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ABSTRACT In 2007, a clutch of approximately 30 fossil eggs reported from the Upper Cretaceous Gaogou Formation, Xiaguan-Gaoqiu Basin, Neixiang County, Henan Province, China was assigned to the oofamily "Crocodiloolithidae". After 11 years, another clutch of 15 eggs of the same type associated with *Mosaiceratops azumai* and from the same county was identified as turtle eggs (*Testudoolithus*), based on the needle-like crystals interpreted as aragonite from their eggshells. Our detailed study of the clutch of 15 eggs and six clutches of the same ootaxon using

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transmitted/polarized light microscope, followed by scanning electron microscope observations combined with electron backscatter diffraction analysis showed that their eggshells are dominated by secondary calcite; with a few pristine aragonite crystals that unequivocally support the turtle affinity of the eggs. Furthermore, superimposed cone-shaped structural units with radial ultrastructures, combined with the extremely thick eggshell challenges the assignment to the oogenus *Testudoolithus*. Hence, we suggest that this ootaxon could represent a new oogenus of Testudoolithidae. Although additional eggshell units were reported in the abnormal fossil turtle egg with multilayered eggshell from the Judith River Formation in Montana, U.S.A, the relatively constant eggshell thickness indicates that the eggs from Neixiang are not pathological. Therefore, this is the first report of superimposed structural units inside normal turtle eggshells, revealing the unique eggshell formation mechanism of the extinct turtle from Neixiang. Moreover, the aragonite discovered in the eggs may extend the temporal record of confirmed aragonite in turtle eggs. The crystallographic comparison between the eggs from Neixiang and the fossil turtle eggshells from Zhejiang Province and Montana revealed the transformation process from aragonite to calcite in fossil turtle eggshell.

Keywords: *Neixiangoolithus yani*; Testudoolithidae; turtle eggs; aragonite; EBSD; Henan

1. Introduction

The ultrastructure of modern turtle eggshells is characterized by radially arranged acicular (needle-like) aragonite crystals (Al-Bahry et al., 2009, 2011; Kusuda et al., 2013). Hirsch (1983, 1996) compared ultrastructures of fossil turtle eggs with those of modern turtle eggs and established the Testudoid basic type, which means that the fossil and modern turtle eggshells share the same overall ultrastructure. In subsequent studies, numerous fossil turtle eggs were reported all over the world (e.g. Jackson et al., 2008; Zelenitsky et al., 2008; Q. Wang et al., 2013; Lawver and Jackson, 2014, 2016, 2017; Imai et al., 2016; Cadena et al., 2019; Moreno-Azanza et al., 2021). However, in contrast to huge diversity of dinosaur eggs (Zhao et al., 2015), fossil turtle eggs were relatively rare in China (Jackson et al., 2008; Q. Wang et al., 2013; Lawver and Jackson, 2014).

In 2007, a clutch of approximately 30 spherical eggs with extremely thick eggshell was reported from Neixiang (Fig. 1). These eggs were 4.0–5.5 cm in diameter and approximately 2.3 mm in eggshell thickness, and were initially identified as crocodile eggs (Fang et al., 2007a, b, 2009). After 11 years, Jackson et al. (2018) published a clutch of 15 spherical eggs associated with a partial skeleton of *Mosaiceratops azumai* (Neoceratopsia; ZMNH M8856) from the Xiaguan-Gaoqiu Basin in

Henan Province, China. The most complete egg of the clutch was 53 × 58 mm in size and 1.5 mm in eggshell thickness (Jackson et al., 2018). The high gas conductance of this ootaxon indicated that the eggs were incubated in a high humidity and low oxygen environment (Jackson et al., 2018; Ke et al., 2021). The thick eggshell could prevent breakage or depredation of the eggs (Ke et al., 2021). Although the studied eggs were highly recrystallized, Jackson et al. (2018) assigned them to the oofamily Testudoolithidae, mainly based on the large size of former organic core and the supposedly acicular crystals at the inner surface of eggshell. Furthermore, they estimated the carapace of the turtle that produced the clutch as 1.5 m, based on a formula that correlated egg size and carapace length (Elgar and Heaphy, 1989; modified by Lawver and Jackson, 2017). The only turtle closest to this size from the Upper Cretaceous of China is *Nanhsiungchelys wuchingensis* of Nanhsiungchelyidae, in which the carapace length is larger than 111 cm. However, this large turtle was recovered from Nanxiong County, Guangdong Province (Ye, 1966; Tong and Li, 2019). On the other hand, a smaller species of Nanhsiungchelyidae, *Yuchelys nanyangensis* where the carapace length was estimated to be 55 cm, was recovered from Neixiang (Tong et al., 2012). Ke et al. (2021) assumed that the eggs from Neixiang were possibly laid by *Yuchelys nanyangensis* , based on the nanhsiungchelyid embryonic remains (identified by maxilla, mandible and scapula) of an egg, which are similar to ZMNH M8856 in egg size and eggshell microstructure.

In this study, we revisited the eggshell micro- and ultrastructure of ZMNH M8856 and the clutches of the same ootaxon housed at the Henan Natural History Museum (HNHM) and Zhejiang Museum of Natural History (ZMNH). We used a combination of transmitted/polarized light microscope (TLM/PLM), detailed scanning electron microscope observations and an electron backscatter diffraction (EBSD) analysis, and investigated whether the “acicular crystals” near the inner surface of the eggshells were acicular aragonite crystals or not. In addition, we discussed the eggshell formation process and parataxonomic position of these eggs based on the extreme thickness and the unique superimposed cone-shaped structural units with radial ultrastructures of their eggshells. Different stages of the transformations from aragonite to calcite in fossil turtle eggshell were also shown in this study, highlighting the importance of the application of electron backscatter diffraction in the studies of fossil turtle eggs.

2. Geological setting

The first-order geotectonic units of Henan Province are the North China Craton and the Yangtze Craton. The Qinling-Dabie orogenic belt is the collision zone between these two cratons (Wu et al., 2019). There are a series of NW-SE-trending fault depression basins in the eastern part of the Qinling-Dabie orogenic belt, such as Xiaguan-Gaoqiu, Xixia, Xichuan, Lingbao and Nanzhao basins (Liang et al., 2009).

