A GRAVID FOSSIL TURTLE FROM THE EARLY CRETACEOUS REVEALS A DIFFERENT EGG DEVELOPMENT STRATEGY TO THAT OF EXTANT MARINE TURTLES

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Abstract: Extant sea turtles develop and lay pliable (flexible) eggs; however, it is unknown whether they inherited this reproductive strategy from their closer fossil relatives or if it represents an evolutionary novelty. Here, we describe the first undisputable gravid marine fossil turtle ever found, from the early Cretaceous of Colombia, belonging to *Desmatochelys padillai* Cadena & Parham, which constitutes a representative of the Protostegidae. Using thin sectioning of one of the eggs, as well as scanning electron microscopy coupled with elemental characterization, cathodoluminescence, and computer tomog-raphy, we established that *Desmatochelys padillai* produced rigid eggs similar to those associated with some extant and fossil freshwater and terrestrial turtles. At least 48 spherical eggs were preserved inside this gravid turtle. We suggest that the development of rigid eggs in the extinct marine turtle *Desmatochelys padillai* resulted as an adaptation for egg-embryo requirements dictated by the physical attributes of the nesting site.

Key words: gravid turtle, fossil eggs, Protostegidae, Testudines, Villa de Leyva.

TURTLES have been a successful group of vertebrates since at least the middle Triassic (*c.* 240 Ma), adapted to almost all types of terrestrial, freshwater and marine environments. However, how the embryological development and reproduction of extant linages of turtles differ from or resemble those of their ancestors remains a poorly understood aspect of turtle evolution. Evidence of reproductive strategies in fossil turtles is restricted to eggs, nests, embryos and coupling pairs (Lawver & Jackson 2014); very few of these findings correspond to gravid mothers.

At present, the fossil record of gravid turtles with their eggs preserved is restricted to the Late Cretaceous (Campanian) *Adocus* sp. (Cryptodira, Pan-Trionychia, Adoci-dae) egg clutch and a gravid individual from the Oldman and Dinosaur Park formations in Alberta, Canada pre-serving 26 eggs, only two of which are relatively uncrushed (Zelenitsky *et al.* 2008), and a specimen of *Basilemys variolosa* (Cryptodira, Nanhsiungchelyidae) from the Upper Cretaceous (upper Campanian) Dinosaur Park Formation, Alberta, Canada, in which some eggs were found by accident inside its carapace (Braman &

Brinkman 2009). Both of these gravid fossil turtles repre-sent freshwater and/or terrestrial forms, and their egg morphologies are consistent with those of extant turtles with the same life-style adaptations (Lawver & Jackson 2014). The egg characteristics of extant (freshwater, ter-restrial and marine) and fossil turtles (freshwater and ter-restrial) have been summarized by Hirsch (1983), Schleich & Kästle (1988), Nuamsukon *et al.* (2009) and Lawver (2017). A potential third example of a gravid fos-sil turtle is a Late Jurassic, eurysternid? (Cryptodira, Eucryptodira) from Solnhofen, Bavaria, Germany. How-ever, investigation of the microstructure revealed com-plete diagenetic alteration of the 'eggshell'. Therefore, the authors conclude that these eggs must be referred to as pseudomorphs because no details of the eggshell microstructure can be ascertained (Joyce & Zelenitsky 2002; Lawver & Jackson 2014), leaving this finding highly controversial.

Here, we describe the first undisputable gravid marine fossil turtle ever found, with eggs preserved inside its vis-ceral cavity, preserved in a shallow-marine rock sequence belonging to the Paja Formation in Villa de Leyva, a town

in Colombia, South America. We attribute these eggs to *Testudoolithus* oospp. Thus, we compare the egg development strategies among the truly marine-adapted extant clades Dermochelyidae and Cheloniidae, and the extinct Protostegidae and suggest possible explanations for the differences among them.

GEOLOGICAL SETTING

The region of Villa de Leyva has yielded important remains of fossil vertebrates, invertebrates and plants from the Early Cretaceous (late Barremian to early lower Aptian, >120 myr in age), dated based on ammonoid biostratigraphy (Reboulet & Hoedemaeker 2006; Reboulet *et al.* 2014). All of these fossils belong to the Paja Formation, which is characterized by a succession of mudstones interbedded with limestones, deposited in an intertidal marine complex (Forero & Sarmiento 1985). Fossil vertebrates from Villa de Leyva include the pliosaurs *Kronosaurus boyacensis* Hampe, 1992 and *Stenorhynchosaurus munozi* Páramo-Fonseca *et al.*, 2016; the first dinosaur from Colombia *Padillasaurus leivaensis* Carballido *et al.*, 2015; the turtle *Desmatochelys padillai* Cadena & Parham, 2015, a basal marine protostegid; *Leyvachelys cipadi* Cadena, 2015, the first sandownid turtle from South America; and several unpublished ichthyosaurs, plesiosaurs and fish. The gravid fossil turtle with its eggs reported herein was discovered by Juan de Dios Parra-Ruge at Monsalve hill, located between the towns of Villa de Leyva and Sutamarchan (Cadena *et al.* 2018, fig. S1A).

MATERIAL AND METHOD

Fossil material and preparation

The fossil turtle specimen was prepared at the Centro de Investigaciones Paleontológicas (CIP). The bone surface of the turtle was originally preserved in a hard orange-reddish layer of iron-rich mudstone. To expose the bone and properly identify the taxonomy, the rock matrix was removed using airscribe tools (Micro Jack; https://www.paleotools.com/) and dental picks.

Light and polarized transmitted microscopy

To establish the microstructural preservation and mineralogical composition of the eggs and the rock matrix, we took one of the four isolated eggs collected together with the specimen that separated during the collection and used mechanical preparation of the shell to produce a thin section at the Paleontological Lab, North Carolina State University, Raleigh, USA. The whole egg with the rock matrix was processed after being embedded in Silmar SIL66 resin. We extracted bubbles using a vacuum system, with subsequent refrigeration for 24 hours to harden the Silmar resin. Then, we cut the sample and mounted it on a glass slide. We used a precision saw and a grinding machine to reach an average thickness of 70 μm. Finally, we observed the thin section and took photographs using an Olympus BX53 polarized light microscope with 2×, 4× and 10× lenses at the Paleontological Lab of Yachay Tech University in San Miguel de Urcuquí, Ecuador.

