demonstrated. It shows that calcite-dominant points under EBSD still preserve 130 small amounts of aragonite (e.g. point i in Fig. 2F, H), consistent with this calcite having 131 originated as aragonite. We additionally obtained Raman spectra with an extended spectral 132 range (1000–1800 cm<sup>-1</sup>) to test the presence of thermally altered organic matter (Choi et al., 133 2020, 2021). As anticipated by the light colour of eggshell, there was no sign of thermally 134 altered organic matter, which would normally be represented by broad graphite (G) and 135 disordered carbon (D) bands at 1200–1700 cm<sup>-1</sup> (Fig. S3; Buseck and Beyssac, 2014; Henry 136 et al., 2019). 137

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139 **5. Discussion** 

143	In this study, advanced analytical techniques typically applied to fossil and modern
144	invertebrates to test for remnant biogenic aragonite (e.g. Balthasar et al., 2011; Janiszewska et
145	al., 2017; Stolarski et al., 2021) were successfully introduced to vertebrate palaeontology via
146	turtle eggshells from the Judith River Formation. A recent study by Moreno-Azanza et al.
147	(2021) suggested the possible presence of aragonite in Eocene (55.8–33.9 Ma) turtle eggshell
148	based on EBSD analysis. However, their results remain ambiguous, as it was unclear whether
149	the signal was biogenic aragonite or indexing artifact due to the low abundance of aragonitic
150	signal (Moreno-Azanza et al., 2021). On the other hand, previous studies used
151	cathodoluminescence (CL) to study the preservation of turtle eggshells (Lawver et al., 2015;
152	Lawver and Jackson, 2016, 2017; Cadena et al., 2019). CL is useful for detecting diagenesis
153	by finding a luminescence signal such as Mn <sup>2+</sup> , which is a proxy for recrystallization (Pérez-
154	Huerta et al., 2018). However, although intense CL reaction may mean that the aragonite is
155	transformed into calcite, the absence of luminescence reaction does not necessarily mean the
156	lack of diagenesis (Pérez-Huerta et al., 2018). In addition, pristine (modern) biogenic CaCO3
157	can show luminescence reaction under CL (Barbin, 2013). Thus, compared to indirect CL
158	approach, EBSD and Raman spectroscopy provide direct evidence of preserved aragonite in
159	fossil turtle eggshells. Finally, XRD is a useful tool, which has previously been used to
160	investigate preservation of aragonite in fossil turtle eggs (see Introduction). However, XRD
161	has relatively low sensitivity so that low abundance of aragonite (~ $1-2\%$ ) may not be always
162	detected and abiogenic secondary aragonite in a material cannot be differentiated by XRD
163	diffractograms alone (Cusack et al., 2008). In addition, XRD analysis is not suitable to
164	measure preservation variations on a spatial scale of micrometres (e.g. Fig. 2) so a XRD

diffractogram is less informative than *in situ* EBSD or Raman maps (Cusack et al., 2008;
Milano et al., 2016).

The presence of well-preserved aragonite in MOR 710 cross-validated by EBSD and 167 Raman spectroscopy (Fig. 2) confidently dates aragonitic turtle eggshell as far back as the 168 Campanian, extending the previous age from Pliocene (Fig. 3). Currently, turtle eggs are 169 170 known from Jurassic to Quaternary deposits (Lawver and Jackson, 2014) but reports of 171 preserved aragonite (both confirmed and alleged ones) are very limited (Fig. 3). We suggest that our approach of combining EBSD and Raman spectroscopy techniques should be 172 extended to spatiotemporally diverse turtle eggs in the world (Fig. 4; Table S1) to ultimately 173 174 trace the history of aragonitic eggshells.