The Xiaguan-Gaoqiu Basin is located in the northern part of Neixiang (Fig. 1). The Upper Cretaceous deposits in this basin are divided into the Gaogou, Majiacun and Sigou formations in ascending order (Pan et al., 2008). The fossil eggs studied here were recovered from the Gaogou Formation (Fig. 1), formerly the Xiaguan Formation in previous works (Liang et al., 2009; Zheng et al., 2015; Jackson et al., 2018). Due to the fact that the egg-bearing strata in the Xiaguan-Gaoqiu Basin are comparable to the Gaogou Formation of Xixia and Xichuan basins, with regard to the features of fossil assemblages, lithology and sedimentary characteristics (Pan et al., 2008), we use the name “Gaogou Formation” in our study. The lower portion of the Gaogou Formation is composed of brown conglomerates and glutenites interbedded with brown fine sandstones and siltstones representing fluvial-alluvial paleoenvironment, while the upper portion is characterized by brown siltstones interbedded with grey fine sandstones and glutenites, deposited in a fluvial-pluvial system. The fossil eggs were found in thick-bedded brown mudstones interbedded with thin-bedded yellowish-brown sandstones from the upper portion of the Gaogou Formation. Meanwhile, the assemblage of fossil eggs represents the typical dinosaurian oofauna that is composed of

Paraspheroolithus, *Ovaloolithus* and *Youngoolithus* (Zhao, 1979a, b; Zhou et al., 2003). In addition, several vertebrate fossils, including Dinosauria *Nanyangosaurus zhugeii*, *Mosaiceratops azumai*, *Baotianmansaurus henanensis* and Testudines *Yuchelys nanyangensis* have been recovered in this region (Xu et al., 2000; Zhang et al., 2009; Tong et al., 2012; Zheng et al., 2015). Although the accurate age of the Gaogou Formation is debatable due to the lack of radiometric dating, it is considered to be Late Cretaceous (early–middle Turonian to the middle Campanian) based on plant and invertebrate fossils (Wang et al., 2013).

3. Material and methods

The egg clutches housed at HNHM and ZMNH are embedded in purplish red, strongly cemented, fine-grained silty sandstones. We investigated several eggshell fragments from the previously studied ZMNH M8856 and six egg clutches of the same ootaxon (41HV0001-3, ZMNH M8709-1, M8709-2, M8854, M8855 and M8880). Then the eggshells were embedded in EXAKT Technovit 7200 one-component resin. Radial and tangential thin sections of the eggshells were prepared by using an EXAKT 300CP and EXAKT 400CS cutting and grinding system (housed at the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP)). The thickness of the thin sections is about 50 μm. The thin sections were viewed under TLM and PLM using a Zeiss

Axio Imager A2 PLM (housed at IVPP). The radial sections of ZMNH M8709-1 and M8855 were polished by P4000 sandpaper and etched by acetic acid (5%) for 3–4 min. The radially fractured eggshells from 41HV0001-3 were etched by hydrochloric acid (1%) for 5 s. All these specimens were coated with gold and viewed under a Zeiss MA EVO25 SEM (housed at IVPP). The settings of the SEM were as follows: acceleration voltage of 10 kV and working distance of 13.5 mm for ZMNH M8709-1; acceleration voltage of 15 kV and working distance of 20.5 mm for ZMNH M8855; acceleration voltage of 5 kV and working distance of 6 mm for 41HV0001-3.

Additional turtle and crocodilian eggshells prepared for a comparative study, including several fossil turtle eggshell fragments (ZMNH GM13203) collected from the upper Lower Cretaceous (Albian) Liangtoutang Formation, Huzhen Basin, Zhejiang Province, China, *Testudoolithus zelenitskyae* (MOR 710) collected from the Upper Cretaceous (Campanian) Judith River Formation, Montana, U.S.A, *Pelodiscus sinensis* (modern turtle; IVPP OV2724), *Alligator sinensis* and *Caiman latirostris* (modern crocodiles; personal collections of Z.K. Zhao and loaned from K. Tanaka and H. Echizenya). The eggshells of ZMNH GM1320 were etched by acetic acid (5%) for 3 min. The modern turtle and crocodilian eggshells were etched by hydrochloric acid (1%) for 4 s and 20 s, respectively. These specimens were viewed under a Zeiss MA

EVO25 SEM.

We prepared EBSD specimens of the fossil and modern turtle (41HV0001-3, ZMNH M8709-1, M8855, M8856, GM13203, MOR 710 and IVPP OV2724), and modern crocodile (*Alligator sinensis* and *Caiman latirostris*) eggshells to compare their crystallography. In particular, EBSD analysis of the fossil turtle eggshells was performed to test the preservation of relic aragonite since EBSD provides a direct way to identify relic aragonite in fossil turtle eggshell (Moreno-Azanza et al., 2021; Choi et al., 2022). We followed the established EBSD specimen preparation method of Moreno-Azanza et al. (2013) and Choi et al. (2019). We used a Symmetry detector (Oxford Instruments) attached to the FE-SEM (JEOL JSM-7100F), housed at the School of Earth and Environmental Sciences, Seoul National University. All specimens were analyzed by AZtec software under 15.0 kV acceleration voltage and 15.0 or 25.0 mm working distance.

4. Systematic paleontology

Oofamily: Testudoolithidae Hirsch, 1996

Crocodiloolithidae Fang et al., 2007a: p.101, fig. 4a; Fang et al., 2007b: p.139, 141, fig. 16; Fang et al., 2009: p.528, fig. 7.

Oogenus: *Neixiangoolithus* Fang, Zhang, Pang, Li, Han, Xie, Yan, Pang, Lv, and Cheng, 2007

Type oospecies. *Neixiangoolithus yani* Fang, Zhang, Pang, Li, Han, Xie, Yan, Pang, Lv, and Cheng, 2007.

Etymology. "*Neixiang*-" in reference to the locality where the eggs were collected; "*oo*", in Greek, means egg; "*lithus*", in Greek, means stone.

Emended diagnosis. Spherical eggs closely packed and arranged irregularly in clutches. Thick eggshell (1.43–2.17 mm) is composed of columnar eggshell units that tightly interlock with each other, but can be separated by slender pore canals. Eggshell unit is composed of superimposed cone-shaped structural units that grew from the concave down accretion lines. Radial ultrastructure and concave down accretion lines appeared in every cone-shaped structural unit.