Computer tomography scanning of the specimen

To establish the total number of eggs preserved inside the shell, as well as their characteristics, internal morphology and preservation, we scanned the whole specimen using computer tomography (CT scanning) at the Hospital San Ignacio X-ray facility, Bogotá, Colombia, under the following settings: zoom factor 1.16×, 12 902 total images, 120 kV, 150 mA, and 2 mm interslice spacing (horizontal view (Cadena *et al.* 2018, video S1), coronal view (Cadena *et al.* 2018, video S2), and sagittal view (Cadena *et al.* 2018, video S3)). We analysed the CT data using iQ-VIEW software (https://www.image-systems.biz).

Scanning electron microscopy and elemental analysis (SEM-EDS)

We performed elemental analysis combined with high-resolution imaging of the eggshell structure and rock matrix of one of the isolated eggs using a scanning electron microscope coupled with an energy-dispersive X-ray spectroscopy analyser, Phenom ProX, at the Paleontological Lab of Yachay Tech University, San Miguel de Urcuquí, Ecuador. SEM-EDS allowed us to explore the mode of preservation of the eggshell ultrastructure and to establish the elemental variation from the top to the base of the eggshell. The eggshell of one of the internal eggs (region without application of stabilizer Paraloid B-72) was carefully removed and transported to the laboratory in sterilized aluminium foil. In the laboratory, we carefully fractured the egg fragment, and one of the smaller pieces (a fresh eggshell surface) was observed and analysed with SEM-EDS. We mounted the sample in a holder and analysed it without adding any coating. Imaging was performed at 5 kV using different magnification settings. During the entire process of collecting the sample, breaking, and placing it in the SEM holder we used sterilized

gloves and avoided any direct contact of the sample with hands. Point and map elemental composition were performed at 15 kV.

Reproductive traits versus phylogeny

To explore the potential phylogenetic role in egg development strategy between fossil and extant lineages of marine turtles (Chelonioidea), we mapped information from the egg-shape, eggshells and clutch size onto the simplified phylogenetic tree of Evers & Benson (2018). Data for Chelonioidea were obtained from: this study (*Desmatochelys padillai*), Solomon & Watt (1985) and Rostal *et al.* (1996) (extant sea turtle *Dermochelys coriacea*); for members of the crown Cheloniidae from: Schleich & Kästle (1988) (*Caretta caretta*), Hirsch (1983) (*Lepidochelys kempii*), Sikiwat *et al.* (2015) (*Chelonia mydas* and *Eretmochelys imbricate*). For other groups inside Testudines we took information from: Vanzolini (2003), Winkler & Sánchez-Villagra (2006), Zelenitsky *et al.* (2008), Lawver & Jackson (2014), Lawver *et al.* (2015) and Erickson & Baccaro (2016).

Cathodoluminiscence analysis

We analysed the same sample with SEM-EDS by mounting it on a stub and studying it in a TESCAN Mira 3 SEM coupled with a cathodoluminescence colour detector and backscattered electrons at 7 kV at the Centro de Nanociencia y Nanotecnología (CENCINAT) of the Universidad de las Fuerzas Armadas (ESPE), Sangolqui, Ecuador.

Institutional abbreviations. FCG-CBP, Fundación Colombiana de Geobiología, Carlos Bernardo Padilla, Colección Centro de Investigaciones Paleontológicas (CIP), Villa de Leyva, Boyacá, Colombia; NMW, Natural History Museum, Vienna, Austria.

SYSTEMATIC PALAEONTOLOGY

Oofamily TESTUDOOLITHIDAE Hirsch, 1996, *sensu*
Jackson *et al.* 2008
Genus TESTUDOOLITHUS Hirsch, 1996, *sensu*
Jackson *et al.* 2008
Testudoolithus oospp.
Figures 1–3

Referred material. FCG-CBP-75 partially preserved carapace and plastron with a clutch containing at least 48 eggs inside the visceral cavity and 4 isolated eggs.

Remarks. The FCG-CBP-75 eggs are attributed to the *Testudoolithus* oogenus by: being spherical–spheroidal in shape and having an eggshell thickness between *c.* 200 and 800 μm. Even though the eggs exhibited some unique characteristics, we avoid erecting a new ootaxon considering that most of them have suffered some degree of diagenesis. The unique combination of characters include: spherical eggs, 32.2–42.8 mm in diameter; 785–820 μm eggshell thickness; shell units with a height-to-width ratio of 1.1:1 to 1.3:1; well-defined pores 180–215 μm in diameter at the eggshell surface.

Description and comparisons. Specimen FCG-CBP-75 resembles the holotype of *Desmatochelys padillai* in many characteristics, including poor lateral ossification of posterior costals exhibiting naked costal ribs, as well as developing dorsal keels at the neural series (Cadena *et al.* 2018, fig. S3) and is similar to many extant sea turtles, such as the *Caretta caretta* specimen NMW 31531, in that the most posterior pair of costal ribs project almost parallel to the middle axis of the shell (Cadena *et al.* 2018, fig. S2E–H). Thus, FCB-CBP-75 resembles other chelonioid turtles in having hyo and hypoplastral star-shaped elements with extremely serrate medial edges and large central fontanelles between them (Fig. 1C–D; see also Hirayama 1997, figs 3–6). Considering all these similarities between specimen FCG-CBP-75, the holotype of *D. padillai*, and other chelonioids, we attribute this specimen to *D. padillai*, and exclude the possibility that it could correspond to a specimen of the sandwonid *Leyvachelys cipadi* (Cadena 2015), which is characterized by a fully ossified carapace. Unfortunately, the preservation of the bone surface is not optimal and does not allow us to clearly recognize any additional features of this specimen, or clearly defined sutural contacts between the preserved bones, particularly between costal and neural bones, or even to establish whether the bones were covered by scutes and left marked sulci between them.