175 The presence of aragonite in MOR 710 supports the hypothesis that eggs of the most recent common ancestor (MRCA; Fig. 5) of all turtles were composed of aragonite, thus, 176 synapomorphic to all turtles (Lawver and Jackson, 2016). Although recently extinct, 177 Meiolania is a non-testudines turtles and had aragonitic eggshells (Fig. 3; Lawver and 178 179 Jackson, 2016). Because all extant Testudinata eggs are, to our knowledge, mainly composed 180 of aragonite, it is most parsimonious to hypothesize that all descendants of the turtle MRCA laid aragonitic eggshells (Fig. 5; Witmer, 1995). The unequivocal presence of aragonite in the 181 182 MOR 710 specimen (Fig. 2) and a Pliocene egg (Mueller-Töwe et al., 2011) and other turtle eggshells alleged to preserve aragonite support this view (Fig. 5). Only fossil turtle eggs with 183 clear presence of aragonite can support the hypothesis above with palaeontological data. 184 Thus, we emphasize that materials with alleged aragonite must be revisited to provide more 185 186 supporting evidence for the history and monophyly of aragonitic eggshell. Potentially contradicting this hypothesis, Cadena et al. (2019) reported calcite from eggs of 187 Desmatochelys (Early Cretaceous sea turtle; Chelonioidea) and interpreted this taxon as 188

having laid calcitic eggs (Fig. 3). However, aragonite is metastable, and therefore, easily
transformed into calcite (Hall and Kennedy, 1967; Hirsch, 1983; Balthasar et al., 2011).
Considering that *Desmatochelys* eggs show rather poor preservation, the presence of
originally calcitic eggs is debatable and further work is needed (ideally, microanalysis by
EBSD or Raman spectroscopy).

Lastly, the crystallographic nature of turtle eggs may have an important implications in the 194 controversial taxonomic affinity of turtles (i.e., either closer to Archosauria or Lepidosauria). 195 Recent studies, especially those using molecular data, support an archosaurian affinity (see 196 Field et al., 2014; Lyson and Bever, 2020). Although turtle eggs are composed of aragonite, 197 198 the crystallographic arrangement is nearly the same as those of archosaur eggshells. 199 Specifically, eggshell deposition starts from membrana testacea (Figs. 2, 3) and grows outward (Packard and DeMarco, 1991). In contrast, eggshells of lepidosaurs such as in 200 201 Gekkota, show the opposite crystallographic arrangement where biomineralization starts from the outer surface and proceeds toward the inner surface (confirmed by EBSD; Choi et al., 202 2018). Both tuatara (Packard et al., 1988; Packard and DeMarco, 1991) and Cretaceous 203 anguimorph eggshells (Fernandez et al., 2015) show similar crystal arrangement with that of 204 gecko eggshells. Thus, between this dichotomic crystallography, turtle eggs clearly align with 205 206 the archosaur-type, independently supporting their archosaur-affinity (Text S2).

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### 208 *5.2 Taphonomic implications*

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It is likely that the Coal Ridge Member of the Judith River Formation provides ideal
conditions for the preservation of aragonite (Rogers et al., 2016) and several factors may

contribute to this. Situated above the mid-Judith discontinuity, the Coal Ridge Member is 212 213 highly fossiliferous in both invertebrates and vertebrates, and characterized by a shallow water table, widespread tidal influence, and low-energy overbank (coastal environment) 214 215 deposition (Rogers et al., 2016). Importantly, Rogers et al. (2016) found that the Coal Ridge 216 Member experienced a two-fold increase in floodplain aggradation compared to the underlying McClelland Ferry Member and this higher rate of aggradation could have 217 impeded flushing of cations, important for geochemical alteration, and encouraged burial and 218 219 fossilization (see Cherns et al., 2008 for the importance of rapid burial for preservation of aragonite). Consistent with this view, aragonite was detected from Judith River Formation 220 221 bivalves and the igloo-shaped traces left on their shells by their parasites (Fricke et al., 2008; Rogers et al., 2010, 2018). Among fossil turtle eggs (Fig. 4; Table S1), those from the 222 Dinosaur Park Formation of Alberta, Canada (equivalent to the Coal Ridge Member; Rogers 223 224 et al., 2016) and the upper Fruitland Formation, New Mexico, USA (equivalent to the Judith River Formation; Rogers et al., 2016), may also represent good candidates for preservation of 225 unaltered aragonite (Zelenitsky et al., 2008; Tanaka et al., 2011; note that Zelenitsky et al. 226 227 (2008) already mentioned the presence of aragonite in the Canadian material although data were not shown). The presence of aragonite in the bivalves from the Fruitland Formation 228 indirectly supports this prediction (Fricke et al., 2009). Notably, the upper Fruitland 229 230 Formation represents well-drained palaeoenvironments (Tanaka et al., 2011) dissimilar to the 231 more mesic Judith River Formation (Rogers et al., 2010). Testing for the presence of aragonite in the Fruitland Formation turtle eggshell would, thereby, provide a chance to 232 233 investigate the presence of aragonite in different terrestrial palaeoenvironments of similar 234 age.