Type locality and horzion. Wugang Village, Xiaguan Town, Neixiang County, Henan Province, China; Turonian–Campanian Gaogou Formation, Upper Cretaceous.

Oospecies: *Neixiangoolithus yani* Fang, Zhang, Pang, Li, Han, Xie, Yan, Pang, Lv, and Cheng, 2007

(Figs. 2–6, S1)

Etymology. Oospecies name in honor of Mr. R. H. Yan for his contributions in the construction of Xixia Dinosaur Egg Fossil Museum (Fang et al., 2007b).

Holotype. 060525-1, a thin section of an eggshell from an uncatalogued clutch of approximately 30 eggs housed at the Geological Museum of

China (Fang et al., 2007b).

Referred specimens. 41HV0001-3, an incomplete clutch of 23 eggs, housed at HNHM; ZMNH M8854, an incomplete clutch of 18 eggs; ZMNH M8855, an incomplete clutch of 34 eggs; ZMNH M8856, an incomplete clutch of 15 eggs; ZMNH M8709-1, an incomplete clutch of 61 eggs; ZMNH M8709-2, an incomplete clutch of 36 eggs; ZMNH M8880, an incomplete clutch of 33 eggs and another incomplete clutch of seven eggs.

Emended diagnosis. Eggs are approximately 5 cm in diameter. Ratio between the height and width of the eggshell unit is 7.2:1 to 9.2:1. Eggshell is 1.43–2.17 mm thick with an average thickness of 1.67 mm.

Type locality and horzion. Same as for oogenus.

Description. The eggs are closely spaced or contacting adjacent ones directly, and are irregularly stacked (Fig. 2). The clutch of ZMNH M8856 contains at least 15 eggs (Jackson et al., 2018). The clutch size of ZMNH M8709-2 (Fig. 2A), M8854, M8855 (Fig. 2B), M8709-1 (Fig. 2C) and 41HV0001-3 (Fig. 2D, E) is 36, 18, 34, 61 and 23, respectively. ZMNH M8880 (Fig. 2F) is composed of a curved clutch of 33 eggs and another clutch of seven eggs in different levels. The distance between the two clutches is less than 10 cm. An isolated egg is in the same level of the smaller clutch.

Perhaps due to lithostatic compaction (e. g. Wilson et al., 2014), the maximum diameters of the eggs in these clutches vary significantly. The diameters of the eggs with shape range from 5.7–6.3 cm in 41HV0001-3, 4.1–6.0 cm in ZMNH M8709-2, 4.0–6.2 cm in ZMNH M8709-1, 4.6–6.1 cm in ZMNH M8880, 5.0–6.9 cm in ZMNH M8854, 4.4–6.1 cm in ZMNH M8855 and 5.3–5.8 cm in ZMNH M8856. Outer surface of the eggs is smooth and glossy (Fig. 2G).

In SEM images of the well-preserved part of radial sections of ZMNH M8855, broad craters where organic cores would have been present (Baird and Solomon, 1979; Al-Bahry et al., 2011), appeared at the inner surface of the eggshell (Fig. 3A). Radial ultrastructures (the needle-like crystals in Jackson et al., 2018, fig. 4D) that originated from the craters, concave down organic membranes and boundaries between the columnar eggshell units were obviously noticed (Fig. 3A). The radial ultrastructure extended towards the outer surface, but is not obvious in the outer part of the eggshell units due to recrystallization (Fig. 3B). More importantly, from the image set at a higher magnification, the radial ultrastructure consists of regularly arranged tabular secondary calcite crystals (Fig. 3C), but not acicular aragonite crystals (see below). In the radial section of ZMNH M8709-1, radial ultrastructures of numerous cone-shaped structural units are distributed throughout the whole eggshell (Fig. 3D).

In radially fractured eggshells of 41HV0001-3, only the craters are visible at the innermost end of the eggshell units. The boundaries between eggshell units are indistinct (Fig. 3E). However, concave down accretion lines were clearly observed in the whole eggshell (Fig. 3E, F). In the outer portion of eggshell, the contours of the eggshell units can be recognized based on the distribution of accretion lines (Fig. 3F).

Most thin sections of the eggshell showed significant recrystallization of the original biomineral (Jackson et al., 2018), and only the boundaries between the eggshell units and the broad craters are visible at the inner surface of eggshell (Fig. 4A). Also, in relatively well-preserved specimens, the cone-shaped structural units with radial ultrastructures are characterized by dark concave down accretion lines (Figs. 4B–F, S1A–C). Due to the accretion lines, these structural units are more conspicuous in the middle portion of eggshell (Figs. 4B, C, S1A). In some portions of the eggshell of ZMNH M8855, the eggshell is separated into two layers at the middle portion of the eggshell (Fig. 4D), while in other portions, the eggshell is continuously intact (Figs. 3B, S1C). In the eggshells of ZMNH M8709-1, well-developed cone-shaped structural units form six to seven layers (Fig. 4E). In some parts of ZMNH M8854, the eggshell unit is composed of two vertical lines of superimposed cone-shaped structural units (Figs. 4F, S1D). Meanwhile, pore canals are straight with constant width in radial views (black arrows in Fig. 4B–D).