Detailed comparisons of fossil ootaxa have been presented (Lawver & Jackson 2014, 2017; Imai *et al.* 2016). Four of these ootaxa resemble *Testudoolithus* oospp. FCG-CBP-75 from *Desmatochelys padillai* in that they are spherical to spheroidal in shape; these ootaxa are: (1) *T. jiangi* (Fang *et al.* 2003; Jackson *et al.* 2008) from the Lower Cretaceous (Albian) of China, potentially from a terrestrially adapted turtle; (2) *T. rigidus* (Hirsch 1996), from several Mesozoic and Cenozoic localities in England, Ethiopia, Spain, France and the USA, also interpreted as laid by terrestrially adapted turtles; (3) *T. lordhowensis* (Lawver & Jackson 2016) from the middle to upper Pleistocene of Australia, laid by *Meiolania platyceps*, a meiolaniform terrestrially adapted turtle; and (4) *T. zelenitskyae* (Lawver & Jackson 2017) from the Upper Cretaceous (Campanian) of the USA and Canada, from a turtle potentially related to the genus *Adocus*, and potentially freshwater-adapted.

Testudoolithus oospp. described herein differs from *T. jiangi* and *T. zelenitskyae* in the shell unit height-to-width ratio, which is 2.5:1 to 3:1 for *T. jiangi* and 3.15:1 to 5.5:1 for *T. zelenitskyae*. It differs from *T. rigidus* in having a thicker eggshell, 220–240 μm in thickness, and in the shell unit height-to-width ratio,

which is 2:1 for the latter. This specimen is smaller than *T. lord-howensis*, which measures 53.9 mm in diameter.

RESULTS

Distribution of the eggs inside the shell

The eggs are preserved randomly inside the visceral cavity of the gravid turtle, from the base (ventral surface) of the carapace to the top (dorsal surface) of the plastron. Some of them are visible on the most dorsal and ventral surfaces of the carapace and plastron in places where the bones have an extremely low degree of ossification, such as at the most lateral portions of costal bones, at sutures between them, or at the fontanelles (Fig. 1A–D). The whole turtle specimen was found fractured in several blocks, which allowed observation of the eggs from different views, as well as study of their preservation (Cadena *et al*. 2018, fig. S1B–E*)*. Four

eggs were collected separately or were dislodged during preparation of the specimen (Fig. 1E). The external surfaces of the eggshells exhibit a micropitted texture (Fig. 1F).

Microstructure and mineralogical composition of the eggshell

We studied a thin section of one of the eggs of *Desmatochelys padillai* using light and polarized transmitted microscopy, which showed that the preservation of the eggshell varies along the surface of the egg, with the presence of calcite crystals and other more iron-rich carbonates (siderite) and haematite (Fig. 2A, B). The calcitic regions of the egg better preserve the details of the eggshell units, superficial pores, and a thin layer that we interpret as the remains of the eggshell membrane. The shell units are slightly taller than wide, with a height-to-width ratio that varies from 1.1:1 to 1.3:1 (Fig. 2C–H). Accretion lines in the eggshell are very abundant at the
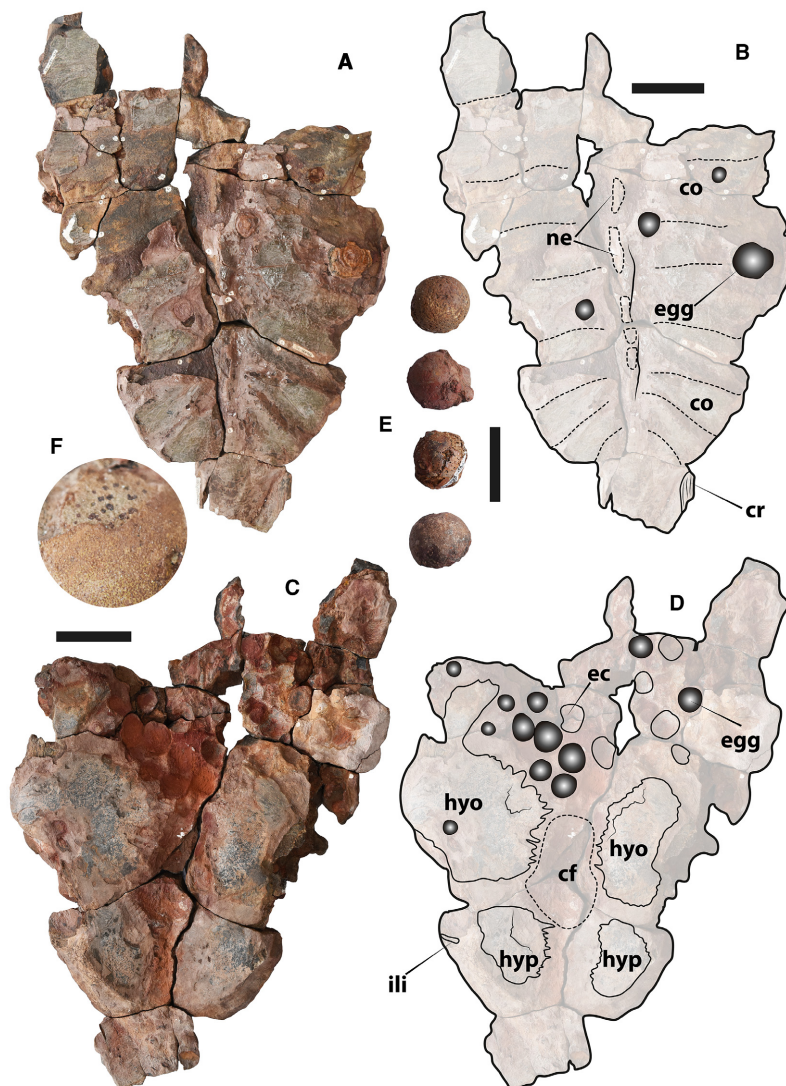
FIG. 1. *Desmatochelys padillai* FCG-CBP-75 and the ootaxon *Testudoolithus* oospp. A–B, carapace in dorsal view, showing some eggs between costal bones or where they are poorly preserved. C–D, plastron in ventral view, showing several eggs and cavities left by the eggs. E, four isolated eggs of *T.* oospp. F, close-up of one of the eggs of *T.* oospp. showing the micropitted texture of the eggshell surface. *Abbreviations*: cf, central fontanelle; co, costal bone; cr, costal rib; ec, egg cavity; hyo, hyoplastron; hyp, hypoplastron; ili, ilium; ne, neural bone. Scale bars represent 10 cm (horizontal bar; A–D); 5 cm (vertical bar; E); 2 cm (horizontal bar; F). Colour online.