The preservation of aragonite in MOR 710 might have been possible due to minimal geothermal influence, which is known to impact fossil preservation (Casella et al., 2017,

2018; Wiemann et al., 2018; Pederson et al., 2019; Choi et al., 2020, 2021). MOR 710 lacks 237 thermally altered organic matter (Fig. S3). Because turtle eggshell contains intracrystalline 238 proteins (Kusuda et al., 2013), the absence of thermally altered organic matter implies very 239 little to no geothermal alteration. In addition, acicular crystals of aragonite begin to lose their 240 241 original structure at about 300°C and convert to calcite (Milano et al., 2016; Milano and Nehrke, 2018). This may occur at temperatures as low as 175°C when exposed to artificial 242 burial fluids (see Casella et al., 2017, 2018). However, MOR 710 preserves the original 243 acicular structure very well (Fig.2; Fig. S1, S2). The well-preserved acicular structure and 244 lack of thermal alteration in the MOR 710 imply that the Coal Ridge Member was ideal for 245 preservation of aragonite. This is further supported by the other aragonitic fossils in the 246 Judith River Formation (Rogers et al., 2010, 2018). 247

That being said, aragonite preservation within a formation can be vary due to diverse 248 249 factors, such as local corrosive pore water chemistry, microbial degradation of organic matter, sedimentation rate, lithology, and geothermal gradient (Gerhardt et al., 2000; Casella et al., 250 2017, 2018; Nohl et al., 2020). Although the Coal Ridge Member of the Judith River 251 Formation and its equivalent formations may be ideal for aragonite preservation, the local 252 factors should not be neglected for more advanced understanding for aragonite preservation, 253 254 especially when turtle eggshells are entirely composed of calcite (e.g. Cadena et al., 2019). Given that local variation within a formation is fully considered, the investigation of 255 aragonite in turtle eggshell would not only be helpful in understanding the paleobiology of 256 turtles, but also informative for clarifying the taphonomic history of spatiotemporally diverse 257 fossil localities (Hall and Kennedy, 1967; Fricke et al., (2008) used the presence of fossil 258 aragonite as a strong indicator for the absence of intensive diagenetic alteration) where turtle 259 eggshell occurs (Fig. 4; Table S1). 260

# **6.** Conclusion

264	The presence of aragonite in fossil turtle eggs can be best demonstrated by a combination
265	of EBSD and Raman spectroscopy, a technique frequently employed by invertebrate
266	palaeontologists. Our results show the preservation of aragonite in the Late Cretaceous turtle
267	egg with firm evidence. This study drastically reduces the large temporal gap that existed
268	between the first osteological record of true turtles and the oldest confirmed aragonitic
269	eggshells. We suggest that the presence of aragonite should be tested whenever fossil eggs of
270	turtles are recovered. The spatiotemporally wide distribution of turtle eggs provides good
271	opportunities to apply our approach. Furthermore, the preservation of aragonite is a
272	taphonomic phenomenon that testifies that the fossil locality has been free from intense
273	diagenetic alteration. Recognition of preserved aragonite in turtle eggs may indicate a locality
274	where superb, pristine palaeoenvironmental information may be collected.
275	
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Al-Bahry, S.N., Mahmoud, I.Y., Al-Amri, I.S., Ba-Omar, T.A., Melgheit, K.O., Al-Kindi, A.Y., 286