EBSD phase maps reveal that small aragonite grains are preserved in

41HV0001-3, ZMNH M8855, M8856, and M8709-1 (Figs. 5A, S2A–C; Table S1). Aragonite acicular crystals in ZMNH M8855 are well-preserved as compared to other specimens. In fact, they account for 1.54 % of the eggshell (Table S1) and most of them occured in the outer portion of the eggshell (Fig. 5A–C). The aragonite grains (Fig. 5B, C) showed a reliable mean angular deviation (MAD) value, a parameter that describes the quality of the electron backscatter diffraction signal. Since the aragonites have a low mean MAD value (0.38), which is comparable to that of calcite (0.31), the identified aragonite grains are not indexing artefacts (see also Moreno-Azanza et al., 2021). The aragonite grains are needle-shaped, while some are intersected by calcite grains (Fig. 5B, C). The *a*-axis of aragonite is also parallel to the eggshell growth direction, which is similar to those of modern turtles (Fig. 5C; see Comparative study below). The IPF Y map of the radial thin section of ZMNH M8709-1 shows various colors (Fig. 5D). Compared to the IPF Y maps of well-preserved fossil eggshells of archosaurs (Grellet-Tinner et al., 2011, 2016; Moreno-Azanza et al., 2013, 2017; Choi et al., 2019, 2020a; Oser et al., 2021), calcite with horizontally laid *c*-axis (blue and green grains in IPF Y map) are widespread in the eggshell. It is similar to the results from a recrystallized fossil eggshell from the Namyang Basin, South Korea (Choi et al., 2019). The contours of the cone-shaped structural units in ZMNH M8709-1 can be identified based on the boundaries of calcite grains (Fig. 5E). The radial ultrastructure grown from the accretion lines are preserved in some cone-shaped structural units, but replaced by blocky calcite grains in others (Fig. 5E). On the other hand, radial ultrastructures composed of calcite grains are found from some of the inner portions of ZMNH M8855, despite the extensive recrystallization of the eggshell, which is characterized by numerous blue and green colored grains in the IPF Y map, and red grains in the phase map, respectively (Fig. 5A, F).

In tangential sections near the inner surface of the eggshells, the eggshell units are round and separated from each other (Fig. 6A). Throughout the inner part of the eggshell, numerous round and irregularly shaped pores were distributed between the round eggshell units (Fig. 6B). Towards the outer surface, the eggshell units fuse together gradually (Fig. 6C). Near the outer surface of the eggshell, the eggshell units are tightly arranged with irregular boundaries and the density of pores reduces significantly (Fig. 6D).

5. Comparative study

5.1 With Crocodilian Eggshells

The Neixiang egg (hereafter, *N. yani*) was originally identified as a fossil crocodilian egg and assigned to a new oofamily “Crocodiloolithidae" when first reported (Fang et al., 2007b). Fang et al.

(2007b) argued that the *N. yani* was composed of calcite crystals, thus excluded the possibility of being laid by turtles, where their eggshells are composed of radial aragonite crystals (Hirsch, 1983). However, the possibility that the calcite crystals of the supposed “crocodilian ootaxon” could have been abiogenic were not considered, but may have been altered from aragonite.

Extant and fossil crocodilian eggshells are characterized by wedge-shaped eggshell units with prominent tabular calcite crystals (Fig. 7A–C; Schleich and Kästle, 1988; Mikhailov, 1997; Jackson and Varricchio, 2010; Marzola et al., 2015; Moreno-Azanza et al., 2015). The pore canals are broad in the inner part of the eggshell, but gradually narrowed towards the outer part of the eggshell. Under PLM and EBSD, irregularly shaped divergent wedges are shown in the whole eggshell (Fig. 7B, C; Hirsch, 1985; Kohring and Hirsch, 1996; Moreno-Azanza et al., 2014; Marzola et al., 2015; Russo et al., 2017; Choi et al., 2020b). Although tabular calcite crystals were found in *N. yani* (Fig. 3C), they were not pristine calcite crystals. Casella et al. (2018a, fig. 4C) reported diagenetic ‘inorganic rhombohedral calcite’ in calcitic brachiopod fossil and its shape is nearly the same as the one in *N. yani* (Fig. 3C). Moreover, in contrast to crocodile eggs, *N. yani* has columnar eggshell units with cone-shaped structural units and the pore canals are straight with constant width in radial views, hence *N. yani* was deemed to not belong to crocodiles (*contra* Fang et al., 2007b).

5.2 With Turtle Eggs

A clutch of *N. yani* associated with a partial skeleton of *Mosaiceratops azumai* (ZMNH M8856) was identified as a clutch of turtle eggs and was assigned to *Testudoolithus* (Jackson et al., 2018). However, Jackson et al. (2018) also noticed that the eggshell of ZMNH M8856 was much thicker than all known oospecies within *Testudoolithus*. Notably, to our knowledge, the eggshell thickness of *N. yani* exceeds all reported thickness of fossil and modern turtle eggs (Elgar and Heaphy, 1989, appendix; Hutterer et al., 1997, table 2; McGee, 2012, appendix A; Lawver and Jackson, 2017, table 1). With regard to egg size, it is comparable to the *Meiolania platyceps* egg from the Pleistocene of Lord Howe Island, Australia, the fossil tortoise eggs from the Miocene and Pliocene deposits of the Canary Islands, and the eggs of several extant species of *Dermochelys*, *Geochelone* and *Gopherus* (Table 1; Elgar and Heaphy, 1989, appendix; Hutterer et al., 1997; Lawver and Jackson, 2016). With regard to clutch size, it is comparable to several trionychid and chelydrid turtles (Table 1; Elgar and Heaphy, 1989, appendix). The largest clutch size of *N. yani* (61 eggs) even exceeded that of *Chelonia depressa*, which is a type of marine turtle with relatively small clutch size (Table 1; Elgar and Heaphy, 1989, appendix).

Although radial ultrastructures of the eggshell units are not clear in ZMNH M8709-2 and 41HV0001-3 (Figs. 3E, F, 4A, B), other specimens exhibited distinct radial ultrastructures throughout the whole eggshell unit (Fig. 4C–F). However, most radial ultrastructures are composed of regularly arranged tabular calcite crystals rather than acicular aragonite or calcite crystals under SEM (Fig. 3A, C). By contrast, the eggshell units of the modern turtle eggshell (*P. sinensis*) consist of radially arranged acicular aragonite crystals (Fig. 7D–F; Hirsch, 1983); in the fossil turtle eggshell fragments (ZMNH GM13203) from the Huzhen Basin, Zhejiang Province, China, the radial ultrastructures are composed of radially arranged acicular calcite crystals, resembling those of the fossil turtle eggs reported from Hokkaido, Japan and Madagascar (Fig. 7G–I; Fukuda and Obata, 1991, fig.8; Lawver et al., 2015, fig.3). However, no aragonite was detected in the phase map of ZMNH GM13203 (Fig. S2D; Table S1).