FIG. 2. Thin section of one of the eggs of *Testudoolithus* oospp. A–B, whole-egg view, with a portion of a costal bone showing its interior and part of its cancellous bone. C–D, close-up of one of the regions of the eggshell (left rectangle in B), showing a remaining portion of the egg membrane. E–F, close-up of one of the regions of the eggshell (right rectangle in B), showing pores and accretion lines around them. G–H, close-up of one of the regions of the eggshell, showing clearly defined shell units, pores, accretion lines and a potential organic core. *Abbreviations*: al, accretion line; c, cavity; ca, calcite; cb, cancellous bone; cv, cavern; es, eggshell; ic, internal cortex; lca, large calcite crystals; mic, micrite; oc, organic core; pc, pore core; si, siderite; smr, shell membrane remain; su, shell unit. Scale bars represent: 1 cm (A, B); 500 μm (C–H). Colour online.
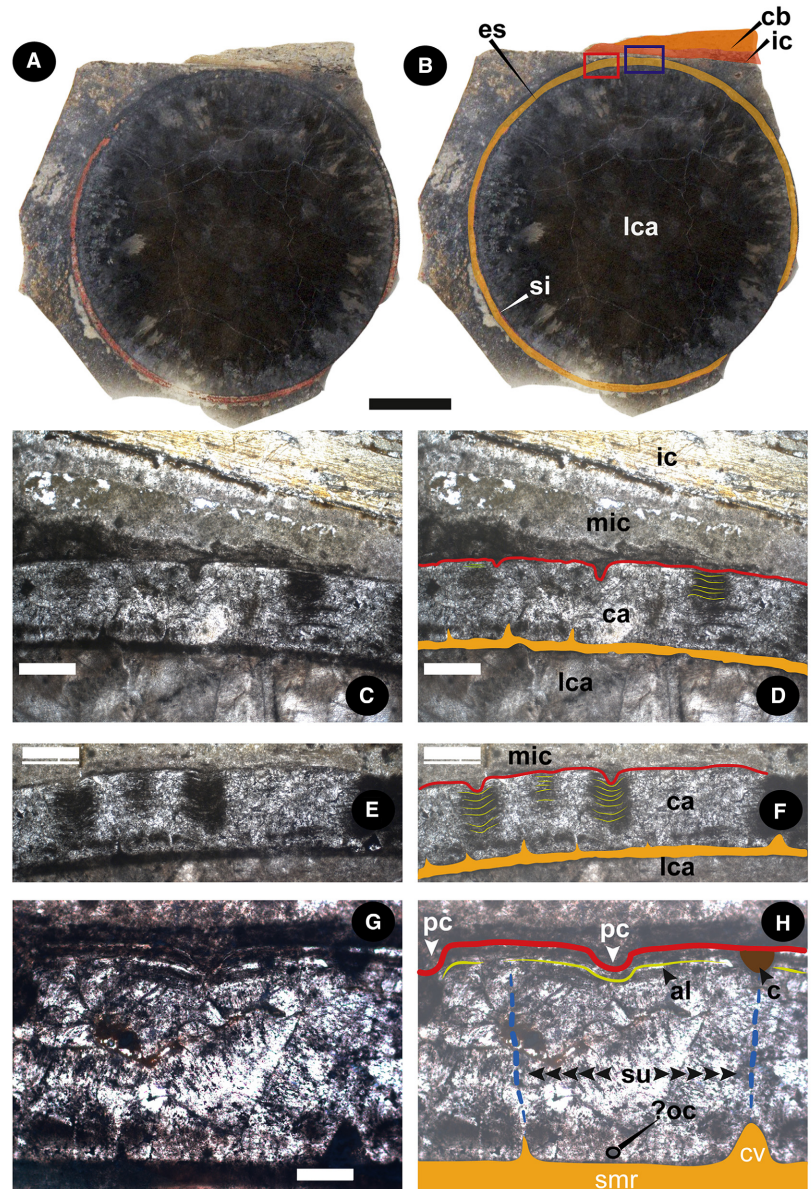

outer portion of the shell units and at the regions where the pores are located (Fig. 2D, F, H). Pores are well defined with an opening diameter between 180 and 215 μm at the eggshell surface (Cadena *et al.* 2018, fig. S2A–D). Some of the eggs are filled with recrystallized calcite (Fig. 2A) and/or a ferroaluminosilicate mineral, which gives them an orange-reddish colour (Fig. 1C).

Three-dimensional exploration and egg counting

Computer tomography (CT-scan) study of the whole gravid turtle revealed the preservation of at least 48 eggs inside the visceral cavity (Fig. 3A, B). Some of the eggs are internally empty, others have a moderate rim of

calcite crystals, and the remaining eggs exhibit their yolk-albumen regions fully recrystallized or with some empty internal pockets (Fig. 3C). Thus, we analysed each of the eggs using the magnification tool of the iQ-VIEW software and found that some of them underwent breaking of their eggshells, with broken fragments preserved toward the inner portion of the egg. We also measured all preserved eggs, and established that their diameter range varied from 32.2 to 42.8 mm (e.g. Fig. 3D).