- 2009. Ultrastructural features and elemental distribution in eggshell during pre and post hatching periods in the green turtle, Chelonia mydas at Ras Al-Hadd, Oman. Tissue Cell 41, 288 214-221. 289
- 290 Baird T., Solomon, S.E., 1979. Calcite and aragonite in the egg shell of Chelonia mydas L.. J. Exp. Mar. Biol. Ecol. 36, 295–303. 291
- 292 Balthasar, U., Cusack, M., Faryma, L., Chung, P., Holmer, L.E., Jin, J., Percival, I.G., Popov,
- L.E., 2011. Relic aragonite from Ordovician-Silurian brachiopods: implications for the 293 evolution of calcification. Geology 39, 967–970. 294
- 295 Barbin, V., 2013. Application of cathodoluminescence microscopy to recent and past
- biological materials: a decade of progress. Mineral. Petrol. 107, 353-362. 296
- 297 Brand, U., 1989. Aragonite-calcite transformation based on Pennsylvanian molluscs. Geol. Soc. Am. Bull. 101, 377-390. 298
- 299 Buseck, P.R., Beyssac, O., 2014. From organic matter to graphite: graphitization. Elements 300 10, 421–426.
- 301 Cadena, E.-A., Parra-Ruge, M.L., Parra-Ruge, J. de D., Padilla-Bernal, S., 2019. A gravid
- fossil turtle from the Early Cretaceous reveals a different egg development strategy to that 302
- of marine turtles. Palaeontology 62, 533-545. 303
- 304 Casella, L.A., Griesshaber, E., Yin, X., Ziegler, A., Mavromatis, V., Müller, D., Ritter, A.-C.,
- Hippler, D., Harper, E.M., Dietzel, M., Immenhauser, A., Schöne, B.R., Angiolini, L., 305

306	Schmahl, W.W., 2017. Experimental diagenesis: insights into aragonite to calcite
307	transformation of Arctica islandica shells by hydrothermal treatment. Biogeosciences 14,
308	1461–1492.
309	Casella, L.A., He, S., Griesshaber, E., Fernández-Díaz, L., Greiner, M., Harper, E.M.,
310	Jackson, D.J., Ziegler, A., Mavromatis, V., Dietzel, M., Eisenhauer, A., Veintemillas-
311	Verdaguer, S., Brand, U., Schmahl, W.W., 2018. Hydrothermal alteration of aragonite
312	bicarbonates: assessment of micro- and nanostructural dissolution-reprecipitation and
313	constraints of diagenetic overprint from quantitative statistical grain-area analysis.
314	Biogeosciences 15, 7451–7484.
315	Cherns, L., Wheeley, J.R., Wright, V.P., 2008. Taphonomic windows and molluscan
316	preservation. Palaeogeogr. Palaeoclimatol. Palaeoecol. 270, 220-229.
317	Choi, S., Han, S., Kim, NH., Lee, YN., 2018. A comparative study of eggshells of Gekkota
318	with morphological, chemical compositional and crystallographic approaches and its
319	evolutionary implications. PLoS One 13, e0199496.
320	Choi, S., Han, S., Lee, YN., 2019. Electron backscatter diffraction (EBSD) analysis of
321	maniraptoran eggshells with important implications for microstructural and taphonomic
322	interpretations. Palaeontology 62, 777-803.
323	Choi, S., Lee, S.K., Kim, NH., Kim, S., Lee, YN., 2020. Raman spectroscopy detects
324	amorphous carbon in an enigmatic egg from the Upper Cretaceous Wido Volcanics of South
325	Korea. Front. Earth Sci. 7, 349.
326	Choi, S., Park, Y., Kweon, J.J., Kim, S., Jung, H., Lee, S.K., Lee, YN., 2021. Fossil
327	eggshells of amniotes as a paleothermometry tool. Palaeogeogr. Palaeoclimatol.
328	Palaeoecol. 571, 110376.

329	Cusack, M., England, J., Dalbeck, P., Tudhope, A.W., Fallick, A.E., Allison, N., 2008.
330	Electron backscatter diffraction (EBSD) as a tool for detection of coral diagenesis. Coral
331	Reefs 27, 905–911.

- Dauphin, Y., Werner, D., Corado, R., Perez- Huerta, A., 2021. Structure and composition of
  the eggshell of a passerine bird, *Setophaga ruticilla* (Linnaeus, 1758). Microsc. Microanal.
  27, 635–644.
- Evers, S.W., Benson, R.B.J., 2019. A new phylogenetic hypothesis of turtles with
- implications for the timing and number of evolutionary transitions to marine lifestyles in

the group. Palaeontology 62, 93–134.