The IPF X map of *P. sinensis* eggshell (Fig. 7F) clearly shows that it is entirely composed of acicular aragonite crystals. The *a*-axis lies roughly parallel to the eggshell growth direction and in this case, *b*- and *c*-axis lie perpendicular to the *a*-axis direction (Parmentier et al., 2007), while little amount of aragonite next to the organic cores have horizontally laid *a*-axis. The IPF Y map of the recrystallized fossil turtle eggshell fragment (ZMNH GM13203) from the Huzhen Basin, Zhejiang

Province shows that it is entirely composed of acicular calcite crystals with various *c*-axis directions (Figs. 7I, S3A). As we described above, the IPF Y map of *N. yani* is almost the same as that of ZMNH GM13203, but most calcite crystals are blocky rather than acicular (Figs. 5D–F, S3B, C). The scattered acicular aragonite grains in the phase and IPF X maps reveal the original shape and arrangement of aragonite crystals of the eggshell units of *N. yani* (Figs. 5A–C, S2A–C).

Despite severe recrystallization, we can conclude that the radial ultrastructures of the eggshells of *N. yani*, which composed of tabular calcite crystals were derived from acicular aragonite crystals. Moreover, the unique characteristics such as the extremely thick eggshell and the superimposed cone-shaped structural units can easily distinguish *N. yani* from the currently known fossil and modern turtle eggs.

6. Discussion

6.1 Cone-shaped structural units of Neixiangoolithus yani

Although the contours of the cone-shaped structural units are unclear in some specimens (Fig. 4A–C), they can be easily distinguished by radial ultrastructures in the relatively well-preserved specimens (Fig. 4D–F). In the outer portion of the eggshells of ZMNH M8855, the cone-shaped structural units form an additional layer in some portions (Fig. 4D), but originated from former organic membranes in the SEM images (Fig. 3A, B). In the eggshells of ZMNH M8709-1, six to seven layers of cone-shaped structural units are distributed in the whole eggshell (Figs. 3D, 4E). But in other specimens, the cone-shaped structural units that originated from the concave down accretion lines appeared occasionally and were randomly distributed in the eggshells (Fig.4B, C). Neither organic membrane nor nucleation center is observed at the innermost end of the cone-shaped structural units (Figs. 3D, 4F).

The superimposed cone-shaped structural units without nucleation centers of *N. yani* are superficially similar to multilayered extant turtle eggshell, but *N. yani* are unlikely to be pathological because all the eggshells collected from different clutches of *N. yani* have a relatively constant thickness (Fig. 4A–E, 1.67 ± 0.21 mm (N=12)). By contrast, the additional layers of extant turtle eggshell are often separated by additional shell membranes, forming an unusually thick eggshell (Ewert et al., 1984; Schleich and Kästle, 1988, pl. 7: figs. 3, 4; Jackson and Varricchio, 2003; Jackson and Schmitt, 2008).

On the other hand, the cone-shaped structural units of *N. yani* do not form distinct layers inside the eggshells, except for those of ZMNH M8855 and M8709-1 (Fig. 4D, E). However, distinct layers similar to those of ZMNH M8855 and M8709-1 were reported as additional eggshell layers in titanosaur, hadrosaur and other fossil turtle eggshells (Vianey-Liaud et al., 1994; type II and III morphologies in Jackson and

Schmitt, 2008; Jackson and Varricchio, 2016; Lawver and Jackson, 2017). In these multilayered eggshells, the internal surface of the additional outer layer conforms closely to the external surface of the inner layer. Nucleation centers were not observed between the outer and inner layers (Vianey-Liaud et al., 1994, fig. 11.8C, D; Jackson and Schmitt, 2008, fig. 2B, C; Lawver and Jackson, 2017, fig. 3F). Based on these descriptions, characteristics of the eggshells of ZMNH M8855 and M8709-1 correspond with the definition of the multilayered fossil eggshell (Jackson and Schmitt, 2008). However, the eggshells of ZMNH M8855 and M8709-1 have similar thickness to other eggshells (Fig. 4A–E). Thus, the layers of cone-shaped structural units should not be considered as additional layers of the eggshells, but could have been the results of interruptions of aragonite deposition. In some portions of the eggshells, only organic matrix (see the organic membranes in Fig. 3A, B) was secreted, thus a significant interval of mineral deposition appeared.

Although turtle eggshell is generally composed of a single layer of eggshell unit, additional eggshell units have been observed in extant and fossil turtle eggshells occasionally (Figs. 7E, I, S4). Even in the eggshell of *Testudoolithus zelenitskyae* with a single layer (Lawver and Jackson, 2017, fig. 3A, B), additional eggshell units are rather common near the outer surface of the eggshell (Fig. S4). The cone-shaped structural units of *N. yani* are similar to the additional eggshell units in arrangement of crystals and absence of nucleation center. It is possible that the "columnar eggshell unit" of *N. yani* is a complex construction composed of superimposed smaller eggshell units. But this hypothesis should be tested with further studies of more well-preserved materials of *N. yani.*

6.2 Parataxonomic assignment and eggshell formation mechanism

Because of the slender interlocking eggshell units and the aragonite crystals in the eggshells, *N. yani* can be unequivocally assigned to the oofamily Testudoolithidae (Jackson et al., 2018). The unique superimposed cone-shaped structural units have not been reported in any known oogenera in Testudoolithidae. They could represent an eggshell formation mechanism differing from that of other fossil and modern turtle eggs: radial ultrastructures composed of radial arranged aragonite crystals form repeatedly during the whole eggshell formation process, leading to an extremely thick eggshell (Fig. 8). In contrast, the eggshells of other fossil and modern turtle eggs are formed by a single layer of radial ultrastructures (every eggshell unit has a single radial ultrastructure; Schleich and Kästle, 1988; Hirsch, 1983, 1996; Lawver and Jackson, 2014, 2016, 2017; Lawver et al., 2015), which lead to relatively thin eggshells. According to the classification principle on oogenus level (Mikhailov et al., 1996), an oogenus should be identified based on egg shape, variation within a certain morphotype (testudoid morphotype herein), pore system and ornamentation on outer surface of eggshell. Although *N. yani* resembles *Testudoolithus* in egg shape, pore system and smooth outer surface, it could represent a new oogenus of Testudoolithidae based on its unique superimposed cone-shaped structural units and extremely thick eggshells, which were derived from a unique eggshell formation mechanism.