CT-scanning also allowed us to better outline the shell bones, particularly the costal and plastral elements of the turtle. The thickness of the costal bones varied between 0.5 and 0.8 cm, and the plastral elements ranged from 0.4 to 0.6 cm (Fig. 3E). Additional features of the shell are also visible, such the central regions of the last series of
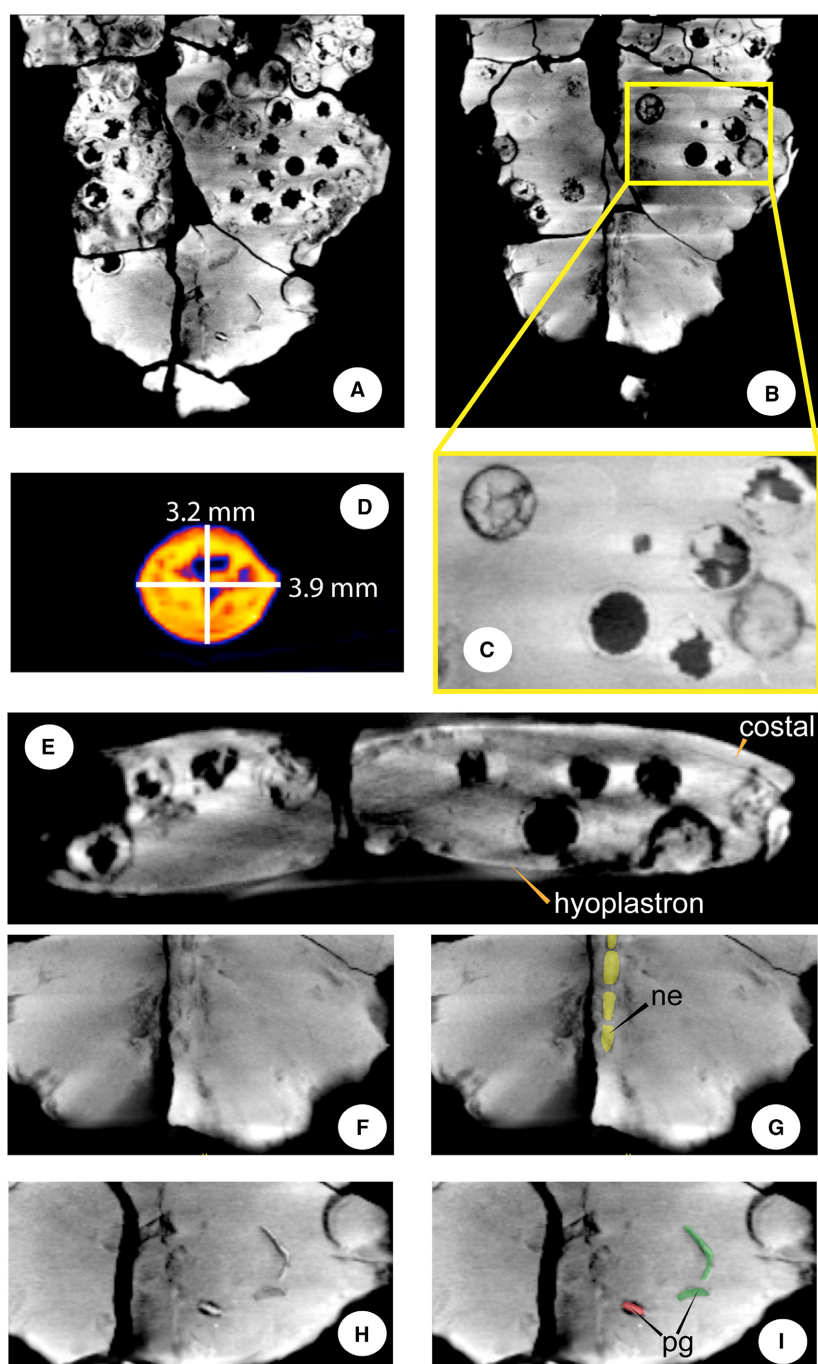
FIG. 3. Computer tomography images of *Desmatochelys padillai* specimen FCG-CBP-75 and its eggs, *Testudoolithus* oospp. (see also Cadena *et al.* 2018, videos S1–3). A–C, two horizontal slides and a close-up of the rectangle in B. D, CT image of one of the isolated eggs using the colour mapping tool in iQ-VIEW to visualize cavities inside the egg, as well as measurements of it. E, one of the coronal slides, showing the eggs, as well as portions of one of the costal and hyoplastron bones. F–G, close-up of the posterior region of the shell, where one of the horizontal slides allows recognition of the posterior series of neural bones. H–I, close-up of the posterior region of the shell, with one slide that allows us to identify portions of the pelvic girdle bones. *Abbreviations*: ne, neural bones; pg, pelvic girdle bones. Colour online.

neural bones (Fig. 3F–G), portions of the pelvic girdle (Fig. 3H–I, Cadena *et al.* 2018, fig. S1B–C), and potential fragments of isolated peripheral bones (Cadena *et al.* 2018, fig. S1D–E).

Eggshell fine-scale observation and elemental composition

We studied the eggshell of one of the eggs from the top (exterior region) to the base (near the shell membrane) (Fig. 4A, B) using SEM, and established that the eggshell lacks well-defined structures or an arrangement of needle-like crystals (aragonite) in the shell units or shell membrane region, with only intercrossing crystals of calcite. Even though an aragonite eggshell is considered to be an autapomorphy of the Testudines (Lawver 2017), both forms of calcium carbonate (calcite and aragonite) can constitute the eggshells of some extant sea turtles (Baird & Solomon 1979; Solomon & Watt 1985). Thus, it is possible that the original mineralogical composition

of the eggs of *Desmatochelys padillai* was calcite rather than aragonite and that the fossil diagenetic processes that affected these eggs involved recrystallization of the original calcite, transformation of calcite to siderite (iron-rich carbonate), and haematite growth (Fig. 4; Cadena *et al.* 2018, fig. S4A–B); other fossil ootaxa exhibit similar diagenetic alterations (Lawver & Jackson 2014).