- 338 Fernandez, V., Buffetaut, E., Suteethorn, V., Rage, J.-C., Tafforeau, P., Kundrát, M. 2015.
- Evidence of egg diversity in squamate evolution from Cretaceous anguimorph embryos.PLoS One 10, e0128610.
- 341 Field, D.J., Gauthier, J.A., King, B.L., Pisani, D., Lyson, T.R., Peterson, K.J., 2014. Toward
- consilience in reptile phylogeny: miRNAs support an archosaur, not lepidosaur, affinity for
  turtles. Evol. Dev. 16, 189–196.
- 344 Fricke, H.C., Rogers, R.R., Backlund, R., Dwyer, C.N., Echt, S., 2008. Preservation of
- 345 primary stable isotope signals in dinosaur remains, and environmental gradients of the
- Late Cretaceous of Montana and Alberta. Palaeogeogr. Palaeoclimatol. Palaeoecol. 266,
  13–27.
- Fricke, H.C., Rogers, R.R., Gates, T.A., 2009 Hadrosaurid migration: inference based on
  stable isotope comparison among Late Cretaceous dinosaur localities. Paleobiology 35,
  270–288.
- Fukuda, Y., Obata, I., 1991. Upper Cretaceous chelonian egg from Hokkaido, Japan. Bull. Natl.

- 352 Sci. Mus. Tokyo Ser. C 17, 111–118.
- 353 Gerhardt, S., Groth, H., Rühlemann, C., Henrich, R., 2000. Aragonite preservation in late
- Quaternary sediment cores on the Brazilian Continental Slope: implications for intermediate
  water circulation. Int. J. Earth Sci. 88, 607–618.
- Hall, A., Kennedy, W.J. 1967. Aragonite in fossils. Proc. R. Soc. B 168, 377–412.
- 357 Hauber, M.E., Dainson, M., Baldassarre, D.T., Hossain, M., Holford, M., Riehl, C., 2018. The
- 358 perceptual and chemical bases of egg discrimination in communally nesting greater anis
- 359 *Crotophaga major*. J. Avian Biol. 49, e01776.
- Henry, D.G., Jarvis, I., Gillmore, G., Stephenson, M., 2019. Raman spectroscopy as a tool to
- determine the thermal maturity of organic matter: application to sedimentary, metamorphic
  and structural geology. Earth-Sci. Rev. 198, 102936.
- Hirsch, K.F., 1983. Contemporary and fossil chelonian eggshells. Copeia 1983, 382–397.
- 364 Hirsch, K.F., 1996. Parataxonomic classification of fossil chelonian and gecko eggs. J.
- 365 Vertebr. Paleontol. 16, 752–762.
- 366 Hirsch, K.F., Lopez-Jurado, L.F., 1987. Pliocene chelonian fossil eggs from Gran Canaria,
  367 Canary Islands. J. Vertebr. Paleontol. 7, 96–99.
- 368 Isaji, S., Matsushita, A., Hirayama, R., 2006. Chelonian eggshells from the Lower Cretaceous
- Kuwajima Formation of the Tetori Group, Central Japan. Paleontol. Res. 10, 29–36.
- Janiszewska, K., Mazur, M., Escrig, S., Meibom, A., Stolarski, J., 2017. Aragonitic
- scleractinian corals in the Cretaceous calcitic sea. Geology 45, 319–322.
- Janiszewska, K., Mazur, M., Machalski, M., Stolarski, J., 2018. From pristine aragonite to
- blocky calcite: exceptional preservation and diagenesis of cephalopod nacre in porous

Cretaceous limestones. PLoS One 13, e0208598.