6.3 Transformation from aragonite to calcite in fossil turtle eggshell

In vertebrate paleontology, transformation from aragonite to calcite was given little attention because of the fact that except for turtles, all other amniotic eggs are composed of calcite (Mikhailov, 1997; Dauphin et al., 2021). Recently, however, analytical approaches have been applied to fossil eggs of turtle to detect *in situ* preservation of aragonite (Moreno-Azanza et al., 2021; Choi et al., 2022; see also Mueller-Töwe et al., 2011 for bulk analysis using XRD). Choi et al. (2022) reported well-preserved aragonite in the holotype of *Testudoolithus zelenitskyae* (MOR 710, Lawver and Jackson, 2017) from the Judith River Formation (mid- to late Campanian; Rogers et al., 2016) of Montana. Although the preservation of aragonite in *N. yani* is not as good as that of *T. zelenitskyae*, the age of *N. yani* (viz. age of the Gaogou Formation) is probably early–middle Turonian to the middle Campanian (Wang et al., 2013), so to which *N. yani* may extend the temporal record of confirmed aragonite in turtle eggs. In addition, because the egg-layer of *N. yani* is likely Nanhsiungchelyidae (Ke et al., 2021), *N. yani* positively supports the view that aragonitic eggshell is synapomorphic to all true turtles (Testudinata; see Lawver and Jackson 2016; Choi et al., 2022, fig. 5) and especially for Cryptodira.

Several authors mentioned the presence of aragonite in fossil eggs of turtles based on the acicular crystals (e.g. Winkler and Sánchez-Villagra, 2006; Buscalioni et al., 2008; Tanaka et al., 2011; Poust, 2017; Jackson et al., 2018). However, we suggest that the term ‘aragonite’ should be used with robust supporting data (e.g. Fig. 5) as pointed out by Bajpai et al. (1997), Prasad et al. (2015), and Vlachos et al. (2015). For example, EBSD image of *N. yani* (Fig. 5) shows that acicular aragonite is nearly transformed into blocky calcite, therefore it is hard to find relic aragonite based on the morphology of crystals in either SEM or PLM images (Figs. 3, 4). By contrast, ZMNH GM13203 is completely transformed into calcite (Fig. S2D; Table S1), but its SEM, PLM, and EBSD images (Figs. 7G–I, S3A) all showed that the calcite still preserves the acicular structure. If the identification of calcium carbonate polymorph is entirely based on the morphology, one may conclude that ZMNH GM13203 is composed of aragonite, which is not true. We suggest the term ‘aragonite’ and ‘acicular ultrastructure’ should not be interchangeable because recrystallized calcite can preserve the acicular ultrastructure (Figs. 7G–I, S3A). That being said, preserved acicular ultrastructure in fossil turtle eggshell may be a good starting point for testing the preservation of aragonite because ultrastructural preservation is a sign of relatively less taphonomic alteration (see below). In short, only through rigorous analytical evidence (e.g. EBSD, Raman spectroscopy, and XRD), the true phase of calcium carbonate in fossils could be unequivocally identified (Hirsch, 1983, 1996; Zelenitsky et al., 2008; Mueller-Töwe et al., 2011; Moreno-Azanza et al., 2021; Choi et al., 2022) and it will bring the valid understanding for the preservation and paleobiology of turtle eggshells.

The transformation from aragonite to calcite in fossil records have been well-studied in invertebrate fossils, both by natural specimens and experimental approaches (Hall and Kennedy, 1967; Balthasar et al., 2011; Milano et al., 2016; Casella et al., 2017, 2018b; Janiszswska et al., 2017, 2018; Pederson et al., 2019). In a hydrothermal experiment, Casella et al. (2017, 2018b) and Pederson et al. (2019) transformed aragonite crystals of modern *Arctica islandica* (Mollusca), *Mytilus edulis* (Mollusca), *Porites* sp. (Coral), and *Haliotis ovina* (Gastropoda) to large and randomly oriented calcite crystals. All these results showed that aragonite is indeed metastable, so with proper experimental setting (e.g. 175 °C with periods of at most 35 days with artificial burial fluid; Casella et al., 2017, 2018b), aragonite could easily be transformed to abiogenic calcite in a geologically shorter time.

The preservation of *N. yani* (Figs. 5, S2A–C, S3B, C; Table S1), *T. zelenitskyae* (Fig. S4; Lawver and Jackson, 2017, fig.3) and ZMNH GM13203 (Figs. 7G–I, S2D, S3A; Table S1) are clearly in different stages of biogenic aragonite degradation, which reflect different taphonomic stages of all specimens (Fig. 9). Casella et al. (2018b) suggested that transformation from (biogenic) aragonite to (abiogenic) calcite in experimental setting follows these stages: (i) decomposition of biopolymers, (ii) homoepitactic overgrowth of aragonite that causes amalgamation of adjacent minerals, (iii) deletion of original microstructure but sustaining the original mineral phase, (iv) replacement of microstructure and phase with newly formed abiogenic calcite. As described in the above, *N. yani* is dominantly composed of randomly oriented blocky calcite crystals and only a few acicular aragonite crystals appeared occasionally (Figs. 5, S2A–C, S3B, C). It is consistent with the stage (iv) of Casella et al. (2018b). In contrast, the fossil turtle eggshell (ZMNH GM13203) from the Huzhen Basin, Zhejiang Province preserved the radial (acicular) ultrastructures of eggshell units, but its radially arranged aragonite crystals were replaced by acicular calcite grains with various *c*-axis directions (Figs. 7G–I, S3A). Because ZMNH GM13203 lost its original phase but was not completely replaced into blocky calcite (Figs. S2D, S3A), ZMNH GM13203 cannot be confidently categorized into either stage (iii) or (iv) of Casella et al. (2018b), but rather between the two stages. In fact, Casella et al. (2018b, p. 7461) also reported that despite the phase change, the newly formed abiogenic calcite often sustains original morphology of the pristine aragonite mineral. This observation is consistent with the case of ZMNH GM13203 and supports the view that ZMNH GM13203 is taphonomically less altered than *N. yani*. In the case of a well-preserved *T. zelenitskyae*, the acicular ultrastructure is composed of aragonite and only shows minor amalgamation, although some parts are transformed into calcite (Choi et al., 2022). Thus, it is most similar to the alteration stage (ii) or (iii) of Casella et al. (2018b).