For the elemental analysis of the eggshell of *Testudoolithus* oospp., we used SEM coupled with energy-dispersive X-ray spectroscopy (EDS). We observed compositional variation from the top to the base of the eggshell in the sample. The top of the eggshell is mostly composed of abundant concentrations of calcium, nitrogen, oxygen and carbon, and traces of aluminium (Table 1; Fig. 4B, C); the latter is abundant only at the most external layer of the eggshell. The occurrence of calcium, carbon and oxygen support the presence of calcium carbonate (calcite form, based on the geometry and morphology of the crystals, resembling character 1; Lawver 2017). The middle portion of the eggshell is exclusively dominated by calcium, carbon, oxygen and nitrogen; again, we interpreted this region as calcium carbonate-dominated (Table 1; Fig. 4B, D). The base of the eggshell is dominated by carbon and oxygen, with minor contents of calcium, iron, silica, aluminium and magnesium (Table 1, Fig. 4B, E). This high carbon content was also detected at the ventral surface of the eggshell (egg membrane surface), together with some small circular bumps, which seem to represent the organic cores where the shell units originated (Table 1; Cadena *et al.* 2018, fig. S5A–B). We hypothesize that the occurrence of high carbon content at the ventral surface together with nitrogen in the middle portion of the eggshell could indicate that organic remains of the original egg membrane may have been preserved in some of these eggs. Richer carbon content at the ventral surface of the eggshell (membrane-inner fibrous layer) in the eggs of the extant leatherback sea turtle *Dermochelys coriacea* has been also reported (Areekijseree *et al.* 2013). Testing this hypothesis based on specific egg antibodies and/or mass spectrometry is beyond the scope of this project, but we hope it will be addressed in future studies.

In addition to the analysis of the eggshell composition, the rock matrix covering the carapace, the plastron and some of the eggs was also analysed using SEM-EDS, which revealed that its composition was dominated by silica, iron, oxygen and aluminium (indicating a ferroaluminosilicate mineral), without any traces of nitrogen or carbon (Table 1; Cadena *et al.* 2018, fig. S5C–D), excluding potential contamination of the entire specimen, shell, eggs and rock matrix by an external organic source.

To evaluate the fossil diagenesis of the eggshell of *Desmatochelys padillai* studied under SEM-EDS, we analysed the same sample using cathodoluminescence. We found that the central region (Fig. 4F) and ventral surface (Cadena *et al.* 2018, fig. S6A) of the eggshell exhibited purple to cyan fluorescence corresponding to wavelengths between 400 and 500 nm (Fig. 4F). Chicken eggshell and some extant and fossil turtles exhibit dull blue to dark green luminescence (England *et al.* 2006; Lawver & Jackson 2016, 2017) which in the case of fossils indicates minimal alteration of the original composition. In contrast, bright orange to red luminescence indicates the opposite (high alteration and replacement) (Lawver & Jackson 2016, 2017).

DISCUSSION

*Differences between extant and extinct marine turtle egg development*

Eggs of extant sea turtles exhibit highly pliable (flexible) shells, shell membranes thicker than the eggshell, shell units with greater width than height, collapsible shells and visible pores (missing in *Caretta caretta*) (Baird & Solomon 1979; Hirsch 1983; Solomon & Watt 1985; Schleich & Kästle 1988; Nuamsukon *et al.* 2009; Lawver & Jackson 2014; Sikiwat *et al.* 2015) (Table 2). In contrast to all of these characteristics, the fossil gravid specimen of *Desmatochelys padillai*, which was also a fully marine-adapted turtle (Cadena & Parham 2015), had rigid eggshells, similar to those exhibited by some extant and fossil freshwater and terrestrial turtles.

Similarities between extinct and extant lineages of sea turtles can also be established from the gravid fossil specimen of *Desmatochelys padillai* described herein. In addition to the egg size and shape (circularity), the high number of eggs (52 in total preserved for *D. padillai*) is similar to the typical numbers of eggs in extant nests of *Dermochelys coriacea* (50–100) (Hendrickson 1980; Wallace *et al.* 2006), which is the extant taxon most closely related to the Protostegidae phylogenetically (Hirayama 1998; Cadena & Parham 2015; Evers & Benson 2018). This similarity indicates that marine protostegid turtles were also ecologically more *r*-selected vertebrates that produced many offspring, and thus increased the possibility of survival of at least few individuals under high rates of mortality due to climatic or ecological factors (Pianka 1970; Hendrickson 1980).

The morphology of the eggs of the fossil gravid specimen of *Desmatochelys padillai* described herein resembles that of the rigid-shelled eggs of the gravid *Adocus* sp. specimen from the late Campanian Judith River Group of Canada (Zelenitsky *et al.* 2008). Therefore, it appears that this particular egg trait (rigid shell) was common to