- Joyce, W.G., Anquetin, J., Cadena, E.-A., Claude, J., Danilov, I.G., Evers, S.W., Ferreira,
- 376 G.S., Gentry, A.D., Georgalis, G.L., Lyson, T.R., Pérez-García, A., Rabi, M., Sterli, J.,
- 377 Vitek, N.S., Parham, J.F., 2021. A nomenclature for fossil and living turtles using
- phylogenetically defined clade names. Swiss J. Palaeontol. 140, 5.
- 379 Ke, Y., Wu, R., Zelenitsky, D.K., Brinkman, D., Hu, J., Zhang, S., Jiang, H., Han, F., 2021. A
- large and unusually thick-shelled turtle egg with embryonic remains from the Upper

381 Cretaceous of China. Proc. R. Soc. B 288, 20211239.

- Kohring, R., 1990. Upper Jurassic chelonian eggshell fragments from the Guimarota mine
  (Central Portugal). J. Vertebr. Paleontol. 10, 128–130.
- Kusuda, S., Yasukawa, Y., Shibata, H., Saito, T., Doi, O., Ohya, Y., Yoshizaki, N. 2013.
- Diversity in the matrix structure of eggshells in the Testudines (Reptilia). Zool. Sci. 30,
  366–374.
- 387 Lawver, D.R., Jackson, F.D., 2014. A review of the fossil record of turtle reproduction: eggs,
- embryos, nests and copulating pairs. Bull. Peabody Mus. Nat. Hist. 55, 215–236.
- Lawver, D.R., Jackson, F.D., 2016. A fossil egg clutch from the stem turtle Meiolania
- 390 *platyceps*: implications for the evolution of turtle reproductive biology. J. Vertebr.
- 391 Paleontol. 36, e1223685.
- Lawyer, D.R., Jackson, F.D., 2017. An accumulation of turtle eggs with embryos from the
- Campanian (Upper Cretaceous) Judith River Formation of Montana. Cretac. Res. 69, 90–
  99.
- Lawver, D.R., Rasoamiaramanana, A.H., Werneburg, I., 2015. An occurrence of fossil eggs

- from the Mesozoic of Madagascar and a detailed observation of eggshell microstructure. J.
  Vertebr. Paleontol. 35, e973030.
- Lyson, T.R., Bever, G.S., 2020. Origin and evolution of the turtle body plan. Annu. Rev. Ecol.
  Evol. Syst. 51, 143–166.
- 400 Mikhailov, K.E., 1997. Fossil and recent eggshell in amniotic vertebrates: fine structure,
- 401 comparative morphology and classification. Spec. Pap. Palaeontol. 56, 1–80.
- 402 Milano, S., Nehrke, G., 2018. Microstructures in relation to temperature-induced aragonite-
- 403 to-calcite transformation in the marine gastropod *Phorcus turbinatus*. PLoS One 13,
  404 e0204577.
- 405 Milano, S., Prendergast, A.L., Schöne, B.R., 2016. Effects of cooking on mollusk shell
- structure and chemistry: implications for archeology and paleoenvironmental
  reconstruction. J. Archaeol. Sci. Rep. 7, 14–26.
- Moreno-Azanza, M., Mariani, E., Bauluz, B., Canudo, J.I., 2013 Growth mechanisms in
  dinosaur eggshells: an insight from electron backscatter diffraction. J. Vertebr. Paleontol. 33,
  121–130.
- 411 Moreno-Azanza, M., Díaz-Berenguer, E., Silva-Casal, R., Pérez-García, A., Badiola, A.,
- 412 Canudo, J.I., 2021. Recognizing a lost nesting ground: first unambiguous Testudines
- 413 eggshells from the Eocene, associated with the pleurodiran *Eocenochelus* (Huesca,
- 414 Northern Spain). Palaeogeogr. Palaeoclimatol. Palaeoecol. 576, 110526.
- 415 Mueller-Töwe, I.J., Kjeldahl-Vallon, T.A., Milàn, J., Vallon, L.H., Theodorou, G., Lindgren,
- 416 J., Roussiakis, S.J., and Bromley, R.G., 2011. First chelonian eggs and carapace fragments
- from the Pliocene of Rhodes, Greece. Neues Jahrb. Geol. Paläontol. Abh. 262, 309–322.

418	Nohl, T., Wetterich, J., Fobbe, N., Munnecke, A., 2020. Lithological dependence of aragonite
419	preservation in monospecific gastropod deposits of the Miocene Mainz Basin:
420	implications for the (dia-)genesis of limestone-marl alternations. J. Sediment. Res. 90,
421	1500–1509.