This finding highlights the importance of the further study of different taphonomic settings of the turtle egg localities in Cretaceous East Asia. Interestingly, aragonite is preserved in *N. yani*, even though the alteration was probably more progressive than that of ZMNH GM13203 (Fig. 9C, D). It means that the preservation of aragonite may not be an "all-or-nothing" phenomenon (*sensu* Janiszewska et al., 2018), where it heavily altered fossil turtle eggs that are mainly composed of secondary calcite grains, which may still preserve relic aragonite (e.g. Figs. 5A–C, 9D, S2A–C; see Janiszewska et al., 2018, p. 17 and references therein). In East Asia, turtle eggs were reported from Cretaceous deposits in Henan (this study; Jackson et al., 2018), Zhejiang (this study; Jackson et al., 2008), and Shandong (Q. Wang et al., 2013) provinces of China, Mongolia (Mikhailov et al., 1994), and Japan (Fukuda and Obata, 1991; Isaji et al., 2006). In Korea, there is a report of turtle egg (Huh and Zelenitsky, 2002), but its preservation is very poor so the identification is highly unreliable. In line with Fukuda and Obata (1991), Isaji et al. (2006) and our result (Fig. S2D; Table S1), which showed that the turtle eggs from Japan and Zhejiang Province are fully composed of calcite, currently, it is likely that *N. yani* from the Henan Province is the only turtle egg in East Asia that preserves aragonite. Nevertheless, we suggest that aforementioned East Asian turtle eggs are worth-revisiting with modern analytical tools because there may be minute amounts of aragonite (Fig. S2A–C; Table S1), which are hardly detectable by conventional techniques (e.g. staining and bulk analysis using XRD). If aragonite can be found, combined with detailed petrographic and sedimentological data from laboratory analyses and fieldwork, they will not only be useful for the paleobiology of turtles, but also helpful for understanding the taphonomic settings of the Cretaceous fossil localities , because preservation of aragonite is highly influenced by paleoenvironments (e.g. Cherns et al., 2008; Janiszewska et al., 2018). Thus, continued investigation for the Cretaceous turtle eggs will extend our understandings for the Cretaceous turtles and preservation of aragonite in the Cretaceous deposits, which was an important period for the evolution of turtles (Evers and Benson, 2019).

7. Conclusions

The appearance of pristine aragonite reveals that the clutches of unusually thick fossil eggs (*Neixiangoolithus yani*) from Neixiang unequivocally belong to turtles. *N. yani* is the first ootaxon in Testudoolithidae that has extremely thick eggshell and superimposed cone-shaped structural units with radial ultrastructures and concave down accretion lines inside its eggshell units, thus making an independent oogenus *Neixiangoolithus*. The crystallographic comparison between *N. yani* and the fossil turtle eggshell fragments from the Huzhen Basin, Zhejiang Province, China and Montana, U.S.A. shows that they are in different stages of the aragonite-calcite transformation, highlighting the importance of further understanding of different taphonomic settings of Cretaceous turtle egg localities in East Asia.

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Figure captions

Fig. 1. (A) Geological map of the fossil egg location (red asterisk) near Xiaguan Town in Neixiang County, Henan Province, China. Abbreviations: Pt$_1$y, Lower Proterozoic Yanlinggou Formation; Pt$_1$g, Lower Proterozoic Guozhuangyan Formation; Pt$_1$s, Lower Proterozoic Shicaogou Formation; Pt$_2$g, Middle Proterozoic Guishan Formation; Pz$_1$b, Lower Paleozoic Baoshuping Formation; Pz$_1$x, Lower Paleozoic Xiaozhai Formation; K$_2$g, Upper Cretaceous Gaogou Formation; $\gamma\pi_5^2$, Yanshan Movement monzonitic porphyry; δ_3^3, Caledonian Movement diorite; $\eta\gamma_4^2$, Variscan monzonitic granite; $\eta\gamma_5^2$, Yanshan Movement monzonitic granite; (B) The position of the Xiaguan-Gaoqiu Basin in Henan Province; (C) The position of B in China. [1.5-column fitting]

Fig. 2. Egg clutches of *Neixiangoolithus yani*. (A) ZMNH M8709-2, an incomplete clutch of 36 eggs; (B) ZMNH M8855, an incomplete clutch of 34 eggs; (C) ZMNH M8709-1, an incomplete clutch of 61 eggs; (D) 41HV0001-3 in top view; (E) 41HV0001-3 in lateral view; **(**F) ZMNH M8880, note two clutches preserved in different levels (black arrows); (G) part of F, ZMNH M8880, showing irregularly stacked eggs. Scale bars equal 10 cm (A–C and F) and 5 cm (D, E and G). [2-column fitting]

Fig. 3. Ultrastructure of *Neixiangoolithus yani*. The white arrows indicate concave down organic membranes; the black arrows indicate concave down accretion lines, note organic membranes are thicker than accretion lines; the black arrowheads indicate the craters at the innermost ends of eggshell units. (A) Innermost ends of eggshell units of ZMNH M8855, showing the radial ultrastructures (Jackson et al., 2018); (B) Middle portion of the eggshell of ZMNH M8855, dashed line indicates the boundary between the portions with (lower part) and without (upper part) clear radial ultrastructures; (C) Tabular secondary calcite crystals in the eggshell units of

41HV0001-3, dashed line indicates the boundary between the tabular secondary calcite crystals aligned in different orientations, supporting the different crystallographic arrangement (Fig. 5); (D) Radial ultrastructures of the cone-shaped structural units of ZMNH M8709-1, dashed lines indicate contours of the cone-shaped structural units; (E) Radially fractured section of 41HV0001-3; (F) An eggshell unit near the outer surface of 41HV0001-3. Scale bars equal 100 μm (A, B, D and F), 20 μm (C) and 200 μm (E). [2-column fitting]