different groups of Cretaceous cryptodiran turtles, as *Adocus* is more closely related to the Trionychia than to the Protostegidae (*D. padillai* described here). In contrast to the large number of eggs laid by extant marine turtles, and *D. padillai*, a total of 19 eggs have been estimated for the *Adocus* sp. specimen (Zelenitsky *et al.* 2008).
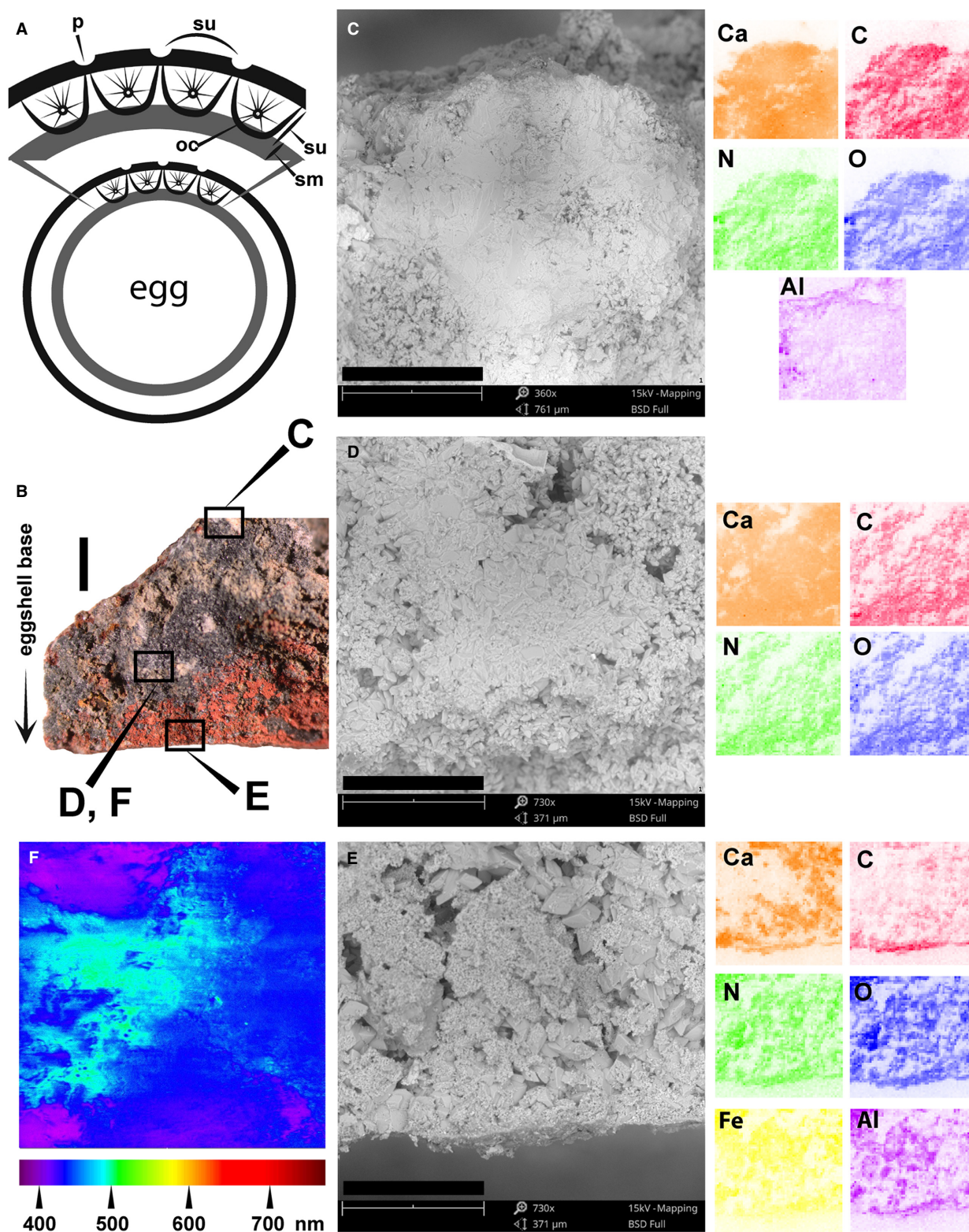
With the establishment of the egg characteristics of the most basal known protostegid *Desmatochelys padillai*, a significant question emerges: why do the eggs of this fully marine-adapted turtle differ so much from those of extant marine turtles? We hypothesize that a combination of phylogenetic and palaeoecological adaptive factors could be involved.

Does phylogeny control egg morphology in marine turtles?

Fossil eggs of *Desmatochelys padillai* show that some egg features have characterized Chelonoidea total-group *sensu stricto* Evers & Benson (2018) for at least the last 125 myr. These are spherical eggs and large clutches of >40 eggs, which we suggest here as synapomorphic for the group (Fig. 5). Instead, the occurrence of rigid-eggs in the oldest chelonioid (*D. padillai*) indicates that either this characteristic is homoplastic and related to particular environmental adaptations of Cretaceous marine chelonioids (Protostegidae) as we discuss in detail in the following paragraph, or that this character represents a reversal in the Protostegidae lineage. More gravid marine fossil turtles or fossil eggs indisputably attributed to chelonioids will have to be found to test or support any of these hypotheses, and to track in detail the egg reproduction strategies and changes of the group during the Mesozoic and Cenozoic.

Role of palaeoenvironment in the eggs of extinct protostegid turtles

The development of rigid eggs in the extinct marine turtle *Desmatochelys padillai* could have been the result of egg-embryo requirements and adaptations according to the physical attributes of the environment and nesting sites, such as: water saturation and gas exchange of the sand where the nest was created; the depth and temperature at which the nest was located; the hydric, thermal and respiratory properties of soil; and the type of sediment at the nesting site (mineral composition, albedo and grain size). All of these factors have been reported as playing key physiological roles in the development of the embryo, particularly in terms of its metabolic rate,

blood chemistry and the diffusion of gases through the eggshell and membranes (Ackerman & Prange 1972; Ackerman 1980, 1997; Hendrickson 1980; Bilinski *et al.* 2001; Wallace *et al.* 2006; Schneider *et al.* 2011; Warner *et al.* 2011).

The thick and rigid structure exhibited by the eggs of the gravid specimen of *Desmatochelys padillai* could also have favoured the development of the embryos,

TABLE 1. Elemental analysis of the eggshell of one of the eggs of *Testudoolithus* oospp. obtained from SEM coupled with an energy-dispersive X-ray spectroscopy (EDS).

Element symbol	Atomic concentration	Weight concentration	Stoichiometric concentration
Top (exterior region) of the eggshell			
O	53.53	49.68	
Ca	9.25	21.50	19.91
N	21.36	17.35	45.96
C	15.39	10.72	33.12
Al	0.47	0.74	1.02
Middle of the eggshell			
O	54.06	49.50	
Ca	10.30	23.63	22.43
N	20.80	16.67	45.29
C	14.83	10.19	32.28
Base (near shell membrane) of the eggshell			
O	53.24	52.63	
C	37.75	28.01	80.72
Fe	1.76	6.08	3.77
Ca	1.58	3.91	3.38
Si	2.17	3.77	4.64
Al	2.05	3.42	4.39
Mg	1.45	2.17	3.10
Eggshell ventral surface			
O	46.04	47.44	
C	46.74	36.15	86.61
Ca	4.43	11.44	8.21
Si	1.57	2.83	2.90
Al	1.23	2.14	2.28
Rock matrix covering the shell and some eggs			
O	61.24	38.82	
Fe	16.53	36.57	42.64
Si	19.35	21.53	49.91
Al	2.89	3.09	7.45

FIG. 4. SEM-EDS imaging-elemental analysis and cathodoluminescence of one of the eggshells of *Testudoolithus* oospp. described herein. A, turtle eggshell microstructure (modified from Lawver & Jackson (2014)). B, general view of the eggshell fragment analysed by SEM without any coating, rectangles indicate the regions of images C–E where elemental analysis were performed: C, top of the eggshell showing rich content of aluminium; D, middle portion of the eggshell, predominantly calcium carbonate; E, base of the eggshell, showing high carbon content at the egg membrane-eggshell contact; greater intensity of colour indicates higher elemental concentration. F, cathodoluminescence showing bright purple to cyan, corresponding to wavelengths between 400 and 500 nm. Details of the EDS elemental analysis of the three regions of the eggshell are shown in Table 1. *Abbreviations*: Al, aluminium; C, carbon; Ca, calcium; Fe, iron; N, nitrogen; O, oxygen, oc, organic core; p, pore; sm, shell membrane; su, shell unit.