- 422 Packard, M.J., DeMarco, V.G., 1991. Eggshell structure and formation in eggs of oviparous
- 423 reptiles. In: Deeming, D.C., Ferguson, M.W.J. (Eds), Egg incubation: its effects on
- 424 embryonic development in birds and reptiles. Cambridge University Press, Cambridge,
  425 UK, pp. 53–69.
- Packard, M.J., Thompson, M.B., Goldie, K.N., Vos, M., 1988. Aspects of shell formation in
  eggs of the tuatara *Sphenodon punctatus*. J. Morphol. 197, 147–157.
- 428 Pederson, C., Mavromatis, V., Dietzel, M., Rollion-Bard, C., Nehrke, G., Jöns, N., Jochum,
  429 K.P., Immenhauser, A., 2019. Diagenesis of mollusc aragonite and the role of fluid
- 430 reservoirs. Earth Planet. Sci. Lett. 514, 130–142.
- 431 Pérez-Huerta, A., Coronado, I., Hegna, T.A., 2018. Understanding biomineralization in the
- 432 fossil record. Earth-Sci. Rev. 179, 95–122.
- 433 Portugal, S.J., Bowen, J., Riehl, C., 2018. A rare mineral, vaterite, acts as a shock absorber in
  434 the eggshell of a communally nesting bird. Ibis 160, 173–178.
- 435 Rogers, R.R., Fricke, H.C., Addona, V., Canavan, R.R., Dwyer, C.N., Harwood, C.L.,
- 436 Koenig, A.E., Murray, R., Thole, J.T., Williams, J., 2010. Using laser ablation-inductively
- 437 coupled plasma-mass spectrometry (LA-ICP-MS) to explore geochemical taphonomy of
- 438 vertebrate fossils in the Upper Cretaceous Two Medicine and Judith River formations of
- 439 Montana. Palaios 25, 183–195.
- 440 Rogers, R.R., Kidwell, S.M., Deino, A.L., Mitchell, J.P., Nelson, K., Thole, J.T., 2016. Age,

- 441 correlation, and lithostratigraphic revision of the Upper Cretaceous (Campanian) Judith
- 442 River Formation in its type area (north-central Montana), with a comparison of low- and
- high-accommodation alluvial records. J. Geol. 124, 99–135.
- 444 Rogers, R.R., Curry Rogers, K.A., Bagley, B.C., Goodin, J.J., Hartman, J.H., Thole, J.T.,
- 445 Zatoń, M., 2018. Pushing the record of trematode parasitism of bivalves upstream and
- back to the Cretaceous. Geology 46, 431–434.
- 447 Schleich, H.H., Kästle, W., 1988. Reptile Egg-Shells SEM Atlas. Gustav Fischer, 123 pp.
- 448 Stolarski, J., Coronado, I., Murphy, J.G., Kitahara, M.V., Janiszewska, K., Mazur, M.,
- 449 Gothmann, A.M., Bouvier, A.-S., Marin-Carbonne, J., Taylor, M.L., Quattrini, A.M.,
- 450 McFadden, C.S., Higgins, J.A., Robinson, L.F., Meibom, A., 2021. A modern scleractinian
- 451 coral with a two-component calcite–aragonite skeleton. Proc. Natl. Acad. Sci. 118,
  452 e2013316117.
- Tanaka, K., Zelenitsky, D.K., Williamson, T., Weil, A., Therrien, F., 2011. Fossil eggshells
  from the Upper Cretaceous (Campanian) Fruitland Formation, New Mexico. Hist. Biol.
  23, 41–55.
- Taylor, P.D., Kudryavtsev, A.B., Schopf, J.W., 2008. Calcite and aragonite distributions in the
  skeletons of biomineralic bryozoans as revealed by Raman spectroscopy. Invertebr. Biol.
  127, 87–97.
- Vuke, S.M., Porter, K.W., Lonn, J.D., Lopez, D.A. and compilers. 2007. Geologic map of
  Montana. Montana Bureau of Mines and Geology Geologic Map, 62, 67 pp.
- 461 Wiemann, J., Fabbri, M., Yang, T.-R., Stein, K., Sander, P.M., Norell, M.A., Briggs, D.E.G.,
- 462 2018. Fossilization transforms vertebrate hard tissue proteins into N-heterocyclic
- 463 polymers. Nat. Commun. 9, 4741.