Fig. 4. Microstructure of *Neixiangoolithus yani* in radial views. The white arrows indicate cone-shaped structural units; the black arrows indicate pore canals. (A) The severely recrystallized specimen ZMNH M8709-2; (B) 41HV0001-3, showing dark accretion lines at the middle part of eggshell; (C) ZMNH M8856; (D) ZMNH M8855, showing the horizontal boundary between two eggshell layers; (E) ZMNH M8709-1, showing six or seven layers of cone-shaped structural units, markings (black bars) at the left side indicate boundaries between adjacent layers of cone-shaped structural units; (F) Enlargement of the cone-shaped structural units of the eggshell of ZMNH M8854, dashed lines indicate the contours of two vertical lines of cone-shaped structural units. Scale bars equal 200 μm (A–E) and 100 μm (F). [1.5-column fitting]

Fig. 5. EBSD images of *Neixiangoolithus yani* in radial views. (A) Phase map of ZMNH M8855, calcite and aragonite grains are red and blue, respectively; (B) Enlargement of the outer portion of ZMNH M8855; (C) IPF X map of the outer portion of ZMNH M8855, showing the fragmentary acicular aragonite grains whose *a*-axes are roughly vertical to the inner and outer surfaces of the eggshell; (D) IPF Y map of ZMNH M8709-1; (E) Enlargement of the cone-shaped structural units of the eggshell of ZMNH M8709-1, dashed lines indicate contours of the cone-shaped structural units; (F) IPF Y map of ZMNH M8855. Scale bars equal 500 μm (A, D and F), 250 μm (E) and 100 μm (B and C). [2-column fitting]

Fig. 6. Microstructure of *Neixiangoolithus yani* (41HV0001-3) in tangential views.

(A) Near the inner surface of the eggshell; (B) Through the inner part of the eggshell; (C) Through the outer part of the eggshell; (D) Near the outer surface of the eggshell. The black arrows indicate the pores. Scale bars equal 200 μm. [2-column fitting]

Fig. 7. Eggshell structures of modern crocodile, modern and fossil turtles. A and B, *Alligator sinensis*. (A) Tabular calcite crystals under SEM; (B) Wedge-like extinction pattern under PLM; (C) *Caiman latirostris*, IPF Y map of radial section. D–F, *Pelodiscus sinensis*. (D) radially arranged acicular aragonite crystals under SEM; (E) undulating extinction pattern under PLM, note the additional eggshell units near the outer surface (white arrows); (F) IPF X map of radial section, showing the radially arranged aragonite crystals, diagnostic characteristic of all turtle eggshell (Hirsch, 1983). G–I, ZMNH GM 13203. (G) Acicular aragonite crystals which were replaced by calcite crystals under SEM; (H) Wedge-like and blocky extinction pattern under PLM (white arrows); (I) IPF Y map of radial section, dashed line indicates contour of the additional eggshell unit. Even though aragonite is entirely converted to calcite, they still partly preserve acicular crystal structure of turtle eggshells (see also Moreno-Azanza et al., 2021 and Choi et al., 2022). Scale bars equal 25 μm (A), 200 μm (B and H), 250 μm (C), 10 μm (D and G), and 100 μm (E, F and I). [2-column fitting]

Fig. 8. Eggshell formation process of *Neixiangoolithus yani*. (A) Eggshell units with radially arranged aragonite crystals grow from shell membrane. Eggshell formation process of modern turtles and *Testudoolithus* terminates at this step; (B) new aragonite crystals grow from accretion lines, forming cone-shaped structural units; (C) cone-shaped structural units form repeatedly, leading to an extremely thick eggshell of *Neixiangoolithus yani*. [1-column fitting]

Fig. 9. Preservations of fossil turtle eggshells (B–D; Late Cretaceous in age) shown by EBSD and schematic images. (A) *Pelodiscus sinensis*. Modern turtle eggshells

have clear grain boundaries and are fully composed of aragonite (colored blue in a schematic view); (B) *Testudoolithus zelenitskyae* (MOR 710; Choi et al., 2022). Aragonite shows amalgamation and some parts of eggshell are transformed into calcite (white arrows; colored red in a schematic view); (C) ZMNH GM13203. Aragonite was fully transformed into calcite and amalgamation has progressed, but acicular ultrastructure has been preserved. (D) *Neixiangoolithus yani*. Most parts of eggshell were transformed into blocky and randomly oriented calcite but relic aragonite (white arrows) is preserved. A to D show the overall progress of taphonomic alteration in order. However, they are not necessarily linear process but show diverse end members of taphonomic alterations (see text). Scale bars equal 100 μm (A), 250 μm (B–D). [2-column fitting]

Fig. S1. Microstructure of *Neixiangoolithus yani* in radial views. (A) 41HV0001-3; (B) ZMNH M8709-1; (C) ZMNH M8855, note the continuously intact eggshell in this portion; (D) ZMNH M8854, showing the eggshell units composed of two vertical lines of cone-shaped structural units (see also in Fig. 8C). The white arrows indicate the dark accretion lines where the cone-shaped structural units grow from; the black arrows indicate the vertical lines of cone-shaped structural units. A to C are under cross polarized light. D is under normal light. Scale bars equal 200 μm. [2-column fitting]

Fig. S2. Phase maps of *Neixiangoolithus yani* (A, ZMNH M8709-1; B, 41HV0001-3; C, ZMNH M8856) and the fossil turtle eggshell fragment from the Huzhen Basin, Zhejiang Province, China (D, ZMNH GM 13203). Calcite and aragonite (white arrows) grains are red and blue, respectively. Scale bars equal 500 μm (A–C) and 250 μm (D). [2-column fitting]

Fig. S3. IPF Y maps of the fossil turtle eggshell fragment from the Huzhen Basin, Zhejiang Province, China (A, ZMNH GM 13203) and *Neixiangoolithus yani* (B, 41HV0001-3; C, ZMNH M8856). Note that ZMNH GM 13203 (A) preserves acicular ultrastructure well, compared to 41HV0001-3 and ZMNH M8856 (B, C). Scale bars equal 250 μm (A) and 500 μm (B, C). [2-column fitting]

Fig. S4. The additional eggshell units of *Testudoolithus zelenitskyae* (MOR 710; white arrows). (A) under cross polarized light; (B) under normal light; (C) IPF X map, note that the presence of aragonites with horizontally laid *a*-axis of an additional eggshell unit. It means a new initiation of aragonite deposition. Scale bars equal 100 μm (A and C) and 40 μm (B). [2-column fitting]