TABLE 2. Comparison of egg characteristics of some extant marine turtles with those of *Desmatochelys padillai* and its ootaxon *Testudoolithus* oospp.

Species	Egg shape	Egg size (mm)	Shell morphotype, composition	Shell unit height-to-width ratio	Shell thickness (µm)
Lepidochelys kempii (Kemp's ridley)	Spherical	38	Pliable, egg membrane ≫eggshell (aragonite)	1:2	120–400
Caretta caretta (loggerhead)	Spherical	39–42	Pliable, egg membrane ≃ eggshell (aragonite)	Loose crystallic mass	80–140
Chelonia mydas	Spherical	–	Pliable, egg membrane ≃ eggshell (aragonite or calcite)	1:2	200
Eretmochelys imbricata	Spherical	–	Pliable, egg membrane ≃ eggshell (aragonite)	1:3	235
Desmatochelys padillai (fossil protostegid)	Spherical	32.2–42.8	Semipliable–rigid, egg membrane ≪eggshell (calcite–siderite)	1.1:1–1.3:1	785–820

Data for *Lepidochelys kempii* taken from Hirsch (1983); for *Caretta caretta* from Schleich & Kästle (1988); for *Chelonia mydas* and *Eretmochelys imbricata* from Sikiwat *et al.* (2015).
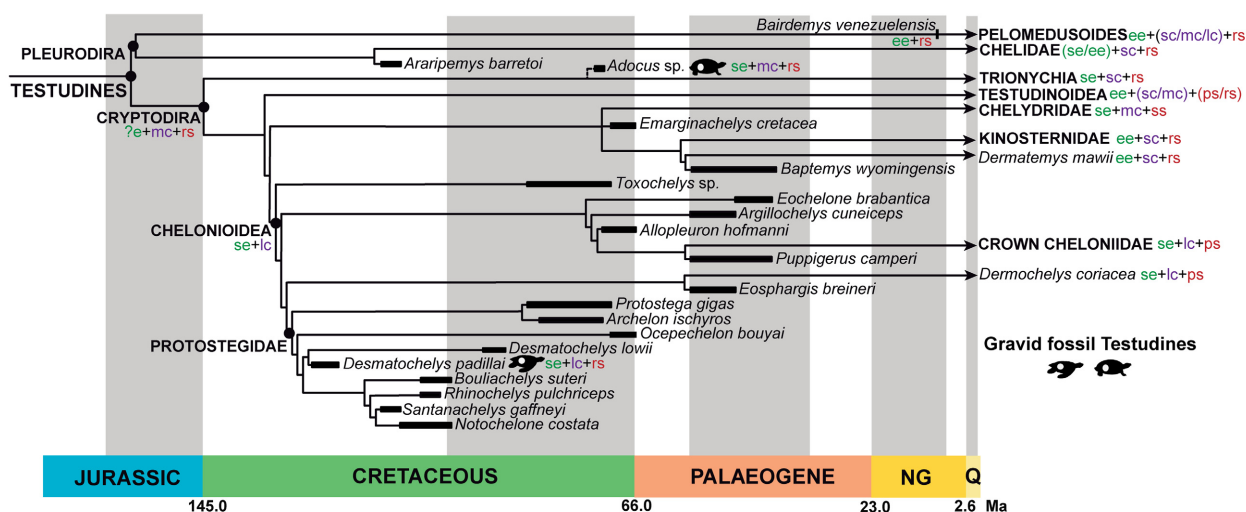
FIG. 5. Distribution of reproductive traits in Testudines. Phylogenetic tree is based and modified from Evers & Benson (2018). *Adocus* sp. was added as sister taxon of Trionychia following Zelenitsky *et al.* (2008). *Abbreviations*: egg shape (ee, elongate eggs; ?e unknown egg shape; se, spherical eggs) + clutch size (sc, small clutch size (<15 eggs); mc, medium clutch size (15–40 eggs); lc, large clutch size (>40 eggs)) + eggshell type (ps, pliable-shelled eggs; rs, rigid-shelled eggs; ss, semipliable-shelled egg); NG, Neogene; Q, Quaternary. Colour online.

providing them with a greater source of calcium, and making them more independent of water conductance and environmental temperature, but maintaining their gas permeability through the pores, as has been documented for similar eggs of some extant reptiles (Grigg & Beard 1985; Packard 1999; Bilinski *et al.* 2001). However, it is relevant to point out that maternal and abiotic controls on egg development and mortality in extant turtles have shown inconsistency of selection operating on egg size, nesting phenology and nesting behaviour (Warner

et al. 2010), which could also have occurred in extinct linages, such as protostegid turtles. Additionally, the rigid eggs of *Desmatochelys padillai* could have provided better protection against potential predators, such as small vertebrates and crabs, which are typical predators of extant sea turtle nests (Spencer 2002; Spencer & Thompson 2003; Bishop *et al.* 2011; Jackson *et al.* 2015) and also comprised components of the palaeoecology of the environments inhabited by *D. padillai* during the Early Cretaceous.

Fossil diagenesis and potential preservation of original egg components

The luminescence colours and intensity observed in the eggshell of *Desmatochelys padillai* allow us to conclude that its central and ventral surface regions suffered minimal diagenetic alteration. This result favours our hypothesis that the highly enriched carbon content on the ventral surface exhibited by the SEM-EDS analysis may be related to preservation of the remains of some of the original organic constituents, which can be tested in the future with immunological, Raman spectroscopy and mass spectrometry techniques.

DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.8br42vr

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