464	Witmer, L.M., 1995. The extant phylogenetic bracket and the importance of reconstructing
465	soft tissues in fossils. In: Thomason, J. (Ed), Functional morphology in vertebrate
466	paleontology. Cambridge University Press, Cambridge, UK, pp. 19-33.
467	Zelenitsky, D.K., Therrien, F., Joyce, W.G., Brinkman, D.B., 2008. First fossil gravid turtle
468	provides insight into the evolution of reproductive traits in turtles. Biol. Lett. 4, 715–718.
469	
470	
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476	
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484 Figure captions

485 Fig. 1. Nonmarine Upper Cretaceous formations in Montana and the fossil locality for MOR 710 (a blue star). Adapted from Vuke et al. (2007). A scale bar equals 100 km. 486 Fig. 2. EBSD and Raman spectroscopy maps for MOR 710 and a modern turtle (Pelodiscus 487 sinensis) eggshell. (A) A phase map of MOR 710. Blue represents aragonite and red 488 calcite. (B) Inverse pole figure (IPF) X map for aragonite of MOR 710. Note the 489 490 preservation of acicular crystals. (C) IPF X map of modern turtle eggshell. (D-F) Raman 491 spectroscopy maps of the area marked in the EBSD maps. D and E show the intensity map for peak of calcite at 280 cm<sup>-1</sup> and that of aragonite at 200 cm<sup>-1</sup>, respectively (Taylor et al., 492 2008; Stolarski et al., 2021). Red region in D represents the dominance of calcite and blue 493 region in E aragonite (caution: blue region in D and red region in E do not mean the 494 presence of aragonite in D and calcite in E, respectively). F, The normalized intensity map 495 based on  $(280 \text{ cm}^{-1})/(200 \text{ cm}^{-1})$  ratio, equivalent to the intensity of calcite/aragonite. 496 Raman spectra acquired from the lowercase Roman numbers are presented in H. Raman 497 498 spectra at point iii indicates the co-occurrence of calcite and aragonite. (G) Interpretation keys for IPF X mappings of aragonite (B, C). Scale bar equal 250 µm (A, B); 100 µm (C); 499 50 μm (D–F). 500 Fig. 3. The confirmed (red symbols; this study; Mueller-Töwe et al., 2011) and alleged (blue 501 symbols; Hirsch, 1983, 1996; Kohring, 1990; Hirsch and Lopez-Jurado, 1987; Zelenitsky 502 et al., 2008) preservation of aragonite in turtle eggshells and simplified phylogeny of 503

- turtles (modified from Evers and Benson, 2019; Joyce et al., 2021). Clades marked with
- arrowheads are extant ones. *Meiolania* was recently extinct (Lawver and Jackson, 2016).
- 506 R, Semi R, and S in the brackets represent rigid, semi-rigid, and soft eggshells as
- presented in the lower left of the figure (after Packard and DeMarco, 1991; MT represents

508	membrana testacea). The two clades marked by orange characters are fossil taxa and their
509	eggs known by gravid females (Zelenitsky et al., 2008; Cadena et al., 2019; Ke et al.,
510	2021).

511 Fig. 4. Spatiotemporal distribution of fossil eggs of turtles in the world. Based on the Table512 S1.

Fig. 5. Importance of aragonite preservation in fossil egg of turtles. (A) Because eggshell of 513 514 Meiolania is composed of aragonite (confirmed aragonite is marked by red symbol; Lawver and Jackson, 2016), Extant Phylogenetic Bracket (EPB) predicts that MRCA of 515 Meiolania and modern turtle (i.e. Testudinata) laid aragonitic eggshell (predicted aragonite 516 517 is marked by blue symbol). (B) EPB predicts (or hypothesizes) that descendants of MRCA of all turtles laid aragonitic eggshells (Witmer, 1995). (C) Investigating the preservation of 518 aragonite in fossil eggs of turtle provides the chances to test the hypothesis of EPB. MOR 519 710 proves that the inference holds true in Cryptodira. Alleged aragonite in other fossil 520 eggs of turtles (Fig. 3) provide more chances to solidify the hypothesis (B) if the 521 522 preservation of aragonite is confirmed by supporting data (e.g. EBSD, Raman

spectroscopy, XRD).